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ABSTRACT BOOK

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Thursday Morning 27th April

PHENOTYPING AND AI

Peter N Robinson

THE HUMAN PHENOTYPE ONTOLOGY: AI AND SEMANTIC ALGORITHMS FOR PHENOTYPE DRIVEN TRANSLATIONAL RESEARCH AND GENOMIC DIAGNOSTICS

Knowledge representation and reasoning is the field of artificial intelligence (AI) dedicated to representing information about a domain to enable computational analysis. An ontology is a form of knowledge representation that formally names concepts and defines their properties and relations to one another. The Human Phenotype Ontology (HPO) is an OWL ontology that models the phenotypic abnormalities seen in human disease, providing a hierarchy of over 16,000 terms (concepts), many of which are computationally defined as OWL class definitions that leverage other open-source ontologies for anatomy, histology, pathology, biochemistry, and gene function to model human phenotypic abnormalities. The computational foundation of the HPO enables sophisticated computational algorithms that are being used for computational deep phenotyping and precision medicine as well as integration of clinical data into translational research. The HPO is being increasingly adopted as a standard for phenotypic abnormalities by diverse groups such as international rare disease organizations, registries, clinical labs, biomedical resources, and clinical software tools and will thereby contribute towards nascent efforts at global data exchange for identifying disease etiologies. In this talk I will explain how HPO-based algorithms for the analysis of clinical data work and show how they can be applied to genomic diagnostics of rare genetic diseases, which is an area where ontologies have made a substantial contribution to translational research and diagnostics. I will focus on how the HPO enables fuzzy, specificity weighted phenotype matching, an approach that drives the Exomiser software which was the best performing software for phenotype-driven genomic analysis in an assessment by the 100,000 Genomes Project. I will present new develops such as the Global Alliance for Genetics and Health (GA4GH) Phenopacket Schema, which will support more detailed computational phenotype analysis in the future. I will present our recent work in using HPO-based unsupervised machine learning to identify generalizable subgroups of long COVID.

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Tzung-Chien Hsieh

GESTALTMATCHER DATABASE – A FAIR DATABASE FOR MEDICAL IMAGING DATA OF RARE DISEASES

Introduction: Next-generation phenotyping (NGP) technology is increasingly used in the diagnostic workup of patients with facial dysmorphism. The performance of these tools increases with the training set's size and diversity, but properly labelled training data is currently the biggest

bottleneck. Therefore, we developed GestaltMatcher Database (GMDB) - a database for medical image data that complies with the FAIR-principles.
Methods: An entry in GMDB consists of a medical image such as a portrait, X-ray or fundoscopy, and machine-readable meta information such as clinical features or a disease-causing mutation. Starting by collecting imaging data from the literature, the GMDB now serves as a new publication medium, allowing to share previously unpublished cases and updating them dynamically after further consultations. Patients can easily provide data due to a patient-centred digital consent system. To enable inter-cohort comparisons, a research platform can compute the pairwise syndromic similarity between hand-picked cases.
Results: At the time of writing GMDB consisted of 8316 cases with 781 different disorders. We collected data from 2038 case reports and 574 individuals that are not published elsewhere. The web interface enables gene- and phenotype-centred queries. GMDB also serves as a repository for medical images that cannot be included in medRxiv. The research app within GMDB was used to generate syndromic similarity matrices to characterise novel phenotypes (e.g. CSNK2B, PSMC3).
Conclusion: GMDB is a database for NGP where data are findable, accessible, interoperable, and reusable. GMDB connects clinicians with a shared interest in particular phenotypes and simultaneously improves the performance of AI.

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Alexander Hustinx

FACE2HPO: CNN-BASED HPO LABELING AND DISORDER CLASSIFICATION OF SYNDROMIC FACES

Background/Objectives: In the last two decades, we have seen an exponential growth of new (unlabeled) data. While most of the data can be labeled by laymen, labeling medical data like syndromic faces requires expert knowledge. On top of helping patients, clinicians are expected to work on this labor-intensive labeling process. In this work, we aim to automatically label syndromic faces with their Human Phenotype Ontology(HPO)-terms, as well as provide new explainable artificial intelligence (XAI) insights.

Methods and results: We present a convolutional neural network (CNN) capable of automatically labeling syndromic faces with their respective HPO-terms. We leverage the tree-like data structure of HPO to allow multiple levels of abstraction, referred to as HPO-levels. These HPO-levels help us deal with data scarcity of underrepresented disorders in the GestaltMatcher DataBase (GMDB). More importantly, the HPO-level can help us give new insights into the decision-making process of our black-box CNN. Previously we had to directly explain a model's diagnosis of disorders, which is extremely difficult and the results were hard to interpret. However, using our HPO-classifier we can directly infer the regions of interest, as well as consider the spatial and descriptive information of the predicted HPO terms.

Conclusion: Not only can this tool facilitate clinicians with their diagnosis and administration, it can also assist GMDB users when adding new patient photos or supplementing old ones with more

information. More importantly, the use of HPO terms in our model inherently makes the process more explainable than most other diagnostic tools.

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Alexander J M Dingemans
PHENOSCORE: AI-BASED PHENOMICS TO CLASSIFY GENETIC VARIANTS

Allgorithms using molecular information to predict the pathogenicity of genetic variants, such as the phyloP-, CADD- and SIFT score, are well established to classify genomic variants. An equivalent score to capture the phenotype is, however, not available, despite clinical features being one of the most significant predictors of pathogenicity of variants. We therefore developed PhenoScore: an open source, artificial intelligence-based phenomics framework, combining facial recognition technology with Human Phenotype Ontology data analysis to quantify phenotypic similarity. We prove PhenoScore's ability to recognize distinct phenotypic entities by establishing recognizable phenotypes for 37 of 40 investigated syndromes against clinical features observed in individuals with other neurodevelopmental disorders and show it is a significant improvement on existing approaches. PhenoScore provides predictions for individuals with variants of unknown significance and enables sophisticated genotype-phenotype studies by testing hypotheses on possible phenotypic (sub)groups. PhenoScore confirmed previously known phenotypic subgroups caused by variants in the same gene for *SATB1*, *SETBP1*, and *DEAF1* and provides objective clinical evidence for two distinct *ADNP*-related phenotypes, already established functionally.

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Short communications session 1

Noraly B. Jonis

HUMAN DISEASE GENES WEBSITE SERIES; DEVELOPMENTS AND ACHIEVEMENTS

As the field of clinical genetics is progressing quickly, an increasing number of genetic disorders are discovered. To address diverse clinical questions and coordinate research activities that arise with the identification of these rare disorders, we developed the Human Disease Genes website series (HDG website series): an international digital library that records detailed information on the clinical phenotype of novel genetic variants in the human genome (https://www.humandiseasegenes.nl/about-us/vision-hdg).

Each gene website is moderated by a dedicated team of clinicians and researchers, focused on specific genes, and provides up-to-date—including unpublished—clinical information. The HDG website series is expanding rapidly with over 1200 genes currently adopted by over a thousand moderators from across the globe, creating new scientific communities. Over 400 sites have been launched and data of over nearly 4000 patients have been collected. These patient data consist not only out of already published data, but also include unpublished patient data, demonstrating the added value of the initiative. Major new developments are taken place within the HDG website series, such as the development of a patient registry and visual representation of the genotype and connected phenotype per gene domain. Additionally, the websites have improved real world value by being able to print the information and patient data present and the ability to contact the moderators directly. In conclusion, the HDG website series provides an easily accessible, open and up-to-date clinical data resource not only for clinicans, researchers but also for individuals/families with pathogenic variants in specific genes. The HDG website series is a dynamic platform that keeps improving and growing, aiding in the improvement of clinical knowledge and management of these rare disorders.

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Maria del Rocio Pérez Baca

A NOVEL NEURODEVELOPMENTAL SYNDROME CAUSED BY LOSS-OF-FUNCTION OF THE ZINC FINGER HOMEOBOX 3 (ZFHX3) GENE

Neurodevelopmental disorders (NDDs) are a heterogeneous disorder which result from impaired development and functioning of the brain, including intellectual disability (ID), affecting approximately 2-5% of children worldwide. In our study, we identify loss-of-function variants in

ZFHX3 as a novel cause for syndromic intellectual disability (ID). ZFHX3, previously known as ATBF1, is a zinc-finger homeodomain transcription factor involved in multiple biological processes including cell differentiation and tumorigenesis. However, its involvement in neuronal development has not been investigated in prior research.
Through international collaborations and GeneMatcher, we collected clinical data of 42 individuals with protein truncating variants (PTVs) or (partial) deletions of *ZFHX3*. Loss-of-function variation of *ZFHX3* consistently associates with (mild) ID, postnatal growth retardation, feeding difficulties, and recognizable facial characteristics as supported by artificial intelligence (Face2Gene).
Publicly available and in-house generated expression data show increased expression of ZFHX3 during human brain development and neuronal differentiation. Immunoprecipitation followed by mass spectrometry in neural stem cells and SH-SY5Y shows that ZFHX3 interacts with the chromatin remodelling BRG1/Brm-associated factor complex and the cleavage and polyadenylation complex. In addition, we identified a specific DNA methylation signature in leukocyte-derived DNA of individuals with aberrations affecting *ZFHX3* (I.e. both (micro)deletions and PTVs). ChIP-seq for ZFHX3 in neural stem cells, reveals that this transcription factor primarily binds to promoter regions of genes associated with axon and neuron projection development. In *Drosophila melanogaster*, *zfh2* is considered the ZFHX3 orthologue. We also show that zfh2 is expressed in the nucleus of neurons in third instar larval brains. Moreover, ectopic expression of zfh2 as well as ZFHX3 in the wing discs result in a similar cleft thorax phenotype in adult flies, confirming their functional conservation and a key role in development. In addition, overall knockdown of zfh2 results in a lethal adult phenotype thereby again providing evidence for its role in development. To conclude, loss-of-function variants in *ZFHX3* are a novel cause for syndromic ID and are associated with a specific DNA methylation profile. Our results indicate a role for ZFHX3 in neuronal development, chromatin remodelling and mRNA processing.

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Thursday Afternoon 27th April

SPEECH AND ASD RELATED DISORDERS

Thomas Bourgeron

PHENOTYPIC EFFECTS OF GENETIC VARIANTS ASSOCIATED WITH AUTISM BEYOND DIAGNOSIS

The heritability of autism is high (>80%), but the genetic architecture is complex made of a combination of common and rare variants. Some conditions such as autism, attention deficit hyperactivity disorders (ADHD), intellectual disability (ID), epilepsy share genetic variants, and the factors contributing to the diversity of the clinical trajectory remain largely unknown. Our previous studies pointed at one biological pathway associated with autism related to synapses. In this presentation, I will discuss our recent results coming from human studies in large populations and genetic isolates as well as mouse studies that shed new light on the inheritance of autism and some of the underlying mechanisms. Finally, I will illustrate how we are currently studying *Resilience* to understand why some carriers of mutations seem to be protected from adverse symptoms while others are severely affected.we are currently studying *Resilience* to understand why some carriers of mutations seem to be protected from adverse symptoms while others are severely affected.

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Bregje van Bon

THE DIFFERENT FACES OF SETBP1

Germline *de novo SETBP1* variants cause clinically distinct and heterogeneous neurodevelopmental disorders. Heterozygous missense variants at a hotspot encoding a canonical degron lead to SETBP1 accumulation and Schinzel-Giedion syndrome (SGS), a rare severe developmental disorder involving multisystem malformations.
Heterozygous loss-of-function variants result in *SETBP1* haploinsufficiency disorder (HD) which is phenotypically much milder than SGS. We have performed several studies to clinically delineate these disorders, which have led to novel guidance and treatment advices. A major challenge remain missense variants outside the degron. Due to the lack of systematic investigation of genotype-phenotype associations of these types of *SETBP1* variants, and limited understanding of the roles of the gene in brain development, the extent of clinical heterogeneity and how this relates to underlying pathophysiological mechanisms remain elusive, imposing challenges for diagnosis and patient care. I will present the clinical characteristics of SGS and *SETBP1*-HD and present an overview of the largest cohort to-date of individuals carrying *SETBP1* missense variants outside the degron (n=18). These findings suggest that such variants cause a clinically and functionally variable

developmental syndrome, showing only partial overlaps with classical SGS and *SETBP1* haploinsufficiency disorder.

Clinical geneticist, Radboudumc Nijmegen, the Netherlands

Angela Morgan
GENETIC BASIS OF SEVERE CHILDHOOD SPEECH DISORDER

Childhood apraxia of speech (CAS), the prototypic severe childhood speech disorder, is characterized by motor programming and planning deficits. Children with CAS have difficulty producing speech sounds accurately, in the right sequence, and with correct prosody. Knowledge of the aetiology of CAS was limited for decades, and largely since the condition was first recognised 70 years ago. The first gene associated with CAS, in the absence of intellectual disability, was *FOXP2*, identified in 2001 in a 3 generation British family. With advances in genetic sequencing and analysis methods, there has been a significant leap in our understanding of the genetic bases of CAS since this first discovery. It is now established that CAS has a strong genetic basis, with a monogenic pathogenic variant identified in a third of cases, with 34 single genes implicated to date. A critical role for chromatin organization and DNA binding has been identified in typical child speech development. Further, CAS-susceptibility genes are shown to be co-expressed during brain development, suggesting that they are part of specific biological pathways. Recent work has also confirmed the increasing overlap between genes conferring risk for a range of neurodevelopmental disorders including CAS, epilepsy, autism spectrum disorder and intellectual disability. Notably, however, patients may also have CAS in isolation without comorbid conditions. Understanding the aetiological basis of CAS is a critical starting point for unravelling the biological basis of communication, a unique human skill. From a clinical perspective, new knowledge in this field is important to end the diagnostic odyssey, identify comorbidities, open doors to further societal support and ensure patients are poised for precision medicine trials.

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Short Communications Session 2

Davide Aprile

BENCHMARKING CEREBELLAR ORGANOIDS DEVELOPMENT AND THEIR USE TO MODEL AUTISM SPECTRUM DISORDER: THE CHD8 PARADIGM

The cerebellum is a brain structure very well renowned for its involvement in the regulation of balance and motor coordination. Other than these, the role of the cerebellum has been extended during the last decade also to non-motor learning, language processing, high cognitive functions and social behavior, and to their relative disturbances such as intellectual disability and autism spectrum disorders (ASD). Technical and ethical boundaries limit the possibility to longitudinally study its growth, and murine models, despite their well-established robustness fail to fully recapitulate the molecular cascade leading to the formation of a human cerebellum. These challenges have been at least partially solved by the introduction of human-derived *in vitro* models called brain organoids. Such self-organizing 3D structures obtained from pluripotent stem cells, are *bona fide* recapitulating key molecular features characterizing the development of their *in vivo* counterpart. Organoids resembling different brain regions can be differentiated by using growth factors and small molecules, and specific patterning methods have been established to induce the formation of cerebellar organoids (CbOs). In this work, by using multiple lines of induced pluripotent stem cells (iPSCs), we longitudinally studied the replicability of a well-established CbOs differentiation protocol, characterizing the expression of cerebellar specific markers during their growth by means of quantitative PCR and imaging. Moreover, we benchmarked their development against fetal cerebellar transcriptomes, comparing data obtained both from bulk and single-cell RNA-sequencing, identifying over time, the expression of key molecular genes typical of specific cell populations belonging to the developing human cerebellum. We also found at specific timepoints an enrichment in the expression canonical ASD-related genes, including the chromodomain helicase DNA-binding protein 8 (CHD8). Lastly, we used CbOs to model ASD of genetic origin: organoids from control $CHD8^{+/+}$ pluripotent stem cells and their $CHD8^{+/E1114X}$ isogenic counterpart, carrying an ASD-associated variant causative of cognitive, motor and sensory impairment were compared. Bulk RNA-sequencing approaches and complementary quantitative PCR paired with immunostaining, showed alterations in the developmental pattern of $CHD^{+/E1114X}$-derived CbOs in respect to their isogenic controls at early stages of differentiation, confirming the suitability of CbOs as a tool to model the cerebellar component of genetic ASD at molecular and cellular level.

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Lisa Hamerlinck

STRUCTURAL VARIANTS DISRUPT A CRITICAL REGULATORY REGION DOWNSTREAM OF FOXG1

The forkhead box G1 (FOXG1) transcription factor is a crucial regulator during embryonic brain development. Pathogenic variants affecting *FOXG1* cause *FOXG1* syndrome, a congenital form of Rett syndrome. Interestingly, 34 reported individuals with *FOXG1* syndrome related features harbor structural variants (SVs) that disrupt the region downstream of *FOXG1*. Yet, the regulatory mechanisms resulting in aberrant *FOXG1* transcription have not been elucidated.
We identified a *de novo* non-coding deletion in a patient with *FOXG1* syndrome, allowing us to narrow down a ~100kb critical regulatory region affected in all patients with SVs 3' of *FOXG1*. By mapping regulatory interactions via UMI-4C in neural stem cells and neurons, we showed that the *FOXG1* promoter interacts with this region during human neuronal development. Using available epigenomics data, Hi-C interaction maps and *in vivo* enhancer assays in zebrafish embryos, we identified multiple regulatory elements in this region, including a cluster of neuronal enhancers and the distal boundary of the *FOXG1*-containing topologically associating domain (TAD). In addition, through Hi-C and UMI-4C on patient cells we found that deletion of the critical regulatory region impacts *FOXG1* interactions and TAD structure. We are currently validating the impact of elements within this region on *FOXG1* transcription through CRISPRCas9 engineered deletions in neural stem cells and neurons.
In summary, we narrowed down a critical regulatory region downstream of *FOXG1* that is affected in a cohort of *FOXG1* syndrome patients, containing both enhancer and architectural regulatory elements. Our results greatly improve the functional annotation and validation of regulatory elements at the *FOXG1* locus, crucial for correct SV interpretation in patients.

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Dale J. Annear

CGG SHORT TANDEM REPEAT EXPANSIONS ARE OVERREPRESENTED IN NEURODEVELOPMENTAL DISORDERS

Short tandem repeat (STR) DNA tracts make up a significant portion of the human genome, accounting for approximately 3%. The trinucleotide CGG STR is a subset of the repeat found throughout the human genome. Unlike most other STRs of alternative nucleotide composition, CGG STRs are highly enriched within and surrounding gene coding regions. CGG STRs stand out due to their association with neurodevelopmental disorders (NDDs). Large expansion mutations of these repeats trigger hypermethylation of the repeat locus which, in turn, results in transcriptional silencing of the associated gene.

By applying whole genome sequencing, we interrogated 5963 CGG STR loci within a large NDD cohort of 15,996 individuals. Within these individuals, we identified a total of 419 large CGG STR expansions at 142 unique CGG STR loci within 118 different genes. Through enrichment analysis, we observed a significant involvement of these genes in DNA binding, regulation, and transcription (padj = 8.31 x 10-8) and expression within brain tissues (cerebral cortex: padj = 3.6 x 10-2, cerebellum: padj = 9.9 x 10-3).
Specifically, within the individuals displaying autism spectrum disorder (ASD), we were able to identify a 2.9 increased odds ratio of large CGG STR expansion incidence in comparison to unaffected parents. We classified CGG repeat expansions within over 30 genes which have been previously linked to disorders with either an autosomal or X-linked dominant inheritance pattern. With the overwhelming majority of these being neurodevelopmental in nature. Furthermore, by crosslinking our repeat expansion results with both publicly available haploinsufficiency data and exonic variant analysis of the expansion-bearing patients we identified several strong candidate genes, such as *RGPD2* and *SAMD1*, that may be unreported in their involvement within recessive NDDs.
Here, we not only highlight strong novel gene candidates for CGG expansion disorders but through gene enrichment, haploinsufficiency, and variant analyses we solidify the link between CGG STRs and NDDs.

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Friday Morning 28th April

THERAPY SESSION

Ype Elgersma

ANGELMAN SYNDROME: SHIFTING FROM MECHANISMS TOWARDS ASO TREATMENT

Angelman syndrome (AS) is a severe neurodevelopmental disorder caused by mutations affecting the maternally inherited UBE3A gene. UBE3A is a ubiquitin ligase that marks proteins for degradation. Although most studies focus on the synaptic function of UBE3A, we showed that UBE3A is highly enriched in the nucleus of mouse and human neurons. Mice lacking the nuclear UBE3A isoform recapitulate the behavioral and electrophysiological phenotypes of AS mice, whereas mice harboring a targeted deletion of the cytosolic UBE3A isoform are unaffected, suggesting that the role of UBE3A is mostly nuclear [2]. In agreement with these findings, we found that many AS-associated UBE3A missense mutations affect nuclear localization of UBE3A. However, the precise role of UBE3A in the nucleus remains to be identified, which hampers the development of therapeutics targeting downstream targets of UBE3A.
A previous study has shown that the paternally inherited (imprinted) UBE3A allele can be activated using antisense oligonucleotides (ASO), which offers great promise of ASO mediated reactivation of UBE3A as a disease modifying treatment for Angelman syndrome [3,4]. However, ASO treated AS mice did show a significant phenotypic rescue, possibly because treatment was initiated in adult mice [5]. We reinvestigated this by ASO treatment of young AS mice. We observed robust UBE3A reinstatement in the brain, with levels up to 90% of wild-type levels in the hippocampus. In addition we observed a significant improvement of several previously established AS phenotypes [6]. These findings are in line with our earlier observations that there is a distinct critical period in which UBE3A fulfills its function [5,7,8]. Although the mechanism underlying this critical period is unclear, our recent data indicates that loss of UBE3A causes stalled striatal maturation, which cannot be reversed by UBE3A gene reinstatement after brain development (9).

1. Avagliano Trezza RA, et al. (2019) Nat Neurosci (DOI: 10.1038/s41593-019-0425-0)
2. Bossuyt SNV, et al (2021) Hum Mol Genet (DOI: 10.1093/hmg/ddab050)
3. Meng L, et al (2015), Nature (10.1038/nature13975)
4. Elgersma, Y et al (2021) Dev Med Child Neurol (DOI:10.1111/dmcn.14831)
5. Silva-Santos S et al. (2015) J Clin Invest (DOI 10.1172/jci80554)
6. Milazzo C et al. (2021) JCI Insight (DOI: 10.1172/jci.insight.145991)
7. Sonzogni, M et al. (2019) 7. Mol Autism (DOI: 10.1186/s13229-019-0277-1)
8. Sonzogni, M et al. (2020) Mol Autism (DOI: 10.1186/s13229-020-00376-9)
9. Rotaru D et al. (2023) JCI Insight (DOI: 10.1172/jci.insight.166073)

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Giuseppe Testa

TRANSLATING BRAIN ORGANOIDS ENDOPHENOTYPES: FROM MECHANISMS TO SCALES

Thanks to the exponential growth of the field, the organoid-based modeling of neuropsychiatric disorders can now tackle some of its most mature and transformative challenges. Among these, a central one is the translation of in vitro endophenotypes back to the in vivo setting, for mechanistic insight and preclinical validation alike. In parallel, new approaches are needed to scale up the disease modeling throughput in order to render experimentally tractable the polygenic architecture of the most prevalent forms of mental illness. Here I discuss recent progress towards these two goals from the work we have been spearheading in the lab by modelling a paradigmatic set of neurodevelopmental disorders caused by point mutations or dosage imbalances in transcription factors and chromatin regulators that operate in inter-related pathways. These include the symmetrically opposite pair of copy number variations (CNV) at 7q11.23 causing Williams-Beuren syndrome (WBS) and 7q11.23 microduplication syndrome (7Dup), and the haploinsufficiencies of ADNP and YY1 that cause, respectively, Helsmoortel-Van der Aa Syndrome (HVDAS) and Gabriele-DeVries syndrome (GADEVS). I will discuss the integration of longitudinally single cell resolved trajectories and transgenic models to establish preclinical actionability, as well as the convergence across molecular mechanisms and endophenotype layers. Finally, I will introduce recent progress in multiplexing brain organoidogenesis for advancing modelling towards population-scale cohorts.

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Short Communications Session 3

Mathijs van der Lei

A COMBINATION OF THE LIVE MOUSE TRACKER (LMT) AND MULTI-ELECTRODE ARRAY (MEA) AS VERSATILE DRUG SCREENING PLATFORM FOR FRAGILE X SYNDROME

Fragile X syndrome (FXS) is the most common inherited cause of intellectual disability and autism, with a prevalence of approximately 1 in 4000–7000 males and 1 in 6000–11000 females. The *Fmr1* knockout (KO) mouse, created over 25 years ago, is the most commonly used and valuable model for FXS. The mouse model parallels the human disorder in many of its aspects and has been extensively used in preclinical studies. Over the past decades multiple affected receptors, signaling pathways, and downstream targets have been discovered in FXS, highlighting the complexity of the molecular basis of this disorder and the challenging perspectives for drug development and potential treatments for patients. In practice, the approach of testing the effectiveness of a novel compound is based on a battery of behavioral tests. This is labor-intensive, time-consuming, costly,

and therefore not fitted to screen for multiple drugs and certainly not for potential combination therapies.

We introduce here a standardized method to measure the effectiveness of different drugs in a uniform and versatile screening protocol consisting of the live mouse tracker (LMT) and high-density multi electrode-array (MEA). The LMT system is used for behavioral analysis and can dissect up to 35 different (social) behaviors of up to four mice from a single 24-hour recording, and provides a first glance at the effectiveness of a drug. For electrophysiological measurements, the MEA system is used to determine the effects of *Fmr1* deletion on electrophysiology and neural network functioning. By combining the LMT and MEA we have developed a versatile, high throughput, innovative and efficient drug screening platform for FXS.

Preliminary data of the LMT showed a significantly further distance travelled by *Fmr1* KO mice compared to WT. In addition, more isolated behavior was recorded in *Fmr1* KO mice, as measured by move alone, stop alone and rear isolated. Furthermore, social behavioral traits like the grouping of three animals, social approach, make contact, side to side contact, oral-oral and oral-genital contact were significantly decreased in *Fmr1* KO mice. Therefore, we conclude that baseline measurement of *Fmr1* KO mice showed hyperactivity and abnormalities in social interactions in our LMT system, potentially compatible with autism observed in patients. These remarkably robust behavioral abnormalities will be used to detect the efficacy of novel compounds. The MEA system showed in a previous study of our lab a clear alteration in the spontaneous firing of *Fmr1* KO networks compared with WT, which displayed progression toward a mature activity pattern through time. *Fmr1* KO networks displayed shorter, sparser, and rarer bursts at early development and longer, denser, and more frequent ones at the matured stage compared with WT. The experimental model of a disease “in a dish” that we employed here easily allows extensive screening of novel therapeutic strategies.

In conclusion, our LMT system is capable of characterizing behavioral abnormalities of *Fmr*1 KO mice in just 24h, and preliminary data of the MEA system showed a clear distinct difference in electrophysiological properties between *Fmr1* KO and WT. Together we have developed a versatile drug screening platform that is currently used for drug testing in our laboratory.

Mathijs van der Lei, Dale Annear, Ellen Elinck & Frank Kooy
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Oliviero Leonardi

SINGLE-CELL DISSECTION OF CHD2 DOSAGE DISRUPTION EFFECTS ON AUTOPHAGY AND CORTICAL DEVELOPMENT

The chromodomain helicase DNA-binding protein 2 (CHD2) belongs to the SNF2 superfamily of ATP-dependent chromatin remodelers, and is critical for the regulation of chromatin organization, gene expression and cell-cycle progression. CHD2 has been associated to proliferation of neural progenitors in the developing mouse cortex, the establishment of long-term memory and to human

cortical interneurons and neural circuits development. Loss-of-function mutations (LoF) resulting in haploinsufficiency of CHD2 are associated with neurodevelopmental conditions (OMIM #602119) ranging from severe epileptic encephalopathy to cognitive impairment to full blown autism spectrum disorder (ASD). While exhibiting evidence of abnormal neural development, CHD2-deficient mouse models do not fully capture the complexity of human pathogenesis, neither do they recapitulate the epileptic phenotype. In this work, we generated human Cortical Brain Organoids (CBO) from isogenic induced pluripotent stem cells harbouring LoF mutations of the CHD2 gene (in both hetero- and homozygosity), to unravel the molecular mechanisms of CHD2 dosage-dependent alterations in neurodevelopment. To this end, we longitudinally profiled, at both bulk and single-cell resolution, the differentiation dynamics of CHD2 mutant CBO and characterized their phenotypes in terms of high-resolution neurodevelopmental trajectories, cell biological and functional phenotypes. Our results reveal a severe transcriptional dysregulation impinging on other major ASD-related genes and regulators of neuronal differentiation, as well as alterations in cell biological and electrophysiological properties. This evidence supports the use of CBO as a valuable tool to model CHD2-related pathogenetic mechanisms, dissect their molecular basis, and provides an important platform to identify novel potential targets for therapeutic intervention.

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# Contributed equally.

Imke M.E. Schuurmans

MODELLING GENETIC DISORDERS OF LYSINE METABOLISM IN A DISH

Pyridoxine-dependent epilepsy (PDE) and glutaric aciduria type 1 (GA1) are two rare neurometabolic disorders of lysine metabolism, caused by pathogenic variants in *ALDH7A1* and *GCDH,* respectively. Deficiency of the encoded enzymes results in accumulation of neurotoxic metabolites causing debilitating neurological sequelae in patients. Currently, no suitable human PDE and GA1 models are available. Therefore, we here aim to develop human cellular PDE and GA1 models to investigate the disease mechanism and identify therapeutic targets. In this study, patient-derived fibroblasts were reprogrammed into induced pluripotent stem cells (iPSCs). In addition, isogenic knock-out (KO) lines derived from control iPSCs have been generated using the CRISPR/Cas9 system. First, by overexpression of NGN2 these iPSC-lines were differentiated towards cortical excitatory neurons and co-cultured with rat astrocytes to support neuronal maturation. The neurons were characterized using multi-electrode arrays to measure the neuronal network activity. Both PDE- and GA1-KO neurons show a significantly increased network burst rate during differentiation. Because *ALDH7A1* and *GCDH* expression is enriched in astrocytes, we have also optimized a protocol to

differentiate iPSCs towards astrocytes. We show that these iPSC-derived astrocytes are both morphologically and functionally mature within 5 weeks of differentiation. In addition, when co-cultured together with cortical excitatory neurons, the iPSC-derived astrocytes support neuronal maturation and synapse formation confirmed by immunostainings with MAP2 and Synapsin1/2 antibodies respectively. Co-cultures of patient-derived neurons and astrocytes will be used for electrophysiological, molecular and metabolic characterization to identify the cell-type specific effect of these two neurometabolic disorders.

Imke M.E. Schuurmans[1,2,3,4], Shan Wang[5,6], Katrin Linda[5,6], Clara D.M. van Karnebeek[4,7], Alejandro Garanto[1,2,3,5] and Nael Nadif Kasri[5,6].
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Friday Afternoon 28th April 2023

Modelling session

Bassem Hassan
THE BRAIN ON TIME: LINKS BETWEEN DEVELOPMENT AND DEGENERATION

Neurodegenerative diseases are characterized by the progressive loss of structure or function of neurons. Although neurodegenerative diseases are generally thought of as late-onset diseases, recent evidence promotes the notion that they might be considered late onset neurodevelopmental disorders. With this view in mind, I will present ourwork on the role of the Alzheimer's disease protein APP in human-specific temporal regulation of neurogenesis, as possibly key to understanding the etiology and pathophysiology of human neurodegenerative disease.

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Sofia Puvogel Lutjens
TOWARDS FUNCTIONAL AND MOLECULAR UNDERSTANDING OF HUMAN-DERIVED NETWORKS IN NEURODEVELOPMENTAL DISORDERS

Human induced pluripotent stem cells (hiPSCs) can differentiate into functional neural networks that harbor the genetic information of the donors. Thereby, they are a useful tool for modeling neural aspects of genetic neurodevelopmental disorders. Our previous studies using hiPSCs-neuronal networks grown in microelectrode arrays (MEAs) identified alterations in network activity as a result of mutations associated with abnormal neurodevelopment. Nonetheless, the molecular changes that cause the deviations in network activity, which may become potential targets for the treatment of neurodevelopmental diseases, remain unknown. In this talk, I will present our ongoing methodological and analytical approach, called MEA-seq, designed to identify relevant transcriptional changes related to functional phenotypes of hiPSC-networks. I will present our results of applying MEA-seq to model neural aspects of Koolen-de Vries, in addition to discussing our current plans to extend this framework to detect functional network heterogeneity and its molecular signature in hiPSCs-derived neural models of multiple neurodevelopmental syndromes.

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CHRISTEL DEPIENNE
PREDICTING GENE-DOSAGE ASSOCIATIONS AT THE GENOME SCALE USING MACHINE LEARNING: THE EXAMPLE OF THE X CHROMOSOME

Disease gene discovery on chromosome X is associated with greater challenges compared to autosomes because X-linked variants can be inherited according to different modes and penetrance

varies according to sex and X chromosome inactivation (XCI) in females. We undertook a systematic analysis of all coding genes on human chromosome X in the aim of predicting gene-disease associations remaining to be discovered on this chromosome. We used OMIM to compare the proportion and characteristics of disease gene and associated disorders on all chromosomes. We observed a higher proportion of disorder-associated genes and an enrichment of genes involved in cognition, language, and seizures on chromosome X compared to autosomes. We used a threshold approach to analyze gene constraints, exon and promoter conservation, expression, and paralogues, and report 127 genes not yet associated with a disorder sharing one or more attributes with the 205 disorder genes known on chromosome X. We then collected 83 variables from genes on all chromosomes, including gene constraints, nucleotide conservation metrics, expression data stratified by sex, gene structure attributes, relative position of the gene on the chromosome and data on paralogues, and used machine learning to predict remaining disorder genes in a more systematic and unbiased fashion. We trained and compared different machine learning classifiers to distinguish disease-associated from dispensable genes. We classify 247 genes, including 115 of the 127, as having high probability of being disease-associated. We provide evidence of an excess of variants in predicted genes in existing databases. Finally, we report damaging variants in *CDK16* and *TRPC5* in patients with intellectual disability or autism spectrum disorders. This study predicts large-scale gene-disease associations that could be used for prioritization of X-linked pathogenic variants.

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Short Communications Session 4

Federica Marinaro

THE "HIGH THROUGHPUT BRAIN ORGANOID LONGITUDINAL PROFILING FROM PATIENT COHORTS OF NEURODEVELOPMENTAL DISORDERS" STUDY: SEEDING AN INTERNATIONAL AND MULTIDISCIPLINARY EFFORT FOR TRANSFORMING OUR UNDERSTANDING OF NEURODEVELOPMENTAL CONDITIONS AND NEURODIVERSITY

Neurodevelopmental disorders (NDDs) are a broad group of conditions characterised by alterations in the development of the central nervous system (CNS), resulting in varying degrees of cognitive and/or behavioural symptoms. These result from a spectrum of genetic disease architectures, from still poorly characterised polygenic loads of low-risk variants to highly penetrant rare variants. Over the past decades, interdisciplinary efforts started identifying convergent molecular fingerprints that allow to group genetic mutations and, still less frequently, polygenic loads. Initiatives such as the New York Stem Cell Foundation (nyscf.org) and the ALS Data Portal (answerals.org) represent tangible examples of how scientific project management can be key to coordinate large-scale collective efforts that combine deep clinical phenotyping of NDDs with in vitro disease modelling at high resolution. By establishing the "High throughput brain organoid longitudinal profiling from patient cohorts of neurodevelopmental disorders" study, spearheaded by Professor Testa's team at the Human Technopole (HT) in Milan (Italy) we integrate leading expertise from national and

international patient cohorts to unravel the developmental dynamics of NDD molecular pathogenesis and the gene-environment interplay. The first enrolled cohort is the one present in Troina (Italy) led by Professor Romano. Building on our long-standing collaborative experience resulting from the engagement in NDD research programs and cohorts (R2D2-MH, REMEND, IMPACT, ADNPinMED, AUTISYN, SELMA EDCMixRISK), we present here the collaboration with the IRCCS Associazione Oasi Maria Santissima cohort of NDD as the core seed of the "High throughput brain organoid longitudinal profiling from patient cohorts of neurodevelopmental disorders" study. Starting from the mapping and benchmarking of clinical (behavioural and IQ measures), imaging (fMRI and EEG) and genomic datasets from NDD patient cohorts, we introduce a study design that pursues deep-molecular resolution assays integrating information from clinical, imaging, and genomic data to profile patient-specific alterations in neurodevelopmental trajectories. Starting from a defining and enabling aspect of the study, namely the reference frame of high-resolution neurodevelopmental alterations profiled in a suite of paradigmatic and standard setting monogenic conditions, the goal is to identify common altered pathways that have the potential to relate to the general spectrum of NDDs, and to reveal convergent actionable hubs of dysregulation. Using a combination of cutting-edge technologies and the experience of a team of professionals from the Automated Stem Cell and Organoid Facility in HT, we will generate brain organoids from NDD patients and their parents to model the wide spectrum of disease and study patient-specific neurodevelopmental dynamics at single cell resolution as well as sequencing of genomes of participants.
By coordinating data and sample flow, integration of cohorts' data with molecular readouts and validation against clinical biomarkers, the "High throughput brain organoid longitudinal profiling from patient cohorts of neurodevelopmental disorders" study aims to enhance our knowledge about these disorders, paving the way toward the amelioration of lifestyle conditions of patients and their families. We will discuss the experience gathered with ongoing and iterative workstreams to inform data security, privacy, ethics, open policy research and integration of the results from this study into informative campaigns globally.

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Xiuming Yuan

HUMAN STEM CELL-DERIVED GLUTAMATERGIC NEURONS DISPLAY HOMEOSTATIC PLASTICITY AT NETWORK AND SINGLE-CELL LEVEL

The mechanisms that underlie homeostatic plasticity have been extensively investigated at single-cell level in animal models, but are less understood at network level. Here, we used microelectrode arrays to characterize neuronal network activity following induction of homeostatic plasticity in co-cultures of human induced pluripotent stem cell (hiPSC)-derived glutamatergic neurons. Chronic suppression of neuronal activity through tetrodotoxin (TTX) elicited a time-dependent network re-

arrangement. We observed increased expression of AMPA receptors and the elongation of axon initial segments to be associated with increased network excitability following TTX treatment. Transcriptomic profiling of TTX-treated neurons revealed up-regulated genes related to extracellular matrix organization, while down-regulated genes related to cell communication. Overall, our study shows that hiPSC-derived neuronal networks provide a reliable *in vitro* platform to measure and characterize homeostatic plasticity at network and single-cell level, and this platform can be extended to investigate altered homeostatic plasticity in brain disorders.

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Claudio D'Incal

THE CHROMATIN REMODELER ADNP REGULATES THE CELLULAR RESPONSE TO AUTOPHAGY AND MITOCHONDRIAL ACTIVITY: A SUGGESTED ROLE OF SIRT1 IN THE CEREBELLUM OF A DECEASED TODDLER WITH HELSMOORTEL-VAN DER AA SYNDROME

Background: Helsmoortel-Van der Aa Syndrome (HVDAS) is a neurodevelopmental disorder with patients presenting with autism, intellectual disability amongst frequent extra-neurological features such as feeding and gastro-intestinal problems, visual impairments, and cardiac abnormalities. All patients exhibit heterozygous *de novo* nonsense and frameshift stop mutations in the *Activity-Dependent Neuroprotective Protein* (*ADNP*) gene, accounting for a worldwide prevalence of 0.2% of all autism cases. ADNP fulfills a chromatin remodeling function and essential for the formation of the brain. In this study, we investigated the cerebellum of a deceased seven-year-old male toddler with the c.1767dupA/p.His559Glnfs*3 *ADNP* mutation.

Results: An extensive evaluation of the clinical presentation of the ADNP toddler showed autistic traits together with motor delays, developmental delays, hypotonia, generalized symptomatic epilepsy, and unusual facial characteristics. During his lifespan, he underwent two liver transplantations and received immunosuppressants. Following the second liver transplantation, the toddler deceased because of multiple organ failure. In the ADNP cerebellum, we observed an unexpected significant increase ($p = 0.0001$, ***) in global *ADNP* mRNA levels compared to an age-matched control subject. Introduction of the c.1676dupA mutation in an *ADNP* expression vector resulted in mislocalization of the mutant protein into the cytoplasm, while the wild-type protein

was still retained inside the nucleus. Western blots of overexpression lysates showed wild-type ADNP at a molecular weight of 150 kDa (above its theoretical molecular weight of 123 kDa) together with a truncated mutant protein after incubation with an N-terminal ADNP antibody. In cerebellar extracts, we could confirm a clear signal for wild-type ADNP at a molecular weight of 150 kDa in the control subject. However, we were not able to obtain an ADNP signal in the patient. Next, subcellular fractionation of HEK293T protein lysates resulted in a significant reduction ($p = 0.03$,***) of the expression of the ADNP mutant compared to the wild-type protein in the chromatin-enriched protein fraction, underlining its chromatin remodeling function. Hence, a genome-wide methylation analysis of the ADNP toddler cerebellum indicated rather CpG hypermethylation with genes converging to pathways related to neurodevelopmental delay. Additionally, transcription factor enrichment analysis showed that *ADNP* was the strongest associated transcription factor controlling the hypomethylated genes. RNA sequencing of the post-mortem autistic brain supports neurodevelopmental delay by downregulation of the WNT signaling pathway (*CTNNB1*, $p = 0.001$; **) and autophagy defects (*BECN1*, $p < 0.0001$; ****), which was also supported in patient-derived lymphoblastoid cell lines. Ultimately, label-free quantification mass spectrometry identified proteins showing downregulation of mitochondria-related and Sirtuin signaling pathways amongst others. Here, a predictive protein-protein network linked different chromatin remodelers (SMARCC2, HDAC2 and YY1) to autophagy-related proteins (LAMP1, BECN1 and LC3), thereby suggesting a central role of the histone deacetylating enzyme SIRT1. Moreover, mitochondrial activity assays in patient derived-fibroblasts and cellular mitochondrial stainings confirmed a reduced mitochondrial activity ($p = 0.01$,*) and cellular mitochondrial distribution opposed to unrelated controls.

Conclusion: This study forms the baseline clinical and molecular characterization of the ADNP toddler cerebellum, providing essential insights in the disease mechanisms and elucidates new targets for therapy development such as the autophagy process or the mitochondria.

Claudio D'Incal[1,2], Joe Ibrahim[1], Kevin De Man[1,2], Kristy Szrama[2], Kaoutar Choukri[2], Lina Bastini[2], Ellen Elinck[1], ADNP Kids Research Foundation[3], Ligia Mateiu[1], Illana Gozes[4], Zlatko Marusic[5], Mirna Anicic[6], Jurica Vukovic[6], Anke Van Dijck[7], Wim Vanden Berghe[2,*] and R. Frank Kooy[1,*]

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Saturday Morning 29th April

EPILEPSY SESSION

Gemma L Carvill

MOLECULAR AND NEURONAL PHENOTYPES OF CHD2 DOSAGE SENSITIVITY IN NEURODEVELOPMENTAL DISORDERS

Pathogenic loss of function variants in *CHD2* are associated with developmental epileptic encephalopathy (DEE), while overexpression is also associated with a severe pediatric neurological disorder. Chromodomain helicase DNA binding protein 2 (CHD2) is a chromatin remodeling protein that alters chromatin structure, leading to changes in gene expression of downstream targets. To discern the role of CHD2 in human DEE, we used genome editing to create heterozygous disruptions of *CHD2* in human induced pluripotent stem cells (iPSCs) and differentiated these lines into cerebral organoids (COs). We performed single cell RNA sequencing (scRNAseq) in Day 48 COs from two WT lines and two *CHD2*$^{+/-}$ lines to determine cell type specific changes in this model of early neurodevelopment. We also carried out CUT&RUN CHD2 in Day 48 *CHD2*$^{+/-}$ COs and WT controls to identify the direct targets of CHD2. Collectively, this data shows distinct roles for CHD2 in proliferating vs post-mitotic cells, and that CHD2 is associated with activating gene expression, but may also act as a repressor. This more nuanced view of the regulatory mechanism by which CHD2 acts are supported by modeling CHD2 overexpression in a haploid cell and line and iPSCs. Our results demonstrate that physiological CHD2 abundance is required in a very narrow range and that perturbations in either direction are associated with aberrant gene expression, neurodevelopment and pediatric onset neurological manifestations. This has important implications for gene targeting precision therapies in CHD2-related epilepsies, but likely other NDD-related genes involved in chromatin remodeling.

Kay-Marie Lamar, Lauren Rylaarsdam, Masha Bandouil, Jung Hwa, Jonathan Gunti, Kristy Zheng, Marta Amador Molina, Alicia Guemez-Gamboa, Jeffrey Calhoun, Gemma L Carvill
Ken and Ruth Davee Department of Neurology, Northwestern University Feinberg School of Medicine, Chicago, IL

Sarah Weckhuyzen

PRECISION THERAPY DEVELOPMENT FOR KCNQ2-ENCEPHALOPATHY: A COORDINATE EUROPEAN EFFORT

KCNQ-associated encephalopathies (KCNQ-E) are a group of severe epilepsies with onset in the first months of life, characterized by treatment resistant seizures and developmental delay. They are caused by mutations in genes encoding voltage-gated potassium channels that are responsible for the M-current, which plays a critical role in the regulation of neuronal excitability. Both a severe lack and excess of channel function can result in the development of KCNQ-E. Seizures in children with KCNQ-E often respond poorly to anti-epileptic drugs, and therapies for the developmental problems

are currently unavailable. Within the EJP-RD funded TreatKCNQ consortium, we use fluorescence-based assays of potassium flux in CHO cells, rodent and human neuronal cultures expressing KCNQ mutations, and a knock-in mouse model of KCNQ2-E to develop improved treatments for KCNQ-E. We are designing and testing safer and more potent analogues of retigabine, a drug that acts on KCNQ-channels but was recently withdrawn from the market due to side effects, and perform high-throughput drug screening to identify novel openers and blockers of KCNQ channels. In parallel, we study the treatment potential of RNA interference using antisense oligonucleotides. This talk will give an update on the intermediate results of the consortium, with a focus on the work performed in our lab in iPSC-derived neuronal cultures.

Principal Investigator - BAULAC LAB Institut du Cerveau, Paris, France - University of Antwerpen - The Netherlands

Heather Mefford

EPIGENETIC APPROACHES TO EPILEPSY DIAGNOSIS

Developmental and epileptic encephalopathy (DEE) is a group of severe pediatric neurological disorders characterized by drug-resistant seizures and developmental delays or regression. Sequencing technologies have identified the genetic cause of DEE in about 50% of patients, thereby increasing the potential for developing much-needed therapies. However, the remaining 50% of patients do not have a known genetic cause. To determine the molecular cause(s) of unsolved DEE, we must examine genetic variants that are beyond the capabilities of current sequencing approaches. DNA methylation is an epigenetic modification of DNA that plays a role in X-chromosome inactivation, imprinting, and regulation of gene expression, and aberrant methylation is found in neurodevelopmental disorders such as Angelman, Prader-Willi, and Fragile X syndromes. Often, rare differentially methylated regions (DMRs) are due to underlying DNA-sequence variations (e.g., GC-rich repeat expansions) that are not detected by standard sequencing technologies. Methylation patterns can also serve as biomarkers of disease; the diagnostic test EpiSign evaluates genome-wide methylation signatures for about 120 monogenic neurodevelopmental disorders (i.e. disorders caused by a single-gene variant), including more than 30 epilepsy syndromes and DEE. To investigate the role of methylation in DEE, we are generating genome-wide methylation data for a large cohort of unsolved patients to identify rare DMRs and methylation signatures. We are validating rare DMRs with long-read sequencing approaches and investigating the effect of DMRs on gene expression using engineered and patient-derived cells. Using the approach, we have identified novel candidate etiologies for DEE.

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Various

Evan E. Eichler
LONG-READ SEQUENCING OF PATIENT GENOMES

The complete resolution of genetic variation is critical to understanding the mutational processes underlying disease-causing variation. I will present our most recent work sequencing patients and controls using both ultra-long Oxford nanopore technology and high-fidelity PacBio long-read sequencing technologies. I will show how long-read sequencing is improving our understanding of de novo mutation and structural variation, providing access to previously understudied genomic regions and potential new insights into genetic susceptibility to disease with specific focus on autism and developmental delay. This work is leading to new genetic associations, the discovery of pathogenic variants previously missed by short-reads, and genomic susceptibility to new forms of recurrent mutation and rearrangement. Assembly-based variant discovery has the potential to provide a complete understanding of human genetic variation at every level and, we predict, will be the future of genetic and clinical based research, essentially transforming the way we discover pathogenic variants.

Department of Genome Sciences and Howard Hughes Medical Institute, University of Washington, Seattle, WA

Jozef Gecz
GENETIC, MOLECULAR AND MOUSE MODEL INVESTIGATIONS OF BROAD NEURODEVELOPMENTAL IMPACT OF DELETERIOUS VARIANTS OF THE TREX mRNA EXPORT COMPLEX SUBUNITS

mRNA export is mediated by the highly conserved multisubunit TREX (Transcription-Export) complex. We studied >60 deleterious variants in X-chromsome THOC2 subunit of TREX implicated in neurodevelopmental disorders (NDD) with intellectual disability (ID) as the core phenotype. We also identified variants in other TREX subunits THOC1, 5, 6, 7, and ALYREF in children with NDDs. We show that THOC2 protein stability is reduced in patient cells with missense variants, while splicing and deletion variants lead to truncated C-terminal RNA binding domain. Inspired by a patient with ID, speech delay, hypotonia, and microcephaly caused by a THOC2 C-terminal truncating variant, we generated a CRISPR-Cas9-edited Thoc2 Exon37-38 deleted mouse (del37-38) model. Like the patient, the del37-38 male mice are proportionally smaller and lighter (~15%) compared to their wild-type littermates and the truncated Thoc2 protein showed increased stability in multiple tissues Behavioral testing (n=14 mice/genotype) using multiple protocols revealed significant deficit in spatial learning, working memory and sensorimotor functions. Histological investigations of E18.5 embryonic and adult mouse brains show significantly compressed cortical (ventricular and marginal zones of E18.5 mice brain) and corpus callosum architecture. *In vitro* primary neuron and neural

stem cell (NSC) cultures from E18.5 embryonic brains of del37-38 males show shorter primary axons and sub-optimal neural migration. Intriguingly, NSCs from del37-38 male mice show higher proliferation rate and premature differentiation but significantly increased cell death. Our combined patient cell, molecular, and Thoc2 mouse model data suggest novel function(s) and specific neurodevelopmental impact of compromised TREX complex beyond its canonical mRNA export function.

Jozef Gecz[1], Rudrarup Bhattacharjee[1], Elizabeth Palmer[2,3], Alison Gardner[1], Renee Carroll[1], Ing Chee Wee[4], Danielle Mazurkiewicz[1], Luis A Perez-Jurado[1,5,6], Tarin Ritchie[1], Mark Corbett[1], Clare L van Eyk[1], Sandra G Piltz[7], Melissa White[7], Lyndsey Collins-Praino[4], Lachlan Jolly[4], Paul Thomas[1,7], Melanie Leffler[2], Michael Field[2], Raman Sharma[1]

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