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Plenary Sessions

PLEN 1

The genetic and molecular basis of neurodevelopmental disorders

Joseph G. Gleeson

Howard Hughes Medical Institute and Rady Children's Institute for Genomic Medicine, University of California, San Diego, USA

To date most successes with next generation sequencing have been in the areas of recessive disease with parental consanguinity, or in de novo disease. Through targeted recruitment of families with pediatric brain disease, our lab has uncovered a range of novel genes underlying recessive, de novo and somatic diseases of the brain including mutations underlying autism, epilepsy and intellectual disability. In collaboration with physician colleagues from the Middle East and colleagues in Europe and the US, we have uncovered novel causes for over 200 individual genetic conditions. Animal and cell biological modeling have focused on mechanisms involving cellular metabolism, non-coding RNA, and cytoskeletal pathways. A surprising discovery, based entirely on the discovery of the cause, is that many of these conditions have potential points of treatment that can be approached by repurposing existing drugs or through nutritional supplementation. Recent efforts are focused on preventing disease through improved genetic diagnosis, detection of mutations in carriers, and identifying sources of somatic mutations.

PLEN 2

Genetic mosaicism in human diseases

Leslie G. Biesecker

National Human Genome Research Institute, NIH, Bethesda, MD USA

The phenomenon of mosaicism has long been recognized in many species, including humans. One of the earliest recognitions was in dermatology, through the readily observable patterns of skin variegation. It is of central importance for geneticists not only as an explanation for variegated patterns, but also for reduced penetrance, variable expressivity, and critically, for recurrence risk estimation. The karyotype was the first genomic assay that provided a molecular basis for mosaicism. SNP-chip assays for copy number variation extended this observation to mosaic copy number variants. Recently, next-generation sequencing has made the detection of single nucleotide detection feasible and routine, though methods are still being developed to refine and routinize this practice. Examples of the importance of these assays for diagnosing and managing human disease will be discussed. The wider implications of mosaicism are that it raises profound questions about the taxonomy of human disease. Historically, the taxonomy of human disease was phenotype-centered. Mosaicism challenges this approach to disease taxonomy and suggests that new approaches and new thinking will be needed going forward.

Sessions

Talk nach 12

Zwei Jahre Europäische Referenznetzwerke (ERNs) für seltene Krankheiten: Warum die Humangenetik aktiv dabei sein muss!

Moderation: Holm Graessner (Tübingen), Thomas Eggermann (Aachen)

Seltene Krankheiten erfordern eine hochspezialisierte Gesundheitsversorgung. Die EU will durch eine Reihe von Maßnahmen erreichen, dass Kräfte und Mittel zur Diagnose und Behandlung solcher Erkrankungen gebündelt werden und Fachwissen über Grenzen hinweg ausgetauscht wird. Mit den Europäischen Referenznetzwerken (ERNs) sollen Gesundheitsdienstleister in der EU, die Expertise und Erfahrungen auf diesem Gebiet haben,

vernetzt werden. Formale Basis der ERNs ist die Richtlinie Patientenmobilität der Europäischen Union (2011/24/EU). Mit Hilfe der ERNs sollen Patientinnen und Patienten in allen EU-Mitgliedstaaten den Zugang zur Diagnose und Behandlung seltener und hochkomplexer Erkrankungen ermöglicht und erleichtert werden. Der Aufbau von ERN begann 2016 mit der ersten Aufforderung der Europäischen Kommission zur Einreichung von Vorschlägen für die Errichtung von ERN, insgesamt wurden 24 ERNs mit ca. 900 hochspezialisierten Einheiten aus 370 Krankenhäusern in 26 Staaten gegründet. Diese ERNs decken thematisch i. d. R. Organsysteme ab und setzen sich im aus klinisch angewandten und diagnostischen Partnern zusammen.

Als interdisziplinäres medizinisches Fach mit Schwerpunkt bei seltenen Erkrankungen spielt die Humangenetik allerdings nur in etwa einem Viertel der ERNs eine wesentliche Rolle. Aufgrund ihres auf den ersten Blick Hauptschwerpunkts in der klinischen Versorgung wurde die Relevanz der ERNs möglicherweise nicht erkannt. Dies gilt insbesondere für Deutschland. In den 24 ERNs sind nur acht deutsche humangenetische Einrichtungen vertreten (jeweils drei in GENTURIS (Genetic cancer risk syndromes), drei in ITHACA (Rare congenital malformations and rare intellectual disability disorders), eins in ERN PaedCan (ERN for Paediatric Oncology), eins in EndoERN (European Reference Network on Rare Endocrine Conditions)). Die Chancen der ERNs liegen in der europäischen Vernetzung, Wissensgenerierung und Wissensverteilung sowie eHealth basierter Fallkonsultation zum Wohle des Patienten.

Die Veranstaltung gibt einleitend einem grundsätzlichen Überblick über die Struktur, Inhalte und Ziele der ERNs. Einen weiteren Schwerpunkt bildet ein Überblick über Teilnahmemöglichkeiten in existierenden (und zukünftigen) ERNs sowie die perspektivischen Vorteile der bearbeiteten Themen (u. a. eHealth, Registries, networking). An einzelnen ERNs, an denen die Referenten beteiligt sind, werden die persönlichen Erfahrungen geschildert, aber auch die Chancen für den jeweiligen Standort im Bereich Diagnostik und insbesondere das Potential für Forschungsaktivitäten und -förderungen dargelegt.

Die Veranstaltung soll zu einem verstärkten Einsatz der deutschen Humangenetiker in ERNs motivieren, die wie die ZSEs auf nationaler Ebene die mittel- und langfristige Voraussetzung für eine relevante Beteiligung des Fachs an der Forschung und Patientenversorgung seltener Erkrankungen ist.

Referenten:

- H. Graessner (Tübingen): Was muss man über ERNs wissen, und welche Chancen bieten sie für die Humangenetik?
- E. Holinski-Feder (München): Warum sind die ERNs für die Forschung und das Einwerbung von Drittmitteln wichtig?

Pro & Contra I

Die genetische Sprechstunde in der Zukunft

Moderation: Prof. Dr. Christian Netzer, Institut für Humangenetik, Uniklinik Köln

Knapp 300 deutsche Fachärzte und Fachärztinnen für Humangenetik sehen sich einer geradezu explosionsartigen Ausweitung genetischer Testmöglichkeiten gegenübergestellt. Diese katapultieren das Fach in zunehmendem Maße mitten in das Herz der klinischen Krankenversorgung: Onkologen, Kinderärzte, Pränatalmediziner – alle benötigen für immer mehr ihrer Patienten niedrigschwellige humangenetische Versorgungsangebote.

Können wir das mit den wenigen vorhandenen Spezialisten und mit dem derzeitigen Konzept der genetischen Beratung alles leisten? Müssen wir uns neu erfinden und/oder unsere Patientenversorgung effizienter gestalten? Brauchen wir die Unterstützung anderer (neuer) Berufsgruppen wie die der „Genetic Counsellors“? Oder würde dies den Abgang auf unser kleines und in Deutschland berufspolitisch immer gefährdetes Fach einleiten? Wie gehen andere Länder, beispielsweise die USA, mit den vom technologischen und wissenschaftlichen Fortschritt getriebenen Herausforderungen für die humangenetische Patientenversorgung um?

Diese Fragen wollen wir mit Impulsreferaten beleuchten und anschließend mit den Referenten und mit dem Publikum diskutieren. Wir hoffen auf eine kontroverse Pro- und Contra-Debatte.

Referenten:

- Prof. Dr. Dr. Ute Moog, Leiterin der Poliklinik am Institut für Humangenetik der Universität Heidelberg und Sprecherin der Kommission für Grundpositionen und Ethische Fragen der GfH.
- Prof. Dr. Christian Schaaf, designierter Direktor des Instituts für Humangenetik der Universität Heidelberg und Visiting Professor am Baylor College of Medicine und am Jan and Dan Duncan Neurological Research Institute, Houston (USA)
- N.N.

Pro & Contra II

Gentests bei multifaktoriellen Erkrankungen

Moderation: Prof. Dr. Johannes Schumacher, Zentrum für Humangenetik, Uniklinikum Marburg

Die Verfügbarkeit immer größerer Datensätze für genomweite Assoziationsstudien (GWAS) lassen in zunehmenden Maße Polygenic Risk Score-(PRS-)Analysen zu. Hierbei werden die Effekte von einzelnen Risikovarianten für multifaktorielle Phänotypen gemeinsam ausgewertet und analysiert. Damit erlauben PRS-Analysen auch immer zuverlässiger, Risiken für multifaktorielle Erkrankungen bei gesunden Personen vorherzusagen.

In vorliegender Veranstaltung sollen die Vor- und Nachteile von PRS-Analysen kritisch diskutiert werden. Unter anderem wird es darum gehen, ob und für welche Phänotypen PRS-Untersuchungen angeboten werden sollten (u. a. actionable und nicht actionable Erkrankungen). Zudem wird diskutiert, in welchem Rahmen PRS-Analysen durchgeführt werden sollten. Gegenstand der Diskussion wird auch sein, inwieweit das Gendiagnostikgesetz (GenDG) in seiner jetzigen Form PRS-Analysen adäquat berücksichtigt. Sollten solche Untersuchungen nur im Rahmen einer genetischen Beratung angeboten werden?

Diese Fragen sollen mit Impulsreferaten beleuchtet und anschließend mit den Referenten sowie dem Publikum diskutiert werden.

Vortragende:

- Prof. Dr. Markus Nöthen, Institut für Humangenetik, Universitätsklinikum Bonn
- Prof. Dr. Michael Krawczak, Institut für Medizinische Informatik und Statistik, Universitätsklinikum Schleswig-Holstein

EDU 1

Knifflige Mutationen, die wir fast verpasst hätten

Moderation: Bernd Wollnik (Göttingen)

Die sichere Interpretation von genetischen Varianten stellt eine Kernexpertise der Humangenetik dar und ist essentiell für eine akkurate molekulare Diagnose. In den vergangenen Jahren hat die Umstellung auf NGS-basierte Hochdurchsatzsequenzierungen sowohl bei wissenschaftlichen Genidentifizierungsstudien als auch im Rahmen molekularer Diagnostik dazu geführt, dass täglich eine immense Anzahl von Varianten eingeschätzt und interpretiert werden muss. Unsere Erfahrungen der vergangenen Jahre haben uns dabei gezeigt, dass es durchaus sehr knifflige Mutationen gibt, die zum Beispiel aufgrund

- ihrer Position im Gen,
 - ihres unsicheren Effektes auf das Transkript/Protein oder
 - einer gewebspezifischen Expression
- schwierig zu finden und auch nicht einfach einzuschätzen sind.

Im Rahmen dieser EDU werden mehrere Experten auf diesem Gebiet eine Vielzahl an diagnostischen und wissenschaftlichen Beispielen zeigen, in denen die Identifizierung ursächlicher Mutationen eine große Herausforderung darstellte.

Vortragende:

- u. a. Rami Abou Jamra (Leipzig), Peter Bauer (Rostock), Alexander Hoi-schen (Nijmegen), Silke Kaulfuß (Göttingen), Dagmar Wieczorek (Düsseldorf)

EDU 2

„Zeig mir Dein Gesicht!“ – Computergestützte Syndromdiagnostik

Moderation: Peter Krawitz (Bonn), Dagmar Wieczorek (Düsseldorf), Jean Tori Pantel (Berlin)

Die computer-gestützte Gesichtserkennung hat in jüngster Zeit aufgrund der Verwendung künstlicher neuronaler Computersoftware-Netzwerke, sogenannter Deep-Learning-Entwicklung und der Verfügbarkeit für großer Datensätze große Fortschritte gemacht.

In dieser EDU-Session werden wir mit unterschiedlichen Softwaretools wie z. B. Face2Gene (FDNA) oder FaceNet (Minerva Initiative) arbeiten, um die Ähnlichkeiten von Portraitfotos anhand von Hunderten wohl definierten Dysmorphie-Syndromen zu vermessen. Neben dem vom Menschen gesteuerten und überwachten Maschinenlernen von künstlichen Intelligenzsystemen, welches zur Lösung von Mehr-Klassen-Problemen (z. B. bekannter OMIM Krankheiten) geeignet ist, werden wir auch auf "nicht-überwachte" Lernansätze künstlicher Intelligenz eingehen, die die Suche nach neuen Phänotypen ermöglichen (clustering of unknown disease-phenotypes).

Der Workshop beschäftigt sich vor allem mit komplexen fazialen Dysmorphie-Fällen bekannter Diagnosen, die uns besonders gute Einblicke in die Funktion von Systemen mit künstlicher Intelligenz geben können. In dieser Sitzung ist es auch möglich, eigene Fälle zu besprechen. Hierzu bitten wir aber um eine Kontaktaufnahme vor Konferenzbeginn (pkrawitz@uni-bonn.de).

Die Quantifizierung dysmorpher Ähnlichkeiten ist auch die Grundlage der phänotyp-getriebenen Bioinformatik (siehe PP4-Kriterien der ACMG-Richtlinien). Wir werden das Konzept der Priorisierung von Exomdaten durch Bildanalyse (PEDIA) im Labor an Beispielfällen veranschaulichen.

QW Genetische Beratung

Qualitätsmanagement Genetische Beratung – Zentrale Rahmenbedingungen und zukünftige Gestaltung

Moderation: Friedmar Kreuz (Dresden), Dieter Schäfer (Frankfurt)

Vorgesehene Themen:

- NIPT/NIPD: Vorschläge zur künftigen Gestaltung & Konsequenzen aus der Umfrage der BVDH-Kommission Genetische Beratung und Klinische Genetik (F. Kreuz)
- Pilot-Ringversuch zur Qualitätssicherung der Humangenetischen Stellungnahme: Erste Ergebnisse (D. Schäfer)

QW Ringversuche

Über das Zusammenwirken von Ringversuchen und Leitlinien. Wer definiert die Standards humangenetischer Diagnostik in Deutschland und wer kontrolliert deren Einhaltung?

Moderation: Andreas Dufke (Tübingen), Dieter Gläser (Neu-Ulm), Jürgen Kunz (Hamburg), Simone Heidemann (Kiel)

In Deutschland werden bereits seit 1993 überregional Ringversuche für die externe Qualitätssicherung angeboten, die von erfahrenen Humangenetikern ehrenamtlich für derzeit zehn verschiedene Bereiche mit hohem persönlichem Engagement durchgeführt werden (siehe u. a. www.bvdh-ringversuche.de sowie www.emqn.org). Seit 2011 sind Umfang und Häufigkeit der Durchführung externer Ringversuche in der RiLiBÄK, Teil B, festgelegt.

Ebenfalls seit 2011 verfügt die Deutsche Humangenetik über eine eigene Normgebung in Form der S2k-Leitlinie "Humangenetische Diagnostik und genetische Beratung", in die auch die langjährigen Erfahrungen aus den Ringversuchen des BVDH und der EMQN eingegangen sind. Die konsensbasierte Leitlinienerstellung folgt einem von der AWMF (www.awmf.org) vorgegebenen, standardisierten Delphi-Verfahren (mehrheitliche Annahme der – nach Erstellung durch Expertengruppen und Möglichkeit zur Kommentierung durch alle GfH-Mitglieder – vorgegebenen Handlungsempfehlungen durch Mandatsträger beteiligter Fachgesellschaften).

Inzwischen haben im Rahmen der Akkreditierung und der Qualitätssicherungsmaßnahmen der Bundesärztekammer die Ringversuche zunehmend an Bedeutung gewonnen.

Die komplexe Verzahnung zwischen Leitlinie und Ringversuch führt immer wieder zu Unsicherheiten bei den Adressaten der Leitlinien bzw. den Ringversuchsteilnehmern. In kurzen Impuls-Referaten sollen die aktuellen Entwicklungen der S2k-Leitlinie und der Ringversuche dargestellt werden. Eine offene Diskussion zwischen Ringversuchsleitern, Ringversuchsteilnehmern und Mitgliedern der Leitlinienkommission ist erstrebenswert um Kompetenzen abzuklären und die künftige Zusammenarbeit zu fördern.

Oral History Workshop Zur Zeitgeschichte der Humangenetik

Moderation: Klaus Zerres, Aachen

- Dr. Birgit Nemecek (Heidelberg): Genetische Prävention. Konzepte von Früherkennung, Vorsorge und Reproduktion in der Bundesrepublik.
- Dr. Matthias Krischel (Düsseldorf): Geschichtsbewusstsein und historische Verantwortung in der deutschen Humangenetik
- Dr. Felicitas Söhner (Düsseldorf): „Natürlich ganz zentral“. Der medizinethische Diskurs zur humangenetischen Beratung in Deutschland, 1970–2010.

Symposia (S1–6)

S1-01

Pre-implantation genetic diagnosis: state-of-the-art

J. R. Vermeesch,

Centre for Human Genetics, KULeuven, Leuven, Belgium

Large scale whole genome and exome sequencing is uncovering a plethora of novel mutations that cause highly penetrant, early-onset, severe, or later-onset life-threatening dominant and recessive disorders. For couples who are known carriers of mutant alleles, preimplantation genetic testing (PGT) enables the detection of genetic disorders in embryos that have been fertilized in vitro, thereby avoiding their transmission to offspring. In addition, the age of pregnancy increases in Western countries, increasing the risks for aneuploidy conceptions. Traditional PGD methods require a mutation and family specific work-up. We and others have developed generic methods that can be readily applied for all transmitted genetic disorders. They are termed karyomapping and haplarithmisis. The methods reconstructs genome-wide haplotype architectures as well as the copy-number and segregational origin of those haplotypes by employing phased parental genotypes and deciphering WGA-distorted SNP B-allele fractions.

I will present the principles, the use and the results of its implementation since clinical implementation. The introduction of genome wide screening of embryos raised novel ethical questions. The principles guiding embryo selection and prioritization that are applied at our center according to the chromosomal content and mutational load of the embryos, will be presented.

I will present novel technical developments broadening the scope of use. In addition, I will present new insights in the origins and mechanisms leading to early meiotic and mitotic errors as well as on the origin of mixoploid and chimaeric embryos.

S1-02

Rapid whole-genome sequencing of critically ill infants

Stephen Kingsmore

Rady Children's Institute for Genomic Medicine, San Diego, USA

Genetic diseases are the leading cause of death in infants in North America, and in neonatal and pediatric intensive care units. Diagnosis of the genetic etiology of an infant's illness enables transition from generic treatment based on a clinical diagnosis to genomic or precision medicine. Since

disease progression can be very rapid in infants, genetic diagnosis and institution of genomic medicine must be equally rapid in order to optimize outcomes and minimize complications. Over the past seven years we have reduced the time from physician order to provisional genetic diagnosis to 19 hours and have examined the clinical utility and cost effectiveness of rapid precision medicine (RPM) in acutely ill infants. I will review the latest methodologic innovations in rapid whole genome sequencing, the results of clinical studies of RPM. In 2019 we will start a project to evaluate RPM in 10,000 acutely ill infants. I will review the goals and rationale for this project.

S1-03

Integration of personal genomics with health care

Katrina Goddard

Kaiser Permanente Center for Health Research, Portland, OR, USA

Increasingly, people have access to health-related information about themselves outside of the healthcare system, including direct to consumer (DTC) genomic tests. Healthcare systems also have a responsibility to be good stewards of resources, while providing high quality care using evidence-based approaches. What are the characteristics of an integrated system that incorporates DTC genomic tests while meeting the stewardship obligations of the healthcare system?

DTC genomic tests need analytical and clinical validity. The test provider should inform the customer about the meaning of their result and provide access to genetic counseling. Understanding includes knowing that a negative result is not protective and recognizing the chance for inaccurate results. Harms must be minimized, and consumer protections should address consent and privacy concerns. The process should ensure equity in access to testing so that health disparities are not created or exacerbated. Genomic information that is shared with the clinician should be relevant to patient care. Finally, healthcare systems need to be prepared to receive patients with DTC genomic test results.

I will discuss the promise and potential for DTC genomic tests to contribute to better outcomes for major health and reproductive issues in the general population.

S2-01

Hereditary predisposition to childhood (brain) tumors—an underappreciated challenge?

Stefan M. Pfister

Hopp Children's Cancer Research Center at the NCT Heidelberg (KITZ), Heidelberg, Germany

It has been well established that pathogenic germline mutations in genes like TP53, PTCH1, SUFU, APC, NF1, SMARCB1, SMARCA4, BRCA2 and the MMR genes are associated with an increased incidence of brain tumors. Most of them are associated with a specific type of brain tumor. It has been estimated that as many as 7–10% of all pediatric tumors (and brain tumors) are based on a highly penetrant germline mutation. However, this might still be an under-appreciation of this enormous clinical and scientific challenge. We have recently shown that ~20% of SHH-driven medulloblastomas have an underlying germline mutation in TP53, PTCH1, SUFU, BRCA2 or PALB2 when only focusing on known cancer predisposition genes. When then doing an unbiased genome-wide screen investigating all genes for truncating germline variants that are exceedingly rare in the normal population, we were able to identify another highly prevalent medulloblastoma predisposition gene, which we are currently further analyzing in terms of co-occurring mutations and functional impact. This new finding increases the rate of SHH medulloblastomas with a clear germline predisposition to ~30%. It is very likely that similar findings will also further increase this proportion in other pediatric cancers. We are trying to address this question by a genome-wide pediatric cancer germline analysis performed on all publicly available data.

S2-02

Germline variants in cancer

Eamonn R Maher

Department of Medical Genetics, University of Cambridge and Cancer Research UK Cambridge Centre, Cambridge, UK

The identification of the molecular monogenic disorders associated with cancer predisposition has provided fundamental insights into the pathogenesis of both familial and sporadic forms of cancers (e.g. the VHL tumour suppressor gene in the pathogenesis of clear cell renal cell carcinoma). To date clinical testing for inherited cancer predisposition syndromes has generally been phenotype-directed using specific gene panels. Comprehensive genetic analysis using whole genome sequencing (WGS) has the potential to detect pathogenic germline variants not detected by conventional clinical testing pathways (Am J Hum Genet. 2018;103:3–18) but also results in increased detection of VUSs and cases in which the clinical phenotype is atypical for the genetic findings. Interpreting the significance of genomic analysis results in such circumstances may require a multifaceted “omic” analysis approach that can aid determination of variant pathogenicity and delineation novel genotype-phenotype correlations (e.g. J Clin Endocrinol Metab. 2017;102:4013–4022; JCO Precision Oncology 2018 <https://doi.org/10.1200/PO.17.00191>).

S2-03

Predicting leukemia risk in healthy individuals

Liran I. Shlush^{1,2}

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Acute myeloid leukemia (AML) is a devastating disease especially among the elderly. Most AML cases present after a chronic latent phase of age-related clonal hematopoiesis (ARCH), yet while the prevalence of ARCH among the elderly is high (~30%), AML remains a rare event. Recently, we succeeded to predict pre-AML cases seven years prior to diagnosis with a sensitivity of ~40% and a specificity of 98.5%. The most accurate early diagnosis was for cases with mutations in the spliceosome. For these patients we have several drugs that might be useful in AML prevention and clinical trials for AML prevention are under preparation.

The future of AML prevention will rely on continuous improvement in sensitivity and specificity of the careening assays that will take into account not just somatic mutation data. Future studies on AML prevention should focus not only on the intrinsic properties of the preleukemic mutations but also on the differentiation problem and the changes in the bone marrow microenvironment.

S3-01

Ultra-long sequencing methods aid assembly of human genomes and microbes

Nicholas Loman

Institute of Microbiology and Infection, School of Biosciences, University of Birmingham, Birmingham, UK

End to end chromosomal assemblies of nearly any species are nearly ready for prime-time due to recent advances in sequencing technologies. We have explored the utility of the Oxford Nanopore range of sequencers (Flongle, MinION, GridION and PromethION) to generate ultra-long read genomic datasets from a range of organisms including humans and microbes. By using a combination of classical DNA extraction techniques, optimised to maintain fragment length, and a modified transposase library preparation method we are able to routinely generate datasets with read N50s of >100 kb, with maximum read lengths between 1 and 2 megabase. These results suggest there is no intrinsic read length limit associated with Oxford Nanopore sequencing, suggesting whole-chromosome-spanning reads are possible. Such “ultra-long” read datasets dramatically improve contig-level statistics following de novo assembly, such as in the example

of the human cell line NA12878. Ultra-long read approaches have great promise for human genomics, however DNA extraction remains challenging for many sample types and a bottleneck to large-scale human genome sequencing efforts. I will discuss some of the potential options for overcoming these challenges and our new Wellcome Trust funded “Long Read Club” (longreadclub.org).

S3-02

Multi-platform discovery of haplotype-resolved structural variation in human genomes

Mark Chaisson

USC University of Southern California, Los Angeles, USA

As whole genome sequencing is being applied to large disease studies, researchers are faced with the challenge of detecting a wide range of variation, including large insertions, deletions, and rearrangements called structural variation (SV) with short read high-throughput sequencing (HTS) data. It is common use the consensus of multiple algorithms to detect SV with minimal false discovery, however the extent of missing variation is unknown. To address this, we applied a suite of approaches: long-read, short-read, strand-specific sequencing technologies, optical mapping, and variant discovery algorithms to comprehensively analyze three human parent-child trios to define the full spectrum of human genetic variation in a haplotype-resolved manner. We identify 818,054 indel variants (<50 bp) and 27,622 SVs (≥50 bp) per human genome. We also discover 156 inversions per genome—most of which previously escaped detection. Fifty-eight of the inversions we discovered intersect with the critical regions of recurrent microdeletion and microduplication syndromes. Taken together, our SV callsets represent a sevenfold increase in SV detection compared to most standard HTS studies, including those from the 1000 Genomes Project. We use these SV callsets to assess the performance of HTS studies including the types of variation that are missed by many current studies, SV detected in functional noncoding DNA, and the combinations of algorithms that most effectively detect SV in HTS reads.

S4-01

Genetics of proteinuric kidney diseases

Matias Simons

Imagine Institute, Hôpital Necker, Paris Descartes University, Paris, France

The term proteinuria describes the loss of serum proteins into the urine due to a failure of glomerular filtration or tubular reabsorption. In the first part of my lecture, I will give an update on the molecular causes of hereditary proteinuric kidney disease. I will also explain how the identified genes have contributed to our mechanistic understanding of glomerular filtration and tubular uptake. In the second part, I will describe our own efforts in the area of tubular proteinuria. I will report on patients with proteinuria and no renal dysfunction due to mutations in the tubular uptake receptor cubilin. These patients have been crucial for our hypothesis on how a reduction in tubular uptake may be renoprotective in conditions of tubular protein overload (Simons, JASN 2018).

I will explain our hypothesis with the help of cellular stress responses, human evolution and population genetics.

S4-02

Simple complex genetics in multi-systemic kidney diseases

Nicholas Katsanis

Center for Human Disease Modeling, Duke University, USA

The generation of 1000s of patient exomes and genomes, while accelerating diagnoses, has also highlighted the complexity of previously-considered “simple” traits. Numerous examples are now reported of patients with deleterious alleles in multiple genes. In some, the phenotype reflects an amalgam of two disorders; in others, it is the product of genetic interactions. Moreover, recent exome- or panel-based resequencing of groups of genes

known to cause a host of dominant or recessive disorders are beginning to report an enrichment of rare variants in patients, highlighting the concept of mutational burden. Bardet-Biedl syndrome (BBS), a founding ciliopathy, has been a model for studying these phenomena and for beginning to understand the contribution of such alleles to non-penetrance and variable expressivity. This is because: (a) the majority of the recessive burden in BBS is now known, with ~80% of BBS patients harboring recessive mutations in 22 genes; (b) most BBS proteins are necessary for cilia structure/function and assemble into defined complexes; and (c) we and others have developed quantitative *in vitro* and *in vivo* tools to assess the total functional output of the cilium; to establish the effect of variants; and to measure genetic interactions. This lecture will cover two major topics. First, I will discuss how it is possible to merge extensive genetic and functional data to build a comprehensive map of mutational distribution in a biological module and how using such approaches we can improve the granularity of understanding the contribution of genetic variants to human disease. Second, I will share our newest observations of how *bona fide* missense pathogenic variants can exert a context-specific pathogenic event that depends both on the splice isoform they map to and also the haplotype they are embedded in. Overall, I hope that these data will inform a robust discussion about causality and genetic burden in rare genetic disorders.

S5-01

Enhancers and Genes—insights into a complicated relationship

Axel Visel

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The human genome harbors tens of thousands of distant-acting gene regulatory sequences that play important roles in the development and function of the human body. Multiple converging lines of evidence from experimental and human genetic studies indicate that both common and rare sequence variants involving enhancers play major roles in Mendelian and complex human disease phenotypes. However, the underlying molecular mechanisms are difficult to study due to our limited understanding of the *in vivo* functions of enhancers, as well as their molecular and functional interactions with their respective target genes. We use a combination of sequence-based molecular approaches (ChIP-seq), large-scale transgenic mouse studies (<http://enhancer.lbl.gov>), and CRISPR genome editing in the mouse model to study the *in vivo* function of enhancers in developmental, evolutionary, and disease-related processes. Using examples from our ongoing work, I will illustrate how these studies provide insight into the function and evolution of distant-acting regulatory sequences and offer a starting point for understanding their role in human disease.

S5-02

A 3D code in the human genome

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Stretched out from end-to-end, the human genome—a sequence of 3 billion chemical letters inscribed in a molecule called DNA—is over 2 meters long. Famously, short stretches of DNA fold into a double helix, which wind around histone proteins to form the 10 nm fiber. But what about longer pieces? Does the genome's fold influence function? How does the information contained in such an ultra-dense packing even remain accessible? In this talk, I describe our work developing 'Hi-C' (Lieberman-Aiden et al., Science, 2009; Aiden, Science, 2011) and more recently 'in-situ Hi-C' (Rao & Huntley et al., Cell, 2014), which use proximity ligation to transform pairs of physically adjacent DNA loci into chimeric DNA sequences. Sequencing a library of such chimeras makes it possible to create genome-wide maps of physical contacts between pairs of loci, revealing features of genome folding in 3D.

Next, I will describe recent work using *in situ* Hi-C to construct haploid and diploid maps of nine cell types. The densest, in human lymphoblas-

toid cells, contains 4.9 billion contacts, achieving 1 kb resolution. We find that genomes are partitioned into contact domains (median length, 185 kb), which are associated with distinct patterns of histone marks and segregate into six subcompartments. We identify ~10,000 loops. These loops frequently link promoters and enhancers, correlate with gene activation, and show conservation across cell types and species. Loop anchors typically occur at domain boundaries and bind the protein CTCF. The CTCF motifs at loop anchors occur predominantly (>90%) in a convergent orientation, with the asymmetric motifs "facing" one another.

Next, I will discuss the biophysical mechanism that underlies chromatin looping. Specifically, our data is consistent with the formation of loops by extrusion (Sanborn & Rao et al., PNAS, 2015). In fact, in many cases, the local structure of Hi-C maps may be predicted *in silico* based on patterns of CTCF binding and an extrusion-based model. Finally, I will show that by modifying CTCF motifs using CRISPR, we can reliably add, move, and delete loops and domains. Thus, it is possible not only to "read" the genome's 3D architecture, but also to write it.

S6-01

Development of antisense-mediated exon skipping for Duchenne: It takes more than an antisense oligonucleotide

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DMD is a severe, progressive muscle-wasting disorder, while Becker muscular dystrophy (BMD) is a milder muscle disease. Both are caused by mutations in dystrophin, a protein, which stabilizes muscle fibers during contraction. In DMD patients mutations disrupt the open reading frame, generating prematurely truncated, nonfunctional dystrophins. In BMD patients, mutations maintain the reading frame allowing production of internally deleted, partly functional dystrophins. The aim of AON-mediated exon skipping for DMD is to restore the reading frame to allow production of a BMD-like, partially functional dystrophin. The approach is mutation specific. However, as mutations cluster in a few hotspots, skipping of some exons applies to larger groups of patients (e.g. exon 51 skipping applies to 13%). Eteplirsen (targeting exon 51) received accelerated approval from FDA in 2016. This lecture will highlight the road from concept to approved drug, which involved not only tests in model systems and patients, but also the development of outcome measures and trilateral education of the stakeholders involved.

S6-02

Regeneration of the epidermis in epidermolysis bullosa using transgenic stem cells

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Laminin beta3-deficient generalized Junctional Epidermolysis Bullosa is the first genetic disease targeted by transplantation of epidermal cultures originated from transgenic epidermal stem cells. A seven-year-old child, carrying a homozygous acceptor splice site mutation (C1977-1G>A, IVS 14-1G>A) within intron 14 of LAMB3 and suffering complete life-threatening epidermal loss on 80% of his body surface, was treated with autologous epidermal cultures transduced with a MLV-derived retroviral vector carrying the LAMB3 cDNA under the control of the viral LTR. Several skin biopsies were taken to perform histological analysis, immunofluorescence, *in situ* hybridization and genome-wide analysis of the retroviral integration sites. The regenerated epidermis was normal-looking, remained mechanically stable throughout the entire follow-up period (almost 3 years) and did not form blisters, even upon shear force. We observed a proper expression and location of laminin 332 in the basal lamina. *In situ* hybridization performed using vector-specific LAMB3 probes showed homogenous expression of LAMB3 mRNA in all epidermal layers, confirming that the regenerated epidermis consists only of transgenic keratinocytes. Histologi-

cal analysis showed a normal and fully differentiated epidermis with a normal dermal-epidermal junction. Electron Microscopy confirmed the presence of well-defined, organized hemidesmosomes comparable to those of healthy controls. The proviral integration pattern was maintained in vivo and epidermal renewal did not cause any clonal selection. Clonal tracing showed that the human epidermis is sustained not by equipotent progenitors, but by a limited number of long-lived stem cells, detected as holoclones, that can extensively self-renew in vitro and in vivo and produce progenitors that replenish terminally differentiated keratinocytes.

SEL Selected Presentations

SEL-01

Do we need to test them all? – 15 % of cancer patients <50 years of age carry pathogenic germline mutations: results from the NCT MASTER cohort

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The MASTER (Molecularly Aided Stratification for Tumor Eradication Research) program at NCT (National Center for Tumor Diseases) Heidelberg and Dresden is one of the largest precision oncology platforms in Germany. Patients with early-onset cancers (<50 years of age) or rare cancers are included for whole exome (WES) or whole genome (WGS) and transcriptome sequencing of tumor tissue and WES/WGS of blood/normal tissue. Treatment recommendations are being discussed in a multidisciplinary molecular tumor board and translated into personalized patient care. In the context of precision oncology, bioinformatics pipelines usually exclude germline variants identified in matched blood samples to select for somatic mutations. However, in the NCT MASTER cohort, we anticipated an enrichment of patients with a possible hereditary background. We therefore manually evaluated >1,700 germline variants in a set of 172 cancer-associated genes in 1,023 MASTER patients in accordance with ACMG criteria. Thereby, we identified (likely) pathogenic germline mutations in 15% of patients, including 10% of patients with an autosomal dominant tumor predisposition syndrome and 1% of patients with two or more pathogenic mutations. Here we will present our results, with particular focus on the broad genotypic and phenotypic spectrum, recurrent mutations and the identification of carriers for rare recessive disorders. We further evaluate the informative value of somatic DNA and RNA sequencing data for the assessment of germline variants, such as loss of heterozygosity, mutational signatures and gene expression in tumor tissue.

These results contributed to treatment recommendations and have important implications for further surveillance of the patients and their family members. Given that current guidelines in the field of hereditary tumor predisposition syndromes specify a 10% risk of identifying a tumor predisposition as the cutoff for testing, our results suggest that every patient with cancer under the age of 50 needs to be offered genetic counseling and next-generation sequencing to search for pathogenic germline mutations in >30 genes.

SEL-02

Biallelic LZTR1 mutations activate RAS-MAPK signaling and cause hypertrophic cardiomyopathy with abnormal calcium handling in iPSC-derived cardiomyocytes of a novel type of Noonan syndrome

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Noonan syndrome (NS) is a multisystemic developmental disorder characterized by its clinical variability with common symptoms such as typical facial dysmorphism, skeletal findings and short stature, intellectual disability as well as congenital heart disease and hypertrophic cardiomyopathy. The disease is causally linked to gain-of-function mutations in a number of genes leading to an increased signal transduction along the RAS-MAP kinase (MAPK) signaling pathway. However, our understanding of the pathophysiological alterations and mechanisms, especially of the associated hypertrophic cardiomyopathy, remains limited. In this study, we present a family with two siblings displaying a novel autosomal recessive form of NS identified by exome sequencing with severe hypertrophic cardiomyopathy caused by biallelic mutations within the leucine zipper like transcription regulator 1 (LZTR1) gene. The generation of induced pluripotent stem cells of the affected siblings allowed us to deeply investigate the disease phenotype in patient-specific cardiomyocytes (iPSC-CMs) on a molecular, cell biological and functional level. Our data demonstrated that the patient-specific iPSC-CMs recapitulate the hypertrophic phenotype in vitro and uncover disturbances in calcium handling, which could be attenuated by verapamil treatment. Long-term verapamil treatment even corrected the hypertrophic morphology and molecular markers for cardiac hypertrophy in affected cardiomyocytes. Furthermore, we identified a link of disrupted LZTR1 function and elevated RAS-MAPK signaling activity. The reported human stem cell model of a novel type of NS will improve our current knowledge of the pathophysiological mechanisms of NS and NS-associated hypertrophy and will further serve as a platform for personalized medicine.

SEL-03

Quantitative Proteomics as a complementary diagnostic tool for Mendelian disorders

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The care of rare Mendelian diseases has been revolutionized by genome sequencing. While in the past it could be a long, frustrating and often losing battle for parents with an affected child to find the cause of their child's suffering, the availability of genome sequencing has made this—at

least conceptually-possible for every patient. However, across a large variety of Mendelian diseases, analysis of the coding sequence does not lead to diagnosis for 50–75% of patients. This indicates that in many cases the pathogenic variant evaded detection, was detected but remained of uncertain significance (VUS), or involved a more complicated interaction. We recently demonstrated the power of combining DNA and RNA sequencing to tackle unsolved WES cases. We identified 3 situations in which to prioritize candidate disease-causing genes for a rare disease. Firstly, genes with expression outside physical range can be identified as expression outliers. Secondly, RNAseq can reveal cases of allele-specific expression. Detection of mono allelic expression can help in re-prioritizing heterozygous rare variants. Thirdly, splicing of a gene can be affected. Still, many pathogenic alterations cannot be seen at the RNA level, but they may affect protein folding and stability. We therefore re-investigated protein levels in fibroblast cell lines using antibodies specific for the proteins affected by pathogenic mutations and found significantly reduced levels in 71 out of 80. The large dynamic range and highly sensitive analytical capabilities of recent MS-based proteomics platforms now allows systematic, near comprehensive, quantification of the proteins expressed in cells. Hence, proteomics has the potential to be an important tool to screen for pathogenic variants. Moreover, most proteins function in multiprotein complexes, the stability of which depends on the availability of all components. We established a protocol for quantitative deep proteome analysis using TMT-10plex labeling and Trinity fractionation combined with the MultiNotch MS3 method for peptide quantification that allowed us to quantify about 8,000 proteins. Proteomics of 100 fibroblast cell lines from patients with suspected mitochondrial disorder quantified more than 700 mitochondrial proteins including two thirds of the known mitochondrial disease with missing values of less than 1%. Proteomic data helped us to interpret the destabilizing effect of several missense VUS and small in frame deletions and insertions that did not alter transcript level. In one case we identified almost all subunits of the mitochondrial ribosome to be strongly reduced indicating a defect in mitochondrial translation. In summary, quantitative proteomics has proven to be a powerful complementary tool to genome and transcriptome sequencing. It delivers functional data supporting interpretation of VUS not only on the level of the affected protein but also on protein complexes sometimes providing insides in disease mechanisms.

SEL-04

The transcriptional landscape of mammalian organogenesis at single cell resolution

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During mammalian organogenesis, the cells of the three germ layers transform into an embryo that includes most major internal and external organs. The key regulators of developmental defects can be studied during this critical window, but conventional approaches lack the throughput and resolution to obtain a global view of the molecular states and trajectories of a rapidly diversifying and expanding number of cell types.

We have developed a three-step combinatorial barcoding method to profile single-cell transcriptomes (sci-RNA-seq3⁺) without requiring physical isolation of each cell. We have used this new method to profile whole mouse embryos staged between 9.5 and 13.5 days of gestation (E9.5 to E13.5) with sci-RNA-seq3, and created a transcriptional atlas of mouse organogenesis at single cell resolution. We identify hundreds of expanding, contracting and transient cell types, many of which are only detected because of the depth of cellular coverage obtained here, and define the corresponding sets of cell type-specific marker genes, several of which we validate by whole mount in situ hybridization. With Monocle 3, we delineate and annotate 56 single cell developmental trajectories of mouse or-

ganogenesis. We explore the dynamics of proliferation and gene expression within cell types over time, including focused analyses of the apical ectodermal ridge, limb mesenchyme and skeletal muscle. These data comprise a foundational resource for mammalian developmental biology, and are made available in a way that will facilitate their ongoing annotation by the research community.

This single cell atlas of the development of wild-type mice also represent a first step towards understanding pleiotropic developmental disorders at the organismal scale and provides rich opportunities for detailed investigations of subtle roles for genes and regulatory sequences involved in developmental defects.

W1 Neurodevelopmental Disorders

W1-01

FBXO11 defects cause a variable neurodevelopmental disorder in humans and neuronal deficits in *Drosophila melanogaster*

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Next-Generation sequencing combined with international data sharing has enormously facilitated identification of new disease associated genes and mutations. This is particularly true for genetically extremely heterogeneous entities such as neurodevelopmental disorders (NDDs). Through exome sequencing and world-wide collaborations we identified and assembled 20 individuals with *de novo* variants in *FBXO11*. They present with mild to severe developmental delay associated with a range of features including short (4/20) or tall (2/20) stature, obesity (5/20), microcephaly (4/19) or macrocephaly (2/19), behavioral problems (17/20), seizures (5/20), cleft lip or palate or bifid uvula (3/20) and minor skeletal anomalies. *FBXO11* encodes a member of the F-Box protein family, constituting

a subunit of an E3-ubiquitin ligase complex. This complex is involved in ubiquitination and proteasomal degradation and thus in controlling critical biological processes by regulating protein turnover. The identified *de novo* aberrations comprise two large deletions, ten likely gene disrupting and eight missense variants distributed throughout *FBXO11*. Structural modeling for missense variants located in the CASH or the Zinc-finger UBR domains suggests destabilization of the protein. This in combination with the observed spectrum and localization of identified variants and the lack of apparent genotype-phenotype correlations is compatible with loss-of-function or haploinsufficiency as an underlying mechanism. Functional analyses in the model organism *Drosophila melanogaster* revealed an essential role of Fbxo11 during development, with complete loss leading to embryonal lethality and partial knockdown resulting in lethality in later developmental stages at the pupal level. Additionally, neuron-specific knockdown of Fbxo11 revealed a mild impairment of gross motor function observed as a deficit in the climbing assay. Further functional studies into the role of Fbxo11 in the fly nervous system are currently ongoing. In summary, we implicate *de novo* missense and likely gene disrupting variants in *FBXO11* in a neurodevelopmental disorder with variable intellectual disability and various other features.

W1-02

RAC3 a novel gene for a distinct neurodevelopmental syndrome

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RAC3 is a member of the Rho GTPase gene family that is expressed in the developing brain and linked to key cellular functions. Members of the Rho family of small GTP-binding proteins act as molecular switches that alternate between GTP-bound (active) and GDP-bound (inactive) states via conformation changes in the conserved Switch I and Switch II domains. Experimentally, it has been shown that RAC3 is involved in neurogenesis, neuronal migration and other aspects of cortical development. *De novo* missense variants in the homolog RAC1 have recently been associated with developmental disorders. Here we report five individuals with *de novo* missense variants in RAC3 identified by genome or exome sequencing. The five cases were identified at different places and brought together by an international data-sharing initiatives (MatchMaker Exchange, ClinVar, Genematcher). Clinical evaluation of the cases revealed a distinct syndrome characterized by severe intellectual disability, abnormal muscle tone and characteristic brain malformations including hypoplasia of the corpus callosum, polymicrogyria and ventriculomegaly. There were variable nonspecific dysmorphic features, including recurrent features like arched and/or medially flared eyebrows. Interestingly, 3 of the 5 patients are sharing the same variant (c.184G>A, p.Glu62Lys) and one more patient was a carrier of the variant p.Gln61Leu. These variants were localized within the Switch II region of RAC3, which forms a 3(10)-helix structure, which changes conformation upon activation. In the 5th patient the variant p.Pro29Leu located within the Switch I domain was found. Moreover, the alteration p.Gln61Leu has been extensively studied in the RAC subfamily, including in RAC3, as an archetype for constitutive protein activation. All detected variants were predicted to be damaging by *in silico* programs and were absent from the gnomAD database. Our findings represent the first evidence that missense variants in RAC3 can cause severe neurodevelopmental phenotypes in humans. A major challenge posed by genome-wide sequencing in clinical diagnostics is how to prove the causality of novel *de novo* variants in plausible candidate disease genes identified in single patients since variant-specific functional experiments are beyond the scope of most clinical laboratories. Our approach illustrates the growing value of aggregation databases such as MatchMaker Exchange and ClinVar.

W1-03

Biallelic mutations in MADD cause a multisystemic disorder including severe developmental delay, hypotonia, and metabolic, hematological and gastrointestinal anomalies

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Using whole-exome sequencing, we identified compound heterozygous mutations in the *MADD* gene [deletion of exons 11–24; p.(Gly305Val)] in two siblings (family 1) who presented with severe developmental delay, muscular hypotonia, exocrine pancreatic insufficiency, low hemoglobin, growth hormone deficiency, and repeated episodes of desaturations. *MADD* encodes the mitogen-activated protein kinase activating death domain protein, a member of the death domain containing protein family (DENN/MADD), which directly interacts with members of the Rab family of small GTPases. As specific guanine nucleotide exchange factor for RAB3, *MADD* is an important regulator of neurotransmitter release and exocytosis. Moreover, under conditions of cytotoxic stress, *MADD* has been shown to be involved in blocking the apoptosis of neuronal cells.

We identified a second family (family 2) with two similarly affected siblings carrying the homozygous splice site mutation c.983 + 1G>A in *MADD*. By analysing fibroblast-derived RNA of one sibling, we found an in-frame deletion of 30 nucleotides in the majority of *MADD* transcripts (appr. 87%), predicted to encode a protein lacking ten evolutionarily highly conserved amino acids in the functional DENN domain [p.(Leu313_Val322del)]. The remaining 13% of transcripts harbored either a 50-bp exonic deletion or a 7-bp insertion of intronic sequence which both are predicted to lead to introduction of a premature stop codon [p.(Val306Glyfs*70) and p.(Val323Argfs*72)].

From a GeneMatcher-enabled international collaboration, we identified two additional individuals affected by severe global developmental delay, muscular hypotonia, and similar metabolic, hematological and gastrointestinal findings as the two aforementioned siblings: one female carried the compound heterozygous *MADD* variants c.979C>T [p.(Arg327*)] and c.1705 + 1G>C, and the other the homozygous missense mutation p.(Leu1040Arg). In contrast to the missense variant p.(Gly305Val) detected in family 1 and the splice site variant c.983 + 1G>A [p.(Leu313_Val322del)] in family 2 the missense variant p.(Leu1040Arg) affects an evolutionarily highly conserved amino acid residue outside the DENN domain.

In conclusion, we have identified *MADD* as new disease gene for a complex multisystemic disorder characterized by developmental delay, muscular hypotonia, exocrine pancreatic insufficiency, gastrointestinal problems ranging from exudative enteropathy to severe constipation, hematological abnormalities, growth hormone deficiency and repeated episodes of desaturations. Four of the six affected individuals died early in life (between the age of 15 month and 7 years) suggesting that the *MADD*-related disorder can be associated with early lethality. Functional studies using fibroblasts of three unrelated patients are ongoing to characterize the cellular and biochemical abnormalities in this model system.

W1-04

Activating mutations in PAK1, encoding p21-activated kinase 1, cause a neurodevelopmental disorderHarms FL.¹, Kloth K.¹, Bley A.², Denecke J.², Santer R.², Lessel D.¹, Hempel M.¹, Kutsche K.¹¹Institute of Human Genetics, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; ²Department of Pediatrics, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

p21-activated kinases (PAKs) are serine/threonine protein kinases acting as effectors of CDC42 and RAC, which are members of the RHO family of small GTPases. PAK1's kinase activity is autoinhibited by homodimerization, whereas CDC42 or RAC1 binding causes PAK1 activation by dimer dissociation. Major functions of the PAKs include actin cytoskeleton reorganization, for example regulation of the cellular protruding activity during cell spreading. We report the *de novo* PAK1 mutations c.392A>G/p.Tyr131Cys and c.1286A>G/p.Tyr429Cys in two unrelated male subjects with developmental delay, secondary macrocephaly, seizures, and ataxic gait. Both mutations affect highly conserved residues in the autoinhibitory domain and the kinase domain of PAK1, respectively. In subject-derived fibroblast we showed enhanced phosphorylation of the PAK1 targets JNK and AKT in cells of one subject and of c-JUN in those of both subjects compared to control subjects that was suggestive of activating PAK1 alleles. We hypothesized that the hyperphosphorylation of PAK1 downstream targets was either due to an increased intrinsic kinase activity of mutant PAK1, a dysbalance between dimeric and monomeric PAK1 forms due to a dimerization defect or a combination of both. To test the intrinsic kinase activity of PAK1, we performed an *in vitro* kinase assay with fibroblasts of the two affected individuals and controls and observed a trend toward enhanced PAK1 activity in subject cells. To study the dimerization capability of the mutant PAK1 proteins, we performed co-immunoprecipitation experiments in HEK293T cells ectopically expressing wild-type together with the PAK1 mutant Tyr131Cys or Tyr429Cys. We demonstrated a significantly reduced dimerization for both PAK1 mutants compared to wild-type PAK1. In addition, we could detect a shift in the PAK1 dimer-monomer equilibrium towards monomers by size-exclusion chromatography in patient cells with the p.Tyr429Cys mutation. In a cell spreading assay, subject-derived fibroblasts showed significant enrichment in cells occupied by filopodia compared to control cells. Interestingly, application of the PAK1 inhibitor FRAX486 completely reversed this cellular phenotype in patient cells. Together, our data reveal that dominantly acting, gain-of-function PAK1 mutations cause a neurodevelopmental phenotype with increased head circumference, possibly by a combined effect of defective homodimerization and enhanced kinase activity of PAK1. This condition, along with the phenotypes associated with RAC1 and CDC42 missense mutations, may emerge as a potentially treatable group of neurodevelopmental disorders and highlight the importance of RHO GTPase members and effectors in neuronal development.

W1-05

De novo mutations in TAOK1 cause neurodevelopmental disordersTrinh J.¹, Dulovic J.¹, Kandaswamy K.², Braathen G.³, Busk O.³, Tveten K.³, Werber M.², Weiss M.², Oprea G.², Kishore S.², Vos M.¹, Rolfs A.^{2,4}, Lohmann K.¹¹Institute of Neurogenetics, University of Lübeck, Lübeck, Germany;²Centogene AG, Rostock, Germany; ³Telemark Hospital, Skien, Norway;⁴University of Rostock, Rostock, Germany

Background: Rare de novo mutations represent a significant cause of neurodevelopmental delay and intellectual disability (ID). Although next generation sequencing technology increased our understanding of genes mutated in neurodevelopmental disorders (NDD), only about 50% of the cases can be genetically explained, underlining that many more genes need to be elucidated.

Methods: We employed whole-exome sequencing in 4785 patient-parent-trios, 2030 of which have NDD. The NDD patients were diagnosed with global developmental delay, seizures, microcephaly, macrocephaly,

motor delay, delayed speech and language development, or ID according to Human Phenotype Ontology (HPO) terms. In a first step to identify novel NDD genes, we used a reported pLI score of ≥ 0.90 on ExAC to select potential candidate genes for NDD since such a value suggests that there is negative selection of loss-of-function variants in these genes in the population without severe paediatric disease. We then compared de novo variants found in these genes in trios with NDD ($n = 2030$) and without NDD ($n = 2755$). To further examine the role of TAOK1, a candidate gene, on neurodevelopment, we used an RNAi-mediated knockdown *Drosophila* model.

Results: This pLI approach revealed 3,230 genes of which 168 genes harbored de novo variants. We focussed on genes with three or more de novo variants among NDD trios and none in non-NDD trios. This resulted in one novel candidate gene for NDD, TAOK1. We identified four patients with novel de novo variants (p.Glu17Gly, p.Lys298Glu, p.Glu781*, c.2362-1G>A) in TAOK1 and an additional carrier (p.Arg641 fs) through GeneMatcher. The patients share a common phenotype of delayed speech and development, muscular hypotonia and intellectual disability. All five variants were considered pathogenic or likely pathogenic according to ACMG guidelines, with a CADD score > 20 and have not been reported in ExAC or GnomAD. Knockdown of TAOK1 in *Drosophila* resulted in slower early development compared to a control line. Consistent with this developmental delay, the majority of the TAOK1-knockdown flies did not survive beyond the 3rd instar larvae stage, and only few reach the pupa stage and did not develop further.

Conclusion: In summary, we provide compelling evidence that de novo mutations in TAOK1 cause a novel form of NDD. Our approach underlines the value of large-scale next generation sequencing on patient-offspring trios for identifying additional causes for NDD.

W1-06

De novo variants in MAPK8IP3 cause intellectual disability with variable brain anomaliesPlatzer K.¹, Sticht H.², Allen W.³, Angione KM.⁴, Bonati MT.⁵, Brasington C.⁶, Cho MT.⁷, Demmer LA.⁶, Falik-Zaccai T.^{8,9}, Gamble CN.¹⁰, Hellenbroich Y.¹¹, Iacone M.¹², Kok F.¹³, Mahida S.¹⁴, Mandel H.⁸, Marquardt T.¹⁵, McWalter K.⁷, Panis B.¹⁶, Pepler A.¹⁷, Pinz H.¹⁸, Ramos L.¹³, Shinde DN.¹⁹, Smith-Hicks C.³, Stegmann APA.²⁰, Stöbe P.¹⁷, Stumpel CTRM.²⁰, Wilson C.³, Lemke JR.¹, Di Donato N.²¹, Miller KG.²², Abou Jamra R.¹

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MAPK8IP3 has been shown to be part of the axonal transport machinery which is essential for the function and maintenance of neurons. It supplies the synapse with newly synthesized proteins and lipids while damaged or misfolded proteins are cleared. Therefore, MAPK8IP3 was an appealing candidate gene for further investigation after the initial identification of a de novo missense variant in an individual with a neurodevelopmental disorder. Through international collaboration and exome sequencing in a cohort that sums up to 27.232 affected individuals, we identified heterozygous de novo variants in MAPK8IP3 in thirteen unrelated individuals with an overlapping phenotype of mild to severe intellectual disability and variable brain malformations such as perisylvian polymicrogyria, cerebral or cerebellar atrophy and hypoplasia of the corpus callosum. De novo variants comprise six missense variants, three of which are recurrent, and three truncating variants. Brain anomalies were consistent among individuals harboring recurrent de novo missense variants, indicating possible variant specific phenotypic effects. We provide several lines of evidence for causation through (1) the significant enrichment of de novo variants in MAPK8IP3 within our cohort using an established framework of gene-specific mutation rates, (2) structural modelling of variants that lie in known (template) structures and (3) through using the CRISPR-Cas9 system to target six conserved amino acid positions in *Caenorhabditis elegans* to demonstrate increased axonal lysosomal density for two and an adverse locomotion phenotype for five of the six investigated human variants in this model. Taken together, we firmly establish heterozygous de novo variants in MAPK8IP3 as a novel cause of a neurodevelopmental disorder.

W2 Cancer Genetics

W2-01

Two-hit inactivation of GPR161 leads to medulloblastoma and reveals a novel tumor predisposition syndrome

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Increasing knowledge on heritable tumor predisposition syndromes changes clinical practice, patient management, and surveillance of individuals being at risk. We here report a novel tumor predisposition syndrome in patients with medulloblastoma, a prevalent and highly malignant brain tumor especially in children. Our study identifies for the first time heterozygous germline variants in GPR161 in seven independent medulloblastoma patients. In tumor tissue a recurrent segmental uniparental disomy leads to a copy-number neutral loss of the second GPR161 allele. This two-hit inactivation is consistent with a loss of tumor suppressor activity of the protein. GPR161 is a ciliary protein and interferes with sonic hedgehog, a known player in the pathogenesis of medulloblastoma. In line with our findings, neuron-specific inactivation of Gpr161 was recently

shown to result in medulloblastoma in homozygous knockout mice. Sonic hedgehog drives the mitotic rate of granule cells of the cerebellum and its prolonged activation by impaired GPR161 function potentially increases cell proliferation and initiates medulloblastoma development. All patients reported here show a genome signature of sonic hedgehog medulloblastoma type 1 which has direct therapeutic implications. Data sets from an international multicentric cohort of more than 1.000 highly genotyped and phenotyped medulloblastoma patients indicate that GPR161 mutations account for 3.5% of all medulloblastoma cases with sonic hedgehog signature. Moreover, the family history of affected individuals may suggest a broader tumor spectrum in adult GPR161 mutation carriers including common tumors such as colorectal cancer. Conservative estimates from available population frequency data suggest that at least 4 million people worldwide are carriers of a tumor predisposing GPR161 variant, underlining the need to investigate the penetrance and entire clinical spectrum of this novel cancer syndrome.

W2-02

B-cell precursor acute lymphoblastic leukemia: atypical chromosomal patterns of aneuploidies indicative of masked hypodiploidy

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Background: Precise genomic characterization is a prerequisite for optimal treatment of B-cell precursor acute lymphoblastic leukemia (pB-ALL). Genome-wide SNParrays are a well-established and easy to integrate diagnostic tool to detect otherwise diagnostically challenging hypodiploid karyotypes (<45 chr), which are associated with high risk of treatment failure and disease relapse. Moreover, masked hypodiploid pB-ALL (representing a chromosomal doubling of the hypodiploid clone) can be reliably detected with this method. We here report on our observations from $n = 447$ cases of pediatric pB-ALL analyzed with genome-wide array-based molecular karyotyping within the BFM-ALL 2009 and BFM-ALL 2017 trials. **Methods:** Patient samples were submitted for array analysis only if they had tested negative for risk stratification relevant markers including translocations (*BCR-ABL1* and *ETV6-RUNX1*), rearrangements (*KMT2A* and *TCF3*), and hypodiploidy (<45 chr) using conventional cytogenetic, FISH or PCR analyses. Molecular karyotyping was conducted using high resolution array-CGH (400k eArray, Agilent) and samples suggestive of masked hypodiploidy were additionally evaluated for uniparental disomies (UPD) using SNParray (CytoScan HD/OncoScan by Affymetrix or CGH+SNP 400k eArray by Agilent). TP53 mutational status of masked hypodiploid cases was assessed by exome sequencing.

Results: Between February 2017 and November 2018, more than $n = 620$ pB-ALL patients were enrolled to the BFM trials and the hierarchical diagnostic procedure identified $n = 447$ samples for microarray analysis. A total of $n = 7$ masked hypodiploidies were discovered of which $n = 3$ appear to have originated from a near-haploid clone (24–31 chr) and $n = 4$ from a low hypodiploid clone (32–39 chr). The existence of the hypodiploid founder clone was verified by DNA index measurement in $n = 4/5$ masked hypodiploidies (two cases had insufficient material to conduct DNA index). Due to a generally low clone size of the hypodiploid clones, validation with FISH probes was only successful for the predominant masked hypodiploid clones. Whole exome sequencing was conducted for $n = 6/7$ cases (one case had insufficient DNA from time point of diagnosis) and identified TP53 variants in four cases (ACMG class 3: $n = 1$; class 4–5: $n = 3$). As an unexpected result, two masked hypodiploidies presented an atypical pattern of numerous trisomies, which accompanied expected UPDs and tetrasomies (arr(1,2,4,5,10q23.33q26.3,11,12,19)x3, (3,7–9,13–17,20,X,Y)x2 hmz, (6,10p15.3q23.33,18,21,22)x4 and arr(1,8,19,21,22)x4, (2–4,7,9,12,13,15–17)x2 hmz, (5,6,10,11,14,18,20,X)x3). Both patients car-

ried *TP53* variants (class 3 and class 5, respectively). The second case displayed a clear hypodiploid population alongside the masked hyperdiploid clone in DNA index measurement.

Conclusion: Integration of SNParray analysis in our diagnostic algorithm identified masked hypodiploidy in seven patients with hyperdiploid clones as detected by conventional karyotyping and FISH. Noteworthy, two cases of masked hypodiploidy presented an atypical pattern of numerous trisomies, which accompanied expected UPDs and tetrasomies. This observation challenges the widely accepted concept of a simple chromosomal doubling of hypodiploid clones and indicates a more complex clonal evolution in masked hypodiploidy for some cases. Retrospective analyses are now warranted, in order to assess the relevance of these possible subgroups within the extremely rare and high risk group of hypodiploid pB-ALL.

W2-03

Integrative genomic profiling elucidates key pathogenic pathways in primary mediastinal large B-cell lymphoma

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Background: Primary mediastinal large B cell lymphoma (PMBCL) represents a rare subtype of Non-Hodgkin lymphoma, accounting for 2–3%. Its characteristic presentation features a mediastinal mass in young adults, with higher prevalence in females. Molecular studies, including global gene expression (GE) profiling, have provided evidence, that PMBCL truly typifies a distinct large cell lymphoma entity, which is more closely related to classical Hodgkin lymphoma (cHL). The increasing notion of the molecular/genetic heterogeneity warrants a comprehensive characterization of lymphoma subtypes. In addition, there is cumulating evidence of the importance of a correct classification for patient risk stratification and therapeutic decision making. Although targeted sequencing studies have revealed a number of key genetic lesions involved in PMBCL pathogenesis, a comprehensive description of genetic alterations and perturbed pathways is still lacking.

Materials and Methods: We performed whole-exome sequencing of 95 PMBCL cases, using the SureSelect Human All Exon V6+UTR bait (Agilent) followed by massively parallel sequencing. For 21 cases paired germline DNA was available. Somatic SNV/indel variants were identified using the intersection of predictions by VarScan, Strelka and MuTect, and annotated with SnpEff. We inform on oncogenic driver genes as identified by MutSigCV and recurrent copy number alterations utilizing CNVkit and GISTIC. Global GE profiling using the DASL platform (Illumina) was available for 69 patients and 90 cases have been molecularly classified using a GE-based assay (Lymph3Cx). The impact of somatic mutations on gene expression was assessed using xseq.

Results: Tumor and normal samples were sequenced to an average coverage of 115x. Analysis of somatic alterations in tumor-normal pairs yielded 50 putative driver genes recurrently mutated in PMBCL. Beside known oncogenic mutations in the JAK-STAT (SOCS1, STAT6, IL4R, PTPN1) and NFkB-pathway (TNFAIP3, NFKBIE, TRAF3) we provide additional evidence of the importance of immune evasion in this disease (CIITA, CD58) and identified the IRF-pathway as a putative novel hallmark (IRF2BP2, IRF4, IRF8, CISH). As expected, the most significant regions of copy number gain predicted by GISTIC were located on 9p (72%), 2p (36%), 6p (47%) and 11q (40%). The most frequent regions of loss were mapped to 10q, 14q, 8p, 1p, and 7p (all >30%). PMBCL driver genes, with the exception of *EZH2*, were significantly more frequently mutated in comparison to diffuse large B-cell lymphoma, whereas only three genes were significantly different between PMBCL and cHL.

Conclusion: We identified candidate driver genes with clear evidence of somatic mutations in PMBCL. A number of these are associated with related

B-cell lymphoma entities. We observed an enrichment of somatic mutations affecting genes involved in the JAK-STAT- and NFkB-pathway, and propose the IRF-pathway being critically involved in PMBCL pathogenesis.

W2-04

Comprehensive molecular analyses of rhabdoid tumors treated according to the consensus regimen of the multinational European Rhabdoid Registry (EU-RHAB) identifies genetic and epigenetic outcome predictors

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Rhabdoid tumors (RTs) are rare pediatric tumors with a very unfavorable prognosis. They most commonly affect young children and may manifest in the brain and spine (atypical teratoid/rhabdoid tumor: AT/RT), the kidneys (RT of the kidneys: RTK) and soft tissue (extracranial malignant RT: eMRT). Due to the rarity of the disease controlled trials are missing. From 2006 up to October 2018 in total 384 patients with RT were included into the EU-RHAB registry, 258 from Germany and 126 from other countries. Of these 244 were AT/RT [median age at diagnosis: 18 (0–211) months], 89 eMRT [median age: 13.5 (0–207) months], 34 RTK [median age: 13 months (2–166)] and 17 had synchronous tumors at various sites [median age: 3 months (0–23) months].

As part of the EU-RHAB diagnostic workup we performed germline and somatic genetic analyses (sequencing, MLPA, FISH, cytogenetics) of the rhabdoid tumor associated genes *SMARCB1* and *SMARCA4*. The genetic analyses were complemented by immunohistochemical (*SMARCB1*/INI1 and *SMARCA4*/BRG) and DNA-methylation (Illumina 450K/EPIC methylation analysis) studies. Here, we evaluated the clinical impact of germline mutations (GLM) in this cohort of RT.

In total 62 of 281 (22%) tested patients from the EU-RHAB registry carried GLM in *SMARCB1* or *SMARCA4* leading to the diagnosis of rhabdoid tumor predisposition syndrome I or II, respectively. GLM were especially enriched in patients with synchronous tumors (88% of patients with GLM) and congenital tumors (66%).

Patients with germline alterations had a very poor survival rate compared to children without GLM (5 year OS ATRT ~35%, eMRT/RTK ~45%, RTPS <10%). Only one of 25 patients with a GLM and congenital tumor was alive at 102 months following diagnosis (Nemes et al., 2018). *SMARCA4*-mutated AT/RT are associated with a higher frequency of inherited germline alterations ($p < 0.01$). Independent of age and GLM *SMARCA4*-deficient AT/RT run a worse prognosis compared to *SMARCB1*-mutated ATRT (OS *SMARCA4*-mutated AT/RT compared to *SMARCB1*-mutated AT/RT 3 months versus 24 months).

Next to genetic analysis 23/35 patients with AT/RT and GLM were analyzed by DNA methylation profiling. Of these 14 belong to the ATRT-SHH subgroup, characterized by supra- and infratentorial location, focal *SMARCB1* aberrations and overexpression of the SHH pathway, 7 belong to the ATRT-TYR group, located mostly infratentorial, associated with broad *SMARCB1* deletions and overexpression of melanosomal genes and 2 belong to the ATRT-MYC subgroup, located mostly supratentorial, with focal *SMARCB1* deletions and overexpression of MYC and HOX cluster genes.

In summary the EU-RHAB registry provides a comprehensive clinical, pathologic and (epi)genetic database as seed for further functional characterization to develop targeted therapeutic strategies especially for patients with GLM in *SMARCB1* and *SMARCA4*, who still face a very poor prognosis.

W2-05

cDNA analyses of the MMR genes MLH1, MSH2, MSH6, and PMS2 investigate the effect of VUS upon splicing, detect unexpected splicing defects, and find allelic losses indicating a germline defect

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Heterozygous germline defects in the DNA mismatch repair (MMR) genes MLH1, MSH2, MSH6, or PMS2 predispose to Lynch Syndrome (LS) with early onset of colorectal cancer and associated tumors. While 53–65% of the patients fulfilling the clinical criteria of LS can be molecularly confirmed by the detection of a pathogenic MMR variant, the other patients remain unsolved including their tumor risk and heritability. Many of these unsolved cases harbour a germline variant of uncertain significance (VUS), whereas for the others no potentially causative variant was detected except for benign variants. Particularly for the unsolved cases with familial tumor clustering the suspicion of a MMR germline defect remains. Based on 900 samples, we established and validated a cDNA analysis for the four MMR full-length transcripts (FLT) for the investigation of the splicing pattern and transcript integrity in patients' cDNA with active and inhibited nonsense-mediated mRNA decay (NMD). On the basis of results from normal and pathogenic controls, we defined cutoff values for alternative versus aberrant splicing, and for biallelic expression versus an allelic loss. Then, we analyzed the effect of VUS upon splicing, including variants conferring a potential splice site defect (SSD). We could verify all variants already predicted to affect splicing as SSD, experimentally categorized VUS with questionable bioinformatic splicing prediction as SSD, and unexpectedly unraveled variants as SSD, which were predicted as truncating or missense variants. We found that remarkable 15% of the putative missense variants are in fact SSD.

Furthermore, we investigated unsolved cases with immunohistochemical MMR protein loss in their tumor in the respective transcript for allelic representation in an informative variant class 1–2 and splicing. In 19% of the unsolved cases we detected an aberrant transcript or allelic loss with our FLT cDNA analyses indicating a germline defect.

Our method helps to enhance diagnostic accuracy by finding MMR variants and defects, and assessing by the mechanism of pathogenicity of VUS and pathogenic variants, which will gain relevance for precision medicine and gene therapy, helps optimizing prediction programs, and calibrating a high-throughput NGS-based mRNA analysis. Splice-neutral variants predicted not to alter the protein can be classified as benign, whereas functional tests are needed for missense variants and in-frame deletions in a next step towards a clinically significant classification.

W2-06

TRIM28 haploinsufficiency predisposes to Wilms tumor

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Wilms tumor is the most common childhood renal tumor. Although most diagnoses are established in sporadic patients, about 2% of cases have one or more affected relatives. A proportion of these familial cases can be ascribed to known syndromes and genes. Nevertheless, the underlying cause of most cases remains unexplained, indicating the existence of other Wilms tumor predisposition genes.

We performed exome sequencing in a family with Wilms tumors in two brothers. One boy was diagnosed with a unilateral tumor at age 5 years and 6 months and the other boy developed bilateral tumors when he was 6 months old. After exclusion of germline mutations in WT1, exome sequencing on lymphocyte-derived DNA from both boys was performed. We identified a shared heterozygous frameshift aberration c.1562_1569dup in TRIM28, which was predicted to result in a premature termination codon after 155 amino acids (p.(Arg524Leufs*155)).

A second family with a frameshifting TRIM28 mutation could be identified by using GeneMatcher.

In this family one girl was diagnosed with bilateral tumors at the age of 1.5 years and her sister developed a unilateral tumor when she was 6 months old. In both families, the TRIM28 variants were maternally inherited. Both mothers were unaffected, indicating incomplete penetrance of germline TRIM28 mutations for the development of nephroblastoma. In the index family, the mutation occurred de novo in the mother, adding further evidence to its causality.

TRIM28 encodes a highly complex scaffold protein involved in many different processes, including gene silencing, DNA repair and maintenance of genomic integrity. The functions of TRIM28 and its major protein interaction partner WTX as well as that of other Wilms tumor genes have been linked to the modulation of cytoplasmic WNT signaling. TRIM28 Loss-of-function (LoF) variants in the ExAC and gnomAD databases are extremely rare. In fact, the high probability of LoF intolerance score (pLI) of 1.00 and high Z-score of 3.16 in ExAC indicated that TRIM28 is highly intolerant for normal variation.

To establish the prevalence of germline TRIM28 mutations in individuals with Wilms tumors, we performed targeted sequencing of further 269 cases. In total, we identified mutations in tumor samples of eight additional individuals, including two nonsense, one splice site and four frameshift mutations. Validation on DNA derived from normal kidney tissue confirmed that all mutations but one were present in a heterozygous state in

the germline. In one individual postzygotic mosaicism was indicated by a mutation allele frequency of about 5–10% in the germline sample. Pathological examination of the tumors of 6 individuals showed diffuse anaplasia in one case and a predominantly epithelial histology in all remaining cases. Four out of seven individuals had bilateral Wilms tumors, and all patients but one exhibited either perilobar nephrogenic rests (PLNR) or nephroblastomatosis. Expression studies on mRNA and protein levels showed a significant reduction of TRIM28, confirming a loss-of-function effect of the germline mutations. Overall, our results confirm TRIM28 as a novel Wilms tumor predisposition gene.

W3 Biological Mechanisms

W3-01

Insights into Liechtenstein-Knorr Syndrome

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Liechtenstein-Knorr syndrome (LIKNS) is an autosomal recessive disorder characterized by early onset of cerebellar ataxia in combination with hearing loss which is associated with mutations in the Solute Carrier Family 9 Member A1 (SLC9A1) gene. SLC9A1 encodes the broadly expressed Na⁺/H⁺ Exchanger 1 (NHE1), which localizes to the plasma membrane and contributes to pH regulation by extruding protons in exchange for Na⁺. We generated a mouse model deficient of Nhe1 resulting in severe ataxia and early mortality. Stained brain sections of Nhe1-WT/KO mice and subcellular fractionation of brains tissue suggests that Nhe1 is enriched at excitatory and inhibitory presynapses. To investigate its role for the neuronal network, we either disrupted Nhe1 specifically in principal neurons (Emx1-Cre) and in a subset of inhibitory neurons (PV-Cre). Surprisingly, Emx1-specific knockout mice did not show any obvious phenotype, while PV-specific knockout mice showed severe ataxia and epilepsy. Electrophysiological characterization of acute brain slices revealed that GABAergic transmission was severely perturbed upon disruption of Nhe1 in PV-positive interneurons. Pointing to a defect of GABA vesicle release, the frequency of miniature inhibitory postsynaptic currents (mIPSCs) was decreased and their amplitudes increased. Alkalization of neurons by trimethylamine normalized mIPSC amplitudes but not frequencies. This suggests that Nhe1 modulates the loading of GABA vesicles in a pH dependent manner, while its effect on release probability is pH independent. Taken together, our data provide first evidence that ataxia in LIKNS is caused by a defect of the inhibitory control of the neuronal network.

W3-02

NEK3 dysfunction is associated with impaired tubulin phosphorylation and causes primary microcephaly in human and mouse

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During human cortical development, most neurons are born at embryonic stages by successive division cycles of neural stem cells. Neurons then migrate and integrate into different cortical layers and areas, which provide the structural basis of sensorimotor processing and intellectual functionality of the cerebral cortex. Defects in neurogenesis lead to malformations in cortical development such as primary microcephaly.

We present a 2-year-old boy with primary microcephaly, hypomyelination of the brain, frontotemporal hypotrophy, and pulmonary stenosis. Multigene panel analysis excluded mutations in all known microcephaly-associated genes. Using trio exome sequencing, we identified a de novo missense mutation, p.Y81C, in NEK3 which encodes a serine/threonine-protein kinase. The tyrosine at position 81 is highly conserved and predicted to impair protein function. In order to explore the effects of NEK3 in cortical development and the genetic mechanisms underlying NEK3 perturbation in primary microcephaly, we generated Nek3 knockout (KO) and Nek3.pY81C mouse mutants by using CRISPR/Cas9 system and a fast microinjection-independent technique. Our brain phenotype analysis indicated that the missense mutation of Nek3 results in altered brain morphology and microcephaly in mouse, possibly by a defect in neuronal differentiation. In addition, our biochemical experiments revealed that NEK3 interacts and controls phosphorylation of many α - and β -tubulin isoforms, and the microcephaly-associated NEK3 mutation resulted in the loss of tubulin phosphorylation.

Our findings show that NEK3 plays important roles during brain development by controlling tubulin phosphorylation and suggests that loss of NEK3-mediated phosphorylation causes primary microcephaly by alteration of tubulin function.

W3-03

In vivo and in vitro characterization of microRNA-149, a candidate for orofacial clefting.

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Nonsyndromic cleft lip with/without cleft palate (nsCL/P) is a common facial malformation with multifactorial etiology. The majority of risk loci known to date map to non-coding regions of the genome, suggesting that the associated variants act through regulatory effects on gene expression. One potential mechanism involved in nsCL/P are micro RNAs (miRNA), which mediate specific posttranscriptional gene regulation. Previous studies have already shown that miRNAs are involved in craniofacial morphogenesis, and that dysregulation of miRNA expression can lead to severe facial malformations.

In the first phase of this project, presented at last year's conference, our group combined array-based miRNA profiling in human neural crest cells (hNCC) with in-house GWAS to identify candidate miRNAs for nsCL/P. hNCC are mesenchymal precursor cells that give rise to the majority of cranial cartilage and bones, rendering them a suitable cellular model to study craniofacial development and disease. Our approach revealed miRNA-149-3p as strong candidate for nsCL/P involvement, based on consistent hNCC-expression across replicates and the presence of associated risk variants within its genomic region.

In the second phase of the project, we aim at understanding the molecular impact of miR-149-3p in the context of craniofacial development. In endothelial cells, it has been shown that miRNA-149 represses GPC1 and FGFR1, two genes known to regulate cellular migration. Using RNA-Seq data from hNCC, we found both genes to be expressed, suggesting that miRNA-149 might act on nsCL/P through a similar network. We chose in vivo and in vitro approaches to characterize the role of miR-149-3p further. In vitro, we modified miRNA-149 abundance levels in hNCC by overexpression and inhibition assays and monitored the migration behavior of hNCC using scratch assays. We found that inhibition of miR-149-3p expression show significantly higher cellular migration rates as compared to control cells. To identify target genes of miR-149-3p, we are currently performing RNA-Seq of differently treated cells at various time-points after treatment. For in vivo analyses we use a zebrafish model in which candidate genes, such as GPC1, are analyzed for expression pattern and molecular effects after knockdown/overexpression. At first, we established a time-course of Sox10-fluorescence in the zebrafish larvae, allowing for tracing the trajectories of hNCC-derived cells. Furthermore, alcian staining is performed to identify phenotypic effects on the palatal structures. Our study will provide unique insights into the molecular effects of miR-149-3p in craniofacial development and the modification of these processes in the context of associated common variation.

W3-04

Unexpected phenotypes and novel pathomechanistic insights in mouse models for autosomal recessive cutis laxa type 2A

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Mutations in *ATP6V0A2*, encoding the $\alpha 2$ subunit of vacuolar(v)-type ATPases, cause autosomal recessive cutis laxa type II A (ARCL2A). Besides the typical wrinkly skin patients also show cobblestone-like frontal cortex malformations, variable developmental delay, seizures, and a congenital defect of glycosylation (CDG). The $\alpha 2$ subunit is mainly localized at the Golgi compartment. V-type ATPases are large multisubunit complexes responsible for proton transport into intracellular organelles. The $\alpha 2$ subunit is responsible for subcellular targeting of the complex and directly involved in proton transport, but also a SNARE-like function in vesicle fusion is discussed.

In order to distinguish these two different mechanisms we generated a knock-out mouse model with a complete loss of $\alpha 2$ and a knock-in mouse model resulting in a stable $\alpha 2$ protein that is not able to transport protons. Both disease models recapitulate the human phenotype. The dermis appeared thinned and dermal decorin showed an abnormal glycosylation. Mutant mice showed seizures and aberrant migration of neurons in the frontal cortex. Additionally we detected a loss of layer I in the frontal cortex and an abnormal distribution of neurons within the different layers. Unexpectedly, we were not able to detect glycosylation abnormalities in brain and serum proteins. Evidence suggests that delayed secretion of extracellular matrix components might cause abnormal neuronal migration. Surprisingly, homozygous male mice turned out to be infertile and histo-

logical investigations of the testis showed globozoospermia. Acrosome formation was disrupted at an early stage similar to defects in other proteins involved in retrograde transport within the Golgi compartment. Electron microscopy revealed swollen Golgi-derived vesicles that are unable to fuse. In line with these observations, we found a delay in anterograde and retrograde Golgi transport in fibroblasts, which was more pronounced in the knock-out, while a pH elevation in the trans-Golgi was more pronounced in the knock-in.

We conclude that both, vesicular trafficking and fusion, as well as altered vesicular pH cause an ARCL2A-like phenotype in *Atp6v0a2* mutant mice that also includes globozoospermia. Unexpectedly, the glycosylation abnormalities, which are pathognomonic for human ARCL2A, are almost absent in our disease models.

W3-05

The FOCAD tumor suppressor is associated with survival in IDH mutant glioma, targets the centrosome, impacts microtubule assembly and mitotic progression

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In search of novel genes associated with glioma tumorigenesis, we have previously shown that *KIAA1797/FOCAD* is frequently deleted in gliomas and that the encoded focadhesin functions as a tumor suppressor impacting proliferation and migration capacity of glioma cells *in vitro* and *in vivo*. In this study, we examine an association of *FOCAD* loss with overall survival of glioma patients, and address the molecular mechanisms that govern the suppressive effect of focadhesin on glioma tumorigenesis. We found *FOCAD* loss to be associated with profoundly inferior outcome in patients with isocitrate dehydrogenase-mutant WHO grade III and IV gliomas. Multivariable analysis of WHO grade III and IV gliomas confirmed *FOCAD* loss as an independent prognostic factor for overall survival. Subsequently, using a yeast two-hybrid screen and pull-down assays, we identified tubulin beta-6 and other members of the tubulin family as novel focadhesin-interacting partners. We demonstrate that tubulins and focadhesin co-localize at the centrosome, and that focadhesin is enriched at interphase and mitotic centrioles and pericentriolar material. Focadhesin also localizes to microtubules and reduces the microtubule assembly rate in glioblastoma cells by impairing recruitment of the focadhesin interaction partner, SLAIN motif-containing protein 2, possibly explaining why focadhesin decreases cell migration. During the cell cycle, focadhesin levels peak prior to mitosis and influence time-dependent mitotic progression, possibly via regulation of polo-like kinase 1, providing a possible explanation for focadhesin-dependent cell growth reduction. We conclude that *FOCAD* loss is associated with significantly decreased overall survival in subsets of glioma patients, a predictive effect that may be linked to our

findings that focadhesin physically interacts with members of the tubulin family, impacts microtubule assembly and mitotic progression.

W3-06

Multisite de novo mutations in human offspring after paternal exposure to ionizing radiation

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It has been recently shown that Ionizing radiation can cause a particular pattern of mutations in DNA. Multisite de novo mutations (MSDNs) are defined as at least two lesions occurring no more than 20 bp apart. During the 1950's to the early 1980's many of the radar technicians of the German "Bundeswehr" and the "NVA" were exposed to high doses of radiation due to suboptimal shielding of the weapon systems they were operating. In this study we perform a genome wide evaluation of the effects of ionizing radiation on the offspring of exposed soldiers based on the occurrence of MSDNs.

For the pilot study, we performed whole genome sequencing and thorough analysis for MSDNs for 18 families of radar soldiers. The same analysis was performed on 28 trios with no known exposure to ionizing radiation. In our analysis we identified 12 MSDNs in 18 offsprings of radar soldiers, with one family accounting for 6 MSDNs in 3 children. While controls showed only 1 MSDN per 5 offsprings in average. All MSDNs in offsprings of radar soldiers were identified on the paternal allele. Phasing was performed using PacBio long-read sequencing. Aside from the MSDNs we found two de novo translocations in offspring of radar soldiers.

In the pilot study we found an increase in MSDNs in the offspring of radar soldiers, which were likely exposed to ionizing radiation compared to the control group. This indicates that MSDNs might be suited to assess the effects of ionizing radiation on human DNA. Based on the findings of the pilot study the German parliament approved an extension of study and requested sequencing of at least 80 further families of radar soldiers in order to verify these results in a statistically significant manner.

W4 Technology and Bioinformatics

W4-01

Exploring unknown genotype-phenotype relationship by next-generation phenotyping approaches

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Introduction: Recent advances in computer vision and machine learning resulted in the next-generation phenotyping (NGP) tools for syndromology. Deep convolutional neural networks such as DeepGestalt in Face2Gene have been trained on thousands of patient photos with confirmed molecular diagnoses and learned phenotype representations of multiple disorders. These systems of artificial intelligence support clinicians in the diagnostic workup of patients and even excel human experts in certain classification

tasks. We therefore wondered whether this unprecedented sensitivity to detect mutation-specific patterns in the facial gestalt could also be used to reveal information about gene function and further explore the unknown genotype and phenotype relationship.

Materials and Methods: We compiled a data set consisting of 679 patients with 105 different monogenic disorders. For each individual in the cohort a frontal photo and the disease-causing mutation are available. We utilized FaceNet to train a convolutional neural network with the photos of 697 patients and constructed the Clinical Face Phenotype Space (CFPS). We further performed clustering analysis in this space to quantify the similarities between each patient.

Results: A prominent cluster was formed by BRAF, PTPN11, NRAS and other genes of the MAPKinase pathway that result in phenotypes such as Noonan, LEOPARD or cardiofaciocutaneous syndrome and which are considered similar. Likewise, many genes linked to inborn errors of metabolism also clustered, mirroring a higher-level feature that is referred to by clinicians as 'coarse facies'. Furthermore, two unrelated patients with a progeria-like facial phenotype of initially unknown molecular cause were located in close proximity to cases with mutations in LMNA and PYCR1. Diagnostic workup later identified the same de novo mutation in a novel disease gene in these two patients with similar function.

Conclusion: We were able to reproduce molecular interactions by information encoded in the facial gestalt by using NGP tools. Moreover, the unknown phenotype and genotype was discovered by clustering patients in the CFPS. Thus, the phenotype space exploration is a promising subject in the characterization of gene function.

W4-02

Identification and characterization of disease-causing genes in non-5q-SMA by next-generation sequencing technology: lessons learned from NeurOmics study

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Spinal muscular atrophy (SMA) is one of the most common neuromuscular disorders (NMDs). It is characterized by the loss of α -motor neurons in the spinal cord causing symmetrical muscle weakness and atrophy of limb and trunk muscles. Most of the individuals with a strong clinical diagnosis of SMA show biallelic mutations causing functional loss of *SMN1* (5q13.2). On the other hand, SMA cases without deletions or point mutations in *SMN1* (non-5q-SMA) constitute a clinically and genetically heterogeneous group of disorders. Non-5q-SMAs have high phenotypic variability and diverse inheritance pattern. Due to phenotypical overlaps between several types of motor neuron disorders, the differential diagnosis of non-5q-SMAs is challenging. Next-generation sequencing (NGS) has become the standard method in the diagnosis of NMDs with high genetic heterogeneity.

For all individuals, *SMN1* deletions had to be excluded by using MLPA. After deep-phenotyping the individuals with negative results, we included 214 patients from 162 families with non-5q-SMA. We used short-read NGS methods in stepwise approach. For 30 patients, we first developed a gene panel restricted to 62 lower motor neuron disease-associated genes (LMND panel). Later, 90 newly recruited patients were tested with a more comprehensive panel harboring 479 known NMD-associated genes (NMD panel). For 6 patients with unusual features, we performed Mendeliome sequencing to investigate all possible Mendelian gene mutations. The unsolved individuals from both panels were subjected to whole ex-

ome or whole genome sequencing (WES/WGS) in presence of the consent for the test.

During the last 22 years, we performed *SMN1* deletion analysis to 3,465 individuals with suspected SMA. In 1,676 (48.4%) of these individuals, no *SMN1* deletion or mutation was detected. LMND panel provided a genetic diagnose to four individuals (13%, 4 of 30). Due to low diagnostic yield from the LMND panel, we continued with larger NMD panel. We tested 55 patients with non-5q-SMA and 35 patients with axonal Charcot-Marie-Tooth neuropathy (CMT). NMD panel provided the genetic diagnose to 31% (17 of 55) non-5q-SMA patients and 63% (22 of 35) of axonal CMT patients. Non-5q-SMA group showed a high genetic heterogeneity, as only 36% of the discovered mutations were found in SMA-associated genes, thus confirms the necessity of using gene panels with expanded gene profile. To improve our diagnostic yield, we performed WES or WGS for individuals that remained unsolved after LMND panel ($n=15$) or NMD panel ($n=9$). WES/WGS revealed the disease-causing mutation in 37% (9 in 24) of the patients previously unsolved by gene panels. By WES/WGS, we identified in various unrelated families six novel genes (*BICD2*, *PIEZO2*, *CHPI*, *VAMPI*, *PRUNE1*, *ADPRHL2*) as a novel disease-causing gene. By Mendeliome sequencing, we identified *MCM3AP* as a novel disease-causing gene autosomal recessive CMT.

Altogether, our data between 2012–2017 corroborated NGS as a powerful method for diagnostic and gene identification purposes in non-5q-SMA. Targeted gene panels with larger gene representation show a robust performance and high diagnostic yield for non-5q-SMA and axonal CMT. Targeted sequencing performs best in clinical conditions with certain phenotypes with highly heterogenic genetic background. For phenotypes that are more complex or for phenotypes with unexpected findings, a more comprehensive WES or WGS methods should be used.

W4-03

A scalable framework to identify regulatory variants at non-coding GWAS loci and its application to orofacial clefting

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For many multifactorial disorders, GWAS and meta-analyses have yielded unprecedented insights into their genetic etiology. Major outcomes have been that associated common risk variants (i) are mainly located in non-coding regions of the human genome, (ii) tend to have low to moderate effect sizes, and (iii) show extended stretches of LD. These findings hamper the identification of regulatory elements and/or causal variants. While approaches such as eQTL analyses have been successful in some disorders, their application is restricted to traits where disease-relevant cell types and tissues are available.

An alternative approach to narrow down the functional candidate regions at GWAS loci is via the identification of rare, deleterious mutations in individual families, and/or the increased burden of low-frequency variants in cases as opposed to controls, consequently, resequencing of large case-control cohorts is required. While whole genome sequencing is currently very cost-intensive in large cohorts, targeted resequencing approaches such as single-molecule molecular inversion probes (smMIPs) have emerged as strong alternative. As the associated regions at GWAS loci can be up to hundreds of kb in size, a selection step to define the regions of interest within a candidate GWAS locus is required. Criteria might include the most associated variants of genetic data, and/or the integration of func-

tional and genomic data such as DNase I hypersensitive or transcription factor binding sites, and histone modifications.

Here we applied the described strategy to non-syndromic cleft lip with or without cleft palate (nsCL/P). nsCL/P is a frequent birth defect with multifactorial etiology, for which about 40 risk loci have been identified by GWAS. With few exceptions, the causative regulatory elements and the functional effect of common risk variants have not yet been pinpointed. While eQTL analyses are not possible, rare variants have been identified (including variants at GWAS risk loci such as the 350dupA-mutation at the *IRF6* locus). We selected three risk loci that had been previously associated with risk for nsCL/P ($P < 10^{-5}$). Using credible SNP regions, twelve candidate elements were identified based on publicly available functional data from human neural crest cells and human craniofacial tissue. smMIPs-based resequencing of these regions (totaling 57 kb) in 2,652 samples (1,061 nsCL/P cases and 1,591 ethnically matched controls) was performed, yielding 2,382 variants in total, and 1,835 variants with a MAF $< 1\%$. Identified variants (common, low-frequency and rare) are currently analyzed individually and in aggregate, including pedigree analyses (where possible). In addition, we are testing different non-coding variant annotation tools in order to separate potentially causative variants from those that are benign. Our framework is scalable to incorporate more regions and can be extended to other disorders where access to relevant tissue is similarly difficult.

W4-04

Capturing epistatic effect with deep learning—a comparison with polygenic risk score on trait prediction from SNP-based genetic data

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Background: Polygenic Risk Scores (PRS) that consider additive effects of multiple variants are now commonly used to test for association of complex disorders. Recent studies even suggest that PRS can be used for risk assessment of complex disorders such as coronary artery disease. However, predictive powers of PRS on other heritable traits such as type 2 diabetes and inflammatory bowel disease have yet to reach a clinically useful level. Since a PRS considers only the additive component of genetic variance, it is unlikely such model can fully account for the genetic architecture of all complex traits. Substantial differences between the heritability estimated through twin studies and SNP-based heritability have been consistently observed in different traits. Part of the so-called “missing heritability” may be attributed to rare variant risk effects as well as presence of non-additive effects.

Deep learning (DL) is a sub-category of machine learning capable of extracting non-linear features. In this study we evaluated the use of DL on capturing variant-variant interaction (epistatic) effects with simulated SNP-based genetic data. **Methods:** As a proof of concept, we simulated panels containing around 20,000 independent SNPs for 20,000 individuals. We considered various proportions of “causal” SNPs to explore the utilities of DL under different signal to noise ratios representing different levels of polygenic signals (i.e. from “oligogenic” to “omnigenic”); one dataset was simulated with only additive effects and one with both additive and epistatic effects. Effect sizes were simulated based on observations from real world datasets of large scale GWAS. PRS prediction and DL were applied on simulated datasets for disease classification and performances were assessed by computing the area under curve (AUC) of receiver operating characteristics. Additionally, we estimated, for each dataset, the empirical maximum AUC for additive effects (AUC_A) and the maximum AUC for additive plus epistatic effects (AUC_{A + E}) by computing PRS including all causal alleles. A simple fully connected network with 3 hidden layers was used in this study and an ensemble of 5 networks was trained.

Results: Deep Learning Classifier (DLC) outperformed PRS in all datasets (average difference in AUC = 0.05); however, the difference was larger in presence of epistasis (presence of epistasis = 0.06 vs absence of epista-

sis=0.04). Under the omnigenic model, the DLC achieved an AUC higher than the empirical maximum AUC considering all additive variants (AUC_[DL] vs AUC_A vs AUC_[A + E] = 0.82 vs 0.80 vs 0.84).

Conclusion: These results suggest that DL approaches are able to capture non-linear relationship in genetic data which would be neglected by additive PRS tests. In particular, by simulating different in-silico datasets representing various level of complexity, we show that DLC outperforms PRS in traits prediction (AUC), especially in the presence of epistatic interactions.

W4-05

Inference of tumor cell-specific transcription factor binding from cell-free DNA

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Alterations in transcription factors are important drivers of tumorigenesis, but non-invasive assays for assessing transcription factor activity are lacking. Here, we evaluated the feasibility of inferring transcription factor binding in solid tumors from their nucleosome footprint in circulating cell-free DNA. We developed a novel analysis pipeline to determine accessibility of transcription factor binding sites and applied it to 244 cell-free DNA samples from patients with prostate, breast and colon cancer. We observed patient-specific as well as tumor-specific patterns. Specifically, inferred binding patterns for the transcription factors AR, HOXB13, and NKX3-1 allowed us to classify patients by tumor type, including subtypes of prostate cancer, which has clinical implications for the management of patients. Our approach for mapping tumor-specific transcription factor binding in vivo based on blood samples makes a key part of the noncoding genome amenable for clinical analysis.

W4-06

Cell type resolution of cis-eQTL in GTEx tissues

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The Genotype-Tissue Expression (GTEx) project has identified expression quantitative trait loci (cis-eQTL) for the majority of genes across a range of human tissues. However, the interpretation of these eQTL has been limited by the complex cell type composition of GTEx tissue samples.

Here, we applied gene expression-based deconvolution approaches to estimate the cellular composition of GTEx tissues, based on gene expression profiles of 17,382 RNA-seq samples from 838 individuals across 49 tissues (v8 data release). We found that highly abundant cell types were robustly estimated across multiple methods and were consistent with cell types expected to be present in different tissues. Cell type composition explained a large part of inter-individual variation in gene expression and

provided insights into cellular events associated to pathological changes in GTEx individuals.

To demonstrate that computationally inferred cell type abundances can be used to identify cell type-dependent eQTL, we used neutrophil estimates from GTEx whole blood RNA-seq data (670 individuals) and neuron estimates from 13 GTEx brain tissues (126–209 individuals) to identify cell type-interacting eQTL (ieQTL). Brain tissues were jointly analyzed using the Multivariate Adaptive Shrinkage (mash) method to improve power and accuracy of effect estimates. We found 1258 and 367 genome-wide significant (FDR 5%; LFSR 1%) ieQTL interacting with neutrophil and neuron abundance respectively; these showed good replication of eQTL identified in purified neutrophil and in external replication data sets. Cell type ieQTL were enriched among tissue-specific eQTL, eQTL with multiple independent signals and in context-specific functional genomic regions. Finally, colocalization analysis of cell type ieQTL with 114 complex traits not only pinpointed the cellular origin of known whole blood and brain eQTL but identified novel colocalized loci that are masked in bulk tissue. We show that cell type ieQTL help to disentangle the allelic heterogeneity of cis-eQTL and improve candidate causal gene detection in disease-associated loci.

Taken together, our results emphasize the importance of identifying genetic effects on gene expression at the cellular level and demonstrate that cell type-specific effects can be uncovered in bulk tissues by integrating increasingly available single-cell and cell type-specific transcriptomic data. The GTEx tissues provide a unique opportunity to expand this approach to immune and stromal cell types in tissues where cell-specific eQTL have not yet been characterized.

W5 Monogenic Diseases 1

W5-01

Scientific yield of diagnostic exome sequencing and systematic scoring of candidate genes in neurodevelopmental disorders

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Introduction: Negative exome sequencing in diagnostic settings produces valuable data that is often neglected. This leads to loss of scientific and clinical value and hinders deciphering the genetics of rare disorders.

Materials and Methods: We performed trio exome sequencing of 450 undiagnosed cases, 384 of these are cases of neurodevelopmental disorders (NDD), and analysis of 203 is completed. Of these, 59% were preceded by a large, but negative diagnostic panel. Sequencing was performed by Centogene (Rostock), CeGaT (Tübingen) or BGI (Hong Kong) using well-established enrichment kits and sequencing platforms. Bioinformatic anal-

yses were performed by Limbus (Rostock). If exome sequencing did not reveal a clear causative variant in an established NDD gene, we searched for candidate genes on scientific basis. To estimate the relevance of identified candidate genes, we established a scoring system with maximum of 13.5 points by using 13 parameters, based on inheritance, gene and variant attributes, and published literature.

Results: A reliable clinical genetic diagnosis was achieved in 60 (29.5%) trios, corresponding to 20% in cases preceded by panel diagnostics and 43% in cases without prescreening. In the remaining 143 (70.5%) trios, re-evaluation revealed potentially causative variants in 146 candidate genes. We applied our scoring system to prioritize the relevance of these variants. The scores varied between 2 and 11.9. The top 10% of the scores (score >9) are *ASIC1*, *TANC2*, *KMT2E*, *GLS*, *ACTL6B*, *GRIN3B*, *CUX1*, *UNC13A*, *GRIA4*, *PUM2*, *TOP1*, *WDFY3*, *NPTX1* and *SPEN*. For the majority of the above mentioned candidates with highest scoring variants as well as for other candidates with score >8 additional cases have been identified through our network of collaborators and through matching platforms. Most of the genes are either published, submitted or the manuscript is in preparation.

Conclusion: Our results illustrate the enormous scientific value of systematic re-evaluation of negative exome sequencing. We assume that deciphering the genetics of monogenic disorders is going to be achieved by the cooperation of multiple small centers, and not by meta-analyses. The latter lose power by concentrating on statistical thresholds and ignoring the specificity of each single case. In Weimar, we are going to also present the remaining data of at least 450 trio exomes.

W5-02

The genetic landscape of mitochondrial disease: exploration of over 1500 cases by whole exome sequencing

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Mitochondrial diseases pose a diagnostic challenge due to clinical and genetic heterogeneity, propelling unbiased whole exome sequencing (WES) into the early diagnostic setting. To date, over 300 disease-associated genes implicated in mitochondrial energy metabolism are recognised, and this number continues to grow. Through global collaboration, initiated by the European Network for Mitochondrial Diseases, GENOMIT, we assimilate data from patients investigated by WES under clinical and/or biochemical suspicion of mitochondrial disease. Systematic analysis of WES data from 1630 paediatric and adult patients thus far, establishes a genetic diagnosis in 752 (46%). Furthermore, though this endeavour and subsequent follow-up studies, over 40 novel disease genes have been discovered. Pathogenic variant(s) harbour within 323 different genes with a diagnosis in 194 genes occurring in single cases only. Diagnoses in 68% of solved cases fall within one of 148 mitochondrial disease-associated genes, with defects in *ACAD9*, *ECHS1*, *FBXL4*, *MT-ATP6* and *MT-ND1* occurring most frequently. Remarkably, the remaining 32% of solved cases fall within non-primarily mitochondrial disease-associated genes (including *TANGO2*, *CAD*, *IARS*, *SLC52A2*), highlighting difficulty in disease definition. This is further reflected by 60 patients with molecularly-verified mitochondrial disease demonstrated normal respiratory chain complex activity and 64 patients, not included in this study, with unsuspected yet genetically-proven mito-

chondrial disease diagnosed in the same time-period. To date almost 40 mitochondrial diseases, potentially amendable to a specific disease-modifying treatment, have been described, such as the cofactor metabolism defects where supplementation can be efficacious. Of our molecularly confirmed mitochondrial disease cases, 99 (19%) fall into this category. However, effective defect-targeted treatments for the majority of mitochondrial diseases remain elusive, thus genotyping is fast becoming the prerequisite for clinical trial inclusion and the development of treatment. This study exemplifies the importance of WES in the diagnosis of suspected mitochondrial disease and emphasises the value of international collaboration in the rare disease setting. By coupling exomes to clinical, biochemical and experimental data, we further elucidate the heterogeneous genetic landscape of mitochondrial disease and prompt careful consideration of diagnostic criteria, by emphasising discrepancies between clinical, biochemical and genetic means of diagnosis and demonstrating the need to consider this entire body of evidence in aggregate. GENOMIT is hence establishing a valuable global registry for both clinical and genomic data which is open to further expansion.

W5-03

A homozygous two exon deletion in UQCRH: matching mouse and human phenotypes

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The ubiquinol:cytochrome c oxidoreductase hinge protein (UQCRH) is a very small subunit of complex III (CIII) of the oxidative phosphorylation (OXPHOS) system. UQCRH connects the subunits cytochrome c with cytochrome c1. Thus, it plays an important role in the assembly of CIII. A disease linked to *UQCRH* has not yet been described, although somatic mutations to *UQCRH* have been linked to different types of cancer.

Whole exome sequencing and autozygosity mapping were performed on two male first cousins from a consanguineous family with recurrent episodes of severe ketoacidosis, excess blood ammonia and hypoglycaemia and signs of encephalopathy. Brain MRIs showed no abnormality and between episodes the health of the two patients was entirely normal. OXPHOS protein levels and complex assembly were analysed by western blot and blue native PAGE. A homozygous *UQCRH* deletion of exons 2 and 3 was detected.

A mouse model was created by deletion of exons 2 and 3 of *Uqcrh* and screening of the mouse phenotypes was performed. The murine presentation was more severe compared to the human, with progressive functional impairment and premature early adult death. Enzymatic activity and protein expression of OXPHOS complexes were investigated in mouse tissue

revealing a significant decrease in CIII activity in heart, brain and liver tissues. Expression analysis of mRNA from mouse liver tissue and mouse embryonic fibroblast (MEFs) showed a reduced expression of *Uqcrlh* transcript, more severe in the homozygous compared to the heterozygous. Proteomic analysis of the heart from WT and homozygous mice, showed reduced CIII expression and altered assembly. Cultured patients' primary fibroblasts also showed incomplete assembly and decreased steady-state protein levels of CIII subunits and low CIII enzyme activity. Moreover, Seahorse analysis of both MEFs and patients' primary and immortalized fibroblast revealed reduced maximal respiration.

Here we describe the first patients with biallelic mutations in *UQCRH*. Notably, biallelic variants in *UQCRC2* and *UQCRB*, two other subunits of complex III, result in a similar episodic clinical presentation.

W5-04

Biallelic Mutations in ADPRHL2, Encoding ADP-Ribosylhydrolase 3, Lead to a Degenerative Pediatric Stress-Induced Epileptic Ataxia Syndrome

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ADP-ribosylation, the addition of poly-ADP ribose (PAR) onto proteins, is a response signal to cellular challenges, such as excitotoxicity or oxidative stress. This process is catalyzed by a group of enzymes referred to as poly(ADP-ribose) polymerases (PARPs). Because the accumulation of proteins with this modification results in cell death, its negative regulation restores cellular homeostasis: a process mediated by poly-ADP ribose glycohydrolases (PARGs) and ADP-ribosylhydrolase proteins (ARHs). Using linkage analysis and exome or genome sequencing, we identified recessive inactivating mutations in *ADPRHL2* in six families. Affected individuals exhibited a pediatric-onset neurodegenerative disorder with progressive brain atrophy, developmental regression, and seizures in association with periods of stress, such as infections. Loss of the *Drosophila* paralog Parg showed lethality in response to oxidative challenge that was rescued by human *ADPRHL2*, suggesting functional conservation. Pharmacological inhibition of PARP also rescued the phenotype, suggesting the possibility of postnatal treatment for this genetic condition.

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W5-05

Aberrant DEGS1 sphingolipid metabolism impairs central and peripheral nervous system function in humans

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Sphingolipids including ceramides are important components of cellular membranes and functionally associated with essential processes such as

cell differentiation, neuronal signaling and myelin sheath formation. Mutations in sphingolipid metabolizing enzymes are associated with a variety of pathological conditions, affecting proper function of both the peripheral and the central nervous system. Besides defects in the catabolism of complex sphingolipids manifesting as lipid storage diseases, defects in the *de-novo* synthesis are also disease-causing. However, the entire spectrum of disorders affecting sphingolipid metabolism remains elusive.

By trio whole-exome sequencing we identified a homozygous variant (p.Ala280Val) in a highly conserved region of *DEGS1*, the gene encoding the sphingolipid metabolism enzyme dihydroceramide desaturase 1 (DEGS1). The affected patient presented with a multisystem neurological phenotype, including developmental delay, leukoencephalopathy, cerebellar ataxia, and peripheral neuropathy. MRI and peripheral nerve biopsies confirmed an underlying hypomyelination and neurodegeneration of the CNS and PNS. In line with the function of *DEGS1*, lipidomics analysis revealed a significantly shifted blood sphingolipid profile in the patient from unsaturated to dihydro-forms of sphingolipids. The same metabolic shift was seen in patient-derived fibroblasts. Additionally, we identified an atypical and potentially toxic sphingolipid metabolite, which is formed as consequence of the altered synthesis pathway. The changes in intracellular sphingolipid profiles were recapitulated in a CRISPR/Cas-based *DEGS1* knockout HAP1-cell model, suggesting a loss-of-function mechanism as the underlying defect due to an altered protein stability.

Our findings identify *DEGS1* for the first time as gene leading to a human disease, i.e. a multisystem neurological sphingolipid disorder. A sphingolipid-rich diet may correct the impaired metabolic profile and improve the clinical outcome of affected patients and could offer a potential treatment of this heritable condition.

W5-06

Disruption of CLRN2, a gene encoding clarin-2, causes autosomal recessive hearing loss in humans and zebrafish

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Non-syndromic hearing loss (NSHL) is the most common hereditary sensory disorder that affects one to two per 1000 newborns. Hereditary hearing loss is characteristically heterogeneous in both clinical and genetic aspects. Up to 80% of NSHL follows an autosomal recessive mode of inheritance. Out of the currently identified genes that are associated with autosomal recessive forms of NSHL, roughly 80% of these genes have been identified by studying families with consanguineous unions.

Homozygosity mapping of an extended consanguineous Iranian family segregating autosomal recessive sensorineural NSHL revealed a 15.2 Mb run of homozygosity on chromosome 4p15.32p15.1. Exome sequencing identified a novel homozygous missense variant contained within the locus affecting the gene clarin-2 (*CLRN2*) that was predicted to be pathogenic via in silico pathogenicity prediction programs. Segregation of the

variant in 14 recruited family members was concordant with autosomal recessive hearing loss. The missense variant was predicted to activate an exonic splice enhancer site. *In vitro* analyses using mini-gene assays revealed two splicing products; one of which was the normally spliced product, the other disclosed defective splicing leading to intron retention and a shift in the reading frame. The mutant *Clrn2*⁺-YFP protein was transiently expressed in zebrafish hair cells and it failed to localize to the hair bundle or plasma membrane.

The *clarin-2* (*CLRN2*) gene encodes a putative four transmembrane protein closely related to tetraspanins. Its amino acid sequence in the human, mouse, and zebrafish is well conserved and mutations in the paralogous gene, *clarin-1* (*CLRN1*), have been causally linked to hearing loss in humans (Usher syndrome IIIA, USH3A), mice, and zebrafish.

To investigate the functional consequence of disrupted *clarin-2*, we generated zebrafish carrying a *clrn2* knockout (KO) allele using CRISPR/Cas9 technology. *clrn2*^{+/KO} zebrafish were normal at all ages; however, *clrn2*^{KO/KO} larvae exhibited poor startle response (poor hearing) and a balance defect 6 days post fertilization. Hair cells in *clrn2*^{KO/KO} zebrafish displayed abnormal morphology with short and disrupted hair bundles and a significantly reduced number of mature ribbon synapses. The mechanotransduction function of *clrn2*^{KO/KO} neuromast hair cells, as reflected by microphonic potentials, was significantly attenuated. Consistent with the *clrn2*^{KO/KO} phenotype, we showed that *clrn2* mRNA is restricted to hair cells within the inner ear. Additionally, *Clrn2*-YFP fusion protein expressed in zebrafish hair cells localized to the hair bundle and plasma membrane. These data demonstrate that *clarin-2* is an essential hair cell protein, and its defect results in loss of hearing and balance in zebrafish.

Our results associate *CLRN2* with autosomal recessive NSHL and link disruption of *CLRN2* to the pathophysiology of hearing loss.

W6 Complex Genetic Diseases

W6-01

A protective LOXL1 variant associated with a decreased risk for pseudoexfoliation syndrome implicates retinoic acid signaling in its pathophysiology

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Background: Pseudoexfoliation (PEX) syndrome is a complex, age related, systemic disorder characterized by deposition of abnormally crosslinked, extracellular material in various ocular tissues. It often results in PEX glaucoma (PEXG), the most common identifiable form of secondary glaucoma, accounting for 25–70% of all open angle glaucoma. To date several large, genome wide association studies, based on a large international collaborative effort, revealed seven associated loci. Among them, LOXL1 is the major genetic and pathophysiologic risk factor for this complex disease. However, all major associated LOXL1 risk variants identified to date, show significant allele reversal between Europeans and other populations, casting doubts on their causative role in disease pathogenesis. Our aim was to identify and functionally characterize regulatory variants at the LOXL1 locus showing consistent association among different ethnic groups which should help clarify PEX pathogenesis.

Methods: Deep sequencing of LOXL1 locus was performed on a total of 3,281 PEX cases and 3,280 controls from Japan, Italy, South-Africa and Russia followed by independent testing of variants using genotype data of a German study group (755 PEX cases and 1,243 controls). Allele-specific binding affinities were analyzed by electrophoretic mobility shift assays (EMSA) using nuclear extracts from different human eye tissues, in-

cluding Tenon's capsule fibroblasts (hTKF), trabecular meshwork cells (hTMC) and non-pigmented ciliary epithelial cells (hNPE). LOXL1 promoter activity was measured using dual luciferase reporter assays in hTKF, hTMC and hNPE. Genome editing of human embryonic kidney cells 293T (HEK293T) was done by CRISPR/Cas9 technique using a dual cut approach. Transcript levels were determined by qPCR in ocular tissues obtained from donor eyes without and with manifest PEX syndrome.

Results: Analysis of sequencing data from different ethnic groups revealed one common variant, rs7173049, near LOXL1 with minor allele G enriched in Italian, Japanese, South-Africa, Russian and German controls, thus consistent associated with a decrease in PEX risk (OR = 0.63, *p* = 6.33 × 10⁻³¹). Functional assays provided evidence for a regulatory activity of the genomic region surrounding rs7173049. Deletion of 200 bp around the rs7173049 SNP, using CRISPR/Cas9 technology, resulted in upregulated expression levels of two neighboring genes, STRA6 (stimulated by retinoic acid receptor 6) and ISLR2 (immunoglobulin superfamily containing leucine-rich repeat protein 2), located within 1 Mb from rs7173049. The protective G allele was found to increase tissue expression levels of STRA6 and ISLR2 by 75 and 100%, respectively. Both genes were significantly downregulated in PEX tissues at the mRNA and protein levels, together with other key components of the retinoic acid signaling pathway including retinol binding proteins and retinoic acid receptors. siRNA-mediated knockdown of RXRa resulted in significant upregulation of LOXL1 and PEX-relevant matrix genes such as elastin and fibrillin-1.

Conclusion: These data indicate an involvement of retinoic acid signaling in PEX pathogenesis and suggest that the intergenic variant rs7173049A>G could exert its protective effect by upregulating the expression of STRA6 and ISLR2 in ocular tissues. The identification of retinoid dysregulation as a contributing pathway to PEX pathophysiology may open up the possibility of new therapeutic interventions.

W6-02

MetaXcan gene-based association analysis yields novel insights into male-pattern baldness biology

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Male-pattern baldness (MPB) is a highly heritable trait and GWAS have implicated more than 130 genomic regions. The majority of associated variants however are located in noncoding regions and their functional relevance remains unclear. Integrative analyses that link genetic variation with biological function are needed to identify relevant genes and mechanisms. Here, we used MetaXcan (Barbeira et al., bioRxiv) on summary statistics from two published large-scale genetic studies on MPB (Heilmann-Heimbach et al., 2017; Hagenaars et al., 2017) and gene-expression data from human hair follicle (own data) and GTEx (skin, adipose tissue, blood) to test for an association between MPB and the genetically determined regulation of gene-expression in these tissues. The analysis identified associations between MPB and expression levels of 30 (hair follicle) to 637 genes (skin). Twenty-seven genes showed significant association in one or more tissues after Bonferroni correction (*P* < 0.05/27300 gene-tissue pairs). These included previously implicated genes (e.g. *WNT10A*, *IRF4*, *TWIST1*) and novel candidate genes such as *SPRR1B* and *TRADD*, which have a reported role in keratinocyte biology and apoptosis, thereby rendering an involvement of these processes in early MPB-pathogenesis likely. The fact that associations with previously implicated genes were detected in subcutaneous adipose tissue (*TWIST1*) and blood (*WNT10A*) lends further support to the hypothesis, that cells in the perifollicular environment play a role in MPB pathogenesis. In summary, our analyses provide evidence for an MPB-associated genetic regulation of plausible candidate genes (*SPRR1B*, *TRADD*, *WNT10A*) and biological processes (apoptosis) and yield novel insights into the functional effects of current GWAS findings.

W6-03

Integration of genetic data and functional data of human craniofacial development reveals insights into biological effects at known and novel risk loci for orofacial clefting

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Human craniofacial development (CD) is a well-orchestrated complex process during embryonic phase. The main cell type involved in this process is neural crest cells (NCC), which subsequently develop into cranial NCC and then generate the majority of craniofacial tissue. Defects in CD might lead to malformations such as orofacial clefts, including non-syndromic cleft lip with/without cleft palate (nsCL/P), one of the most common human birth defects. Although about 40 genetic risk factors of nsCL/P have been recently identified by GWAS, the translation of these findings into biologically meaningful results has largely been hampered by the lack of relevant human tissue, and the position of associated variants within non-coding regions. In the present study we generated the largest nsCL/P genotype data set to date and used comprehensive epigenetic and functional data from NCCs, cranial NCCs, and embryonic facial tissue, to gain insights into molecular mechanisms underlying CD and nsCL/P. Our meta-analysis, which included 1,248 cases, 2,887 controls and 2,789 trios from diverse ethnicities, revealed five novel nsCL/P risk loci at genome-wide significance. To elucidate relevant molecular mechanisms underlying the genetic associations we assessed publically available data sets from NCC, cNCC and craniofacial tissue. These data included expression data, histone modifications, chromatin accessibility, and interaction data. After rigorous quality control, alignments, peak calling, epigenetic imputation and chromatin segmentations, we first analyzed the 45 nsCL/P risk loci. Our results indicate that for about 50% of the now 45 nsCL/P risk loci, functional data in NCC and craniofacial tissue yield insights into enhancer-gene interactions and allow for prioritization of candidate genes. These loci include the 1p22 locus, where our analysis confirms craniofacial enhancers within introns of *ABCA4* regulating the expression of nearby located *ARHGAP29* in NCC and craniofacial tissue. Notably, our data also demonstrate that the more distantly located *ABCD3* becomes activated at later stages of CD, suggesting a yet unknown role of *ABCD3* at this locus. Similarly, at the novel 1p36 locus, our data show evidence of interaction with the promoters of several genes in both NCC and craniofacial tissue (*CAPZB*, *MINOS1*, *NBL1*). However, the data also demonstrate that the adjacent *HTR6* gene is activated in NCC but gets repressed at later stages of CD.

Our data provides new insights into the etiology of nsCL/P. Additionally, this is the first systematic integration of functional data from relevant embryonic cell types to understand the biological impact of the associated loci. Although analyses are still ongoing, our data strongly indicate that at some loci, several genes become activated and repressed in a time- and tissue-specific manner. This, in turn, implies that the “one locus—one candidate gene” theory might be too simplistic, at least for nsCL/P.

W6-04

Identification of functionally relevant loci to Barrett's esophagus and esophageal adenocarcinoma: cross-referencing of eQTL data from disease-relevant tissues with genetic association data

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Esophageal adenocarcinoma (EA) and its precancerous condition Barrett's esophagus (BE) are multifactorial diseases with rising prevalence rates in Western populations. A recent meta-analysis of genome-wide association studies (GWAS) data identified 14 BE/EA risk variants. However, most are located in intergenic or intronic regions of the genome, which renders identification of the disease-relevant genes and underlying pathomechanisms via GWAS data alone virtually impossible. Since many non-coding GWAS risk variants exert their effects via gene regulatory mechanisms, expression quantitative trait loci (eQTL) analyses make an important contribution to the elucidation of multifactorial disease etiology. The aim of the present study was to identify loci of functional relevance to BE/EA through the integration of eQTL- and genetic association data. For this purpose, eQTL data from disease-relevant tissues (esophageal mucosa, gastroesophageal junction) generated within the context of the Genotype-Tissue Expression (GTEx) project were cross-referenced with the GWAS meta-analysis data. Of the 14 previously identified risk variants, six eQTLs were identified in esophageal mucosa and/or gastroesophageal junction tissue, which have not been linked to BE/EA so far. The regulated genes included *C2orf43/LADH*, *ZFP57*, and *SLC9A3*. In addition, 16 variants representing eQTLs in esophageal mucosa and/or gastroesophageal junction showed evidence for association in the GWAS meta-analysis ($5 \times 10^{-08} < P < 5 \times 10^{-05}$). To follow up these variants, a genetic association study was performed in a replication sample comprising 1,993 BE/EA cases and 967 controls from Central Europe followed by a combined analysis with the GWAS meta-analysis data. Replication was achieved for two variants, which represent eQTLs for the expression of *CFDP1* and *SLC22A3*, respectively. In summary, the present approach identified genes whose expression was regulated by risk variants in disease-relevant tissues. These findings may facilitate the elucidation of BE/EA pathophysiology on a functional level.

W6-05

Dissecting the polygenic component of Barrett's esophagus and esophageal adenocarcinoma

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Esophageal adenocarcinoma (EA) is among the most common causes of cancer-related death worldwide and is therefore a major global health challenge. Barrett's esophagus (BE) is the premalignant precursor of EA, though only a small fraction of individuals with BE (estimated to be around 1%) will progress into EA. We performed linkage disequilibrium (LD) score regression analysis on the largest available GWAS meta-analysis including 6,167 patients with BE, 4,112 individuals with EA, and 17,159 controls (CTRL) showed a relatively high SNP-heritability (h^2) both for BE ($h^2=0.2533$, $se=0.0347$) and EA ($h^2=0.1923$, $se=0.0242$). We observed a large genetic correlation between BE and EA ($rg=0.85$, $se=0.06$) in the analyzed cohort. The combined meta-analysis data (BE and EA vs CTRL) revealed a $h^2=0.2706$ ($se=0.03$). Given this genetic background we evaluated the performance of Polygenic Risk Score (PRS) modeling for BE and EA. PRS have been calculated as the sum of the imputed dosage of risk alleles in a specific cohort (Bonn genotypes) weighted by the effect size estimates from an independent cohort (BEACON association statistics). PRS was calculated after clumping (to filter for independent genetic signal) considering different p -value thresholds for variant selection. The best fitting model was retained to calculate the strength of association, using p -value and Nagelkerke's R^2 of logistic regression including 4 PCA component to adjust for population ancestry. We identified a strong association of PRS comparing both BE against CTRL ($p=3.7E-5$, $R^2=0.56\%$, p -threshold=0.017) and EA against CTRL ($p=3.5E-7$, $R^2=0.67\%$, p -threshold=0.037). The association was stronger for the larger combined PRS analysis between BE and EA against CTRL ($p=5E-13$, $R^2=1.09\%$, p -threshold=0.038). However, we were not able to find a significant association for BE against EA using PRS ($p=0.09$, $R^2=0.15\%$, p -threshold=0.008). By means of Functional Mapping and Annotation of Genome-Wide Association Studies (FUMA) platform, we observed a tissue enrichment among BE/EA meta-analysis signal for genes specifically expressed in Esophagus suggesting that a strong component of polygenic signal might be mediated through the regulation of tissue specific expression. To test this hypothesis, we also performed an association test considering tissue specific gene-expression PRS (GE-PRS). GE-PRS was computed as the sum of gene expression z -scores (PrediXcan on Bonn genotyping data) weighted by their association size effects (computed by MetaXcan for the BEACON summary statistics). Noteworthy, GE-PRS in esophagus was associated with the case/control status ($p=1.2E-8$, $R^2=0.4\%$, p -threshold=0.82). In conclusion, our work suggests that BE and EA have a strong shared polygenic component. Interestingly, the genetic effect might be exerted by tissue-specific gene expression regulation.

W6-06

Genome-wide association study identifies 30 loci associated with bipolar disorder

Forstner AJ on behalf of the Bipolar Disorder Working Group of the Psychiatric Genomics Consortium.

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Bipolar disorder (BD) is a severe disorder of mood that is characterized by recurrent episodes of depression and mania. With a lifetime prevalence of

around 1% in the general population, BD is a common condition. The elevated morbidity and mortality, the generally early age at onset in young adulthood and the chronic course of BD make it a major public health problem and a leading cause of the global burden of disease. BD shows high heritability estimates of around 60–85%. Previous genome-wide association studies (GWAS) have identified a number of susceptibility genes contributing to BD. However, the disease relevant pathways and underlying molecular networks are still largely unknown.

In the present study, the Bipolar Disorder Working Group of the Psychiatric Genomics Consortium (PGC) performed the largest GWAS of BD to date comprising more than 20,000 patients and 31,000 controls of European descent. In our study, we combined GWAS data of 32 cohorts from 14 different countries in Europe, North America and Australia. The genotype data were processed using the PGC Ricopili pipeline for standardized quality control, imputation, and analysis. Genotype imputation was performed using the 1,000 Genomes Project reference panel. The association results of the different discovery cohorts were combined using an inverse-variance weighted fixed effects model. 822 variants with a p value $<1 \times 10^{-4}$ were followed up in an independent sample of 9,412 BD patients and 137,760 controls.

In the combined analysis, 30 independent genomic loci reached genome-wide significance ($p < 5 \times 10^{-8}$), of which 20 were novel. The associated loci contain genes that encode synaptic components (ANK3, RIMS1), ion channels and neurotransmitter transporters (e.g. CACNA1C, GRI-N2A, SCN2A, SLC4A1).

Using LD score regression, the calculated SNP-based heritability for BD was 17–23%. In addition, we found significant genetic correlations between BD and other psychiatric diseases including schizophrenia, major depression, autism spectrum disorder and anorexia nervosa. Polygenic risk score analyses revealed that BD type I (characterized by depressive and manic episodes) was strongly genetically correlated with schizophrenia, whereas BD type II (depressive and hypomanic episodes) was correlated with major depressive disorder. We also conducted summary Mendelian randomization analyses to integrate expression quantitative trait loci (eQTL) data with the results of our BD GWAS and identified 21 significant genes after correction for multiple testing including GNL3. Pathway analysis using MAGMA revealed nine significantly enriched gene-sets including endocannabinoid signaling and regulation of insulin secretion. Our findings address key clinical questions, give insights into the extensive polygenic architecture and the involved pathways of BD and provide further evidence that BD belongs to a spectrum of highly correlated neuropsychiatric disorders.

W7 Modelling Neurogenetic diseases

W7-01

A genetic model of hereditary spastic paraplegia (SPG15/Spastizin) reveals myelination defects in adult zebrafish

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Hereditary Spastic Paraplegia (HSP) comprises a heterogeneous group of inherited neurodegenerative disorders. The common symptom of HSPs is the progressive paralysis of the legs. More than 60 genes have been isolated, which cause HSP. Nonetheless, the cellular mechanism, by which their disruption causes neurodegeneration, is not clear, because animal models are rare.

Here, we generate the first genetic model of HSP in the zebrafish by disrupting the Spastizin/ZFYVE26 locus, which causes Kjellin syndrome (SPG15) in patients. To look for the expression of the protein in zebra-

fish, we generated a Spastizin antibody. When we labeled the central nervous system (CNS) of spastizin mutant fish, we did not detect the protein suggesting that the spastizin allele eliminates its expression. Fascinatingly, when we analyzed Spastizin protein in wild types, the expression was restricted to the Mauthner cells. Mauthner cells are motor neurons in the hindbrain of fishes forming giant axon bundles in the CNS. Unexpectedly, we noted during the preparation of histological sections that the Mauthner bundles were frequently lost suggesting a structural difference between wild-type and mutant axons. Indeed, with high-pressure freezing electron microscopy we discover that the myelin sheath of mutants frequently shows defects in enveloping the Mauthner axons in concentric rings. These results suggest that Spastizin plays a critical role for the function of Mauthner neurons.

Decades of behavioral experiments emphasized the role of Mauthner neurons in the escape reflex aka startle response of teleosts. To analyze the physiological consequences of the neuronal defect, we analyzed the startle response of larval and adult mutants but did not discover any differences. In patients, SPG15 leads to a spastic gait, but zebrafish do not have legs, which would allow us to quantify their motility. We therefore developed a novel videotracker algorithm to quantify the undulatory movements of the zebrafish body during swimming. Excitingly, this sensitive approach detected a paralysis in the bending of their caudal body segments. These data indicate that the failure of myelination of neurons may lead to the observed motility defects in mutant fish providing an interesting mechanism for HSP in patients. Taken together, our results establish the first genetic zebrafish model for HSP. This will allow us in the future to analyze cellular changes e.g. mitochondrial activity in HSP neurons in the physiological context of an entire animal. More importantly, the zebrafish is extensively used for drug screens providing a feasible approach to initiate a therapeutic strategy for HSP.

W7-02

A mouse model for SPG48 reveals a block of autophagic flux upon disruption of adaptor protein complex 5

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Hereditary spastic paraplegia (HSP) is characterized by a length-dependent degeneration of corticospinal axons resulting in a progressive spastic gait disorder. With more than 80 genetically distinct forms (SPGs) and variable additional manifestations, HSP is highly heterogeneous. SPG48 follows an autosomal recessive trait and is caused by mutations in the zeta subunit of the adaptor protein complex 5 (AP-5). Previous data suggest that AP-5 is related to the retrieval between late endosomes and the trans-Golgi network (TGN). Here, we report a mouse model for SPG48, which displays a late-onset phenotype characterized by motor impairment and degeneration of corticospinal axons. Our analysis of knockout mouse embryonic fibroblasts (MEFs) supports a trafficking defect from late endosomes to the TGN and, in addition, it reveals structural alterations of the Golgi apparatus. While basal autophagy was not affected in AP5 zeta KO MEFs, the abundance of LC3-II was significantly increased in AP-5 zeta knockout MEFs under conditions of induced autophagy. Because LC3-II abundance did not differ between genotypes upon starvation while inhibiting lysosomal function by Bafilomycin A1 administration, a block in the autophagic flux appeared likely. In agreement, LC3- and Lamp1-positive puncta, indicative for autolysosomes, were significantly decreased in starved AP-5 zeta KO MEFs, while autophagosomes (LC3 puncta) were increased. Moreover, the number of Lamp1-positive tubules was reduced in MEFs upon prolonged starvation suggesting a defect in the recycling of lysosomes from autolysosomes (autophagic lysosome reformation). In primary neuron cultures the degradation of an aggregation prone Huntingtin-variant was delayed upon disruption of AP-5 zeta. In agreement

with our in vitro findings, the numbers of autophagosomes and autolysosomes were increased in Purkinje neurons already in young knockout mice, while changes in the Golgi apparatus were only evident in aged mice. We hence, conclude that disruption of AP-5 results in defective autophagy and ALR, which leads to the accumulation of autophagic material and axon degeneration. This scenario may be aggravated by alterations of the Golgi apparatus.

W7-03

Modelling FOXP1-associated autism in the mouse reveals functional defects in brain and gut

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Dysfunction of motor, social, sensory and cognitive aspects play a major role in autism spectrum disorder (ASD). Frequently the associated gastrointestinal dysfunction is overlooked. FOXP1 syndrome is characterized by autistic traits, social deficits, language impairment and intellectual disability. Brain dysfunction in FOXP1 deficiency has been previously studied in some detail. Feeding difficulties and constipation, together with other gastrointestinal problems were also reported in these patients. Whether the observed symptoms are caused by primary gastrointestinal impairment or rather due to an epiphenomenon caused by altered behaviour or side effects of psychotropic medication has remained unclear so far.

In order to address this, we have investigated the gastrointestinal tract of *Foxp1*^{+/-} mice reflecting human *FOXP1* haploinsufficiency. Expression of *Foxp1* mRNA and protein in wildtype mice was detected in all gut sections tested (oesophagus, fundus, corpus, duodenum, ileum and colon) and found to be reduced in *Foxp1*^{+/-} mice. Significant defects in morphology and function were observed in the proximal and distal part of the GI tract. Both oesophagus and colon exhibit a pronounced atrophy of the tunica muscularis. *Foxp1*^{+/-} animals also show altered feeding behaviour with reduced food and water intake compared to wild type mice. Furthermore, *Foxp1*^{+/-} mice display pronounced defects in nitric oxide-induced relaxation of the lower oesophagus sphincter (achalasia), which may well explain the feeding difficulties in *Foxp1*^{+/-} mice and children with FOXP1 deficiency. In addition to oesophageal achalasia, *Foxp1*^{+/-} animals exhibit a significant prolongation of total gut transit. Spatiotemporal maps depicting the contraction patterns of the gut revealed strongly disturbed contractility and peristalsis of the colon in *Foxp1*^{+/-} mice. Overall, our findings demonstrate for the first time that the observed gastrointestinal disturbances in patients with FOXP1 syndrome may be caused by impaired gut motility, specifically in the oesophagus and colon.

W7-04

Inhibition of histone deacetylation improves Birk-Barel intellectual disability dysmorphism syndrome in mice

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Kcnk9/CNK9 is a paternally silenced and maternally expressed imprinted gene in mouse and human, whose heterozygous pathogenic variants on the maternal allele are causative for the maternally inherited Birk-Barel intellectual disability dysmorphism syndrome (BBIDS). It encodes a two-pore domain potassium (K2P)-channel and is involved in the modulation of the resting membrane potential and excitability of neurons. In a study

comparing full *Kcnk9* knock-out animals (*Kcnk9KO^{hom}*) with maternal allele *Kcnk9* knock-out animals (*Kcnk9KO^{mat}*) and wildtype littermates in a behavioral battery, we surprisingly found an intermediate phenotype in nocturnal locomotor activity in *Kcnk9KO^{mat}* animals. Furthermore, electrophysiological analysis of tyrosine hydroxylase (TH)-positive neurons in the locus coeruleus (LC), a region involved in regulation of arousal and nocturnal activity in mice, showed significantly increased spontaneous firing frequency only during the active dark phase for full knock-out mice in contrast to wildtype mice, while it was fully rescued in the *Kcnk9KO^{mat}* animals. Allele-specific expression analysis of *Kcnk9* mRNA in (C57BL/6JxCast/Ei)F1 hybrid animals revealed non-canonical imprinting of *Kcnk9* in the brain with significant residual paternal expression of up to 14% of total *Kcnk9* expression particularly in the LC. *Kcnk9KO^{mat}* animals displayed paternal *Kcnk9* expression in the brain further increased compared to that of (C57BL/6xCast/Ei)F1 wild-type animals suggesting a regulatory feedback-loop between maternal and paternal *Kcnk9* expression. Daily intraperitoneal injection of the second-generation histone deacetylase (HDAC) inhibitor CI-994 could increase this expression up to three-fold and resulted in a full rescue of the behavioral phenotype of *Kcnk9KO^{mat}* animals. This rescue was accompanied by an increase of histone 3 lysine 27 (H3K27ac) acetylation at the promoter and intronic region of *Kcnk9* in LC tissue samples. Taken together, our findings demonstrate that the paternal allele of *Kcnk9* is not fully silenced in the brain, further de-repressed in the case of loss of the maternal allele and substantially activated upon exogenous epigenetic modulation with CI-994 leading to a full rescue of the behavioral phenotype of *Kcnk9KO^{mat}* animals after treatment. Thus, we provide evidence for a promising therapeutic effect of the class I HDAC inhibitor CI-994 in a mouse model for BBIDDS and introduce the first epigenetic therapy to improve cognitive dysfunctions in a mouse model of an imprinting disorder.

W7-05

A screen for transcriptional and genetic interactions of genes associated with neurodevelopmental disorders reveals common transcriptional targets and genetic interactions between *Ube3a* and *Mef2* in *Drosophila melanogaster*

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Neurodevelopmental disorders (NDDs) including intellectual disability (ID) are clinically and genetically extremely heterogeneous with more than thousand genes implicated to date. Within this large group, phenotypically and biologically coherent modules can be increasingly delineated, as overlapping phenotypes are often caused by mutations in genes involved in the same networks, complexes or pathways.

We selected five genes (*TCF4*, *MEF2C*, *UBE3A*, *ZEB2* and *ATRX*) implicated in clinically overlapping, syndromic forms of severe ID with epilepsy that are often considered as close differential diagnoses. Most of these genes are involved in transcriptional regulation.

We assessed potential genetic interactions of orthologues of these five genes in *Drosophila melanogaster*. We used the UAS/GAL4 system to induce knockdown or overexpression of each single gene and in pairwise combinations. Modification of a phenotype resulting from deregulation of a single gene A by simultaneous deregulation of a second gene B indicates genetic interaction between gene A and B. Several parameters such as lethality, wing and eye morphology, neuromuscular junction morphology and bang sensitivity and climbing behavior were assessed. We found stringent evidence for genetic interaction between *Ube3a* and *Mef2*: pairwise knockdown and pairwise overexpression as well as a combination of *Ube3a*-overexpression and *Mef2*-knockdown resulted in either more severe or milder eye phenotypes compared to single knockdown or overexpression of any of the two genes.

Additionally, we performed transcriptome analysis on RNA from patient blood samples to investigate possible common transcriptional targets of the four transcriptional regulators *TCF4*, *ZEB2* (3 individuals, each),

MEF2C, and *ATRX* (1 individual, each). In all patient groups, markedly more genes were down-regulated than up-regulated (*TCF4*: 132 vs. 75, *ZEB2*: 1163 vs. 506, *MEF2C*: 3779 vs. 2311, *ATRX*: 1314 vs. 498), indicating a shared role as transcriptional activators for all four proteins. Pairwise comparison of deregulated genes in the four patient groups revealed significant overlap, indicating common transcriptional targets. Moreover, we found significant enrichment of known ID genes among the deregulated genes, indicating central roles of the four proteins in neurodevelopment. 629 genes were commonly deregulated in at least three of the four patient groups. These genes were enriched for gene ontology processes such as cell communication and (intracellular) signal transduction.

Systematic assessment of five genes implicated in overlapping human neurodevelopmental disorders revealed commonly deregulated target genes involved in neurodevelopment and genetic interactions between *Ube3a* and *Mef2* in the *Drosophila* model system. Our findings thus confirm and characterize the molecular commonalities underlying clinically overlapping syndromic forms of severe ID and are a further step towards elucidating the pathophysiology of these disorders.

W7-06

Baraitser Winter cerebrofrontofacial syndrome patient iPSC-derived cerebral organoids show abnormal neuronal development

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Baraitser Winter cerebrofrontofacial syndrome (BWCF) is caused by missense mutations in one of the two cytoplasmatic actin genes *ACTB* and *ACTG1*. BWCF presents with multiple disabilities as well as cortical malformations, e.g. pachygyria, which is considered to be a neuronal migration disorder. However, the precise pathomechanism of actin-associated cortical malformations remains unknown. Therefore, we aimed to validate brain organoids as a model system for actinopathies and use them to characterize the pathomechanism of cortical malformations of BWCF.

For generating cerebral organoids, we used iPSC lines from two BWCF patients carrying following mutations (1) NM_001101.3(*ACTB*): c.359C>T, p.(Thr120Ile), and (2) NM_001614.3 (*ACTG1*): c.608C>T, p.(Thr203Met). We used the commercially available iPSC lines SC102A-1 (System Biosciences), 409B2 (RIKEN BRC cell bank) as normal controls. Additionally, we performed CRISPR repair of the nucleotide substitutions to obtain isogenic control organoids. The differentiation was performed according to the self-patterning Lancaster et al., 2013 protocol to generate cerebral organoids. We also applied the pre-patterning Qian et al., 2016 protocol to generate forebrain-specific organoids. Organoids were fixed, cryosectioned and stained for an immunohistochemical analysis after 30, 50 and 70 days in culture.

First, we could confirm that brain organoids are suitable cellular system to model actinopathies since the actin isoform expression is similar to human fetal cortex. BWCF organoids showed abnormal morphology and altered distribution of both actin isoforms. The ventricular zone of BWCF organoids was thinner and had decreased number of neural progenitors (Sox2⁺) as well as decreased numbers of intermediate progenitors (Tbr2⁺) in the subventricular zone. We also observed an increased accumulation of intermediate progenitors in cortical plate, more pronounced with *ACTG1* mutation.

The adhesion junction belt was highly abnormal in the presence of *ACTB* and *ACTG1* mutations. Most intriguingly we demonstrated an inverted production of deep and upper layer neurons. Compared to the control, BWCF organoids show less Ctip2 (deep layer marker) and more SatB2

(upper layer marker) at early developmental stages. This indicates that first cells are formed for the upper layer and cells for the deeper layer are formed at later stages, an order opposite to the normal cortical development. Taking together, we showed that BWCF mutations lead to defects in adhesion junction belt resulting in diminished VZ and abnormal cell composition and reverted production of deep and upper layer neurons correlating with abnormal cortical layering observed in BWCF patients. Our work provides a new evidence that developmental mechanisms of cortical malformations are considerably more complex than a current simplified classification in malformations of cellular division, migration and postmitotic organization.

W8 Monogenic Diseases 2

W8-01

CAPZB—a potential key player in cardiac development and congenital heart defects

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Congenital heart defects (CHD) are one of the most common birth defects in humans with an incidence of 8 per 1000 live births. In 25% of the cases, the CHD is life-threatening and needs intervention directly after birth or in the first year of life. The aetiology of CHD is still poorly understood. Here we present a new candidate gene for CHD—CAPZB—, which was identified in four patients with CHD. CAPZB encodes the beta subunit of the fast growing-end actin binding protein, which belongs to the F-actin capping protein family. It regulates the assembly and disassembly of actin filaments, modulates the cytoskeleton as well as tethers actin filaments to the Z-line of the sarcomere and is therefore essential for muscle development. Using exome sequencing, we identified de novo missense mutations in CAPZB in two patients with non-syndromic CHD (hypoplastic left heart syndrome and pulmonary atresia with ventricular septal defect) and two patients with syndromic CHD (atrial septal defect and ventricular defect). To understand the functional relevance of the identified CAPZB mutations we generated a mutant zebrafish model by a retroviral insertion in the second intron. In addition, we modeled the human CAPZB mutations in transgenic zebrafish. We performed cardiac output measurements on zebrafish larvae at different developmental stages. Additionally, to identify physiological and morphological defects, we performed immunofluorescence staining, electron microscopy and optical mapping. To determine differentially regulated genes influenced by CAPZB mutation we performed RNA-Seq on 48 hours (hpf) old zebrafish larvae.

Interestingly, zebrafish larvae being homozygous for the mutation (capzb^{-/-}) died after 100 hours post fertilisation (hpf). Heterozygous zebrafish (capzb^{+/-}) survived until adulthood and were used for husbandry. Mutant capzb^{-/-} zebrafish larvae developed microcephaly, short stature, hypoplastic jaw and an elongated, unlooped heart at 96 hpf compared to their heterozygous capzb^{+/-} and wildtype siblings. Cardiac output measurements showed an impaired contractility in capzb^{-/-} compared to capzb^{+/-} and wildtype larvae at 72 hpf, which was significantly reduced after 96 hpf. Moreover, using immunofluorescence staining, electron microscopy and optical mapping capzb^{-/-} larvae showed physiological and morphological defects after 72 hpf compared to capzb^{+/-} and wildtype siblings.

In addition, RNA-Seq revealed differentially expressed actin binding genes in capzb^{-/-} larvae, suggesting a migration defect.

Taken together, loss of CAPZB in zebrafish results in physiological and morphological defects, which phenocopy part of the observed human CHD phenotypes of the identified patients. These results highlight the importance of CAPZB as a new candidate gene for CHD and show its role during early cardiac development.

W8-02

Bi-allelic mutations in LSS, encoding lanosterol synthase, cause autosomal recessive hypotrichosis simplex

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Hypotrichosis simplex (HS) is a rare form of hereditary alopecia characterized by childhood-onset of diffuse and progressive scalp and body hair loss. Although research has identified a number of causal genes, genetic etiology in about 50% of HS cases remains unknown. The present report describes the identification via whole exome sequencing of five different mutations in the gene LSS in three unrelated families with unexplained, potentially autosomal recessive HS. Affected individuals showed sparse to absent, lanugo-like scalp hair, sparse and brittle eyebrows, sparse eyelashes and body hair. The LSS gene encodes lanosterol synthase (LSS), which is a key enzyme in the cholesterol biosynthetic pathway. This pathway plays an important role in hair follicle biology. After localizing LSS protein expression in the hair shaft and bulb of the hair follicle, the impact of the mutations on keratinocytes was analyzed using immunoblotting and immunofluorescence. Interestingly, wild-type LSS was localized in the endoplasmic reticulum (ER), whereas mutant LSS proteins were localized in part outside of the ER. A plausible hypothesis is that this mislocalization has potential deleterious implications for hair follicle cells. Immunoblotting revealed no differences in the overall level of wild-type and mutant protein. Analyses of blood cholesterol levels revealed no decrease in cholesterol or cholesterol intermediates, thus supporting the previously proposed hypothesis of an alternative cholesterol pathway. The identification of LSS as causal gene for autosomal recessive HS highlights the importance of the cholesterol pathway in hair follicle biology, and may facilitate novel therapeutic approaches for hair loss disorders in general.

W8-03

The role of NARF, a novel progeria-associated gene in aging processes

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The world is aging rapidly. People aged 60 and older make up 12.3 per cent of the global population and, by 2050, this number will rise to almost 22 per cent. So far, we have only limited knowledge on specific aging-associated processes, mostly due to the lack of proper models for systematic investigations. The aging process is complex and covers events inside single

cells, interactions between different cells and organs as well as interplay of organisms and environment. In light of this definition it is extremely difficult to study aging using appropriate *in vivo* and *in vitro* systems. The molecular analysis of premature aging diseases (also known as progeroid syndromes) offers the opportunity to identify novel key players in aging. Here, we present a 4½-year-old girl diagnosed with a congenital, segmental progeria syndrome. Trio whole-exome sequencing (WES) identified a *de novo* mutation, located in the *Nuclear Prelamin A Recognition Factor* (*NARF*) gene. The c.1100A>G mutation changes a highly conserved histidine at position 367 to arginine (p.His367Arg) and is predicted to be damaging. Interestingly, the identical amino-acid position in *Narf* has been described to be mutated in a *C. elegans* model for lifespan alterations. *NARF* protein was shown before to interact with prelamin A, which is mutated in Hutchinson-Gilford progeria syndrome. To figure out the potential function of *NARF*, we compared patient's fibroblasts with corresponding healthy controls. We observed a potential role of *NARF* in nuclear envelope morphology as well as genome instability, two well-known mechanisms observed in other progeroid syndromes. Next, we showed that the c.1100A>G mutation neither affects *NARF* transcript levels or composition, nor *NARF* protein stability. Instead, the mutated form of *NARF* changes the nuclear localization typical of WT-*NARF* protein and is found only in a diffused manner in the cytoplasm. Furthermore, we identified two novel interaction partners of *NARF*: *NARF* itself and *CBX5*. This finding suggests that *NARF* forms homodimers important for nuclear localization and that a dominant negative effect of mutated *NARF* on WT protein prevents *NARF* complex nuclear translocation.

W8-04

Specific combinations of biallelic POLR3A variants cause Wiedemann-Rautenstrauch syndrome

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Wiedemann-Rautenstrauch syndrome (WRS) is a well-known neonatal form of segmental progeria characterized by growth retardation, sparse scalp hair, generalised lipodystrophy with characteristic local fatty tissue accumulations and specific facial dysmorphism.

To understand its molecular cause we performed exome sequencing in two families and targeted sequencing in 10 additional WRS families. *In silico* modelling studies and transcript processing analyses were performed to explore the structural and functional consequences of the identified variants. We now present the identification of causative biallelic *POLR3A* variants in eight individuals with WRS strongly indicating that WRS is a genetically homogeneous disorder caused by defective *POLR3A* function. Multiple variants were found to affect *POLR3A* transcript processing and were mostly located in deep intronic regions and suggested to impair correct *POLR3A* splicing. Indeed, extensive RNA analyses showed altered transcripts in various patients. All WRS-associated *POLR3A* amino acid changes were predicted to perturb substantially *POLR3A* structure/function. In the four remaining families, only monoallelic *POLR3A* causative

variants have been yet detected suggesting deeper intronic or regulatory mutations on the second allele.

POLR3A, which encodes the largest subunit of the DNA-dependent RNA polymerase III, constitutes together with *POLR3B* the active site of this complex. Biallelic putative loss-of-function/hypomorphic mutations in both *POLR3A* and *POLR3B* are known to cause two forms of hypomyelinating leukodystrophy, with or without oligodontia and/or hypogonadotropic hypogonadism. As isolated functional sites in *POLR3A* cannot explain the phenotype variability in *POLR3A*-related disorders, we suggest that specific combinations of compound heterozygous variants, indicated by recurrent haplotypes specifically occurring in individuals with WRS, must be present to cause the WRS phenotype.

W8-05

The homozygous variant c.797G>A/p.(Cys266Tyr) in *PISD* is associated with a spondyloepimetaphyseal dysplasia with large epiphyses and disturbed mitochondrial function

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Spondyloepimetaphyseal dysplasias (SEMD) are a group of genetically heterogeneous skeletal disorders characterized by abnormal vertebral bodies and epimetaphyseal abnormalities. We investigated two families with a new SEMD type with one proband each. They showed mild facial dysmorphism, flat vertebral bodies (platyspondyly), large epiphyses, metaphyseal dysplasia, and hallux valgus as common clinical features. By trio exome sequencing, the homozygous missense variant c.797G>A/p.(Cys266Tyr) in *PISD* was found in both affected individuals. Based on exome data analyses for homozygous regions, the two patients shared a single homozygous block on chromosome 22 including *PISD*, indicating their remote consanguinity. *PISD* encodes phosphatidylserine decarboxylase that is localized in the inner mitochondrial membrane and catalyzes the decarboxylation of phosphatidylserine to phosphatidylethanolamine (PE) in mammalian cells. PE occurs at high abundance in the mitochondrial membranes, and structural properties of this phospholipid play an important role for various mitochondrial functions. To characterize the functional effects of the missense variant in *PISD* we used fibroblasts derived from one patient and healthy individuals. Western blot analysis revealed no difference in the *PISD* protein amount in patient- and control-derived fibroblasts. In contrast to fibroblasts from healthy individuals, patient fibroblasts showed fragmented mitochondrial morphology. Treatment of cells with MG-132 or staurosporine to induce oxidative stress and activation of the intrinsic apoptosis pathway revealed significantly decreased cell viability with increased caspase-3 and -7 activation in patient fibroblasts. We did not observe any statistically significant difference in caspase-3 and -7 activation between three control and patient cells when cells were exposed to TNF-alpha and cycloheximide to stimulate extrinsic apoptosis. Remarkably, ethanolamine supplementation largely restored cell viability and enhanced apoptosis in MG-132-stressed patient cells. Our data demonstrate that the bi-allelic hypomorphic *PISD* variant p.(Cys266Tyr) is associated with a novel SEMD form, which may be treatable with PE or ethanolamine administration.

W8-06

Rare deleterious heterozygous GDF6 variants in patients with renal anomalies

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Congenital renal anomalies represent a significant health burden since they are a common cause of chronic kidney disease in children and adolescents. Although around 50 genes are known to cause renal malformation when mutated, most cases remain genetically unsolved when analyzing known genes. In search of novel genes causing renal anomalies, we studied a family with one sibling affected by crossed fused renal ectopia as well as multiple skeletal abnormalities including malformations of vertebral bodies, scoliosis and atresia of the external auditory canal with auricle dysplasia, while a preauricular pit was diagnosed in another sibling and their mother. In the three affected family members, whole-exome sequencing yielded a rare heterozygous missense variant in the growth differentiation factor 6 (*GDF6*) gene known to cause autosomal-dominant Klippel-Feil syndrome type 1 (KFS1). While KFS1 is characterized by vertebral fusion, scoliosis and hearing impairment, renal anomalies have not been described as a hallmark. To assess whether *GDF6*, encoding a member of the bone morphogenetic protein family, plays a role in the pathogenesis of renal malformation, we performed mutational analysis of 193 further patients with renal anomalies. In this cohort, we identified a second patient carrying the same missense variant, resulting in a significant frequency increase of this variant in patients with renal anomalies versus controls (1000 genomes project), and a third patient carrying another rare *GDF6* missense variant. RNA *in situ* hybridization on sections of murine embryos revealed expression of *Gdf6* in the developing urogenital system, i.e. the nephric duct and ureteric tip, starting embryonic day 11.5. After generating a CRISPR/Cas9-derived *Gdf6* knockout in murine inner medullary collecting duct (mIMCD3) cells, we could show that loss of *Gdf6* function *in vitro* attenuated mIMCD3 cell migration, a phenotype rescued by re-expression of human wildtype *GDF6* but not by expression of detected human *GDF6* mutants. As *gdf6* expression was also observed in the developing pronephros of *Xenopus laevis*, this animal model was used for *in vivo* studies of *gdf6* knockdown, resulting in an impairment of pronephros development in embryos with downregulated *gdf6* expression. Altogether, we demonstrate rare heterozygous *GDF6* variants in 1.6% of patients with renal malformations, and provide evidence for an involvement of *GDF6* in early nephrogenesis using *in vitro* and *in vivo* models.

W9 Clinical Genetics

W9-01

Genome-wide non-invasive prenatal testing in single- and multiple-pregnancies at any risk: identification of maternal polymorphisms to reduce the number of unnecessary invasive confirmation testing

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Non-invasive prenatal testing (NIPT) using cell free DNA (cfDNA) from maternal plasma measuring relative whole-chromosome dosages of chromosomes 13, 18, and 21 after low-coverage sequencing has rapidly changed the landscape of prenatal care. However, sensitivity and specificity of such standard NIPTs are limited and genome-wide copy number profiling NIPTs are expected to take non-invasive prenatal testing to the next level. Since prospective results and outcome data are still scarce on the latter, we assessed the performance of genome-wide copy number profiling NIPT in singleton and multiple gestational pregnancies at any risk, including comprehensive follow-up. In a total of 3053 prospective cases, 89.3% asked for genome-wide NIPT. We achieved a result in 98.2% of total cases and detected confirmed chromosomal pathologies in 47 (1.6%) samples, five of which would have remained undetected in standard NIPTs. The abnormalities observed in NIPT was not detected in the fetal tissue in another 11 cases, but was still of clinical relevance in at least 4 of these cases. 7.4% of tests showed small segmental anomalies, of which the majority were (likely) benign maternal copy number variants as determined by chromosomal microarray analysis on maternal leucocytes. Identification of the latter therefore seems crucial in order to avoid false positive results and unnecessary invasive testing. Copy number profiling NIPT showed a higher sensitivity and specificity than standard NIPT, and including retrospective data on 116 additional cases, we found that segmental aberrations sizing at least 11 Mb can be reliably detected, while the recurrent 2.5 Mb 22q11.2 deletion requires a fetal fraction of above 10% to be detectable if the mother is not a carrier.

Finally, interpretation of rare autosomal trisomies or large segmental aberrations indicated by NIPT but neither detectable in amniocytes nor in maternal leucocytes needs caution. Such aberrations may represent signs of maternal hematologic and other (pre-)malignancies or confined placental mosaicism associated with a high risk of fetal anomalies.

W9-02

Results of preimplantation genetic diagnosis on blastocysts for monogenic disorders at our PGD center in Regensburg

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Preimplantation genetic diagnosis (PGD) in Germany had been restricted to polar body analysis until 2010. Since 2015 PGD on trophectoderm cells now is possible in concordance with the modified German embryo protection law (ESchG). We here present the results for 304 consecutive PGD cycles at our PGD center in Regensburg over the last 18 years on first and second polar bodies (PBD) or trophectoderm samples (TED), respectively. Our data include 246 cycles of polar body diagnosis (PBD) for

121 families at high-risk for monogenic disorders, which are compared to our preliminary results obtained for 58 PGD cycles on blastocysts for 22 different monogenic disorders in 39 families. For each family, mutation specific assays were established including 3–6 closely linked informative microsatellite markers and direct mutation detection, whenever possible. Our data confirm an overall increased rate of clinical pregnancies of 51,5% per transfer cycle for TED, for non-repeat disorders overall even higher (56,0%), when compared to polar body diagnosis (26,6%). With a slightly lower number of required TED treatment cycles (1,46 per family) a clinical pregnancy could be obtained for 43,6% of the families, with about half of the diagnosed blastocysts suitable for transfer still in cryoconservation. In contrast, 2,04 PBD treatment cycles per family resulted in a clinical pregnancy for 45,8% of the families requesting PBD.

On average 4,35 blastocysts were available per PGD diagnostic cycle. As expected, the rate of diagnostic cycles with transfer was lower (63,8% vs. 84,5% per PBD diagnostic cycle), reflecting a blastocyst formation rate of only 37,9% (blastocysts suitable for trophectoderm biopsy per mature oocyte). However, when comparing the overall clinical pregnancy rate as fetal heart beat per started diagnostic cycle, overall PBD still has a lower clinical pregnancy rate (22,3%) when compared to TED (29,3%).

Based on these preliminary data we now recommend TED as the preferred method for couples interested in PGD. Our data confirm TED to be a robust PGD procedure for various monogenic disorders with clinical pregnancy rates superior to those obtained by PBD.

W9-03

Additional genomic findings identified in 31 cardiovascular genes among 2347 diagnostic exomes

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Background: Whole exome sequencing (WES) is a routine molecular diagnostic test for patients with genetic disorders. This genomic technique leads also to the detection of variants potentially causing diseases not related to the primary disease under investigation, namely “additional genomic findings” (AGFs). The American College of Medical Genetics and Genomics (ACMG) has recommended the return of AGFs from a list of 56 actionable genes of which more than half (31/56) are associated with cardiovascular disorders.

Methods: Clinical diagnostic WES was performed in 2437 individuals without a cardiovascular disorder as primary disease under investigation. After applying standard quality thresholds and a frequency filter of minor allele frequency <1% using exome control databases, we identified and investigated variants present in 31 ACMG actionable cardiovascular genes and reported in HGMD (Human Gene Mutation Database) and/or ClinVar.

Results: We detected a total of 2254 variants in the 31 ACMG actionable cardiovascular genes. Among these, 1082 variants were present in HGMD and/or ClinVar databases and 26 were labeled as likely pathogenic or pathogenic in ClinVar. Six variants reached the assignment of pathogenicity by two independent reviewers and were located in genes associated with channelopathies, cardiomyopathies or familial hypercholesterolemia. The clinical evaluations did not reveal the presence of cardiovascular disorders.

Conclusions: Our data provide an estimation of the burden of AGFs in cardiovascular-related genes among 2437 diagnostic exomes and highlight the difficulties in the assignment of variant pathogenicity. The low a priori probability of pathogenicity of AGFs and the incomplete penetrance of the disorders make variant interpretation particularly challenging.

W9-04

Exome sequencing uncovers multilocus genomic variation and unexpected molecular heterogeneity in patients with retinal degeneration and hearing impairment

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The prevalence of co-occurring visual and hearing impairment has been described between 1:29.000 to 100.000. It is well known that about 50% of the combined sensory impairment is associated with Usher syndrome and due to the enormous genetic heterogeneity, the remaining cases represent a diagnostic and therapeutic challenge. In a time when a successful gene therapy for a form of retinal degeneration has been described and clinical trials are currently underway, molecular genetic diagnoses play an important role in terms of prognosis, preventive measures and possible inclusion in clinical trials. For these purposes, using ES (Exome Sequencing) we analyzed 54 unrelated patients with co-occurring retinal degeneration and hearing impairment without intellectual disability. In many cases, the available clinical data were comprised of slit-lamp anterior segment, fundus examinations, fundus autofluorescence, optical coherence tomography, Infrared, visual field test, sustained color vision test, and electroretinogram. Likely causative variants (LCV) were found in 94.4% of the cases. Unexpectedly, 13% of the probands showed extensive clinical heterogeneity including coenzyme Q10 deficiency, peroxisome biogenesis disorder 7A, Scheie syndrome, three patients with mutations in ALMS1, associated with Alström syndrome and one patient was identified with a LCV in FLNB, whose symptoms were so mild that the diagnosis was not previously suspected. Besides the fact that hearing loss and retinal degeneration are not one of the most frequent manifestations in some of these conditions, it is interesting to note that they were the cardinal symptoms that motivated the study of these patients, which highlights the extreme clinical variability of some nosological entities. Only partial elucidation was possible in two cases, since independent mutations in PRPF8 andTECTA respectively were found.

Additionally, an astonishing combination of mutations in genes associated with hearing loss and retinal degeneration were identified in 7.4% of the cases. Co-occurrence of LCVs in genes such as RHO and KCNQ4 in a patient or CHD7 and ABCA4 in another proband reproduces the findings of Posey et al. regarding the concept of multiple molecular diagnoses to determine the degree of overlap between two Mendelian diseases in the same patient.

As expected, the most commonly associated etiology of this cohort was Usher syndrome, accounting for 70.4% of the cases. As anticipated, the patients with LCV in MYO7A and USH2A were the most frequent subgroups representing 27.8% and 25.9% of diagnoses, respectively. Cystic macular edema was more frequent and severe in patients with LCV in MYO7A. In addition to the worst best corrected visual acuity, 46.6% of the affected individuals showed vestibular impairment.

Our findings show the numerous benefits of the clinical implementation of WES, leading to a reduction of overall costs, limiting the diagnostic odyssey experienced by patients, improving prognostic accuracy and opening potential avenues to targeted therapies.

W9-05

Mutations in KYNU cause Catel-Manzke-like syndrome

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Catel-Manzke syndrome (MIM 616145) is characterized by the combination of Pierre-Robin sequence and radial deviation, shortening as well as clinodactyly of the index fingers, due to an accessory ossification center between the second metacarpals and proximal phalanges (Manzke dysostosis). It is classified as typical Catel-Manzke syndrome and Catel-Manzke-like syndrome. Mutations in TGDS have been identified as the cause of typical Catel-Manzke syndrome, whereas the cause of Catel-Manzke-like syndrome is unknown in most cases. The objective of this study was the identification of a molecular basis of Catel-Manzke-like syndrome. We performed a chromosome microarray and/or exome sequencing in three unrelated patients with Manzke dysostosis, severe heart defect, short stature and developmental delay, all of whom were initially given a clinical diagnosis of Catel-Manzke syndrome. Two of the patients had Pierre-Robin sequence without cleft palate and butterfly vertebrae. In one patient, we detected a large deletion of exons 1–8 and the missense variant NM_003937.2:c.1282C>T (p.Arg428Trp) in KYNU, whereas homozygous missense variants in KYNU were found in the other two patients (c.989G>A (p.Arg330Gln) and c.326G>C (p.Trp109Ser)). Urine organic acid analysis was performed in two of the three patients and showed excretion of xanthurenic acid. Biallelic loss-of-function mutations in KYNU were recently described in two patients as a cause of nicotinamide adenine dinucleotide (NAD⁺) deficiency with vertebral, cardiac, renal, and limb defects; however, neither Manzke dysostosis nor Pierre-Robin sequence were present in those patients. We hypothesize that the missense variants in our patients cause milder NAD⁺ deficiency than nonsense variants, and only this leads to the very specific hand malformation by disturbing TGDS function, an NAD⁺ dependent enzyme. In conclusion, we present unrelated patients identified with mutations in KYNU leading to xanthurenic aciduria as a cause of Catel-Manzke-like syndrome. We suggest performance of urine organic acid analysis as a new, easy available diagnostic tool in patients with Catel-Manzke syndrome, particularly in those with cardiac or vertebral defects or without mutations in TGDS.

W9-06

Genetic variants in components of the NALCN-UNC80-UNC79 ion channel complex cause a broad clinical phenotype (NALCN channelopathies)

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NALCN is a conserved cation channel, which conducts a permanent sodium leak current and regulates resting membrane potential and neuronal excitability. It is part of a large ion channel complex, the “NALCN channelosome”, consisting of multiple proteins including UNC80 and UNC79. The predominant neuronal expression pattern and its function suggest an important role in neuronal function and disease. So far, biallelic NALCN and UNC80 variants have been described in a small number of individuals leading to infantile hypotonia, psychomotor retardation, and characteristic facies 1 (IHPRF1, OMIM 615419) and 2 (IHPRF2, OMIM 616801), respectively. Heterozygous de novo NALCN missense variants in the S5/S6 pore-forming segments lead to congenital contractures of the limbs and face, hypotonia, and developmental delay (CLIFAHDD, OMIM 616266) with some clinical overlap. In this study, we present detailed clinical information of 16 novel individuals with biallelic NALCN variants, 1 individual with a heterozygous de novo NALCN missense variant and an interesting clinical phenotype without contractures, and 12 individuals with biallelic UNC80 variants. We report for the first time a missense NALCN variant located in the predicted S6 pore-forming unit inherited in an autosomal-recessive manner leading to mild IHPRF1. We show evidence of clinical variability, especially among IHPRF1-affected individuals, and discuss differences between the IHPRF1- and IHPRF2-phenotypes. In summary, we provide a comprehensive overview of IHPRF1 and IHPRF2 phenotypes based on the largest cohort of individuals reported so far and provide additional insights into the clinical phenotypes of these neurodevelopmental diseases to help improve counseling of affected families.

Poster

P-Basic Mechanisms and Epigenetics

P-BasEpi-001

Vitamin B12 supplementation affects TrkB signaling via DNA methylation

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Depression is a serious, debilitating condition and the leading cause of disability worldwide. While several groups of antidepressants are now available, they are only effective in a minority of cases and take several weeks to fully function, indicating an urgent need to identify alternative pathways of antidepressant action. While most research into risk factors for depression focuses on stress, dietary factors have a strong link with depression. This includes vitamins, which provide essential cofactors for chromatin regulation. Vitamin B12 (cobalamin) is an essential nutrient, which can only be synthesized by bacteria. There is evidence that vitamin B12-supplementation may reduce depression risk, and helps to reverse the prodepressive effects of early life stress in animal models. Despite this behavioural evidence for a connection between vitamin B12 and depression risk, it is unclear how vitamin B12 metabolism is coupled to depressive symptoms or antidepressant action on a molecular level.

We here present evidence that acute vitamin B12-supplementation selectively alters DNA methylation and gene expression of the antidepressant-associated gene *TrkB*. The patterns of *TrkB* regulation by vitamin B12 are opposite to changes observed post-mortem in suicide completers. CRISPR-dCas9 epigenome editing of DNA methylation on the *TrkB* gene indicated a causal link between epigenetic and transcriptional changes of *TrkB*. Supplementation with folic acid did not affect *TrkB* DNA methylation or gene expression. Taken together these data suggest that acute vitamin B12 supplementation can positively regulate the expression of the antidepressant-linked gene *TrkB*. Our findings encourage further investigation of vitamin B12-supplementation as a novel model for fast-acting antidepressant action.

P-BasEpi-002

Sex hormones regulate SHANK expression

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Autism spectrum disorders (ASD) have a higher prevalence in male individuals compared to females, with a ratio of affected boys compared to girls of 4:1 for ASD and 11:1 for Asperger syndrome. Mutations in the SHANK genes (comprising SHANK1, SHANK2 and SHANK3) coding for postsynaptic scaffolding proteins have been tightly associated with ASD. As early brain development is strongly influenced by sex hormones, we investigated the effect of dihydrotestosterone (DHT) and 17 β -estradiol on SHANK expression in a human neuroblastoma cell model. Both sex hormones had a significant impact on the expression of all three SHANK genes, which could be effectively blocked by androgen and estrogen receptor antagonists. In neuron-specific androgen receptor knock-out mice (ArNesCre), we found a nominal significant reduction of all Shank genes at postnatal day 7.5 in the cortex. In the developing cortex of wild-type (WT) CD1 mice, a sex-differential protein expression was identified for all Shanks at embryonic day 17.5 and postnatal day 7.5 with significantly higher protein levels in male compared to female mice. Together, we could show that SHANK expression is influenced by sex hormones leading to a sex-differential expression, thus providing novel insights into the sex bias in ASD.

P-BasEpi-003

Common genetic variation at the Angelman syndrome imprinting centre affects the imprinting of chromosome 15

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Angelman syndrome (AS) is a neurogenetic imprinting disorder characterised by severe intellectual disability with absence of speech, microcephaly, ataxia, seizures and a happy demeanour. It is caused by the loss of function of the *UBE3A* gene, which in brain is expressed from the maternal allele only. In approximately 3–5% of AS patients, the disease is due to an imprinting defect. These patients lack DNA methylation of the maternal SNRPN promoter so that a large SNRPN sense/*UBE3A* antisense transcript (*SNHG14*) is expressed, which silences the *UBE3A* gene. In very rare cases, the imprinting defect is caused by a deletion of the AS imprinting center (AS-IC) 35 kb upstream of the SNRPN promoter and associated with an increased recurrence risk, if the mother carries the deletion. In 183 patients with an imprinting defect but without an AS-IC deletion, we did not detect any point mutation in the AS-IC. However, the AS-IC harbors several common DNA polymorphisms (five SNPs and one TATG insertion/deletion polymorphism), which constitute five common haplotypes (Zogel et al. 2006). We investigated whether any of these haplotypes might be associated with an increased risk for an imprinting defect. For this analysis we used the transmission disequilibrium test (TDT), which is a family-based association test that measures the over-transmission of an allele or haplotype from heterozygous parents to affected offspring. In 48 parent-child trios tested previously, we had found a trend for maternal over-transmission of the haplotype H-AS3 ($p=0.058$). We have now extended this cohort to 119 AS-ID trios. Again, we observed maternal over-transmission of haplotype H-AS3 ($p=0.0073$), supporting the trend seen in the smaller cohort from the previous study. Interestingly, haplotype H-AS3 is the only haplotype that includes the TATG deletion allele. We conclude that this haplotype and possibly the TATG deletion, which removes a SOX2 binding site, increases the risk for a maternal imprinting defect and Angelman syndrome. Our data strengthen the notion that the AS-IC is important for establishing or maintaining DNA methylation at the SNRPN promoter and show for the first time that common genetic variation can affect genomic imprinting.

P-BasEpi-004

Targeted methyl-Seq quantification by NGS technology for routine diagnostic of FSHD

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Facioscapulohumeral Muscular Dystrophy (FSHD) is associated with hypomethylation of the D4Z4 microsatellite, leading to an increased expression of the *DUX4* gene which is suggested to cause progressive muscle atrophy and ultimately FSHD pathology. Chromatin changes are caused by contractions to less than 11 units of the D4Z4 repeat (FSHD1) or by pathogenic variants in chromatin regulatory proteins (FSHD2). We developed a novel NGS-based method in an attempt to diagnose FSHD and distinguish FSHD1 from FSHD2 in a single approach. Enrichment of 4qA (A) and 4qAL (AL) associated D4Z4 repeats as well as all 4q35 D4Z4 repeat units (*DUX4*) was performed using nested PCRs after bisulfite conversion of genomic DNA (EpiTect). DNA libraries were prepared with the NEBNext Ultra DNA Library Prep Kit and sequenced on an Illumina MiSeq System. Reads were mapped to A and AL using bwameth. Mean methylation levels were calculated with an in-house script. We analyzed 21 patients with a confirmed FSHD diagnosis and 21 negative controls. We could confirm the hypomethylation status in all patients. Low methylation levels tend to

correlate with a more severe clinical disease status. Due to a chromatin dysregulation DUX4 is hypomethylated in FSHD2 as well; as expected we could confirm lower methylation levels in DUX4 in FSHD2-patients compared to FSHD1. In conclusion, sensitive detection of methylation levels in the D4Z4 array may give insight into the clinical progression levels more exactly than currently available methods. Additionally, a much higher yield and throughput in FSHD diagnostics may be achieved.

P-BasEpi-005

A novel pathway links caffeine effects with the circadian CLOCK system and mediates antidepressant action

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Category: Basic mechanisms and Epigenetics!!!

Depression is one of the most common psychiatric illnesses, affecting nearly 20% of the population. Sleep disorders and excessive drowsiness are often symptoms of depression. Although adenosine antagonists (eg, caffeine) alter sleep, their role in depression is understudied. Evidence from animal studies suggests that antagonists of the adenosine 2A (A2A) receptor, including caffeine, may help alleviate a variety of depression-like symptoms, but the underlying mechanisms are poorly understood. Here we propose a novel pathway, which links caffeine action with the circadian regulation of the genome via the dopaminergic key protein DARPP-32 (Dopamine- and cAMP regulated phosphoprotein). Additionally, this pathway mediates caffeine-induced antidepressant effects in the awake-phase of the circadian cycle. Specifically, our results show that CLOCK (Circadian Locomotor Output Cycles Kaput), a key regulator of circadian gene expression, is directly bound by phospho-T75-DARPP-32. The DARPP-32 binding interrupts the interaction of CLOCK with its partner BMAL1 (aryl hydrocarbon receptor nuclear translocator-like protein 1, also known as ARNTL), which is necessary for subsequent binding to the genome. The CLOCK-DARPP-32 interaction depends on the phosphorylation of the amino acid threonine 75 (T75) in DARPP-32, which is blocked by the A2A receptor, e.g. after administration of caffeine. T75A point mutant mice show specific behavioral changes indicative of decreased caffeine effects and abnormal circadian adaptations. Under standard conditions they have no circadian or depression phenotypes. However, in the awake phase of the circadian cycle, they show a reduced effect of caffeine on the CLOCK:BMAL1 binding to the chromatin. This is accompanied with a lack of caffeine-induced antidepressant effects in T75A-mutants in the awake phase. The studies reported here strongly suggest a role of the A2A receptor T75-DARPP-32-CLOCK signaling pathway in depressive and circadian behavior. Accurately characterizing this pathway will contribute to the understanding of caffeine-induced antidepressant responses and circadian alterations.

P-BasEpi-006

Impact of CRBN, PSMD5 and CD38 promoter/enhancer methylation on drug resistance in multiple myeloma: potential epigenetic marker panel

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Background: Multiple myeloma (MM) is a malignant plasma cell dyscrasia with 120,000 newly diagnosed patients per year worldwide. Recent approv-

al of drugs like immunomodulators (IMiDs), proteasome inhibitors (PIs) or monoclonal antibodies (MAs) have significantly prolonged survival, but the disease remains incurable as virtually all patients evolve drug resistance under treatment and die from refractory disease. The underlying mechanisms appear to be complex with genomic mutations just explaining few percentages of cases.

Aim: To study the role of epigenetic gene dysregulation in the development of MM drug resistance and to establish a comprehensive and clinically oriented panel of DNA methylation markers for MM diagnosis and prognosis.

Material/Method: Seven genes of relevant drug-response pathways and three prominent tumor genes were investigated at single molecule resolution using targeted deep bisulfite sequencing in human primary tumor cells (138+ purified). In total, 62 newly diagnosed and 84 relapse samples (25 partly at sequential time points) were sequenced, as well as 61 paired peripheral blood mononuclear cells (PBMCs) and 32 healthy controls. All assays were tested with artificially methylated control samples 0%, 25%, 50%, 75% and 100% and approved to be reliable ($R^2 = 0.97$).

Results: Aberrant methylation patterns were seen in three therapy associated gene loci, PSMD5 promoter, CRBN enhancer (enh) and CD38 enh. In contrast, tumor suppressor and oncogenes remained epigenetically stable. For CD38 enh, the MM patients were hypermethylated with $31\% \pm 0.17$ compared to the paired PBMCs that showed in average $60\% \pm 0.12$. For PSMD5 promoter and CRBN enh on the other hand, all PBMCs displayed an unmethylated state ($2.0\% \pm 0.01$ and $6.0\% \pm 0.02$), whereas the MM cells were hypermethylated with significantly increasing ($p \leq 0.001$) methylation profiles from diagnosis (PSMD5: $2.0\% \pm 0.02$; CRBN enh: 22.7 ± 0.20) to relapse (PI resistant patients PSMD5: $18\% \pm 0.08$; IMiD resistant patients: CRBN enh: $44\% \pm 0.26$).

Summary: For all three drug classes, genes encoding the primary therapy binding target were affected by epigenetic alterations. There is evidence in the literature that IMiD resistant patients have decreased CRBN expression (Dimopoulos et al. 2018; Franssen et al. 2018). The same was shown for PSMD5 levels in PI resistant patients (Tsvetkov et al. 2017). DNA promoter and enhancer methylation are known to be effective tools for the downregulation of gene expression. The accumulation of epimutated MM clones might explain the drug resistance of the affected patients over a drug fitness selection process. Recovering native gene expression (e.g. via demethylating agents) might be a valid therapy strategy to explore.

P-BasEpi-007

Estrogen-mediated regulation of FGF5 and its role for male-pattern baldness

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Male-pattern baldness (MPB) is the most common form of hair loss in men and is characterized by a progressive loss of hair from the scalp that occurs in a distinct pattern. The pathogenesis of MPB is driven by androgens based on a genetic predisposition. Genome-wide association studies have identified >130 risk loci that implicate several plausible candidate genes. However, for the majority of loci the exact biological mechanisms through which the associated variants affect relevant genes remain elusive. An interesting example is a genome-wide significant risk locus on chromosome 4q21.21 that is located intronically in *FGF5* (fibroblast growth factor 5). Research has shown that FGF5 regulates human hair length by stimulating the entry into the regression phase of the hair cycle, which determines the end of the hair growth phase (Higgins et al., 2014). This renders *FGF5* a plausible candidate gene for MPB since a premature stop of hair growth is one of the characteristic pathophysiological changes observed in MPB affected hair follicles.

To gain a deeper understanding of the regulatory architecture of the *FGF5* risk locus and its relevance to MPB development the HaploRegv4.0 database was queried to identify potential regulatory elements. The database

research revealed that the MPB lead SNP rs4690116 is located in an estrogen receptor 1 (ESR1) binding site. This is of particular interest, as estrogens have a reported role in hair growth control and act as opponents of androgens, the key hormonal mediators in MPB development. We therefore hypothesized that the MPB risk allele at 4q21.21 may affect ESR1 binding affinity at the *FGF5*-locus, which might eventually result in an insufficient inhibition of the hair growth regulator *FGF5*. The consequently higher levels of *FGF5* would lead to a premature entering of the regression phase, a characteristic change in hair cycle dynamics that is observed in MPB-affected hair follicles.

To test this hypothesis, we designed an *in vitro* luciferase assay, where we are co-transfecting eukaryotic cells with (i) a luciferase construct that contains an *FGF5* promoter sequence and the 4q21.21-ESR1 binding site with either the risk (A) or the non-risk (T) allele for rs4690116 and (ii) an ESR1-expression vector. Stimulation with estrogen (10 nM) is used to induce ESR1 function. To compare the results in cells that exert endogenous and non-endogenous expression of *ESR1* the experiments are performed in HEK293T (*ESR1*^{-/-}) and CHO (*ESR1*^{+/+}) cells. First results in HEK293T cells indicate an inhibition of the *FGF5* promoter under estrogen stimulation. Detailed results including results from CHO cells will be presented at the conference. These data will elucidate the underlying molecular mechanism at the *FGF5*-locus and will thereby contribute to a deeper understanding of the yet unknown molecular causes of the hair cycle abnormalities seen in MPB.

P-BasEpi-008

Regulation of CHRM3 gene expression by tumor suppressive miR-129-5p in hepatocellular carcinoma

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Background and aims: Histone deacetylases (HDACs) are frequently overexpressed in hepatocellular carcinoma (HCC) causing a global loss of histone acetylation and silencing of specific genomic regions. As modulation of microRNA (miR) expression is a potential strategy for liver cancer therapy, we aimed to identify miRs that are affected by histone deacetylation and to investigate their functional effects in HCC tumorigenesis.

Methods: HCC cell lines (HLE, HLF, Huh7, HepG2, Hep3B) and immortalized liver cell lines (THLE-2 and THLE-3) were incubated with HDAC inhibitors trichostatin A, vorinostat and romidepsin to induce histone acetylation. Differentially expressed miRs were identified by miR-microarrays and miR-sequencing and findings were validated by qRT-PCR. After transfection of miR mimics, tumor-relevant functional effects were analyzed using *in vitro* assays. Putative target genes were examined by siRNA-mediated knockdown, western blotting, luciferase reporter assay and ribonuclease protein immunoprecipitation (RIP) with subsequent qRT-PCR.

Results: miR-129-5p was identified as strongly upregulated after HDAC inhibitor-induced histone acetylation in HCC cell lines. Transfection of miR-129-5p inhibited cell proliferation and migration and induced apoptosis of HCC cells. One predicted target gene of miR-129-5p which may mediate the tumor suppressive effects is the muscarinic acetylcholine receptor M3 (*CHRM3*). This G-protein-coupled receptor stimulates matrix metalloproteinase-7 (MMP7) -dependent cell proliferation and migration by transactivating epidermal growth factor receptors (EGFR). In primary HCC, an overexpression of *CHRM3* has become evident. Transfection of HCC cells with miR-129-5p reduced expression of *CHRM3*. This regulation of *CHRM3* by the epigenetically regulated miR-129-5p is likely to be conducted directly. Moreover, the expression of *MMP-7* and *MMP-1*, which are potential downstream targets of *CHRM3*, is also regulated by miR-129-5p.

Conclusions: Histone deacetylation is an important epigenetic modification that is altered in many cancer types, including HCC. We demonstrated that miR-129-5p is re-induced after HDAC inhibition and acts as a tumor suppressor in liver cancer by causing cell death and inhibiting cell migration. Our results indicate that miR-129-5p may be considered for miRNA replacement therapy to prevent HCC progression and metastasis.

P-BasEpi-009

DNA methylation analysis of imprinted regions in *Nlrp2* knockout mice and their offspring

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Mutations in *NLRP2* and *NLRP7* in women have been associated with multilocus imprinting disorders in their children. These changes have been classified as maternal effect mutations showing no effect in the mutation carriers themselves but an impact on the offspring. Human *NLRP7* evolutionary originates from a duplication of an ancestral *NLRP2* homolog. The mouse genome contains the homologue of human *NLRP2*, i. e. *Nlrp2*, but lacks the homologue to human *NLRP7*. Mahadevan et al. [1] described that female mice in which a 'reporter-tagged tm1a' [2] cassette was inserted in the *Nlrp2* locus are subfertile and their offspring showed abnormal DNA methylation at imprinted loci. Kuchmiy et al. [3] published an *Nlrp2* knockout (ko) mouse model, the reproductive potential of which is normal in young mice but gets worse with increasing age.

We analyzed the DNA methylation status of imprinted loci in the *Nlrp2* ko mice developed by Kuchmiy et al. and their offspring. We isolated DNA from tail and/or spleen of (a) 25 *Nlrp2* homozygous wild type (wt/wt) mice bred from *Nlrp2* wt/wt parents, (b) 18 *Nlrp2* ko/ko mice bred from *Nlrp2* ko/ko parents and (c) 31 *Nlrp2* ko/wt heterozygous mice. The latter group of *Nlrp2* ko/wt heterozygous mice derived from crossing *Nlrp2* wt/wt to *Nlrp2* ko/ko mice, with 20 of them inheriting the ko allele from the mother and 11 from the father. DNA methylation was measured by bisulfite pyrosequencing in eleven regions previously published to be imprinted in mice [4]. A subset of spleen samples was also analyzed by targeted next generation bisulfite sequencing of these loci. Taking into account a total of 973 data points in which the DNA methylation was measured by both techniques, we demonstrated a good correlation between both methods ($r = 0.817$).

The methylation data of the samples from *Nlrp2* wt/wt mice was used to define a tissue- and technique-specific range of normal DNA methylation (nmr). We compared the methylation values of the samples from *Nlrp2* wt/ko and ko/ko mice with the tissue- and technique-specific nmr. Locus-specific DNA methylation values were compared between groups using the Kruskal-Wallis test.

After correction for multiple testing, we detected neither significant single nor multi-locus DNA methylation differences at imprinted regions between the three genotypes. Moreover, there was no obvious dependency of the DNA methylation at imprinted loci from the maternal *Nlrp2* genotype. Summarized, our results do not provide evidence for altered DNA methylation of imprinted loci in *Nlrp2* deficient mice. The discrepancy between our results and the previously published results [1] might be related to the analysis of mice with a reporter-tagged *Nlrp2* locus with knockout potential in the reported study versus the analysis of mice with a genuine *Nlrp2* knockout allele reported here.

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P-BasEpi-010

Analysis of long-range methylation of imprinted loci by nanopore sequencing

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Genomic imprinting is characterized by an allele specific gene expression in which either the maternal or the paternal origin of the allele determines the activity. Monoallelic expression mediated by epigenetic mod-

ifications includes DNA methylation, changes in chromatin structure or histone modifications. Defective imprinting is associated with congenital disorders like Silver-Russell-, Beckwith-Wiedemann-, Prader-Willi-, and Angelman syndrome. Although methylation patterns of imprinted loci can be analyzed in bisulfite converted DNA with next generation sequencing (NGS), information about methylation site dependencies over long distance are lost due to the short-read length. Third generations sequencing (TGS) in contrast overcomes these limitations because of the enormous read lengths. Nanopore sequencing, one of the TGS techniques, does not depend on a polymerase and allows sequencing of native DNA and thereby enables the recognition of all types of base modification. We here utilized nanopore sequencing for the analysis of differentially methylated regions (DMRs) in the human genome. For a targeted analysis, DMR regions were amplified from bisulfite converted DNA by long-range PCR. The PCR products were labeled with barcodes (EXP-NBD103 or SQK-PBK004) and further processed by the 1D Ligation sequencing kit (SQK-LSK-108) to generate the final nanopore sequencing library. Samples of healthy controls, Silver-Russell, Beckwith-Wiedemann and Multi-Locus Imprinting Disturbances patients were analyzed by this approach. Data from the targeted bisulfite sequencing analysis for some of these patients was compared to available whole genome data sets generated on GridION and PromethION platforms from Oxford nanopore technologies. In summary, long-read nanopore sequencing provides additional insight into long-range genome methylation.

P-BasEpi-011

Specific changes in hippocampal-cortical projections underlying intellectual disability in patients with tuberous sclerosis

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Tuberous sclerosis (TS) is an autosomal dominant genetic disease caused by mutations in either the TSC1 or the TSC2 gene. Mutations are thought to result in an increase in mTOR (mechanistic target of rapamycin) activity and an upregulation of downstream signalling. The mTOR kinase is the most important regulator of local dendritic and presynaptic protein translation in the brain and controls the synthesis of several proteins involved in synaptic function and plasticity.

Together with autism, epilepsy and benign tumours in several organic systems, intellectual disability (ID) is a characteristic feature of TS. About half of the patients with TS show a significant predisposition for ID with an IQ less than 70. However, not much is known about the neurophysiological mechanisms underlying ID in TS patients. To investigate the development and mechanisms of ID, we are using a heterozygous Tsc2 knockout (KO) mouse model that resembles the heterozygosity of Tsc2 mutations in TS patients.

Previous studies showed, that memory deficits and ID in TS patients do not occur due to hippocampus damage—cognitive test battery consisting of Morris Water Maze (MWM) and a 24 hours novel object recognition test (NORT), both testing for hippocampal function, was unaffected.

Interestingly, by using a 7 days NORT and a test battery for episodic memory however, a clear decline in 8 months old animals in both tests was observed, while 3–4 months old animals were unaffected. The 7 days NORT measures memory consolidation, a brain function that relies on projections between the hippocampus, the entorhinal and the prefrontal cortex. Similarly, episodic memory is a domain of brain function that depends on hippocampal-entorhinal projections. So far, our data show that ID in TS (i) develops over time and (ii) is caused by a decline of projections between the hippocampus and cortical regions.

In order to get a deeper insight into the mechanisms underlying this decline we performed neuroanatomical tract-tracing using fluorescent Alexa Fluor conjugates of cholera toxin subunit B in mouse brains of 8 months old Tsc2+/- and Tsc2+/+ mice to check for projection deficits between dorsal hippocampus, ventral hippocampus and entorhinal cortex in vivo. We are currently analyzing the microscopy outcome by a semi-automated

image processing. If we can confirm our hypothesis, we are one step closer to understand the mechanism behind the TS phenotype and these data could open new avenues for the development of novel therapeutic strategies for patients with Tuberous Sclerosis.

P-BasEpi-012

Whole genome DNA methylation analysis of sperm of normozoospermic and oligoasthenoteratozoospermic men

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It has been suggested that sperm of subfertile men have an increased rate of aberrant methylation at imprinted genes such as MEST and H19. In a series of 104 swim-up sperm samples from men with oligoasthenoteratozoospermia (OAT), we determined MEST methylation levels by bisulfite pyrosequencing. In samples with highest MEST methylation (7–34%), we confirmed aberrant MEST and H19 methylation levels by targeted deep bisulfite sequencing (DBS). In order to determine whether other imprinted and non-imprinted loci were also affected, we performed whole genome bisulfite sequencing (WGBS) at 13–16X coverage in five of these samples as well as in five OAT and five normozoospermic samples with MEST methylation <2% as determined by DBS. We found that the five OAT samples with aberrant MEST and H19 methylation—but not the other samples—had abnormal methylation levels at all of the 50 known maternally and paternally methylated imprinting control regions and that the degree of aberrant methylation at these regions was highly correlated within each sample. In order to investigate whether these results reflected epigenetic germ line mosaicism or contamination by somatic DNA, we made an inventory of soma-germ cell specific methylation differences. For this we analysed in-house WGBS data of isogenic blood and sperm samples of 12 normozoospermic men with two different bioinformatic tools (camel and metilene) to identify methylation differences. By defining a differentially methylated region (DMR) as a region of at least 10 CpGs with a methylation difference of at least 80%, we detected 33,457 DMRs, of which 6,328 overlap the promoter of 6,029 genes. Of these genes, 699 genes were among 2,446 genes previously shown to be highly expressed in mitotic and meiotic germ cells and spermatids, as compared to testicular somatic cells (Chalmel et al. 2012). In line with the expression analysis, almost all of these gene promoters were methylated in blood and unmethylated in sperm. Analysis of the methylation levels of these regions in the five OAT samples with aberrant methylation at all imprinted loci revealed abnormal methylation levels at all of these loci and in each sample to a degree similar to the abnormal methylation levels of the imprinted regions. Since it is unlikely that sperm can develop from germ cells in which hundreds of essential mitotic, meiotic and spermatid genes are silenced by DNA methylation, we conclude that a fraction of OAT samples—despite swim-up purification—is contaminated with somatic DNA rather than carrying imprinting defects.

In summary, our work provides a comprehensive list of spermatogenesis genes regulated by promoter methylation and emphasizes the need for further studies and the development of biomarkers to exclude somatic contamination of sperm samples.

P-BasEpi-013

Chromosomal fragile sites as markers of cancerogenic rearrangements—aspects of nuclear architecture and molecular localization

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Chromosomal fragile sites (CFSs) are genomic regions prone to form gaps or breaks on metaphase chromosomes. As CFSs often appear as hot spots for copy number changes in cancer cells, it is of high clinical relevance to map CFSs genome-wide for better understanding of complex aberrant karyotypes and to predict their clinical prognosis. For now, CFSs have mainly been analysed in human lymphocytes but the method is also established for other tissues and model systems. The mapping usually followed the traditional method by metaphase spreading and molecular cytogenetic definition. A new approach has become available recently (Pentzold C et al., *Nucleic Acids Res.* 2018 Feb 16;46(3); Okamoto Y et al., *Nucleic Acids Res.* 2018 Apr 6;46(6)) to standardize this mapping to a high throughput and high resolution Next Generation Sequencing(NGS)-based genome-wide application. The aim of this project is to establish this novel method in human cells, implement it for tumor material to overcome conventional metaphase spreading and morphological definition by a state-of-the-art NGS approach. To expand the view on CFSs by specific functional analyses in disease-derived cell lines, aspects of nuclear localization as well as molecular positioning of CFSs will be investigated. Overall, the project aims to identify the connection between the fragility of certain genomic regions and cancer predisposing and premature aging related disorders. Early and late replicating genomic regions appear compartmentalized in the nucleus with late replicating regions being situated at the nuclear periphery (Ryba T et al., *Genome Res.* 2010 Jun;20(6)). Since CFSs are replicating late (Laird CD et al., *Fragile sites in human chromosomes as regions of late-replicating DNA.* 1987), this would predict a localization of fragile sites into peripheral nuclear compartments. Interestingly, for FRA6E, the CFS associated with the *PARK2 (PRKN)* gene, it was proven to be affiliated to the nuclear lamina (Mitsui J et al., *Am J Hum Genet.* 2010 Jul 9;87(1)). Moreover, the nuclear position of chromosomal regions does not only tell something about the replication status but also about the transcriptional activity of the residing genes. Nuclear lamina associated domains (LADs) are predominantly defined by a low transcription rate (Guelen L et al., *Nature.* 2008 Jun 12;453(7197)). This fact implies that CFSs should be located in transcriptionally repressed or lowly transcribed genomic regions if mainly situated at the nuclear periphery. The nevertheless occurring transcription of late replicating regions and large replication origin-scarce genes (Letessier A et al., *Nature.* 2011 Feb 3;470(7332); Oz-eri-Galai E et al., *Mol Cell.* 2011 Jul 8;43(1)) would thereby predict these regions to act as CFSs. Like CFSs, also LADs can span genomic regions of several Mb (Guelen L et al., *Nature.* 2008 Jun 12;453(7197)). AT richness is another common feature of LADs and CFSs (Durkin SG et al., *Annu Rev Genet.* 2007;41; Zhang H et al., *Mol Cell.* 2007 Aug 3;27(3); Zlotorynski E et al., *Mol Cell Biol.* 2003 Oct;23(20); Meuleman W et al., *Genome Res.* 2013 Feb;23(2)).

It needs further investigation whether CFSs and LADs represent the same or at least overlapping genomic regions and how mutations in the *LMNA* gene lead to cellular catastrophe disrupting the lamin A protein which is a major part of the nuclear envelope maintaining nuclear architecture and also DNA repair (Ding SL et al., *Clin Interv Aging.* 2008;3(3)).

P-BasEpi-014

Genetic linkage between cardiac autonomic dysfunction and schizophrenia

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Cardiovascular disease (CVD) has been identified as one of the leading causes of death in schizophrenic patients, mainly contributing to a shortened life expectancy of about 15–20 years. Beyond the effects accounted for by lifestyle and antipsychotic medication, cardiac autonomic dysfunction (CADF), which is mainly characterized by increased heart rates, reduced vagal modulation, reduced baroreflex sensitivity and increased variability of the QT interval, has been considered as an independent predictor for CVD-mortality. Those CADF-features are not only observed in patients suffering from acute psychosis, but also in patients that are in remission.

A comparable pattern was demonstrated in healthy first-degree relatives, too. Due to the tremendous complexity of brain structure, a huge number of genes might be involved, so that it seems to be viable to analyze more elementary features of schizophrenia, like CADF, to shed new light in underlying pathophysiological mechanisms.

In a preliminary work, we first focused on *HCN1*, which encodes hyperpolarization-activated cation channels, regulating both neuronal excitability and cardiac pacemaking. Based on the Genome-wide association study (GWAS) and its biological functions, *HCN1* has been suggested to be of particular interest with respect to current hypotheses of schizophrenia aetiology and treatment.

We observed significant increased heart rates and a decrease of vagal modulation in patients who are heterozygous or homozygous for rs16902086, which is located in intronic region of *HCN1*. rs16902086 has been most recently reported to be associated on a genome-wide significant level in the largest study of schizophrenia thus far. Furthermore, CADF was significantly deteriorated in patients who are homozygous for rs16902086.

To provide a better understanding in the contribution of *HCN1* to the genetic linkage between schizophrenia and CADF, further analyses are strongly indicated. In addition to *HCN1*, there are various genes that are located in schizophrenia-associated loci, encoding numerous ion channel subtypes, which contribute to the electrophysiological functions of cells not only in the brain but also the heart. We designed a NGS-custom panel containing 50 genes associated with schizophrenia and/or CADF. To evaluate CADF-severity, various autonomic measures have been previously obtained at rest for 30 minutes from the included 150 unmedicated patients with schizophrenia and 150 age- and gender-matched healthy controls. The clinical relevance of rare genetic variants will be classified according to ACMG-guidelines (class 1–5). Finally, the relation between genetic variation and CADF-severity will be estimated.

P-BasEpi-015

A combined epigenome- and transcriptome-wide association study of the oral mucosa assigns CYP1B1 a central role for maintaining health at the oral interface in smokers

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The oral cavity is the gateway to the gastrointestinal and respiratory tracts. The oral mucosa has an important role in maintaining barrier integrity. Smoking is a strong environmental risk factor for the common oral inflammatory disease periodontitis and oral cancer. It was demonstrated that cigarette smoke affects gene methylation and expression in various tissues. Epigenome-wide association studies (EWAS) combined with whole transcriptome RNA sequencing (RNA-Seq) enable the unbiased and systematic identification of biologically active differences in the methylation patterns of regulatory elements between individuals. We aimed to identify the putative differences in the epigenome and transcriptome of the healthy oral mucosa between smokers and nonsmokers. These could indicate clinically relevant changes before the manifestation of a disease. Using *ex-vivo* tissue samples of the oral mucosa of healthy individuals, we performed an EWAS in smokers ($n = 18$) and non-smokers ($n = 24$). 15 CpG sites showed a genome-wide significant association of smoke packyear (SPY) levels with changes in methylation values in an analysis of covariance (ANCOVA). The most significant association located to the gene *CYP1B1* (cytochrome P450 family 1 subfamily B member 1), with $p = 1.1 \times 10^{-11}$. RNA-Seq with a coverage of 16 Mio 50 bp single-end reads per sample in the same biopsies validated the results from the EWAS and showed significantly increased expression of *CYP1B1* in smokers compared to non-smokers ($p = 5.8 \times 10^{-8}$). In conclusion, this study identified *CYP1B1* as having a strong tissue-specific and epigenetically altered activity in smokers compared to non-smokers.

P-BasEpi-016**MLXIPL controls brown adipose tissue whitening**

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In light of the rediscovery of brown fat in adult humans a decade ago, the remarkable capacity of caloric breakdown in brown adipocytes is believed to represent a valuable therapeutic target for treating obesity, dyslipidemia and atherosclerosis. At thermoneutrality, which is the predominant housing environment of humans, brown adipocytes degrade mitochondria and accumulate lipids and become a morphologically white-like, energy storing cell. However, mechanisms determining this thermogenic involution of brown fat, a process called whitening, are poorly understood. Genome wide association studies revealed correlations of MLXIPL variants with plasma triglyceride levels, a parameter which can be highly influenced by brown fat. Here we show that MLXIPL, a transcription factor regulating a set of genes essential for the de novo synthesis of fatty acids, is critical for brown fat whitening. MLXIPL-deficient mice are resistant to excessive lipid accumulation in brown adipocytes, thermoneutrality-induced mitochondrial degradation and thus, retain the high oxidative capacity of active brown fat. Moreover, we show that the pharmacological inhibition of Acetyl-CoA-Carboxylase, which gene is transcribed MLXIPL-dependent, phenocopies the genetic knockout of MLXIPL. Therefore, knockdown of MLXIPL or pharmacological inhibition of Acetyl-CoA-Carboxylase might represent a valuable target for sustained brown fat activity and might help understanding the development and treatment of obesity-related metabolic disease.

P-BasEpi-017**miRNAs of the miR-15 family regulate expression of the neuronal migration gene Pafah1b1**

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PAFAH1B1, also called the lissencephaly gene encodes LIS1 which plays crucial roles during neuronal migration and brain development. PAFAH1B1 is a dosage sensitive gene. Deletions or point mutations in PAFAH1B1 cause a variety of neuronal migration defects ranging from lissencephaly to subcortical band heterotopia. PAFAH1B1 duplications result in neurodevelopmental abnormalities such as microcephaly. This suggests that, during neurodevelopment, expression of PAFAH1B1 underlies complex regulatory mechanisms.

MiRNAs are small noncoding RNAs that bind to the 3'-untranslated regions (3'UTRs) of their target mRNAs and fine-tune their expression. Here, we hypothesized that, during neurodevelopment, miRNAs bind to the 3'UTR of PAFAH1B1 and fine-tune its expression. By comparing human and rat miRNA expression data and using different miRNA prediction tools including TargetScan and miRwalk 10 we identified ten candidate miRNAs. In Luciferase reporter assays, one of these ten miRNAs, miR-16, interacted with two of the three candidate binding sites in the murine Pafah1b1 3'UTR. The microRNA miR-16 is a member of the miR-15 miRNA family, which in addition to miR-16 contains miR-15, miR-195 and miR-497. MiRNAs of the miR-15 family are best known for their anti-proliferative effects in tu-

mor cells and neural progenitors. Their functions in postmitotic cells such as neurons are, however, unknown. When analyzing the expression of miR-15 and miR-16 by miRNA-seq and RT-qPCR we found a high expression of these miRNAs not only in neural progenitors but also in neurons. To investigate the effect of miR-16 and miR-15 on neuronal migration and the expression of Pafah1b1 in the mouse model, we are currently performing in vivo overexpression- and knock down experiments via in utero electroporation.

P-BasEpi-018**The mTOR pathway orchestrates the expression of gene regulatory networks during neuronal differentiation**

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Dysfunction of the mTOR signaling pathway is thought to be the root cause for several neurodevelopmental disorders such as autism spectrum disorder (ASD), intellectual disability (ID) and epilepsy. Although it has been known for many years that mTOR regulates local mRNA translation in neuronal dendrites, few targets have been identified and specific mechanisms remain largely unexplored. Identifying these targets and mechanisms will be important to understand neuronal function as well as the pathogenesis of neurodevelopmental disorders associated with changes in mTOR activity. To identify new mTOR targets we treated differentiating neurons with the mTOR inhibitor Temsirolimus and carried out RNA sequencing. Surprisingly, a high number of genes were found to be differentially expressed between mock and Temsirolimus treated neurons suggesting that in neurons mTOR not only regulates translation but also mRNA stability and transcription. Gene ontology and pathway analysis of the upregulated genes revealed a strong enrichment for genes related to activity induced transcription. Down-regulated genes were involved in the cholesterol and other metabolic pathways. Currently, we are carrying out further analyses to elucidate the molecular mechanism by which mTOR controls expression of these genes. To study gene changes at the transcriptional and epigenetic level we are performing ATAC- and Pyro-sequencing. Moreover, to test for changes in mRNA stability we are performing Actinomycin D treatment followed by RT-qPCR.

P-BasEpi-019**Does the human protective SMA modifier Plastin 3 escape X-chromosome inactivation?**

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Spinal muscular atrophy (SMA) is a devastating motor neuron disorder caused by homozygous loss of the Survival of Motor Neuron 1 (SMN1) gene and insufficient functional SMN protein produced by the SMN2 gene. Several families with asymptomatic individuals, which share the SMN1 deletion and the same copy number of SMN2 as their affected siblings have been identified. The genetic modifier Plastin 3 (PLS3) has been found to be upregulated in asymptomatic women. PLS3 is a Ca²⁺-dependent F-actin binding and -bundling protein, which is able to restore F-actin levels in SMA condition. By this, it is able to rescue axonal growth defects, the structure and function of the neuromuscular junctions and motor neuron function in several SMA model systems. PLS3 is upregulated in approximately five percent of the healthy human population, while the reason for the upregulation is still elusive. Interestingly, all asymptomatic individuals in seven PLS3-discordant families are women. As PLS3 is an X-linked gene, we hypothesized that PLS3 might escape the X-chromosomal inactivation (XCI). By analysing transcriptomes of single cell clones of iPSC-derived motor neurons of asymptomatic individuals, we were able to detect biallelic expression of PLS3 indicating that the gene escapes XCI. PLS3 (hg 19, ChrX:114,827,819–114,885,179 bp) is an X-linked gene, which is located adjacent to the unique

macrosatellite DXZ4 (hg 19, ChrX:114,867,433–114,919,088 bp). This macrosatellite has a highly variable repeat number in humans ranging from 50 to 100 copies of a 3 kb repeat monomer, and is essential for XCI. Surprisingly, DXZ4 is associated with heterochromatic epigenetic features on the active X-chromosomes, while it has euchromatic features on the inactive X-chromosomes and the locus of DXZ4 is therefore open for transcription. Based on this, we hypothesized that the repeat number of DXZ4 can modulate the expression of PLS3 and other genes in its molecular neighbourhood on the inactivated X-chromosome in women facilitating the escape of those genes from XCI. By molecular combing, we analysed the repeat number of DXZ4 comparing samples from women and men that differ in their PLS3 expression. This method uses multi-colour DNA probes to mark repeat regions on linear stretched DNA molecules. By this, we were able to quantify the repeat number of DXZ4 in SMA affected and healthy individuals and compared the results to PLS3 expression levels measured by quantitative RT-PCR. We were able to distinguish both alleles in women. Women with a high PLS3 expression showed a copy number of 89 to 100 repeats for at least one allele. The larger of the two alleles in women with a low PLS3 expression showed a copy number of 43 to 66 repeats. We correlated the expression levels of PLS3 to the DXZ4 copy numbers and found a significant correlation for samples from women. In males a non-significant correlation was found.

P-BasEpi-020

mTOR inhibition prevents impairments in social interaction and cognitive functions of stressed mice

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Background: Anhedonia, or diminished interest or pleasure in rewarding activities, characterizes depression and reflects deficits in brain reward circuitries. An ethologically valid animal model that has been shown to be effective in studying depression is the chronic social defeat stress model. In this model, C57BL/6J mice are subjected to chronic social defeat stress induced by CD-1 aggressor mice. Exposure to chronic social defeat (CSD) causes long-lasting impairments in social interaction (social avoidance) and hedonic behavior in most tested model. However, a small subset of animals is resilient to these impairments and maintains normal behavior. In resilient animals active compensatory mechanisms and changes within regions of the mesocorticolimbic system can be found. The signaling pathways that transduce these changes remain unknown. Our candidate of interest is the mTOR (mechanistic Target of Rapamycin) kinase. It is already known to be a key mediator of local protein translation in neurons transducing neuronal stimulation into response pathways. Earlier studies already indicate that chronic application of mTOR inhibitors (e.g., rapamycin) induces pro-resilient effects. We therefore propose that chronic application of the mTOR inhibitor Rapamycin increases the rate of resilient animals in social interaction and memory consolidation of stressed mice.

Methods: We examined the influence of treatment with mTOR-inhibitor Rapamycin on behavioral outcome in social interaction testing and 24 h Novel Object recognition testing after CSD. mTOR inhibition was checked by Western Blot analysis of members of the mTOR signaling cascade in the prefrontal cortex, hippocampus and the nucleus accumbens.

RESULTS: Stressed animals that were treated with vehicle show significantly reduced social interaction compared to non-stressed controls that were treated with vehicle. For the Rapamycin treated animals no significant difference between stressed and non-stressed was found. Overall the cohort treated with Rapamycin shows significantly higher social interaction scores than the vehicle treated cohort. Compared to non-stressed controls, stressed mice showed significantly reduced Discrimination Indices (DI). There is no significant difference within the Rapamycin cohort. Comparison of Social Interaction (SI) and NOR performance (DI) in individual animals shows that social behavior and memory consolidation do not

show any correlation. Western Blot showed a decrease in phosphorylation of mTOR target S6 in Rapamycin treated animals.

Conclusions: Our data show that treatment with the mTOR-inhibitor Rapamycin increases the number of animals with resilient behavior in both domains, social interaction and object recognition/memory consolidation. Comparison of both domains shows that they are independent.

P-BasEpi-021

Characterisation of a novel deletion in distal imprinting center 1 (11p15.5) through Oxford Nanopore Technologies: Insights in the regulation of imprinting

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Deletions of the imprinting center 1 (IC1) in 11p15.5 are rare and their clinical significance is not only influenced by their parental origin but also by their exact genomic localisation. In case the maternal IC1 allele is affected, the deletion is associated with the overgrowth disorder Beckwith-Wiedemann syndrome (BWS) and a methylation testing pattern corresponding to a gain of methylation (GOM) of the IC1. Alterations of the paternal IC1 can have different effects depending on the localisation and the affected putative methylation regulating transcription factors binding sites. It has been suggested that distal deletions of the paternal allele are associated with a normal IC1 methylation and phenotype, whereas proximal alterations cause a loss of methylation (LOM) and Silver-Russell syndrome (SRS) features. In a patient referred for molecular BWS testing and his mother, a deletion within the IC1 was identified by MLPA. It was associated with a GOM in line with maternal transmission. Accordingly, the deletion was also detectable in the maternal grandmother, but here the paternal chromosome 11p15.5 was affected and a IC1 LOM was observed. We report for the first time both GOM and LOM of the IC1 in the same family, caused by transmission of a 2.2 kb deletion in 11p15.5. Nanopore sequencing could determine the precise localisation of the deletion and provides further insights in the regulation of imprinting in the IC1.

P-Cancer Genetics

P-CancG-022

Characterization of a large novel duplication in BRCA2: how a VUS becomes a pathogenic variant

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Aim: Reclassification of a novel variant of uncertain significance (VUS) in the *BRCA2* gene, a duplication of exons 22–24, in a patient with bilateral hereditary breast cancer, as a pathogenic variant using detailed molecular genetic characterization.

Background: The patient was diagnosed with bilateral breast cancer at 35 and 44 years of age. In her family, two uncles (testicular cancer and brain tumor) and one cousin (sarcoma, deceased at 28 years of age) suffered from cancer. *BRCA1/BRCA2* testing in 2010 using DHPLC/HRM prescreening, Sanger sequencing and MLPA for *BRCA1* was uneventful. In 2018, Li-Fraumeni syndrome was ruled out by negative TP53 testing, and MLPA testing for structural variants in *BRCA2* was ordered.

Methods: The *BRCA2* duplication was identified with routine laboratory testing by MLPA analysis (kits P045 and P077, MRC Holland, Amsterdam, Netherlands) and next generation sequencing (TruSight Cancer Sequencing Panel, Illumina, San Diego, California, USA). For detailed characterization and exact breakpoint determination the whole *BRCA2* locus including intronic regions was enriched using a custom SureSelect design (Agilent Technologies, Waldbronn, Germany). Deep sequencing was performed on an Illumina Next Seq sequencer. Subsequent sequence analysis

was performed using megSAP (<https://github.com/imgag/megSAP>) with an integrated CNV detector (CnvHunter; <https://github.com/imgag/ngs-bits>). Results were validated by Sanger sequencing.

Results: Initially, a duplication of exons 22–24 in *BRCA2* was identified by MLPA analysis. By means of deep targeted sequencing and split-read detection, the exact location of the breakpoints was identified. The duplication of exons 22–24 was oriented in tandem. In detail, the breakpoints were located in intron 21 and in intron 24 (HGVS: NM_000059.3(BRCA2):c.8754+651_9256+6112dup (Chr13(GrCh37):g.32951579_32960394 dup) about 8815 bp), respectively.

Conclusion: The duplication of *BRCA2* exons 22–24, identified via MLPA, were initially classified as a VUS for the lack of further information on this newly identified alteration. By breakpoint identification, a tandem orientation of this duplication was verified. This duplication results in a disruption of the reading frame, most likely leading to a nonsense mediated decay of the mRNA. With this new information, we reclassified the structural variant as a pathogenic. Our strategy could help to reclassify other *BRCA2* duplications, currently graded as a VUS.

P-CancG-023

Breast cancer and kidney tumor (oncocytoma): an atypical manifestation of Cowden-like syndrome

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We report on a young woman, age of 38, asking for the genetic testing at diagnosis breast cancer (low difference invasive ductal breast cancer with medullary features, triple-negative). She received neoadjuvant chemotherapy and mastectomy.

At age of 28 the patient had subarachnoid hemorrhage from an aneurysm. It was a titanium skull roof plastic implanted. She has also hypertension and hypothyroidism. She was 34 years old when a kidney tumor (oncocytoma) was removed.

The patients' mother had at the age of 34 skin cancer. Her father died within one year after liver carcinoma diagnosis, at age of 77.

An oncocytoma is a tumor made up of oncocytes, epithelial cells characterized by an excessive amount of mitochondria, resulting in an abundant acidophilic, granular cytoplasm. Renal oncocytoma is thought to arise from the intercalated cells of collecting ducts of the kidney. It represents 5% to 15% of surgically resected renal neoplasms.

With prevalent of 12% breast cancer is the most common cancer in woman. To determine the mutation we performed panel-based (Illumina) germline mutation testing of 12 high and moderate penetrance breast cancer genes (*BRCA1*, *BRCA2*, *ATM*, *CDH1*, *CHEK2*, *NBN*, *PALB2*, *RAD51C*, *RAD51D*, *TP53*, *PTEN* and *BRIP1*). Data was analysed with GensearchNGS (PhenoSystems). We found no mutation in routine examination. In a second analysis, we focused on susceptibility rare genes with low or moderate penetrance. It was identified one deletion of exons 3–8 of *SDHB*-gene, thus, the diagnosis Cowden like syndrome was set.

Classical Cowden syndrome is characterized by occurrence of breast cancer, thyroid cancer, endometrial carcinoma and macrocephaly. Germline mutations in *PTEN* were found in 85% of families with Cowden syndrome (CS). About 10% of CS individuals without a detected *PTEN* mutation carry germline *SDHx* variants. CS cases are defined by the strict International Cowden Consortium criteria. When individuals have features of CS but do not meet these criteria, they are referred to as CS-like (CSL) and necessarily represent a heterogeneous series. Germline heterozygous *SDHx* mutations result in hereditary pheochromocytoma-paraganglioma (PCC/PGL) syndrome. Approximately 1–5% of carriers of *SDHB* mutations have been found to have renal cell carcinoma, breast cancer or papillary thyroid cancer.

Conclusion: Germline mutation-negative breast cancer individuals should be offered *SDH* testing, especially in the setting of thyroid and kidney cancer. Carrier of *SDHx* mutation should be screened beyond CS and PCC/PGL clinical practice guidelines.

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P-CancG-024

Pathogenic germline mutations in *BRCA1*, *BRCA2* and *CHEK2* in a single patient with pancreatic acinar cell carcinoma

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Here we report on a family with hereditary breast and ovarian cancer with a known *BRCA1* frameshift mutation (NM_007294.3: c.5266dupC, p.(Gln1756Profs*74)). The index patient's son was diagnosed with pancreatic acinar cell carcinoma at 34 years of age soon after the molecular testing in the family. Pancreatic acinar cell carcinoma is a very rare tumor with a poor prognosis and limited treatment options for the patient. Therefore, testing of the tumor tissue with regards to possible targeted therapeutic options in the future (PARB-inhibitor therapy) was recommended.

Using a multi-gene panel we surprisingly not only identified the familial *BRCA1* mutation in the tumor tissue in heterozygous state but additionally found a frameshift mutation in *BRCA2* (NM_000059.3: c.3708dupA, p.(Ala1237Serfs*6)) in 50% of the reads as well as the well-known *CHEK2*-founder mutation (NM_007194.3: c.1100delC, p.(Thr367Metfs*15)) in 50% and an *ATM* stop mutation (NM_000051.3: c.2426CG; p.(Ser809*)) in 23% of reads. The mutations in *BRCA1*, *CHEK2* and *BRCA2* could all be confirmed to be germline mutations, while the *ATM* mutation was of somatic origin. Of interest the patient's pedigree showed a single case of breast cancer in his deceased paternal grandfather at 60 years of age that had not further been investigated.

This case challenges the current concept of targeted testing in hereditary cancer predisposition families. We question whether panel sequencing should be performed as predictive testing in families with a cancer predisposition evident in two branches of the family. It also poses the question of adequate clinical surveillance and predictive testing algorithms in a family with three heterozygous pathogenic germline variants in genes encoding for proteins that are involved in DNA repair mechanisms.

P-CancG-025

A complex founder mutation in *BRCA1* in 3 families from the Upper Palatinate in Bavaria

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Several founder mutations have been described in the *BRCA1* and *BRCA2* high risk susceptibility genes for hereditary breast and ovarian cancer (HBOC). Here, we report a novel complex allele in *BRCA1* that was found to cosegregate in three seemingly unrelated families from the Upper Palatinate region in Bavaria.

Family A: In 2013, a 53-year-old woman presented in counselling with the initial diagnosis of ovarian cancer (serous cystadenocarcinoma, FIGO IIIb) at the age of 42 years. One sister of the patient was diagnosed with ovarian carcinoma at the age of 48, another sister with breast cancer at

age 67. The other five sisters, two brothers and their descendants were tumor-free. Breast cancer also was detected in two paternal aunts (at the age of 38 and 41 years, respectively).

Molecular testing of the BRCA1 and BRCA2 genes revealed a heterozygous c.5564T>A (p.Ile1855Lys) missense mutation, a heterozygous c.5569delC (p.Gln1857ArgfsX65) deletion and a missense c.5572A>T (p.Ile1858Phe) mutation, all localized within exon 24 of the BRCA1 gene. Further analysis demonstrated a cis position (complex allele) for the three mutations. This complex allele has not been reported previously in the literature. In the patient's sister with breast cancer, we also identified this mutation.

Family B: In 2015, a 35-year-old healthy woman was in genetic counseling, whose mother fell ill with breast cancer at the age of 29 and died at the age of 32. The 82-year-old maternal grandmother developed a cervical carcinoma at around the age of 50. One sister of this grandmother had breast cancer at the age of 65, and two other sisters reportedly had "abdominal cancer". We offered genetic testing of the BRCA1 and BRCA2 genes in the maternal grandmother as an obligatory carrier. The complex allele c.5564T>A/c.5569delC/c.5572A>T was identified heterozygously. Similar to family A, the three mutations occur in the patient also in the cis position. Presymptomatic DNA testing in the 35-year-old granddaughter detected also the complex BRCA1 allele.

Family C: In 2018, DNA testing identified the complex BRCA1 allele in two sisters of family C, one of whom was diagnosed with a triple negative breast cancer at the age of 43 while the other suffered from breast cancer at the age of 46.

The three families A, B, and C are living in the same district of Upper Palatinate in Bavaria suggesting a common founder. Mutation c.5564T>A was classified as likely pathogenic [IARC Class 4 (International agency for research on cancer)]. The c.5569delC mutation leads to a frame shift in protein translation, ultimately resulting in a stop codon and pathogenic truncation of the protein. Consequently, this mutation was classified as pathogenic (IARC Class 5). Finally, the missense c.5572A>T mutation is located downstream of the frameshift mutation, no further deleterious effect on the protein function is expected. Therefore, this was classified as likely benign (IARC Class 2).

From these 3 families 7 women were positive for this complex allele, 3 of them show no sign of breast/ovarian cancer so far. Two of them are age 31, 40 respectively and have an increased risk to develop breast- or ovarian cancer. The healthy 83-years old woman is indicating the reduced penetrance of BRCA1-mutation.

This mutation is a founder mutation in Upper Palatinate region in Bavaria, but its overall frequency appears to be low. Given the high genetic heterogeneity of gene mutations in HBOC, full gene panel testing is still highly recommended.

P-CancG-026

The mutational landscape of MED12 alterations in uterine smooth muscle tumors and fibroepithelial tumors of the breast

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Large subsets of uterine smooth muscle tumors and fibroepithelial tumors of the breast share a common type of driver mutations i.e. those affecting Mediator Complex Subunit 12 (MED12). In the latter case the mutations seem to be restricted to the stromal part of the tumors and, in general, epithelial tumors so far seem to lack frequent subtypes with MED12 mutations. In uterine smooth muscle tumors they occur particularly frequent among multiple leiomyomas despite an independent monoclonal origin of the single nodules. Accordingly, five or even more uterine leiomyomas with different MED12 mutations are not infrequently seen in one patient. Based on our own cases and tumors reported in the literature we have carried out an in depth analysis of the mutational landscape of MED12 alterations found in these tumors. Most frequently, base exchanges affecting codon 44 of the gene can be observed. Next frequently, simple in-frame deletions are found where the deleted segments strongly vary in their size

but are clustered mainly around codon 44 with the majority of them removing this codon. Nevertheless, deletions and indels only flanking that region either at its 3' or 5' side are not associated with a significantly lower growth rate or other clinical findings distinguishing them from those deletions affecting the mutational hotspot or single base exchanges within codon 44. The results of genomic array hybridization reveal that the large majority of tumors bearing a MED12 mutation are not characterized by other genomic alterations as e.g. signs of "healed" chromothripsis which is often seen in tumors with rearrangements of High Mobility Group Protein AT-Hook 2 (HMGA2), constituting the second most frequent driver mutation in uterine leiomyomas.

However, the mutational landscape of MED12 in uterine smooth muscle tumors and fibroepithelial tumors of the breast may reflect the action of a so far unknown factor favoring site specific mutations. Once exposed to such factor, effects resembling field tumorigenesis may result.

P-CancG-027

Immunohistochemical detection of BRCA1 and BRCA2 expression as prognostic biomarker for ovarian cancer therapy

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Purpose: Decrease or loss of expression of BRCA1 and BRCA2 are associated with cancer development and tumor progression in ovarian cancer. In this study, we retrospectively evaluated samples from ovarian cancer patients and correlated both BRCA1 and BRCA2 protein expression levels with the clinical and pathological outcome to evaluate the use of BRCA1/2 immunohistochemistry as a putative biomarker.

Methods: This study included 97 ovarian cancer tissue samples from the Institute of Pathology and the Department of Gynecological Oncology at the University Medical Center Schleswig-Holstein, Kiel, Germany. Samples were prepared and stained using antibodies targeting BRCA1 or BRCA2. Results were scored based on the ratio of positively stained cells.

Results: A statistically significant decrease in the occurrence of ascites in the sample cohort was observed for patients with focal BRCA1 expression ($n=6$, Fisher's exact test, $p<0.03$) or positive BRCA2 expression ($n=25$, Fisher's exact test, $p<0.02$). The data also suggest that patients with at least weak expression of both BRCA1 and BRCA2 have a prolonged PFI compared to patients lacking the expression of either one BRCA genes (46.8 months; Mann-Whitney-U-Test, $p<0.05$).

Conclusions: Our study provides evidence that the immunohistochemical detection of BRCA1 and BRCA2 protein expression levels might serve as a prognostic biomarker for ovarian cancer therapy. This is in concordance with the published evidence, that patients with deleterious BRCA1 mutation had a better prognosis.

P-CancG-028

Association between survival in ovarian cancer patients and tumor associated lymphocytes and macrophages

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Background: Epithelial ovarian carcinoma (EOC) is one of the most dangerous cancers among women in western countries because of the high

mortality risk. In Germany the incidence of newly diagnosed EOC was 7250 cases per year in 2014, with 5354 deaths (Robert Koch Institute). New therapeutic agents are needed to improve survival. Immune checkpoint inhibitors, as anti-PD-L1 (programmed death-ligand 1) antibodies, a drug to overcome the immune suppression by cancer cells, are under evaluation. Therefore, the aims of this study were to evaluate a prognostic impact of tumor-infiltrating lymphocytes (TILs) and tumor-associated macrophages (TAMs) in ovarian cancer patients.

Methods: All patients in this study are derived from the Department of Gynecology and Obstetrics registry, UKSH Campus Kiel, Germany. The study includes only patients with invasive ovarian carcinoma, borderline tumors were excluded.

Immunohistochemistry was performed on paraffin-embedded tissue specimens with specific antibodies for CD3+, CD8+, CD68+, CD163+, FOXP3 and CD25+ cells. After staining, the number of positive reactions with the specific antibodies was determined. The staining intensity of the TILs and TAMs were counted in the intraepithelial and stromal compartments. Pairwise correlation between staining was estimated using Spearman's rank correlation coefficient. Associations between staining and clinical characteristics such as grading and cisplatin treatment sensitivity were investigated using Wilcoxon tests. Survival analyses for time to relapse and tumor death were performed using univariate Cox regression models.

Results: The study includes 69 patients with ovarian cancer. The age ranged from 29–83 years with a median age of 60. The majority of patients had FIGO Stage III (53.7%), high tumor grade (62.7%) and were of serous histology (52.2%). 30 patients died because of disease (43.5%).

A strong positive correlation between CD3 and CD8 stromal and epithelial staining was observed. Staining of CD8+/CD163+ and CD3+/CD68+ in the epithelial compartment were also positively correlated. Intraepithelial CD68 positivity was significantly stronger in tumors with higher grading (p value = 0.0448). No significant correlation to Platin-sensitivity was detected. Stronger staining of epithelial CD68 was significantly associated with earlier relapse (p value = 0.02).

Conclusion: Our results are in line with published results that TILs and intratumoral macrophages could be of prognostic value for ovarian cancer patients. The positive correlation of epithelial staining give hint, that intraepithelial immune response plays a role in ovarian cancer. The results would further be compared with staining against PD-L1, which is under the way.

P-CancG-029

Molecular profiling of circulating tumor DNA in plasma of patients with renal cancer

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Renal cell cancer (RCC) represents the most lethal urological malignancy. Extensive genetic heterogeneity offers a particular challenge, confounding treatment decisions. Although the cell-free circulating tumor DNA (ctDNA) has been demonstrated to allow non-invasive detection and tracking of various cancers, the utility of ctDNA in RCC has not been well established.

Therefore, we characterised the levels and composition of ctDNA in the plasma of 43 patients with metastatic clear cell RCCs. Untargeted approaches, including shallow Whole Genome Sequencing (sWGS), and modified Fast Aneuploidy Screen Test-Sequencing System (mFAST-SeqS) were initially applied. These methods revealed that ctDNA levels are lower in RCC than other cancers of similar stage, detecting ctDNA in only 2 out of 43 patients (4.7%). However, in silico size selection of fragments smaller than 150 bp resulted in a ctDNA substantially higher detection rates consistent with previous reports that tumor-derived fragments might be smaller compared to cell-free DNA of normal cells. Moreover, high-resolution mutation analysis was performed using Qiagen's QIASeq custom capture panel and enabled detection of tumor-specific mutations at base-

line in 18% (8/43) of patients. Furthermore, we had access to longitudinally obtained plasma samples for 37 of our 43 (86%) patients with a median follow-up period of 6 months range, 0.4–19.2. The QIASeq panel was applied to follow-up samples of 6 patients of which mutations were identified at baseline. For most of these patients, ctDNA was elevated at treatment initiation but decreased with response. At the time of progression, or when a response could not be achieved, ctDNA increased or remained elevated. This data, generated using state-of-the-art techniques, confirm that ctDNA can be detected in blood of RCC patients and there is potential for clinical utility. However, as RCC present significantly lower tumor fractions than other tumor types, improved isolation and detection methods are needed before this can be realised.

P-CancG-030

KMT2A gene rearrangements displaying an important molecular marker in the diagnostic and prognostic of acute leukemia

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KMT2A gene alterations can be found in 10% of acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) cases, each. However, certain KMT2A alterations are independent, unfavorable prognostic markers, and such patients are usually treated according to high-risk protocols. For this reason, identifying KMT2A alterations has relevant implications for therapy decision-making. In the present study, ten cases with acute leukemia (ALL and AML) were retrospectively studied in detail by molecular cytogenetic and molecular approaches, i.e. multitude multi-color banding (mMCB) and locus-specific probes and high resolution array-comparative genomic hybridization (aCGH). FISH-banding (mMCB) identified cryptic and complex chromosomal rearrangements involving chromosome 11 in all ten studied cases. Dual-color-FISH using a commercial available break-apart rearrangement probe specific for KMT2A locus (LSI KMT2A, Abbott/Vysis) revealed in eight cases reciprocal translocations (cases 3–10) and in two cases (cases 1–2) an insertion of the KMT2A gene into another chromosome. Gene fusion, as a result of chromosomal translocation as well as insertion, is an important event in leukemogenesis. Two clinical subgroups have a high frequency of 11q23 aberration: one is infants (<1 year) with KMT2A rearrangements (frequency ~50%) and the other group is adult “secondary leukemia”, potentially after treatment with DNA topoisomerase II inhibitors. The present study highlights that KMT2A gene rearrangements should be considered and tested, especially in case of a normal cytogenetic result in patients with AML M4/M5 and B-cell precursor acute lymphoblastic leukemia (BCP-ALL), being characterized by extreme leukocytosis.

P-CancG-031

Deciphering the structural variants in T-prolymphocytic leukemia by whole genome and transcriptome sequencing

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T-cell prolymphocytic leukemia (T-PLL) is a rare T-cell leukemia. T-PLL is mostly observed in patients with an average age at diagnosis of 65 years. The genetic hallmark of these tumors is an inv(14)(q11;q32)/t(14;14)(q11;q32) involving the TCR alpha/delta locus with TCL1 genes. Few T-PLL cases harbor a translocation t(X;14)(q28;q11) involving *MTCP1* which is the paralog of the *TCL1A*. Additional recurrent secondary copy number alterations (CNA) observed in T-PLL include aberrations of chromosome 8, deletions of 12p13 and 22q. Nevertheless, genome wide sequencing analyses on structural variants (SVs) are yet rare. The aim of the present study was to identify genomic and transcriptomic fusions in T-PLL using sequencing technologies.

We recruited 13 T-PLL samples which were analyzed by whole-genome sequencing ($n=13$) and stranded RNA-Sequencing ($n=8$). In addition, we included a described model cell line for T-PLL (SUPT-11), which was also analyzed by whole-genome sequencing. SVs were retrieved using the SOPHIA algorithm and verified by fusion crossing PCR and subsequently using Sanger sequencing.

First, we characterized the breakpoint locations of the hallmark alterations in T-PLL. We identified inv(14), t(14;14) and t(X;14) in 10, 2 and 1 case, respectively, which is in line with the previous molecular cytogenetic results. We then mapped the breakpoints of the inv(14) and t(14;14) affecting the TCL1 gene locus. The breakpoints of the inversions were located in 9 cases at a median distance of 41,719 bp (range 5,745–132,339 bps) upstream of *TCL1A*, disrupting e.g. *TCL1B*. In one case of inv(14) the breakpoint disrupted the exon 4 of *TCL1A*. In contrast, the breakpoints of the both t(14;14) cases mapped 7,634 and 128,460 bps downstream of *TCL1A*, disrupting intron 2 of *BX247990* in one case. The breakpoint on chromosome X in cases with t(X;14) mapped to the intron 1 of *MTCP1*. Using the stranded RNA-seq data, we analyzed if the hallmark aberrations altered the expression of the genes located in the proximity of the breakpoints. Notably, in contrast to the other genes (like *TCL1B*, *TCL16* and *BX247990*) in the 14q32 region affected by the SV, *TCL1A* was the only gene with higher expression in the cases with inv(14)/t(14;14) as compared to normal T-cells. Similarly, *MTCP1* was higher expressed in the case with t(X;14) respectively. Next, we did a global analysis of the fusion transcripts in T-PLL using the stranded RNA-seq data. In 8 cases analyzed, we identified a median of 31 (range 17–34) fusion transcripts. Whether these fusion

transcripts are specific to T-PLL or arise due to genomic SVs is currently under investigation.

Based on the combined genomic breakpoint and RNA expression analysis we corroborate that *TCL1A* and *MTCP1* are the target genes of the hallmark alteration in T-PLL. Further, we have identified a series of fusion transcripts in the T-PLL cases which are currently under investigation for their pathogenetic role in T-PLL.

P-CancG-032

Variant profiling of colorectal adenomas from patients with MSH3-related adenomatous polyposis

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Aim: Recently, biallelic *MSH3* germline mutations were described to cause a novel, rare, autosomal recessive subtype of adenomatous polyposis. In the present study we comprehensively analyzed the variant spectrum of colorectal adenomas from patients with biallelic *MSH3* germline mutations to characterize mutational signatures and to identify potential driver genes and pathways of *MSH3*-related tumorigenesis.

Method: We performed whole exome sequencing (WES) and array-based copy number variant (CNV) analysis of 2–3 adenomas and matched normal tissue in three patients with biallelic *MSH3* mutations from two families. For data analysis and filtering for somatic variants an in-house bioinformatics pipeline was applied.

Results: The amount of all somatic variants in the *MSH3* deficient adenomas (36–120) and the pattern of single nucleotide variants (SNVs) seem to be similar to sporadic adenomas, whereas the fraction (16–40%) of small insertions/deletions (Indels) is higher. Interestingly, pathogenic somatic *APC* mutations were found in all adenomas, and the vast majority (6 of 7) are Indels, which seem to occur preferentially (4 of 6) in short nucleotide tandem repeats compared to published somatic and germline *APC* Indels. Only three of seven *APC* mutations lie within the published mutational cluster region. On average, 26 large deletions were found in each of three fresh-frozen adenomas from one patient. Besides *APC*, five more genes harbor truncating mutations, missense variants, or deletions in at least two polyps, including the potential tumor suppressor gene *ELF3*. Of the genes suspected to be drivers in early adenomas, only *KRAS* and *TMPRSS13* showed each one missense mutation. In line with the early stage of carcinogenesis, the adenomas are lacking mutations in driver genes found frequently mutated in colorectal carcinomas, like *TP53*, *PIK3CA* and *SMAD4*. A second hit was only found in *APC*.

Conclusions: To our knowledge, effects of germline *MSH3* deficiency on the mutational spectrum and profiles have not yet been described, neither in humans nor in mice. Our preliminary data demonstrate that *MSH3*-related carcinogenesis seem to follow mainly the classical *APC*-driven pathway. We found similar mutation patterns of SNVs in *MSH3*-deficient polyps compared to sporadic adenomas, however, in line with the specific function of *MSH3* in the mismatch repair system, we observed a higher proportion of Indels.

P-CancG-033**Exome sequencing identified potential causative candidate genes for serrated polyposis syndrome**

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Aim: Serrated polyposis syndrome (SPS) is a poorly defined colorectal cancer predisposition syndrome characterized by the occurrence of multiple and/or large serrated lesions throughout the colon. To date, only few molecular signatures have been described and the etiology of the syndrome has not been identified in the vast majority of patients. The aim of this study is to identify causal variants for SPS.

Methods: To uncover predisposing causative variants, the exomes of 49 unexplained SPS index patients have been sequenced (Illumina HiSeq) using leukocyte DNA. For data analysis and filtering, the GATK software and the Varbank2 software were applied. The germline variants were filtered for rare (biallelic: MAF ≤ 1%, monoallelic: 0.1% according to gnomAD and an in-house database) loss of function (LoF) variants. Regarding splice sites, variants up to +/-10 nucleotides from the exon boundaries were analyzed if they showed a combined predicted change of splice efficiency of at least 50% in the mutation analysis software Alamut[®]. The pLi-score was used to further characterize the genes. A pathway analysis was performed using the reactome software.

Results: After stringent filtering steps and manual inspection, homozygous LoF variants were found in three genes none of which being recurrently mutated. No potentially compound heterozygous variants were identified. All in all, 548 genes harbored heterozygous LoF variants. 25 genes were recurrently mutated. In 18 of these genes, two patients carried different variants, and one gene was mutated in three patients. 31/551 genes were extremely LoF intolerant indicated by pLi-score ≥ 0.9; all of these genes were mutated in one patient only. According to the reactome pathway analysis, 10/551 genes are involved in DNA repair, 12 in cell cycle checkpoint control, and one gene in programmed cell death. Most interestingly, different LoF variants in two patients were found in one gene functioning in the canonical wnt signalling pathway, as does RNF43, in which pathogenic variants have been shown to be causal for SPS.

Conclusions: Exome sequencing identifies potentially causative germline variants underlying the susceptibility to SPS, however, the preliminary data indicate considerable genetic heterogeneity. The current work-up consists of the validation of variants by Sanger sequencing, screening of additional SPS patients for the most interesting variants, analyzing the segregation with the phenotype where applicable, and the inclusion of missense variants and CNVs. Additionally, a rare variant analysis and burden tests will be conducted.

P-CancG-034**Phenotype studies of Fanconi anemia patients belonging to the rare complementation groups FA-J, FA-M and FA-N**

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Fanconi Anemia (FA) is a rare autosomal recessive and X-linked chromosomal instability disorder characterized by progressive bone marrow failure, physical abnormalities and an increased risk for developing cancer. Long-term genotype-phenotype studies exist for the main FA subgroups while data on rare subgroups is limited, e.g. FA-J (~2% of all FA patients, *FANCI/BRIP1* gene), FA-M (<1%, *FANCM* gene) or FA-N (<1%, *FANCN/PALB2* gene). This knowledge has an impact for both the management of FA patients and the understanding of the biological and clinical signifi-

cance of disease-causing mutations. Moreover, heterozygous mutations in *FANCI/BRIP1* and *FANCN/PALB2* are associated with an increased risk for breast and other cancers.

We report on 17 FA patients from 10 families that were assigned to three of the rare FA subgroups (≤2%), namely FA-J (n=10), FA-M (n=1) or FA-N (n=4). All patients with confirmed biallelic mutations in *FANCN/PALB2* or *FANCM* showed a high risk for developing solid tumors (e.g. Wilms tumor or squamous cell carcinoma) early in their lives rather than for progressive bone marrow failure, which is a hallmark of FA. On cellular level all patient-derived fibroblasts showed a high sensitivity towards DNA-damaging agents like mitomycin C (MMC).

Interestingly, biallelic mutations in *FANCI/BRIP1*, a DNA repair associated DEAH-box DNA helicase, caused only mild MMC sensitivity in two cases. Notably, all of our FA-J patients display a very mild hematological phenotype.

Although clinical heterogeneity is well-known for FA and no defined genotype-phenotype correlations have been found for most FA subgroups, our data suggest that this might be different for some rare groups. A detailed examination of the underlying mutations could provide further insights into the molecular functions of the corresponding protein.

P-CancG-035**The identification of pathogenic variants in BRCA1/2 negative, high risk, hereditary breast and/or ovarian cancer patients: high frequency of FANCM pathogenic variants**

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NGS-based multiple gene panel resequencing in combination with a high resolution CGH-array was used to identify genetic risk factors for hereditary breast and/or ovarian cancer in 237 high risk patients who were previously tested negative for pathogenic *BRCA1/2* variants. All patients were screened for pathogenic variants in 94 different cancer predisposing genes. We identified 32 pathogenic variants in 14 different genes (*ATM*, *BLM*, *BRCA1*, *CDH1*, *CHEK2*, *FANCG*, *FANCM*, *FH*, *HRAS*, *PALB2*, *PMS2*, *PTEN*, *RAD51C* and *NBN*) in 30 patients (12.7%). Two pathogenic *BRCA1* variants that were previously undetected due to less comprehensive and sensitive methods were found. Five pathogenic variants are novel, three of which occur in genes yet unrelated to hereditary breast and/or ovarian cancer (*FANCG*, *FH* and *HRAS*). In our cohort we discovered a remarkably high frequency of truncating variants in *FANCM* (2.1%), which has recently been suggested as a susceptibility gene for hereditary breast cancer. Two patients of our cohort carried two different pathogenic variants each and ten other patients in whom a pathogenic variant was confirmed also harbored a variant of unknown significance in a breast and ovarian cancer susceptibility gene. We were able to identify pathogenic variants predisposing for tumor formation in 12.3% of *BRCA1/2* negative breast and/or ovarian cancer patients.

P-CancG-036**Gold standard MLPA technique pushed to the next level: digitalMLPA in routine cancer diagnostics**

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Copy number variations (CNVs) are an important pathomechanism in numerous genetic diseases including cancer, like hereditary breast and ovarian cancer (HBOC) and hereditary non-polyposis colorectal cancer (HNPCC). Next-generation sequencing (NGS) is now a routinely used diagnostic technique and many algorithms promising to detect CNVs from NGS data have been published. Some of these need a certain minimal number of samples in the same NGS run to work reliably, others need positive controls and others don't work in mixed panel NGS runs. In the end, multiplex ligation-dependent probe amplification (MLPA) is still the gold standard to reliably detect CNVs. Up to now, only one, or a small number of genes could be combined in one MLPA reaction which made standard MLPAs laborious and cost-intensive if all genes of a more comprehensive cancer gene panel should be covered. Here, we present data of a so called "digitalMLPA" panel of MRC-Holland (Amsterdam, Netherlands), which includes 26 cancer-related genes and additional regions and which combines the advantages of the gold-standard MLPA technique and high-throughput NGS diagnostics. Additionally, sample tracking by single nucleotide polymorphism (SNP) identification, and a large number of control probes are included in digitalMLPA to ensure security in high-throughput diagnostics. More than 1.500 HBOC and HNPCC samples were analyzed using this new technique which makes our study the most extensive so far published. Whereas only conventional MLPAs for the HBOC genes BRCA1, BRCA2, CHEK2, RAD51C and PALB2 are performed routinely in most diagnostic labs, we generated data for many other genes, including RAD51D, TP53, ATM, CDH1, and NBN. Thereby, we detected a remarkable number of disease-associated CNVs in those genes which would have remained unseen by analyzing only the first five genes. DigitalMLPA proved to be the next level in robust, reliable, routine CNV diagnostics with great advantages in the lab and for the patients.

P-CancG-037

Analysis of BRCA1 and BRCA2 mutations and gene expression in hematological malignancies—implications for differential involvement in pathogenesis of lymphoid neoplasms

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Loss of activity by (germline) mutations (mut) or downregulation (DR) of gene expression (GE) of the tumor suppressor genes BRCA1 and BRCA2 (breast cancer 1 and 2) correlates with an increased risk of breast and ovarian cancer. Mut in or reduced GE of BRCA1/2 have also been implicated in some hematological malignancies, although data are so far scarce. Thus we aimed at investigating BRCA1/2 mut and GE in 3340 patients with different hematological malignancies by whole genome sequencing (WGS) and RNA sequencing.

We identified 14/3340 patients diagnosed with a variety of hematological neoplasms with a pathogenic (according to ClinVar) BRCA1/2 mut (BRCA1: $n = 11$; BRCA2: $n = 3$; incidence: 0.42%). Analysis of the mut frequencies of 1067 cancer associated genes revealed RNF213 (6/14, 43%), ARID1B, CREBBP (5/14, 36%, respectively), JAK3, TET2, NUMA1, POLD1, ZFXH3 (4/14, 29%), ATM, KMT2D and POLE (3/14, 21%) as most frequently mutated genes in cases with BRCA1/2 mut. Three of them (ATM, POLD1, POLE) are known to be directly involved in DNA repair. Comparison of mut frequencies of these 11 genes in cases with/without BRCA1/2 mut revealed JAK3 and POLD1 to be significantly more abundant in BRCA1/2 mut (JAK3: 29% vs. 9%, $p = 0.028$; POLD1: 29% vs. 8%, $p = 0.026$). GE analyses of BRCA1/2 showed DR of BRCA1 and/or BRCA2 in several lymphoid malignancies: for BRCA1, the strongest reduction was detected in PPBL (persistent polyclonal B-cell lymphocytosis; normalized BRCA1 expression: 1.5; control: 5.2), CLL (chronic lymphocytic leukemia; 1.6), T-PLL (T-cell prolymphocytic leukemia; 1.7) and MZL (marginal zone lymphoma; 2.2). DR of BRCA2 was less pronounced (control: 6.3; PPBL: 3.5; HCL, hairy cell leukemia: 4.0; T-PLL: 4.1; MZL: 4.6). For further analysis, the 50 lymphoid cases with the lowest and highest BRCA1/2 GE (BRCA1/2 high/low) were selected: these included for BRCA1 low (me-

dian GE: 0.8) mainly CLL (33/50), T-PLL (4/50) and PPBL (4/50), whereas BRCA1 high (median: 5.4) comprised B-ALL (23/50), T-ALL (15/50) and Burkitt lymphoma (7/50). BRCA2 low (median: 3.4) included only 2/50 CLL cases, as mainly PPBL (14/50), T-PLL (10/50), multiple myeloma (9/50) and HCL (9/50) cases showed a low BRCA2 GE. BRCA2 high (median: 6.6) included T-ALL (21/50), B-ALL (16/50) and Burkitt lymphoma (4/50). Comparison of BRCA1/2 low/high revealed several significant differences in detected gene mut: for BRCA1 low vs. high, JAK3 was more frequently mutated in low (18% vs. 4%, $p = 0.05$), whereas KAT6B (4% vs. 24%, $p = 0.008$), BCL11B (0% vs. 18%, $p = 0.003$) and PHF6 (0% vs. 18%, $p = 0.003$) were more abundant in high. For BRCA2, results were similar (JAK3: 20% vs. 4%, $p = 0.028$; BCL11B: 2% vs. 18%, $p = 0.025$; PHF6: 0% vs. 24%, $p < 0.001$), moreover BRAF (20% vs. 2%, $p = 0.008$) and STAT5B (16% vs. 0%, $p = 0.006$) were more frequently mutated in low. Thus, we conclude that not molecular mut in, but DR of BRCA1/2 in CLL, T-PLL and MZL could play a more important role in pathogenesis than so far expected.

P-CancG-038

Gene expression profiling in aggressive digital papillary adenocarcinoma sheds light on the architecture of a rare sweat gland carcinoma

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Background: Sweat gland carcinomas are rare cutaneous adnexal malignancies. Aggressive digital papillary adenocarcinoma (ADPA) represents a very rare sub-entity, thought to arise almost exclusively from sweat glands of the fingers and toes. The aetiology of sweat gland carcinomas and ADPA is largely unknown. ADPAs are most likely driven by somatic mutations. However, somatic mutation patterns are largely unexplored, creating barriers to the development of effective therapeutic approaches to the treatment of ADPA.

Objective: To investigate the transcriptome profile of ADPA using a sample of eight formalin-fixed, paraffin embedded (FFPE) tissue samples of ADPA and healthy control tissue.

Methods: Transcriptome profiling was performed using the Affymetrix PrimeView Human Gene Expression Microarray and findings were validated via reverse-transcription of RNA and real-time qPCR.

Results: Transcriptome analyses showed increased tumour expression of 2,266 genes, with significant involvement of cell cycle, ribosomal and crucial cancer pathways. Our results furthermore point to tumour-overexpression of FGFR2 ($p = 0.001$).

Conclusions: Our results indicate the involvement of crucial oncogenic driver pathways, highlighting cell cycle and ribosomal pathways in the aetiology of ADPA. Suggested tumour-overexpression of FGFR2 raises the hope that targeting the FGF/FGFR axis might be a promising treatment for ADPA and probably for the overall group of sweat gland carcinomas.

P-CancG-039**Genetic and epigenetic characterization of different subtypes of primary cutaneous follicle center lymphoma**

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Primary cutaneous follicle center B-cell lymphoma (PCFCL) is an indolent follicular B-cell lymphoma predominantly located in the skin. A rare variant of PCFCL, termed PCFCL spindle-cell variant (sc-PCFCL) has a more aggressive histopathologic appearance. Little is known about the mutational landscape of PCFCL. Hence, the aim of this analysis was the characterization of the genetic and epigenetic landscape of the two PCFCL subtypes. We recruited 11 cases of PCFCL and 9 cases of sc-PCFCL (9 initial, 1 relapse sample) all being *BCL2* and *BCL6* break negative. To analyze the chromosomal imbalances we performed OncoScan™ CNV FFPE array in 9 PCFCL and 8 sc-PCFCL cases. The mutational landscape was studied by whole exome sequencing of 7 PCFCL and 6 sc-PCFCL cases as well as whole genome sequencing of 2 PCFCL cases (analyzed within the frame of the ICGC MMML-Seq project). The DNA methylation was analyzed using the HumanMethylation450 BeadChip array in 7 PCFCL and 5 sc-PCFCL cases (4 initial, 1 relapse sample). The epigenetic profile was compared to various mature B-cell lymphoma subtypes including 86 follicular lymphoma (FL, analyzed within the ICGC MMML-Seq project, Vogt et al., 2018) as well as to 94 benign B-cell populations from different developmental stages recently published.

First, we analyzed the pattern of chromosomal imbalance and detected a comparable number of copy number alterations (CNA) in both PCFCL variants with a median of 6 (range 0–17) CNA in PCFCL and 5.5 (range 2–24) CNA in sc-PCFCL. We could identify 18 minimal altered regions affected in at least 3 of 17 samples. None of these CNA occurred unique to one of the two subtypes.

Next, we analyzed the mutational landscape using the sequencing data. The mutational load was comparable in both variants with a median of 122 potential protein-changing mutations in PCFCL (range 78–208 mutations) and 155 in sc-PCFCL (range 87–369). Overall 56 genes were mutated in ≥2 PCFCL cases including *CREBBP* (4/9), *IRF8* (3/9), *EP300* (2/9) and *EZH2* (2/9) which have been described to be frequently mutated in nodal FL. In contrast, 32 genes were mutated in ≥2 sc-PCFCL cases including *FAT1* (4/6) and *AP3B1* (3/6) whereas the genes recurrently mutated in nodal FL were not recurrently or not at all mutated in the sc-PCFCL cases. Analysis of the DNA methylation data showed in both PCFCL variants significantly higher mean DNA methylation values than in nodal FL ($p=0.0037$). Moreover, we found 644 loci to be slightly differentially methylated between PCFCL and sc-PCFCL ($p < 0.01$, $\sigma/\sigma_{\max}=0.4$).

In conclusion, we could show that PCFCL and sc-PCFCL resemble one another with respect to the chromosomal imbalance and epigenetic profile. However, the mutational landscape differs between both variants, with PCFCL being more similar to nodal FL. Hence, our data supports the classification of the sc-variant as subtype of PCFCL according to the WHO classification. However, the histopathological variants show molecular differences.

P-CancG-040**The mutational landscape of Burkitt-like lymphoma with 11q aberration is distinct from that of Burkitt lymphoma**

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The recent revision of the “WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues” has introduced a provisional lymphoma category named Burkitt-like lymphoma with 11q aberration (mnBLL, 11q). It comprises cases similar to Burkitt lymphoma (BL) on morphological, immunophenotypic and gene expression level but lacking the Burkitt translocation resulting in *IG-MYC* juxtaposition characteristic for Burkitt lymphoma. Instead, mnBLL, 11q, are characterized by a peculiar imbalance pattern of gains and losses on chromosome 11.

The mutational landscape of mnBLL, 11q, has not yet been described. Thus, we here aimed at characterizing the genomic alterations of this novel lymphoma category.

To this end, we retrospectively collected 15 mnBLL, 11q, which were all shown by FISH to lack an *IG-MYC* translocation, and applied a combination of whole-exome sequencing as well as chromosomal imbalance profiling using OncoScan™ FFPE arrays.

Besides the peculiar 11q aberration pattern imbalance mapping revealed recurrent (partial) trisomy 12 (7/15 cases, minimally gained region in 12q13.11-q24.32), gain in 7q34-qter and loss in 13q32.3-q34 (both 3/15 cases).

Exome sequencing identified 47 genes recurrently affected by protein-changing mutations (each >3/15 cases). Strikingly, we did not detect recurrent mutations in genes of the ID3-TCF3 axis or SWI/SNF complex that are frequently altered in BL, or in genes frequently mutated in germinal center derived B-cell lymphomas like *KMT2D* or *CREBBP*. An exception is *GNA13* being mutated in 7/15 mnBLL, 11q, cases, which is reported to be frequently altered in B-cell lymphomas. Strikingly, in 4/15 mnBLL, 11q, cases we identified potential protein-changing mutations of *NFRKB* which is located within the recurrent region of loss in 11q24.3-qter. Interestingly, 3/4 *NFRKB* mutations were stopgain mutations and accordingly *NFRKB* function is supposed to be bi-allelically lost in these three cases, i.e. by deletion of one allele and mutation of the other. *NFRKB*

belongs to the INO80 chromatin remodeler complex and the mutations are predicted to hamper its function.

We conclude that the genomic landscape of mnBLL,11q, differs from that of BL both at the chromosomal and mutational level. Our findings implicate that mnBLL,11q, is a lymphoma category distinct from Burkitt lymphoma at the molecular level.

R. W. and J. S. share the first authorship.

P-CancG-041

Central neurocytoma: SNP array analyses and subtelomere FISH

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Introduction: According to the World Health Organization (WHO) classification of 2016, the central neurocytoma (CN) is an uncommon intraventricular neoplasm composed of uniform round cells with a neuronal immunophenotype and low proliferation index and histologically classified as WHO grade II. It is usually located in the region of foramen of Monro, predominantly affects young adults, and has a favourable prognosis. However, some CN occur as histological “atypical” variant, combined with increasing proliferation and poor clinical outcome. Therefore, detailed genetic knowledge could be helpful to check a potential atypical behavior in CN and to elucidate causal genetic events for tumorigenesis in CN.

Methods: Cytogenetic analyses from blood, tumor tissue and primary tumor cells of an intraventricular neurocytoma WHO grade II by genome-wide high-density single nucleotide polymorphism array (SNP array; Affymetrix CytoScan[®] HD) and subtelomere FISH (ToTelVysion Multi-colour DNA probes, Abbott) were performed.

Results: Applying these genetic techniques, we confirmed known chromosomal aberrations such as mosaic gains of the chromosomal regions 10q26.13-q26.3, 11q24.3-q25, 13q33.3-q34, 18q22.3-q23 and mosaic losses of the chromosomal regions 17p13.3-p13.2 and 19q13.11-q13.43. Interestingly, we identified six not previously described chromosomal aberrations with mosaic gains of 1p36.33-p36.31, 2q37.1-q37.3, 6q27, 12p13.33-p13.31, 20q13.31-q13.33, and mosaic loss of 19p13.3-p12. Using the database “Atlas of Genetics and Cytogenetics in Oncology and Haematology” 42 brain tumor genes were identified within the affected chromosomal regions. These 42 cancer genes are involved in different signal transduction pathways and pathways in relation to cancer. Regarding to the 6 identified chromosomal regions, which have not been previously described for CN, the following 8 brain tumor genes were detected: MIR200A, GPC1, THBS2, FOXM1, PTBP1, VAV1, NTSR1, and PTK6. Pathway analyses of these 8 brain tumor genes suggested possible influences predominantly in focal adhesion and signal transduction pathways.

Conclusions: Although our results provide additional evidence in the genetic characterization for CN, further analyses of an extended cohort are necessary to get more genetic information for this rare tumor identity.

P-CancG-042

Epigenetic and genetic alterations of ITIH5 during cervical carcinogenesis

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Introduction: Progression from human papillomavirus-induced premalignant cervical intraepithelial neoplasia (CIN) to cervical cancer (CxCa) is driven by genetic and epigenetic events.

Previous studies revealed a progressive down-regulation of the gene ITIH5 in the course of cervical carcinogenesis. Promoter methylation in conjunction with deletions in the gene region of ITIH5 seem to contribute to notable ITIH5 downregulation as shown in in vitro studies using cervical cell lines and cryosections of cervical lesions. Aim of the current study is to investigate (epi)genetic alterations in cervical scrapes of CIN of different severity.

Materials and Methods: In order to investigate the genetic status of ITIH5, cervical scrapes of patients with histologically confirmed low grade lesions (CIN1) ($n=10$) and high grade lesions (CIN2–3) ($n=30$) are analysed using a specific probe for ITIH5 and a control probe for centromere 10 for direct interphase fluorescence in situ hybridization (I-FISH).

In parallel, ITIH5 promoter methylation is investigated by qualitative and quantitative methylation specific PCR (qMSP) in the same clinical probes.

Results: In CIN1, dual I-FISH revealed a copy number loss of ITIH5 in two cases and promoter methylation in one out of ten cases. Preliminary results for high grade CIN showed a higher number of cases with copy number loss of ITIH5 and ITIH5 DNA methylation as compared to low grade lesions. Additionally, in some cases of high grade lesions a loss of centromere 10 was detected. Investigations are still ongoing.

Discussion: Results so far indicated that both, promoter methylation and deletion of ITIH5 contribute to its progressive downregulation during cervical carcinogenesis. A combined screening of the methylation pattern and the investigation of the copy number state of ITIH5 may help in the early detection of HPV-induced genetic instabilities as well as genetic modifications and might be utilized as a novel molecular marker for progression.

P-Clinical Genetics Genetic Counselling and Prenatal Diagnosis

P-ClinG-043

Novel VPS33B mutation in a patient with autosomal recessive keratoderma-ichthyosis-deafness (ARKID) syndrome

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Autosomal recessive keratoderma-ichthyosis-deafness (ARKID) syndrome is a rare multisystem disorder caused by biallelic mutations in VPS33B; only three patients have been reported to date. ARKID syndrome is allelic to arthrogryposis-renal dysfunction-cholestasis (ARC) syndrome (MIM #208085), a severe disorder with early lethality whose phenotypic characteristics also include ichthyosis, hearing loss, severe failure to thrive,

platelet dysfunction and osteopenia. We report on an 11-year-old male patient with ARKID syndrome and compound heterozygous VPS33B mutations, one of which [c.1440delG; p.(Arg481Glyfs*11)] was novel. Clinical features of this patient included ichthyosis, palmoplantar keratosis, hearing loss, intellectual disability, unilateral hip dislocation, microcephaly and short stature. He also had copper hepatopathy and exocrine pancreatic insufficiency, features that have not been associated with neither ARKID nor ARC syndrome so far. The patient broadens the clinical and molecular spectrum of ARKID syndrome and contributes to genotype-phenotype associations of this rare disorder.

P-ClinG-044

Mosaic functional disomy Xp in a Turner patient with developmental delay, severe language impairment and seizures

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An infant with a prenatal CVS-karyotyping diagnosis of Turner syndrome, 46,X,i(X)(q10), presented with global developmental delay, seizures, and hypotonia. As the phenotype was not explained by Turner syndrome, SNP array analysis of whole blood cell DNA was performed. Extra material of the complete short arm (Xp) of the X chromosome was detected in a mosaic state. Conventional G-banded chromosome analysis in lymphocytes revealed two types of chromosomal rearrangements: 76% of the cells had the prenatally diagnosed isochromosome Xq, while the remaining 24% of cells showed monosomy of a complete chr. X plus Xp translocated onto chr.13 (Karyotype: mos 46,X,i(X)(q10)[76]/45,X,der(13)t(X;13)(p10;q10)[24]). The results were confirmed by fluorescence in situ hybridization (FISH). Trio exome analysis did not detect any additional pathogenic variants influencing the phenotype.

Turner syndrome patients with typical karyotypes such as 46,X,i(X)(q10) may have subtle impairments of non-verbal cognition but usually do not have severe language impairment. Untypical findings in Turner syndrome call for further genetic investigations (Jones et al. 2018). Indeed, in a subset of patient cells, we observed disomy Xp which can be assumed to be functional since the translocated Xp lacked the X-inactivation centre (XIC) and therefore could not be inactivated. Inactivation of the other X chromosome would have led to negative selection of the respective cells due to the absence of Xq genetic activity.

Only a few comparable cases have been described in the literature so far. The corresponding phenotype, although variable, shows a characteristic pattern of symptoms including developmental delay, cognitive impairment, and seizures. At the age of 5 yrs, our patient also had global developmental delay with severe language impairment and sleep disturbance besides the typical growth deficit of Turner patients. As compared to a published case (Gustashaw et al. 1994) with the same but non-mosaic translocation of Xp to chr.13, the neurological phenotype appeared to be less severe, possibly due to a moderating effect of the mosaic subset of cells with the typical Turner karyotype.

P-ClinG-045

Cleidocranial dysplasia in a female due to a chromosome translocation 46,XX,t(2;6)(p11.2;p21.1)

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Cleidocranial dysplasia (CCD) is a rare autosomal dominant congenital disorder of bone development characterized by persistently open or delayed closure of cranial sutures and wormian bones, hypoplastic and/or aplastic clavicles, wide pubic symphysis, short stature and dental anomalies. *RUNX2* (*CBFA1*) was identified in 1997 as the responsible gene.

RUNX2 is essential for differentiation of osteoblast during both intramembranous and endochondral ossification. As a transcription factor it is involved in bone formation upregulating the expression of bone matrix genes including collagen I among others. Moreover, it is responsible for regulating expression of mesenchymal tissue, controlling differentiation of dental epithelium. Missense mutations in the runt domain of *RUNX2* are most commonly described in patients with classic CCD. Besides point mutations, intragenic microdeletions and microduplications of the genomic region containing *RUNX2* have been reported (Jaruga et al., 2016). In this report we describe a girl with the clinical diagnosis of CCD without a mutation in *RUNX2* but with an apparently balanced translocation t(2;6)(p11.2;p21.1).

The 12-months old girl was referred to us with motor development delay, weakness of the shoulder girdle and wide open sutures. Prior microarray analysis had revealed a *de novo* and apparently balanced translocation 46,XX,t(2;6)(p11.2;p21.1) which had initially not been considered to be the cause of the girl's symptoms. Upon clinical examination CCD was diagnosed according to her typical phenotype but neither *RUNX2* sequencing nor MLPA revealed a disease-causing sequence variant or deletion/duplication, respectively.

There are only four reports of translocations either disrupting the *RUNX2* gene or causing position effects resulting in haploinsufficiency for this gene in the literature. Additional cis-acting regulatory sequences located 200 and 400 kb upstream of exon 1 have been identified that act as enhancers of the *RUNX2* gene (Stock and Otto et al., 2005). After reviewing the literature and the microarray analysis and sequencing data we hypothesize that the translocation of the *RUNX2* gene on chromosome 6 to chromosome 2 in our patient has disrupted a 5'-control element, such as an enhancer, resulting in haploinsufficiency for *RUNX2* and the CCD phenotype.

P-ClinG-046

Limits of Clinical Phenotyping—Two Patients with Substantially Diverse Phenotypes and Rubinstein-Taybi Syndrome Due to Deleterious Mutations in EP300 Gene

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Expanding number of NGS-exome analyses is accompanied by increasing rate of the diagnosed patients, whose clinical symptoms fulfil just a small fragment of cardinal criteria of the given syndrome, but the molecular diagnosis is explicit. An experienced syndromologist or digital programme has in such a case only a little chance to assess the right diagnosis.

The authors refer on two patients with a Rubinstein-Taybi syndrome (RTS) with significantly different phenotypes. The substantial differences applied to nearly all important physical characteristics—the height, weight, head circumference, habitus, facial features and birth defects. Relatively comparable, although not equal, were the birth measurements, early psychomotor development and cognitive abilities. In the first patient, the clinical diagnosis was supposed already before the exome analysis. In the second patient, the diagnosis of RTS was (despite a bit broad halluces and down-slanted palpebral fissures) not considered, due to missing most of the cardinal features of RTS. Only the exome analysis surprisingly resolved the diagnosis by finding a *de novo* 1 bp deletion in Exon 3 of the gene *EP300*. In the first patient, a *de novo* nonsense Mutation in Exon 18 had been identified. None of the two mutations had been described yet.

The exome analysis represents a substantial advantage for the patients with clinically not specific features, mainly when it provides an explicit interpretable result. Nevertheless, the role of a clinical geneticist-syndromologist remains still important, for the need to characterize the patients' phenotype in detail and to score the "phenotypical consistency" with published data, what is of particular importance in not so explicit interpretable DNA variations.

P-ClinG-047

Experience after 2 years of NIPT (Non-Invasive Prenatal Testing)

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From the blood of pregnant women with an increased risk of fetal aneuploidy, the PraenaTest® (Lifecodexx) determines either trisomy 21, 18 and 13 or, in addition to the three trisomies, gonosomal aneuploidies (Turner's, Triple X, Klinefelter's and XYY syndromes) from 9 + 0 p.m. until the 30th week of pregnancy. The PraenaTest® was launched by Lifecodexx in Europe in August 2012 and is performed by massively parallel sequencing (MPS) of cell-free DNA from maternal plasma. Since May 2016, the PraenaTest® is carried out by a physician-led laboratory and under the responsibility of specialists in human genetics (Ärzteverbund praena bayern). The data presented here were collected between May 2016 and October 2018 as part of the laboratory routine for the detection of trisomies 13, 18 and 21 and the gonosomal aneuploidies. Test accuracy, quality parameters and selected case studies are discussed.

In total, 91.3% of analyses showed inconspicuous results (i. e. z-score within the normal range) following the initial analysis of the first of two test tubes. 6.05% of the analyses revealed a z-score in the non-evaluable range (grey-zone) and had to be repeated. In 1.42% of analyses the z-scores were outside of the defined limits indicating a high-risk for chromosomal aneuploidy.

1.21% of the samples did not reach the necessary quality threshold for analysis and had to be left without results.

NIPT results that are discordant with results in diagnostic prenatal tests (CVS or amniocentesis) were most frequently reported for gonosomal aneuploidies and may include statistical/technical false positive/negative, and biological factors such as confined placental mosaicism, vanishing twin pregnancies, maternal mosaicism and maternal malignancy.

In summary, PraenaTest® is a screening test which needs to be offered in the context of genetic counselling. It does not replace the precision achieved with a diagnostic test, and it should not replace the differentiated fetal ultrasound examination of the fetus.

P-ClinG-048

Do X-linked inherited *DKC1* mutations cause cancer susceptibility in women?

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Background: Mutations in the *DKC1* gene have been reported to cause dyskeratosis congenita (DC), a rare disease due to a dysfunctional telomere maintenance. DC is characterized by the triad of dysplastic nails, lacy reticular pigmentation mostly of the neck and upper chest and oral leukoplakia. Additionally, patients show an increased risk of solid tumours as well as diseases of the haematopoietic system and pulmonary fibrosis. As *DKC1* mutations are inherited in a X-linked recessive mode, males are known to be the affected sex. But female carriers have already been described to suffer from mucocutaneous features likely based on a skewed X-inactivation. We now discovered indications of cancer susceptibility in female carriers too.

Clinical case: Our index patient is a 47-year old male diagnosed with DC and suffering from nail dysplasia along with lacy reticular pigmentation since his teens. He was additionally diagnosed with stomach cancer at the age of 47. Molecular genetic testing via PCR followed by sanger sequencing showed a mutation in the *DKC1* gene (NM_001363: c.1050G>C, p.Met350Ile). This mutation has already been identified as a pathogenetic

variant on the protein level leading to DC. The proband's mother died at the age of 64. She was diagnosed with kidney cancer at the age of 54 and with lung cancer at 64 although she never smoked. Her 79-year old sister showed very dry and scaly skin all over the body. Her son, suffering also from DC, deceased at the age of 47. He presented the classical triad of DC and first symptoms were recognised in his teens. At the age of 44 he moreover suffered from stomach and oesophageal cancer. Another aunt died aged 67 and was diagnosed with kidney cancer at the age of 52 and with pancreatic cancer one year before she deceased. Her 51-year old daughter also carries the *DKC1* mutation and shows no signs of DC or neoplasia at this stage.

Discussion: DC causes cancer susceptibility as seen in our two male patients. Surprisingly also the female carriers developed several solid malignant tumours shortly after the age of 50. It appears that female carriers of a *DKC1* mutation are also exposed to cancer susceptibility. Therefore, female carriers might profit from intense cancer screening.

P-ClinG-049

Cells, SNPs, and babies–4 years of successful PGT in Freiburg.

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Preimplantation genetic testing (PGT) in Germany is ruled by the Preimplantation Genetic Testing Regulation which is in force since February 2014. In the federal state of Baden-Württemberg, PGT is legal since July 2015. Here, we summarise and analyse the work of the PGT center Freiburg since its opening in July 2015. The centre offers PGT by STR analysis, SNP array, and NGS. Our review of the performance of the PGT center Freiburg is based on 51 couples having obtained approval for PGT by the PGT ethics committee. 37 couples requested PGT because of monogenic diseases, 14 PGTs were requested because of chromosomal structural rearrangements or recurrent miscarriage. To date, reproductive outcomes could be followed up for 43 couples. 84 embryos were transferred in total, resulting in 32 pregnancies (implantation rate: 38%) and 30 births to 28 couples (live birth rate: 35%).

P-ClinG-050

Late-onset MTHFR deficiency: a treatable diagnosis revealed through next-generation sequencing analysis for complicated spastic paraplegia

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Severe methylenetetrahydrofolate reductase deficiency usually presents with developmental delay, seizures and additional neurological symptoms in early childhood. However, rare late-onset forms with manifestation in adolescence or early adulthood have been described. We present here a 31-year-old female patient from a consanguineous family who developed rapidly progressive spastic gait, complete loss of vision, severe cognitive deficits and epileptic seizures during several months, starting from age 30. Brain MRI showed confluent periventricular and subcortical hyperintensities, probably corresponding to diffuse demyelination. Clinically, a complex form of hereditary spastic paraplegia was suspected and a panel analysis based on exome sequencing was performed. While no disease-causing mutation in a known SPG gene was discovered, the patient carried the mutation c.973C>T, p.(Arg325Cys) in the *MTHFR* gene in homozygous state, suggesting late-onset MTHFR deficiency as causative for her symptoms. Subsequent comprehensive clinical and neurological analyses confirmed this diagnosis; especially homocysteine blood levels were highly elevated (256.1 µmol/l; <9 µmol/l). High dosage betan combined with folic acid,

vitamin B6, B12 and methionine has been started and resulted in rapid and significant clinical improvement in parallel to a decrease of homocysteine levels to 80.7 µmol/l.

The parents of our patient were confirmed as heterozygote carriers of the *MTHFR* mutation. Since two brothers additionally showed mild psychomotor retardation and epilepsy starting from early childhood, further segregation analyses were performed.

Our results confirm late-onset *MTHFR* deficiency as an important treatable differential diagnosis for complex SPG and suggest that homocysteine screening should be considered as a relevant diagnostic test for these patients.

P-ClinG-051

An intronic *COH1* mutation generates a cryptic splice site and causes Cohen syndrome in a large Jordanian family

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Cohen syndrome is a rare recessive disorder characterized by microcephaly, facial abnormalities, truncal obesity, myopia and pigmentary retinopathy. More than 100 different loss of function mutations in *COH1* have been described in literature so far. Interestingly, a considerably large number of these patients have only one molecularly confirmed mutation. Here we present an 11-year-old Jordanian patient clinically diagnosed with Cohen syndrome. Additionally, three maternal cousins also meet the clinical characteristics of this condition.

To decipher the molecular cause of the condition we performed Array-CGH and found a paternally inherited deletion of about 200 kb, affecting the *COH1* exons 18–23. Unfortunately, the second mutation was not detectable for many years. We performed Sanger sequencing and found an intronic alteration 23 base pairs upstream of exon 24 (*COH1*: c.3446-23T>G; NM_017890.4), which was predicted to form a cryptic acceptor splice site. To further investigate the consequence of this variant, expression and splicing studies were carried out on RNA from patients' skin fibroblast in comparison to controls. Quantitative RT-PCR showed a significant decrease of *COH1* mRNA expression. Furthermore, direct sequencing of cDNA confirmed that splicing occurs at the upstream cryptic splice acceptor site, resulting in an inclusion of 22 intronic bases and thereby to a frameshift causing a premature termination codon (p.Gly1149Valfs*9). Segregation analysis revealed that the mother was a heterozygous carrier and the three affected maternal cousins were homozygous for the intronic splice site mutation.

We identified the molecular cause of Cohen syndrome in a large Jordanian family. The index patient carries a copy number alteration and a deep intronic splice site mutation in *COH1*. Our data show causality of both alterations and strongly suggest the expansion of the classical diagnostic strategy to copy number alterations and deep intronic splice site mutations in molecularly unconfirmed patients affected by Cohen syndrome.

P-ClinG-052

NFIX-associated overgrowth: two new patients with Malan syndrome

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Malan syndrome (also called Sotos syndrome 2) is a rare condition characterised by overgrowth, a recognisable facial appearance, intellectual disability, and unusual behavior characterised especially by anxiety. Malan syndrome is caused by heterozygous mutations in the *NFIX*-gene, mostly located in the 5' part of the gene with a cluster in exon 2. However, muta-

tions further downstream have scarcely been described. Up to now about 80 patients with Malan syndrome have been reported. Both our patients present with overgrowth and the typical facial gestalt comprising a long triangular face, a broad forehead, downslanting palpebral fissures, a thin nasal bridge, and a small chin. While most mutations published so far are located in exon 2, our first patient carries an intronic splice mutation in intron 4 of *NFIX* (c.721 + 1G>A). The second patient carries a stop-mutation located in exon 2 of *NFIX* (c.333C>A p.(Cys111*)). We will present genetic and detailed clinical data of two new patients with Malan syndrome and thus further delineate the phenotypic and genotypic spectrum of Malan syndrome.

P-ClinG-053

Our experience with whole exome next-generation sequencing

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Introduction: Whole exome sequencing (WES) with NGS (next-generation-sequencing) has opened up new possibilities for the search for disease-causing mutations in disorders with common clinical features but a heterogeneous genetic background. The identification of the underlying genetic defect provides a clear diagnosis for patients influencing their management and it might be the prerequisite for prenatal or preimplantation decisions in the affected family. However, many new challenges have been introduced, with consequences including new requirements for interpretation of results. We report on our experience with whole exome sequencing of 280 patients in clinical diagnostics.

Patients and methods: 280 patients (age 0–81 years; mean 21.5) with intellectual disability (syndromic: 55 (ID s); non-syndromic: 48 (ID ns)), neuromuscular symptoms (NM) (42), epilepsy (E) (13), disorder of the eyes (EYE) (12), metabolic disorders (MD) (11), cancer (C) (11) and other diseases (O) (88) were analyzed with WES. For exome enrichment the SureSelect Human All Exome V6 or V7 kit from Agilent was used. In 120 of these patients (42%) chromosomal rearrangements and copy number variations had been excluded by high resolution SNP array analysis. Sequencing was performed on an Illumina NextSeq500 system (2 × 126bp PE). Raw reads were mapped to the reference genome (hg19) using BWA-MEM and variant discovery was performed according to GATKs best practice guidelines. The resulting VCF files were annotated with the ENSEMBL VEP. All detected variants were checked against dbSNP147-150, ClinVar, the 1000 Genomes Project, the gnomAD (exomes and genomes) population database, the OMIM catalog, the professional version of the HGMD, and other sources in a self-implemented user interface. For exclusion of technical artifacts and segregation testing of all likely disease-causing variants, PCR and Sanger sequencing were performed according to standard protocols.

Results: With an overall detection rate of 34.3% (ID s: 38.2%; ID ns: 20.8%; NM: 40.5%; E: 38.5%; EYE: 41.7%; MD: 27.3%; C: 18.2%; O: 37.5%) WES is an important tool in clinical diagnostics of monogenetic diseases in patients with excluded CNVs. We identified 37 pathologic (class 5) mutations in a cohort of 280 patients (ID s: 6/55; ID ns: 4/48; NM: 5/42; E: 3/13 EYE: 4/12; MD: 1/11; C: 2/11; O: 12/88). Class 4 mutations in genes likely associated with the patients phenotype have been identified in 59/280 patients (ID s: 15/55; ID ns: 6/48; NM: 12/42; E: 2/13 EYE: 1/12; MD: 2/11; C: 0/11; O: 21/88). Further 33 variants were classified as variants of unknown significance (class 3) because of up to now lacking evidence of pathogenicity. These results show the usefulness of WES. Especially in patients with childhood intellectual disability, it can offer a possibility for risk prediction and prenatal and preimplantation choices for the family.

P-ClinG-054

A diabetes insipidus family in which affected family members present two genetically different forms: central and nephrogenic

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Diabetes insipidus is categorized into two groups—central and nephrogenic; each can have congenital and acquired causes. For the congenital forms, the associations between genes and phenotypes have been well established based on previously reported patients: pathogenic variants in the AVP gene (arginine vasopressin, OMIM *192340) are known to cause central diabetes insipidus in autosomal dominant manner. Approximately 90% of nephrogenic diabetes insipidus patients are males with the X-linked recessive form, which is caused by a defect in the vasopressin V2 receptor (encoded by AVPR2 gene, OMIM *300538) in renal collecting duct cells. Remaining 10% of patients are caused by pathogenic variants in the AQP2 gene (aquaporin-2 water channel, OMIM *107777) in autosomal recessive or dominant manner. Central and nephrogenic diabetes insipidus are two distinct disorders associated with totally different genes. If more than one family member is affected, normally only one type of diabetes insipidus is presented, and subsequently the disease-causing variant can be detected in the corresponding gene. Here, we report on a diabetes insipidus family with eight affected members, including father and daughter counselled by us. All family members, except the daughter, were affected with central diabetes insipidus, responded very well to treatment with vasopressin (antidiuretic hormone, ADH). In the father, a heterozygous pathogenic AVP variant c.232_234del (p.Glu78del) was identified and thereby confirmed the clinical diagnosis. In contrast, the daughter presented nephrogenic diabetes insipidus, which did not respond to ADH treatment. Exome sequencing revealed the presence of one heterozygous variant, c.962A>T, p.Asn321Ile in the AVPR2 gene, and the absence of the AVP variant. We also observed an abnormal X-Inactivation pattern in the daughter, indicating an abnormal skewing of the X-chromosomes. A second mutation of the AVPR2 gene was not found (results of deletion/duplication analysis pending). Findings of the AVP pathogenic variant confirmed the central diabetes insipidus in the father as well as in other family members. For the daughter, based on her clinical phenotype and absence of the AVP variant, we hypothesize that the AVPR2 variant is very likely disease-causing. This report illustrates the presence of two clinically similar but genetically different forms of diabetes insipidus, central and nephrogenic, in a single family, and emphasizes the importance of clinical phenotypes in interpretation of genetic data.

P-ClinG-055

Novel autosomal recessive loss-of-function variants in CDC14A are associated with sensorineural hearing loss in Iranian and Pakistani patients

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Hearing loss (HL) is a highly heterogeneous disease and is one of the most prevalent sensory disorders in humans. About one in 500–1000 children

is affected at birth. To date, approximately 100 genes have been identified which are responsible for non-syndromic hearing loss. The aim of the current study was to identify likely pathogenic variants in known and novel genes associated with HL in selected Iranian and Pakistani families by exome sequencing and bioinformatics analysis. Analysis of an Iranian and Pakistani proband with consanguineous parents revealed two novel homozygous likely pathogenic loss-of-function variants in the gene *CDC14A* (DFNB32/105) which encodes the Cell Division Cycle 14A protein and is associated with autosomal recessive HL. The encoded CDC14A protein localizes to the kinocilia of inner hair cells, basal bodies and to sound-transducing stereocilia. Since its discovery as a HL gene in 2016, only nine variants responsible for hearing impairment have been reported so far. Furthermore, five of these variants in *CDC14A* have been discovered causing male infertility accompanied by a hearing loss phenotype. We identified a homozygous c.1041dup (NM_033312.2, p.Ser348Glnfs*2) in a 13.6 Mb homozygous interval on Chr1 in two affected patients from a Pakistani family which is responsible for causing a frameshift resulting in the generation of a premature stop codon. Both affected patients suffer from congenital, bilateral, stable, severe-to-profound hearing loss accompanied by compound myopic astigmatism and glaucoma. The second identified homozygous splice mutation c.1421+2T>C (NM_033312.2) in two affected patients from an Iranian family is thought to be responsible for the loss of a donor splice site in exon 14 based on in-silico prediction tools. The affected patients suffer from congenital, sensorineural, bilateral, non-progressive, profound hearing loss. Both the splice- and the frameshift-mutations likely result in an altered reading frame leading to a potentially truncated or modified defective protein. All affected individuals in our families are female. Our findings expand the spectrum of clinically relevant mutations related to sensorineural hearing loss in the gene *CDC14A* and reinforce its importance in the auditory process.

P-ClinG-056

Hereditary neuropathies and differential diagnoses: Experiences from more than 400 cases of next-generation sequencing based genetic panel diagnostics

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Peripheral neuropathies or polyneuropathies (PNP) are a heterogeneous group of disorders of the peripheral motor, sensory and autonomic nerves with an estimated prevalence of 5–8 per cent in the general population and a higher prevalence among the elderly. PNPs can have a multitude of etiologies and present with overlapping phenotypes; hereditary forms include Charcot-Marie-Tooth disease/hereditary motor sensory neuropathy (CMT/HMSN), hereditary motor sensory and autonomic neuropathies (HS(A)N), hereditary motor neuropathies (HMN) and small fiber neuropathies (SFN). Mutations in far more than 100 different genes have been described so far and more are yet to be discovered; hereditary neuropathies may follow all different patterns of inheritance. In some instances, mutations in neuropathy-associated genes may also be responsible for other clinical entities such as spastic paraplegia (HSP), spinal muscular atrophy (SMA), muscular dystrophy or myopathy. Using next generation sequencing (NGS), diagnostics has become easier, more straightforward and less time-consuming. Although in the majority of cases no causal treatment is yet available, knowing the cause of the disorder provides relief for patients and it allows conclusions regarding possible risks for family members. Learning about the underlying pathology might also provide options for treatment in the near future. Based on our experiences from more than 400 cases of genetic panel diagnostics, we suggest an algorithm for molecular diagnostic work-up and present a summary of the results. Approx-

mately 15 per cent of all cases analysed by NGS showed a clear pathogenic variant; in approximately 20 per cent of patients one or more variants of unknown significance (class III) were detected which prompted us to ask for further clinical (and/or molecular) investigations, segregation analyses or functional analyses. Detection rates varied considerably among the various subgroups with higher rates in the more defined entities. Some unexpected results will be discussed.

P-ClinG-057

Pseudohypoparathyroidism and related disorders: first international consensus statement on diagnosis and management

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Pseudohypoparathyroidism (PHP) and related disorders refer to heterogeneous diseases characterized by physical findings, differently associated in each subtype. The group includes short bones, short stature, a stocky build, subcutaneous ectopic ossifications, hypocalcemia, hyperphosphatemia, and elevated PTH and TSH levels. PHP and related disorders vary with regards to presentation and severity between affected individuals with considerable clinical and molecular overlap. The diagnosis is often delayed due to lack of awareness. The first international consensus statement published recently (Nat Rev Endocrinol. 2018 Aug;14(8):476–500. <https://doi.org/10.1038/s41574-018-0042-0>) will be presented. It covers recommendations for both diagnosis and management of patients affected with PHP and related disorders. Clinical diagnosis is based on major criteria including resistance to the parathyroid hormone, subcutaneous ossifications, brachydactyly and early onset obesity. This diagnosis should be confirmed by a molecular analysis. The management requires a multidisciplinary team including a transition program. Specific features should be screened and carefully managed such as parathyroid hormone resistance, TSH resistance, growth, gonadal function, bone mineralization, brachydactyly, oral health, weight gain, lipid profile and hypertension. Subcutaneous ossifications and neurocognitive impairment represent heavy burdens for the patients, and have been addressed by experts despite the lack of clinical trials. Overall, a coordinated approach from infancy through adulthood should help us to improve the care for these disorders. After first international consensus statements on Silver-Russell and Beckwith-Wiedemann syndromes, the PHP guideline represent the internationally consented recommendations and suggestions for the third imprinting disorders and will significantly contribute to the diagnosis and management of these congenital entities.

P-ClinG-058

Is there an unusual inheritance in epilepsy?

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Epileptic seizures are neurological disorders characterized by variable clinical presentation and a broad range of genetic causes bringing the molecular diagnostic into a special challenge. There are now hundreds of genes identified to be associated with epilepsy; therefore, the high throughput sequencing technologies becomes essential in these diagnostics. Usually monogenic inheritance is described with exception of febrile seizures and photosensitive epilepsy as well.

For some years, we have already been using the next generation sequencing technique based on targeted panels covering more than 300 genes. Therefore, the required orders comprise different epilepsy forms.

Interestingly, 44% of all patients had one or more variants reported. However, only 10% had a distinct epilepsy causing mutation. Whereas 34% of patients carried a variant of unknown significance (VUS) including 12% of people with more than one variant. Looking in detail, the similar statistic yielded for patients with epileptic encephalopathy: 48%, 12%, 36% and 12% had one or more variants, had a disease causing mutation, and had VUS and patients with more than one variant, respectively. About 42% of patients with idiopathic epilepsy carried variants, at which 9% had a disease causing mutation and 33% had VUS comprising 13% of patients had again more than one variant. There are up to six variants in one person. Indeed no groups of genes, e.g. encoding specific ion channels, could not be determined.

These proportions become different, if the clinical symptoms are highly specific for an epileptic disease. For example, 57% of patients with a Rett like pathology had a variant, whereas 29% of people had a disease causing mutation as well as a VUS. Certainly, no patient had more than one VUS. Latter was also seen in patients with Rolando epilepsy, generalized epilepsy with febrile seizures plus, nocturnal frontal lobe epilepsy and nuclear gene-encoded Leigh syndrome.

In conclusion, the more complex the seizures or rather nonspecific clinical symptoms occur, the more complex are the identified genetic variants as seen in patients with idiopathic epilepsy and epileptic encephalopathy. This raises the question of a digenic or polygenic inheritance of these entities.

P-ClinG-059

Two novel cases affected by autosomal recessive or dominant cutis laxa type 3

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Mutations in *ALDH18A1*, encoding the mitochondrial enzyme pyruvate-5-carboxylate synthase (P5CS), lead to a broad spectrum of rare genetic conditions. Besides hereditary spastic paraplegia, *ALDH18A1* mutations are causative for autosomal recessive (ARCL3) and dominant (ADCL3) cutis laxa with progeroid features. These patients show lax, thin and translucent skin, a variable degree of intellectual disability, a typical facial gestalt and lipoatrophy leading to an overall progeroid appearance. Here we report on two affected individuals from Italy and Sri Lanka, respectively, meeting the main clinical criteria for ARCL3/ADCL3. In the Italian patient we identified the *ALDH18A1* (NM_002860) mutation c.1273C>T; p.(Arg425Cys) in a homozygous state. This mutation has been described previously in a case with classical features of ARCL3. Interestingly, in the case from Sri Lanka, we found the heterozygous alteration c.343G>A; p.(Val115Met). This position is highly conserved during evolution, not present in the ExAc/1000 Genomes data and has occurred *de novo* in the affected individual. To investigate the consequence of the mutation p.(Arg425Cys) in vitro, we performed immunofluorescence and immunoblot analyses in patient derived fibroblasts. The mutation has no impact on protein stability and localization implying an enzymatic dysfunction. In summary, we identified two novel cases affected by *ALDH18A1*-related cutis laxa and found the third residue affected by a *de novo* mutation causative for ADCL3. Further research is pending to decipher the consequence of numerous mutations affecting P5CS providing novel insights into the pathophysiology of this complex disease spectrum.

P-ClinG-060

Father-to-son transmission of Silver-Russell-phenotype due to a heterozygous HMGA2 loss of function deletion

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Silver-Russell syndrome (SRS) is a clinically and molecularly heterogeneous growth disorder mainly characterized by severe intrauterine and postnatal growth retardation. The clinical diagnosis can be confirmed molecularly in about 60% (loss of methylation of the H19/IGF2:IG-DMR on 11p15.5 (50%); maternal uniparental disomy of chromosome 7 (5–10%)). In addition to these two major molecular alterations, numerous structural chromosomal variants and rare monogenetic defects (e.g. IGF2, CDKN1C mutations) have been identified in patients with the clinical diagnosis of SRS according to the Netchine-Harbison clinical scoring system (NH-CSS). First mutations and exon deletions of the high-mobility-group-A-2 gene (HMGA2) have been described in 6 patients. For four patients clinical data were available and all of them fulfilled a positive NH-CSS for SRS. HMGA2 acts as an architectural transcription factor and has been shown to regulate the expression of the fetal growth factor IGF2. Here we report on a father with the clinical diagnosis of SRS who asked for molecular confirmation of his short-stature syndrome after birth of his similarly affected son. Whereas the typical molecular SRS alterations were excluded, the diagnostic workup showed a deletion of exons 1–3 of the HMGA2 gene in both affected individuals. This familial case report underlines the need for further investigations in patients fulfilling the NH-CSS criteria but lacking a molecular confirmation of their SRS diagnosis. In contrast to the majority of the classical (imprinting) causes of SRS HMGA2 mutations bear a 50% risk for transmission to offspring which should be considered in context of family planning of SRS patients. Thus in patients without signs of body asymmetry and negative SRS molecular genetic test results, investigation of HMGA2 should be included in the diagnostic workup.

P-ClinG-061

A new patient with compound heterozygous splicing variants in KIAA1109 refines the clinical picture and broadens the mutational spectrum of Alkuraya-Kucinkas syndrome (ALKKUCS)

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Biallelic loss of function (LoF) and missense mutations in KIAA1109 (OMIM *611565) encoding a evolutionary conserved protein with yet poorly understood function are described as causative of the recently characterised Alkuraya-Kucinkas Syndrome (ALKKUCS, OMIM # 617822). Patients with this rare autosomal recessive and often lethal disorder generally share a clinical picture of profound developmental delay, infantile epilepsy, ventriculomegaly, cerebellar hypoplasia, cerebral parenchymal rarefaction and arthrogryposis. So far only 13 patients have been described in a single report with a spectrum of severity ranging from death in utero or soon after birth in association with LoF mutations to several neurodevelopmental disease and brain anomalies in individuals with missense variants compatible with life. Using exome sequencing we identified a boy born to healthy non-consanguineous German parents with severe neurodevelopmental delay, hypotonia, Dandy-Walker malformation, hydrocephalus, cerebral atrophy, epilepsy, respiratory insufficiency, hypothyroidism and dysmorphic facial features but no arthrogryposis and KIAA1109 compound heterozygosity for a nonsense and a splice variant inducing skipping of an exon. Through comparison with the hitherto described patients and characterisation of the splicing mutations in this pa-

tient we expand and refine the phenotypic and mutational spectrum of ALKKUCS.

P-ClinG-062

Pan-European Network EnGagE–Enhancing Psychiatric Genetic Counselling, Testing, and Training in Europe

EnGagE ...

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Psychiatric disorders such as schizophrenia and bipolar disorder are common and highly heritable. Despite this high heritability it has taken many years to begin to identify underlying genetic risk factors. However, in the last decade major advances in psychiatric genetics have been driven by both technological advances (e.g. cost effective array-based genome-wide genotyping and next-generation sequencing technologies) and the establishment of large-scale, international consortia.

Of particular relevance has been the identification of copy number variants (CNVs), such as 22q11.2 deletions, that confer substantial risk for psychiatric disorders. Findings such as these have sparked an interest in implementing genetic testing for patients with these disorders in routine clinical care. Psychiatric Genetic Testing (PsyGT) is a newly emerging and interdisciplinary field.

Within the recently European Union funded Network EnGagE, preclinical and clinical researchers from the fields of neuroscience, psychiatric genetics, psychosocial research, and ethics; clinicians from the fields of psychiatry, psychology, and medical genetics; genetic counsellors, scientists from diagnostic genetic testing laboratories and patient representatives have assembled to facilitate pan-European research relevant to PsyGT and to help pave the way for its clinical implementation. This unique, Network (currently members from 27 countries) has been set up in September 2018 and will address: (i) setting up an infrastructure in multiple European countries to provide genetic counselling and testing to patients with a psychiatric disorder and their family members; (ii) establishing a list of genes/CNVs for which it is scientifically/clinically appropriate to offer genetic testing; (iii) defining patient inclusion criteria for e.g. CNV testing; (iv) discussing the potential challenges of PsyGT (e.g. informed consent; reporting back incidental findings to a patient with a psychiatric disorder); (v) promoting research into the potential positive and negative effects of PsyGT for patients and their family members; (vi) increasing the “psychiatric genomic literacy” among all relevant stakeholders as genetic counsellors and psychiatrists report that they feel ill-equipped to educate patients and their family members about the genetics of psychiatric disorders.

P-ClinG-063

Uniparental isodisomy as a cause of recessive Mendelian disease: A diagnostic pitfall with a quick and easy solution in medium/large NGS analyses

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Complete uniparental isodisomy (iUPD)—the presence of two identical chromosomes in an individual that originate from only a single parental homologue—is an underestimated cause of recessive Mendelian disease in humans. Correctly identifying iUPD in an index patient is of enormous consequence to correctly counselling the family/couple, as the recurrence risk for siblings is reduced from 25% to usually <1%. In medium/large-scale NGS analyses, complete iUPD can be rapidly and straightforwardly inferred from a singleton dataset (index patient only) through a simple chromosome- and genotype-filtering step in less than one minute. The opportunities of iUPD-detection in medium/large-scale NGS analyses are presented by example of a case of fetal CHRNA2-associated multiple

pterygium syndrome due to complete maternal iUPD. Using computer simulations for several detection thresholds, we validate and estimate sensitivity, specificity, positive (PPV) and negative predictive values (NPV) of our proposed screening method for reliable detection of complete iUPD in NGS variant call data (.vcf file). When screening for complete iUPD, our models suggest that a >85% proportion of homozygous calls on a single chromosome with ≥ 30 sufficiently interspaced called variants results in a sensitivity of 97.9% and specificity of 99.7%. The PPV is 95.1%, the NPV 99.9%. When this threshold is exceeded for a chromosome on which a patient harbours an apparently homozygous disease-associated variant, it should be sufficient cause to discuss iUPD as a plausible or probable pathomechanism in the genetic analysis report, even when parental segregation has not (yet) been performed.

P-ClinG-064

Breast Cancer Risk in BRCA1/2 Mutation Carriers and High-Risk Non-carriers: A Prospective Cohort Study

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The German Consortium for Hereditary Breast and Ovarian Cancer (GC-HBOC) offers an intensified multimodality breast cancer surveillance program to women with proven pathogenic BRCA1/2 mutations and women from families tested negative for BRCA1/2 mutations who are at elevated breast cancer risk based on a risk prediction model. In the present study we characterized FBC and CBC risks in BRCA1 and BRCA2 mutation carriers and, more importantly, in the large group of women from families in which no pathogenic BRCA1/2 mutation could be identified. The two cohorts were prospectively followed up for incident FBC ($n = 4,380$; 16,398 person-years) and CBC ($n = 2,993$; 10,090 person-years), respectively, within the framework of a structured breast imaging. Absolute cumulative FBC risks at age 60 were 61.8% (95%CI 52.8–70.9%) for BRCA1 mutation carriers, 43.2% (95%CI 32.1–56.3%) for BRCA2 mutation carriers, and 15.7% (95%CI 11.9–20.4%) for high-risk non-carriers. FBC risk was significantly higher in all three risk groups compared to the general population. Cumulative CBC risk 10 years after first breast cancer was 25.1% (95%CI 19.6–31.9%) for BRCA1 mutation carriers, 6.6% (95%CI 3.4–12.5%) for BRCA2 mutation carriers, and 3.6% (95%CI 2.2–5.7%) for non-carriers. FBC risk in high-risk women from BRCA1/2-negative families was considerably lower than in BRCA1/2 mutation carriers, but elevated compared to the general population. CBC risk in high-risk women from BRCA1/2-negative families was similar compared to the general population. Our results provide the basis for risk-adjusted early detection programs.

P-ClinG-065

Identification of compound heterozygous AP4E1 mutations causing rare spastic paraplegia-51

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A biallelic disruption of AP4E1 (Adaptor-related Protein Complex 4, Epsilon-1 Subunit; OMIM 607244) is associated with autosomal recessive spastic paraplegia-51 (SPG51; OMIM 613744). To the best of our knowledge, only four affected families have been reported so far. SPG51 is characterized by severe intellectual disability with poor or absent speech development, microcephaly, seizures and neonatal hypotonia that progresses to hypertonia and spasticity. All variants found in these four families are homozygous mutations leading to a truncated protein (one frameshift, one nonsense, one splice site mutation and a partial gene deletion).

Here, we report the first case of SPG51 with European descent and the fifth family worldwide with SPG51. By whole-exome analysis we detected two AP4E1 mutations in a compound heterozygous state in this patient. He inherited a nonsense mutation (NM_007347.4: c.992C>A) in exon 9 from his mother and a frameshift mutation (NM_007347.4: c.556dup) in exon 6 from the father, resulting in a premature stop codon in both copies of the gene [p.(Ser331Ter) and p.(Arg186LysfsTer16)]. He is the child of healthy non-consanguineous parents from Germany. At the age of 2 years 7 months he presented with severe intellectual disability, poor speech development, and spasticity of the extremities; signs consistent with the reported phenotype of patients with SPG51. Due to its rarity, our findings may help to specify and expand the genotypic and phenotypic spectrum of SPG51.

P-ClinG-066

Is there a locus for Muscular Dystrophy Congenital Type 1 B (MDC1B; OMIM 604801) on chromosome 1q42?

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Congenital muscular dystrophies are a genetically as well as clinically highly heterogeneous group of disorders. Genomewide linkage analysis and sequencing of the underlying genes is a powerful tool to identify new disease loci and subsequently novel disease genes. In 1998, Muntoni et al. described a form of congenital muscular dystrophy with proximal muscle weakness, muscle hypertrophy and early respiratory failure in a consanguineous family from the United Arab Emirates (Muntoni et al. *Eur. J. Paediat. Neurol.* 1:19–26, 1998). Subsequent genetic analysis suggested a maximum two-point LOD Score of 2.85 for marker D1S213 in 1q42; in addition the data from another family of German descent with similar clinical and pathological features allowed to achieve a higher level of significance, with a cumulative LOD Score of 3.57 (Brockington et al. *Am. J. Hum. Genet.* 66:428–435, 2000). Since 2000, this locus is known as MDC1B in the OMIM database, the affected gene however remains unknown. Next Generation Sequencing-based panel analysis was initiated after follow-up genetic counselling of the German family in our clinic. Intriguingly, pathogenic sequence variants in the Muscular Dystrophy-Dystroglycanopathy Type C5 (MDDGC5) -causing gene *FKRP* on 19q13.3 were identified as the underlying cause of disease in this German family. While additional genetic studies in the consanguineous family from UAE, did not uncover pathogenic or likely pathogenic variants our results question the existence of the MDC1B locus on 1q42.

P-ClinG-067

Prenatal diagnosis of TSC2/PKD1 contiguous gene syndrome in a fetus with a cardiac rhabdomyoma

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We present a case of prenatal diagnosis of TSC2/PKD1 contiguous gene syndrome (TPCG-syndrome) in a fetus with a cardiac rhabdomyoma (RM).

The woman was referred to us in the 21st week of her first pregnancy because of an external diagnosis of a tumor in the cardiac septum. Detailed ultrasound examination showed an echogenic tumor in the right ventricle (DD: RM) with a size of 1.5 cm.

Because of this ultrasound finding of a potential RM, tuberous sclerosis (TS) was a possible differential diagnosis. Amniocentesis was performed and karyotyping, Array-CGH analysis and molecular testing of the TSC1 and TSC2 gene were initiated.

Karyotyping with FISH showed a terminal deletion of chromosome 16 (karyotype: 46,XX,ish del(16)(p13.3)(16pTEL05-,16qTEL013+)dn). The Array-CGH analysis demonstrated a complex rearrangement of chromosome 16 in the region p13.3. It consists of a deletion of approximately 2.17 Mb (arr [GRCh37] 16p13.3 (96766_2174541) x1), followed by an interstitial duplication of approximately 1 Mb (arr [GRCh37] 16p13.3 (2188712_3111327) x3) of the short arm of chromosome 16.

Haploinsufficiency of TSC2 and PKD1 genes, located within the deleted region on chromosome 16p13.3, has been implicated in the pathophysiology of TPCG-syndrome. Affected patients show phenotypic abnormalities of TS (multiple hamartomas or benign tumors, possible mental retardation) and autosomal dominant polycystic kidney disease (ADPKD). Whether the identified duplication contributes to the phenotype is unclear. The duplicated region contains three disease-associated genes (ABCA3, TBC1D24 and THOC6). The associated diseases usually follow an autosomal recessive inheritance, so that in the presented case an additional phenotype due to the duplication appears unlikely.

The pregnancy was terminated in the 24th week and pathological examination of the fetus confirmed a 1.5 cm tumor in the right ventricle.

In summary the clinical findings and the genetic detection of a terminal deletion of chromosome 16 confirm with high probability the diagnosis of a TPCG-syndrome for the fetus. This case exemplifies that chromosomal aberrations can be the cause of a TS like phenotype and should be kept in mind in cases of prenatal suspicion of TS.

P-ClinG-068

Whole exome sequencing reveals digenic cause of dilated cardiomyopathy

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Introduction: A 14 years old girl was admitted to the pediatric intensive care unit (PICU) from an external hospital. The day before she had been found unconscious and was resuscitated by her family for about ten minutes before the ambulance arrived. Because of ventricular fibrillation the emergency physician performed defibrillation three times. Circulation could be restored under high dosage catecholamine therapy. Medical history revealed that she had been diagnosed with dilated cardiomyopathy with prolapse of the mitral valve at the age of nine and had been under surveillance since then. Because of this and a marfanoid habitus Marfan syndrome had been suspected but had never been tested genetically. The last cardiologic examination four weeks before the incident had shown a reduced left ventricular ejection fraction. Echocardiography at admission to the PICU showed left ventricular dilation and highly compromised ejection fraction. Electrocardiography showed arrhythmias with prolongation of the QT-interval under therapy with amiodarone. Myocardial biopsy revealed evidence of a preceeding myocarditis with persistence of Parvovirus-B19. Her status improved under intensive care therapy and she was discharged from hospital about three weeks later. Neurologic status at discharge showed a slight impairment in short-term memory. Before discharge an internal cardiac defibrillator (ICD) was implanted.

Methods and results: Whole exome sequencing (SureSelectXT Human All Exon v6) was performed and proprietary protocols were used to call, filter and classify variants. Phenotype based variant prioritization showed a heterozygous pathogenic variant in *FBN1*, however, it also revealed a heterozygous pathogenic variant in *RBM20*.

Discussion: The *FBN1* mutation confirmed the suspicion of an underlying fibrillinopathy but the clinical presentation in this patient appeared significantly more severe and rather unusual. This could be attributed to a preceeding myocarditis, however, the incident could also be explained by an

arrhythmic event. *RBM20*-associated cardiomyopathies carry an increased risk for arrhythmias in comparison to other hereditary cardiomyopathies. This should be considered by supervising clinicians.

Conclusion: Whole exome sequencing in this case could not only confirm the primary suspicion of an underlying fibrillinopathy but also revealed a second genetic cause of disease that goes along with an increased arrhythmia risk. This information is important for prognostic evaluations as it enables a better risk assessment for the developing of arrhythmias and cardiomyopathy for the patient and genetic testing of relatives. A single gene testing approach (*FBN1* testing) would have confirmed the diagnosis but would have missed this valuable information for the patient's prognosis and for her family. Thus, whole exome sequencing should be considered especially in cases with uncommon presentation of severity of disease.

P-ClinG-069

Clinical diagnosis of PCSK1 deficiency in a child with a homozygous deletion of PCSK1 exons 1–9

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Background: Homozygous and compound heterozygous variants in the proprotein convertase subtilisin/kexin type 1 (*PCSK1*) gene have been established as rare cause of monogenic childhood obesity linked with severe hormone disturbances (ORPHA:71528).

Results: Here, we report on a 5-year-old boy who presented with malabsorptive diarrhea and the need for parenteral nutrition during his first 18 months of life. At the age of about two years the patient developed hyperphagia, followed by weight gain and obesity. Several episodes of pronounced hypoglycemia were observed during the postprandial period. Hypothyroidism was diagnosed as well. On clinical examination at an age of 55 months, his body mass index (BMI) was 22.1 kg/m². Based on the clinical presentation, the patient was suspected for *PCSK1* deficiency. Genetic analyses of a blood sample of the patient demonstrated a novel, homozygous deletion of about 249 kb within the *PCSK1* gene involving exons 1–9: arr[GRCh37] 5q15(95742125_95990869)x0. His parents were heterozygous carriers of the deletion in *PCSK1*, the mother presenting with class II obesity (BMI 38.1 kg/m²) and the father with overweight (BMI 27.5 kg/m²).

Conclusions: We conclude that genetic analyses of the *PCSK1* gene should be considered in children, who present with a combination of endocrinopathy and feeding difficulties in infancy followed by hyperphagia and obesity in young childhood. Regular follow-up of endocrine parameters and individualized hormone therapy adapted to the individual patient, where appropriate, is indicated. Selective agonists of the melanocortin 4 receptor (MC4R) currently being tested in clinical trials may in future expand the therapeutic options for *PCSK1* deficiency. Thus, affected patients should be identified early in the clinical course to ensure appropriate treatment.

P-ClinG-070

Alazami-Syndrome as a rare cause of syndromic intellectual disability–Report of a novel mutation in LARP7

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One function of the ubiquitously expressed LARP7 is to stabilize a non-coding RNA, the 7SK-ncRNA, and to protect it from degradation. Non-coding RNAs play an important role in gene regulation. The protein complex containing LARP7 and 7SK-ncRNA keeps the transcription factor P-TEFb in an inactive state and thus inhibits elongation of transcripts

by the RNA Polymerase II. However a more critical and complex role of LARP7 in gene regulation is suspected.

Deleterious homozygous mutations in LARP7 have first been reported in patients with primordial dwarfism, mental retardation and facial dysmorphisms by Alazami et al. in 2012. Until today a few more families with Alazami-Syndrome have been described widening the clinical phenotype. We report on two children born to consanguineous parents presenting with a syndromic form of intellectual disability. The index patient, a ten year old girl, presented with mental retardation, psychotic symptoms, short stature, microcephaly, submucous cleft palate, scoliosis and facial dysmorphisms. Her three year old brother showed global developmental delay with short stature, microcephaly, multicystic renal dysplasia, foot deformities and strabismus.

By Whole Exome Sequencing a homozygous mutation in the LARP7 gene was identified in the index patient: c.1669-1_1761del. This deletion of four base pairs affects the acceptor splice site of exon 13 and according to prediction tools results in skipping of this exon. The exact consequence of the mutation cannot be predicted as exon 13 is the last exon of the LARP7 gene, but a loss of function has to be assumed. Autosomal-recessive inheritance and segregation of the mutation could be confirmed by Sanger-sequencing.

In conclusion Whole Exome Sequencing provides a reasonable diagnostic option for syndromic patients without distinct phenotype. Further functional studies are intended to specify the effect of above mentioned mutation in LARP7 on its protein function.

P-ClinG-071

Phenotype of males and females with C-terminal truncating mutations in MECP2

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MECP2-related disorders are inherited in an X-linked manner. In the past, pathogenic MECP2 mutations were considered to be lethal in males.

In females, MECP2-related disorders include classic Rett syndrome, variant Rett syndrome and mild learning disabilities. In affected males, the severe neonatal encephalopathy resulting in death before the age of two years is thought to be the most common phenotype, but also mild phenotypes are reported depending on the mutation type and modifying factors. This wide spectrum of symptoms often hampers the correct diagnosis.

Here we report four families with different frameshift mutations in the last exon of MECP2. These mutations are not a target of nonsense-mediated decay but result in a deletion of the C-terminal end of the resulting protein. In three of the four families the mutations were inherited from the heterozygous mutation carrying mother. In family 4 the mutation is most likely *de novo*.

In family 1 the index patient, a 13 years old boy, shows an intellectual disability, language impairment, gait abnormalities, hyperactivity, aggressive behavior and problems with fine motor skills. Washing hand movements developed over time. The mutation-carrying mother is rather simpleminded, but has until now no other symptoms. The mutation-carrying 3 years old brother of the index patient shows developmental delay and muscle weakness.

The index patient of family 2 is a 65 years old woman who, when she was approximately 50 years old, developed choreatic movements, temporary gait disturbances, as well as problems of short-term memory. She has no intellectual deficits but has had epileptic seizures in the past. Her adult daughter is rather simpleminded without neurological symptoms. Both adult sons have a severe intellectual disability and epilepsy.

In family 3 two brothers, 34 and 39 years of age, show an epilepsy with focal seizure onset and a moderate intellectual disability. Interestingly the seizure disorder of the younger brother is highly drug-resistant, whilst the older is seizure free in monotherapy since early adulthood. The

mutation-carrying mother appears to show no obvious symptoms of a MECP2-related disorder until now.

Mutations in MECP2 (NM_004992.3) are c.1105_1144del, p.(His369Cysfs*27), c.1165_1217del, p.(Pro389Glyfs*29) and c.1099_1164delins77, p.(His367Glyfs*7) in family 1, 2 and 3, respectively.

All male mutation carriers in these three families have an intellectual disability plus varying neurological symptoms and obviously a life expectancy into adulthood.

In contrast, the 18 month old female index patient of family 4 with a similar mutation, c.1157_1200del, p.(Leu386Glnfs*4), showed a classical Rett syndrome phenotype with global developmental delay, muscular hypotonia, stereotypic movements, ataxia, secondary microcephaly and focal epilepsy. Beside classical Rett syndrome, MECP2 mutations should be considered in phenotypes of intellectual disability as well as other neurologic manifestations.

P-ClinG-072

A fourth mutation in TACO1: A new phenotype different from the previously described

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Cytochrome c oxidase, the last enzyme of the respiratory chain, catalyzes the reduction of molecular oxygen by reduced cytochrome c. TACO1 is a mitochondrial translational activator required for efficient translation of cytochrome c oxidase subunit I, a component of complex IV of the mitochondrial respiratory complex. Cytochrome c oxidase deficiency is clinically heterogeneous, ranging from isolated myopathy to severe multisystem disease, with onset from infancy to adulthood. To date, according to the HGMD professional 2018.3, there are three reported mutations and seven reported patients associated with TACO1. All patients identified up to now showed juvenile-onset Leigh syndrome phenotype.

In this present paper, we report the child with severe findings from birth such as; developmental delay, no speech, spastic tetraparesis, pyramidal and extra-pyramidal signs, wheelchair dependent, seizures, atrophy of occipital lobes, respiratory failure, capillary hemangioma and renal cyst of consanguineous family homozygous for c.814G>A in TACO1 from Turkey. To date this variant is not described homozygous or heterozygous in Exome Variant Server (EVS) and 1000 Genomes Project. For the in-silico prediction tools this variant is a disease causing (PolyPhen: Probably damaging, MutationTaster: Disease causing). For the confirmation of this variant, DNA was extracted from venous blood lymphocytes and this variant validated by conventional Sanger sequencing. The same mutation was also performing from parents' blood. The result of parents showed heterozygous c.814G>A mutation.

P-ClinG-073

First mutation in TSHZ1 in a family with Rasmussen syndrome

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In 1979, Rasmussen reported of a family with congenital bilateral vertical talus, bilateral external auditory canal atresia and increased interocular distance. In 2002, Rasmussen syndrome was mentioned for the first time by Julia et al. describing a patient with clinical features similar to the patients reported in 1979. However, genetic causes are still unknown. In 2011, Feenstra et al. reported of families with deletion or mutations in TSHZ1 causing congenital aural atresia. Individuals with microdeletion in 18q22.3 encompassing TSHZ1 also showed vertical talus. Here, we describe for the first time a family with vertical talus and mutation only affecting TSHZ1. We report on a family with an eleven year old boy, who was born at term after uneventful pregnancy. Body measurements were in normal range.

The boy had bilateral atresia of the external auditory canal and vertical talus (rocker bottom feet). The patient presented with mild intellectual disability and facial features including epicanthus, hypertelorism, broad nasal root, prominent forehead as well as small deep-seated ears.

The parents are non-consanguineous. The mother had learning difficulties as well as vertical talus and bilateral atresia of the auditory canal. The grandfather had congenital external auditory canal atresia like several members of the family of the mother, too. In addition, the daughter of one cousin had vertical talus and agenesis of external auditory canal.

After normal results in karyotyping, FISH-analysis for the Wolf-Hirschhorn region and array-CGH a suspicion of Rasmussen syndrome was made based on described clinical features. Targeted sequencing analysis of *TSHZ1* identified a heterozygous frameshift mutation c.718delA;p.Arg240Glyfs*10 in the index patient and his mother resulting in a premature stop of translation.

Systematic review of the literature showed that affected persons of two families with mutations in *TSHZ1* show atresia of external auditory canal but vertical talus has not been described in these patients. Therefore, we show for the first time the combination of vertical talus and congenital aural atresia due to mutation in *TSHZ1*.

Therefore, our findings support the hypothesis that heterozygous mutations in *TSHZ1* cause Rasmussen syndrome with incomplete penetrance and variable clinical expressivity.

P-ClinG-074

Biallelic truncating AEBP1 variant causing connective tissue disorder with features of Ehlers-Danlos and Marfan Syndrome

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Ehlers-Danlos Syndrome (EDS) is a clinically and genetically heterogeneous group of connective tissue disorders (CTDs). Affected individuals typically present with skin hyperextensibility, joint hypermobility and tissue fragility. Thirteen EDS subtypes have been proposed so far, based on specific clinical criteria. Causative variants in 55 genes coding for extra cellular matrix proteins (ECM) or mediating their biosynthesis, trafficking, secretion or assembly have been associated with EDS. Despite the wide application of targeted sequencing of these genes, in many cases no pathogenic variant can be identified. Whole exome sequencing (WES) has been used in research settings to screen for genetic causes in undiagnosed EDS. Indeed, a novel autosomal-recessive EDS subtype caused by biallelic variants in the *AEBP1* gene was recently identified based on WES.

The *AEBP1* gene encodes the ECM-associated adipocyte enhancer-binding protein 1 (AEBP1; also "aortic carboxypeptidase-like protein" (ACLP)) which is abundantly expressed in tissues with high collagen content such as blood vessels, skin and connective tissue. Thus far, only four individuals with *AEBP1*-associated CTD from three unrelated families have been reported. Clinical features included joint hypermobility, predisposition for dislocations, skin hyperextensibility, redundant skin, poor wound healing, atypical scarring and osteopenia.

Using clinical exome sequencing in a female individual with previously unsolved CTD, we identified a homozygous nonsense variant c.917dup, p.Tyr306* in exon 6 of *AEBP1*. Chromosomal microarray analysis excluded a microdeletion and showed no evidence for close consanguinity. Segregation testing confirmed homozygosity in the clinically affected brother and heterozygous carrier status in the healthy mother. RT-PCR analysis on PAXgene stabilized fresh blood of the mother revealed a monoallelic expression of the normal transcript supporting a nonsense-mediated mRNA decay and functional nullizygosity as disease mechanism.

The herein described siblings shared most of the previously reported *AEBP1*-associated clinical features. In addition, they presented with prematurely aged appearance, redundant skin lesions of the buttocks, pronounced hypermobility of the fingers and early onset kyphoscoliosis.

Our results confirm that autosomal-recessive inherited LOF variants in *AEBP1* are associated with clinical features of different EDS subtypes, but also of the marfanoid spectrum. As identification of further individuals is necessary to inform the clinical characterization, we stress the value of exome sequencing for cases negative in gene panel sequencing.

P-ClinG-075

Maternal Uniparental Isodisomy causes Ribose-5-phosphate Isomerase Deficiency in a child with developmental delay, epilepsy and leukoencephalopathy

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Ribose-5-phosphate isomerase deficiency (RPID) is an autosomal recessive inherited metabolic disorder of the pentose phosphate pathway (PPP), caused by pathogenic variants in *RPIA*. Affected patients show psychomotor delay, epilepsy, leukoencephalopathy and abnormal polyol metabolism. Since the first report of this entity in 1999 only two additional cases were described. Hence, RPID belongs to the exceptionally rare disorders. Here we report a 6 year old girl, who was born at term after an uneventful pregnancy. Postnatally she suffered from intermittent respiratory failure, but no infection could be revealed. At the age of one month she presented with muscular hypotonia. Within the first 7 months she developed epileptic seizures. Despite intensive anticonvulsant medication the patient continues to suffer from both focal and generalized epileptic seizures from once daily to once weekly. Magnetic resonance imaging at age of 9 months identified a nonspecific cystic leukoencephalopathy. To date, she continues to show severe psychomotor retardation, is able to crawl and to produce some single words.

We performed trio exome sequencing of the index and her parents and identified a homozygous donor splice site variant c.596+3A>G in *RPIA*, which is classified as VUS. This variant has not been reported in the general population and mutation databases to date. *In silico* programs predict a pathogenic effect of the variant. Segregation analysis of the parents revealed a heterozygous maternal origin, whereas the variant was not confirmed in the father although paternity was confirmed. Therefore, the suspicion of a maternal UPD(2) in the patient arose as explanation for a homozygous variant. Chromosomal SNP-array analysis confirmed a complete maternal uniparental isodisomy of chromosome 2.

Taken together, we present the fourth described case with Ribose-5-phosphate isomerase deficiency. Our analysis emphasize the importance of early and large genetic testing of unclear cases since very rare but less specific disorders, such as RPID, would otherwise be missed.

P-ClinG-076

Diagnostic exome sequencing identifies a novel mutation in the maternally imprinted gene CDKN1C as a cause of short stature and Silver-Russell phenotype in mother and son

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The 3-year-old male patient is the only child of his parents. His mother had been clinically diagnosed with Silver-Russell-Syndrome due to short stature (final height of 140 cm after treatment with growth hormone during childhood) and characteristic phenotypic features such as prominent forehead, clinodactyly of fifth fingers and a shortened os metatarsale IV. During pregnancy growth retardation was observed in the boy. At 33 + 1

weeks of gestation caesarean section was carried out (birth weight: 770 g, –3.95 SDS; length 31.5 cm, –5.42 SDS; head circumference 25.5 cm, –3.32 SDS). There were no major health problems during the neonatal period, motor development was slightly delayed. In addition to short stature a triangular face and broad forehead were recognized. As epigenetic analyses for Silver-Russell-Syndrome and PIK3R1 sequencing/MLPA (SHORT syndrome) were negative, diagnostic exome sequencing was performed leading to the identification of a novel mutation in the *CDKN1C* gene in mother and son (c.835C>A, p.Arg279Ser).

The gene *CDKN1C* (cyclin-dependent kinase Inhibitor 1C) is located on chromosome 11p15.5, a region which contains several imprinted genes. *CDKN1C* is a negative regulator of cellular proliferation and is expressed from the maternal allele. Depending on the mutation type (loss-of-function vs. gain-of-function) various growth disorders including Beckwith-Wiedemann Syndrome (BWS), Silver-Russell-Syndrome (SRS) and IMaGe syndrome may be the result of alterations in the *CDKN1C* gene. IMaGe syndrome is the predominant phenotype of *CDKN1C* gain-of-function mutations, the acronym denotes the major findings of Intrauterine growth restriction (IUGR), Metaphyseal dysplasia, Adrenal hypoplasia congenita, and Genitourinary abnormalities (in males). However, the phenotypic spectrum is wide. Considering the 34 patients in 21 families reported so far, a first approach for genotype-phenotype correlations emphasizes the significance of amino acids Ala263 to Glu285. The mutation identified in our family (p.Arg279Ser) leads to a SRS phenotype (no signs of adrenal hypoplasia/dysfunction are present in mother and son) and affects the same amino acid position, for which the only other *CDKN1C*-variant associated SRS phenotype has been reported so far (p.Arg279Leu). Therefore, the mutation found in our family may help providing additional insight into the phenotypic variability of *CDKN1C* gain-of-function mutations.

P-ClinG-077

The classical-like EDS—a subtype of the Ehlers-Danlos syndromes with subtle, but distinctive peculiarities

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Ehlers-Danlos syndrome (EDS) is a heterogeneous group of connective tissue disorders. According to the “2017 international classification of the Ehlers-Danlos syndromes” (Malfait F et al.), EDS is comprised of 13 clinical subtypes, some of which can be caused by a pathogenic genetic alteration in one of several genes. According to the 2017 classification, EDS type 2 is referred to as classical-like EDS (cEDS), and is also known as tenascin-X deficient EDS in the literature. Patients with cEDS have symptoms quite similar to patients with EDS type 1 (classical EDS or cEDS). But patients with cEDS show specific clinical features that may even allow the clinical discrimination of this subtype, not only from the cEDS subtype, but also from all other EDS subtypes, by means of physical examination alone.

The cEDS is associated with biallelic pathogenic changes in the *TNXB* gene, which is the only gene associated with this EDS subtype, resulting in a complete loss of the tenascin X protein. Due to the high homology of part of the *TNXB* gene with the pseudogene *TNXA*, it can be very difficult to detect pathogenic variants in the *TNXB* gene, and according to the actual EDS guideline, routine testing for *TNXB* variants is not recommended. Therefore, the ability to clinically discriminate the cEDS from the other EDS subtypes may be essential for making the right diagnosis.

Hence, with this case report, presenting a 9-year old female patient with impressive and characteristic clinical features, we want to highlight the subtle, but distinctive symptoms of the EDS subtype 2 and illustrate the importance of detailed clinical assessment as well as subtleties and difficulties in confirming the diagnosis by genetic analysis.

P-ClinG-078

Prenatal diagnosis of Chudley-McCullough syndrome in a fetus with ventriculomegaly, agenesis of the corpus callosum and interhemispheric arachnoid cyst

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A 29 year old woman was referred to us in the 33rd week of her third pregnancy because of progressive ventriculomegaly. The woman has two healthy children. Consanguinity was negated. A detailed ultrasound examination revealed a severe ventriculomegaly (ventricle width >20 mm), the suspicion of a complete or partial agenesis of the corpus callosum and an interhemispheric arachnoid cyst. These findings were confirmed by a fetal MRI of the brain which was performed in the 34th week of gestation. There was no evidence of additional brain anomalies at that time. Cytogenetic chromosome analysis after amniocentesis revealed a normal male karyotype. A chromosomal microarray has not been performed. Using an NGS based multigene panel encompassing 262 brain malformations-associated genes a novel homozygous mutation c.592C>T (p.Arg198Ter) in the fetus *GPSM2* gene has been detected. As expected and in accordance with an autosomal recessive inheritance the pregnant woman and her partner (the parents!) were tested heterozygous for the corresponding mutation in the *GPSM2* gene. *Gpsm2*, which is a receptor independent activator of G-protein-signaling, is involved in signaling pathways that control mitotic spindle dynamics and thereby asymmetric cell division, polarity, growth and differentiation in mammalian cells (Beer-Hammer et al., 2018). Biallelic mutations in the *GPSM2* gene have first been described as a cause of profound non-syndromic hearing loss (Walsh et al., 2010). Subsequent brain imaging of these individuals showed additional distinct brain anomalies leading to the diagnosis of Chudley-McCullough syndrome (CMS) (Doherty et al., 2012; Chudley and McCullough et al., 1997). CMS is characterized by sensorineural hearing loss, ventriculomegaly, partial or complete agenesis of the corpus callosum, frontal neuronal migrational anomalies, cerebellar dysplasia and interhemispheric arachnoid cysts. Psychomotor development is generally normal. Affected patients usually don't show neurological abnormalities like seizures. In most cases hearing loss is treated with cochlear implants. Sometimes ventricular shunting is required (Chapman et al., 2016; Diaz-Horta et al., 2012;). In summary the application of an NGS based sequencing panel led to the prenatal diagnosis of Chudley-McCullough syndrome. Consequently adequate counseling concerning prognosis, recurrence risk and obstetrical management was provided to the pregnant woman and the involved obstetricians and pediatricists respectively.

P-ClinG-079

Familial Xp11.22 microdeletion including *SHROOM4* and *CLCN5* is associated with intellectual disability, short stature, microcephaly and Dent disease

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Dent disease, short stature, short stature, psychomotor delay and minor facial anomalies were recently reported to be caused by two interstitial microdeletions Xp11.22 including the *CLCN5* and *SHROOM4* genes in a male patient. Dent disease characterized by a specific renal phenotype is caused by truncating mutations of *CLCN5* in the majority of affected cases. Here, we present the clinical and molecular data of a second such male individual affected with renal and neurological manifestations.

Molecular karyotyping revealed a hemizygous interstitial microdeletion Xp11.23p.11.22 of about 700 kb. The clinically unaffected mother also carried this microdeletion.

In total six genes were deleted. Among them is CLCN5, which explains the renal phenotype of Dent disease in our patient. SHROOM4, which is involved in neuronal development and was shown to be associated with intellectual disability, was partially deleted. Therefore, SHROOM4 is a candidate gene to explain the further clinical manifestation of our patient. Additionally, whole exome sequencing was performed in the index patient, identifying no other potential mutation in genes related to intellectual disability.

We summarize the clinical findings in our and the previously reported patient with an Xp11.22 microdeletion, expanding the phenotypic spectrum of such microdeletions.

P-ClinG-080

Genetical, clinical and functional analysis of a large international cohort of patients with autosomal recessive congenital ichthyosis due to mutations in NIPAL4

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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. To date, ten genes have been identified to be causative for ARCI: *TGM1*, *ALOX12B*, *ALOXE3*, *NIPAL4/ICHTHYIN*, *CYP4F22*, *ABCA12*, *PNPLA1*, *CERS3*, *SDR9C7* and *SULT2B1*. In this study we present a large cohort of 101 families affected with ARCI carrying mutations in *NIPAL4* (MIM: 609383). For mutation analysis we performed Sanger sequencing 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). We identified 16 novel mutations and increase the total number of pathogenic mutations in *NIPAL4* to 34. Ultrastructural analysis of biopsies from six patients showed morphological abnormalities consistent with an ARCI EM type III. One patient with a homozygous splice site mutation, which leads to a loss of *NIPAL4* mRNA, showed additional ultrastructural aberrations together with a more severe clinical phenotype. Closer examination of clinical characteristics points towards a correlation of nonsense and splice site mutations to a more severe phenotype. Our study gives insights to the distribution of mutations within *NIPAL4*, the frequency of occurrence of mutations, potential hot spot mutations and genotype-phenotype correlations.

P-ClinG-081

In a girl with features of Saethre-Chotzen syndrome array analysis reveals three submicroscopic deletions, one of them involving the TWIST1 gene, in a cytogenetically putative „balanced“ translocation t(1;7)

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We describe a female patient who is the first child of healthy non-consanguineous parents. In the 17th gestational week prenatal ultrasound

demonstrated intrauterine growth retardation and, four weeks later, additionally a severe heart defect that turned out to be a malalignment ventricular septal defect. The girl was born after 34 + 6 weeks of gestation with short stature, low birth weight, and microcephaly. She displayed the following facial features: brachyrrhynchia with large anterior and posterior fontanelles, blepharophimosis, hypertelorism, downslanting palpebral fissures, shallow orbits, depressed nasal bridge, broad nasal tip, anteverted nares, and long philtrum. Bilateral coronary suture synostosis was confirmed. Moreover, she demonstrated syndactyly of the 2nd and the 3rd fingers as well as of the 2nd and the 3rd toes.

Routine chromosome banding analysis (550 band level per haploid karyotype) revealed a female karyotype with a reciprocal translocation between the long arm of chromosome 1 and the short arm of chromosome 7: 46,XX,t(1;7)(q42.13~3;p15.1~3). The translocation was confirmed by FISH using subtelomeric probes for 1qter and 7pter in combination with chromosome specific painting probes. Both parents showed normal karyotypes by conventional cytogenetic and FISH analysis. In order to check for submicroscopic imbalances, we performed a genome-wide array analysis with the Affymetrix CytoScan HD Oligo/SNP-Array. The array data revealed a 2.7 Mb deletion in the chromosomal region 1q43, a 4.2 Mb deletion of chromosomal material 7p21.1-p15.3, and a 2.2 Mb deletion of the chromosomal region 7p15.3. The 4.2 Mb deletion of 7p21.1-p15.3 included the *TWIST1* gene leading to a haploinsufficiency of *TWIST1*. Heterozygous genetic changes of this gene are known to cause Saethre-Chotzen syndrome, which fits to most of the patients' symptoms.

To our knowledge, this is the first report of an unbalanced translocation t(1;7) with three deletions at the breakpoints of the translocation, one of them including the *TWIST1* gene, in a patient with a phenotype of Saethre-Chotzen syndrome. Standard karyotyping identified a putative „balanced“ translocation t(1;7) and genome-wide array analysis was necessary to detect submicroscopic deletions. Therefore, this is another example that in phenotypically abnormal patients with de novo reciprocal translocations further diagnostics like array analysis should be performed to exclude clinically relevant submicroscopic copy number variations.

Note: Jakob-Obeid, K. and Schwaibold, E. M. C. contributed equally to this work.

P-ClinG-082

A stop mutation in the COL4A2-Gene (c.4357G>T; p.Gly1453*) for the first time associated with acute aortic dissection

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Background: Thoracic aortic aneurysms (TAAs) have genetic and non-genetic causes (e.g. atherosclerosis, arterial hypertension). If they occur in young age or in many family members, a genetic cause is suspected. A familial accumulation is observed in about 20% of patients. Many gene mutations leading to familial TAAs and dissection have been described. A causative mutation is identified in only 20% of familial TAAs, it is therefore presumed that there are many other unknown genes associated with the disease.

Case: A 45-year-old male patient presented to our praxis after being discharged from the hospital, where he had been admitted because of an acute Type-A aortic dissection, extending to the brachiocephalic trunk, left subclavian artery, descending and abdominal aorta up to the right common iliac artery, ending at the right iliac bifurcation. Interestingly, the ascending aorta had a maximum diameter of 44 mm at the time of dissection, far below the limit of 55 mm for an elective surgery. The aortic valve was tricuspid. An emergency surgical treatment with resection of the aortic arch and the ascending aorta and repair using a vascular graft was performed. The patient was discharged ten days later and sought genetic counseling because he was worried for his two sons. Regarding his family history, he stated that his brother had been admitted in hospital 15 years ago because

of strong back pain and died one hour later at the age of 42 years, no autopsy was performed.

The first results of our molecular genetic analysis using standard TAA-panels were negative. However, given the family history and the extension of the aortic dissection, we still suspected a genetic cause of the disease. A new analysis was carried out using candidate genes of connective tissue diseases, which have never been published before in combination with aortic diseases. A heterozygous stop mutation in the COL4A2-gene (c.4357G>T; p.Gly1453*) was found, which probably leads to a nonsense mediated mRNA-decay.

The COL4A2-gene (chromosome 13q34) encodes the alpha-2 chain of type IV collagen, which is the main structural component of basement membranes. Mutations in the COL4A2-gene have been shown to cause cerebrovascular disease including porencephaly and intracerebral hemorrhage. They also affected the blood vessel stability in a mouse model, but to the best of our knowledge there has been no connection of a mutation in this gene to an aortic disease until now. Because of the nonsense type of the mutation, we believe that it is pathogenic and might contribute to the aortic dissection, although the actual data are inconclusive as to whether it is the cause of the serious collagen tissue disease of this patient.

Conclusion: In TAA-patients with strong clinical suspicion of genetic cause of their disease and negative results of the standard TAA-panels, it is important to extend the molecular analysis testing genes of connective tissue diseases as well.

P-ClinG-083

16p13.11 microdeletion uncovers a hypomorphic MYH11 allele in a patient with megacystis-microcolon-intestinal-hypoperistalsis syndrome (MMIHS)

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Background: Megacystis-microcolon-intestinal hypoperistalsis syndrome (MMIHS) is a rare condition that affects smooth muscle cells. Sporadic cases have been linked to heterozygous mutations in the ACTG2-gene. More recently MMIHS has been associated with biallelic null alleles in MYH11 in two patients. Mouse models deficient for myh11 reflect the human clinical presentation. Here, we report on the genetic findings in a child with MMIHS and additional clinical features.

Methods: We performed Sanger sequencing of ACTG2 and MYH11 in a child with MMIHS phenotype; copy number variations were analyzed by CGH-array. Origin of MYH11 aberrations was determined by Sanger sequencing and CGH-array in the healthy parents.

Results: We uncovered a novel heterozygous likely pathogenic variant [c.379C>T; p.(Pro127Ser)] in MYH11 and a heterozygous 1.3 Mb deletion in 16p13.11 encompassing MYH11 in a girl with MMIHS who is in addition affected by growth hormone deficiency and central hypothyroidism. Moreover, the girl has a likely benign 1 Mb duplication in 16p24.1. Her mother carries the 1.3 deletion in 16p13.11, whereas her father is heterozygous for the c.379C>T [p.(Pro127Ser)] change. Molecular modeling demonstrates that proline 127 is crucial for the formation of the ATP binding pocket of the MYH11 motor domain.

Conclusion: In light of the asymptomatic father of the patient, we suggest that MYH11 c.379C>T [p.(Pro127Ser)] constitutes a hypomorphic loss-of-function mutation that exerts its pathogenic effect only in the absence of a functional wild-type protein. The MMIHS phenotype in the patient results from compound heterozygosity for a MYH11 null allele and a hypomorphic MYH11 missense variant. Her non-MMIHS associated symptoms are most likely caused by the highly variable 16p13.11 microdeletion syndrome. ACTG2 mutation-negative patients with MMIHS should be screened both for MYH11 sequence alterations and copy number imbalances.

P-ClinG-084

Rothmund-Thomson Syndrome

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Rothmund-Thomson syndrome (RTS, OMIM#268400) is a rare autosomal recessive disorder characterized by poikiloderma, skeletal defects and increased risk of malignant disease. The disease is caused by homozygous or compound heterozygous mutations in the DNA helicase gene RECQL4. Here we report about a three year old girl with short stature, congenital absence of the thumbs, marked poikiloderma, and normal mental development. At the first time, whole exome sequencing showed no abnormal results. However, extended analysis revealed a heterozygous deletion from exon 13 to exon 18 in the RECQL4 gene and a heterozygous 11-bp deletion in intron 8 (c.1483 + 27_1483 + 37del, p.?). A similar large homozygous deletion of exon 13–18 was recently described in a patient with Baller-Gerold syndrome from Japan and interestingly, the mother of our patient also comes from Japan, suggesting that this deletion may not be rare in Japan. The RECQL4 gene structure is unusual because it contains 21 exons in only 6 kb of genomic DNA sequence. The 11 bp-deletion reduces the 77-bp intron 8 to 66 bp in length, leaving the 3' and 5' consensus splice sites intact. Wang et al. (2002) found the same, homozygous reduced 66-bp intron in a proband with RTS and hypothesized that the shortening to this small size is incompatible with proper splicing.

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P-ClinG-085

Functional characterization of a novel CSF1R mutation causing hereditary diffuse leukoencephalopathy with spheroids

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Background: Colony-stimulating factor 1 receptor is a tyrosine kinase transmembrane protein that mediates proliferation, differentiation and survival of monocytes/macrophages and microglia. CSF1R gene mutations cause hereditary diffuse leukoencephalopathy with spheroids (HDLS), an autosomal-dominantly inherited microgliopathy, leading to early onset dementia with high lethality.

Methods: By interdisciplinary assessment of a complex neuropsychiatric condition in a 44-year old female patient we narrowed down the genetic diagnostic to CSF1R gene sequencing. Flow cytometric analyses of uncultivated peripheral blood monocytes were conducted sequentially to measure the cell surface CSF1 receptor and autophosphorylation levels. Monocyte subpopulations were monitored during disease progression.

Results: We identified a novel heterozygous deletion-insertion mutation c.2527_2530delinsGGCA, p.(Ile843_Leu844delinsGlyIle) in our patient with initial signs of HDLS. Marginally elevated cell surface CSF1 receptor levels with increased Tyr723 autophosphorylation indicated enhanced receptor activity. Furthermore, we observed a shift in monocyte subpopulations during disease course.

Conclusion: Our data indicate a mutation-related CSF1R gain-of-function, accompanied by an altered composition of the peripheral innate immune

cells in our patient with HDLS. Since pharmacological targeting of CSF1R with tyrosine kinase inhibitors prevent microglial proliferation and disease progression in a murine model with Alzheimer's-like pathology, our findings suggest a potential pharmacological benefit of CSF1R-inhibition in patients with HDLS.

P-ClinG-086

Syndromal intellectual disability associated with cleft palate, congenital heart defect, behavioural anomalies and craniofacial dysmorphism due to a de novo MEIS2 mutation

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Intellectual disability (ID) has an estimated prevalence of 1.5–2%. Whole exome sequencing (WES) studies have identified a multitude of novel causative gene defects and have shown that a large proportion of sporadic ID cases results from *de novo* mutations. Here, we report on a 9-year-old girl, born to healthy non-consanguineous parents after uneventful pregnancy with normal birth measurements. A median cleft palate as well as a VSD and PFO had to be surgically corrected in infancy. Her psychomotor development was delayed (crawling at 24 months, unsupported walking at 3.5 years) and her speech development severely impaired. At present, she can only speak 10 words and uses a “talker” or sign language to communicate. Apart from ID, she has behavioural anomalies with episodes of aggressiveness, autistic features, and sleep disturbance. At the age of 9 years, she has a mild short stature (height –2.3 SD), a normal weight (BMI 14.5 kg/m²) and a normal head circumference (–1.5 SD). She has muscular hypotonia, scoliosis, and hypermobile joints. Her facial phenotype is characterized by arched eyebrows, mildly upslanted and long palpebral fissures, prominent nasal tip and columella and large, protruding ears with underdeveloped anthelices. Because of her clinical signs and especially her facial phenotype, Kabuki syndrome was initially suspected as clinical diagnosis, but analyses of *KMT2D* and *KDM6A* (for sequence and dosage anomalies) were normal. A microarray analysis did not identify a causal aberration. Trio whole exome sequencing revealed a *de novo* missense mutation in *MEIS2* (c.998G>A, p.(Arg333Lys)). *MEIS2* is a homeodomain-containing transcription factor of the TALE superfamily. Haploinsufficiency of *MEIS2* had been discussed as the mostly likely mechanism of the microdeletion 5q14-associated complex phenotype with ID, cleft palate, and heart defect. Only very recently, two studies including in total 15 individuals with intragenic *MEIS2* variants leading to haploinsufficiency were published (Douglas *et al.*, 2018, Verheije *et al.*, 2018), adding this gene to the growing number of genes underlying syndromal ID. We present the clinical phenotype of our patient and discuss it in comparison with the recent reports.

P-ClinG-087

Unusual presentation of two affected siblings with pontocerebellar hypoplasia type 1B broadens our understanding of this rare disorder

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Pontocerebellar hypoplasia (PCH) comprises a heterogeneous group of well-defined neurodevelopmental disorders with variable clinical course and prognosis. PCH1B (OMIM 614678) represents a rare autosomal recessive EXOSC3-related sub-entity, characterized by hypoplasia of cerebellar structures and pons, degeneration of the anterior horn cells and motoric as well as neurocognitive impairment. Phenotypic expression is variable with an overall poor outcome. Current research suggests clear genotype-phenotype correlations among EXOSC3-mutation carriers. Homozygosity for the EXOSC3 variant c.395A>C, p.(Asp132Ala) is proposed to lead to a rather mild phenotype compared to compound-heterozygous EXOSC3 mutation carriers with lethal neurological disease in very early childhood. In this study, we report two siblings (21- and 8-year-old) affected by PCH1B with an unusual presentation. We identified compound-heterozygosity for the well-established EXOSC3 variant c.395A>C, p.(Asp132Ala) and the novel variant c.572G>A, p.(Gly191Asp), expanding the genetic spectrum. Phenotypic presentation of the siblings was strikingly different from that of literature reports with a surprisingly mild disease manifestation; the longest by far reported survival, and an unexpected intra-familial variability. This study demonstrates the extensive clinical heterogeneity and the broad phenotypic spectrum associated with PCH1B. Enlargement of sample sizes and reports of novel individuals will be essential for the delineation of associated phenotypes.

P-ClinG-088

Molecular Diagnosis of Familial Hypercholesterolemia in Austria

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Introduction: Familial Hypercholesterolemia (FH) is a common metabolic disorder characterized by high levels of low-density lipoprotein (LDL). Incidence is about 1:300–500. Most cases are due to heterozygous variants in the gene coding for the LDL receptor protein (*LDLR*), but pathogenic variants in *APOB*, *PCSK9* and *LDLRAP1* are also causal for FH. The molecular diagnosis of FH is important regarding the risk for cardiovascular disease and the appropriate therapy. Carrier diagnosis in relatives is necessary to prevent cardiovascular events in time. In this study we summarized analyzed FH patients in Innsbruck to estimate the significance of molecular diagnosis and to discover and classify new pathogenic variants. **Methods:** From 2010 to Oct 2018 286 patients from Austria with suspicion of FH (adults with LDL-Cholesterol level >160 mg/dL, children >150 mg/dL; clinical assessment using the standard scoring system) were analyzed in Innsbruck for variants in *LDLR*, *PCSK9*, *APOB* and *LDLRAP1* by massive parallel sequencing (Nextera and NextSeq) and/or Sanger sequencing and Multiplex Ligation Dependent Probe Amplification (MLPA). Software analysis by SeqPilot (JSI), additionally analysis of copy number variations with panelcn.MOPS was performed and variants have been classified according to the 5-class-system by Plon *et al.* (2008).

Results: In total, 289 patients from Austria with suspected FH were analyzed in Innsbruck between 2010 and October 2018. Thereof, 125 carry *LDLR*-mutations (*n*=289), 13 carry *APOB*-mutations (*n*=210), 1 carried a *PCSK9*-mutation (*n*=171), 3 carry *LDLRAP1*-mutations (*n*=170) (numbers include family members). Altogether, 26 families with 50 relatives in

total were tested for the familial mutation. 82% of the relatives carried the familial mutation, 18% do not carry the familial mutation. In our cohort, we found 13 novel mutations (*LDLR*: $n=8$; *APOB*: $n=4$; *LDLRAP1*: $n=1$), not yet published in the literature or in mutation databases.

Discussion: The overall advantage of molecular diagnostics of FH for relatives is the possibility of an early diagnosis on the molecular level. Another advantage regarding the treatment is to find prediction strategies for statin responders, to optimize the use and dosage of cholesterol-lowering drugs. Additional genes will need to be investigated in the future regarding patients showing high amounts of LDL-C without detection of mutations in the above-mentioned genes. By means of genetic testing possible genotype–phenotype correlations regarding the age at diagnosis, LDL-C values, clinical phenotype (score assessment) and pharmacogenomics can be facilitated.

Conclusion: The clinical diagnosis can be made by LDL-C measurements and score assessment, but individuals with borderline values as well as relatives may be more accurately determined by molecular means. Knowing the genotype will help to discover further lipid-lowering therapy options, because of the already known correlation of the type of *LDLR*-mutations with drug response.

P-ClinG-089

POMGNT2-associated Walker-Warburg Syndrome

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We present a case of POMGNT2-associated Walker-Warburg Syndrome. Our patient is an 11 year old boy with congenital hydrocephalus and high creatine kinase levels. The boy's mother has been referred to us again pregnant in the 16th week to investigate the recurrence risk for a next child with syndromal hydrocephalus. Our patient is the fifth child of a consanguineous couple of syrian origin and has three healthy siblings. One of his sisters was affected by a syndromal hydrocephalus and died at the age of eight. One pregnancy was terminated in Syria by late fetocid in the 39th week because of a congenital fetal hydrocephalus. In the mother's family there are additional children of consanguineous parents affected with hydrocephalus. Our patient presented the following anomalies: macrocephalus, hydrocephalus with mental retardation and tetraplegia. External diagnostic test showed a normal karyotype. Gene analysis of DMD- and FKFR-genes did not detect any mutations.

We performed a clinical exome analysis (TruSight One Sequencing Panel, Illumina) in the index-patient and detected a homozygous mutation c.511G>A, p.Asp171Asn in his POMGNT2-gene. Both parents and all healthy siblings were heterozygous carriers for the mutation in POMGNT2-gene. POMGNT2 is an endoplasmic reticulum (ER) protein which is involved in O-mannosyl glycosylation. Defects in the glycosylation of α -dystroglycan lead either to a subgroup of muscular dystrophies with or without mental retardation or to a severe phenotype known as Walker-Warburg Syndrome (or muscle-eye-brain disease; Endo et al., 2015). This case report expands the phenotypic spectrum associated with POMGNT2-mutations.

P-ClinG-090

Divergent phenotype in a patient with *ASTN1* frameshift variants: epilepsy and developmental delay but no structural brain anomalies

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Background: Astrotactin 1, a neuronal adhesion molecule, is required for cerebral cortical development. The coding gene, *ASTN1*, is regarded as candidate gene for structural brain anomalies and neurological impair-

ment. Only few well characterized patients with variants in *ASTN1* have been reported so far: two sisters with homozygous missense mutations in *ASTN1* presented with corpus callosum dysgenesis, developmental delay and microcephaly. One patient with compound heterozygous missense variants in *ASTN1* had diffuse polymicrogyria in brain MRI, developmental delay, epilepsy and spastic tetraplegia.

Case report: We report on a 14 months old boy with global developmental delay, muscular hypotonia and epilepsy with spasms and atonic seizures starting at the age of nine months. His dysmorphic features comprised slightly dysplastic and low set ears, an epicanthus and a small chin. 3 Tesla brain MRI and metabolic work up were unremarkable. EEG first showed multifocal susceptibility to seizures and subsequently also signs of hypersarrhythmia.

Methods: Assuming a genetic cause of the disease, we performed Whole Exome Sequencing (WES) in the patient and his parents.

Results: WES revealed compound heterozygous frameshift variants in *ASTN1* in our patient (c.838_839delGA [p.(Glu280Lysfs*11)] and c.3125delAinsGACCACAAGTG [p.(Glu1042Glyfs*28)]). The parents are heterozygous carriers for one of the variants, respectively. *ASTN1* variants were confirmed by Sanger sequencing.

Conclusion: This is the first report of a patient with biallelic *ASTN1* frameshift variants. In contrast to previously reported patients with biallelic missense *ASTN1* variants our patient was not affected by brain anomalies but shared the known *ASTN1*-associated symptoms developmental delay and epilepsy. We speculate that biallelic loss-of-function variants in *ASTN1* might lead to a milder phenotype without structural brain anomalies. However, the full clinical phenotype will be determined over the course of time.

P-ClinG-091

Case report: Two brothers with PQBP1-Stopvariation leading to Renpenning Syndrome

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Renpenning Syndrome is a X-chromosomal recessive disorder caused by pathogenic variations in PQBP1-Gene. PQBP1-Gene is a regulatory protein for translational processes and splicing. Intellectual disability (95%), microcephaly (87%), small and slim stature (84%), triangular, long face (63%), dysplastic ears (50%), neurological pathologies (49%) and minor distinctive features characterize Renpenning Syndrome.

Here we present two brothers, who were seen at age 11 (*2006) and 14 (*2003) in December 2017. Both presented with intellectual disability, microcephaly, developmental delay, a small and slim stature, triangular, long face and dysplastic ears. The older brother additionally presented horseshoe kidney and status post maldescent testis. The younger presented a ventricle septum defect, which was hemodynamically non-relevant.

The family history was inconspicuous regarding retardation syndromes in men. The boys have an older healthy sister and a sister, who was born in 2001 and died 2003 presumably due to spongy cardiomyopathy and a younger healthy paternal half-sister.

After clinical genetic counselling, we performed chromosomal analysis, exclusion of FMR-1-Variants, Array-CGH and panel-analysis for X-chromosomal recessive microcephaly.

In both brothers and the mother the pathogenic variant c.586C>T, p.(Arg196*), Exon 5 in the PQBP1 gene was found in hemizygous pattern. Additionally, a maternal deletion Xp22.11 (116–137 kb) was found in both brothers. In the DECIPHER database (accessed: 08 Jan 2018) four patients with a comparable de novo deletion are listed, showing global developmental delay and speech developmental delay. According to the DGV database (accessed: 08 Jan 2018), a comparable deletion was detected in 1 of 873 individuals in an unremarkable cohort. Segregation analyses showed the maternal (grand-)father to be a healthy carrier of the deletion Xp22.11. On the evidence of the literature and the segregation analyses the deletion Xp22.11 was classified as a benign, not disease-causing deletion.

Phenotypic presentation of both brothers with microcephaly, small stature, intellectual disability and the minor frequent features are thus in good agreement with the features of the PQBP1 gene variations described in the literature. Comparative photos from the literature show a special agreement of the facial features. Renpenning syndrome is a rare disease (1:1 Mio.) with distinctive characteristic features.

P-ClinG-092

Clinical and Molecular Characterization of an Amelogenesis imperfecta Family

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Introduction: Inherited Amelogenesis imperfecta (AI) constitute a clinically and genetically heterogeneous group of conditions characterized by inherited developmental enamel defects. Over 20 different genes have been described to cause AI, inherited either autosomal dominant, autosomal recessive or X-chromosomal. Homozygous or compound heterozygous mutations in the gene *SLC24A4* cause AI type II A5 (AI2A5; OMIM #615887). Patients with mutations in *SLC24A4* have an isolated AI of the hypomaturation-type (discolored enamel, soft and premature loss of enamel, caries). To date not more than five families have been described with mutations in *SLC24A4*.

Case Report: We investigated a consanguineous family with isolated AI (discolored enamel and enamel hypoplasia, caries); the father and four children are affected, while the mother and one child show an attenuated phenotype. Enrichment of target gene exons and adjacent exon-flanking regions (± 15 bp) from genomic DNA by Nextera® Rapid Capture (TruSight™ One Expanded Panel, Illumina®) and massive parallel sequencing (NextSeq, Illumina). Alignment of sequences to the human reference sequence GRCh37 (hg19). Data analysis with SeqNext and evaluation of the variants with varSEAK [JSI medical systems GmbH]. Variant filtering: (1) HPO term: AI (HP:0000705), (2) autosomal recessive inheritance, (3) MAF <1%, (4) coverage >20x, (5) stop, missense and splice site variants, (6) not classified as benign or likely benign in mutation database (ClinVar, in house).

The analysis revealed a homozygous missense mutation c.1604G>A in *SLC24A4* in the two affected siblings. The expected effect is an amino acid change (p.Gly535Asp) which concerns a highly conserved amino acid; the *in silico* prediction programs (GVGD, SIFT und PolyPhen) rate the mutation as pathogenic. This mutation is not yet described in the literature. Interestingly, the affected father carries the mutation c.1604G>A homozygous, too. The mother is heterozygous carrier of the mutation. Six teeth from four affected individuals were available for histologic analysis. Briefly, the teeth were dehydrated and stained with basic. Teeth were embedded in methylmethacrylate and sawed perpendicular to the occlusal surface in order to produce slices with a thickness of 25–30 μ m, which were polished and contrast-stained with acetic light green. Histological dental investigations confirmed the clinical diagnosis of hypoplastic and hypomaturation AI. A reduced thickness of the enamel with pits and grooves is visible. The enamel presents with a countless number of cracks. The dentin has a normal structure.

Conclusion: *SLC24A4*-dependent amelogenesis imperfecta is a rare cause of familial enamel disorders. The condition is best detected through massive parallel exome or panel sequencing. The treatment is symptomatic.

P-ClinG-093

C9orf72 expansion is associated with accelerated decline of respiratory function and decreased survival in amyotrophic lateral sclerosis

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Introduction: Respiratory insufficiency is the main cause of death in amyotrophic lateral sclerosis (ALS). As the C9orf72 repeat expansion represents the most common genetic risk factor for this disease, we studied whether C9orf72 modulates respiratory function and survival.

Methods: Demographic and clinical data, and C9orf72 status were collected from 372 ALS patients followed in our center. Multiple regressions controlling for the C9orf72 expansion, diagnosis delay, region of onset, age, gender, and comorbid frontotemporal dementia were performed to evaluate the functional and respiratory status of the patients at baseline and during disease progression—assessed using the global ALSFRS-R score and its respiratory subscore, and the predicted forced vital capacity (%FVC). A Cox regression controlling for the same variables was carried out to analyze survival.

Results: At baseline, 32/372 (8.60%) patients carried the C9orf72 repeat expansion. During disease progression, the ALSFRS-R_{global} and ALSFRS-R_{respiratory} scores were not significantly influenced by the mutation ($p > 0.8$ and $p > 0.3$, resp.). On the contrary, we found that the C9orf72 mutation is an independent risk factor for a faster %FVC decline ($p = 0.001$) and shorter survival ($p = 0.002$).

Conclusions: In ALS patients the C9orf72 expansion is independently associated with accelerated respiratory dysfunction and thus with a shorter survival. This finding indicates a new pathogenic mechanism of C9orf72 in ALS.

P-ClinG-094

A novel homozygous likely pathogenic variant in LRP4 in tree fetuses with severe distal limb malformation and renal agenesis—the severe end of the phenotype?

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Biallelic variants in the LRP4 gene (OMIM 604270) have been identified in patients with autosomal recessive Cenani-Lenz syndactyly syndrome. Characteristic clinical findings in the patients reported in the literature include skeletal anomalies with short and/or fused ulnae and radius, syndactyly, fused metacarpals and malformed phalanges of hands and feet, genitourinary anomalies with renal hypoplasia/aplasia in about 50% of the cases, and craniofacial anomalies including micrognathia, prominent forehead and hypertelorism.

Here we report on a Lebanese consanguineous couple with three discontinued pregnancies due to severe fetal anomalies detected by prenatal ultrasound. The first pregnancy was terminated at 24 weeks of gestation due to ultrasonographic detection of severe limb malformations with absent radii, ulnae, tibia and fibulae, and renal agenesis with anhydramnios, and dolichocephalus. The second pregnancy was terminated at 13 + 2 weeks of gestation. The fetus showed absent radii, ulnae, tibiae and fibulae, and renal agenesis, and in addition an occipital encephalocele. The fetus of the

third pregnancy again showed the same skeletal and renal anomalies, the pregnancy was terminated at 13 + 0 weeks of gestation. Fetal pathologic investigation further revealed anal atresia in the fetuses of the second and third pregnancies and median cleft lip- and palate with retrognathia in the fetus of the third pregnancy.

Next generation sequencing analysis revealed a homozygous novel LRP4 variant c.1894C>T, p.(R632C) in the fetus of the second pregnancy. Sanger sequencing confirmed the variant in the other two affected fetuses in homozygous state as well as in both healthy parents in heterozygous state. Genetic results allowed for correct assigning of the clinical findings to a specific but rare genetic disease. The findings in this family possibly present the severe (lethal) end of the phenotype of Cenani-Lenz syndactyly syndrome with severe distal limb reduction defects and renal agenesis. Further, the fetal pathological findings of anal atresia and occipital encephalocele may further expand the phenotype.

P-ClinG-095

Ataxia Charlevoix-Saguenay: more frequent than expected in the German population?

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We report on a 24-year-old young woman presenting with a progressive weakness in the legs, pes equinus, hyperreflexia and spasticity. The first symptoms arose at the age of 15 months when she started to walk with difficulties and unbalancing. In the following years she developed a spasticity and furthermore a dysarthria, particular in connection with excitement. No abnormalities of the heart, eyes, ears or urinary tract are known. During her childhood a causative infantile brain damage due to Rhesus incompatibility was suspected. Her older brother showed the same symptoms but stronger pronounced. The sister and the non-consanguineous parents are healthy.

Given the background of the family history first an autosomal recessive spastic paraplegia was taken into account as underlying cause for the symptoms of the woman and her brother. Sequence analysis and MLPA of the gene *SPG7* generated inconspicuous results and there was no GAA trinucleotide repeat expansion in intron 1 of the *FXN* gene.

Extended panel analysis revealed homozygosity for the mutation c.12160C>T (p.Gln4054*) in the gene *SACS* (Sacsin). Segregation analyses of the parents and the brother are in progress. This pathogenic mutation has already been described (Vermeer et al. 2008).

Pathogenic mutations in *SACS* cause autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) (Engert et al. 2000), first described in the Charlevoix-Saguenay region of Quebec (Bouchard et al. 1978), outside Quebec it is a very rare disease. ARSACS is characterized by early-onset (12–18 months) cerebellar ataxia (vermis atrophy and hypointensities in magnetic resonance imaging), pyramidal spasticity of the lower limb and peripheral neuropathy. Further symptoms, e.g. dysarthria, dysphagia, retinal hypermyelination, nystagmus, urge incontinence and, very rarely, mitral valve prolapse may occur (Bouchard et al. 1991, Vermeer et al. 2008). The phenotype is variable (Baets et al. 2010). In individuals who do not originate from Quebec a later-onset of symptoms and absence of retinal abnormalities are described.

The protein encoded by *SACS* (Sacsin) is probably involved in mitochondrial network organization interacting with dynamin-related protein 1 (Drp1), which mediates mitochondrial fission to maintain mitochondrial health. Mitochondrial dysfunction and impairment of dendritic arbor organization likely lead to Purkinje cell loss, described in ARSACS patients and saccin-knockout mice (Bradshaw et al. 2016, Girard et al. 2012). We report on a rare case of ARSACS in a German patient, illustrating that in the differential diagnosis of an early-onset ataxia in combination with lower limb spasticity, ARSACS should also be considered.

P-ClinG-096

PIEZO1 mutations in a foetus with hepatomegaly and hydrops fetalis

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Hepatomegaly is a condition in which the liver is enlarged beyond its normal size. In disease, hepatomegaly could be already existent during embryonic development and could be revealed by prenatal ultrasound examination. Prenatal enlargement of the liver is particularly suggestive of glycogen storage disease (GSD), which is characterized by accumulation of excessive amounts of glycogen in the liver. The standard genetic diagnostic procedure for identifying the underlying putative cause of the hepatomegaly also includes sequence analysis of genes known to be involved in the glycogen storage pathway.

Here, we report on a 29-year old pregnant woman whose foetus had clinical presentation of hepatomegaly and hydrops fetalis detected via ultrasound examination at 20 + 2 weeks of gestation. We performed clinical exome sequencing targeting the coding regions of 6700 disease-associated genes. Analysis of NGS data did not reveal any genetic abnormalities in genes of the glycogen storage pathway. However, we identified two rare heterozygous mutations in the *PIEZO1* gene. The gene encodes a transmembrane protein acting as mechanically activated ion channel in the plasma membrane of different cell types. Mechanotransduction is an essential mechanism regulating physiological processes, starting from embryonic development. Homozygous or compound heterozygous mutations in the *PIEZO1* gene are reported to cause generalized lymphatic dysplasia (GLD) with non-immune hydrops fetalis. Heterozygous missense mutations are causative for dehydrated hereditary stomatocytosis (DHS) with or without pseudohyperkalemia and/or perinatal edema. At least some patients with DHS or GLD present with hepatomegaly and/or splenomegaly. Segregation analysis revealed the clinically unaffected father of the foetus to be heterozygous carrier of one of the detected rare *PIEZO1* mutations. Hence, a dominant pathogenic effect of this mutation is very unlikely. Additionally, this finding confirmed paternity. The second observed *PIEZO1* mutation was detected by segregation analysis to be *de novo*. To our knowledge, this mutation has not been described previously in databases and literature.

Since it remains unclear if and in what extend these two mutations in the *PIEZO1* gene are causative for the abnormalities seen in the ultrasound of the foetus, the involvement of *PIEZO1* mutations in prenatal hepatomegaly development should be further investigated in future studies.

P-ClinG-097

White-Sutton syndrome (MRD37) due to a truncating mutation in the POGZ gene

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White-Sutton syndrome is a distinct intellectual disability syndrome (MRD37, MIM 616364) caused by heterozygous mutation in the *POGZ* gene. In addition to delayed psychomotor development and intellectual disability, patients show a variable constellation of abnormalities and dysmorphic features. This may include muscular hypotonia, microcephaly, short stature, visual defects, hearing impairment, gastrointestinal difficulties, and autism or autistic features. However, in most cases the abnormalities and dysmorphic features alone do not unequivocally predict a particular type of syndromic intellectual disability. The first truncating mutations in *POGZ* were reported only in 2014 and the description of the clinical phenotype followed in 2016 (White et al.).

We report on a three years old girl with delayed motor and speech development (DD), moderate intellectual disability (ID), muscular hypotonia, abnormal findings on brain MRI (enlarged ventricles, subdural hygroma,

periventricular gliotic spots), pronounced hyperopia (+8,5 dpt./+10 dpt.), ataxic gait and stereotypic hand movements. Physical examinations at the age of 6 months, 1 year and 3 years showed brachycephaly, short neck, flat face, high forehead, palpebral ptosis, telecanthus, short palpebral fissures, long philtrum, hypotonic facies with open mouth, pointed chin, posteriorly rotated ears, persistent edema on the back of the hands and feet, slightly broadened great toe and sandal gaps.

Conventional cytogenetic analysis displayed a normal female karyotype (46,XX) and array CGH analysis did not detect any submicroscopic genomic imbalances. Testing for congenital disorders of glycosylation (CDG) and extended screening for metabolic disease were without pathological findings. Initially targeted DNA-analyses of several genes showed normal results (15q11-13 methylation, UBE3A, FMR1, CREBBP, EP300, KMT2A, KMT2D, SETBP1, SLC9A6 and PLA2G6).

Due to the clearly visible syndromic intellectual disability and the lack of an initially detectable cause, we decided to perform clinical exome sequencing. Exome sequencing revealed a heterozygous de novo nonsense mutation in POGZ: NM_015100.3: c.3001C>T (p.Arg1001*). According to the ACMG guidelines this mutation is to be classified as pathogenic (PVS1, PM2, PM6). The same mutation was previously reported in two other unrelated patients with syndromic DD/ID (Stessman et al. 2016). The clinical phenotype of our patient agrees well with these two as well as with other patients carrying POGZ mutations, confirming White-Sutton syndrome.

P-ClinG-098

A case of Joubert syndrome caused by digenic inheritance of TMEM67 and NPHP1 variants

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Background: Joubert syndrome and related disorders (JSRD, ORPHA 140874) are characterized by hypoplasia of the cerebellar vermis, detectable as molar tooth sign (MTS) in magnetic resonance imaging (MRI). Moreover, JSRD patients show developmental delay and congenital malformations. Pathogenic variants in at least 30 genes including *TMEM67*, *C5orf42*, *CC2D2A*, *CEP290*, and *AH11* as the most common ones, have been associated with JSRD, a genetically heterogeneous group of ciliopathies. Digenic heterozygous inheritance has also been reported (*CC2D2A/CEP41*, *KIF7/CEP41*).

Materials and Methods: Here, we describe a clinical case of Joubert syndrome of a patient presenting with developmental delay, an irregular breathing pattern, an MTS on MRI, and bilateral retinochoroidal coloboma.

Results: A trio exome analysis of the patient and his parents was performed. A known heterozygous pathogenic variant c.1027G>A p.Gly343Arg within the *NPHP1* gene (NM_000272.3) was detected as a paternally inherited variant, in addition to a known heterozygous pathogenic variant c.2498T>C p.Ile833Thr within the *TMEM67* gene (NM_153704.5) identified as a heterozygous maternally inherited variant.

Conclusions: Here, we describe a case of a clinical Joubert syndrome with heterozygous pathogenic variants in both the *NPHP1* and *TMEM67* genes. Since digenic heterozygous Joubert cases have been reported previously, we propose a digenic inheritance of Joubert syndrome caused by heterozygous pathogenic variants in *TMEM67* and *NPHP1* genes.

P-ClinG-099

Utilisation of genetic counselling services in Germany before and after the enactment of the Gendiagnostikgesetz (GenDG): results of the GenBIn study

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Background: The Gendiagnostikgesetz (GenDG) stipulates the requirements of good practice in regard to safety, patient rights to know, patient rights to decide and requirements for genetic counselling. Empirical data on the utilisation of genetic counselling services since the enactment of the GenDG (February 1, 2010) are lacking.

Objectives: Development of a database that provides both (a) baseline data on the utilisation of genetic counselling services prior to the enactment (status quo ante) and (b) data on utilisation after the enactment.

Methods: Development of a standardised data collection tool for documenting in-person genetic counselling cases in order to assess and compare utilisation and counselling workload in 2011 and in 2016/2017. To assess change variables were compared by bivariate statistical analysis.

Results: 26 genetic counselling centres volunteered in 2013/2014 to collect data on counselling cases seen in 2011, 34 centres provided real time data for cases seen in 2016/2017 (19 centres based at medical schools, 2 centres based at teaching hospitals and 13 private practice centres). 85% of the counselling centres that participated in the 2011 data collection also provided data for 2016/2017. In total the GenBIn database contains data on 5256 counselling cases and as such is providing an unprecedented wealth of data. Compared to 2011 the relative share of referrals by obstetricians & gynecologists has decreased in 2016/–2017 (by 20 percentage points in private practice), whilst referrals by other specialists and self-referral have increased. The scope of indications for genetic counselling has changed. Referral indications for prenatal counselling have decreased, counselling for familial cancer and other familial disorders has increased. Mean waiting time has increased from 41,18 in 2011 to 47,14 days in 2016/2017. Total time spent on a case (workload per case) including pre- and post-counselling activities has increased. The number of genetic tests ordered has increased.

Conclusions: The GenBIn data document a steadily rising demand for genetic counselling services (reported in detail at the GfH meeting 2018) and indicate a shift in indications for referral to counselling services and topics addressed in counselling sessions. The observed change is probably due to a multiplicity of underlying causes and cannot solely be attributed to the enactment of the GenDG. Advances in genetic testing technology, growing awareness among healthcare providers (GenDG) and among patients (increased self-referrals) all together are resulting in higher patient volumes, increased workload and capacity constraints for genetic counselling services.

P-ClinG-100

Whole-exome sequencing identifies a recurrent SOX2 deletion in a patient with global developmental delay, dystonia and ataxia in the absence of major ocular malformations

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Neurodevelopmental disorders such as intellectual disability and movement disorders in children are genetically extremely heterogeneous. Al-

though a monogenic cause is often assumed, incomplete penetrance and variable expressivity of known Mendelian disorders can complicate a phenotype-driven diagnostic approach. We here report on an 11-year-old girl who presented with global developmental delay and a complex movement disorder with features of dystonia and ataxia. She was born at term after uneventful pregnancy as the 2nd child of healthy non-consanguineous German parents and started to walk independently at the age of 30 months. At the age of 4¹¹/₁₂ years, a dystonic and ataxic gait with flinging movements and a mixed receptive-expressive language disease were observed. Over the following years, her walking difficulties were progressive, and she had special educational needs due to severe learning difficulties. Magnetic resonance imaging, cerebrospinal fluid analyses, and a metabolic screen gave normal results. Trio-based whole exome sequencing identified no candidate gene in an autosomal recessive inheritance model but finally revealed a *de novo* variant in *SOX2* which is a well-known disease gene for syndromic congenital eye malformations. Notably, the identified 20-bp deletion [c.70_89del; p.(Asn24Argfs*65)] is listed in ClinVar as pathogenic and had been described several times in patients with anophthalmia or microphthalmia. An ophthalmological examination of our index case demonstrated bilateral scotomas, macrodiscs with abnormal non-glaucomatous excavations and hypoplastic lacrimal ducts but no other ocular malformations. As intellectual disability and movement disorders belong to the spectrum of symptoms observed in patients with *SOX2*-related ocular malformations, the identified variant was classified as pathogenic for the phenotype of our index case. However, this is a very rare case of a c.70_89del *SOX2* mutation carrier without a prominent eye phenotype. The case presented here demonstrates that the use of exome sequencing in a genotype-first approach does not only help to identify a causative variant but also to refine the characteristic phenotypic spectrum associated with known disease genes.

P-ClinG-101

*** Next-generation sequencing of 32 genes associated with hereditary aortopathies and related disorders of connective tissue in a cohort of 199 patients

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Heritable factors play an important etiologic role in connective tissue disorders with vascular involvement, and a genetic diagnosis is getting increasingly important for gene-tailored, personalized patient management. Given the pronounced and still growing genetic heterogeneity, we performed genetic testing of 31 and 32 disease-associated genes by using targeted next generation sequencing (tNGS) and whole exome sequencing (WES), respectively. We examined a clinically relevant cohort of 199 indi-

viduals with familial or sporadic aortopathy and/or related disorder of connective tissue and classified and refined sequence variants with minor allele frequency $\leq 0.1\%$ according to their likelihood for pathogenicity. We identified ≥ 1 pathogenic mutation (PM; in *FBN1*, *LOX*, *SMAD3*, *TGFBR1*, or *TGFBR2*) in 23 patients (11.6%) and ≥ 1 likely pathogenic mutation (LPM; in *COL3A1*, *FBN1*, *FBN2*, *LOX*, *MYH11*, *NOTCH1*, *SMAD3*, or *TGFBR1*) in 13 individuals (6.5%), together resulting in 18.1% diagnostic yield. 14 of the 41 PM/LPM were novel. Most PM or LPM were detected in *FBN1* followed by *SMAD3*, *NOTCH1* and, *ex aequo*, *FBN2*, *LOX* and *TGFBR1*. Of the PM/LPM-negative patients 39 (19.6%) showed ≥ 1 variant of uncertain significance (VUS; in 19 different genes), 12 of these were novel; most VUS were identified in *FBN2*, *DCHS1*, *COL3A1* and *LTBP2*. 21 patients had concomitant reportable variants (i.e. PM, LPM and VUS). In-depth evaluation of reported and calculated variant classes for all detected variants resulted in the reclassification of 20.3% of variants. Thus, both variant classification and refinement are essential for shaping the mutational spectra of disease genes, thereby improving clinical sensitivity. Taken together, an obligate stringent multigene analysis, i.e. either tNGS or WES, is a powerful tool for identifying the genetic cause of clinically related disorders of connective tissue. Nonetheless, the relatively high rate of PM/LPM/VUS-negative patients underscores the existence of yet unknown disease loci and/or oligogenic/polygenic inheritance.

P-ClinG-102

RIT1-associated Noonan syndrome in prenatal diagnostics

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Noonan syndrome occurs with a prevalence of 1:1.000–1:2.500 live births and is highly variable in its clinical characteristics. Patients with Noonan syndrome generally show a combination of abnormalities. Most patients show congenital heart defects (80%), mostly pulmonary stenosis (20%–50%) and hypertrophic cardiomyopathy (20%–30%). In addition, patients with Noonan syndrome appear with short stature as well as facial dysmorphic features and some patients also exhibit a delay in psychomotor and language development of variable degree. Noonan syndrome caused by mutations in the *RIT1* gene (Noonan syndrome 8) is furthermore characterized by a significantly increased risk of hypertrophic cardiomyopathy (70%), which may be present at birth or develop in infancy or childhood. Here, we report three cases of prenatal diagnostics of Noonan syndrome after abnormalities in prenatal ultrasound examination. Gene panel analysis was performed either with native chorionic villi samples or cultured amniotic fluid cells. In all cases maternal contamination of fetal DNA was excluded by marker analysis. The first case presented with fetal hydrops and an increased nuchal translucency detected at 11th week of gestation. Later in pregnancy intrauterine fetal death occurred. The second case presented with an increased nuchal translucency and lateral neck cysts on both sides detected at 18th week of gestation. The third case also presented with an increased nuchal translucency and lateral neck cysts on both sides detected at 12th week of gestation. In none of these cases congenital heart defects were traceable at the time of the ultrasound examination. In all three cases we detected heterozygous missense mutations in the *RIT1* gene, which were previously described as causative for Noonan syndrome in the literature and mutation databases. All mutations were confirmed by Sanger sequencing. The analysis of the parents showed wildtype sequences in all cases. Therefore, the mutation in the *RIT1* gene most likely occurred *de-novo* in the fetus. According to the literature, in about 50% of affected patients molecular genetic testing identifies a pathogenic variant in the *PTPN11* gene, whereas mutations in the *RIT1* gene are responsible in only 5% of live birth Noonan patients. The results of our prenatal gene panel analysis reveals that especially in cases with increased nuchal translucency without congenital heart defects, one should consider evaluation of the less common causes of Noonan syndrome besides mutations in the *PTPN11* gene. It might also raise the imagination that mutations in the *PTNP11* gene may not present with an increased nuchal translucency in prenatal ultrasound examination in a greater amount of cases than expected.

P-ClinG-103

A novel frameshift mutation in CHD8 gene causes a subtype of Autism spectrum disorder

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Autism spectrum disorder (ASD) is neurodevelopmental disorder with a broad range of potential genetic causes. The CHD8 gene encodes a Chromodomain helicase DNA-binding domain protein 8, and plays a crucial role as chromatin remodelling factor and transcriptional regulator. Loss of function mutations in the CHD8 gene result are well known reasons for a ASD subtype (AUTS18, OMIM # 615032) with hypotonia, speech delay, macrocephaly, variable facial dysmorphism, gastrointestinal problems and autism as main features. Here, we report a 2 3/12-year old boy with a complex developmental disorder. Particularly, the language development is delayed and he exhibits hypotonia with pathological movement pattern, craniofacial features, such as long face with a prominent broad forehead, high hairline, supraorbital ridge, pointed chin, triangular-shaped open mouth, posteriorly rotated ears (more pronounced on the right side), tall stature and difficulties falling asleep. The patient has neither structural nor EEG brain abnormalities. We performed multi-gene panel testing via sequencing by synthesis, including candidate genes contributing to complex developmental disorders with speech delay, mild forms of autism and facial dysmorphism. We revealed the novel frameshift mutation c.1220delG in exon 3 of the CHD8 gene. This one-base deletion occurred de novo since it is not present in the parents. The truncating mutation results in premature termination of the protein after 12 amino acids. Truncating mutations in the CHD8 gene have been described to be causative for autism spectrum disorders. Due to the deleterious impact of the mutation and the segregation analysis, there is a strong evidence for the disease causing effect of the truncating mutation in the CHD8 gene.

P-ClinG-104

Master of Science in Genetic and Genomic Counselling: introduction of the German training programme in German language starting in Innsbruck 2019

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Genetic counselling in Germany, Austria and Switzerland is by law an exclusive task of medical doctors qualified in human genetics. However, genetic and genomic analyses are becoming a major part of all aspects of medicine, and there is a rising demand for competent communication of the background, aims and consequences of genetic tests in the individual context. Professionally trained Genetic Counsellors can help in meeting the demand for informed decision-making and consent for genetic testing. While MSc programmes in Genetic and Genomic Counselling have been established worldwide for non-medical staff, and many have been approved by the European Board of Medical Genetics (EBMG), there is as yet no corresponding education programme in German-speaking countries. The Innsbruck MSc programme for Genetic and Genomic Counselling at the Medical University Innsbruck follows the structure of the European Core Curriculum. It addresses mainly professionals with BSc or higher degrees in Biological or Biomedical Sciences, Psychology or related fields, as well as academically trained paramedical staff. The students are taught all practical, scientific, social and communicative skills needed in Genetic and Genomic Counselling, preparing them for an occupation in an interdisciplinary team under specialist medical genetic supervision.

The programme is organized as an extra-occupational (berufsbegleitendes) training comprising 100 European Credit Transfer System (ECTS) points in 5 semesters. Core modules in semester 1 include genetic and genomic concepts, statistics, laboratory and scientific approaches, and communication processes and skills. From semester 2 onwards there will

be a broad education in the diagnostic and predictive setting of Genetic and Genomic Counselling, integrating ethical, legal and psychosocial aspects of genetic services. A major component is the Practical Placement module (30 ECTS) in a human genetics institute or practice. Knowledge and skills that the students have developed in theory and seminars will be applied and assessed in a clinical genetic counselling service in different cities or regions. This will take place in 3 modules over 3 semesters and will involve participation in the genetic counselling of clients. Experience shows that students can provide valuable support in clinical practice from an early stage of their training. The MSc finishes with a Master thesis in a counselling related topic.

We believe that the expertise of Genetic and Genomic Counsellors will also be useful in the German language countries and will be welcomed by many institutes and practices also for their Practical Placement modules. We herewith would like to introduce the concepts of our MSc programme to the members of the German Society for Human Genetics and invite students to join the course.

P-ClinG-105

Expansion of the clinical and genetic spectrum of nemaline myopathy 10 by a series of Austrian and German patients

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Nemaline myopathy (NEM) is a genetically heterogeneous congenital myopathy with the characteristic finding of electron-dense 'nemaline bodies' in myofibers. In 2014, 21 patients from 14 families with a severe form of congenital NEM and disrupting biallelic mutations in LMOD3 (MIM*616112) were reported. This form is denoted as NEM10 (MIM #616165). Following the first patients series, 2 further families with fatal NEM10 were published.

Here we describe 4 unrelated patients with clinically, ultrastructurally, and genetically confirmed NEM10 based on 2 previously undescribed missense mutations within LMOD3 presenting with a mild and stable phenotype. Despite impaired postnatal adaptation and the presence of muscular hypotonia from birth, our patients developed remarkably well and stabilized during the first year of life. Severe weakness of facial, oral, and pharyngeal muscles was present in all patients. All had swallowing difficulties after birth, making transient nasogastric tube feeding necessary in 3 of them. Motor milestones were delayed in 2 patients, all achieved independent walking. The patients survived into adulthood and were between 18 and 30 years of age when last examined. Three of 4 patients remained ambulatory, and all showed marked facial weakness and had dysarthric speech. Muscle biopsy specimens gave evidence of nemaline bodies. All patients were unrelated but originated from Austria (Tyrol and Upper Austria) and Southern Germany (Bavaria).

Two patients carried the missense mutation c.1648C>T, p.(Leu550Phe) of the LMOD3 gene on both alleles. Two patients carried the identical missense mutation c.1648C>T, p.(Leu550Phe) in trans with another missense variant (c.1004A>G, p.Gln335Arg). Both variants segregated in the 4 families corresponding to an autosomal recessive mode of inheritance. Compared with the severe clinical picture of NEM10 comprising a substantial risk of early death, our patients showed a chronic disease course after being critically ill in the neonatal period. As the missense variant c.1648C>T is located further downstream compared to all previously published LMOD3 mutations, it might be associated with higher protein ex-

pression compared to the reported loss-of-function mutations. The apparent clusters of two mild mutations in Germany and Austria in four unrelated families may be explained by a founder effect.

P-ClinG-106

Hallermann-Streiff syndrome: A missing molecular link for a highly recognizable syndrome

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Hallermann-Streiff syndrome (HSS; OMIM 234100) is a very rare congenital disorder with a highly recognizable phenotype, mainly characterized by typical craniofacial dysmorphism, eye malformations, a distinctive facial appearance, abnormalities of hair and skin, short stature, and might also present with aspects of premature aging. While intensively and critically reviewing the literature, we realized that a substantial number of patients described as having HSS might actually represent only a "spectrum disorder" or even another unrelated clinical entity. Despite extensive NGS-based screenings, the molecular basis of HSS is still unknown. To substantially improve the chance of identifying the missing molecular link, it is necessary to precisely define the "classical" HSS phenotype. We therefore present here, the clinical results of a systematic analysis of the current literature and our own large cohort of patients with HSS, which we collected and clinically ascertained during the last years. We included only the classic cases that fit the phenotypes of the original patients reported by Hallermann in 1948, Streiff in 1950, François in 1958 and Cohen in 1991. Our extensive exome/genome sequencing approaches in our unique cohort are still ongoing, but the fact that we did not find causative mutations in genes associated with clinical overlapping syndromes (e.g. *LMNA*, *ZMPSTE24*, *POLR3A*, *SLC25A24*, *GJA1*, *CEP152*, *TCOF1*) underlines the specificity of HSS diagnosis in cases with classic phenotypic expression. Our comprehensive phenotyping presented here, will improve gene identification in HSS using innovative molecular strategies.

P-ClinG-107

Design of questionnaires for a survey on attitudes towards the potential role and detection of fetal epigenetic changes

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Prenatal epigenetic programming can influence the development of predispositions and diseases later in life. Malfunctioning epigenetic programming is held responsible to contribute to maladaptation in utero and to several diseases developing later in life, e.g. metabolic disorders or increased risk of cancer. Since the pathogenicity of many epigenetic modifications has not been proven or reveals completely unknown, the discourse how maternal impacts during pregnancy can affect the child's health, is sometimes controversial. Technical possibilities are progressing and epigenetic tests, pre- and postnatally, may soon provide additional layers of predictive information, complementary to genetic information, but in addition

one may draw conclusions on someone's past exposures or disrupters of epigenetic mechanisms. Meanwhile, hardly any studies on the ethics of epigenetic testing exist.

To address the ethical issues associated with prenatal programming and the non-invasive recognition of fetal epigenetic patterns and alterations, we have designed questionnaires as the key component of a survey among parents in situations where epigenetic testing might be potentially applicable in the future. The questions aim at assessing the respondents understanding of (epi)genetic effects on health and disease as well as at determining their attitudes on fetal genetic and epigenetic diagnostics.

Our approach to the construction of questionnaires was inspired through a study by Lakeman et al. [1] on preconceptional screening for cystic fibrosis and haemoglobinopathies. As in [1], the implemented scales are based on the Theory of Planned Behavior (TPB) and complemented by further variables. In detail, we focused on the roles of personal attitudes, social influence, perceived behavioral control (which together form the theoretical basis of TPB), perceived benefits, reproductive consequences, severity of disorders, risk perception, and stigmatization.

In our study, the corresponding questions are embedded in three exemplary case reports: (1) on a fetally diagnosed Beckwith-Wiedemann imprinting disorder (representing a specific diagnosis with potential epigenetic components), (2) on an unspecific whole-genome analysis, and (3) on a targeted search for epigenetic causes of diabetes and obesity.

Additionally, participants will answer short questions that will function as a summary assessment of attitudes on fetal epigenetic diagnostic. Finally, we examine to what extent demographic differences are related to (epi)genetic knowledge and attitudes towards prenatal (epi)genetic testing. Most individual items will be measured on a 5-point Likert scale.

At the moment, we are preparing the application for ethical approval and plan to invite further institutions to this survey.

References

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P-ClinG-108

Preimplantation Genetic Diagnosis for monogenic disorders and concurrent aneuploidy testing at PID-Zentrum Munich

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Introduction: Women requesting preimplantation genetic diagnosis for monogenic inherited disorders (PGT-M) usually do not show reduced fertility; thus, an ample amount of oocytes is available for PGT-M in most of the cases. However, oocytes may show numerical aberrations of chromosomes (aneuploidies) regardless of the haplotype of the gene locus being tested. As aneuploidies increase with maternal age, preimplantation genetic testing for aneuploidies (PGT-A) may be meaningful.

PGT is subject to strict legal regulation in Germany and can be performed only at a licensed institution following approval of an ethics committee. Concurrent testing for monogenic disorders and aneuploidies must be explicitly approved by the ethics committee.

At the MGZ–Medical Genetics Center's PID-Zentrum Munich, PGT-M is conducted using Karyomapping (Illumina), which is based on SNP microarray haplotyping. Single nucleotide polymorphisms (SNPs) enable determination of the gene's haplotype, i.e., the parental origin of the alleles and phase (heterozygous affected, homozygous affected, wildtype) of the trophoblast sample tested. Simultaneously, SNPs allow analysis of the copy number of chromosomes and display aneuploidies of meiotic chromosome errors (monosomies and trisomies). Validation of Karyomapping results is accomplished by next-generation sequencing (VeriSeq, Illumina).

Materials and Methods: 125 trophoblast samples of 37 PGT-M cycles were investigated at PID-Zentrum Munich (mean maternal age 34 years). Whole genome amplification (WGA, SureMDA, Illumina, Repli-G, Qiagen) was followed by Karyomapping. Samples that were transferable based on the haplotype but showed an aberrant profile for a chromosome copy

number underwent additional VeriSeq analysis. Data analysis was done using BlueFuse Multi software (Illumina).

Results: 67 of 125 (54.5%) trophoctoderm samples were transferable after Karyomapping analysis according to the gene's haplotype. 23 (34%) of the 67 trophoctoderm samples resulted in suspicion of an aneuploid profile. These samples were validated by VeriSeq, which confirmed all of the 23 suspected aneuploidies.

Discussion: Transferable embryos may have aneuploidies after PGT-M. Concurrent analysis of both the haplotype of the gene and the copy number of the chromosomes in a trophoctoderm sample leads to a more accurate determination of the embryo's genotype. This method is therefore a meaningful complement to PGT-M analysis and may well lead to an increased probability of giving birth to an unaffected child.

P-ClinG-109

Whole COL3A1 gene deletion in a patient with vascular Ehlers-Danlos syndrome and atypical clinical presentation

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The Ehlers-Danlos syndromes constitute a group of connective tissue disorders characterized by heterogeneous clinical expression and a broad range of genetic causes. Pathogenic variants in the COL3A1 gene cause vascular Ehlers-Danlos syndrome (vEDS) with substantial tissue fragility. Patients with vEDS have a significantly increased risk of arterial, intestinal and uterine rupture and the rate of major complications exceeds 80% until the age of 40 years.

We report on a 17-year-old young man who presented with arachnodactyly and notably progressive contractures of the fingers and toes, as well as tall thin build and reduced upper to lower segment ratio. Juvenile idiopathic arthritis was diagnosed at the age of eleven years, but anti-rheumatic medication was lacking effect. Differential diagnosis after clinical genetic assessment comprised congenital contractual arachnodactyly or SMAD3-associated Loeys-Dietz syndrome due to obvious contractures. Surprisingly, multi-gene panel testing and corresponding CNV analysis revealed a complete COL3A1 gene deletion. As shown by array CGH, the deletion interval does not include other genes. The 48-year-old mother of the index patient also carries this deletion, featuring long thin fingers and easy bruising, but no major vEDS complication has occurred so far. The maternal grandfather of the index patient experienced cerebral aneurysm rupture at the age of 59 years. However, he has not been available for genetic testing up to now.

To our knowledge, this is the second report of a patient with a whole COL3A1 gene deletion, but the first detailed clinical description of a family with vEDS caused by this pathogenic variant leading to true haploinsufficiency.

P-ClinG-110

A rare cause for prenatal onset growth retardation: Mulibrey nanism in two brothers with compound heterozygous insertion and deletion in TRIM37

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Growth is a complex dynamic progress with high heritability. A diagnosis of short stature is based on the extent and type of growth retardation as well as additional clinical characteristics. Among syndromes with short stature as the main clinical feature, Mulibrey (MUScle, LIver, BRain and EYes) nanism is a rare autosomal-recessive disorder. It is characterized by prenatal onset growth retardation (nanism), dysmorphic features, multi-

ple organ abnormalities and an elevated risk of neoplasia. The underlying gene, TRIM37, encodes for a member of the tripartite motif (TRIM) family localized in peroxisomes and possesses ubiquitin-ligase activity. Thus far, about 130 patients with TRIM37 mutations have been reported, including 90 affected persons from Finland.

By performing WES we detected a compound heterozygous 2 bp deletion in exon 20 (c.2377_2378del) and 1 bp insertion in exon 21 (c.2461dup) of the TRIM37 gene in two affected brothers of non-consanguineous parents. Both variants were not previously reported. Expression analysis by qPCR in lymphocytes from one of the patients showed significantly reduced TRIM37 expression levels of 0.65 compared to controls. This result might indicate that some of the truncated transcripts might be translated into an altered TRIM37 protein.

Both patients presented with prenatal onset growth failure without postnatal catch-up growth (−4.0 SD and −3.6 SD), failure to thrive, mildly disproportionate stature and facial features, such as dolichocephaly, triangularity, a prominent forehead and a flat nasal bridge. Neither of the brothers showed intellectual disability. One patient developed Wilm's tumor at the age of 2 years, as it is shown to occur in about 4% of Mulibrey nanism patients. The other patient developed transient hyperphosphatasia and anemia. Even though endocrinology screening was normal, low IGF1-levels and primordial growth retardation led to the initiation of growth hormone treatment prior to the genetic diagnosis. In both brothers growth hormone therapy showed only minor effects on growth development and final adult height prediction. This goes in line with previous observations of the only moderate impact of GH treatment in patients with Mulibrey nanism or other forms of skeletal dysplasias on adult height of +0.6 SDS (Karlberg et al. 2007).

Although rare, Mulibrey nanism is an important differential diagnosis to other SGA phenotypes such as Silver-Russell-syndrome or 3M-syndrome. Early diagnosis in these patients is necessary for proper treatment of feeding difficulties, detection of constrictive pericarditis and the elevated risk of neoplasias. This report further underlines the importance of WES in patients with idiopathic short stature.

P-ClinG-111

Nemaline Myopathy Type 3: congenital tetraparesis associated with two novel compound heterozygous Mutations in ACTA1 Gene

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Nemaline myopathy (NM) is a form of congenital myopathy characterized by the presence of nemaline rods in muscle fibers and can be caused by mutations in 11 different genes. Disease severity is variable and unpredictable, with prognosis ranging from neonatal death to almost normal motor function. Pathogenic variants in ACTA1 lead to autosomal dominant or recessive NM type 3 and similar myopathies. Autosomal dominant origin has a variable penetrance and a milder phenotype than the autosomal recessive one, which leads to a congenital and severe phenotype. Here, we describe two novel compound heterozygous variants in the ACTA1 gene which we identified in a young man with NM type 3.

The patient described here is the son of healthy non-consanguineous parents and has a healthy twin-sister. He showed onset of myopathic symptoms *in utero*. After birth, he suffered from respiratory failure and a tetraparesis. Available muscle biopsies from the left quadriceps muscle at the age of 5 months were investigated by standard histological and electron microscopic techniques. The expression of various proteins was determined by immunohistochemistry. The results were suspicious, but not indicative. At the age of 20 years, we undertook targeted next-generation sequencing. We searched for variants in a set of 59 genes known to be causative for congenital myopathies using the patient's DNA isolated from whole blood. Putative pathogenic variants were tested for segregation in the DNA of the unaffected parents and his twin sister. Two compound heterozygous variants in ACTA1 were identified in the patient's DNA. The parents and the sister each carried one of the two mutations.

Our patient shares many features with other reported cases, especially with patients with recessive NM Type 3, who are devoid of skeletal muscle α -actin, but who show normal heart α -actin expression.

P-ClinG-112

Unbalanced translocation der(13)t(2;13)(p25.2;q34) in a boy with a Kabuki-like phenotype and in his sister with syndromic developmental delay

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Kabuki syndrome is a rare autosomal dominant or X-linked disorder caused by heterozygous KMT2D or hemizygous KDM6A mutations. Main symptoms are intellectual disability of varying degree, postnatal growth deficiency, a distinct facial gestalt including long palpebral fissures, arched eyebrows with sparing in the lateral one-third, short columella, prominent ears, and persistence of fetal fingertip pads.

Our index patient was a 5-year old boy with several, in particular facial features indicative of Kabuki syndrome. He presented with intellectual disability (IQ 53), muscular hypotonia, relative microcephaly (height: 115,5 cm, +2 SD; head circumference: 50 cm, P15), and dysmorphic features including epicanthus, long palpebral fissures, sparse lateral eyebrows, prominent nasal root, short trapezoid philtrum, inverted nipples and fetal fingertip pads. Sequencing of KMT2D and KDM6A revealed no putative pathogenic mutation. Subsequent DNA array analysis showed a double segment chromosomal imbalance with a terminal microduplication 2p25.2–2pter and a terminal microdeletion 13q34–13qter. Metaphase FISH analysis confirmed the presence of a derivative chromosome 13. The mother was found to carry a balanced translocation t(2;13).

Only few patients with terminal microduplications 2p are reported to date. Common features are developmental delay, craniofacial dysmorphic features and cardiovascular malformations. One child with a complex mosaic duplication-triplication 2p25 is reported with overlapping features of CHARGE and Kabuki syndrome. The phenotype of terminal microdeletions 13q is very variable, but most patients present with intellectual disability, microcephaly and muscular hypotonia.

At the time of the initial genetic consultation, the boy's mother was in the 35th week of gestation of her second pregnancy. Delivery was uneventful, birth height was in the low normal range (48 cm; P7), birth weight (2460 g; -2,2 SD; 100 g < P3) and head circumference (31 cm; -2,8 SD; 1,2 cm < P3) were reduced. In the first month of life, pronounced muscular hypotonia became evident. From 7 months, the girl was able to roll over, but crawling or sitting was not possible at the age of 9 months, the date of our last examination. Further, she also had microcephaly and unspecific dysmorphic features including low set rotated ears, prominent nasal root, retrogenia, inverted nipples, single transverse palmar crease, swollen feet and overriding toes. She bore only little facial resemblance to her brother, her clinical features at this young age were not suggestive of Kabuki syndrome. Nevertheless, FISH analysis revealed the same unbalanced translocation der(13)t(2;13) as in her brother.

The case emphasizes the importance of DNA array analysis or quantitative analysis of sequencing data in children with developmental delay even if there is a strong suspicion of a monogenic syndrome. Further, there is some indication that microduplications 2p may cause a Kabuki-like phenotype.

P-ClinG-113

Ethical decision-making competence regarding genome editing by CRISPR/Cas9 of adolescents and medical students

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Theoretical background: The CRISPR/Cas9 technology may be used for gene therapy in the near future. Therefore, it seems essential to enhance lively public debate as to the ethical consequences of genome editing. Young people may use this technology in the future and face these ethical issues.

To facilitate participation of adolescents in public debates, the competence field of ethical decision-making is fixed as a part of the educational standards for the natural science curricula in Germany. In the present study, we intend to investigate ethical decision-making competences of medical students and adolescents in the context of genome editing.

Research design and sample: The Oldenburg model of decision-making competences is a structural model displaying eight sub-competences, each describing three levels. The sub-competences include perception and awareness of moral relevance as well as of sources of the own attitude, assessment, judgement, argumentation, consequence reflection, change of perspective and basic ethical knowledge with regard to a bioethical context.

Based on a paper-pencil test with open questions on authentic ethical dilemmas, regarding genome editing for somatic or germline gene therapy for RUNX1-associated familial leukemia, the above-mentioned sub-competences are recorded in a sample of 50 medical students of Hannover Medical School and 50 adolescents from advanced level of a secondary school in Oldenburg.

The responses of participants are analyzed using the structured qualitative content analysis according to Mayring (2015). When analyzing the test responses, points are awarded for the dimensions of decision-making competence. The test scores allow quantitative analysis of the study results.

Results and conclusion: First results of the study show that adolescents as well as medical students are open-minded towards genome editing in case of healing severe diseases. Some students see great chances of genome editing, but the effects and the possibilities of the new method appear frightening to them. Therefore, these students were not able to decide for or against the use of genome editing. Missing guarantees and experiences fortify the contra position. Most of the students advocate genome editing, but see clear limits for its usage. None of them agree with the usage in general. With calculated averages, each participant is classified to the levels of decision-making competence. Most students reach level 2. Only a few are in level 3 (best level). Especially in the sub-competences of consequence reflection, change of perspective and assessment the adolescents as well as the medical students have deficits. First results of this study suggest that education on ethical decision-making competence and/or on basics about the CRISPR/Cas based genome editing should be further improved in school and university.

P-ClinG-114

Undetected glycogen storage disease as a cause for sudden death—Case report and review of the literature

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We report the case of an 8 year old girl, who suddenly died with no obvious cause identified by autopsy. Pregnancy had been unremarkable. At 5 months of age she was hospitalized due to acute respiratory distress syndrome and was found to have muscular hypotonia. Neuropediatric examinations at 7 months lead to the suspicion of a mitochondrial depletion syndrome, but molecular testing revealed no causative mutation. At the age of 6 years, a developmental delay was obvious, especially regarding speech development, but also attention and fine motor skills were affected. She visited school and received continuous help for her learning difficulties. At age 8 years and 3 months, she suddenly collapsed during a family celebration and died. Prior to this event, she had felt weaker than usual, and someone had to carry her bag on the way to school. Since no obvious cause of death had been identified upon autopsy, molecular testing initially focussed on cardiac arrhythmias, but revealed normal results. Consanguinity of the parents prompted us to analyse a clinical exome for homozygous mutations in genes with likely effects on cardiac and muscu-

lar function. We detected a homozygous GYS1 frameshift mutation, which we regard as disease causing in this patient. We will discuss this case with respect to the literature

P-ClinG-115

Mutations in GABAA receptor subunits lead to a broad spectrum of epilepsy and cognitive impairment

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GABA_A receptors are primarily involved in inhibitory neurotransmission in the central nervous system. The most abundant receptor consists of two $\alpha 1$, $\beta 2$ and $\gamma 2$ subunits, each encoded by *GABRA1*, *GABRB1* and *GABRG2*, respectively. Missense variants in *GABRA1* cause juvenile myoclonic, childhood absences and idiopathic generalized epilepsy as well as early infantile epileptic encephalopathy. Missense variants in *GABRG2* result in a broad clinical spectrum, ranging from benign febrile seizures and childhood absence epilepsy to severe epileptic encephalopathy. Here we describe three individuals with very variable epilepsy and cognitive impairment, harbouring missense substitutions in either *GABRA1* or *GABRG2*. Individual 1 is a 19 year-old male with infantile generalized tonic-clonic seizures, autistic traits and mild cognitive impairment of non-consanguineous healthy parents. Onset of seizures was at 8 months, during childhood he had 2–3 seizures per year and later he solely experienced numerous dizziness episodes. His 26 year-old sister (individual 2) had onset of epilepsy at 9 months and currently has monthly seizures which are difficult to treat. She had only mild impaired speech development and learning difficulties. By exome sequencing in individual 1 we identified the heterozygous missense variant c.640C>T, p.(Arg214Cys) in *GABRA1*, classified as pathogenic in ClinVar. Sanger sequencing from lymphocytes confirmed the variant in the affected sister but in none of the parents, indicating germline mosaicism.

Individual 3 was a 20 year-old male of non-consanguineous parents, who presented with drug-resistant epilepsy and profound intellectual disability (no speech or mobility). Seizure onset was at 5 months and he currently has about eight myoclonic and rarely tonic-clonic seizures per day. He also exhibited microcephaly and hyperbreathing episodes during daytime, resembling Pitt-Hopkins syndrome. A deletion or mutation in *TCF4* was excluded. Exome sequencing revealed the novel heterozygous missense variant c.844C>A, p.(Pro282Thr) in *GABRG2*, which was not present in ExAC and predicted as deleterious. The father was a not carrier and genetic material of the deceased mother was not available. She was reported to have multiple febrile seizures in childhood. Although de novo occurrence in individual 3 is highly likely, as previously described in all patients with severe epileptic encephalopathy, maternal inheritance cannot be excluded. Our observations confirm that mutations in *GABRA1* or *GABRG2* result in extremely variable epilepsy and cognitive impairment and that germline mosaicism should be considered in affected siblings.

P-ClinG-116

TAF1-associated intellectual disability with thoracic deformity and distal arthrogryposis

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We report on a 21-year-old male affected by a severe developmental disorder, distal arthrogryposis, contractures of the knees, severe scoliosis and kyphosis, hip dysplasia, postnatal progressive microcephaly and facial dysmorphism (long face, strabismus, synophrys, hypertelorism, downslanted palpebral fissures, bulbous nose, long philtrum, protruding ears). Growth parameters at birth (41th week of gestation) were within

normal ranges (10th percentile). Poor feeding, neuromuscular developmental delay, progressive microcephaly and dystrophy were noted in the first year of life. Walking was achieved at the age of 4 years. Speech was not acquired. Difficulty with chewing was reported. The young man is fed, he is not toilet trained. Eye examination showed strabismus divergens alternans, myopia and astigmatism. Recurrent otitis media was observed in early childhood. MRT of the brain in the first year of life showed no structural abnormalities.

Chromosomal analysis revealed a familial Robertson translocation 13/14, which was also ascertained in the unaffected brother and the mother. Uniparental disomy was excluded and array CGH and FMR1-analysis revealed inconspicuous results. Clinical exome analysis showed a previously unreported de novo missense mutation in the TAF1 gene (c.3604C>T, p.Arg1202Cys), which was classified as probably pathogenic. TAF1 is one of the largest subunits of a transcription factor (transcription factor 2 D, TFIID), required by DNA polymerase II (Irvin et al.2005). There are three different phenotypes associated with mutations affecting this gene: a duplication syndrome affecting TAF1 (Kaya et al. 2011), dystonia-parkinsonism due to repeat expansion (Makino et al. 2007) and mental retardation, X-linked, syndromic 33 due to missense or splice site mutations (O'Rawe et al. 2015). Most mutations are de novo and female carriers demonstrate highly skewed X-inactivation.

Most of the observed phenotypic features in our patient were previously reported in other patients with mental retardation, X-linked, syndromic 33. Cognitive impairment ranges from mild to severe. Further documented abnormalities in this syndrome are hypoplasia of corpus callosum, hearing impairment and a characteristic gluteal crease with sacral caudal remnant. Some males were also affected by spastic diplegia and dystonic movements. In addition, our patient showed distal arthrogryposis, which has not been associated with TAF1 mutations yet.

P-ClinG-117

Whole exome sequencing: Identification of a genetic cause of hearing impairment in 26% of analyzed GJB2-negative patients

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Aim: Explore the diagnostic yield of exome sequencing in GJB2-negative patients with hearing impairment

Background: Hearing impairment (HI) is the most common sensory disorder worldwide with a prevalence of 1–5 in 1000 newborns and a rising number to 3.5 in 1000 in adolescents. Approximately 80% of the prelingual deafness cases have a monogenetic etiology. Although it has not yet been conclusively shown, it is only likely that genetics also plays an important role in hearing impairment in the elderly.

Patients: Our cohort includes 95 unrelated children and adults with HI which were negative for pathogenic variants in the most common causative genes GJB2. Furthermore these patients were negative for medical history related to potential causes of acquired HI (infection, trauma, ototoxic drugs and perinatal complications) and exclusively affected by a cochlear disorder.

Methods: Using whole exome sequencing (WES) the search for causative variants was first focused on 148 genes already associated with HI and which were listed in the Deafness Variation Database before we supplemented the gene panel with additional 681 genes (listed in the Human Phenotype Ontology database based on hearing impairment). All variants were classified according to the guidelines of the American College of Medical Genetics.

Results: In 25 of 95 patients we identified variants considered causally related to the HL phenotype (pathogenic or likely pathogenic) in 23 different genes (*COL11A2*, *WFS1*, *DFNB59*, *FGF3*, *PCDH15*, *POU4F3*, *OTOF*, *LHFPL5*, *PEX6*, *OTOF*, *CHD7*, *DSPP*, *MARVELD2*, *MITF*, *C10orf2*, *CRYM*, *P2RX2*, *TMCI*, *CLND14*, *MYO15A*, *PROK2*, *BBS9*, *ILDR1*), confirming the genetic heterogeneity of hereditary HI. 8 of these patients received a genetic diagnosis with a complex clinically unrecognized syndrome in which HI is only one feature. These syndromes include Usher syndrome

(1 patient), Waardenburg syndrome (2 patients), CHARGE syndrome (1 patient), Warsaw breakage syndrome (1 patient), Kallmann-syndrome 1 (1 patient) and Bardet- Biedl-syndrome (1 patient).

Conclusion: WES of 95 patients with possibly hereditary HI leads to the identification of a genetic cause in 26% of analyzed patients. This result absolutely supports WES to use as a diagnostic tool in patients with HI, analyzing at least a panel of 148 genes.

P-ClinG-118

*** Mitochondrial DNA mutation analysis from exome sequencing—towards a holistic approach in diagnostics

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Purpose: Next generation sequencing, especially exome sequencing (ES), has revolutionised the diagnostics for mitochondrial disorders. However, the nuclear and mitochondrial DNA (mtDNA) are investigated with separate tests, increasing costs and duration of diagnostics. The full potential of ES is often not exploited as the additional analysis of “off-target reads” deriving from the mtDNA can be used to analyse both genomes.

Methods: We performed mtDNA analysis by ES of 2,111 cases (various, mostly neurological, referral indications) in a clinical setting. We further assessed the recall rate and precision as well as the estimation of heteroplasmy by ES data by comparison with targeted mtDNA next generation sequencing in 49 cases.

Results: ES identified confirmed pathogenic mtDNA point mutations in 38 individuals, increasing the diagnostic yield by nearly 2%. Analysis of mtDNA variants by ES has a high recall rate ($96.2 \pm 5.6\%$) and an excellent precision ($99.5 \pm 2.2\%$) when compared to the gold standard of targeted mitochondrial DNA next generation sequencing. ES estimates heteroplasmy levels with an average difference of $6.6 \pm 3.8\%$, sufficient for clinical decision making.

Conclusion: Taken together, the mtDNA analysis from ES is of sufficient quality for clinical diagnostics, and should be included in the standard diagnostic exome analysis.

P-ClinG-119

Identification of a new BVES variant associated with cardiac arrhythmia—a case report

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The tight-junction-associated transmembrane protein Blood vessel epicardial substance (Bves) is expressed in cardiac and skeletal tissue. *Bves* $-/-$ mice show an age-dependent decline in cardiac pacemaking function as well as abnormalities in sinus node structure and stress-induced sinus node dysfunction. Furthermore, *Bves* plays a role during muscle regeneration in adult mice. In humans, *BVES* is associated with cardiac arrhythmia with increased serum creatinine kinase. However, the relationship between phenotype and gene is provisional. To our knowledge, so far only one homozygous missense variant in *BVES* has been identified in a family with cardiac arrhythmia and late-onset limb-girdle muscular dystrophy. Here we present the first patient who harbors a homozygous nonsense variant in *BVES* and provide clinical information for this consanguineous multiplex pedigree with two affected siblings. Segregation analysis confirmed heterozygosity for the *BVES* variant in both parents. The variant is absent from non-public and public databases such as the genome aggregation database (gnomAD). Affected patients pre-

sented with cardiac arrhythmia with second/third degree atrioventricular block and sick sinus syndrome, elevated levels of serum creatinine kinase.

P-ClinG-120

*** Lessons from exome sequencing in prenatally diagnosed congenital heart defects—a basis for prenatal testing

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Objectives: We aimed to illuminate the clinical utility of exome sequencing (ES) in cases with prenatally diagnosed congenital heart defects (CHDs).

Methods: In the present study, we retrospectively analysed the diagnostic yield as well as the percentage of non-conclusive findings and incidental findings in 30 cases with prenatally diagnosed CHDs in which we performed ES. In most cases, ES was done as parent-child trios.

Results: A definite genetic diagnosis was established in 20% (6/30) of cases and potential causative findings were reported in 6.7% (2/30). Non-conclusive results were found in 4 out of 30 cases (13.3%). Incidental findings were reported in 10% (3/30) of cases. There was a phenotypic discrepancy between the reported prenatal and postnatal findings, in 40% (8/20) of individuals. However, none of these additional, postnatal findings influenced the genetic diagnosis.

Conclusions: Our study shows that ES in prenatally diagnosed CHDs results in a comparably high diagnostic yield. There was a significant proportion of cases in which incidental findings and variants of unknown significance in known disease genes were found, as well as potentially pathogenic variants in novel disease genes. These kind of findings can bedevil genetic counselling and decision making for pregnancy termination. Our findings also contribute to the range of prenatal findings in genetic disorders, which are unknown in most cases.

P-ClinG-121

Identification of a mutation in TFAP2A in a fetus with polycystic degeneration of the kidneys

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Congenital anomalies of the kidney and urinary tract (CAKUT) are a relatively frequent prenatal diagnosis with an incidence of 3–6 in 1000 in live and stillborn infants and describe a broad spectrum of different malformations with renal agenesis or renal dysplasia/polycystic degeneration of the kidneys. Most common monogenic causes of severe early polycystic degeneration of the kidneys, prenatally or in early childhood, are mutations in *PKHD1* or *HNF1B*. Furthermore, a number of syndromic diseases such as ciliopathies including Bardet Biedl syndrome, are reported to cause multicystic kidney dysplasia.

We present the case of an 18 week fetus of healthy, non-consanguineous parents. Prenatal ultrasound showed extensive cystic degeneration of both kidneys and Potter's sequence. Predicted poor prognosis led to termination of the pregnancy. No other malformations were detected by ultrasound or postmortem. Trio exome sequencing in the fetus revealed the heterozygous de novo missense variant c.763A>G (p.(Arg255Gly)) in *TFAP2A*,

previously described as disease causing in a patient with branchiooculofacial syndrome.

Branchiooculofacial syndrome (BOFS) is caused by heterozygous germline mutations in transcription factor AP alpha 2 (*TFAP2A*). BOFS is characterized by growth retardation, branchial sinus defects, cleft lip with or without cleft palate, ocular anomalies and ear malformations. In addition, limb and renal malformations have been described as facultative symptoms. Accordingly, *TFAP2A* is widely expressed and plays a significant role in embryonic/fetal development.

To the best of our knowledge this is the first description of prenatal renal cysts as a leading symptom of BOFS. We conclude that the clinical spectrum of mutations in *TFAP2A* is broader than previously described. Prenatal testing via panel or exome sequencing in cases with multicystic kidney dysplasia is highly relevant for peri-/postnatal care but also family planning.

P-ClinG-122

Detection of pericentric inversion in DMD by Whole Genome Sequencing

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The dystrophinopathies, namely Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) are rare, x-linked, recessive muscular dystrophies caused by mutations in the large dystrophin gene (*DMD*). Standard diagnostics consists of multiplex ligation-dependent probe amplification (MLPA) and sequencing of all coding exons. The most common types of mutations are large deletions, point mutations and duplications. Although *DMD* is well studied, 2% to 7% of patients do not receive a molecular diagnosis. This could be due to deep intronic mutations that affect splicing or due to other types of structural abnormalities apart from CNVs such as inversions, which are a very rare cause of DMD.

Here, we present the case of a young boy with clinical signs of DMD and high elevated CK levels. His brother was also suffering from muscular dystrophy and a lack of dystrophin was proven in his muscle tissue. No large deletions or duplications nor other mutations in the coding sequence were detected in the index patient. His brother had no genetic diagnosis either. Since no muscle tissue of the patient was available for RNA analysis to search for effects on splicing, we sequenced the whole genome (WGS) of the patient using a Nextera library preparation (Illumina). We analysed only the sequencing data of the complete *DMD* gene using the software GenesearchNGS (PhenoSystems). Few rare intronic variants in *DMD* were detected but *in-silico* predictions did not produce distinctive results. Therefore, we analysed the coverage data of the *DMD* gene and detected a seemingly large deletion (5,4 kb) in the intron 44. A closer look at the alignment showed hybrid-reads that could be found at the beginning and end of the deletion, consisting of partial *DMD* sequence and an unknown sequence. A BLAST-search of the unknown sequences from both ends of the alignment gap mapped to a genomic position at Xq13.3 only a few nucleotides apart from each other. The orientation of the sequences led to the assumption of an inversion. Sanger sequencing of the breakpoints confirmed the inversion. As the inversion's breakpoints are located in the intron 44 of *DMD* (Xp21.1) and a genomic region in Xq13.3, it represents a pericentric inversion affecting a large portion of the c-terminal dystrophin. Hence, the detected inversion is very likely the cause of the patient's phenotype. To sum up we present the case of a boy suffering from DMD due to a pericentric inversion of the X chromosome including *DMD*. Pericentric inversions are a very rare cause of DMD and only few cases are reported in the literature. To the best of our knowledge, we present the first case of a *DMD* inversion that was detected by whole genome sequencing.

P-Complex Diseases Population and Evolutionary Genetics and Genetic Epidemiology

P-Compl-123

Barrett's esophagus–functional annotation of genetic associations using tissue-specific gene expression and the FUMA platform

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Barrett's esophagus (BE) is defined as a condition of metaplasia in which the normal stratified squamous epithelium of the esophagus is replaced by metaplastic columnar epithelium. Its public health importance relates to its association with esophageal adenocarcinoma (EA), a cancer with a mortality rate greater than 85%. The etiology of both BE and EA is multifactorial, and genetic analyses have identified 17 risk loci so far. All risk loci are located in non-coding genomic regions. Therefore, our understanding of the underlying biological mechanisms of these associations is incomplete. Functional data on the underlying risk genes and on disease pathomechanisms are highly warranted to improve the understanding of BE pathophysiology, and to gain insights into biological mechanisms that promote the progression from healthy esophageal epithelium into BE towards EA. Here, we aimed at (i) characterizing BE/EA genetic associations using eQTL data from two relevant tissues, and (ii) investigating the biological function of eQTL genes.

For this purpose, we generated two eQTL datasets from tissue biopsies derived from healthy gastric cardia tissue, the region where the lower esophagus merges into the stomach ($n = 148$ samples), and from esophageal metaplastic tissue ($n = 83$ samples). As an important feature, these eQTL datasets represent healthy and metaplastic states, respectively, rendering a systematic investigation of biological pathways involved in the development of BE possible.

The eQTL analysis revealed 145,011 significant cis eQTLs in cardia tissue and 50,517 significant cis eQTLs in metaplasia tissue. We cross-referenced the eQTL data with genetic association data of the BE/EA meta-analysis. Here, we identified cis eQTL effects for genome-wide significantly associated BE/EA risk variants on 2p24 regulating the expression of *C2orf43/LDAH* in cardia tissue. In metaplasia, only suggestively associated variants ($P < 5 \times 10^{-6}$) were identified as cis eQTLs, with regulating effects on *SMPD2* on 6q21 and *NADSYN1* on 11q13.

Using the GENE2FUNC function of the FUMA (Functional Mapping and Annotation of Genome-Wide Association Studies) web application, we aimed at obtaining further insights into putative biological mechanisms involved in the development of BE. For this, we performed gene set en-

richment tests with genes that showed eQTL effects (eGenes) in (i) cardia tissue only ($n=2,557$), (ii) metaplasia tissue only ($n=1,028$), and (iii) both tissues ($n=585$). Interestingly, we observed an enrichment of metaplasia eGenes in immunological pathways, whereas cardia eGenes revealed an enrichment in metabolic processes. eGenes that are expressed in both tissues revealed enrichment in pathways involved in degradation of the extracellular matrix.

In summary, the present study generated two unique eQTL datasets from cardia and metaplasia tissue to obtain a better understanding of genes and pathways that are involved in the development of BE. Our results suggest specific biological pathways playing a role in healthy and disease state of the relevant tissues that are involved in the pathophysiology of BE.

P-Compl-124

Analysis of the allelic spectrum of the newly identified schizophrenia risk locus Xq28 using high-throughput, multiplex targeted sequencing

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Schizophrenia (SCZ) is a severe neuropsychiatric disorder with an estimated heritability of 60–80%. Gender-specific differences between male and female patients are well known and include differences in the age-of-onset, clinical course and outcome, and a second incidence peak for women after the entry into menopause.

Copy number variants (CNVs) in specific chromosomal loci are well established risk factors for SCZ. Recently, the CNV Working Group of the Psychiatric Genomic Consortium published the world's largest genome-wide CNV analysis, to date. This impressive analysis encloses the data from 21,094 SCZ cases and 20,227 controls. In total, genome-wide significant evidence was obtained for eight loci, and suggestive support was detected for further 8 loci.

The latter included the first risk-associated locus ever reported on the X chromosome. Duplications in Xq28, distal confer risk to SCZ in both genders (odds ratio=8.9). In the literature, duplications of distal Xq28 have been described in association with developmental delay both in female and male patients. So far, 26 duplication carriers are characterized clinically of which 12 individuals are reported to have behavioural or psychiatric conditions.

The SCZ-associated risk locus Xq28, distal spans eight genes: F8, FUNDC2, CMC4, MTCP1, BRCC3, VBP1, RAB39B and CLIC2. Due to shared genomic breakpoints of the duplications identified so far, it is unclear which gene/s in this locus is/are relevant for the development of SCZ. The aim of this project was to analyse the allelic spectrum of the genes located in newly identified SCZ risk locus Xq28, distal, using high-throughput Next Generation Sequencing. The identification of an increased burden of rare and potentially pathogenic mutations in the patient cohort compared to the control cohort in any of the eight candidate gene/s, will

be additional evidence for the involvement of this gene/s in the pathogenesis of SCZ.

Single molecule molecular inversion probes were designed for all protein coding regions of the implicated genes (in total 61 exons and their flanking sequences). Using an Illumina HiSeq2500, the genes are currently sequenced in 2,000 patients with SCZ and 2,000 population-based controls. Subsequently, statistical gene-burden analyses will be performed. The full results will be presented at the upcoming conference.

P-Compl-125

Challenges in analysis of antihypertensive exposures and birth defect rates in women with chronic hypertension: an evaluation of the German Embryotox Database

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Introduction: Treatment of hypertension during pregnancy is essentially different from treatment in non-pregnant women. Since Cooper et al. observed an increased risk for congenital malformations after first trimester use of angiotensin-converting enzyme inhibitors (ACEIs) in 2006, a new debate on the safety of antihypertensive drugs (AH) during the first trimester arose. Various studies reported increased risks for (specific) birth defects in women with hypertensive disorders or after use of AHs. To assess possible teratogenic effects we analyzed AH exposure patterns and birth defect rates after use of common AHs.

Methods: 1501 prospective requests to Embryotox from 2000–2015 related to treatment of hypertension were included. Of these, 1152 pregnancies with completed follow-up were analyzed for AH exposure pattern including preconceptional AH use, discontinuation or switching of AHs and number of concomitantly used AHs. In addition, birth defect rates after first trimester exposure to commonly used AHs (i. e. methyldopa ($n=261$), metoprolol ($n=215$), bisoprolol ($n=203$), ACEIs ($n=329$) and angiotensin receptor blockers (ARBs, $n=215$) were determined in separate analyses.

Results: Nearly 40% of hypertensive women were treated with renin-angiotensin system (RAS)-inhibitors before conception. For these, the number of concomitant antihypertensive drugs was higher than for women without RAS-inhibitors. In general, changes in antihypertensive treatment resulted in a reduction of the number of concomitantly used AH and a shift towards drugs of choice like methyldopa and metoprolol. Rates of major birth defects were generally higher in hypertensive pregnancies compared to non-hypertensive, but a significant increase in major birth defects was only observed in the largest sub-sample of ACEI exposed pregnancies. The risk of birth defects seemed to increase with ARB treatment duration within the first trimester, although pregnancies exposed later than 20 gestational weeks had been excluded.

Conclusion: Treatment changes from non-recommended to recommended AHs during first trimester reflect the well-known proportion of unplanned pregnancies. Chronic hypertension or unmeasured confounders may have contributed to the observed higher risk of birth defects after exposure to individual AHs. However, it is difficult to disentangle the treatment effect from the effect of the underlying maternal condition. Possible effects of gestational age at treatment discontinuation or switching to other AHs as well as concomitant AHs have to be investigated more in-depth in future studies.

P-Compl-126

WES and large-scale resequencing in cleft lip with/without cleft palate patients provides further evidence for a causative role of CDH1

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Nonsyndromic cleft lip with or without cleft palate (nsCL/P) belongs to the most common congenital malformations and has a multifactorial etiology. To date, 39 risk loci have been identified for nsCL/P, explaining about 25% of its heritability. We hypothesize that some of the remaining genetic liability is explained by rare dominant de novo mutations.

In order to identify such rare de novo events, we performed whole exome sequencing (WES) in 50 nsCL/P patients and their unaffected parents. This resulted in 33 rare de novo events in 33 genes. Among these 33 candidate genes is *CDH1*, a gene that is already well known to be associated with nsCL/P. A de novo in-frame deletion in *CDH1* was identified in one of the 50 index patients in the WES.

To find further support for a contribution of the 33 candidate genes to nsCL/P, and to strengthen our hypothesis of dominant de novo events adding to nsCL/P etiology, the candidate genes were subjected to a multiplex resequencing study with single molecule molecular inversion probes (smMIPs). The independent resequencing cohort comprised nsCL/P patients and controls of Arabian, Mexican and European ancestry ($n_{\text{cases}} = 1,013$, $n_{\text{controls}} = 1,591$). The assay was designed using the MIPgen pipeline and was successful for 32 genes. Libraries were sequenced on HiSeq 2500 v4 with 2×125 bp read length. Raw reads were aligned with BWA and variants were called with UnifiedGenotyper. Downstream analysis included filtering for CADD ≥ 15 and MAF $\leq 0.1\%$. Only those variants not present in any of the controls were used for further analysis which included manual inspection of reads and segregation analysis.

When we checked for variants found in *CDH1* in the resequencing cohort, 8 further rare missense variants and 1 rare stopgain in *CDH1* were identified, none of them present in any of our controls. The stopgain in *CDH1* was identified in an Arabian patient, but the inheritance status of the variant could not be checked as no parental DNA is available.

Of note, the stopgain is located in Exon 11 of 15 and leads to a loss of the cytosolic binding domains of the CDH1 binding partners CTNND1 and CTNNB1. This protein complex is part of the epithelial adhesion complex and was recently shown to lead to nsCL/P when disrupted in a mouse model. However, as this stopgain is not located in the last exon, nonsense mediated decay and haploinsufficiency are probably more likely than a truncated protein to result from this mutation.

In summary, our WES and our resequencing approach support the previously described contribution of rare *CDH1* variants to nsCL/P development. Further analyses of our data are on the way, aiming at identification of rare de novo/co-segregating variants in genes unknown to cause nsCL/P yet.

P-Compl-127

Identification of rare high penetrance variants at bipolar disorder risk loci using next generation sequencing

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Bipolar disorder (BD) is a severe psychiatric disorder with a lifetime prevalence of around 1–2% and a high heritability of around 70%. The disease is mainly characterized by the alternation of depressive and (hypo-) manic episodes, which affect perception, emotion and social behaviour. Recently, the Psychiatric Genomics Consortium (PGC) conducted the largest genome-wide association study of BD to date comprising 29,764 patients and 169,118 controls. In this study, 30 independent genomic loci with genome-wide significance were identified. As common variants with small effects only explain a part of the genetic causes of BD, rare, highly penetrant variants, which cannot be detected by the application of current genotyping techniques, have been suggested to contribute to the development of BD.

The aim of the present study was to identify rare sequence variants in protein coding genes that are located at the 30 genome-wide significant BD risk loci. To prioritize the candidate genes, we systematically analyzed the 30 loci using publicly available platforms including the UCSC Genome Browser. In eight of the 30 loci, there was only one protein coding gene within a 200,000 bp window up- and downstream of the lead SNP. The eight implicated genes (*ADCY2*, *ANK3*, *CACNA1C*, *FSTL5*, *GRI-N2A*, *RIMS1*, *SHANK2*, *THSD7A*) were selected for resequencing. Using data generated by the Genotype-Tissue Expression (GTEx) project, we further investigated whether the lead SNPs had expression quantitative trait (eQTL) effects in brain tissue. We found eQTL effects of the associated SNPs for 12 genes (*ADD3*, *ASB16*, *CDAN1*, *CILP2*, *GLYCTK*, *GNL3*, *HAPLN4*, *LMAN2L*, *NMB*, *PACS1*, *PLEKHO1* and *PPM1M*). Interestingly, *ANK3*, *CACNA1C*, *GRIN2A*, *RIMS1* and *SHANK2* are already known to be involved in synaptic processes.

The 20 selected genes were investigated by resequencing in 1000 BD patients and 1200 controls of German ancestry. For next generation sequencing, we chose the single molecule molecular inversion probes (smMIP) method which enables multiplex targeted resequencing in large case-control cohorts. The primer sequences were designed using the MIPGen software (Boyle et al., 2014). A test run was accomplished to determine how each of the primers performed, and the performance was then optimized in a rebalancing run. The sequencing protocol was started with an optimized smMIP primer pool that covers all exons of the candidate genes and ± 6 bp flanking sequences. Resequencing is currently being performed

on the Illumina HiSeq2500. In addition to the investigation of single variants, we will also perform gene burden tests. The results will be presented at the upcoming congress.

P-Compl-128

*** The highly pleiotropic variant rs2168518:G>A in the seed region of miR-4513 is associated with age-related macular degeneration (AMD) at genome-wide significance

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Purpose: Investigations into complex diseases represent a challenging task due to their complex nature of disease risk and pathology, their high prevalence as well as the frequent lack of treatment options. Therefore, several studies have highlighted the potential of microRNAs (miRNAs) as innovative therapeutic applications based on their functional role in human pathologies. Recently, the genetic variant rs2168518:G>A within the seed region of hsa-miR-4513 has been associated with AMD, blood lipid levels and blood pressure, although with only marginal significance. Seed regions of miRNAs play a crucial role in target gene recognition and binding. Variations in these important regions may lead to altered gene expression and therefore may affect corresponding phenotypes. So far, the biological function of rs2168518:G is unknown, as well as the impact of risk variant rs2168518:A.

Methods: We performed the association of rs2168518:G>A with late-stage AMD in a dataset of 63,155 individuals, including the currently largest data set of a genome-wide association study for AMD from the International AMD Genomics Consortium (IAMDGC) and in the Genetic Epidemiology Research on Aging (GERA) Cohort. We further performed subgroup analyses of different AMD subtypes, as well as different age and gender groups within the IAMDGC data set. To investigate pleiotropic effects of rs2168518:G>A we calculated associations with 40 different phenotypes in the electronic medical records and genomics (eMERGE) data set, containing 55,029 individuals.

Results: We replicated the findings from previous studies and report rs2168518:G>A to be associated with late-stage AMD with genome-wide significance. Moreover, subgroup analyses revealed that rs2168518:G>A is mainly associated with the choroidal neovascular form of late stage AMD. A stronger association of rs2168518:G>A with AMD could be detected in individuals under 75 years of age, compared to those over 75 years, and in males in contrast to females. Investigations addressing the pleiotropic effect of rs2168518:G>A revealed that 24 out of 40 phenotypes within the eMERGE data set are associated with this genetic variant, including e.g. blood lipid levels, blood pressure, several eye phenotypes as well as cardiac diseases.

Conclusion: Taken together, the seed polymorphism rs2168518 is genome-wide associated with late stage AMD, especially with its neovascular form. The high pleiotropic effect of this polymorphism is remarkable and highlights the outstanding role of this miRNA in a large spectrum of different traits and diseases.

P-Compl-129

Multiplex targeted sequencing in schizophrenia candidate genes

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Schizophrenia (SCZ) is a multifactorial disorder with a complex genetic architecture. Common variants in more than 150 genomic loci surpass the threshold for genome-wide significance and are therefore associated with the disorder. Genes in which these variants are located are strong candidate genes for SCZ.

It is now an important and logical step to screen these susceptibility genes for causal variants with higher penetrance. The identification of such rare variants would extend the allelic spectrum of the gene and thus provide additional evidence for its relevance in disease development. Furthermore, the identification of potentially causal variants with higher penetrance is important for functional analyses (e.g. zebrafish studies, functional analyses in induced pluripotent stem cells).

Therefore, the aim of the present study was to sequence selected candidate genes for SCZ in order to further assess their relevance for the disorder and to identify rarer variants for functional follow-up analyses.

All genes in which a genome-wide significant single nucleotide polymorphism was directly located, were considered for our analyses. As the aim of our study was to identify rare, potentially damaging variants, we restricted our sequencing efforts to genes that were *in silico* predicted to be intolerant to mutation (Residual Variation Intolerance Score ≤ 10). In total, 31 genes fulfilled the study inclusion criteria. These included: *CACNA1C*, *CACNA1I*, *CSMD1*, *CUL3*, *GRIN2A*, *SATB2*, *STAG1*, and *ZNF536*.

For all protein coding regions of these 31 genes, single molecule molecular inversion probes were designed. Using an Illumina HiSeq2500, the genes were analyzed in 2,000 patients with SCZ and 2,000 population-based controls. Currently, we are performing statistical gene-burden analyses. The full results will be presented at the upcoming conference.

P-Compl-130

Livedoid vasculopathy—genetic and non genetic risk factors

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Background: Livedoid vasculopathy (LV) has been revealed to be associated with hypercoagulability. However, relevant genetic and exogenous thrombophilic factors are not fully determined.

Objective: To evaluate the frequency of hyperhomocysteinemia (HHCE) and MTHFR-genotypes in LV patients, to clarify the association with further hypercoagulative factors.

Material & Methods: The plasma homocysteine level was measured in 42 LV patients. Polymorphism of MTHFR 677C>T and 1298A>C, -675 5G/4G and PAI1 -844A>G, F2 20210G>A, F5 Leiden mutation and biochemical parameters for hypercoagulability were analyzed.

Results: 62% of LV patients revealed a mild HHCE. Polymorphisms of MTHFR were observed in 75% and 56% of the patients with and without HHCE. The polymorphism 4G in PAI1 was identified in patients with (100%) and without (83%) HHCE. All LV patients with renal failure had mild HHCE. High comorbidities of hypertension (99%) and diabetic type 2 (44%) were noted.

Conclusion: HHCE seems to play a major pathogenetic role in LV patients. High prevalences of further procoagulative factors might support the view that LV is a "complex disease".

P-Compl-131

Design of a candidate smMIPS sequencing study in patients with hereditary angioedema of unknown cause (U-HAE)

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Hereditary angioedema (HAE) is a rare, potentially life-threatening disease with an estimated prevalence of 1:50,000 that is characterized by recurrent episodes of subcutaneous or submucosal angioedema. Transmitted as an autosomal dominant trait, there are five types of HAE with known genetic defects. The most common forms, HAE-I and HAE-II, are caused by a broad spectrum of mutations scattered all over *SERPINE1*, resulting in decreased antigenic levels of C1-INH (HAE-I) or normal antigenic but decreased functional levels of C1-INH (HAE-II). HAE-III is caused by different mutations in exon 9 of *FXII* and explains about 20–25% of the cases with normal C1-INH. Two further forms of HAE have recently been linked to mutations in *ANGPT1* (HAE-ANGPT1, Bafunno et al. 2017) and *PLG* (HAE-PLG, Bork et al. 2017). Both genes broaden the concept of the underlying pathophysiology of HAE. While *PLG* underlines the relevance of the contact system and fibrinolytic pathway in the development of HAE, *ANGPT1* indicates that a vasculature pathway (angiopoietin/tie2 pathway) might play a role in the pathophysiology of HAE. Despite these successes in identifying causes of HAE, there still remains a significant fraction of patients and families where no causative mutation can be detected (HAE with unknown cause, U-HAE).

Therefore, to further elucidate the underlying pathophysiological causes of HAE, we conducted a systematic literature search and pathway analysis. Following this approach, we identified genes that are related to known disease causing genes and pathways in HAE, like *PLAT* and *PLAU*, encoding for two activators of plasminogen or *PRCP* and *HSP90AB1*, encoding for two F12 independent activators of the contact system. We further identified genes that are associated with bradykinin, like for example *ACE* and *MME*, two genes that are involved in the degradation of bradykinin as well as genes that are subsequently activated by bradykinin, like *TAC1*, a peptide which is known to cause vasodilatation. Altogether we selected 29 candidate genes, including the four genes with previously reported variants in order to validate the diagnose of U-HAE. Out of this 29 candidate genes we successfully designed a gene panel, using the single-molecule molecular inversion probes (smMIPs) technology. The panel is currently sequenced in our cohort of 121 HAE patients with normal C1-INH, whose clinical picture of HAE is not explained by mutations in *FXII* and are therefore considered patients with U-HAE.

The study will help to identify potential new disease genes and pathways that may play a role in the development or clinical heterogeneity of HAE. Sequencing is performed on a Illumina MiSeq platform, results will be presented at the upcoming conference.

P-Compl-132

Functional characterization of a regulatory element associated with alopecia areata

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Alopecia areata (AA) is a genetic complex hair loss disorder characterized by patchy areas of hair loss. The hypothesis that AA has an autoimmune background has already been supported by numerous genetic studies. However, to date the identified loci only explain a limited proportion of disease heritability, meaning that additional factors may play a role in the development of AA and other autoimmune diseases. The goal of the current project was to identify and to functionally characterize regulatory elements (REs) which might be of relevance for AA.

Based on data of a recent meta-analysis on AA, we performed a genome-wide in-silico analysis and mapped SNPs to predicted REs. We identified and further cloned a T-cell specific RE, which contains the identified lead SNP, into a pGL4.23 vector to perform luciferase-assays (LA) in T-cell specific Jurkat cells. Further, we analyzed the effect of the reference and alternative allele on gene transcription in association with a minimal promoter or with the promoter of a predicted target gene. The SNP, fulfilling our criteria, was located outside the polymorphic and gene-dense HLA region in a predicted T-cell specific RE, which might influence the expression of the nearby AA candidate gene *IL2RA*. Besides AA, this gene has previously already been associated with other autoimmune disorders, such as multiple sclerosis, type 1 diabetes, systemic lupus and rheumatoid arthritis. Virtual 4C analysis showed structural interaction of the RE and the possible target gene *IL2RA*. Supportingly, computational analysis of CCCTC-binding factor sites revealed a co-localization of *IL2RA* and the RE in the same CTCF flanked domain. LAs of the cloned RE showed a significant decrease of gene expression compared to controls, most probably consistent with silencing properties of the investigated RE. Furthermore, gene expression was significantly suppressed after integration of the validated *IL2RA* promoter. However, introduction of the identified alternative allele led to significantly less suppression of the reporter gene. Through further fragmentation of the RE, we were able to localize the most relevant subunit concerning the silencing properties. A possible influence of the alternative allele on altered responsiveness to binding transcription factors is subject of current research.

Taken together, with our experiments we could characterize a silencing regulatory element and prove an allele-dependent effect of the examined regulatory region. Regulation of the *IL2RA* gene might play a relevant role in the development of regulatory and effector T-cells in AA, both known for their crucial role in the pathogenesis of autoimmune diseases.

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P-Compl-133

Sex, ADHD symptoms & CHRNA5 genotype influence reaction time but not response inhibition

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Attention deficit hyperactivity disorder (ADHD) often appears together with inappropriate reactions. As such, people showing symptoms of ADHD present an impairment of reaction time and response inhibition. These executive functions are influenced by nicotinic acetylcholine receptors (nAChR) as mediators of cholinergic signalling, and show differences between both sexes. We examined the effects of two functional polymorphisms rs3841324 (S/L) and rs16969968 (G/A) of the cholinergic gene *CHRNA5*, ADHD symptoms and sex on response inhibition/reaction time in the Stop Signal Task. This task reliably measures response inhibition, response latency and response variability. In the analyses, 183 Caucasian par-

ticipants (52.4% females) were included. We performed a multivariate analysis of covariance (MANCOVA) with a 2 sex (female vs. male) $\times$ 2 ADHD symptoms (high vs. low) $\times$ 2 rs16969968 (GG vs. GA;AA) $\times$ 2 rs3841324 (SS vs. SL; LL) mixed-design. In participants carrying the diplotype (SS_GG), men with ADHD symptoms responded faster, while men without ADHD symptoms were slower than women ($F = 5.313$; $p = 0.023$; $\eta^2 = 0.034$). Although explorative, this interaction on reaction time but not response inhibition extend previous findings, suggesting a moderating effect of ADHD symptoms in men carrying the CHRNA5 diplotype SS_GG and might inspire research on genotype- and gender-specific ADHD medication.

P-Compl-134

Elucidation of biological mechanisms underlying schizophrenia by leveraging comorbid conditions

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Individuals with psychiatric disorders such as schizophrenia (SCZ) have increased rates of autoimmune comorbidities such as inflammatory bowel diseases (IBD). On the other hand, psychiatric comorbidity in patients with IBD is well known. Furthermore, analyses based on large-scale genome-wide association studies have demonstrated a genetic correlation between SCZ and IBD. These cross-phenotype associations indicate that SCZ and IBD share common genetic pathways.

In order to identify and characterize disease-relevant molecular mechanisms that are common between SCZ and IBD, we performed RNA-sequencing in 500 patients with IBD, 500 patients with SCZ, and 500 population-based controls. Expression analysis was performed using the Lexogen QuantSeq 3'mRNA fwd library preparation kit. Sequencing was then conducted on a HiSeq2500v4 system in high-output mode using on average 10 million 50 bp single-reads.

Currently, we are (i) comparing the gene expression profiles of each patient cohort to the profile of the control cohort; and (ii) conducting differential gene expression analyses across diagnostic boundaries. The results will be followed up via pathway analyses in order to shed light on the underlying biological mechanisms. We hope that with gene expression analysis we can find an association with respect to SCZ-IBD genetic correlation. The analyses are ongoing and will be presented at the conference.

P-Compl-135

PAH gene variants: a two-level view into human population history

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Autosomal gene variants are rarely used for detailed population genetic studies. Common recessive disease mutations can provide information

on the migration history of human peoples, but the use of non-pathogenic autosomal variants for population genetics is hampered by occurrence of recombination and the difficulty to determine haplotypes over large gene regions. We and others previously showed that the regional distribution of PAH gene mutations causing the autosomal recessive disease phenylketonuria (PKU) reflects the history of European populations. High stability of haplotypes associated with ancient PKU mutations indicates that recombination is a rare event in the PAH gene. In order to further investigate the implications of these findings we embarked on a comprehensive mutation-haplotype study in PKU and control samples as well as large public whole genome datasets. Molecular haplotyping of 22 single nucleotide polymorphisms (SNPs) in the distal PAH gene by long-range PCR in conjunction with family analyses in European PKU patients and controls identified five distinct haplotypes that account for >97% of patient/control chromosomes. Extension of the study to >100 variants, and examination of available genomic data of world-wide human populations, primates, and Neanderthal/Denisova, revealed just six major haplotypes in four highly different branches that are found throughout the world and make up 96–99% of all PAH alleles in non-African world populations. Construction of a global evolutionary tree of PAH haplotype emergence indicates that the split into the distinct PAH haplotype branches is very ancient and dates back to the beginning of human development in Africa, long before the emergence of Neanderthal and Denisovan. Examination of the regional origin and haplotype association of the common European PAH mutations indicates that all major PAH haplotypes were present in various European regions in prehistoric times. PAH haplotype analysis allows unique insights both into world population history and the genetic constitution of PAH mutation founders living in definable regions many millennia ago.

P-Cytogenetics and CNVs

P-CytoG-136

Mosaic der(13;18) with additional deletions of chromosome 18q

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Mosaicism for unbalanced structural chromosome aberrations of autosomes is rarely described.

We report on a four-year-old girl with muscle hypotonia, mild developmental delay, craniofacial dysmorphism and a suspected GLUT1 deficiency. At the time of genetic consultation, the mother was pregnant for the second time. To provide a quick clarification of the girl's clinical symptoms and in regard to potential prenatal diagnostic testing, a next-generation sequencing gene panel analysis, including GLUT1 deficiency and differential diagnosis, was performed. This revealed a partial deletion of the TCF4 gene in 18q21.2 as well as a deletion of the NEDD4L gene in 18q21.3. The data suggested mosaicism of the deleted alleles with an amount of approximately 25%, confirmed by multiplex ligation-dependent probe amplification analysis. Heterozygous deletions and mutations in the TCF4 gene cause Pitt-Hopkins syndrome (OMIM #610954).

Further analysis including karyotyping and fluorescence in situ hybridization confirmed mosaicism, showing a cell line with a dicentric derivative chromosome resulting from a translocation involving chromosome 13 and 18 in 23% of all analysed metaphases. This translocation led to a terminal deletion of 18q and included an additional interstitial deletion in 18q12. Array-CGH analysis confirmed an interstitial deletion in 18q12.1 of approximately 6.5 Mb and a terminal deletion 18q21.2→qter of approximately 25 Mb including the genes TCF4 and NEDD4L, specifying breakpoints and sizes of both mosaic deletions.

Our results indicate a complex clinical picture consisting of Pitt-Hopkins syndrome signs including possible symptoms associated with chromosome 18q deletion syndrome (OMIM #601808) in the patient. To our

knowledge, no other case comprising both deletions was previously described.

The clinical features could differ due to size and position of the deleted regions as well as to mosaic level and distribution in different tissues. Our results suggest that the presence of mosaicism of the 18q deletions could explain the mild clinical features of the four-year-old girl. Since the chromosome abnormality occurred post-zygotic, no prenatal diagnostic testing in regard to the current pregnancy of the mother was conducted.

P-CytoG-137

Diagnostic yield of CNV analysis in 1000 clinical exomes

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Background: As technology evolved to detect genetic alterations, more and more forms of structural changes in the human genome were identified and investigated for their causality in relation to genetic diseases. The inclusion of copy number information in the process of evaluating NGS data in clinical routine allows using a single diagnostic platform to study genetic diseases.

Purpose: While many studies are investigating the sensitivity and specificity of tools for the detection of copy number variants (CNVs) from NGS data, we present here the true diagnostic yield of this method in a pure clinical routine diagnostic setting.

Methods: The probes of 1000 individuals affected by a range of genetic disorders were sequenced using the TruSight One panel (Illumina, 4813 genes) on the NextSeq550 platform (Illumina). Read-depth based CNV analysis was carried out by the VARVIS software (Limbus Medical Technologies GmbH, Rostock). Validation of clinically relevant CNVs was performed using qPCR, MLPA, or SNP array.

Results: In total, in 31 (3.1%) of the individuals a CNV was identified that was considered to be causative for the patient's phenotype. The size of the detected CNVs was between 176 bp and 12 mb. In 3 individuals, the combined analysis of CNV and single nucleotide variants in one gene led to the identification of a biallelic pathomechanism. Of 22 cases with a pathogenic CNV, for which both NGS and array have been performed, only one CNV was recognized in the array, but could not be detected in the clinical exome as the corresponding region was not covered. Of the remaining 9 cases, no array has been performed, since validation using qPCR or MLPA seemed to be more appropriate due to the size of the CNV.

Conclusion: Our results show that CNV screening enriches the clinical evaluation of clinical exomes and partially leads to the detection of changes that cannot be reliably detected in array diagnostics. Since chromosome analysis and array diagnostics are usually chosen as the first diagnostic steps, the additional clarification rate of 3.1% is an underestimate. A step-wise approach with NGS based methods that can identify most pathogenic changes in their potential as the last stage seems obsolete in this context, especially in the diagnosis of heterogeneous diseases.

P-CytoG-138

TBX4 single gene duplications as potential cause of non-syndromic autosomal dominant clubfoot

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Introduction: Mutations of genes in the TBX4-PITX1 pathway have been associated with congenital clubfoot (Alvarado et al. 2010). Loss of function TBX4 mutations (17q23.2) are associated with Small Patella Syndrome (SPS) (Bongers et al. 2004). By contrast microduplications of 17q23 of greater than 2 Mb down to at least 350 kb have been identified in families with idiopathic non-syndromic clubfoot, narrowing the pathogenic im-

pact down to three genes including TBX4 (Alvarado et al. 2010, Lu et al. 2012, Peterson et al. 2014).

Patients and Methods: A 28-year-old otherwise healthy young man presented with congenital idiopathic clubfoot of the left side. His mother, maternal aunt, grandmother and cousin are all affected by bilateral club feet. The patient's personal and family history points to a non-syndromic condition. We performed chromosome and microarray analysis on a 180 K-Chip (Agilent) and Sanger sequencing of the TBX4, PITX1, SOX9, HOXD10 and FLNB genes.

Results: We identified a 0.1 Mb duplication of the chromosomal region 17q23.2 comprising the entire TBX4 gene. We rated this to be a possibly disease-causing variant, initiated segregation analysis in the patient's family and referred the patient for extensive radiographic evaluation of the lower extremities, spine and palate. Sequencing of TBX4 and PITX1 furthered normal results. The sequencing of SOX9, HOXD10, FLNB, the radiographic evaluation and segregation were still in progress at the time of submission.

Conclusion: This is the first report of a patient with non-syndromic idiopathic clubfoot carrying a single gene TBX4 duplication. An in-depth work-up of the familial phenotypes and genotypes will show whether this duplication is a likely candidate for causing non-syndromic autosomal dominant clubfoot.

P-CytoG-139

Cryptic unbalanced translocations causing intellectual disability or congenital malformations can be surprisingly large

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Unbalanced reciprocal translocations are a frequent cause of intellectual disability and are usually diagnosed by conventional cytogenetic diagnostics. However, some of them can only be detected by e.g. FISH/MLPA analyzing the subtelomeric chromosome regions or by chromosomal microarrays (CMA) and are thus cryptic.

Here, we present three examples of cryptic unbalanced translocations detected by CMA after conventional cytogenetic analyses with normal results at our institute in the last year. In Patient 1, a 10 year old girl with developmental delay, low normal growth parameters and slight facial dysmorphisms, CMA detected a 3.6 megabase (Mb) terminal deletion of 21q and a 5.0 Mb terminal duplication of 2q. FISH demonstrated an unbalanced translocation and detected the corresponding balanced translocation in the mother who incidentally also carried a second, microscopically visible balanced translocation. In contrast to the first patient, the imbalances in patients 2 and 3 were surprisingly large: Patient 2 was a 10 month old boy with, amongst others, postnatal growth retardation, muscular ventricular septal defects, choanal atresia, laryngomalacia and dysmorphisms. CMA detected an 11.4 Mb terminal deletion of 10p and an 8.9 Mb terminal duplication of 13q. FISH demonstrated an unbalanced translocation and the corresponding balanced translocation in the mother. Patient 3 was a 15 year old boy with developmental delay, short stature, microcephaly, seizures, renal agenesis, cerebral palsy and dysmorphisms. CMA detected a 10.4 Mb terminal deletion of 9p and an 11.4 Mb terminal duplication of 12p. Further diagnostics of the family are ongoing.

Especially Patient 2 and Patient 3 are instructive examples of unbalanced translocations: Both carried imbalances of more than 10 Mb which theoretically should be detectable by GTG banding with 550 band resolution. However, in both cases the partial monosomies and trisomies are comparable in size and affect regions with very similar, maybe even identical banding patterns and thus escaped detection by banding methods. Our observations emphasize the importance of CMA not only in the detection of microdeletions and duplications, but also of large chromosomal imbalances in patients with intellectual disability or congenital malformations.

P-CytoG-140**Array-CGH first: Is a conventional chromosome analysis out in era of molecular karyotyping and NGS?**

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Karyotyping is a simple genetic tool to detect aneuploidies and larger chromosome rearrangements. Array-CGH points out smallest microdeletions and -duplications and next generation sequencing (NGS) rapidly increased genetic diagnoses in the last few years, especially in patients with intellectual disability. International guidelines in several countries advise starting with array-CGH then to proceed further to NGS. In particular, both techniques fail to detect balanced chromosomal translocations, inversions and mosaicism, karyotype can.

Here we report about five clinically affected patients with apparently balanced chromosomal abnormalities. All patients had undergone a detailed clinical examination and genetic counselling. Further array-CGH showed normal results of the specific breakpoints in all cases. Cumulative comprehensive molecular investigations were performed. 1: A girl with wrinkled skin, congenital cataract but normal development, revealed a X-Autosome translocation t(X;2). 2: This patient with developmental delay and muscular hypotonia showed a translocation t(6;14). 3: A boy showed a paracentric inversion 1q in addition to a Klinefelter-Syndrome. He displayed severe phenotype with hydrocephalus, spasms and neurological salience. 4: This patient had a pericentric inversion of chromosome 14 with epilepsy, hypotonia and developmental delay. 5: A girl with intellectual disability showed a balanced Robertsonian translocation between chromosomes 13 and 14.

Our cases suggest, if started with array-CGH first, balanced translocations would never have been found. Interestingly, additionally breakpoint analysis, performed in an external laboratory of case 1 and 2, showed probably cause for the phenotype.

Fact is, that array-CGH and NGS are major steps in genetic testing to solve unclear phenotypes. If no molecular reason is found, karyotype remains a powerful and cheap technique to provide right diagnosis for Mendelian disease, diagnosis of sex chromosome abnormalities and detection of possibly inherited familial translocations like our case 3 and 5.

P-CytoG-141**Is the 1p36 deletion syndrome (MIM #607872) a contiguous gene deletion syndrome or caused by haploinsufficiency of SKI?**

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Terminal deletions on the short arm of chromosome 1 belong to the most frequent terminal deletions in patients with developmental delay. Causative variants may vary significantly in size which in turn may have an effect on clinical presentation. Among the most common clinical features of 1p36 microdeletion are deep set eyes, growth retardation, straight eyebrows, microcephaly, developmental delay, hypotonia, seizures, cardiac defects, and cleft lip/palate. We report on a patient with a previously suspected diagnosis of 1p36 deletion syndrome. Molecular karyotyping did not reveal any copy number change that could have been considered as the cause of the phenotype. Mendeliome sequencing revealed a loss of function mutation in SKI. Missense mutations of this gene have been identified as a cause of Shprintzen-Goldberg syndrome (MIM #182212). Based on the comparison of our patient to individuals from the DECIPHER-database with a loss of functions variant in this gene we raise the question whether the 1p36 deletion syndrome is a contiguous gene deletion syndrome as commonly suggested or rather caused by haploinsufficiency of SKI. We compare clinical features of patients with different loss of function variants in SKI and compare them to 1p36 deletion associated phenotypes.

P-CytoG-142**Mosaic supernumerary marker chromosome 13 leading to 13q11q13.1 tetrasomy in a boy with developmental delay, expressive speech disorder, autism spectrum disease and additional hemophilia A**

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Introduction: A small supernumerary marker chromosome (sSMC) is a structurally abnormal chromosome most often lacking a distinct banding pattern and is rarely identifiable by conventional banding cytogenetic analysis. The application of newer molecular cytogenetic approaches has gained great importance in elucidating the origin and composition of an sSMC. The phenotypic effects of sSMCs depend on the size, genetic content, and the level of mosaicism. The presence of an sSMC causes partial tri- or tetrasomy, and 70% of the sSMC carriers are clinically normal, while 30% have phenotypic abnormalities. In 70% of the cases the sSMC is de novo.

Clinical report: We report on a 3-year-old boy with hemophilia A in whom developmental delay with expressive language disorder, ASD, hyperactivity and hetero-aggressive behavior was first noted at 2 years of age. His physical exam revealed normal anthropomorphic measurements (weight 75th PC, length 50th PC, head circumference 75th PC) without major facial dysmorphism or malformations.

Methods and Results: R-banded analysis of chromosomes from the patient's peripheral blood sample revealed an abnormal male karyotype with a sSMC in mosaic form: 47,XY+mar[6]/46,XY[19]. To elucidate the origin and composition of the marker chromosome microarray analysis was performed in an external laboratory and showed four copies of 14.078 Mb at 13q11q13.1 (19058717–33130933) probably in mosaic form leading to partial tetrasomy of the region. Subsequent FISH analysis with whole chromosome paint 13 (Metasystems Inc., Altlusheim, Germany) confirmed the origin as derivative chromosome 13. To further elucidate the structure of the sSMC as a possible isochromosome 13 with breakpoints at 13q13.1 FISH analysis with BAC-clones (Empire Genetics Inc., Cedarhurst, NY, USA) is in progress.

Conclusion: To our knowledge this is the first report of partial tetrasomy of 13q11q13.1 caused by a sSMC 13. Interestingly, only one case is reported in the literature so far with sSMC 13 and a 2.2 Mb duplication at 13q11q12.11 showing similar features as our patient including developmental delay, severe oral and verbal apraxia and ASD. Similarly, only a few patients with partially overlapping triplication of this region are listed in DECIPHER: all share as main features developmental delay and delayed speech and language development. Our case demonstrates that sSMC can be correlated with phenotypic abnormalities even in low mosaic form. For the detection of these markers and their origin, karyotyping, FISH technique and microarray analysis should be performed supplementally.

P-CytoG-143**Chromosomal rearrangements and CNVs identified in craniosynostosis cases**

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Craniosynostosis, the premature fusion of one or more cranial sutures, is a common birth defect (approx. 1:2500 newborns) occurring either as an isolated malformation or in a syndromic context. Causes for craniosynostosis can be environmental influences or genetic changes including point mutations or chromosomal aberrations. Single nucleotide variants

(SNVs)/mutations in known craniosynostosis genes i.e. *FGFR2*, *FGFR3* and *TWIST1* which are detectable by sequencing approaches account for only 20% of craniosynostosis cases. Besides these SNVs, a significant subgroup (15%) of the craniosynostosis patients with additional clinical features i.e. syndromic craniosynostosis show chromosomal rearrangements. Due to the small number of patients with identical chromosomal aberrations reported in the literature, it is often difficult to determine the critical region or gene which contributes to the phenotype and perform a specific diagnostic analysis. Thus, high-resolution array CGH was chosen as our diagnostic genome-wide CNV screening approach for identification of CNVs in a cohort of 33 clinically well characterized craniosynostosis patients.

In total we detected likely pathogenic CNVs in 18% of our cohort. These CNVs encompass known craniosynostosis genes like *MSX2* and *TCF12* and novel craniosynostosis candidate genes, respectively. Three patients carried unbalanced translocations as confirmed by FISH and in three cases we detected with interstitial gains and losses. Clinically these patients presented with isolated as well as syndromic forms of craniosynostosis. Our results emphasize the importance of chromosomal rearrangements and CNVs as a genetic cause of craniosynostosis, particularly in case of unclear syndrome classification due to clinical variability. Furthermore, genes which reside in or are disrupted by rare CNVs represent candidate genes for craniosynostosis.

In addition to sequencing approaches CNV screening methods like array CGH should be included in the genetic testing algorithm for craniosynostosis patients. This will enable diagnosis in a significant portion of yet unsolved syndromic as well as non-syndromic cases.

P-CytoG-144

From human cytogenetics to human chromosomics

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The designation “chromosomics” was introduced by Prof. Uwe Clausen (30. April 1945–20. July 2008) back in 2005. Chromosomics is about chromosomes, their three-dimensional positioning in the interphase nucleus, the consequences plasticity of chromosomal subregions and genes interacting have, the influence of these chromatin-modification-mediated on functions of cells and even individuals, as well as about evolution and disease. Here it will be highlighted how the growing insights into human chromosome structure finally led to a “chromosomic view” on the three-dimensional constitution and plasticity of genes in interphase nuclei. The progress achieved in recent years includes e.g. the detection of chromosome-chromosome-interactions which, if damaged lead to malfunctions and diseases. However, chromosomics is not progressing in some parts at present, as research interest shifted from single cell to high throughput, genomic approaches. Chromosomics and its potential have been predicted correctly in 2005 by Uwe Claussen. Thus, at present reconsiderations of taking the chromosome and the single cell into human genetic research focus again, are urgently necessary.

P-CytoG-145

An interstitial 1.3 Mb de novo deletion containing the gene *NAA15* in a patient with intellectual disability and syndromic appearance

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Chromosomal abnormalities are well-known causes of isolated or syndromic intellectual disability. Although chromosomal deletions are frequent causes of intellectual disability, each deletion can be unique or rare. It is often challenging to find sufficient clinical data on specific chromosomal deletions to offer comprehensive genetic counselling to the affected family.

We present a 3-year-old patient born to healthy non-consanguineous parents from Germany. He presented with primary microcephaly, developmental delay, short stature, dystrophy and a syndromic facial appearance. Conventional karyotyping in our patient was apparently normal. Array-CGH analysis revealed a 1.3 Mb deletion in 4q31.1 which was confirmed using quantitative PCR (qPCR). Both parents showed normal karyotypes and no deletion in 4q31.1 was identified using qPCR.

The 1.3 Mb deletion identified in our patient contains the genes *CCRN4L*, *ELF2*, *C4orf49*, *NDUFC1*, *NAA15*, *RAB33B*, *SETD7*, *MGST2* and *MAML3*. Heterozygous mutations in *NAA15* (OMIM 608000) are associated with intellectual disability, dysmorphic features, autism, and speech delay. Biallelic mutations in *RAB33B* (OMIM 605950) are associated with Smith-McCort-Dysplasia. The function of the remaining seven genes is not fully known.

We provide detailed clinical and genetic data on a patient with a 1.3 Mb deletion of 4q31.1 adding further information on alterations of *NAA15* and expanding the clinical and genetic spectrum of genetic variation concerning *NAA15*.

P-CytoG-146

Two new cases of interstitial deletion in 2q23.1 encompassing the gene *MBD5*

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Microdeletions of 2q23.1 involving the *MBD5* gene (OMIM 611472) and loss of function mutations of this gene cause *MBD5*-associated neurodevelopmental disorders (MAND). Patients carrying deletions encompassing the *MBD5*-Lokus show a variable phenotype including developmental delay, motor delay, severe speech impairment, seizures, dysmorphic features, autistic-like behavioral problems and muscular hypotonia. The size of the described deletions varies (38 kb to 19 Mb) with the smallest region of overlap contains only the *MBD5* gene. The protein encoded by *MBD5* is expressed in brain and is involved in transcriptional regulation interacting with e.g. *MECP2*. Patients with pathogenic variants in *MBD5* typically have a milder phenotype than patients harbouring deletions in 2q23.1 suggesting that haploinsufficiency of *MBD5* plays a critical role in the overall phenotype but other genes might also contribute to the clinical variability. Here we report two cases of microdeletion 2q23.1 with different deletion size. Case one is a 17 year old boy with intellectual disability and profound psychomotor delay. Since age of ten he lost the ability to walk. He also has severe language impairment, epilepsy, and a profound cachexia. Array CGH analysis revealed an interstitial deletion of 4.1 Mb of 2q22.3–2q23.3 which was confirmed by FISH analysis. Case two is a nineteen months old boy with developmental delay, epilepsy and hydronephrosis. After birth, hypotonia, feeding difficulties and a neonatal seizure occurred. He has a pronounced facial phenotype with narrow and short palpebral fissures, an epicanthus, long eyelashes, small nose, broad nasal bridge, low-set ears with large lobes, open-mouth with tented upper lip and a small chin. He also has small hands and feet with 2/3-syndaktyly. Array CGH analysis uncovered an interstitial 6.5 Mb deletion of 2q22.3–q24.1 which was confirmed in the child and excluded in parents by FISH analyses. Our cases clearly show that the phenotype of patients with 2q23.1 deletion syndrome can differ. The overlapping deletion region in our cases includes 12 genes encompassing the OMIM genes *MBD5*, *ORC4*, *EPC2* and *KIF5C*. The latter three genes are also strongly supposed to contribute to the clinical phenotype. In patient 2, the deletion region includes 11 more genes including the OMIM genes *CACNB4* and *NEB*, the *FMNL2* gene which is highly expressed in fetal brain and functions in morphogenesis, cytokinesis and cell polarity and the *ARL5A* gene which is a GTP binding protein and may play a role in embryonic development. Our cases suggest that further deleted genes might also influence the extended clinical phenotype associated with this deletion syndrome.

P-CytoG-147**Interactive online karyotyping to teach cytogenetics in undergraduate medical education**

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Teaching cytogenetics in undergraduate medical education is a demanding task. In most medical faculties cytogenetics is being taught by paper-based karyotyping using chromosomes which have to be cut from printed metaphases. Students consider this approach old-fashioned and outdated, and the handling of the chromosomes is cumbersome and error-prone. Therefore, we created an internet browser based online karyotyping module using an e-learning course development software (articulate storyline®). The chromosomes of a karyotype are presented as draggable objects and can be moved by mouse or by gestures when using a touch screen. To complete a karyotype the chromosomes have to be moved to the correct position of a karyogram form. The students submit the completed karyogram to the system. The karyogram is rejected if any chromosome has not been placed at its correct position, and the students have to correct the mistake before they resubmit the karyogram. If the karyogram has been accepted, the students have to write the correct karyotype. The karyotype is rejected if it is not written according to ISCN. A course containing seven different karyotypes including numerical as well as structural chromosomal changes was developed and was integrated in the learning management system ILIAS as a sharable content object reference (SCORM) module. In a practical lesson, which was attended by 150 students, each student accessed the course using his own mobile electronic device via WIFI. The students were assisted by cytogeneticists so that each student was able to complete at least one online karyotype. Thus, the online karyotyping course proved to be a robust and platform independent tool for teaching cytogenetics. The system may also be used to address different levels of cytogenetic skills and/or specific learning objectives such as complex karyotypes in acute leukemias, for instance, in graduate medical education and cytogenetic specialization. Supported by the eLearning Foederfonds of the Heinrich-Heine-Universität, Düsseldorf

P-CytoG-148**Atypical 15q Duplication Syndrome and the importance of chromosome analysis**Teichmann AC.¹, Zacher P.², Siebert G.¹, Scheduikat A.¹, Abou Jamra R.¹¹Institute of Human Genetics, University of Leipzig Hospitals and Clinics, Leipzig, Germany; ²Sächsisches Epilepsiezentrum Radeberg, Germany

The 15q Duplication Syndrome is characterized by severe muscular hypotonia in infancy, followed by global developmental delay, intellectual disability, autism spectrum disorder, and epilepsy including infantile spasms. If the duplication comprises two extra copies of 15q11.2-q13.1 (tetrasomy), than these typically arise by a *de novo* maternal isodiscentric 15q11.2-q13.1 supernumerary chromosome idic(15), also known as inverted duplication 15 (inv dup(15)).

We report on two patients, both with moderate intellectual disability and focal epilepsies. Additionally, the first patient, a 43 year old female, presented with behavioral abnormality, while the second, a 28 year old female, presented with ASD. Physical clinical examination was otherwise unremarkable.

SNP-array of the first patient revealed two additional copies of the region 15q11.1–15q13.2 (10.3 Mb) and one additional copy of the region 15q13.2–15q13.3 (1.9 Mb). In the second patient we identified two additional copies of the region 15q11.1–15q12 (7.8 Mb). Conventional chromosome analysis showed a supernumerary marker chromosome in both cases. In order to examine the conformation of the marker chromosomes, we performed FISH analysis by using the Prader-Willi-/Angelman-syndrome FISH probes. As expected, the first patient showed a dicentric chromosome, with two distal signals for the centromere 15 probe and in between two signals for the LSI-probe *SNRPN*. Surprisingly, in the second patient we observed a different staining pattern. The marker chromosome

exhibited two distal signals of the LSI-probe *SNRPN* and one centromere 15 signal in between, therefore an incomplete isochromosome 15.

This atypical 15q Duplication Syndrome shows that only conventional chromosome analysis displays the conformation of the supernumerary chromosome and its origin. The usual mechanism of formation of the bisatellited dicentric marker is a translocation event between homologs during maternal meiosis, due to apposition of low copy repeats (LCR) that are located within 15q11-q14. After formation, there is a non-disjunction event and a centromere inactivation. Maternal idic(15) has been *de novo* in all affected individuals reported to date. In contrast, isochromosomes can arise by a variety of mechanisms, at diverse times and places. The most classical and simple mode of formation is a misdivision at the centromere, which can be understood as a horizontal rather than a vertical division. Independent of the underlying mechanism, the process could occur amongst other things in a premeiotic gametocyte, which would lead to a gonadal mosaicism and therefore has a recurrence risk for further pregnancies. So only the conventional chromosome analysis in combination with FISH analysis reveals the structure of the triplication seen in the array-analysis and suspected as a marker chromosome. But more importantly, only by knowing the conformation of the marker chromosome an estimation of the recurrence risk is possible.

P-CytoG-149**Supernumerary ring chromosome 19 mosaicism in a child with epileptic encephalopathy**Vibe E.¹, Heinrich U.¹, Krimmel E.-M.¹, Wahl D.², Rost I.¹¹Center for Human Genetics and Laboratory Diagnostics Dr. Klein, Dr. Rost and Colleagues, Martinsried, Germany; ²Medical Practice for Genetic Counseling and Psychotherapy, Augsburg, Germany

We report on a 1 year old female patient with focal, corticomedullary dysplasia in the left brain, therapy-refractory epilepsy, global development delay, secondary microcephaly and strabismus. The girl is the second child of healthy, non-consanguineous parents. She was born at 41st week's gestation. At the age of eight months, she had first isolated muscle twitches. Her EEG had showed hypsarrhythmia.

In order to clarify the cause of the epilepsy and development delay, chromosomal analysis was performed from cultured peripheral lymphocytes where a ring chromosome 19 mosaic was detected. The girl had the additional ring chromosome in 19,6% of the peripheral blood cells and in 22,5% of the oral mucosa cells examined; ring chromosome mosaic was confirmed by fluorescence in situ hybridization (FISH). The final karyotype was described as 47,XX,+r(19)(p12q13.2)[17]/47,XX,+dic r(19)[1]/46,XX[17]. Maternal karyotype was normal. The father is presently being tested.

Subsequent micro-array analysis showed a 17,73 Mb duplication 19p12q13.2. The duplicated region contains 223 protein-coding genes (Gene Distiller 2014) including the *SCN1B* gene (OMIM 600235), which is regarded as a potential candidate gene for epilepsy. Voltage-gated sodium channels are essential for the generation and propagation of action potentials in striated muscle and neuronal tissues. In the literature, loss-of-function mutations in the *SCN1B* gene are associated with disorders such as Brugada syndrome and generalized epilepsy. An additional ring chromosome 19 contains another gene copy of the *SCN1B* gene. The effect of the triple dose of the *SCN1B* gene is not cleared.

Epilepsy has been less commonly associated with syndromes of ring chromosome. In the literature, only ring chromosome 20 and 14 are described. This case detected that a combination of conventional and molecular cytogenetic methods is helpful to explain the symptoms of the patient.

P-CytoG-150

Two maternal interstitial paracentric inversions causative for the formation of concurrent duplications and deletions in descendants

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Paracentric inversions occur after a two-break event on the same chromosome arm with a subsequent insertion of the rotated intercalary segment. These are rare intrachromosomal rearrangements, even scarcer are cases with duplications and/or deletions.

Here we report two cases of interstitial paracentric inversions with concurrent breakpoint deletions and duplications that resulted from a balanced paracentric inversion in one of the parents.

The first patient was a 23 months old girl with intellectual disability and developmental delay. After GTG-banding showed a normal female karyotype, array-CGH was performed and revealed a loss of 1.6 Mb in the chromosomal region 15q25.2 and a subsequent gain of 0.6 Mb in 15q25.2-q25.3. FISH using BAC clones confirmed the interstitial deletion in 15q25.2 and the duplication of 15q25.2-q25.3 on the derivative chromosome 15. Chromosomal analysis and FISH of the mother revealed a small interstitial paracentric inversion on chromosome 15 (inv(15)(q25.2q25.3)). The inversion was visualized by FISH, the normally more distal localized signal for 15q25.3 presented proximal to the 15q25.2 signal on the derivative chromosome 15. The father showed an apparently normal male karyotype (46,XY) and demonstrated no indication of a structural rearrangement in 15q25.2-q25.3.

The second patient, also a 23 months old girl, was presented to our institute because of cardiac defect and developmental delay. GTG-banding showed an abnormal karyotype with an interstitial deletion in 8p21-p23. Array-CGH revealed a gain of 4.4 Mb in the chromosomal region 8p23.3-p23.1 as well as a loss of 15.1 Mb in 8p23.1-p21.1. FISH with BAC clones confirmed the imbalance. To clarify the origin of these abnormalities, FISH was also performed on the parent's chromosomes. While the father had an apparently normal karyotype (46,XY), the mother demonstrated the aberrant karyotype: 45,XX,der(13;14)(q10;q10).ish inv(8)(p21.1p23.2). FISH signals for 8p23.1 and 8p21.2 were interchanged on one of her chromosomes 8, revealing an interstitial paracentric inversion that was not visible after GTG-banding. Additionally to this, she also carries a Robertsonian translocation which she did not transmit.

In conclusion: we demonstrated two cases of inverted duplications and deletions arising from a maternal paracentric inversion. The patient's aberrations were not or only in part visible after GTG-banding, only array-CGH in combination with FISH was able to reveal its full extent. The presented cases also underline the importance of additional analysis of the parents to determine the underlying mechanism of the chromosomal aberration, especially in regard of the risk of reoccurrence in further offspring.

P-Monogenic Disease from Gene Identification to Molecular Mechanism

P-MonoG-151

Cardiac Anomaly–Telangiectasia–BRachydactyly–Ectodermal Dysplasia (CATBRED) syndrome is caused by heterozygous de novo mutations in Protein Kinase D1

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We describe two unrelated patients that show similar clinical features including diffuse telangiectasia, brachydactyly, features of ectodermal dysplasia and a congenital heart disease. Using trio-based whole exome sequencing, we identified heterozygous *de novo* mutations in *Protein Kinase D1* (*PRKD1*) in both patients. *PRKD1* is a member of the Protein Kinase D (PKD) family of serine/threonine protein kinases and is involved in diverse cellular processes like cell differentiation and proliferation and cell migration as well as vesicle transport and angiogenesis. We identified the two missense mutations NM_002742.2:c.1774G>C, NP_002733.2:p.(Gly592Arg) and NM_002742.2:c.1808G>A, NP_002733.2:p.(Arg603His). Biochemical analysis using *in vitro* kinase assays with recombinant proteins showed that the mutation c.1808G>A, p.(Arg603His) represents a gain-of-function mutation encoding an enzyme with a constitutive, lipid-independent catalytic activity. The mutation c.1774G>C, p.(Gly592Arg) shows a defect in substrate phosphorylation representing a loss-of-function mutation. Previously, heterozygous *de novo* mutations in *PRKD1* have been described in 3 patients from a cohort of 610 patients with syndromic congenital heart defects (Sifrim *et al.*, 2016). The ectodermal features and the cardiac features of our patients are similar to those reported in two out of three patients carrying heterozygous *de novo* mutations described by Sifrim *et al.* (2016). The skeletal phenotype may have also been present in the patients from Sifrim *et al.*, although this cannot be clearly determined based on the available information. Telangiectasia however, has not been described yet. The present cases therefore represent a new syndrome, Cardiac Anomaly–Telangiectasia–BRachydactyly–Ectodermal Dysplasia (CATBRED), caused by heterozygous *de novo* mutation in *PRKD1*.

P-MonoG-152

A recurrent de novo mutation and a second variant of unknown significance in ZSWIM6 in a boy with severe intellectual disability, microcephaly, strabism and hyperopia

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Recently, a recurrent de novo nonsense variant (c.2737C>T [p.Arg913Ter]) in the penultimate exon of *ZSWIM6* was reported to cause intellectual disability and additional central and peripheral nervous system symptoms by Palmer *et al.* (Am J Hum Genet. 2017 Dec 7;101(6):995–1005), but not frontonasal or limb malformations, which was the phenotype initially discovered with a recurrent missense mutation (c.3487C>T [p.Arg1163Trp]) by Smith *et al.* (Am J Hum Genet. 2014 Aug 7;95(2):235–40.). We present the phenotype and genotype of a boy with severe intellectual disability, strabism, hyperopia, microcephaly and undescended testis. The molecular karyotype was normal. Exome analysis revealed the recurrent de novo

nonsense variant (c.2737C>T [p.Arg913Ter]) in the penultimate exon of ZSWIM6 and in addition a heterozygous missense variant (c.3119G>A [p.Arg1040His]) that was predicted by several tools to be probably pathogenic. This variant is present in dbSNP (rs192222164) and in gnomAD ($f=00.0003779$) and was inherited from the unaffected mother. The phenotype of our patient as evident until now fits into the spectrum described with the recurrent de novo nonsense variant. It remains to be elucidated whether the maternally inherited missense variant might be relevant in a possible recessive mode of inheritance. Anyway, the missense mutation of our patient complicated the interpretation of the mode of inheritance in our patient. Our findings widen the spectrum of genotypes and phenotypes associated with ZSWIM6.

P-MonoG-153

The homozygous variant p.(L126P) in *CACNB4* impairs nuclear targeting of the encoded β_{4b} subunit of PQ-type calcium channels and is associated with intellectual disability, epilepsy, movement disorder and cerebellar atrophy

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PQ-type channels are the principal presynaptic calcium channels functioning in rapid release of neurotransmitter in the central and peripheral nervous system. In brain PQ-type calcium channels are largely composed of the pore-forming $\text{Ca}_v2.1$ α_1 subunit and the auxiliary $\alpha_2\delta$ -2 and β_{4a} subunits. These auxiliary subunits regulate the amplitude, kinetics, and voltage-dependence of calcium currents. β_{4a} is encoded by *CACNB4* which is primarily expressed in the brain. Autosomal dominant variants in *CACNB4* have been proposed to be associated with epilepsy and episodic ataxia. *CACNB4* pre-mRNA undergoes extensive alternative splicing to produce multiple β_{4a} protein variants (β_{4ab} , β_{4cb} , β_{4db} , β_{4eb}) with isoform-specific subcellular localization and functions. For example, β_{4ab} and β_{4cb} play dual roles in channel modulation and gene regulation. In electrically inactive neurons β_{4ab} is targeted to the nucleus and regulates the transcription of multiple target genes.

Using whole-exome sequencing in a cohort of 450 pediatric patients with neurodevelopmental disorders we identified two siblings from a consanguineous couple who inherited the homozygous missense variant c.377T>C/p.(L126P) in *CACNB4*. The 15-year-old boy and the 22-year-old female had severe intellectual disability, psychomotor retardation, blindness, epilepsy, a dystonic and athetoid movement disorder and cerebellar atrophy. The variant was absent from gnomAD and predicted to have an impact on protein function by three *in silico* tools. To investigate the functional consequences of the *CACNB4* variant p.(L126P), we studied the corresponding rat β_{4ab} mutant L125P on association of this subunit with native channel complexes and activity-dependent nuclear targeting. V5-tagged β_{4ab} wildtype was associated with GFP- $\text{Ca}_v1.2$ in skeletal muscle triads and with endogenous channels in presynaptic boutons of hippocampal neurons. In contrast, V5-tagged β_{4ab} -L125P mutant completely failed to incorporate into the calcium channel complexes. Wild-type V5- β_{4ab} was specifically targeted to the nuclei of quiescent excitatory cells. However, the p.L125P mutation abolished nuclear targeting of V5- β_{4ab} in cultured myotubes and hippocampal neurons. Next, we co-expressed wild-type and mutant β_{4ab} with the neuronal $\text{Ca}_v2.1$ channel in tsA201 cells and investigated the whole-cell current properties. Surprisingly, the p.L125P mutation had no effect on current amplitude and voltage-dependence of $\text{Ca}_v2.1$ channel activation. These data indicate that the p.L125P mutation disrupts stable association of β_{4ab} with calcium channels, but has no effect on membrane incorporation and current modulation. In summary, our functional data suggest that the homozygous *CACNB4* p.(L126P) variant caused the severe neurological phenotype in the

two siblings, most likely by abolishing nuclear translocation of β_{4ab} due to impaired interaction with yet to be identified nuclear targeting proteins.

P-MonoG-154

*** A correlation between genotype, phenotype and cellular patho-mechanism in REEP1-associated axonopathies

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Degenerative genetic disorders that affect long axons are referred to as hereditary axonopathies. If they mainly affect upper motor neuron (UMN) axons they manifest as hereditary spastic paraplegia (HSP). Truncating mutations of the Receptor Expression Enhancing Protein 1 (REEP1) are a rather frequent cause of autosomal dominant HSP. Surprisingly, some REEP1 mutations rather manifest as a hereditary motor neuropathy at lower motor neuron (LMN) axons. REEP1 contains an N-terminally located ER-targeting domain, a more centrally located second hydrophobic domain that is thought to form a hairpin-like structure which dips into the ER membrane thus inducing ER curvature and a cytoplasmic C-terminal part. In vitro overexpression of protein variants associated with UMN disease suggest loss-of-function as disease causing, while LMN disease associated variants resulted in ER-derived REEP1 aggregates. We here show that the wild-type protein is prevented from forming protein aggregates because of its C-terminal interaction with microtubules, which is destroyed in protein variants associated with LMN disease. Confirming a loss-of-function mechanism for UMN disease REEP1 knockout mice show a reduced ER complexity. Currently we are analyzing a knock-in mouse model for a REEP1 variant causing LMN disease. Our findings so far suggest that the wide clinical spectrum associated with heterozygous REEP1 variants is explained by the action of two distinct patho-mechanisms: truncating variants confer a loss-of-function and act via haploinsufficiency which mainly manifests in upper motor neurons, while non-truncating variants in the C-terminus confer an aggregation mediated toxic gain-of-function which primarily affects lower motor neurons. Understanding the basis for the differential vulnerability may open up novel therapeutic strategies for hereditary axonopathies.

P-MonoG-155

Personalised therapeutic intervention guided by genetic diagnosis in early-onset movement disorders

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Background: Rapid progress has recently been made in the elucidation of the genetic basis of childhood-onset monogenetic movement disorders due to the power of genomic sequencing methodologies. This allows us now to investigate genotype-phenotype correlation and develop specific treatment approaches for genetically defined disease entities.

Objective: The objective was to systemically recruit patients with childhood-onset inherited dystonia and to elucidate the genetic basis and to investigate natural history as well as the therapeutic outcome to medication and deep brain stimulation of Globus Pallidus internus (GPi-DBS).

Results: We report six patients presenting with global motor retardation, reduced muscle tone and recurrent episodes of severe, hyperkinesia with dystonia, choreoathetosis, and ballism since early childhood. We identified GNAO1 in six patients, those following mutations were novel: splice site mutation (c.723+1G>T), novel missense mutation (c.610G>C; p.Gly204Arg) (Koy et al., 2018).

We have also identified three patients with childhood-onset IGD and hyperkinesia harbouring disease-causing mutations in KMT2B. In a progressive disease course, symptoms spread from lower limbs to generalized dystonia with dysarthria, dysphonia, intellectual disability, orofacial dyskinesia, as well as dysmorphic features. WES showed a mutation (c.7463A>G, p.Y2488C) localized in the highly-conserved FYRC domain likely to alter protein-protein interactions, leading to instability. Mutations leading to predictively unstable transcripts (c.3596_3697insC, p.M1202Dfs*22; c.4229delA, p.Q1410Rfs*12) are likely to be subject to degradation by non-sense mediated decay.

Discussion: GNAO1 codes for α subunit of Go (Go α), mainly expressed in the brain, modulating neurotransmitter release by mediating the presynaptic auto-inhibitory effect of several neurotransmitters on their receptors. By intervention with GPI-DBS the severe paroxysmal hyperkinetic exacerbations could be stopped in five patients harboring GNAO1 mutations. In the KMT2B patients, there was only limited benefit after GPI-DBS.

We hypothesize that deficient KMT2B proteostasis leads to altered signaling in pallidostriatal connectivity in the direct pathway of basal ganglia as motor performances improved and progression of dystonia symptoms slowed down after implantation of GPI-DBS. Pharmacotherapy with an anticholinergic medication (Trihexyphenidyl) distinctly reduced dystonia in two patients with KMT2B mutations.

Conclusion: In complex movement disorders of unsolved etiology, clinical WES can rapidly unveil pathogenic networks. If unmasked early, GPI-DBS and specific pharmacotherapy can be effective or even life-saving treatment options for patients with GNAO1- and KMT2B-associated early-onset movement disorders.

P-MonoG-156

Genomic landscape of glycosylation defects leading to secondary Dystroglycanopathies

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A frequent type of Muscular Dystrophy (MD) is caused by mutations in genes which are involved in the glycosylation pathway of α -dystroglycan (α DG), also called secondary dystroglycanopathies (α DGpathies). α DG has a particular O-mannosylation which is necessary for its function as a glycosylated extracellular matrix receptor in brain and muscle. α DGpathies, can lead to a wide spectrum of MDs including from a lethal Walker-Warburg-Syndrome (WWS) to milder Muscle-Eye-Brain disease and to the mildest Limb-Muscular Dystrophies (LGMD). Despite mutations in 18 genes for secondary DGpathies have been described, including the new identified DGpathy gene INPP5K (Osborn DPS, et al., 2017).

Our aim is to decipher the key genes involved in the maintenance and function of the ECM- α DG-dystrophin axis by discovery of dystroglycanopathy associated genes. Until now, 80 patients from 69 families mainly from Turkey (64%), Europe (14%), Saudi-Arabia (11%) or Brasil (9%) were included in our study. The average age of the patients is 6,4-years-old, starts by 6 month up to 39-years-old, most of them being symptomatic before 6 months of age. Consanguinity was present in 35 (35/80) families. The phenotype includes everything from the mildest LGMD with no MR to the

severe WWS. Molecular work-up included Mendeliome or whole exome sequencing including CNV analysis. In 86% of the cases we have found the disease causing mutation. Total mutations (solved cases/novel mutations) in POMT1 were the most prevalent ($n=12/2$) in our cohort, followed by POMT2 ($n=11/2$) and POMGNT1 ($n=11/1$), LAMA2 ($n=9/3$), FKRP ($n=6/2$), TMEM5 ($n=4/2$), ISPD ($n=4/1$), GMPPB ($n=4/2$), FKTN ($n=2/1$), POMK ($n=2/1$), INPP5K ($n=2/2$), POMGNT2 ($n=1$) and B4GAT1 ($n=1/1$). This lead to 21 novel mutations in 12 of the 18 known genes, including LAMA2, which is not a dystroglycanopathy gene, but its related to α DG. Our evaluations showed some remarkable cases in our cohort. Another patient showed LGMD and mirror movements with a novel homozygous missense mutation in POMK. It is known to be highly expressed in the human brain and muscle during early development and phosphorylates to the first mannose on O-mannosyl glycan of α DG (Ardici et al., 2017). We found a novel homozygous, frameshift mutation c.1144_1145delT (p.F382Sfs*23) in one of our patients. Knock out mutations in B4GAT1 result in early embryonic lethality (Wright et al., 2012), resemble our patient WWS phenotype.

Remarkably, we identified mutations in GMPPB, expanding the phenotype congenital myasthenic syndrome (CMS) combined with high CK-levels. (Wunderlich et al., 2018). This CMS is amenable for treatment with acetylcholinesterase inhibitors.

P-MonoG-157

Genotype-Phenotype correlations in novel form of PYROXD1-related congenital myopathy

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Recently mutations in the gene PYROXD1 (pyridine nucleotide-disulphide oxidoreductase domain 1) were shown in nine patients of five families to cause early onset myopathy with internalized nuclei and myofibrillar disorganization (Gina L. O'Grady, et al., 2016). Here we present two unrelated cases with mutations in PYROXD1.

The first case is a female patient from a consanguineous family. She walked at 12 months of age, but presented at six years of age with generalized weakness more proximal than distal, including facial weakness and swallowing difficulties but no ptosis or ophthalmoplegia. Cardiac function was normal. A muscle biopsy showed cores in the muscle fibers and a type 2 myofibers atrophy. An affected sibling, an older brother, died of cardiomyopathy at 14 years, the other affected sibling had severe weakness at 38 years of age and can still walk with support. Until now, she is the oldest affected patient with a PYROXD1 mutation, indicating a slow disease progression. Whole exome sequencing (WES) revealed a co-segregating homozygous missense variant in PYROXD1 (NM_024854, c.464A>G, p.N155S).

The second case is a male patient, only child of consanguineous parents from Turkey, who was diagnosed with congenital myopathy with normal CK levels. Muscle biopsy showed dystrophic changes with prominent interstitial fatty infiltration and marked variation in fiber size. Internal nuclei, mild endomysial fibrosis and type 1 atrophic fibers. WES lead to the identification of two variants in PYROXD1, a known missense mutation (c.464A>G, p.N155S) and a novel frameshift variant (c.329_332delTCTG, p.L112Vfs*8), confirmed by sanger sequencing. This patient is at the severe end of the clinical spectrum as to one of the PYROXD1 patient in Gina L. O'Grady, et al., 2016, which are non-consanguineous, Persian Jewish patients, with the founder mutation and a splice-site mutation.

The presented patients show an overlapping PYROXD1 phenotype, with mild limb girdle and facial weakness, nasal speech and slow progress. This phenotype is similar to patients with centronuclear myopathy without ophthalmoplegia. This second report strengthens the association of recessive mutations in PYROXD1 and early-onset myopathy with inter-

nalized nuclei. In human muscle and zebrafish myofibers immunolocalization studies demonstrate that PYROXD1 localizes to the nucleus and to the sarcomer. Surprisingly, a mild length-dependent axonal sensory neuropathy was also shown for PYROXD1 patient earlier and deep tendon reflexes were absent in all patients including ours. PYROXD1 is a nuclear-cytoplasmic pyridine nucleotide-disulphide reductase (PNDR). PNDRs are flavoproteins (FAD-binding) and might catalyze pyridine-nucleotide-dependent (NAD/NADH) reduction of thiol residues in other proteins. The underlying mechanisms leading to muscle degeneration remains elusive and represent a novel pathway.

P-MonoG-158

A novel strategy to find promising candidate genes for spermatogenic failure

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Infertility affects ~15% of couples in industrialized countries. Azoospermia, which refers to the complete absence of sperm in the ejaculate, affects ~15% of all infertile men. Despite it being the most severe form of male infertility, the majority of genetic causes for azoospermia remains unknown to date. Over 900 genes are suspected to be exclusively and ~2000 genes moderately to highly expressed in the testis. Employing a random candidate gene approach to find genetic causes for non-obstructive azoospermia (NOA) has therefore been tedious and largely unsuccessful, substantiating the necessity for more systematic approaches, e. g. un-biased genome-wide analyses like whole exome sequencing (WES) in combination with bioinformatic tools to prioritize genes. In this study, the focus was primarily on the most severe testicular phenotype of Sertoli-cell-only syndrome (SCOS) with a complete lack of germ cells.

In order to find promising new candidate genes for male infertility, different bioinformatic tools were applied to genomic data of 180 SCOS patients out of a well-phenotyped cohort of 517 male infertility patients on whom we had performed WES. All filtering was conducted using our in-house software Sciobase and a novel data analysis tool implemented as an R Shiny app. First, the patients' variants were filtered by minor allele frequency (MAF) in 1000Genomes, ExAc and gnomAD genomic databases, with MAF ≤1% for heterozygous and MAF ≤5% for homozygous variants. Variants were also filtered by predicted potentially severe consequences (stop gain, frameshift, predicted splice site effect), leaving 2361 genes. Next, tissue expression in testis of ≥50% compared to the tissue with highest expression was verified via GTEx Portal, resulting in 835 genes. Genes also harboring mutations in fertile controls were excluded, yielding 748 candidate genes for further bioinformatic analysis. Finally, gene set enrichment analysis was performed using the programs Ingenuity Pathway Analysis®, Reactome Pathway Database, DAVID, PANTHER, GOrilla, REVIGO, Enrichr, ToppFun and g:Profiler.

According to these tools, several potentially spermatogenesis-related biological processes were overrepresented in our gene set, e. g. anchoring of the basal body to the plasma membrane, cilium assembly, recruitment of mitotic centrosome proteins and telomere C-strand synthesis.

We are currently ranking the candidate genes by published evidence. Also, verification of mutations in fertile controls and additional NOA cohorts is being carried out, followed by functional analyses of the most promising new candidate genes for SCOS.

This work was carried out within the frame of the DFG Clinical Research Unit "Male Germ Cells: from Genes to Function" (CRU 326).

P-MonoG-159

Pathogenic variants of USP7 cause a neurodevelopmental disorder with speech delays, altered behavior, and neurologic anomalies

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Purpose: Haploinsufficiency of *USP7*, located at chromosome 16p13.2, has recently been reported in seven individuals with neurodevelopmental phenotypes, including developmental delay/intellectual disability (DD/ID), autism spectrum disorder (ASD), seizures, and hypogonadism. Further, *USP7* was identified to critically incorporate into the MUST complex (MAGEL2-USP7-TRIM27), such that mutation of *USP7* leads to altered endosomal F-actin polymerization and dysregulated protein recycling.

Methods: We report 16 newly identified individuals with heterozygous *USP7* variants, identified by genome or exome sequencing or by chromosome microarray analysis. Clinical features were evaluated by review of medical records. Additional clinical information was obtained on the seven previously reported individuals to fully elucidate the phenotypic expression associated with *USP7* haploinsufficiency.

Results: The clinical manifestations of these 23 individuals suggest a clinically recognizable syndrome in the first years of life, characterized by DD/ID, hypotonia, eye anomalies, feeding difficulties, GERD, behavioral anomalies, and ASD, and more specific phenotypes of speech delays including a nonverbal phenotype and abnormal brain MRI findings.

Conclusion: The consistency of clinical features among all individuals presented here, regardless of type of de novo *USP7* variant, supports the pathogenicity of these variants and refines the clinical impact faced by affected individuals and caregivers.

P-MonoG-160

*** Structured assessment of 24 candidate genes validates TEX11, TEX14, TEX15, NR5A1, FANCM and DMRT1 as clinically relevant for non-obstructive azoospermia

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Infertility affects 10–15% of all couples. In about half of them, a descriptive cause can be established in the male partner, mostly reduced sperm output, of which azoospermia, the complete lack of spermatozoa in the ejaculate, represents the most severe form. Non-obstructive azoospermia (NOA) comprises several subforms of spermatogenic failure (SpGF), such as meiotic arrest (MeiA) and Sertoli-Cell-Only syndrome (SCOS). Most of these phenotypes are suspected to be of genetic origin. However, aside from chromosomal aberrations and Y-chromosomal AZF deletions, monogenic causes are poorly understood. Still, a number of candidate genes have been proposed to be associated with SpGF and are listed in Online Mendelian Inheritance in Men (OMIM).

In this study, we systematically analyzed 24 genes reported to be related to NOA in OMIM. Variants in these genes were detected in whole exome sequencing (WES) data from a large cohort of 482 well-phenotyped infertile patients (azoospermia, *N* = 94; cryptozoospermia, *N* = 52; mixed atrophy, *N* = 93; SCOS, *N* = 179; MeiA, *N* = 43; other arrest, *N* = 23) for whom other causes for their infertility, like aberrations of the karyotype or Y-chromosomal microdeletions, had been excluded. Only novel or rare coding variants (MAF ≤1% for autosomal dominant [AD] or MAF ≤5% for autosomal or X-linked recessive [AR, XLR] inheritance) were taken into account. In the next step, pathogenicity of missense variants was predicted by Polyphen-2, Sift and MutationTaster, while potential splice site effects of synonymous and splice site affecting variants were assessed using Alamut® Visual. Remaining variants were verified by Sanger sequencing. Further-

more, if DNA was available, family members were sequenced to demonstrate the inheritance of the variants.

Out of the 24 genes listed in OMIM, *TEX11*, *TEX14*, *TEX15*, *NR5A1*, *FANCM* and *DMRT1* (in order of variant prevalence) were validated as clinically relevant genes causing NOA with strong evidence (Smith *et al.*, Hum Mutat 2017). Specifically, we identified six patients carrying *TEX11* (XLR) variants in addition to three variants which we published earlier (Yatsenko *et al.*, N Eng J Med 2015). Seven patients with SCOS or MeIA carried compound heterozygous ($N=6$) or homozygous ($N=1$) *TEX14* (AR) variants. Homozygous ($N=1$ patient) or compound heterozygous ($N=4$ patients) *TEX15* (AR) variants were detected in five patients. Four patients carried *NR5A1* (AD) and two *DMRT1* (AD) defects. Variants in *FANCM* (AR) were found in two patients with crypto- and azoospermia, respectively.

This is the first report of concise exome sequencing in a large group of infertile males. Our results clearly demonstrate that the above-mentioned six genes have now reached a sufficient level of evidence to be prioritized for clinical analyses.

This work was carried out within the frame of the DFG Clinical Research Unit “Male Germ Cells: from Genes to Function” (CRU 326).

P-MonoG-161

Retinal RNA expression profile in the *Ccdc66*-deficient mouse model for retinal degeneration

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The human Retinitis pigmentosa (RP) phenotype (Gerding *et al.* 2011) is similar to the *Ccdc66*-deficient (*Ccdc66*^{-/-}) mouse model exhibiting slow retinal degeneration. To gain insights into the molecular mechanisms that govern *Ccdc66*-deficient degeneration, RNA gene expression profiles at P10 and P28 are analyzed in retinal tissue of *Ccdc66*^{-/-} and wildtype mice (GeneChip Mouse Gene 2.0 ST Array, Affymetrix) followed by confirmation using quantitative real-time PCR (qRT-PCR). The results indicate that 1) the *Ccdc66*-deficient mouse model reveals early changes in retinal RNA gene expression already at P10 and the highest number of genes differential expressed at P28. 2) Most expression differences at P28 (selected genes were confirmed by qRT-PCR) were associated with genes of the extracellular matrix. Further differentially regulated genes are associated with retinal degeneration, transcription factors and proteolysis. Interestingly, in early retinal development at P10, expression of a gene involved in angiogenesis seems to be altered by a lack of *Ccdc66*. In summary, RNA expression profiles reveal early modulation of cellular processes by *Ccdc66*-deficiency, especially in the gene expression of extracellular matrix proteins. These results revealed candidate genes that enable further studies on the role of genes and processes involved in early *Ccdc66*-triggered retinal degeneration. Moreover, it represents a basis for further studies examining the pathway of retinal degeneration in the mammalian retina including man- and possibly contribute to future studies in man and human disease.

Ccdc66 null mutation causes retinal degeneration and dysfunction. Gerding W.M., Schreiber S., Schulte-Middelmann T., de Castro Marques A., Atorf J., Akkad D.A., Dekomien G., Kremers J., Dermietzel R., Gal A., Rülcke T., Ibrahim S., Epplen J.T., Petrasch-Parwez E.

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P-MonoG-162

Solve-RD—solving the unsolved rare diseases

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Solve-RD is a H2020 funded flagship EU project that brings together 21 partners from 10 countries and which will be running from 2018 to 2022. The main ambitions are (i) to solve large numbers of RD, for which a molecular cause is not known yet, by sophisticated combined Omics approaches, and (ii) to improve diagnostics of RD patients through a “genetic knowledge web”. Solve-RD will pursue a clear visionary and integrated “beyond the exome” approach. The entire Solve-RD project has been motivated, designed and put together by a core group of four European Reference Networks. Solve-RD will deliver 7 main implementation steps: (i) Collect Phenotypes, (ii) New phenotype patterns, (iii) Re-analyse exomes/genomes, (iv) Novel molecular strategies, (v) Functional analysis, (iv) Clinical utility and (vii) Towards therapy. For analysis Solve-RD will apply data driven and expert driven approaches. We anticipate to increase diagnostic yield from 19.000 unsolved exomes/genomes by about 3–5%. Cohort specific innovative -omics strategies will be pursued, also addressing cost-effective issues. Analysis of more than 800 patients with highly peculiar phenotypes will highly increase the chance to find novel disease genes and novel disease mechanisms. We anticipate to solve more than 2000 cases. Finding further matching patients will be secured by newly developed matchmaking approaches and by screening using MIPs technology in the more than 20.000 unclassified patients. For the first time in Europe we will also implement a novel brokerage structure connecting clinicians, gene discoverer and basic researcher to quickly verify novel genes and disease mechanisms.

P-MonoG-163

Homozygous missense mutation in Desmoglein 1 (DSG1) causing SAM-syndrome (Severe dermatitis–multiple Allergies–Metabolic wasting syndrome)

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SAM-syndrome (MIM #615508) (Severe dermatitis, multiple Allergies and Metabolic wasting) is a rare autosomal recessive inherited genodermatosis. Common features are congenital erythroderma with severe psoriasiform dermatitis, ichthyosis, severe yellowish palmoplantar keratoderma, elevated immunoglobulin E levels, multiple food allergies and metabolic wasting. Other variable features may include hypotrichosis, nail dystrophy, recurrent infections, mild global developmental delay, eosinophilia, nyctagmus, growth impairment and cardiac defects. SAM-syndrome is caused by biallelic loss-of-function mutations in *DSG1* encoding desmoglein 1. One case of SAM syndrome is described in the literature due to heterozygous mutation in *DSP* encoding desmoplakin.

We describe a family from Syria with three affected siblings and consanguineous parents. The index patient was a one year old girl described to have ichthyosis, subaortal ventricular septal defect and intracerebral hemorrhaging, elevated triglyceride levels and notably vacuolated granulocytes in a blood smear. Her brothers (7 and 8 years old) presented with ichthyosis, distinct palmoplantar keratosis, short stature and woolly hair. The parents were reported to be healthy. Due to the vacuolated granulocytes the initial suspected diagnosis was Chananin-Dorfman-syndrome. However, no mutations in the gene *ABHD5* could be identified. We performed NGS-multi-gene-panel analysis and identified the homozygous missense mutation c.909G>C, p.(Trp303Cys) in exon 8 of the *DSG1* gene in the index patient. Both brothers carry the same homozygous mutation in *DSG1* and the parents are heterozygous carriers each. Immunohistochemical analyses showed a reduced expression of DSG1 and a lack of membrane staining supporting the *DSG1* mutation being causative for the phenotype of the siblings. To date, only truncating mutations in *DSG1* have been reported in patients with autosomal recessive SAM-syndrome. Usually, monoallelic truncating mutations in *DSG1* cause autosomal dominant striate palmoplantar keratoderma, however, recently one heterozygous missense mutation in *DSG1* has been described in the context of striate palmoplantar kera-

toderma. This is the first case of a biallelic missense *DSGI* mutation causing SAM-syndrome and underlines its extensive phenotypic heterogeneity.

P-MonoG-164

Key function of the conserved p.Arg108 residue in S1PR2 (DFNB68) and its significance in sensorineural hearing loss

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Non syndromic hearing loss (NSHL) follows a recessive pattern of inheritance in 70% of cases. In countries with a high rate of consanguineous marriages, there is an increased prevalence of inherited hearing loss (HL). Overall, there are more than 100 genes associated with NSHL. *S1PR2* (OMIM: 605111), the sphingosine-1-phosphate receptor 2, is associated with autosomal recessive HL (DFNB68) and was classified as a HL-associated gene through the investigation of two Pakistani families with a consanguineous background. This gene is part of the sphingosine-1-phosphate (S1P) signaling pathway. *S1pr2* mutant mice elicit abnormal endocochlear potential, as well as degeneration of the organ of Corti and stria vascularis and had profound HL.

In our study, we identified an individual in a five generation Iranian family with multiple consanguineous marriages. Affected individuals presented a novel pathogenic variant c.323G>A, p.Arg108Gln in *S1PR2*. The gDNA from the index was subjected to exome sequencing library preparation using the Nextera Rapid Capture Exome kit and was sequenced with a NextSeq500 benchtop sequencer (Illumina). Variant and copy number variation analysis were performed using GensearchNGS and an in-house exome analysis pipeline. Segregation analysis of the variant included the index, three additional affected individuals and seven healthy individuals of the family. The affected individuals presented the pathogenic c.323G>A variant in a homozygous state, whereas the healthy parents and sibling were carriers. All affected individuals presented profound HL, which was also observed in the previously described Pakistani families.

The p.Arg108 position seems to play a key role in normal hearing. For further understanding of the p. 108 position and the resulting impact on the *S1PR2* protein structure, 3D homology modelling was performed. We could identify a dysfunctional ligand receptor interaction caused by loss of binding efficiency in this position. This underscores the importance of the p. 108 amino acid for supporting proper S1P signaling. This case confirms *S1PR2* as a recessive HL-associated gene.

P-MonoG-165

Generation of CRISPR/Cas9 induced MID1 knockout iPS cells

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Hemizygous loss of function of X-chromosomal genes is one of the most frequent causes for genetic intellectual disability (ID). Many mutations in different genes have been described to be responsible for the disease phenotype but the underlying neuronal mechanisms are often still unknown.

To gain a better understanding of these neuronal mechanisms induced pluripotent stem cells (iPSCs) that are differentiated into neuronal precursor cells (NPCs) and neurons are a promising tool for translating research, in particular when combined with CRISPR/Cas9 genome editing to generate isogenic controls.

The Opitz BBB/G syndrome (OS) is characterized by a number of ventral midline defects combined with developmental delay and ID. OS is caused by mutations in the X-chromosomal gene *MID1*, that encodes for a ubiquitin ligase. *MID1* regulates mTOR dependent local protein synthesis and therefore plays an important role in synaptic plasticity, learning and memory.

We have generated iPSCs from fibroblasts of a 26-year-old male control using a Sendaiviral transfection of the classic KMOS factors. For CRISPR/Cas9 genome editing we have electroporated a sgRNA together with a Cas9-GFP plasmid into the iPSCs. After FACS sorting the cells were expanded until ready to be analyzed.

Using this approach, we identified one cell line that carries a 2 bp deletion between the RING-domain and the BBox1 subunit leading to a frameshift and an early stop codon (c.204_205delAG). Mutation taster classifies this mutation as disease causing and predicts the RNA to be degraded by nonsense-mediated RNA decay (NMD). Absence of the protein was confirmed by Western Blot and the effects of NMD are currently being analyzed by RT-qPCR. We are also performing whole-exome sequencing at the moment to identify possible off-target effects in the edited cells.

The next steps will be to characterize the edited iPSCs in comparison to their wildtype isogenic controls as well as NPCs generated from either of these cells and see if there are any OS-related differences. For this we will perform proliferation assays, migration assays, cell size measurements and Western Blots to measure the activity of the mTOR downstream target S6. We also plan to generate cerebral organoids from these iPSCs, as we have already seen differences between organoids generated from female iPSCs that either expressed a mutant or a wildtype *MID1*. With these experiments we hope to get a better understanding of the underlying neuronal mechanisms of OS and hope to set up a workflow that can easily be transferred on other genes.

P-MonoG-166

Variant analysis in patients with Retinitis Pigmentosa using a MIP-based next generation sequencing approach

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Retinitis pigmentosa (RP) is a clinically and genetically heterogeneous group of inherited retinal diseases (IRD). Patients experience night blindness, visual field constriction, and eventually loss of central vision. RP can be inherited as an autosomal recessive, autosomal dominant and X-linked recessive trait. To date, more than 45 genes have been implicated in the pathogenesis of RP.

This genetic heterogeneity hampers molecular testing of RP patients as standard targeted next generation sequencing (NGS) still involves high costs and the reimbursement to the patient is often not guaranteed. We therefore sought for a cost-effective and sensitive approach to obtain a molecular diagnosis for 480 patients that had been initially diagnosed with either sporadic or autosomal-recessive RP at the University Eye Hospital Tuebingen.

A total of 108 genes implicated in IRD were analyzed using a targeted NGS procedure based on molecular inversion probes (MIPs). Sequencing and variant filtering led to the identification of putative pathogenic variants in 188/480 cases, resulting in a detection rate of 39%. All putative disease-associated variants were validated by Sanger sequencing. If possible, biallelism in recessive cases was demonstrated using segregation analyses or allelic cloning.

In detail, we identified 230 distinct likely pathogenic variants in 42 IRD-associated genes. Of these 45% have not been reported before, suggesting

that the mutation spectrum of RP and other IRD genes is far from being saturated. Most of the identified mutations are thereby located in USH2A (21%), ABCA4 (9%) and RP1 (7%). 16% of the cases were considered to be partially solved. In these cases, we could either detect only one heterozygous pathogenic variant in genes with an autosomal recessive trait or variants with an uncertain pathogenicity (i.e. missense variants). 45% of the cases remained unsolved as no pathogenic variant explaining the disease phenotype could be identified.

Our detection rate of 39% is somewhat lower in comparison with prior studies using custom IRD panels. This may be explained by several target regions with low or no sequence coverage and undetected copy number variations. In addition, patients might harbor pathogenic variants (e.g. non-canonical splice site variants) in intronic regions which are not covered by the MIP-Panel. Moreover, unsolved cases might result from the presence of mutations in genes not yet associated with inherited retinal diseases.

The MIP technology we used can be as low as € 80 per sample per gene panel, which is 10 to 20 times lower than the price tag for other NGS-based sequencing procedures. In summary, we could demonstrate that MIPs are a cost-efficient and sensitive tool for analyzing RP.

P-MonoG-167

Mutations in PIGU impair the function of the GPI transamidase complex causing developmental delay, seizures, cerebellar atrophy and a characteristic facial gestalt

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We report homozygous mutation in PIGU, a gene that encodes the fifth component of the GPI transamidase complex, that has yet not been associated with global developmental delay, severe to profound intellectual disability, muscular hypotonia, seizures, a hypoplastic corpus callosum, scoliosis and facial dysmorphism.

The pentameric protein complex responsible for loading the glycosylphosphatidylinositol (GPI) anchor with GPI anchored proteins (GPI-APs) in the ER is encoded by PIGT, PIGS, PIGK, GPAA1, and PIGU. Mutations in 18 of the 28 genes that are implicated in the biogenesis of the GPI anchor, have been identified as cause of GPI biosynthesis deficiencies (GPIBDs) in humans associated with intellectual disability and seizures as the cardinal feature. We identified two homozygous missense mutations in PIGU (phosphatidylinositolglycan biosynthesis classes U) (c.209T>A and c.1149C>A) by WES in five individuals from three unrelated families. Using flow cytometry a characteristic profile for transamidase deficiency consisting of reduced cell surface levels of FLAER, CD16, and CD24, but not CD55 and CD59 on granulocytes and free GPI anchor detected by T5 antibody on B-cells, was determined that was similar to patients with mutations in PIGT, supporting pathogenicity of the mutations.

Moreover, automated facial analysis revealed a shared characteristic facial gestalt between individuals with mutations in PIGU, PIGT, and GPAA1. In conclusion, we identified rare mutations in PIGU in patients with clinical features of GPI biosynthesis defects (GPIBDs). Flow cytometry and computer-assisted facial image analysis provide further evidence for the pathogenicity of the underlying mutations and also demonstrate how to discriminate GPIBDs. Our findings strengthen the role of the transamidase complex in the development of brain, skeletal system and the face and expand the clinical spectrum of disorders belonging to the group of GPIBDs.

P-MonoG-168

Novel missense variant in the LOX gene causes multiple aneurysms of the thoracic aorta and other vessels

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Introduction: Heritable Thoracic Aortic Disease (HTAD) is a significant cause of sudden death and is an example of a disease where predictive genetic testing can be lifesaving. Our knowledge about the genetic causes has expanded significantly due to recent advances in diagnostic sequencing technologies. Still, approximately 70% of individuals with HTAD do not have a pathogenic variant in one of the known genes. In this study, we present a patient with multiple aneurysms caused by a heterozygous missense variant in LOX gene, which codes for lysyl oxidase, an extracellular copper enzyme that initiates crosslinking of collagens and elastin. Mutations in LOX have been recently described in association with aneurysm disease.

Methods-Results: Genetic analysis through whole exome sequencing identified a heterozygous novel missense variant in LOX (c.871T>A p.[Cys291Ser]) in a 53-year old patient with a history of multiple aneurysms affecting the whole thoracic aorta as well as other vessels (e.g. politeal artery, coronary arteries). The variant affects a highly conserved amino acid residue in the catalytic domain of the encoded enzyme. Histological analysis of an aortic specimen (HE, PSR, Orcein staining, Trichrome staining) revealed a disturbed aortic wall organization with fragmented elastic fibers and an increased collagen deposition in comparison to the human control tissue. To examine the pathogenic relevance of the detected variant, we performed in vitro transfection studies in HeLa cells with wildtype as well as mutant recombinant expressed LOX proteins. By means of a LOX specific enzyme assay we detected a reduced activity of the enzyme lysyl oxidase indicating a partial loss of function of the mutant LOX. Thus, we could prove the pathogenic relevance of the detected variant.

Conclusion: Although LOX codes for an enzyme, the few reported cases as well as our example are concordant with an autosomal dominant inheritance pattern due to loss-of-function variants. Pathogenic variants in LOX gene have only recently been reported in association with aortic disease. The findings of this study demonstrate that comprehensive genetic studies are often required to accurately define the cause of aneurysm patients. Furthermore, gene panels for hereditary aneurysmal disease should routinely include the LOX gene.

P-MonoG-169

First replication of an association between PIGP and epileptic encephalopathy

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Introduction: Epileptic encephalopathy is a group of childhood disorders characterized by early-onset epileptic seizures and developmental delay. Although usually caused by heterozygous de novo mutations, some EEs have been associated with recessively inherited glycosylphosphatidylinositol deficiencies, in which responsible genes (e.g. PIGA, PIGC, PIGG etc.) encode anchor proteins that tether other human proteins to the cell

membrane. The first family with mutations in the gene PIGP was recently reported by Johnstone et al. with two affected siblings presenting with infantile seizures and developmental regression. One frameshift and one missense mutation (in a compound heterozygous state) in PIGP were found to segregate with the disease.

Patients and Methods: We clinically and genetically characterize a further patient with PIGP-related EE. Diagnostic exome sequencing was used in the affected individual to identify the underlying genetic defect.

Results: This female patient was born from healthy, non-consanguineous parents at 40 weeks gestation. Epileptic seizures first occurred at 5 months of age and psychomotor development had been delayed ever since. During seizures, the patient presented with increased muscle tone, cyanosis, eye deviation, eyelid myoclonia and oral automatisms. Electroencephalography suggested a left temporal seizure onset. The response to antiepileptic treatment with topiramate, levetiracetam and midazolam was poor. Reduced muscle tone and visual impairment was noted interictally. MRI of the brain showed hyperintense pontine lesions and a generalized cerebral atrophy. Using exome sequencing, the homozygous c.456delA;p.Glu-153AsnFs*34 (NM_153681.2) mutation in PIGP was detected, establishing the diagnosis "Epileptic encephalopathy, early infantile, 55" (MIM# 617599). This one-base deletion causes a frameshift predicted to result in a truncated protein. 5 heterozygous carriers were found in more than 15,500 exomes of the in-house database (Munich Exome Server) and 9 heterozygous carriers were found in the Genome Aggregation Database (gnomAD). Homozygous carriers were absent from both control databases.

Conclusion: To our knowledge, this is the first replication that biallelic mutations in PIGP cause early-infantile epileptic encephalopathy, which strongly corroborates its role as a human disease gene.

P-MonoG-170

Genotype-phenotype correlations in fast myosin related congenital myopathy

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Myosin heavy chains form a major structural component of the striated muscle contractile apparatus. The myosin heavy chain 2 gene (MYH2) encodes the isoform, which is expressed in fast type 2A muscle fibers in skeletal muscle.

Recently heterozygous, compound heterozygous, and homozygous mutations in MYH2 were linked with proximal myopathy and ophthalmoplegia (MYPOP), a relatively mild muscle disorder characterized by mild-to-moderate muscle weakness and ophthalmoplegia with childhood onset and slow or no progression.

Here we present three unrelated cases with severe MYH2-related congenital myopathy.

An 11-year-old boy of Serbian descent presented with severe tetraplegia, respiratory insufficiency, inability to speak and ophthalmoplegia. The boy was never able to sit without support. He developed ventilator dependent respiratory insufficiency. Symptoms had a neonatal onset with a history of neonatal asphyxia, hypoxic-ischemic encephalopathy and a slow psychomotor development. The CK levels were normal. An electromyoneurography showed no signs of neuropathy and signs of myopathy, as accelerated recruitment of motor units without activity in the thigh muscles. We performed mendeliome sequencing and found a heterozygous mutation in MYH2 (c.3112G>T, p.D1038Y) in the coiled coil tail that may disrupt the coil. We classified it as likely pathogenic (ACMG 2015).

The second patient is a 6-year-old girl of Turkish descent that presented with axial muscle weakness, myopathy and mild ophthalmoplegia. The girl is able to walk, but has difficulties running and climbing stairs. Also chewing and swallowing is affected. In early childhood she had delayed motor milestones. The CK levels were normal. Mendeliome sequencing revealed

two compound heterozygous missense mutations in MYH2, confirmed by Sanger sequencing of the parents. The first mutation is c.326G>A, p.R109H, in the myosin motor head domain. The second mutation is (c.1416+5G>T) in an intronic splice region. The ACMG criteria prediction is likely pathogenic.

A 44-year-old patient of Sri Lankan descent showed symptoms as, external ophthalmoplegia, dysphagia, and tetraparesis. Symptoms have first been noted in childhood with progression around age 30. Increased CK levels of 400–600 U/l and myopathic electromyography were detected. Muscle biopsy revealed signs of chronic necrotizing myopathy with focal myofibrillary changes, varied fiber size, and marked type 1 fiber predominance. We found a homozygous mutation in the MYH2 (c.4046T>C, p.L1349P) via whole exome sequencing. This mutation is predicted to disrupt the tail coiled coil. We classified the variant as likely pathogenic (ACMG2015). Summarized the phenotype of patient 1 and patient 3 suffering from a severe tetraparesis with inability to walk exceeds the clinical findings reported so far. The typical muscle weakness together with marked type 1 fiber predominance and ophthalmoplegia fits to a recessive fast myosin heavy chain 2 myopathy well.

P-MonoG-171

A young patient of disproportionate short stature suffering of glaucoma was diagnosed to have Weill-Marchesani 4 syndrome by WES however the cause of his additional progeroid phenotype is still unclear

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A currently 19-year-old patient of Nepalesian descent of 149 cm length and 44 kg weight lives in Austria since his first year of age with his adoptive parents, whereas his healthy sister lives in Germany. Since the patient has a short stature and a dry and stiff skin that increasingly shows a reticulated pattern of hyperpigmentation and because ridged and concave dystrophic nails of finger and toes are present, an anomaly of connective tissue structures was considered. Because of his bilateral chronic glaucoma, a therapy is required. He also developed progressive hypotrichosis of the scalp and became already nearly bald. His broad forehead with wrinkled skin did already early suggested, that a kind of progeroid phenotype could present. At an age of 13 years the LMNA-gene was sequenced and no mutation was detected. In addition, the SPINK5-gene was analyzed to rule out a Netherton syndrome as well as sequencing and MLPA analysis of the ZMPSTE24-Gen was performed to rule out a Mandibulo-akral Dysplasia. After another genetic counseling, the option of whole exome sequencing (WES) was discussed with the patient and was finally requested by him. An Illumina NextSeq device was used and several bioinformatics software programs including JSI SEQUENCE PILOT version 5.00 and Golden Helix were utilized at a high coverage. By this approach it was possible now to identify two compound heterozygous missense mutations in the ADAMTS17-gene including the already known rs570605751 making it likely that the patient has Weill-Marchesani 4 syndrome since several of his clinical features are part of this syndrome. Many additional heterozygous variants were found in other genes including CIDEA, PLEC, LPAR6, RAD50, NKX3-2, CPS1, ADAMTSL4, but thus far, we were not able yet to identify causal variants that could explain the obvious additional progeroid phenotype of our patient that is certainly not related to Weill-Marchesani 4 syndrome. So further efforts like whole genome sequencing (WGS) with highly advanced techniques like those from PacBio or Oxford Nanopore should be considered to identify the genetic basis of the progeroid phenotype, a very rare and heterogenic disorder, in this patient.

P-MonoG-172

Nuclear envelope abnormalities in myotonic dystrophy primary myoblasts

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Myotonic dystrophies (DM) are slowly progressing multisystemic diseases with a predominant muscular dystrophy—making DM the most frequent muscular dystrophy in adulthood. DM is caused by heterozygous DNA-repeat expansions in the DMPK gene (DM1) or the CNBP gene (DM2). The repeat-containing RNA accumulates in ribonuclear foci and splicing factors are sequestered to these foci, resulting in abnormal regulation of alternative splicing.

In primary patient myoblast cell cultures we observed invaginations of the nuclear envelope (NE). These abnormalities correlate in DM1 with repeat length—which in turn correlates with disease severity. Searching for the reason of these abnormalities we screened proteins of the nuclear lamina and NE transmembrane proteins (NETs). This revealed down-regulation of the lamins A and B1 as well as different isoforms of the NET nesprin 1. Lamins and nesprins are having functions in the mechanical stability of the nucleus; therefore their mis-regulation is likely directly involved in the observed nuclear abnormalities. This implies possible shared pathomechanism between DM1 and nuclear envelope linked disease.

P-MonoG-173

Protein degradation pathways differ in mutant BEST1 leading to autosomal dominant or autosomal recessive bestrophinopathies

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Purpose: Bestrophin 1 (BEST 1) encodes an integral membrane protein localized to the basolateral membrane of the retinal pigment epithelium (RPE) in the ocular tissues. Functional BEST1 is a Ca^{2+} -regulated volume-sensitive chloride channel, composed of five homomeric BEST1 subunits. Pathogenic mutations in the BEST1 gene have been associated with distinct retinopathies, including the autosomal dominant Best vitelliform macular dystrophy (BD) and the autosomal recessive Bestrophinopathy (ARB).

Methods: To understand the functional mechanisms underlying the dominant and recessive forms of BEST1-associated pathology, we generated RPE cells differentiated from induced pluripotent stem (iPSC-RPE) cells from BD patients (heterozygous for Q238R or Ile295del), ARB patients (compound heterozygous for N99K/R141H or A195V/L197PX26) and their healthy parents (heterozygous mutation carriers for one mutation, respectively). We analyzed BEST1 protein stability and degradation pathways by treating the cells for 12 hours with cycloheximide, a potent protein synthesis inhibitor, and selective inhibitors of the endo-lysosomal (e.g. chloroquine) or proteasomal (MG132) degradation pathway.

Results: Western Blot analysis revealed that upon treatment with cycloheximide normal BEST1 protein is stable up to 12 hours in control iPSC-RPE cells. However, mutant BEST1 is strongly degraded—with recessive iPSC-RPE cells revealing even less protein expression than the dominant ones. In BD iPSC-RPE cells, mutant BEST1 is prone to degradation via the endo-lysosomal degradation pathway whereas the recessive BEST1 mutations are degraded by the proteasome.

Conclusion: Autosomal dominant mutations in BD are degraded via the late endo-lysosomal degradation pathway. As a result, increased formation of non-functional BEST1 channels are likely to occur due to a roughly equimolar incorporation of normal and mutant BEST1 subunits into the channel complex. In contrast, recessive ARB mutations trigger a fast protein degradation process in the proteasome, thereby strongly favoring a decreased stoichiometry of mutant versus normal BEST1 subunits in the assembly of the homo-pentameric BEST1 chloride channel. Our results suggest that the site and speed of subcellular protein degradation account for the distinct retinal disease phenotypes in BD and ARB.

P-MonoG-174

Novel mutations in the GJC2 gene associated with Pelizaeus-Merzbacher-like disease

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Inherited white matter disorders of the central nervous system often are degenerative and progressive clinical entities of different age of onset. They can be classified into myelin disorders, including hypomyelination, dysmyelination, demyelination, and myelin vacuolization, as well as astrocytopathies, leuko-axonopathies, microgliopathies, and leuko-vasculopathies. Hypomyelinating leukodystrophies are mainly represented by innate Pelizaeus-Merzbacher disease (PMD) and Pelizaeus-Merzbacher-like disease (PMLD1). PMD and PMLD1 are clinically similar disorders manifesting common symptoms of developmental delay, nystagmus, spasticity, hypotonia, ataxia, and hypomyelination visible on brain magnetic resonance imaging. These disorders are distinguishable from each other on the molecular level, as they are associated with mutations in different genes. PMLD1 is caused by homozygous and/or compound heterozygous mutations in the *GJC2* gene, encoding the gap junction gamma-2.

Herein, we present novel pathologic variants in the *GJC2* gene associated with the PMLD1-phenotypes.

Next generation sequencing approaches were used to rapidly identify pathogenic variants in two unrelated, infantile patients affected with PMLD1. The first PMLD1-affected child showed compound heterozygous mutations, a duplication (NM_020435.3: c.392dupC, p.H132Afs*6), and a deletion (NM_020435.3: c.989delC, p.P330Rfs*141), both changing reading frames of the *GJC2* gene. The second patient affected with PMLD1 revealed different compound heterozygous variants in the *GJC2* gene, including a nonsense (NM_020435.3: c.291C>G; p.Y97*), and a missense (NM_020435.3: c.716T>C, p.V239A) variant. Sanger sequencing performed in the index patients and their available family members showed co-segregations of the identified pathogenic variants in the *GJC2* gene with both PMLD1-phenotypes.

Multiple-species alignments of the GJC2 proteins showed highly conserved amino acid residues located at positions 97, 132, 239, and 330. *In silico* predictions of the GJC2 protein domains showed that the deletion and the duplication identified in the first patient (p.H132Afs*6 and p.P330Rfs*141) were located in the low complexity region, while the nonsense and missense variants (p.Y97* and p.V239A) detected in the second patient were located in the transmembrane region and the connexin-CCC domain of the GJC2 protein. These computer predictions pointed towards the importance of amino acid positions in the GJC2 protein that were mutated in the analysed PMLD1-affected patients.

Our clinical and molecular findings agree with previously reported PMLD1-cases. They may further extend the knowledge of phenotype-genotype associations in PMLD1-affected patients. These data may be helpful in a genetic counselling, as well as in the development of targeted therapies in the future.

P-MonoG-175**Dissecting TSC2-mutated renal and hepatic angiomyolipomas in an individual with ARID1B-associated intellectual disability**

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Several subunits of the SWI/SNF chromatin remodeling complex are implicated in both cancer and neurodevelopmental disorders (NDD). Though there is no clinical evidence for an increased tumor risk in individuals with NDDs due to germline mutations in most of these genes so far, this has been repeatedly proposed and discussed. A young woman with NDD due to a *de novo* mutation in *ARID1B* now presented with a large renal (>19 cm in diameter) and multiple hepatic angiomyolipomas (AMLs) but no other signs of tuberous sclerosis.

We analyzed tumor and healthy tissue samples with exome and panel sequencing. Additionally to the previously known, germline *ARID1B* variant we identified a post-zygotic truncating *TSC2* variant in both renal and hepatic AMLs but not in any of the healthy tissues. We did not detect any further, obvious tumor driver events. The identification of a passenger variant in *SIPA1L3* in both AMLs points to a common clonal origin. Metastasis of the renal AML into the liver is unlikely on the basis of discordant histopathological features. Our findings therefore point to very low-grade mosaicism for the *TSC2* variant, possibly in a yet unknown mesenchymal precursor cell that expanded clonally during tumor development. A possible contribution of the germline *ARID1B* variant to the tumorigenesis remains unclear but cannot be excluded given the absence of any other evident tumor drivers in the AMLs.

This unique case highlights the blurred line between tumor genetics and post-zygotic events that can complicate exact molecular diagnoses in patients with rare manifestations. It also demonstrates the relevance of multiple disorders in a single individual, the challenges of detecting low-grade mosaicisms, and the importance of proper diagnosis for treatment and surveillance.

P-MonoG-176**Identification of novel loss-of-function mutations in two independent patients with Deficiency of Adenosine Deaminase 2**

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Deficiency of adenosine deaminase 2 (DADA2) is an autosomal recessive disorder that manifests with fever, rash, hypocellular bone marrow, early-onset vasculitis, and propensity to ischemic and hemorrhagic stroke. Over 60 pathogenic mutations, mostly missense variants, have been identified to date. Here, we report two novel loss-of-function mutations in the *ADA2* gene in two unrelated families.

The first index patient is a 5-year-old female who was born to a consanguineous Pakistani family and presents with features consistent with Diamond-Blackfan anemia (DBA). Sequencing analysis did not identify pathogenic mutations in DBA associated genes and a chromosomal microarray did not detect duplications or deletions. Interestingly, several regions of homozygosity were identified in the proband, one of which comprises the *ADA2* gene locus. However, subsequent Sanger sequencing of *ADA2* did not identify a pathogenic variant in the patient. Multiplex ligation-dependent probe amplification analysis (MLPA) identified a homozygous duplication of a region comprising exon 7 of *ADA2* in the proband, her affected sister, her affected father, and a heterozygous duplication of this region in the proband's mother.

Subsequent qRT-PCR experiments confirmed a complete loss of RNA-expression in the individuals homozygous for the duplication. In agreement with the mother's carrier status, she showed approximately 50% reduction

in *ADA2* RNA. Reduced *ADA2* enzyme activity corroborated the qRT-PCR findings. Long range PCR and long read sequencing analysis were performed to localize the breakpoints of the duplication.

The second index patient is a 17-year-old female who presents with a history of ischemic strokes, livedo and vasculitis. She was found to be heterozygous for a known pathogenic variant in *ADA2* (c.1358A>G, p.Y453C). This variant was inherited from the proband's father and is also present in her three at the time unaffected siblings. The *ADA2* enzyme activity assay revealed that the proband and two of her siblings have low to absent *ADA2* enzyme activity. Her parents, as well as a third sibling, exhibit *ADA2* enzyme levels in the carrier range. Haplotype analyses revealed that the three children with low *ADA2* activity inherited the same allele from their mother while the fourth sibling, who exhibits *ADA2* enzyme levels in the carrier range, received the other maternal allele. Whole genome sequencing of the mother and the 3 affected children identified a novel canonical splice site variant (*ADA2*: c.-47 + 2T>C) present in all four individuals. Subsequent Sanger sequencing analysis confirmed the segregation of this variant with the disease. This variant is absent from population databases and predicted to effect splicing by several in silico algorithm. RNA expression analysis showed that the affected individuals express only 50% of the normal *ADA2* mRNA and subsequent cDNA sequencing confirmed that only the allele carrying the pathogenic c.1358A>G, p.Y453C variant is expressed.

P-MonoG-177***** A de novo MEPCE nonsense variant in a patient with intellectual disability and epilepsy leads to a decreased amount of core components of the 7SK snRNP complex controlling RNA polymerase II elongation**

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In eukaryotes, synthesis of precursor mRNAs by RNA polymerase II (RNAP II) is essentially regulated by many factors. During the transcriptional process, RNAP II is paused proximal to the promoter by negative elongation factors. The release of RNAP II is mediated by phosphorylation through the positive transcription elongation factor b (P-TEFb) consisting of Cyclin-T1 (CT1) and the cyclin-dependent kinase 9. P-TEFb in turn is under control by the inhibitory 7SK small nuclear ribonucleoprotein (snRNP) complex. The 7SK snRNP core consists of the 7SK ncRNA that is permanently bound to MEPCE and LARP7. MEPCE stabilizes the 7SK ncRNA by 5' cap methylation, and LARP7 protects the 7SK ncRNA by binding its 3' end. P-TEFb inhibition and incorporation in the 7SK snRNP complex is ensued by HEXIM1/2 dimers. Biallelic loss-of-function variants in *LARP7* underlie Alazami syndrome that is characterized by primordial dwarfism, distinct facial features and severe intellectual disability. Loss of *LARP7* causes depletion of the 7SK ncRNA in patient-derived fibroblasts.

We report a 5-year-old male patient with global developmental delay, moderate intellectual disability and intractable epilepsy starting at the age of 3 years. During the course of the disease he showed a decline in physical capacities and cognitive abilities and developed an impulsive behavior with extreme tantrums. Trio whole exome sequencing identified the *de novo* nonsense variant c.1552C>T/p.(Arg518*) in *MEPCE* in the affected boy. Absence of the variant from gnomAD and intolerance of *MEPCE* to protein-truncating variation (pLI score: 1.00) suggested that the *de novo* variant may underlie the patient's phenotype. We determined the amount of *MEPCE* transcripts by quantitative real-time PCR (RT-qPCR) and found the level to be reduced to 51% and 64% in patient fibroblasts compared to fibroblasts of a control and the patient's mother, respectively, suggesting nonsense-mediated mRNA decay of the mutant transcripts. By immunoblotting, we demonstrated a decreased MEPCE amount by ~50% in patient compared to control cells. Similarly, the level of *LARP7* was decreased by 30% in patient fibroblasts. To study if a reduction in MEPCE and *LARP7* protein levels may trigger 7SK ncRNA degradation,

we performed RT-qPCR analysis and detected a decrease in the 7SK ncRNA amount by ~60% in patient compared to control cells. As 7SK ncRNA degradation may be followed by destabilization of the 7SK snRNP complex and release of P-TEFb from the 7SK snRNP, we immunoprecipitated the CT1 subunit of P-TEFb from fibroblasts and detected HEXIM1 in the precipitates. The amount of co-IPed HEXIM1 in patient cells was reduced to 74% and 63% of that of control and patient's mother fibroblasts, respectively. Together, our data suggest that the assembly of the canonical 7SK snRNP complex is disturbed in patient cells, possibly leading to decreased portion of P-TEFb bound to 7SK snRNP and subsequent stimulation of RNAP II pause release by P-TEFb.

P-MonoG-178

Confirmation of a locus for primary hyperhidrosis mapping to chromosome 2

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Introduction: Primary hyperhidrosis (OMIM 114110) is a genetically influenced condition characterized by excessive sweating. The prevalence is about 4.8% in the general population. The cause of the disorder still remains unclear but an autosomal dominant mode of inheritance, incomplete penetrance and variable phenotypes have been reported.

Material and Methods: Nine pedigrees (49 affected and 56 non-affected individuals in total) were recruited. Clinically characterisation has been performed at the German Hyperhidrosis Centre, Munich, and by using physiological and psychological questionnaires. Genome-wide parametric linkage analysis with Merlin was performed based on the Illumina Human-Core-24 genome-wide SNP array. Haplotypes were constructed using easyLINKAGE and visualized by HaploPainter. Whole exome-sequencing with 100× coverage in 31 selected members (24 affected, 7 not affected) of our pedigrees was achieved by next-generation sequencing.

Results: Three pedigrees map to a shared chromosomal locus on 2q21.2–2q23.3, with a genome-wide significant LOD Score of >3.5. The chromosomal region identified by our study overlaps and confirms a locus at chromosome 2q22.1–2q31.1 reported previously. Other pedigrees did not support that locus but provide suggestive evidence for linkage at multiple sites, thus pointing to considerable genetic heterogeneity. Our whole-exome sequencing did not reveal any causative variant, suggesting that variants or mutations located outside of the coding regions might be involved in the molecular pathogenesis of primary focal hyperhidrosis.

Discussion: To conclude, we confirmed a locus for hyperhidrosis on chromosome 2q21.2–2q23.3 in three pedigrees and provide evidence for genetic heterogeneity. Based on our evaluation of whole-exome data, we suggest a strategy based on whole-genome or targeted next generation sequencing to identify causative genes or variants.

P-MonoG-179

The diagnostic challenge of mild MPS IVA: a case report

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Mucopolysaccharidosis type IVA (MPS IVA), also known as Morquio syndrome A, is an autosomal recessive multisystem disorder characterized by bone and joint involvement, valvular heart disease, hearing impairment, corneal clouding, respiratory compromise and normal intelligence. MPS IVA is caused by deficient activity of the enzyme N-acetylgalactosamine-6-sulfate sulfatase (GALNS). There have been 180 different

mutations of the GALNS gene reported, resulting in a wide spectrum of disease severity. We present a 29-year old man with epiphyseal abnormalities, platyspondyly and short stature, first noted at the age of 2 years. Galactosamine-6-sulfate sulfatase activity was normal in childhood. Later on, he developed a waddling gait, instability of his thoracolumbar spine and joint pain. At the age of 17 cataracts and corneal epithelial dysplasia were diagnosed. We performed an NGS-based analysis of 414 genes related to bone diseases and detected compound heterozygous variants in GALNS (NM_000512.4). Beside the known disease-causing mutation c.337A>T; p.Ile113Phe in exon 4 we found the variant c.192A>C; p.Ala64Ala in exon 2. This so far not described synonymous variant is predicted to activate a cryptic splice donor site. Reassessment of his galactosamine-6-sulfate sulfatase activity showed reduced values and confirmed the diagnosis of MPS IV. Confirmation of the deleterious effect of the splice variant is currently ongoing. This study expands the genetic spectrum of GALNS mutations and emphasizes that mild forms with initially normal enzyme activity can be misdiagnosed as spondyloepiphyseal dysplasia in clinical practice.

P-MonoG-180

CRISPR/Cas9 genome editing reveals that biallelic CCM3 mutations cause a clonogenic survival advantage and endothelial cell stiffening

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CCM3 regulates blood-brain-barrier integrity and vascular maturation *in vivo*. CCM3 loss-of-function variants predispose to cerebral cavernous malformations (CCM). Using a viral- and plasmid-free crRNA:tracrRNA:-Cas9 ribonucleoprotein approach, we introduced biallelic protein-truncating CCM3 variants into human endothelial cells (ECs) and established a novel model which mimics complete CCM3 inactivation in cavernous ECs of heterozygous mutation carriers. This model enabled us to study the molecular and functional effects of long-term CCM3 inactivation in human ECs. Notably, induction of apoptosis, spheroid formation, sprouting and migration were significantly impaired upon prolonged CCM3 deficiency. In agreement with the hypothesis of a clonogenic survival advantage and diminished replicative senescence of CCM3^{-/-} ECs, small RNA profiling disclosed that CCM3 modulates the expression of miRNAs that are associated with endothelial aging. Real-time deformability cytometry also revealed that loss of CCM3 induces profound changes in cell morphology and mechanics: CCM3-deficient ECs have an increased cell area and elastic modulus. In conclusion, CRISPR/Cas9 genome editing provides new insight into the consequences of long-term CCM3 inactivation in human ECs and supports the hypothesis that clonal expansion of CCM3-deficient dysfunctional ECs contributes to CCM formation.

P-MonoG-181

Precise correction of a CCM1 frameshift mutation in patient-derived blood outgrowth endothelial cells via non-viral and plasmid-free CRISPR/Cas9 genome editing

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The CRISPR/Cas9 system has opened new perspectives for basic research and raised hopes for somatic correction of disease-causing mutations. However, precise genome editing in endothelial and other hard-

to-transfect cells remains challenging. In a proof-of-principle study, we isolated blood outgrowth endothelial cells from an individual with multiple symptomatic cerebral cavernous malformations (CCMs) and established a highly efficient CRISPR/Cas9-mediated homology directed repair strategy to correct the proband's 1-bp deletion within *CCM1*. To minimize the risk of off-target mutations, a high-fidelity Cas9 variant was transfected into patient-derived blood outgrowth endothelial cells using a crRNA:tracrRNA:Cas9 ribonucleoprotein (RNP) complex and a single-strand DNA oligonucleotide as donor template. Amplicon deep sequencing demonstrated precise correction of the *CCM1* mutant allele in nearly 33% of all cells. In another one third of cells, the open reading frame was restored by a 1-bp duplication on the mutant allele. Notably, no CRISPR/Cas9-induced variants were identified in predicted off-target loci. The approach presented here demonstrates the feasibility of RNP-mediated CRISPR/Cas9 genome editing for precise gene correction in patient-derived endothelial cells and can be applied to various Mendelian disorders since the establishment of isogenic controls is an important prerequisite for personalized disease models.

P-MonoG-182

Homozygous frame shift variant in *ATP7B* exon 1 leads to bypass of nonsense-mediated mRNA decay and to a protein capable of copper export

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Wilson disease (WD) is an autosomal recessive disease of copper excess due to pathogenic variants in the *ATP7B* gene coding for a copper-transporting ATPase. We present a 5-year-old girl with the homozygous frame shift variant NM_000053.3:c.19_20del in exon 1 of *ATP7B*, detected by whole exome sequencing as a secondary finding. The variant leads to a premature termination codon in exon 2. The girl exhibited no WD symptoms and no abnormalities in liver biopsy. *ATP7B* liver mRNA expression was comparable to healthy controls suggesting that nonsense-mediated mRNA decay (NMD) could be bypassed by the mechanism of translation reinitiation.

To verify this hypothesis, a CMV driven *ATP7B* minigene (pcDNA3) was equipped with the authentic *ATP7B* 5'UTR and a truncated intron 2. We introduced c.19_20del by site directed mutagenesis and overexpressed the constructs in HEK293T cells. We analyzed *ATP7B* expression by qRT-PCR, northern and western blot and examined protein function by copper export capacity assays.

Northern blot, qRT-PCR and western blot revealed that c.19_20del *ATP7B* mRNA and protein is expressed in size and amount comparable to wild-type. Copper export capacity was also comparable to wild-type. Our results indicate that c.19_20del in *ATP7B* is able to bypass NMD by translation reinitiation, demonstrating that the classification of truncating variants as pathogenic without additional investigations should be done carefully.

P-MonoG-183

Expanding the phenotypical spectrum of *BRD4* defects

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The growth of an individual is a variable and highly heterogeneous trait. Under the hypothesis that idiopathic short stature may be caused by rare, highly penetrant Mendelian variants we performed WES in a study group of individuals with a height below 2 standard deviations of the mean (SDS). In 2 independent non-consanguineous families we identified 2 missense heterozygous *de novo* variants in *BRD4* (c.1856G>T; c.2513A>T).

Bromodomain Protein 4 (*BRD4*) is a member of the bromodomain (BET) protein family involved in binding to hyperacetylated genomic regions of promoters and enhancers. *BRD4* mediates CDK9 activity to influence transcription elongation by RNA polymerase II and thus regulates gene expression, cell differentiation and cell cycle. Mice with heterozygous loss-of-function mutations in *Brd4* were reported with severe prenatal-onset of growth deficit, craniofacial abnormalities and low body fat. Recently, three patients with frameshift and missense variants were reported with a Cornelia de Lange-like phenotype including distinct facial gestalt, growth deficit, microcephaly and intellectual disability (Olley et al. 2018). Based on this observation, we identified 1 additional patient with a heterozygous *de novo* frameshift (c.2728delC) variant in *BRD4*. The patient with the frameshift variant and the patient with the c.1856G>T variant both presented with intellectual disability and Cornelia de Lange-like facial gestalt. Microcephaly, but not short stature, was only present in the patient with the frameshift variant.

Protein structure modelling indicates that variant c.1856G>T (p.Ser619I-so) likely affects binding of *BRD4* to its ligands, whereas c.2513A>T (p.His838Leu) does not alter the protein structure. Expression analysis however showed that both missense variants lead to a reduced expression. In contrast, *BRD4* expression levels were unaltered in RNA of the patient with the frameshift variant, excluding mRNA decay and supporting the hypothesis of a shorter functionally impaired protein by the c.2728delC variant indicating a loss-of-function effect of all three identified variants. Immunofluorescence analysis of lymphoblastoid cells from both patients with missense variants confirmed a significantly reduced cellular growth and smaller cell size when compared to controls. Transcriptional profiling to assess the effect of *BRD4* on effector proteins (CDK9, CCND1/2, PCNA, DNTTIP1/2, TOP2A und SPA-1) highlighted a complex dysregulation. These observations were verified by qPCR in CRISPR/Cas9 mediated *BRD4* defects in HEK293.

In conclusion, our findings expand the clinical spectrum of mutations in *BRD4* ranging from idiopathic short stature without distinct facial gestalt to a Cornelia de Lange-like phenotype. Furthermore, we confirm the proposed loss-of-function effects of *BRD4* mutations. We hypothesize that the variability of the phenotype might in part be explained by different effects of the variants on target effector genes.

P-MonoG-184

Is Kabuki syndrome another neurocrestopathy?

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Kabuki syndrome is an autosomal dominant developmental disorder characterized by a typical facial gestalt in combination with short stature, intellectual disability, skeletal findings and additional features like cardiac and urogenital malformations, cleft palate, hearing loss and ophthalmological anomalies. The major genetic cause of the disease are mutations in KMT2D, a gene encoding a histone H3 lysine 4 (H3K4) methyltransferase belonging to the group of chromatin regulators. To identify KMT2D target genes, we performed RNA sequencing on wild-type, KMT2D heterozygous (Mll4+/-) and KMT2D deficient (Mll4-/-) mouse embryos at day 9.5. We find that many of the dysregulated genes are related to neural crest cell induction and migration. Experimental analyses on *Xenopus laevis* demonstrate that KMT2D is expressed in neural crest (NC) cells at pre-migratory and migratory stages. Furthermore, KMT2D loss of function induces severe impairment of NC formation and NC cell migration. Our data link a chromatin modifier to neural crest development and human disease, providing first evidence that Kabuki syndrome may belong to the group of neurocrestopathies.

P-MonoG-185

Identification of the expression pattern of DYNC2LI1 deficiency in short-rib-polydactyly syndrome

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Primary cilia are organelles, present on almost all vertebrate cells. They are composed of the axoneme and the basal body and present various ciliary membrane receptors receiving extracellular signals. Those signals are transmitted into the cell, where they modulate different intracellular signaling pathways. Defects in cilia formation, maintenance and functionality are associated with developmental defects, like brain malformations, polydactyly, kidney cysts and skeletal abnormalities. This phenotypic spectrum is observed among patients with short-rib-polydactyly syndromes (SRPS), which presents the most frequent lethal autosomal recessive osteochondrodysplasia. In preceding work, we found diallelic mutations in the basal body protein NEK1 as an underlying cause of SRPS II. In a yeast two-hybrid screen, we identified DYNC2LI1 as interaction partner of NEK1. DYNC2LI1 is a part of the dynein-2 complex, involved in the retrograde intraflagellar transport (IFT). It is ubiquitously expressed in adult and fetal tissues, with the highest expression levels in chondrocytes. We have shown that defects of DYNC2LI1 lead to significantly reduced cilia length and altered cilia morphology with broadened ciliary tips, confirming the retrograde IFT defect.

As defects in other members of the retrograde IFT (DYNC2H1, WDR34, WDR60) have been identified in patients with overlapping clinical features, we now aimed to identify the potential underlying ciliary transduction pathways involved. Based on the observation of a loss-of-function effect of the DYNC2LI1 mutations, we used CRISPR/Cas9 mediated genomic editing to create DYNC2LI1 deficient HEK293T cell lines. Cell lines were sorted and sequenced to identify variants in DYNC2LI1. In 55 of 122 cell lines we detected NHEJ-based frameshift mutations predicted to disrupt protein function. Reduced DYNC2LI1 expression and protein levels were confirmed by qPCR and Western blot, respectively. We received 3 cell lines with significantly reduced expression levels for further analysis. The cell lines were either cultured under starvation conditions to initiate primary cilia formation or under regular growth conditions. We performed single cell sequencing for the mutated cell lines and cell lines without induced mutations for both conditions. Currently, analysis of involved genes and transduction pathways is ongoing. Here we hope

to gain insights in the differential expression of genes during ciliogenesis and cell proliferation to specify potential functions associated with the short-rib-polydactyly phenotype.

P-MonoG-186

A founder mutation uncovers MPZL2 (DFNB111) as a novel autosomal recessive non-syndromic moderate hearing loss gene

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Hereditary hearing loss (HL) is the most prevalent sensory deficit with a predominantly genetic background. Non-syndromic HL is inherited in an autosomal recessive pattern in 75–80% of affected individuals. Although it exhibits a high degree of clinical heterogeneity, autosomal recessive HL is generally characterized with a prelingual onset, non-progressive course, and severe-to-profound intensity. Current diagnostic rates around 50% assert that the genetic landscape of HL is poorly characterized. A major priority in the field is the identification of all genes involved in biologically crucial functions of normal hearing.

Analysis of exome and genome sequencing data identified a homozygous truncating c.72del (p.Ile24Metfs*22) variant in the gene MPZL2 in two unrelated Turkish families. MPZL2 encodes myelin protein zero-like 2, an epithelial junction protein, and maps to the autosomal recessive deafness locus DFNB111 on chromosome 11q23.3. Screening a combined Turkish and Iranian HL patient cohort totaling nearly 1000 individuals uncovered a third family and the first with an Iranian ethnicity, also presenting the c.72del homozygous variant. All three families indicated parental consanguinity.

Altogether, the variant segregated in eight affected individuals in the three families who all reported non-progressive HL. Seven of these individuals exhibited sloping audioprofiles revealing moderate sensorineural HL. A single individual disclosed asymmetrical (borderline severe) flat audioprofiles, suggesting the possibility of a genetic modifier in MPZL2-associated HL. Haplotype analysis in these three multiplex families implicated that the truncating homozygous variant originated from a single founder. Mpzl2 is expressed in both the outer and inner auditory hair cells in the P0 mouse. Expression appeared in an asymmetric subcellular location with a “vesicle like” structure toward the apical hair cells and at the cell membrane toward the basolateral portion.

Our combined in-house cohort consisting of 704 Turkish and 1300 Iranian individuals yielded a c.72del minor allele frequency of 0.00142 and 0.000384, respectively. Surprisingly, the gnomAD minor allele frequency of this variant is 0.00127 in Ashkenazi Jews and 0.00127 in Europeans, suggesting that it may be an important cause of HL in these respective populations. Its high minor allele frequency also poses a challenge in a molecular genetic diagnostic setting and leaves open the possibility for unintentional filtering from high-throughput sequencing datasets if minor allele frequency thresholds follow the most recent Expert Specification of the ACMG/AMP Variant Interpretation Guidelines for Genetic Hearing Loss PM2 population frequency criteria for autosomal recessive HL. Moreover, autosomal recessive HL caused by variants in *MPZL2* is one of the few genes associated with moderate HL, adding to the relatively short list of genes associated with moderate autosomal recessive HL.

P-MonoG-187

*** IP3 Receptor Degradation—A Mutational Hotspot for Hereditary Ataxia and Motor Neuron Disease

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Alterations of Ca²⁺ homeostasis have been implicated in a wide range of neurodegenerative diseases. Ca²⁺ efflux from the endoplasmic reticulum into the cytoplasm is controlled by binding of inositol 1,4,5-trisphosphate to its receptor. Activated inositol 1,4,5-trisphosphate receptors are then rapidly degraded by the endoplasmic reticulum associated degradation pathway. Mutations in genes encoding the neuronal isoform of the inositol 1,4,5-trisphosphate receptor (ITPR1) and those involved in inositol 1,4,5-trisphosphate receptor degradation (ERLIN1, ERLIN2) are known to cause Hereditary Spastic Paraplegia and Cerebellar Ataxia. We now show that mutations in the ubiquitin E3 ligase gene *RNF170*, which targets inositol 1,4,5-trisphosphate receptors for degradation cause autosomal recessive Hereditary Spastic Paraplegia in four unrelated families and demonstrate the functional consequences of mutations in patient fibroblasts and gene knockdown in zebrafish. Our novel findings highlight inositol 1,4,5-trisphosphate signalling as a key pathway for Hereditary Spastic Paraplegias and Cerebellar Ataxias and thus prioritize this pathway for therapeutic interventions.

P-MonoG-188

PIK3CA mutations in three children with megalencephaly capillary malformation (MCAP)

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Megalencephaly capillary malformation (MCAP; MIM 602501) is a disorder of segmental brain overgrowth including progressive megalencephaly,

ventriculomegaly, cerebellar tonsillar ectopia, polymicrogyria and further brain abnormalities. Patients show seizures, developmental delay and intellectual disability. MCAP is associated with additional features such as cutaneous vascular (mainly capillary) malformations and cutis marmorata, syndactyly, polydactyly, connective tissue dysplasia and hemihyperplasia. Previously, somatic mutations have been identified in the gene *PIK3CA* (MIM 171834) encoding the phosphoinositide 3-kinase (PI3K) catalytic subunit alpha of the PI3K-AKT pathway demonstrating MCAP as subset of the *PIK3CA*-related overgrowth spectrum (PROS).

We had the opportunity to identify *PIK3CA* mutations in three patients clinically diagnosed with MCAP. Patient 1 and 3 were macrosomic at birth. Cranial hemihypertrophy and sonographic brain asymmetry, an extensive facial naevus flammeus, cutis marmorata and hypoglycemic episodes were manifest in patient 1 and a naevus flammeus of the philtrum was manifest in patient 3. The latter patient developed progressive macrocephaly including megalencephaly, ventriculomegaly and triventricular hydrocephalus due to cerebellar tonsillar ectopia. Patient 2 was described with classical MCAP.

Mutation analysis of *PIK3CA* was performed by conventional Sanger sequencing or by applying a TruSeq Custom Amplicon Low Input Library Prep Kit (Illumina) on a Next-generation sequencing platform (MiSeq, Illumina). In patients 1 and 2 we identified somatic *PIK3CA* missense mutations c.2176G>A (p.Glu726Lys) and c.3104C>T (p.Ala1035Val), respectively, in DNA obtained from skin fibroblasts (mutant allele ratio [MAR] 30–35%) or saliva (MAR 35%) and blood (MAR 7%). Both *PIK3CA* mutations have been recently reported in MCAP with p.Glu726Lys reported most recurrently as mosaic and once as constitutional mutation. In the third patient *PIK3CA* missense mutation c.1049A>G (p.Asp350Gly) was detected at a MRA of 50% in DNA from blood suggesting a possible constitutional mutation. This novel *PIK3CA* missense mutation is positioned at an amino acid residue where different missense changes in mosaic patterns have been previously identified in MCAP. In addition, these changes were in proximity to mutations in the C2-domain of PI3K-alpha found in MCAP. We have observed none of three identified *PIK3CA* mutations in other disorders of PROS suggesting a distinct mutation profile in MCAP compared to the hot-spot mutations detected in segmental overgrowth other than MCAP.

P-MonoG-189

Variants of the gene *EDAR* underlying homozygous cases of autosomal recessive hypohidrotic ectodermal dysplasia

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Hypohidrotic ectodermal dysplasia (HED) is a rare genetic condition resulting from defective development of ectodermal derivatives, such as hair, teeth, and sweat glands. Autosomal recessive (AR) forms of HED may be caused by pathogenic variants of the gene *EDAR* that encodes a receptor involved in the NF-κB signaling pathway. The full-blown HED phenotype is observed only in individuals homozygous for such variants and therefore more frequent in consanguineous families. Here we describe three cases of AR-HED in families of Turkish, Austrian, and German-American origin (with or without known consanguinity) carrying very rare *EDAR* variants. In these cases, two out-of-frame deletions and a missense mutation were shown to be most likely disease-causing due to reduced availability of the respective mRNA or impaired interaction of the encoded protein with its binding partner leading to diminished signal transduction. The same missense mutation, c.1258C>T (p.Arg420Trp), has actually been reported to be a variant restricted to the Icelandic population and associated with non-syndromic tooth agenesis but not HED. As our patient has no known relationship to Icelandic individuals and displays a rather severe HED phenotype, we suggest that this variant represents a more widespread missense mutation, possibly with variable penetrance.

P-MonoG-190

Mutations in M1AP and MSH5 as novel cause for male infertility due to meiotic arrest

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Around 7% of all men are infertile. Revealing the underlying causes for male infertility aims to improve counselling and care for these men. Azoospermia, the lack of spermatozoa in the ejaculate, is assumed to be of genetic origin in a large fraction. Routinely, patients with azoospermia are screened for chromosomal aberrations and Y-chromosomal microdeletions. However, also monogenic alterations may cause male infertility. Some patients undergo testicular biopsy for testicular sperm extraction. By characterizing the patient's histological phenotype, meiotic arrest (MeiA) is a condition found in 2–10% of patients. If MeiA is complete, testicular sperm extraction (TESE) will not be successful. Recent studies implicate that MeiA in men and premature ovarian failure (POF) in women share common genetic causes.

In our study, 38 patients with complete bilateral MeiA and negative sperm retrieval results in TESE procedures underwent whole-exome-sequencing after excluding causes for infertility in the patient's history, aberrations of the karyotype, or Y-chromosomal microdeletions. We screened our data focusing on rare biallelic mutations [maximum allele frequency (MAF) <5% in all databases], which are clearly pathogenic (stop-, frameshift-, and splice site-mutations).

We detected two novel genes associated with MeiA in men. Both genes are expressed specifically in the testis. Three unrelated men were homozygous for the same frameshift mutation c.676dup (p.Trp226LeufsTer4) in *M1AP* (MAF=0.4%, gnomAD). *M1AP* has been described to cause MeiA in mice but up to now no pathogenic variants were described in men. The *M1AP* protein is needed for pairing of homologue chromosomes and repair of double-strand-breaks during meiosis. Additionally, we found a patient with MeiA and a novel homozygous frameshift mutation c.75dup (p.Ser26GlnfsTer42) in *MSH5*. Mutations in *MSH5* have already been described to cause POF in women, but no pathogenic mutations have been described in men yet. After screening our cohort of 462 additional patients with non-obstructive azoospermia, who did not undergo a testicular biopsy, we detected two more patients with a different novel homozygous frameshift-mutation of *MSH5* (c.1857del, p.Ala620GlnfsTer9).

Our data suggest that nonsense mutations in *M1AP* and *MSH5* cause autosomal-recessive MeiA. Functional studies to corroborate this finding are on their way. In the future, *M1AP* and *MSH5* may be screened in azoospermic patients before testicular biopsy. This may prevent unnecessary surgical procedures if there is no chance of finding mature spermatozoa in the testis.

This work was carried out within the frame of the DFG Clinical Research Unit 'Male Germ Cells: from Genes to Function' (CRU 326).

P-MonoG-191

Polymorphisms of IL8 and TNF- α genes as cystic fibrosis modifiers

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Background: Manifestation and course of cystic fibrosis (CF) depend on CFTR gene mutations, environmental factors and modifier genes. Crucial role in modifying the severity of disease is indicated on inflammatory

cytokines. Recent scientific outcomes concerning the role of modifiers in CF are inconclusive.

Aim: In our study, we have verified the hypothesis, that single nucleotide polymorphisms (SNPs) of two cytokine genes, IL8 and TNF- α , may play a role as potential modifiers of CF severity from digestive and pulmonary system.

Materials & Methods: We performed genotyping of four SNPs, rs4073 (-251T>A), rs2227306 (781C>T), rs2227307 (396T>G) in IL8 and -238G>A (rs361525) in TNF- α gene using pyrosequencing. We have examined the correlation between those genetic changes and the clinical outcome concerning the respiratory system (as FEV1%, FVC%, DLCO, RV%, lung transplantation), digestive system (pancreatic insufficiency, liver damage) and also BMI, age of diagnosis, genetic background and microbiological data in the group of 55 CF Polish patients, searching for genetic determinants of the manifestation of disease.

Results: We observed, that severe manifestation of CF from pulmonary system was presented in 44 patients (80%) and from the digestive system in 11 individuals (20%). We proved, that variant rs361525 in TNF- α gene correlate with better pulmonary function ($p=0.03$) and with reduced risk of death ($p=0.03$) in the studied group of patients. Furthermore, both changes, rs4073 and rs2227306 in IL8 gene correlated with liver cirrhosis ($p=0.02$; $p=0.01$ respectively), whereas variants rs4073 and rs2227307 determine the occurrence of severe digestive manifestation ($p=0.03$; $p=0.01$).

Conclusions: Genotype of TNF- α gene influence pulmonary function in CF patients, whereas variants in IL8 gene make worse digestive manifestation of the disease.

P-Technology and Bioinformatics

P-Techno-192

Mitochondrial and Nuclear Disease-Panel (Mito-aND-Panel): Combined Sequencing of Mitochondrial and Nuclear DNA by a Cost-Effective and Sensitive NGS-based Method

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Background: The diagnosis of mitochondrial disorders is challenging because of the clinical variability and genetic heterogeneity of these conditions. "Next Generation Sequencing" (NGS) technology offers a robust high-throughput platform for nuclear and mitochondrial DNA (mtDNA) analyses.

Method: We developed a custom Agilent SureSelect Mitochondrial and Nuclear Disease-Panel (Mito-aND-Panel) capture kit that allows parallel enrichment for subsequent NGS-based sequence analysis of nuclear mitochondrial disease-related genes and the complete mtDNA genome. Sequencing of enriched mtDNA simultaneously with nuclear genes was compared with the separated sequencing of the mitochondrial genome and whole exome sequencing (WES).

Results: The "Mito-aND-Panel" permits accurate detection of low-level mtDNA heteroplasmy due to a very high sequencing depth compared to standard diagnostic procedures using Sanger sequencing/SNaPshot and WES which is crucial to identify maternally inherited mitochondrial disorders.

Conclusion: We established a NGS-based method with combined sequencing of the complete mtDNA and nuclear genes which enables a more sensitive heteroplasmy detection of mtDNA mutations compared to traditional methods. Because the method promotes the analysis of mtDNA variants in large cohorts, it is cost-effective and simple to setup, we anticipate this

is a highly relevant method for sequence-based genetic diagnosis in clinical diagnostic applications.

P-Techno-193

Intronic variants validation using mini-gene splicing assay in unsolved whole exome sequencing genetic diagnosis

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Introduction/Background: Recognition of well-known intronic consensus sequences by the splicing machinery is the basis for the correct removal of introns from the pre-mRNA (Cooper and Mattox 1997; Hastings et al. 2001). Impairments in this process can lead to the production of aberrant mRNAs. It has been reported that a significant number of disease causing mutations either disrupts consensus splicing sequences or creates cryptic splice sites and consequently affects pre-mRNA splicing (Krawczak et al. 1992). Identifying these mutations and assessing their potential contribution to disease development is important for routine diagnostics. To assess the potential pathogenicity of splicing mutations in silico prediction tools can be used that analyze their potential effects on splicing (Houdayer et al. 2012). By re-analyzing whole exome sequencing data of unsolved cases we identified several intronic mutations potentially affecting splicing. Using the in silico prediction tools and mini gene splicing assays we here investigated the potential effects caused by the presence of intronic mutations on the splicing of adjacent exons.

Patients and Methods: A combination of splice site prediction tools MaxEntScan (Yeo and Burge 2004) and SpliceSiteFinder (Shapiro et al. 1987; Zhang et al. 1998) with a mutant score reduction of 15% and 5% (as recommended by Houdayer et al. 2012) using Alamut Software (Interactive Biosoftware) were used to further investigate potential splicing variant candidates of the fifty-one unsolved whole exome sequencing data at the Institute of Human Genetics at the University Medical Center Mainz. To validate the effect of the variant on the RNA level a mini-gene splicing assay were conducted using the exon trap vector pET01 (MoBiTec, Göttingen, Germany) and transfected into HEK293 cells. Total RNA was extracted and RT-PCR experiments were performed.

Results: A total of 3 different splice site variants were chosen: one hemizygous intronic missense variant located in the donor splice site of intron 3 of the BCORL1-Gen and predicted to have an exon skipping effect in a boy with severe microcephaly, congenital heart defects, short stature and severe global developmental delay; one heterozygous intronic missense variant located in the donor splice site of intron 5 of the HCN1-Gen found in a girl with epilepsy, sleep disturbance and speech delay, which was inherited from the affected mother. A 10 bp heterozygous intronic deletion located close to the donor splice site of exon 21 of the COL3A1-Gen and Acknowledgements predicted to have an exon skipping effect were found in a girl, whose mother died due to a brain haemorrhage. The variant were inherited from the mother to both of her children, the girl and her brother. Complete validation of the effects of these variants by performing mini gene assays is still in progress. We are grateful to the patients and families for their cooperation.

P-Techno-194

A concept of Integrated Diagnostics to improve patient care

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The implementation of Next-generation sequencing in routine diagnostics led to a drastic shift in genetic testing. With the help of these technology it is now possible, through new assays like whole-exome and whole

genome sequencing, RNA profiling, microbiome sequence or liquid biopsy, to further elucidate prior unclear cases. To unlock the complete potential of these high throughput tests it is necessary to combine the results with other laboratory tests to complement the information and provide a holistic view of the patient and the respective disorder. In state-of-the-art diagnostics the integration of different data sources, both external and internal, from different tests, workflows and clinical specialties are vital for optimal patient care.

One example for this integrated approach is the diagnostic routing for Non Small Cell Lung Carcinoma (NSCLC) where data from different tests and the cooperation between different clinical specialties are combined for an optimal performance. In pathology, tumor diagnosis and classification of the tumor tissue is performed on the basis of the primary or re-biopsy material. After morphological examination, multigene-panel sequencing is used to identify driver and passenger mutations. Based on the results, a molecular profile is created with the aim of identifying therapeutic options. The molecular profile can be used in a follow up by liquid biopsy or liquid profiling from peripheral blood. Targeted ctDNA Analysis is performed for the monitoring of minimal residual disease or therapy resistance. Tumor markers complement and increase the sensitivity of the follow-up. In case of clinical deterioration, therapy/drug resistance or conspicuous imaging results, a re-biopsy with morphological examination and molecular tumor profile is recommended.

In order to integrate and combine these different results new software tools are necessary to fully exploit the complementary data. Here we present MIDAS (Multiple Integration of Data Annotation Software), a central software system for data integration in a diagnostic laboratory. The goal of MIDAS is to construct a modular software system to integrate data from to aid the interpretation and build a comprehensive knowledge base. The MIDAS software may thus serve as central information system for all diagnostic patient data.

P-Techno-195

Performance of bioinformatic tools for NGS-based classification of germline variants

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The complexity of genetic medicine has greatly increased in the past few years, mainly due to the clinical implementation of next-generation sequencing (NGS). Genetic variants can be associated with monogenic diseases or with unknown and/or complex phenotypes. Missense variants with low impact on protein level or in poorly characterized genes tend to be classified as variants of unknown significance (VUS) leading to inconclusive diagnostic findings.

To meet the challenge of variant classification, in particular with BRCA1/2 VUS variants, and to support the genotype/phenotype correlation, we developed two bioinformatic tools: one supporting BRCA1/2 variants classification and one supporting all Mendelian disorders. The variant interpretation relies on two independent classification methods: Consensus classification (i.e., a summary of classifications available for a given variant from different sources) and ACMG classification based on public and proprietary data sources. In order to pre-validate the classification methods, we compared our BRCA tool results with classifications provided by various published hereditary breast and ovarian cancer studies (Hirotsu et al. (2015); Arai et al. (2017), Nagakomi et al., (2017)).

We show that the results of the automated, ACMG-guided interpretation of BRCA1/2 variants are comparable to the reference clinical classification of pathogenicity. The algorithm is designed to be conservative: of 108 pathogenic variants, BRCA tool classified 80 as pathogenic, 27 as likely pathogenic, and 1 as a VUS. The comparison in the benign category showed that 15 of 26 benign variants were classified as VUS, a discrepancy due to lack of public population-frequency and patient-family history data. In

parallel, with the Mendelian tool we compared the consensus classification with a study by Trujillano et al. (PMID: 27848944), in which 98 germline variants spread over 95 genes with a large phenotypic spectrum and from different ethnicities were classified. The consensus classification classified 59/98 as pathogenic and 7/98 as likely pathogenic, a result identical to the reference classification. Pre-calculated variant classifications were found to be accurately associated to the corresponding hereditary disease, which can significantly support the diagnostic process.

In addition, the tool reports a rough confidence level for each final verdict based on ClinVar rating stars. For the 59 variants classified as pathogenic, confidence levels are distributed as follows: 42 low, 14 medium, and 3 high. Missense variants are in general difficult to assess with regard to pathogenicity and correlation with the phenotype, so we evaluated them closely, taking the functional interpretation and the phylogenetic conservation scores into consideration. Further refinement of the methodology in close collaboration with laboratory partners is in progress.

In summary, although patient clinical information and family history are key parameters to holistically classify a variant, we have demonstrated that our tools allow germline variant classification, even without these data. Combining public and proprietary curated variant databases, they provide standardized profiling of all detected variants.

P-Techno-196

Development of a low-frequency variant calling tool for the analysis of tumor samples processed with ultra-deep next-generation sequencing (NGS)

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Background: Solid tumor samples are a mixture of cancer and stromal cells. The high genomic intra-tumor heterogeneity and tumor-purity makes the analysis of the tumor and the detection of the driver mutations challenging. Ultra-deep NGS of tumor tissue enables us to detect variants in the tumor genome even at very low frequencies and is the most promising technology for de novo mutation detection. All mutations regardless of the variant allele frequency (VAF) or genomic region are represented in the raw data given enough read depth. However, confident variant calling is not trivial due to noise in the sequencing data.

Method: We developed a low-frequency variant calling tool, LowFreq 1.0, which was integrated into our germline NGS analysis pipeline, allowing the detection of both high- and low-frequency variants. The tool does not require a matched-tumor sample and is a heuristic-based method, which integrates additional information describing the read mapping (e.g. uniformity and correlation scores) and quality parameters. First, the tumor DNA heterogeneity was simulated in the laboratory by mixing two reference DNAs (NA12560, NA12561) at seven different dilutions followed by sequencing on an Illumina NextSeq 500 (>1000X mean coverage, 1557 protein-coding genes). Resulting raw data was used as training data for the method development. Second, the robustness of the pipeline was evaluated using in silico simulated tumor data (mixture of RM8398 and NA12889 at ratio 1.5%, 2.5%, 3%, 5%, 10%, 15% and 20%), covering 93 cancer-related genes. The sequencing data were simulated in 100X step increments up to 1500X, in order to determine the limit of reliable variant calling. Furthermore, we aimed to decrease the false positive rate by implementing a more specific variant filter (i.e. distinction of true low-frequency variants from artifacts, which are known to often be platform-specific sequencing errors). In a diagnostic application setting, real tumor samples were sequenced on an Illumina MiSeq at high coverage for the target region of 7 cancer-related genes. These data served as a proof of concept to assess the mutation detection rate and to improve the artifact filtering based on real platform-specific data.

Results: We detect variants down to 3% (expected VAF) with a sensitivity of 91% and PPV of 81% at a 1400X mean coverage. Variants with 5% or 10% VAF were detected with the same sensitivity at lower coverage values

of 700X and 300X respectively. The false positive rate did not increase with the mean coverage. The analysis of tumor samples indicate that platform specific artifacts could be filtered out by using the VAF dispersion. True low-frequency variants in MLH1 and PMS2 revealed a highly dispersed measured VAF across different samples for the same variant. Conversely, variant calls present across multiple samples in BRCA1, BRCA2 and MLH1, which consistently show a low VAF dispersion, are more likely to be artifacts. These variants are often located in homopolymer regions or do not match population database entries.

P-Techno-197

OPN1 Diagnostics using Single-Molecule Long-Read Sequencing

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Aim: OPN1LW (opsin, long-wave sensitive) and OPN1 MW (opsin, mid-wave sensitive) encode the red and green cone opsin, respectively. The genes are located in tandem on chromosome Xq28 and show 98% homology. In individuals with normal color vision the opsin gene locus consists of a locus control region (LCR), a single copy of OPN1LW and one or multiple copies of OPN1 MW. Due to location and homology of the genes, homologous and non-homologous recombination leading to gene conversions or hybrid genes occur frequently, but also SNVs disrupting the opsin gene locus have been described. Pathogenic variants in either or both genes can lead to a range of different types of visual impairment from colorblindness to legal blindness.

OPN1 diagnostics is hampered by the high homology and the variable copy number of the genes. Analysis is currently done using gene-specific long-range (LR)-PCRs, shearing and sequencing on an Ion Torrent PGM combined with MLPA. Short read sequencing technology hinders interpretation, and results are sometimes not definite. Accordingly, the Radboudumc is currently one of few laboratories that are offering diagnostic sequencing of OPN1. Here, we investigate whether long-range PCR in combination with single-molecule real-time sequencing improves molecular diagnostics of OPN1.

Method: OPN1LW and OPN1 MW were amplified using two gene-specific LR-PCRs of ~16 kb. In order to allow circular consensus sequencing (CCS), a method that increases the accuracy of long-reads from 85% to >99%, we cut the amplicon-size to ~8 kb by restriction enzyme digestion. The 8 kb amplicons were pooled equimolarly, and underwent PacBio library preparation. Sequencing was done on a Sequel system, and CCS analysis including mapping was performed in SMRTLink 5.1.

Results: A cohort of 50 archived clinical samples, sent to the Radboudumc for OPN1 diagnostics in the years 2013–2018, was selected for sequencing. These included samples with known mutations, samples with previous indefinite sequencing results, and samples with suspected mutations in OPN1 that are not identified yet. Currently, sequencing and data analysis are ongoing, and data is available for the first 16 samples. In those, pathogenic variants (e.g. the OPN1 associated LIAVA or MIAVA combinations) are detected correctly. We further can identify hybrid genes (e.g. ex 1 + 2 of OPN1LW, ex 3 of OPN1 MW, and ex 4–6 of OPN1LW), and due to intronic differences we can also discriminate between one and two copies of OPN1 MW without using MLPA. Whether more than two copies of OPN1 MW can be determined correctly, and whether the residual cases can be solved needs to be determined.

Conclusions: Although this study is ongoing, we show that long read sequencing is capable to overcome (certain) limitations in diagnostic OPN1 testing in comparison to standard short-read sequencing. Therefore, long read sequencing is able to deliver improved OPN1 diagnostics.

P-Techno-198**Phenotero: annotate as you write**

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In clinical genetics, the Human Phenotype Ontology as well as disease ontologies are often used for deep phenotyping of patients and coding of clinical diagnoses. However, assigning ontology classes to patient descriptions is often disconnected from writing patient reports or manuscripts in word processing software. This additional workload and the requirement to install dedicated software may discourage usage of ontologies for parts of the target audience.

Here we present Phenotero, a freely available and simple solution to annotate patient phenotypes and diseases at the time of writing clinical reports or manuscripts. We adopt Zotero, a citation management software to create a tool which allows to reference classes from ontologies within text at the time of writing. We expect this approach to decrease the additional workload to a minimum while ensuring high quality associations with ontology classes. Standardised collection of phenotypic information at the time of describing the patient allows for streamlining the clinic workflow and efficient data entry. It will subsequently promote clinical and molecular diagnosis with the ultimate goal of better understanding genetic diseases. Thus, we hope that Phenotero eases the usage of ontologies and controlled vocabularies in the field of clinical genetics.

P-Techno-199**NGS in diagnostics: where things can go wrong**

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Although the next-generation sequencing (NGS) technology has been around for a number of years, it is still a new technology in continuous development. As a consequence, we still get occasionally surprised by errors which can occur at any step of the process and that according to Murphy's Law and our own experience will occur ("Anything that can go wrong will go wrong").

Because there is currently no platform for laboratories to share such issues, every laboratory has to identify and correct its own errors. The variable amounts and types of damage, if ignored, can negatively affect the final results. The impact on downstream applications such as sequencing can be profound: from simple library failures to libraries that produce spurious data, leading to misinterpretation of the results. Samples may get swapped, samples may get contaminated and during sample preparation sequences might get lost/amplified. The sequencing technology may have systematic errors and when the data go through analysis pipelines things may go wrong during mapping against a reference sequence, variant calling, variant annotation, variant interpretation, variant description and database searches. As a consequence, both false positive (non-existing variants called) and false negative (true variants missed) calls will be made.

This presentation will illustrate the problems by showing some of the errors that were encountered in the past years in our NGS routine diagnostic laboratory at MGZ (about 6,000 NGS analyses per year), discuss how these can be prevented, and most importantly, how errors can be detected. Rigorous evaluation and validation of NGS workflows and External Quality Assessment (EQA) are key requirements for using NGS technologies in routine molecular diagnostics.

We think that learning from each other's mistakes will help to improve the opportunities and simplify the challenges of the daily NGS routine.

P-Techno-200**Semi-Automatic Classification of Genetic Variants: The MIDAS CLA Module**

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Next-Generation Sequencing has brought vast opportunities to clinical interpretation of genetic information, through the ability to simultaneously sequence all genes contributing to a certain indication at a speed and cost superior to traditional sequencing approaches. Yet data analysis and especially the classification and interpretation of genetic variants are bottlenecks in state-of-the-art clinical diagnostics.

We designed MIDAS (Multiple Integration of Data Annotation Software), a central software system for data integration in a diagnostic laboratory. MIDAS is a modular constructed software system to integrate data from Laboratory Information Management System (LIMS), data from the routine Sanger sequencing workflow as well as NGS sequencing results. MIDAS' variant database module encapsulates a classification tool (CLA) that enables the evaluation of genetic variants identified in Mendelian disorders as 'pathogenic', 'likely pathogenic', 'uncertain significance', 'likely benign', and 'benign'. The semi-automated implementation of this classification tool is based on the variant classification guidelines set by the American College of Medical Genetics and Genomics (ACMG; Richards et. al Standards and Guidelines for Interpretation of Sequence Variants, 2015). These guidelines provide two sets of criteria: one for the classification of pathogenic and one for classification of benign variants. Each pathogenic criterion is either weighted as very strong (PVS1), strong (PS1–4), moderate (PM1–6) or supporting (PP1–5). Each benign criterion is either weighted as stand-alone (BA1), strong (BS1–4) or supporting (BP1–6). Finally the rules for combining the selected criteria, given by Richards et. al, are applied to classify the variant.

Researchers can use provided information about the variant from external databases, such as HGMD, ClinVar, gnomAD or OMIM, for the selection of applicable classification criteria. The classification tool in MIDAS offers a thematic categorization of all classification criteria and their linked information from external sources to quickly find the desired information necessary for a certain classification criterion. To examine, for example, whether a variant is absent from controls (PM2), the classification tool in MIDAS under the aspect 'population' will report all gnomAD database occurrences of the selected variant and automatically select PM2 criteria when no hits are found. All met classification criteria and metadata such as name of the author and date of the classification are saved to later track the history of a variants classification.

P-Techno-201**Improving Molecular Diagnostics of Autosomal Dominant Polycystic Kidney Disease by Using Long-Read Nanopore® Sequencing**

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Molecular genetic diagnostics of autosomal dominant polycystic kidney disease (ADPKD) can be challenging due to six highly homologous pseudogenes of PKD1, its allelic heterogeneity and high GC content. In particular the correct alignment of possible disease-causing sequence variants to either PKD1 or its pseudogenes is nearly impossible with short read sequencing data in certain parts of the coding sequence. In order to improve the detection of disease-relevant sequence variants or to assign disease-relevant variants to the canonical transcript it remained necessary to carry out very labor intensive PKD1 specific long-range PCRs (LR-PCR), nested PCR, and conventional Sanger sequencing in addition to short read NGS. We here show that long-read sequencing of LR-PCR products spanning the coding region of PKD1 is a reliable strategy to overcome the above

mentioned limitations. We present a Nanopore® sequencing based approach for comprehensive analysis of the PKD1 coding region that allows a reliable detection of single nucleotide variation (SNV). The presented method is time- and cost-efficient, scalable, allows the detection of low-grade mosaicism, and delivers results after only a few hours of sequencing.

P-Techno-202

*** RegulationSpotter: Interpretation and annotation of extratranscriptic DNA variants

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In the quest to elucidate the causes of rare genetic disorders, high-throughput DNA sequencing techniques such as Whole Genome Sequencing (WGS) have found their way into the realm of clinical medicine and routine diagnostics, with the UK100K project as the best-known example. Unfortunately, many tools for the evaluation and prioritisation of non-coding variants output hard to interpret scores instead of human-readable annotations. However, the comprehensible and understandable presentation of analysis results is a crucial aspect in successfully translating WGS from basic science to patient-driven applications. We think that software should allow clinicians and life scientists to actively participate in the data analysis, as their knowledge about patients' phenotypes and biological concepts is indispensable for a meaningful, in-depth interpretation of variants. We present RegulationSpotter, a web-based tool for the user-friendly annotation and interpretation of non-coding variants located outside of protein-coding transcript regions (extratranscriptic variants). Users can upload single variants or complete VCF files and limit their analysis to variants located within candidate genes, including interacting genomic elements. Additionally, coding and non-coding variants within protein-coding transcripts will automatically be analysed by MutationTaster. RegulationSpotter handles and displays 125 different genomic features from various sources such as ENCODE, FANTOM and other initiatives. For a meaningful interpretation, we give a classification of the functional impact of each variant, which can be either 'functional' or 'non-functional'. The classification is based on a simple numeric score reflecting the nature of the functional genomic elements a variant is located in. Our main focus lies however not on prediction, but on providing background information for clinicians and life scientists in a simple, convenient, and user-friendly way. RegulationSpotter can easily be used as a downstream application for other prediction programs such as CADD, GWAVA or the Genomiser. Our website provides a thorough documentation as well as tutorials. RegulationSpotter is freely available at <http://www.regulationspotter.org>.

P-Techno-203

ePOSSUM2: A web app for predicting the impact of DNA variants on transcription factor binding

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Modelling gene regulation is a major challenge in biomedical research. Mutations in transcription factor binding sites may cause the misregulation of genes, eventually leading to disease. The impact of DNA variants on transcription factor binding is modeled in silico using binding matrices, but it remains unclear whether these are capable of accurately representing in vivo binding. In 2016, we published a study that compared the ability of 179 binding models for 82 human transcription factors to detect experimentally verified "real" in vivo binding sites derived from ENCODE ChIP-seq data.

Our results showed that several models do not reliably detect experimentally verified binding sites. Using these results, we created ePOSSUM, a web application for predicting the impact of DNA variants on transcription factor binding.

Here we present an update of the application, ePOSSUM2. ePOSSUM2 has been rebuilt from the ground up with a modern C++ backend that runs many computations in milliseconds where ePOSSUM takes seconds. In ePOSSUM2, web users can easily upload VCF files to identify and rank variants that most likely affect gene expression. ePOSSUM2 supports more than 4,000 different binding models, including transcription factor flexible models. It also features an improved log-weighted matching algorithm that takes into account the wild-type dinucleotide distribution surrounding matching sites. For each binding model, ePOSSUM2 constructs a Bayes classifier and reports the likelihood that the user-supplied variant leads to the gain or loss of a transcription factor binding site. Because of the poor performance of several binding models in detecting experimentally verified sites, a negative or positive predictive value is reported. ePOSSUM2 is freely available at: <http://www.mutationdistiller.org/ePOSSUM2>

P-Techno-204

*** Gene expression imputation identifies candidate genes and a novel susceptibility locus for age-related macular degeneration (AMD)

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Purpose: In late stage age-related macular degeneration (AMD), genome-wide association studies (GWAS) have identified 52 independent gene variant signals in 34 genomic loci. While GWAS do not generally provide detailed information about the biological function of a particular polymorphism, Gamazon et al. (2015) proposed a gene-based association method called PrediXcan to address this issue. The approach estimates the component of gene expression determined by an individual's genetic profile. Subsequently, it correlates 'imputed' gene expression with the phenotype under investigation. This algorithm has the potential to identify susceptibility loci that were missed in previous GWAS analyses and to suggest candidate causal genes. Here, we used PrediXcan to estimate tissue-specific gene expression of European individuals, which participated in the latest AMD GWAS (Fritsche et al., 2016). As presently no eQTL data of eye samples are available, we have chosen to present the results of liver tissue, a primary site of gene transcription for at least two pathways relevant to AMD aetiology, namely the complement system and cholesterol metabolism.

Methods: We applied PrediXcan algorithms on the genotype information available through the IAMDGC data set and imputed gene expression data of liver tissue. Input included over 12 million genetic variants from 16,473 late-stage AMD cases and 19,033 healthy controls. After gene expression imputation of 3,330 genes, we applied a linear regression model to identify AMD correlated genes. To this end, we used the GTEx database to delineate genes showing local eQTL in liver tissue and to correlate this information with the respective AMD-associated variants.

Results: Overall, we identified 12 genes for which imputed gene expression was significantly correlated (p -value <0.001) with AMD status. Among these, 11 genes were located in well-known AMD loci. The largest effects (estimate 0.228–0.342, SE: 0.004–0.007) have been observed within the complement factor H related (CFHR) genes *CFHR1*, *CFHR3* and *CFHR4*. All of these encode for secreted proteins and likely play an important role in AMD pathology. Furthermore, *LIPC* revealed a higher expression in AMD cases (estimate 0.037, SE: 0.005) compared to controls, further reinforcing the notion that cholesterol metabolism is essential in AMD pathogenesis. Finally, we identified *C1orf21* on chromosome 1q25.3 as a potentially new susceptibility locus for AMD (estimate –0.029, SE: 0.005). This gene has a significant eQTL in liver tissue with rs10797981 (p -value: 6.6e–11) which in turn is known to be associated with AMD (p -value: 2.3e–06), although not with genome-wide significance.

Conclusions: PrediXcan identified 12 genetically regulated genes in the IAMGDC data for which expression is correlated to AMD status in liver. Furthermore, we were able to identify *C1orf21* as a novel AMD susceptibility locus. This analysis will be expanded to additional tissues of interest for AMD pathology.

P-Techno-205

An integrative web platform for user-friendly application of risk prediction models in hereditary cancer predisposition

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Background and Objective: Predicting individual mutation and cancer risks is essential to assist genetic counsellors in clinical decision making for patients with a hereditary cancer predisposition. Worldwide a variety of statistical models and empirical data for risk prediction have been developed and published for hereditary breast and ovarian cancer (HBOC), and hereditary non-polyposis colorectal cancer (HNPCC/Lynch syndrome, LS). However, only few models have so far been implemented in convenient and easy-to-use computer applications. We therefore aimed to develop user-friendly applications of selected HBOC and LS risk prediction models, and to make them available through the “Leipzig Health Atlas” (LHA), a web-based multifunctional platform to share research data, novel ontologies, models and software tools with the medical and scientific community. LHA is a project funded within the BMBF initiative “iDSem-Integrative data semantics in system medicine”.

Methods and Results: We selected a total of six statistical models and empirical datasets relevant for HBOC and LS: 1) the Manchester Scoring System, 2) the “Mutation Frequency Explorer” of the German Consortium for Hereditary Breast and Ovarian Cancer (GC-HBOC), 3) an extended version of the Claus model, 4) MMRpredict, 5) PREMM_{1,2,6}, and 6) PREMM₅. The Manchester Scoring System allows calculation of BRCA1/2 mutation probabilities based on aggregated family history. The “Mutation Frequency Explorer” allows flexible assessment of mutation risks in BRCA1/2 and other genes for different sets of familial cancer histories based on a large dataset from the GC-HBOC. The extended Claus model (as implemented in the commercial pedigree drawing software Cyrillic 2.1.3, which is no longer supported and no longer works on newer operating systems) predicts both mutation and breast cancer risks based on structured pedigree data. MMRpredict, PREMM 1,2,6, and PREMM 5 predict mutation risks in mismatch repair genes for patients from families suspected of having LS. All models were implemented using the statistical software “R” and the R-package “Shiny”. “Shiny” allows the development of interactive applications by incorporating “R” with HTML and other web technologies. The Shiny apps are accessible on the website of the “Leipzig Health Atlas” (<https://www.health-atlas.de>) for registered researchers and genetic counselors.

Conclusions: The risk prediction apps allow convenient calculation of mutation or cancer risks for an advice-seeking individual based on pedigree data or aggregated information on the familial cancer history. Target users should be specialized health professionals (physicians and genetic counselors) and scientists to ensure correct handling of the tools and careful interpretation of results.

P-Techno-206

flowCat: Automated classification of B-cell lymphoma sub types

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Leukaemia and lymphoma are haematological disorders of the bone marrow and lymphatic system involving malignant neoplasms of leukocytes.

There exists a large variety of subtypes, which can differ significantly in their therapeutical consequences and their prognosis. Differentiations between subtypes can only be done on the basis of histological findings in the affected tissues, karyotyping, genetic sequencing or immunological analysis via flow cytometry.

Multi-channel flow cytometry (MFC) is an integral diagnostic method in diagnosis and monitoring of haematological disorders. With the availability of ever more powerful measuring devices, a large number of computational approaches have been developed to automate the manual process of gating and interpreting cell populations in flow cytometry data. We extended previous works on the classification of flow cytometry data and developed a framework to analyze peripheral blood or bone marrow samples of patients with suspected B-cell lymphomas.

We created an automated approach to directly classify to diagnosis labels from flow cytometry data without further human processing. Our approach achieved overall weighted f1-scores of 0.92 in an 8-class classification.

MFC data (FCS files) was obtained from routine diagnostic MFC of 16543 patients and included 9 different B-cell lymphoma subtypes distributed as follows: chronic lymphocytic leukemia (CLL, *n*=3356), prolymphocytic leukemia (PL, *n*=597), follicular lymphoma (FL, *n*=218), hairy cell leukemia (HCL, *n*=187), lymphoplasmacytic lymphoma (LPL, *n*=597), mantle cell lymphoma (MCL, *n*=415), marginal zone lymphoma (MZL, *n*=968), monoclonal B-cell lymphocytosis (MBL, *n*=1458), and non-pathological normal cases (*n*=8442). Two 11-colour MFC tubes were used to measure up to 50,000 cells per case.

Compensated and transformed flow cytometry data from a single case is used to train a self-organizing map, which is used as input to a convolutional neural network generating class predictions. The model was trained from scratch with 10% of our initial dataset used for validation.

In order to visualize the decision process, we utilized a saliency analysis of the trained model, which is used to label the original input data to define populations of special interest to the resulting classification decision. We demonstrate the feasibility of automated classification to external information in the form of subtype labels in dataset both larger in the number of samples and classes than previously described data with a high accuracy. Even with classifiers getting more reliable, proper visualization of the decision process will remain integral to enable the applicability of such approaches to real-world medical practice as well as robust detection of aberrant information.

P-Techno-207

Interphase cells for fluorescence in situ hybridization from human skin

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It is well known that metaphase cells can exclusively be obtained and studied after time consuming cell culture. Still, interphase cells can be gained from many different cells and even without cell culture after short preparation. Here we present an approach by which interphase cells can be easily obtained from skin surface, practically without causing any pain or damage to the patient. The procedure is simple, and is initiated by adding some microliters of liquid to the patient's skin (e.g. the forearm) and subsequently carefully scratching along the wetted skin region by the edge of a slide collects skin cells together with the fluid. Applying this simple cell collection approach, several hundreds of skin-cells/interphases are obtained. The result may be checked in phase contrast microscope and regions with highest density of cells can be selected for interphase-fluorescence in situ hybridization (FISH). Finally, the cells obtained in the described way can be used for DNA-based molecular genetic tests. Here this straightforward method for acquisition of interphase cells is presented. It may be used to complement the available possibilities for the analyses of different tissues in case of questionable low level mosaics detected in peripheral blood testing or buccal mucosa.

P-Therapy for Genetic Diseases

P-Therap-208

Molecular diagnosis of cerebral folate transport deficiency due to FOLR1 compound heterozygous mutations and treatment with folinic acid

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Here we report on a 14-month-old boy of non consanguineous parents who presented prenatally with severe microcephaly. He was born at 38 weeks of gestation with a birth weight of 2430 g (4th centile, a length of 44 cm (–2,9SD) and a HC of 29,5 cm (–3,6 SD). Seizures (due to a bilateral temporooccipital focus) started at the age of 6 months. On MRI, cerebellar hypoplasia and ventricular enlargement were observed and abnormal myelination or leucodystrophy were suspected. At the age of 8 months, he showed muscular hypotonia and opisthotonus and had not reached any motor milestones. At that time, he measured 64 cm (2nd centile), weighed 8300 g (43th centile) and had a HC of 40 cm (–4 SD).

An NGS-panel analysis of 55 genes related to syndromic or non-syndromic microcephaly did not detect any pathogenic mutation. When the patient was 12 months old, we initiated trio exome sequencing (Agilent SureSelect Human All Exon V7), by which we detected two compound heterozygous mutations in FOLR1, c.324G>C, (maternal, predicted to be pathogenic) and c.373C>T, (paternal, known pathogenic mutation). Our molecular diagnosis was confirmed by a reduced 5-methyltetrahydrofolate (5MTHF) level in cerebral spinal fluid (<5 nmol/l in affected patients).

Homozygous or compound heterozygous mutations in FOLR1 cause neurodegeneration due to cerebral folate transport deficiency (OMIM 613068), which is a treatable neurodegenerative disorder associated with disturbed myelin metabolism (Steinfeld R et al., 2009). The phenotype is associated with severe global developmental delay or arrest and proceeds to neurodegeneration in preschool age if untreated. High-dose oral or intravenous treatment with folinic acid in the second year of life has been reported to stop disease progression or even to lead to complete recovery of symptoms (Steinfeld R et al., 2009, Delmelle et al., 2016).

Here, we point out the striking importance of a fast working NGS pipeline to diagnose patients with treatable rare genetic diseases at early ages. This holds true especially for diseases which are not detectable by newborn screening. In this case, the diagnosis was made within 23 days upon reception of the DNA sample for whole exome sequencing, enabling immediate initiation of folinic acid treatment at the age of 13 months. The patient is currently being followed up for clinical outcome.

P-Therap-209

Tau based therapeutic interventions in cellular models of Alzheimer disease and rare tauopathies

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Alzheimer Disease (AD) and Frontotemporal Dementia (FTD) are devastating neurological disorders characterized by severe cognitive decline until complete dependency in their late stages, and impose a tremendous burden on families, caregivers and society. There are no treatments that can effectively stop or reverse the disease progression. The best molecular and histopathological correlate of the declining cognitive functions of these forms of dementia is loss of synapses and intraneuronal accumulations of the protein Tau. This Tau pathology spreads in a very defined fashion throughout the brain (Braak stages) in AD, but the susceptible neuronal cell types remain unknown. Traditionally, Tau is considered an axonal protein important for microtubule stabilization. Central nervous

system Tau is composed of 6 splice-isoforms, which are differentially expressed throughout the brain, likely due to different axonal makeup of the neuronal subtypes. Some forms of FTD are caused by exonic mutations of Tau (FTDP17), while others only show a change in the isoform ratio (e.g. Pick Disease and Corticobasal Degeneration). Yet, the functions of the different isoforms and their contribution to disease pathology is unknown and understudied.

The 6 isoforms of Tau are differentially expressed during development, and also expressed in different ratios in different parts of the brain. Using primary rodent neurons, we have previously shown that the different isoforms of Tau are axodendritically differentially distributed, and that this localization is disturbed upon exposure of cells to AD-like stress. Preliminary data suggests that the least expressed human Tau isoform (2N4R) is responsible for Tau synaptotoxicity, as it is localized in dendrites (Zempel et al., 2017) and restore Tau toxicity when reintroduced into TauKO neurons (unpublished data). Very recently, the beginning of Tau pathology in human brain was found to begin in dendrites of subsets of unidentified neurons (Braak & del Tredici, 2018), in agreement with our proposed model of isoform specific mediation of Tau toxicity (Zempel, 2018, under review). Cellular models are needed to pin down the responsible isoform of Tau, the expression pattern of Tau and the susceptibility of different neuronal cell types for Tau pathology in order to develop novel therapeutic approaches.

With this project we aim to investigate the contribution of the different human isoforms of Tau in primary neurons and human induced pluripotent stem cells (iPSC) derived neurons. We aim to establish the neuronal differentiation of existing iPSCs to investigate the expression pattern and localization of the different Tau isoforms, and identify their contribution to the dementia causing synaptotoxicity. Using state of the art microscopy techniques as well as transcriptomic-based cell cluster analysis, we will identify the susceptible neuronal populations. Once the isoform and the susceptibility genes/neuronal subtypes are identified, we will use both antibody-based as well as RNAi-based methods to reduce the toxic isoform of Tau. The identification of the molecular and genetic make-up of neurons suffering from Tau pathology and synaptic loss will directly translate into novel therapeutic approaches.

P-Therap-210

*** Comprehensive genetic characterization and evaluation of clinical response in a neurofibromatosis type 2 patient treated with bevacizumab

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Neurofibromatosis type 2 (NF2) is a tumor predisposition syndrome characterized by the growth of nervous system tumors, notably schwannomas of cranial, spinal and peripheral nerves, meningiomas, and gliomas. Heterozygous pathogenic variants in the NF2 gene are known to cause NF2, whereby a somatic mosaicism is present in around 25% of patients. However, in patients with schwannomatosis, heterozygous SMARCB1 or LZTR1 pathogenic variants have also been identified. For the treatment of vestibular schwannomas (VS), the efficiency of bevacizumab, a monoclonal antibody against the angiogenic factor VEGF, has been described in some cases. Here, we report on a thirtythree-year-old male patient with nine intracranial schwannomas, seven spinal schwannomas and one intracranial meningioma. At the age of 25 years, bilateral VS were diagnosed in the patient as a cause of his progressive hearing loss. Given this diagnostic hallmark, the patient fulfilled the National Institutes of Health criteria for NF2, which classify bilateral VS as an independent main criterion. To identify the genetic basis of the patient's phenotype we performed target-

ed next generation sequencing (NGS) using the TruSight Cancer Sequencing Panel, which includes *NF2* and *SMARCB1*, on DNA from peripheral blood and oral mucosa of the patient followed by Sanger sequencing of *LZTR1*. No disease causing variants in *NF2*, *SMARCB1* or *LZTR1* were detected in these tissues. Subsequent Sanger sequencing on DNA of three formalin-fixed, paraffin-embedded schwannomas of the patient identified a known pathogenic nonsense variant in exon 8, c.784C>T; p.(R262*), of *NF2* (NM_000268.3, GRCh37/hg19). Consistent with somatic mosaicism, re-evaluation of NGS data with a mean coverage of over 500 confirmed that the variant was not detectable in DNA from blood or oral mucosa. Based on evidence for the efficiency of bevacizumab in the treatment of VS, the patient underwent an off-label therapy with a dose of 5 mg/kg bevacizumab. To determine treatment response with respect to hearing capacity and growth of cranial and spinal tumors, we evaluated pure-tone audiometry as well as cranial and spinal MRI scans from five pre-therapeutic and two therapeutic years. Preliminary data show an average hearing improvement of 10 dB after 12 months of treatment compared with baseline pure-tone audiometry. Eight tumors could be assessed by volumetric measurement, and all of them progressed prior to treatment. Five of seven schwannomas (71%) showed volume reduction of at least 20% after 15 months of treatment, while one schwannoma and the meningioma did not display tumor shrinkage. In conclusion, in a patient with clinical NF2 and the genetic diagnosis of somatic *NF2* mosaicism, an off-label therapy with bevacizumab was efficient with respect to hearing improvement and reduction in the volume of spinal and cranial schwannomas over a period of at least 15 months.

P-Therap-211

*** Developing a Scalable Drug Screening Pipeline for Bestrophin-1 (BEST1)

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Purpose: Human BEST1 is a chloride channel controlled by Ca^{2+} and cell volume and is localized to the basolateral membrane of the retinal pigment epithelium (RPE) in the posterior eye. So far, there is no therapy for BEST1-associated disease of which the most common is Best vitelliform macular dystrophy, also known as Best disease (BD). Our current efforts are directed towards developing drug screening pipelines which target both, BEST1 localization correction (correctors) and BEST1 channel function enhancement (potentiators).

Methods: We established a cellular model, stably expressing wildtype BEST1 or BD-associated BEST1 mutations in MDCKII cell lines. To screen for BEST1 correctors, we designed a BEST1 localization assay based on immunocytochemistry and converted it to a 96 well plate format applying robotic process automation. The microscopy readout and statistical analysis were performed automated by a high content imaging system (Opera[®]). To identify potentiator molecules, we developed the halide assay by using the BEST1-expressing MDCKII cell model in the presence of yellow fluorescent protein (YFP)-based halide sensor. Cells were stimulated by extracellular addition of iodide known to pass the plasma membrane through anion channels consequently quenching intracellular YFP fluorescence. Variations in YFP fluorescence levels as a marker for BEST1 function were recorded in 96 well plates by a plate reader setup. A small-scale 2,560 compound library, commercially available as Spectrum Collection (MicroSource Discovery Systems, Gaylordsville, USA) was used for initial screening performances.

Results: Consistent with endogenous BEST1 in human RPE, wildtype BEST1 shows authentic localization at the plasma membrane in the MDCKII cell model, while several BEST1 mutant forms are grossly mislocalized to the cytoplasm. Based on a defined algorithm, wildtype-like plasma membrane and mutant-like intracellular BEST1 localization can reliably be distinguished in a cell monolayer by automated microscopy analysis. The halide assay revealed reproducible halide permeability across wells and, as a control, reliably detected MDCKII cells overexpressing wild type BEST1 by a decrease of YFP fluorescence to 70% following a 60 second stimula-

tion. Under these conditions, cells expressing mutant BEST1 showed 85% of default YFP fluorescence. Five compounds were detected enhancing halide permeability without a negative effect on cell layer integrity or default YFP fluorescence intensity. Two of these revealed an enhancing effect on BEST1 channel conductance across several BEST1 mutations.

Conclusion: The current study established two assays well suited for high-scale compound screening for BEST1 correctors or potentiators. These pipelines provide the basis for screening large chemical compound libraries in search for future therapies for BEST1-associated retinal diseases.

P-Therap-212

Long term chemoprevention with sulindac in patients with familial adenomatous polyposis coli

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Aim: Several studies on effectiveness of non-steroidal anti-inflammatory drugs (NSAIDs) at reducing polyp burden in patients with familial adenomatous polyposis (FAP) point towards a decrease in number and size of polyps. However, no data on effectiveness and safety over long periods of time have been available so far. This study assesses the long-term benefit of sulindac in a large cohort of patients with FAP with and without prior colectomy.

Methods: We evaluated patients with adenomatous polyposis (with pathogenic APC or MUTYH germline mutations or the classical clinical picture of FAP) taking sulindac; the total absolute observation period exceeded 340 patient-years with a mean of more than 8 years per patient. 17 patients had an intact colon.

Results: The majority of patients showed a fast (i. e. rapid decrease of polyp burden; 57.8%), and slow response (17.8%) on sulindac treatment, and 22.2% had stable polyp burden allowing conservative treatment. Only one patient had slow progress of colonic polyp burden. There also was an effect on upper GI polyp burden with a stable course or improvement in 69% of patients. There were no serious side-effects. Detailed patient specific data will be presented.

Conclusion: Sulindac can be considered an effective and safe drug for long-term use in FAP patients, postponing or even preventing surgical procedures for the majority of patients and thereby maintaining higher quality of life in young adulthood. The occurrence of carcinomas in situ (all within the first 5 years of sulindac therapy) suggests some adenomas had reached a "point of no return" prior to initiation of sulindac therapy, making close colonoscopy intervals necessary. This study can serve as a pilot study for further prospective trials as currently under way.

P-Therap-213

Eteplirsen, a Phosphorodiamidate Morpholino Oligomer for Duchenne Muscular Dystrophy: Longitudinal Comparison to External Controls on Six-Minute Walk Test and Loss of Ambulation

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Objective: Eteplirsen is a phosphorodiamidate morpholino oligomer (PMO) that binds to and excludes exon 51 from dystrophin pre-mRNA, allowing the production of internally shortened dystrophin protein in patients with Duchenne muscular dystrophy (DMD). We evaluated ambulatory function in DMD patients in a phase 2 study of eteplirsen and compared their progression with that of an external control group with

comparable age, glucocorticoid use, and genotype from a DMD natural history study.

Methods: An analysis of performance on the 6-Minute Walk Test (6MWT) over 4 years compared DMD patients aged 7–13 years treated with intravenous eteplirsen 30 or 50 mg/kg/wk ($n = 12$) with comparable untreated external controls ($n = 12$).

Results: At Years 3 and 4, a statistically significant treatment benefit versus external controls of 147 m ($P = 0.007$) and 165 m ($P = 0.001$), respectively, on the 6MWT was observed in eteplirsen patients. Kaplan-Meier estimates of loss of ambulation (LOA) at Year 4 showed that 88% of external controls lost ambulation versus 17% of eteplirsen patients (log-rank, $P = 0.011$). Median age at LOA in external controls was 13.0 years. Eteplirsen patients did not reach a median age of LOA, as 10/12 patients were still ambulatory at Year 4; however, median age of eteplirsen patients at Year 4 was 13.4 years.

Conclusion: A statistically significant treatment advantage was observed for the 6MWT and for LOA following 4 years of eteplirsen treatment, suggesting that eteplirsen may delay the progression of ambulatory function decline in DMD.

P-Therap-214

NGS study of glucocorticoids response in IBD patients

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Glucocorticoids (GCS) are first-line drugs for the achievement of remission in exacerbations of inflammatory bowel diseases (IBD). On average, only every second adult patient with IBD is sensitive to GCS, close to 20% of subjects are steroid-resistant, and approximately 30–40% of cases become dependent on these drugs. Despite a long history of GCS use in various clinical areas and a lot of research, they still generate the challenge for personalized medicine, which currently focuses on therapy effects prediction. Genetic background can influence the response to treatment. Based on the current knowledge, we composed a panel of 22 candidate genes involved in GCS action: NR3C1, IL1A, IL1B, IL2, IL4, IL8, IL10, NFKBIA, TNF, LTA, HSP90AA1, HSPA4, STIP1, FKBP5, NLRP1, MAPK14, JUN, MMIF, CYP3A4, CYP3A5, ABCB1 and IPO13. This study aimed to design and validate the amplicon NGS methodology to determine genetic predictors of patients variable reaction to GCS therapy. We performed the analysis of genes mentioned above in group 139 clinically diagnosed IBD patients: 77 (55.4%) individuals with Crohn's disease (CD) and 62 (44.6%) individuals with ulcerative colitis (UC), treated with methylprednisolone and hydrocortisone. Seventy seven (55.4%) patients were classified as steroid sensitive, 38 (27.3%) steroid-dependent and 24 (17.3%) resistance. Our NGS results indicated 430 ± 63 polymorphisms per sample. Among 121 different consequence DNA variants in total group, we selected 31 changes for correlation analysis, which occurred at a frequency above 2%. Statistically significant association of specific variants with GCS response was detected. The results demonstrate that substitution p.Cys13Ser (rs61745470) in the HSPA4 gene ($p = 0.027$) and p.Glu31Ter (rs188378669) in the IL8 gene ($p = 0.042$) significantly related with GCS-resistance by CD patients. There was also a significant statistical relationship between the occurrence of the variant p.Asn363Ser (rs6195) in the NR3C1 gene and GCS-sensitivity in patients with IBD. Besides, a correlation was observed—on the verge of statistical significance ($p = 0.05$), between the genotype CC in position p.Gly412Gly (rs1128503) in the ABCB1 gene and the correct response to GCS in IBD patients. These results confirm that multigenetic factors may affect the GCS treatment effect in IBD and further investigation on the larger cohort for predicting response are necessary. The Polish National Science Centre supported the research (grant no. 2015/17/N/N25/00689).

P-Therap-215

Paving the way—Transcriptome analysis in Huntington's Disease after metformin treatment

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Huntington's Disease (HD) is an autosomal dominant neurodegenerative disorder, which is caused by a glutamine (CAG) trinucleotide repeat expansion in the exon 1 of the Huntingtin gene (*HTT*). Within the prodromal phase patients develop mood swings, personality changes and subtle cognitive impairment. Later on the disease affects motor abilities and is furthermore characterized by cognitive decline. Understanding molecular mechanisms including changes in gene expression is a very important step for developing effective treatment strategies. We have shown previously that mediated by mTOR signaling, translation of mRNAs carrying expanded CAG repeats is increased (Krauss et al., 2013) and that the anti-diabetic drug metformin antagonizes mTOR signaling in neurons *in vitro* and *in vivo* (Kickstein et al., 2010). Additionally, we have shown recently that metformin by interfering with the mTOR kinase and its opposing phosphatase, PP2A, regulates local protein synthesis in the brain and is able to suppress the production of disease making protein in early HD (Arnoux et al., 2018). Furthermore three weeks treatment with metformin in the drinking water rescues early network aberrations and leads to a significant improvement of early cognitive symptoms in the HdhCAG150 animal model.

Mutant HTT protein is considered to disturb normal gene expression through binding of transcription factors in the nucleus. Therefore we analysed brain samples (striatum and cortex) from 12 weeks old WT and HdhCAG150 after three weeks of metformin treatment compared to controls by RNA sequencing to investigate the differences in the transcriptome and the effect of metformin in early stages of HD. Additionally, we will analyse 8 months old animals (Hdh150 and WT) after metformin treatment over a period of 5 months to analyze effects of long-term metformin treatment on later-stage HD. Together with our recently published data this will pave the way for a prove of principle metformin trials in HD patients.

Aartsma-Rus A.	S6-01
Abad C.	P-MonoG-186
Abicht A.	P-BasEpi-004, P-ClinG-047, P-ClinG-095, P-Techno-192
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Adam R.	P-CancG-032, P-CancG-033
Adham I.	SEL-02
Adjaye J.	P-CancG-038
Agaimy A.	W2-06, P-MonoG-175
Aguet F.	W4-06
Aguilar-Pimentel J. A.	W5-03
Ahmadiéh H.	W9-04
Ahnert P.	P-CancG-041
Ahting U.	P-ClinG-118
Aiden E. L.	S5-02
Akbani AQ.	P-BasEpi-001
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Aksentijevich I.	P-MonoG-176
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Alagramam KN.	W5-06
Alawi M.	P-ClinG-101
Albrecht B.	W9-06
Albus M.	P-Compl-127
Aldhorae K.	P-Compl-126
Alfadhel M.	W1-03
Al-Gazali L.	W8-04
Alhaddad B.	W5-02, W9-03
Alhashem A.	W9-06
Alkuraya F.	W1-03, P-MonoG-156
Allen W.	W1-06
Alper O.	P-MonoG-186
Al-Rikabi A. B. H.	P-Techno-207
Al-Sannaa N.	W9-06
Alt K.	P-ClinG-096
Alter S.	P-ClinG-043, P-ClinG-080, P-MonoG-151, P-MonoG-163
Altmüller J.	SEL-02, W3-02, W4-02, W5- 04, W8-02, W8-03, W8-04, P-BasEpi-019, P-CancG-031, P-CancG-033, P-CancG-032, P-CancG-039, P-CancG-040, P-ClinG-086, P-ClinG-101, P-ClinG-106, P-MonoG-155, P-MonoG-156, P-MonoG-170, P-Therap-208
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Amarie OV.	W5-03
Ameling S.	P-MonoG-180
Ammerpohl O.	P-CancG-039
Anders S.	P-ClinG-058, P-ClinG-109, P-CytoG-136
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Andre S.	W7-01
Andres S.	P-ClinG-044

Angione KM.	W1-06
Ardicli D.	P-MonoG-156
Arelin M.	W5-01, P-ClinG-075
Aretz S.	P-CancG-032, P-CancG-033
Argyriou L.	P-Therap-208
Arlt A.	P-BasEpi-011, P-BasEpi-020
Arnold A.	P-Techno-196
Arnold N.	P-CancG-027, P-CancG-028
Asadollahi R.	W1-01
Aschauer D.	P-BasEpi-011
Auber B.	P-CancG-022, P-CancG-035, P-ClinG-077, P-ClinG-117, P-MonoG-182, P-Therap-210
Aung T.	W6-01
Au-Yeung R.	P-CancG-040
Ayzenberg I.	P-ClinG-050
Azaiez H.	W5-06
Azzarello-Burri S.	W1-01, W9-01

Babcock H.	W9-05
Bachmann S.	P-Therap-209
Backsch C.	P-CancG-042
Bademci G.	P-MonoG-186
Bader I.	P-MonoG-152
Bagowski C.	P-ClinG-067
Bagowski C. P.	P-ClinG-089
Bah M.	W9-06
Bahena-Carbajal P.	W9-04
Bailey J.	W2-05
Bakhtiari S.	P-MonoG-187
Baldinger R.	W9-01
Ballin N.	P-ClinG-080
Bamborschke D.	P-MonoG-156
Bär KJ.	P-BasEpi-014
Barbarics B.	SEL-02
Barrio Garcia S.	P-BasEpi-006
Bartaun C.	P-CytoG-136
Bartelt A.	P-BasEpi-016
Bartolomaeus B.	W5-01
Bartolomaeus T.	P-CytoG-137
Bartsch O.	P-ClinG-053, P-ClinG-054
Bartusel M.	W6-03
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Battaglia A.	W1-01
Batura-Gabryel H.	P-MonoG-191
Bauckhage C.	W4-01
Bauer M.	P-Compl-127
Bauer P.	W9-06
Bauer S.	P-ClinG-082
Bauernhofer T.	W4-05
Bauerschlag D.	P-CancG-027, P-CancG-028
Baumann M.	P-ClinG-105
Baumann U.	P-MonoG-182
Baumer A.	W8-02
Baune B.	P-Compl-124, P-Compl-129
Baydan F.	P-MonoG-157
Beblo S.	P-ClinG-075
Beck E.	P-Compl-125
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Beck M.	P-ClinG-111
Becker J.	W6-04, P-Compl-123, P-CytoG-139
Becker K.	W5-04, P-ClinG-095, P-MonoG-156, P-MonoG-157, P-MonoG-170
Becker L.	W5-03
Becker T.	P-Compl-126
Beck-Wödl S.	P-ClinG-076
Beer K.	P-CancG-042
Beetz C.	P-MonoG-154
Beetz R.	P-ClinG-054
Begemann M.	W2-01, W5-05, P-BasEpi-010, P-BasEpi-021, P-ClinG-056, P-ClinG-060
Behboud H.	W9-04
Behnecke A.	P-ClinG-045
Behr J.	SEL-03
Behunova J.	P-ClinG-046
Bej S.	P-Compl-134
Bekhit A.	P-MonoG-178
Beleggia F.	W8-03, W8-04
Belic J.	W4-05
Benet-Pages A.	P-BasEpi-004, P-ClinG-047, P-Techno-196, P-Techno-192, P-Techno-199
Bens S.	W2-04, P-BasEpi-009, P-CancG-040
Bergmann A.	W2-02
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