1 Julian E. Alecu, MD
Neurology
Harvard Medical School, Boston Children's Hospital

Quantitative natural history modeling of the HPDL-related neurodegenerative disease reveals genotype-phenotype correlations

Objective: To delineate the genotypic and phenotypic spectra of patients with biallelic pathogenic HPDL variants, quantitatively model the disorder's natural history, and uncover genotype-phenotype correlations.

Methods: A cross-sectional analysis of 90 published and one novel case was performed, employing a Human Phenotype Ontology-based systematic approach. Unsupervised phenotypic clustering (via multiple correspondence analysis, hierarchical clustering on principal components, and k-means consolidation) was used alongside in silico analyses and three-dimensional modeling of the HPDL-encoded enzyme to identify distinct patient subgroups and clinical trajectories.

Results: The study quantitatively models the natural history of HPDL-related neurodegenerative disease in a global cohort, clarifying the disease's molecular and phenotypic spectrum and identifying three distinct patient subgroups characterized by significant differences in age at onset, clinical phenotype, developmental trajectories, and survival rates. It also establishes genotype-phenotype associations, finding that presence of a predicted moderately pathogenic missense variant in at least one allele typically leads to a milder, predominantly spastic paraplegic phenotype (OR = 12.4, $p < 0.0001$) with later disease onset (11 years [IQR = 11] vs. 6 months [IQR = 11], $p < 0.0001$), whereas biallelic, highly pathogenic missense or protein truncating variants are associated with a more severe phenotype and reduced life expectancy (median survival = 11.0 years).

Conclusions: Quantitative and unbiased natural history modeling in HPDL-related disease reveals significant genotype-phenotype associations, providing a foundation for variant interpretation, anticipatory guidance and choice of outcome measures in future prospective and functional studies.

2 Jonathan Alexander, PhD
Neuroscience
Tufts University

Inhibition of GSK3α,β rescues cognitive phenotypes in a preclinical mouse model of CTNNB1 Syndrome

CTNNB1 syndrome is a rare monogenetic disorder caused by CTNNB1 de novo pathogenic heterozygous loss-of-function variants that result in cognitive and motor disabilities. Treatment is currently lacking; our study addresses this critical need. CTNNB1 encodes β-catenin which is essential for normal brain function via its dual roles in cadherin-based synaptic adhesion complexes and canonical Wnt signal transduction. We have generated a Ctnnb1 germline heterozygous mouse line that displays cognitive and motor deficits, resembling key features of CTNNB1 syndrome in humans. Compared with wildtype littermates, Ctnnb1 heterozygous mice also exhibit decreases in brain β-catenin, β-catenin association with N-cadherin, Wnt target gene expression, and Na/K ATPases, key regulators of changes in ion gradients during high activity. Consistently, hippocampal neuron functional properties and excitability are altered. Most important, we identify a highly selective inhibitor of glycogen synthase kinase (GSK)3α,β that significantly normalizes the phenotypes to closely meet wildtype littermate levels. Our data provide new insights into brain molecular and functional changes, and the first evidence for an efficacious treatment with therapeutic potential for individuals with CTNNB1 syndrome.

3

Nicole Battaglia, BS
Neurobiology
Boston Children's Hospital

The Demographic, Clinical and Molecular Spectrum of Childhood-onset Movement Disorders Evaluated at an Academic Tertiary Care Movement Disorders Program

Objective: To describe the demographics, referral patterns, predominant phenomenology, etiology, and concurrent medical conditions in patients evaluated at an academic tertiary care movement disorders program during its establishment.

Methods: A retrospective chart review was conducted for all patients referred to our movement disorders program between July 2022 and March 2024.

Results: A total of 321 unique patients were included, with a total of 579 visits. Patients were referred regionally, nationally, and internationally. 92.5% had been previously evaluated by a neurologist, and 71.3% were referred to establish care. The majority of patients (51.4%) had an onset of symptoms before the age of 2. The predominant clinical phenomenology was dystonia (26.8%), followed by spasticity (22.4%), ataxia (8.4%) and stereotypies (8.1%). Approximately half of the patients (47.9%) had a mixed movement disorder with more than one phenomenology. A genetic or presumed genetic etiology was identified in 68.9% of cases. Among acquired movement disorders, 45.1% were attributed to vascular/ischemic causes. Common comorbidities included neurobehavioral symptoms (65.3%), seizures (28.7%), and psychiatric comorbidities (18.9%, mainly anxiety and depression). Eight patients underwent deep brain stimulation. 34.9% were enrolled in research studies.

Conclusion: Establishing a dedicated tertiary care movement disorders program is essential for improving care, fostering subspecialty training and advancing scientific progress in the field of pediatric movement disorders.

4

Alexandra Brooks, MD, PhD
Neurobiology
Boston Children's Hospital

Further Characterization of GIGFY1-related Neurodevelopmental Disorder

Heterozygous loss-of-function (LoF) of GIGYF1 has been implicated in neurodevelopmental disorders (NDDs), especially autism spectrum disorder (ASD), in several large-scale sequencing studies, supported by behavioral phenotypes in LoF zebrafish and mouse models. However, there is a need to fully characterize the variant and phenotypic spectrum of this newly emerging monogenic disorder. We identified a previously unreported heterozygous splice site variant (c.483-1G>C, not maternally inherited) in a 16-year-old male with ASD, attention-deficit hyperactivity disorder (ADHD), minor dysmorphic features, and transposition of the great arteries on genome sequencing. Through GeneMatcher and DECIPHER, we obtained information on 12 additional individuals, aged 4 to 26 years, with GIGYF1 LoF variants (4 nonsense, 4 small insertion-deletions, 2 duplications, and 1 frameshift, and 1 undetermined at this time), of which nine were de novo and three had unknown inheritance. Of these, all individuals had a neurodevelopmental phenotype, with the most prevalent being ASD (92%), followed by intellectual disability (78%) ranging from mild to severe. Additionally, 73% had a history of global developmental delay, with both motor delays (58%) and significant speech delays (82%). Many of the individuals (80%) also experienced behavioral issues, including self-injurious behavior and inattention/hyperactivity. Congenital heart disease was not identified in any of these individuals. Notably, dysmorphic facial features were reported in 88% —including synophrys, a prominent forehead, and a tented/thin upper lip—previously unreported in the literature. Here, we better delineate this newly emerging disorder, highlighting its association with ASD, intellectual disability, behavioral dysregulation, and distinctive facial features.

5

Alexandra K Brooks, MD, PhD
Neurology
Boston Children's Hospital

Expanding the clinical phenotype of SPG73: A case study of two families with progressive spasticity/weakness and concurrent neurodevelopmental/neurobehavioral disorders

Background: Hereditary spastic paraplegias (HSPs) represent an expanding group of neurological disorders characterized by progressive spasticity and weakness of the lower limbs. The spectrum encompasses various clinical presentations, ranging from pure forms with isolated motor impairment to complicated forms involving additional central or peripheral nervous system manifestations. With over 80 subtypes identified to date, the genetic landscape of HSP continues to expand.

Over the past decade, a small number of studies have identified autosomal-dominant variants in the carnitine palmitoyl-transferase gene CPT1C as the cause of hereditary spastic paraplegia type 73 (SPG73). To date, SPG73 has been associated with slowly progressive weakness and spasticity in the lower limbs, suggesting a relatively benign clinical course. Notably, affected individuals typically maintain normal cognitive function and lack documented behavioral concerns, indicating a pure form of HSP. Expanding the phenotypic and molecular spectrum of SPG73, we report two separate families with novel variants in CPT1C, where the affected patients also experienced neurodevelopmental and neurobehavioral issues.

Case Report: Here, we present two unrelated families with two male patients with inherited pathogenic CPT1C variants from their mothers in an autosomal-dominant manner. Both experienced progressive lower limb spasticity, however our patients also exhibited impairments in cognition, seizures, neurobehavioral issues, and/or psychiatric symptoms. Patient #1 (NM_001378488.1: c.1922_1926del: p.Ile641SerfsTer8) developed spasticity at 4 years of age and was diagnosed with anxiety and attention-deficit/hyperactivity disorder (ADHD). Patient #2 (NM_001378488.1: c.1885-1G>C: p.?) age of onset was at 14 years of age and presented with a notable regression in cognitive functioning, marked by the loss of previously acquired skills and knowledge and a new-onset refractory status epilepticus that led to the diagnosis of epilepsy. Furthermore, there were prominent behavioral concerns culminating in additional diagnoses of ADHD, anxiety disorder, depressive disorder, disruptive mood dysregulation disorder, conduct disorder, and autism spectrum disorder.

Conclusion: These findings underscore the complexity of genetic interactions underlying HSP. Furthermore, they contribute to a broader understanding of the clinical phenotype associated with SPG73, highlighting the diverse manifestations of this condition and expanding upon the previously accepted classical pure motor definition of SPG73.

6

Gabrielle Burkholz, MSPH
Simons Searchlight
Boston Children's Hospital

Simons Searchlight: A Global Research and Patient Education Resource for Rare Genetic Neurodevelopmental Disorders

Simons Searchlight is an international research program with the mission to shed light on rare genetic neurodevelopmental disorders (NDDs) by collecting high-quality standardized natural history data and building strong partnerships between academic researchers, industry, and families. Simons Searchlight collects caregiver- and patient-reported medical, developmental, behavioral, and biospecimen data across 180+ rare genetic neurodevelopmental disorders (NDDs). Simons Searchlight has enrolled 6,000+ registrants including 4,500+ individuals with genetic laboratory reports that have been submitted and reviewed by certified genetic counselors, 3,500+ individuals with medical history and/or other surveys completed, and 1,000+ individuals with biospecimens from which 150+ induced pluripotent stem cell (iPSC) lines have been created for distribution to interested researchers. Phenotypic survey data is collected via distribution of in-house and standardized surveys that focus on medical history, previous developmental and psychiatric diagnoses, medication use, seizure history, development and behavior, communication, sleep, quality of life, and more. Standardized assessments include the Vineland Adaptive Behavior Scales 3 (Vineland-3), Child Behavior Checklists (CBCL), Children's Sleep Habits Questionnaire (CSHQ), Social Responsiveness Scale-2, School Age (SRS-2), Social Communication Questionnaire (SCQ), QI-Disability, and PedsQL Family Impact Module. Over 100 peer-reviewed publications have included analyses of Simons Searchlight

data from external investigators, and clinicians including genetic counselors and geneticists have referred many patients to publicly available Simons Searchlight data summaries and gene guides for patient education on rare genetic NDDs. Qualified researchers can apply to access and analyze Simons Searchlight genetic, phenotypic, and biospecimen data to generate scientific and clinical insights by applying via SFARI Base at base.sfari.org. Ultimately, natural history data collected systematically over time can be used to inform clinical trial design and therapeutic development in rare genetic NDDs.

7

Marie Canty, BS
Developmental Medicine
Boston Children's Hospital

Strengths and Concerns in Young Children with Down Syndrome: A Qualitative analysis of common themes from a clinical sample

Introduction: In addition to intellectual disability, children and adolescents with Down syndrome can have multiple medical and behavioral issues and associated neurodevelopmental disabilities. Therefore, it is essential for clinicians to understand caregiver perceptions of their child's challenges, but also their strengths. Family-centered care requires clinicians to understand and prioritize issues most important to families to personalize recommendations and demonstrate an investment in the family-clinician partnership.

Methods: Caregivers were prompted to list three strengths and three concerns about their child as part of a standardized intake procedure prior to their clinic visit at the Boston Children's Hospital Down Syndrome Program. Caregiver-reported strengths and concerns from 2018-2023 for a sample of 261 unique children aged 0-3 years (mean age=1.58 years, SD=0.82) were analyzed qualitatively by two coders. An inductive codebook was developed, and inter-coder reliability was calculated (Cohen's kappa, κ). To address discrepancies between coders, the average number of references for common themes are presented.

Results: Most common strengths (κ=0.66-0.87) referenced child personality and attributes (e.g. happy, curious, n=207), gross motor skill development (n=75), and interpersonal relationships (n=74). Most common concerns (κ=0.80-0.83) referenced medical complexity or co-occurring diagnoses (n=175), personal daily living skills (n=76), and feeding (n=76).

Conclusions: Caregiver-reported strengths and concerns referenced medical, behavioral, and psychosocial factors related to their child's profile and family system. While reviewing the strengths and challenges of an individual child supports quality care for that specific child, reviewing responses from a large group of caregivers can help clinicians contextualize the concerns of individual caregivers and improve recommendations and anticipatory guidance.

8

Ross Carson, MD
Neurology
Boston Children's Hospital

Clinical Validation of SCN1A variant-phenotype association tool.

Objective: Early diagnosis of pathogenic variants in the sodium channel alpha 1 subunit (SCN1A) gene has significant management implications. The most common phenotypes associated with Loss of Function of SCN1A are Dravet Syndrome (DS) and Genetic Epilepsy with Febrile Seizures Plus (GEFS+), but it can be difficult to determine the phenotype early in the disease course. A prediction tool was published by Brunklaus in 2022 using age of seizure onset and genetic variant. The goal is to validate this tool in our patient population and examine additional clinical variables to aid in early diagnosis.

Methods: We performed a retrospective review of patients with SCN1A variants at Boston Children's Hospital. Inclusion criteria included having a variant in SCN1A and meeting modified delphi criteria. SNC1A variants were examined with the novel prediction tool. Additional early manifesting clinical variables were examined.

Results: Of the 121 patients identified, 73 met inclusion criteria (50 DS and 23 GEFS+). The prediction tool had a sensitivity of 1, specify of 0.43, positive predictive value of 0.79, and negative predictive value of 1 for diagnosing DS in our population. Changing the threshold for DS diagnosis significantly alters these characteristics. Additional early variables of significance:

history of status epilepticus (SE), SE before 24 months of age, and multiple seizure characteristics.

Conclusions: The prediction tool published by Brunklaus in 2022 has low specificity for diagnosing DS in our population. Changing the threshold for DS diagnosis changes performance characteristics. Additional consideration of other early markers could improve performance.

9 **Ross Carson, MD**
Neurology
Boston Children's Hospital

Genetic Testing Delays in Dravet Syndrome: Barriers and Opportunities for Improvement

Objective: Early diagnosis of Dravet Syndrome (DS) has significant management implications. Early diagnosis influences antiseizure medication choice, inclusion in clinical trials, appropriate counseling, and access to resources. Genetic testing is an essential part of diagnosing DS but there are many potential barriers. The goal of this project is to examine factors leading to delays in genetic testing for patients with DS.

Methods: We performed a retrospective chart review of patients with SCN1A variants seen at Boston Children's Hospital between 2000 and 2023. Patients were included if they met modified Delphi diagnostic criteria for DS and had available genetic testing. We collected information regarding clinical presentation and socioeconomic variables. These factors were correlated with time delay from the first documented seizure to genetic testing.

Results: Of the 157 patients identified, 69 met inclusion criteria. Average duration of testing delay among all patients was 43 months. The delay to testing for all patients decreased over time. Clinical factors significantly correlated with testing delays included lack of early status epilepticus (SE) and patient age. Socioeconomic variables significantly correlated with testing delays included needing an English language interpreter and having Medicaid-equivalent or safety-net insurance. Interestingly, neither race nor social deprivation index were significantly correlated with delays in genetic testing in our population.

Conclusions: Patients with DS without an early presentation for status epilepticus, non-English speaking families, and families with Medicaid-equivalent insurance experienced delays in genetic testing. Purposefully addressing these factors will be important for early diagnosis and subsequent management.

10 **Xinlan Chen, BA**
Neurobiology
Harvard Medical School

Mini-PCDH15b Gene Therapy Rescues Visual Deficits in a Zebrafish Model of Usher Syndrome Type 1F

Usher syndrome is a severe hereditary condition causing deafness and blindness. Mutations in PCDH15 cause Usher syndrome type 1F (USH1F), manifesting as profound deafness and lack of balance, and blindness developing later. Currently, cochlear implants are the only treatment for USH1F, and no treatment exists for blindness.

Gene addition therapy is an attractive treatment, however the PCDH15 coding sequence of ~5.8 kb is too large to fit into a single AAV capsid. We engineered a mini-PCDH15 gene in which 5 extracellular cadherin repeats were deleted, but which demonstrated proper protein localization and rescue of hearing in mouse models of USH1F.

To test mini-PCDH15 gene therapy for blindness, we used a zebrafish USH1F model, which has a 7-bp deletion in exon 8, leading to a premature stop codon. No Pcdh15b is expressed. Using a transposon-insertion strategy at the one-cell stage we introduced mini-Pcdh15b into Pcdh15b-mutant zebrafish.

We assayed rescue of visual function with electroretinogram (ERG) and optokinetic reflex (OKR) tests. We performed immunofluorescence and electron microscopy to localize the mini-Pcdh15b within photoreceptors in treated fish, and compared results with untreated mutant fish.

Pcdh15b-mutant zebrafish exhibit an early and progressive defect in photoreceptor morphology and visual function. Immunohistochemistry and electron microscopy of mutant photoreceptors revealed abnormal calyceal processes and

distorted outer segments. In 7-dpf mutant larvae, ERGs showed attenuated a- and b-wave amplitudes, and OKR responses were less robust.

Pcdh15b mutant zebrafish expressing mini-Pcdh15b demonstrated rescue of vision to wild type levels, as assessed with ERG and OKR recording. With immunofluorescence and immunogold SEM, strong mini-Pcdh15 signal was detected along calyceal processes of photoreceptors. Immunohistochemical and SEM analysis showed robust rescue of photoreceptor morphology, comparable to that in normal larvae.

These results suggest that a mini-PCDH15 gene therapy is a promising approach for the treatment of progressive blindness in human Usher 1F.

11

Kevin Cho
Neurobiology
Brigham and Women's Hospital, Harvard University

Harnessing Small Molecule Compounds for microRNA-Induced Differentiation of Treatment-Resistant Neuroblastoma

Neuroblastoma is the most prevalent extracranial cancer in children. High-risk neuroblastoma has a five-year survival rate of less than 50%, with ~40% of these patients experiencing relapse despite modern multimodal therapies. In particular, the cellular heterogeneity of neuroblastoma complicates clinical treatments, as the mesenchymal (MES)-type cells exhibit abnormal resistance to standard treatments, including chemotherapy, retinoid therapy, and immunotherapy, compared to the adrenergic (ADRN)-type cells. Therefore, there is an urgent need for novel treatments to effectively target treatment-resistant MES-type neuroblastoma cells. Our previous findings supported that upregulating microRNA miR-124—an established endogenous driver of neuronal differentiation—via small molecules PP121 and bufalin effectively promoted the long-term differentiation of mixed SK-N-AS neuroblastoma cells. Therefore, we hypothesized that miR-124 induction by PP121/bufalin could similarly differentiate other MES cell lines, including those with MYC amplification, a major risk factor in neuroblastoma. We first verified the differentiation-inducing role of miR-124 by showing that transfecting GI-MEN, SH-EP (non-MYC-amplified), KP-N-S19s, and CHP-212 (MYC-amplified) cells with miR-124 mimics significantly decreased cell proliferation as measured by the WST-1 viability assay, and the reduction of proliferation markers by qPCR and immunofluorescence staining (IF). To extend the PP121/bufalin differentiation protocol to other MES-type neuroblastoma cells, we screened for optimal doses for KP-N-S19s and CHP-212 cells and confirmed that 2.5 μM PP121 and 10 nm bufalin doses were optimal for inducing stable cell differentiation. PP121/bufalin-induced differentiation also upregulated miR-124 and downregulated miR-124 targets as measured by qPCR and IF. In conclusion, we demonstrated that miR-124 transfection and PP121/bufalin treatment effectively differentiated MYC-amplified MES cells, further supporting the potential of these miR-124 upregulating small molecule compounds in treating resistant neuroblastoma. Future research will focus on examining the mechanisms underlying PP121/bufalin-induced differentiation, particularly the role of epigenetic regulation.

12

Alexander Cohen, MD, PhD
Neurology
Boston Children's Hospital

Transforming Research Laboratories with TNC DoCS Services

In the ever-evolving digital research landscape, effective data management is paramount. The newly launched Data Organization Collaborative Service (TNC DoCS) at the Rosamund Stone Zander Translational Neuroscience Center provides specialized support for research laboratories aiming to excel in digital data management. TNC DoCS offers a suite of services including One-on-One Consultation for digital transformation, an ESP Database for participant tracking, and REDCap Streamlining Services for enhanced data collection. These services are designed to support researchers at any stage of their projects, promoting the adoption of advanced digital research methodologies.

One-on-One Consultation services offer personalized technical guidance to facilitate digital transformation within research labs. By automating repetitive tasks and processes, researchers can focus more on advancing scientific goals. The ESP

Database, currently in development, revolutionizes participant tracking by reducing study team workloads and ensuring strong privacy protocols. REDCap Streamlining Services, also in development, enhance data collection through reproducible protocols, version control, and data cleaning tools, fostering interdisciplinary collaboration and innovation.

Completed projects highlight TNC DoCS's impact on research efficiency. Notable initiatives include tailoring data backup automation pipelines for the Human Neuron Core and Biorepository Core, designing an automated EEG data collection system for Maski's Lab, offering Docker training for pipeline containerization in the Epilepsy Surgery Outcomes/CRL project, and preparing data for NDA submission in The Emotion Project. The comprehensive support offered by TNC DoCS enables research laboratories to navigate the complexities of digital data management, ensuring accuracy, efficiency, and innovation in scientific endeavors.

13 **Angelica D'Amore, PhD**
Neurobiology
Boston Children's Hospital

Phenotypic rescue via mTOR inhibition in neuron-specific PTEN knockout mice reveals AKT and mTORC1-site specific changes

Phosphatase and Tensin Homolog deleted on Chromosome Ten (PTEN) is a tumor suppressor gene encoding a dual protein and lipid phosphatase. PTEN dephosphorylates phosphatidylinositol-3,4,5-triphosphate (PIP3), directly antagonizing phosphatidylinositol-3-kinase (PI3K)/Akt/mechanistic target of rapamycin (mTOR)-mediated signaling pathway necessary for growth suppression. Germline mutations in PTEN are associated with PTEN Hamartoma Tumor Syndrome (PHTS), a spectrum of hereditary cancer syndromes characterized by the development of hamartomas in the body. Notably, PHTS exhibits high rate of neurological comorbidities, including macrocephaly (43-100%), autism spectrum disorder (ASD) (14-50%), and intellectual disability (ID) (31-67%). However, the relevance of neuronal PTEN mutations in PHTS neuropathological manifestations remains unclear. In this study, we aimed to: 1) generate Synapsin- 1Cre;PTENf/f (Syn-PTEN KO), a novel neuronal-specific PTEN knockout mouse model, 2) characterize Syn-PTEN KO mice somatic development, survival, and molecular changes including AKT/mTOR signaling and transcriptomic analyses, and 3) generate primary neuronal cultures derived from Syn-PTEN KO mice and characterize their function in vitro. Mice and neuronal cultures were then treated with an mTOR inhibitor (everolimus) to test for phenotypic rescue. We crossed PTENf/f (JAX 006440) and Synapsin-1Cre (JAX 003966) to generate Syn- PTEN KO mice. For in vivo characterization, we measured body weight, brain:body weight ratio and survival. Everolimus (3mg/kg) was administered to HOM mice via intraperitoneal (IP) injection starting at postnatal days P7, three times a week. Brain tissues were dissected for western blot and RNA-sequencing. For in vitro characterization, primary neuronal cultures dissected from HOM, HET and WT pups at embryonic day 18.5 were used. Immunofluorescence staining for Synapsin1/PSD95/MAP2 expression was performed to quantify synapses in vitro. Firing activity parameters were measured at baseline (vehicle-treated) and after everolimus treatment using the multi-electrode-array (MEA) platform. We found significant reduction in body weight, increased brain:body weight ratio and shortened lifespan in HOM Syn-PTEN KO mice compared to HET or WT littermate control. Everolimus significantly extended lifespan of HOM mice, but body weight did not improve. Primary neuronal cultures from HOM pups showed significantly increased weighted mean firing rate and number of bursts compared to HET or WT control neurons. This network hyperactivity phenotype was partially rescued with everolimus treatment. Synaptic markers (Synapsin1/PSD95) were increased in neurons derived from HOM pups, and their expression levels were normalized upon everolimus treatment. RNA-seq analyses revealed ~600 differential regulated genes in HOM Syn-Cre/PTEN KO mice compared to control mice. Gene ontology analyses indicated that myelin and extracellular matrix (ECM) are among the major subclasses of upregulated genes. Accordingly, immunostaining confirmed significant increase in myelin and perineuronal nets (PNN), a specialized ECM enwrapping parvalbumin-expressing interneurons, in HOM mice. We successfully generated a novel neuronal-specific PTEN knock-out mouse model, the Syn-PTEN KO. Characterization of Syn-PTEN mice revealed profound neurological and molecular pathology culminating to premature lethality in these mice. Everolimus only partially rescued the discovered Syn-PTEN phenotypes. Alternative approach targeting upstream of mTOR might be tested for better phenotypic rescue in the future.

14 **Raymond Belanger Deloge, MS, CGC**
RSZ TNC
Boston Children's Hospital

GENE TARGET 2.0: A refined tool for evaluating monogenic neurodevelopmental disorders for therapeutic development.

Recently, a framework to evaluate the translational building blocks for gene-based therapeutic development for monogenic neurodevelopmental disorders (NDDs) was proposed (Chopra et al, 2022). This framework, called GENE TARGET (GT) was designed to identify gaps in the translational pipeline for a given disorder to inform prioritization of future research efforts for both academic translational research centers and patient advocacy groups (PAGs). Considerations falling into the domains of genetic mechanism, preclinical modeling, clinical trial, and ethical factors were scored. The composite score, Gene Target Suitability (GTS Score), with a maximum possible score of 40, quantified therapeutic readiness for a given monogenic NDD. Given the preliminary nature of this framework, it has not been subjected to validation or systemic use in the translational research setting, and inter-user variability in scoring is unknown. Here, we used GT to evaluate 50 NDD gene-disease pairs nominated by collaborators including Simons Searchlight, researchers, and PAGs. Through this exercise, we identified strengths and limitations of the GT framework which informed iterative refinements of the tool for real-world applications, culminating in GT 2.0. Additional numerical score categories were added to 3 criteria to capture more disease-gene pair nuances. For 2 criteria, score descriptions were clarified, but the numerical scoring categories remained the same. The remaining 5 criteria did not change. A resource guide was developed to aid users in their search for information when generating GT 2.0 scores. Currently, we are evaluating the inter-user variability of GT 2.0 using a subset of 20 NDD genes and 5 raters. Although the refined framework remains focused on gene-based therapeutic suitability for scoring purposes, points to consider for medicinal chemistry approaches to treatment will be outlined. GT 2.0 will serve as a more refined, validated tool for the evaluation of monogenic NDDs for therapeutic readiness.

15 **Iris den Hertog, BS**
Neurobiology
Boston Children's Hospital

Epileptogenic Tubers in Tuberous Sclerosis Complex Epilepsy Surgery map to a Common Brain Network

Purpose: Among patients with Tuberous Sclerosis Complex (TSC), 60 to 70% has refractory epilepsy. The success of epilepsy surgery in children with TSC hinges on accurate identification of the epileptogenic tuber. This study aims to evaluate whether epileptogenic tubers are more functionally connected to epilepsy network nodes than non-epileptogenic tubers.

Method: We applied Lesion Network Mapping (LNM) to identify the functional connectivity of each tuber to the basal ganglia, brainstem, and cerebellum—epilepsy network nodes that are more associated with symptomatic epilepsy. We collected demographic, clinical, and MRI data from 7 institutions. We manually segmented tubers on the preoperative MRI and registered them to the MNI152 common atlas space. First, in a hypothesis-free analysis, we identified the common network nodes of all resected tubers using voxel-wise nonparametric permutation analysis of linear models (PALM) with 2,000 permutations. Then, we used region-of-interest-to-region-of-interest (ROI-to-ROI) analysis to compare the connectivity of epileptogenic versus non-epileptogenic tubers to the predefined epilepsy network nodes. Based on this ROI-to-ROI functional connectivity, we made individualized epileptogenicity risk maps.

Results: There were 59 children (0-18 years), with 61 resected tubers and more than 1,000 tubers designated as non-epileptogenic. In the hypothesis-free analysis, resected tubers mapped to the same epilepsy network nodes as found in prior work: the vermis of the cerebellum, the pons, and the bilateral globus pallidus ($p < 0.05$). The ROI-to-ROI connectivity analysis confirmed that epileptogenic tubers were more connected to these network nodes as compared to non-epileptogenic tubers ($p = 0.029$). We illustrated the functional connectivity of tubers with individualized risk maps.

Conclusion: Resected tubers map to the previously reported epilepsy network nodes. For pediatric epilepsy surgery candidates with multiple lesions, these findings allow for the generation of an individualized tuber epileptogenicity risk map.

16 **Chen Ding, PhD**
RSZ TNC
Boston Children's Hospital

Sarm1 knockout rescues age-related retinal ganglion cell (RGC) degeneration in a novel mouse model of Autosomal Dominant Optic Atrophy (ADOA)

Autosomal Dominant Optic Atrophy (ADOA) is the most prevalent inherited optic neuropathy. Patients with ADOA typically experience vision loss in the first decade of life due to selective degeneration of retinal ganglion cells (RGCs), with a gradual progression to legal blindness over subsequent years. The majority of ADOA cases result from mutations in the OPA1 gene, which encodes a conserved GTPase required for mitochondrial inner membrane fusion and cristae remodeling. Homozygous for loss of OPA1 are embryonic lethal, while ADOA human patients are heterozygous. Defective OPA1 in cells leads to fragmented mitochondria, abnormal cristae structure, reduced ATP production, increased oxidative stress, and mitochondrial DNA loss. Currently, no therapies exist for ADOA, and its disease mechanism remains poorly understood. We have developed a novel mouse model of ADOA carrying the pathogenic OPA1R290Q/+ mutation. Our data show that OPA1R290Q/+ mice exhibit many features of human ADOA, including RGC death, optic nerve degeneration, demyelination, and impaired RGC responses. At the cellular level, the OPA1R290Q/+ cells display highly fragmented mitochondria, decreased respiration, and signs of oxidative stress. We then investigated the role of Sarm1, a conserved NADase and a major pro-degenerative factor in neurons, in mediating RGC degeneration in ADOA. Sarm1 knockout has been shown to prevent neurodegeneration in various pathological conditions, including glaucoma and mitochondrial stress models. Our results demonstrate that Sarm1 knockout in OPA1R290Q/+ mice significantly reduces age-dependent RGC loss. This suggests that Sarm1 activation drives RGC degeneration in ADOA. We are currently exploring how OPA1-induced mitochondrial dysfunctions activate Sarm1. Overall, our study indicates that the OPA1R290Q/+ mouse model recapitulates human ADOA and that targeting Sarm1 may offer potential therapeutic benefits for ADOA patients.

17 **Katie Donovan, BS**
Neurobiology
Harvard University, Boston Children's Hospital

The role of HSP27 in neuronal primary cilia dysfunction in tuberous sclerosis complex

Tuberous sclerosis complex (TSC) is a genetic disorder caused by heterozygous inactivating variants in TSC1 or TSC2. TSC is characterized by the growth of benign tumors in multiple organs, as well as neurological manifestations such as refractory epilepsy, autism spectrum disorder, and intellectual disability. TSC1 and TSC2 form a protein complex with TBC1D7 that acts as an upstream negative regulator of the mechanistic target of rapamycin complex 1 (mTORC1), and mTORC1 hyperactivation is thought to be a key pathogenic driver of TSC. Treatments that inhibit mTORC1 cause arrest or regression of tumors in TSC but have shown limited efficacy in treating epilepsy and no efficacy in treating the neurocognitive symptoms of TSC, necessitating the need for further research. Disruption of primary cilia has emerged as one potential candidate for mediating neuronal dysfunction in TSC downstream of mTORC1. The primary cilium is an immotile organelle that extends from the plasma membrane and contains a distinct composition of transmembrane receptors, rendering it a signaling hub. We have found that Tsc2-deficient neurons are less likely to be ciliated, and their remaining cilia are longer, which correlates with cytomegaly. We observed that Heat shock protein 27 (HSP27) is increased at the transcript and protein level in Tsc2-deficient cortical cultures, and that it is primarily localized to astrocytes. Ubiquitous shRNA knockdown of Hsp27 in Tsc2-deficient cortical cultures rescued neuronal ciliation, while ablation of glia did not. This result suggests that Hsp27 knockdown rescues neuronal ciliation through a cell-autonomous mechanism. Further elucidation of this pathway will yield novel insights into the pathogenesis of neurological disorders in TSC and facilitate development of therapeutic strategies.

18 **Kasey Edwards, BA**
Cure AP-4
https://cureap4.org/

Patient Advocacy Group Partnership:Cure AP-4

For more information about the work of Cure AP-4 and its partners, please visit https://cureap4.org/.

19 **Darius Ebrahimi-Fakhari, MD, PhD**
Neurology
Boston Children's Hospital

Using Lipid Droplets as a Cellular Phenotype in RINT1-Deficient Cells for High-Throughput Small Molecule Screening

Objectives: Develop a robust platform using RINT1-deficient fibroblasts for high-throughput small molecule (HTSM) screening, utilizing lipid droplets as the primary readout.

Background: Bi-allelic loss-of-function variants in the RINT1 gene are linked to acute liver failure and complex hereditary spastic paraplegia (HSP) in children. This study investigates cellular phenotypic differences in patient-derived cells, focusing on detectable changes in lipid metabolism to establish a platform for a cell-based phenotypic small molecule screen.

Methods: A high-throughput platform was used to image RINT1-deficient fibroblasts and controls in 96-well microplates. Automated and standardized image analysis reliably identified nuclei and cell boundaries using DAPI and phalloidin staining. Lipid droplets were stained with Dojindo Lipi-Green, and their characteristics were quantified using the Harmony Image Analysis Software. Two lipid droplet stains, Lipi-Green and BODIPY, were tested during development.

Results: RINT1-deficient fibroblasts exhibited significantly fewer lipid droplets per cell compared to control cells, with a small difference in droplet size. Patient cells showed a mean of 13.8 ± 4.76 (SD) LDs per cell per well, compared to 31.4 ± 8.2 (SD) in control wells, resulting in a 56% difference. Control cells had an average lipid droplet area of 1.23 ± 0.055 (SD) μm^2, while RINT1-deficient fibroblasts averaged 1.05 ± 0.052 (SD) μm^2, a 14% difference. Z-Prime STD and Z-Prime robust values ranged from -0.09 to -1.5, and SSMD values ranged from -1.7 to -3.05, indicating high variability and insufficient assay quality.

Conclusion: Despite extensive optimization, the high variability in lipid droplet numbers across wells challenges the development of a reliable high-throughput assay based on this readout. The current assay is unsuitable for high-throughput screening. Future research should explore alternative cellular phenotypes or readouts to establish a more robust screening platform for RINT1 deficiency.

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Patient Advocacy Group Partnership: TSC Alliance

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21 **Isabel Gisser, BA**
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Second hit mutations in TSC2 alter neuronal differentiation of surrounding cells via secreted factors

Tuberous Sclerosis Complex (TSC) is an autosomal dominant genetic disorder caused by heterozygous variants in either TSC1 or TSC2 and is associated with epilepsy, autism, and intellectual disability. Patients display focal cortical dysplasias or cortical tubers in the brain, which are characterized by fewer neurons, astrocytosis, and dysmorphic cells. Patients also present with benign tumors found in several organs, including the heart and kidneys. As opposed to benign tumors in TSC that frequently show second hit mutations in TSC1 or TSC2, only a minority of cortical tubers show these changes. This suggests that second hit mutations are either not necessary for cortical tuber formation or that these genetic alterations affect only a small percentage of cells.

We have previously demonstrated that iPSC-derived neurons with biallelic mutations in TSC2 (TSC2-/-) display gene expression changes found in cortical tubers, despite most cells in the tuber being heterozygous for TSC2 (TSC2+/-). We have also shown that in mixed-genotype organoid models containing both TSC2+/- and TSC2-/- cells, there is a significant reduction in developing cortical pyramidal neurons and an increase in a specific subset of outer radial glia when compared to organoids comprised of TSC2+/- and CRISPR-corrected TSC2+/+ cells. Further, TSC2+/- cells at the neuroprogenitor stage showed reduced TBR2 expression when exposed to TSC2-/- cells, consistent with impairment of cortical neuron differentiation pathways. Therefore, we hypothesized that second hit TSC2-/- cells may be capable of altering surrounding cells via secreted factors, leading to cortical tuber formation.

To determine whether this effect was mediated by secreted factors, we performed neuroprogenitor differentiation in transwell plates and observed that TSC2+/- cells show decreased TBR2 mRNA expression when cultured with TSC2-/- cells. We then isolated extracellular vesicles from organoids with all three TSC2 genotypes and characterized them with proteomics. Interestingly, there is a significant enrichment of regulators of the actin cytoskeleton, including angiomotin (AMOT), implicating cellular remodeling in aberrant neuronal differentiation induced by TSC2-/- cells. Our data suggest that a small percentage of cells with second hit mutations can alter neuronal differentiation in neighboring cells via extracellular vesicles, providing a mechanism for amplifying the effect of second hit mutations and leading to cortical tuber formation.

22 **Madison, Glass, PhD**
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in vitro modeling of CDKL5 Deficiency Disorder (CDD) reveals cortical neuron specific early hyperexcitability

CDKL5 deficiency disorder (CDD) is a rare epileptic encephalopathy resulting from variants in cyclin-dependent kinase-like 5 (CDKL5) that lead to impaired kinase activity or loss of function. In addition to infantile spasms, CDD patients also show severe developmental delays and loss of motor function. To study how CDKL5 variants impact human neuronal activity, gene expression and morphology, CDD patient-derived induced pluripotent stem cells and their isogenic controls were differentiated into excitatory neurons using either an NGN2 direct induction protocol or a guided cortical organoid differentiation. Patient-derived neurons from both differentiation paradigms had decreased phosphorylated EB2, a known molecular target of CDKL5. Induced neurons showed no detectable differences between cases and isogenic controls in network activity using a multielectrode array, or in MAP2+ neurite length, and only 3 genes were differentially expressed. However, patient-derived neurons from the organoid differentiation showed increased synchrony and weighted means firing rate on the multielectrode array within the first month of network maturation. CDD patient-derived cortical neurons had lower expression of CDKL5, possibly due to nonsense mediated decay, and lower expression of HS3ST1, a 3-O-sulfotransferase that modifies disaccharide side chains on heparan sulfate proteoglycans, which may change the extracellular matrix around the synapse and contribute to hyperexcitability. We identified donor specific differences in gene expression in these neurons, including changes in genes associated with glutamate receptor activity in one donor. Similar to the induced neurons, there

were no differences in neurite length across or within donor patient-control cell lines. Induced neurons have poor cortical specification while the organoid derived neurons expressed cortical markers, suggesting that the changes in neuronal excitability and gene expression are cell-type specific. Examining molecular mechanisms of early hyperexcitability in cortical neurons is a promising avenue for identification of CDD therapeutics.

23 **Julia Grocott, BS**
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Shank3 loss in hippocampal GABAergic cells results in epileptiform activity

Heterozygous loss of Shank3 in humans is associated with epilepsy, but mouse models with even two mutated/deleted alleles fail to capture this phenotype, hampering the development of therapies. Since Shank3 is a key scaffolding protein at excitatory synapses onto both glutamatergic and GABAergic cells, we hypothesized that selective deletion from GABAergic cells would result in an epileptic phenotype, owing to the expected impairment of excitatory drive only onto the inhibitory cells but intact excitatory communication. To this end, we utilized a new conditional mouse model that results in the deletion of exons 4 to 22 in the Shank3 gene in the presence of Cre-recombinase. Cre was delivered to the hippocampus via a low titer AAV with a Dlx enhancer element to restrict expression to GABAergic cells in wild-type, heterozygous, and homozygous Shank3 mice. Using two 32-channel silicon probes, we recorded hippocampal local field potential from both the injected and uninjected hemispheres in awake behaving mice. We observed profound seizure activity in both heterozygous and homozygous mice, but not wild-type. Moreover, epileptiform events propagated to both hemispheres, suggesting widespread circuit impairment. While this work establishes a new mouse model for Shank3-related epilepsy to test various treatment strategies, it also supports that cell type-specific differences are key regulators of how Shank3 loss manifests at the circuit level.

24 **Ryan Guardado, BS**
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Prospective Biobanking for the Acceleration of Research on Pediatric Neurological Disorders

Background: Pediatric epileptic disorders represent a significant burden on global health, while also profoundly devastating the quality of life of affected individuals and their families. Despite the impressive recent advances in the areas of diagnostic testing, clinical evaluation, and medical interventions for many of these illnesses, the limited availability of high-quality, diverse biospecimens available for research purposes remains a major challenge in executing rigorous basic science and translational research studies. The Boston Children's Hospital (BCH) Rosamund Stone Zander Translational Neuroscience Center (RSZ TNC) neurological biorepository was established to address this gap by providing researchers with donated brain tissue resected from patients during eligible epilepsy neurosurgeries, thereby accelerating discovery and innovation in a wide range of studies. This review is aimed at examining the success of impacting research by investigating key metrics and outcomes relating to sample collections, research collaborations, publications, quality control, and compliance.

Methods: The RSZ TNC biorepository facilitates the advancement of translational neuroscience research headed by BCH investigators through the employment of comprehensive and systematic methods of patient recruitment, specimen procurement, sample processing, storage, sample distribution, and data collection.

Results: Over the past 14 years, the RSZ TNC biorepository has enrolled 1393 patients into our Phenotyping and Banking Core for Neurological Disorders IRB, which has allowed us to collect resected brain tissue samples from 495 patients, amounting to over 2000 specimens. More recently, the biorepository started collecting cerebrospinal fluid (CSF) samples to accelerate biomarker discovery research and has already banked 185 samples in the last 2 years. In total the biorepository samples have been utilized to support 54 BCH investigator-led projects.

Conclusions: The RSZ TNC biorepository provides resources that have proven to be instrumental in the advancement of translational neuroscience research. Additionally, it has laid down a foundational framework for other leading institutions to guide the establishment of their own biorepositories.

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Patient Advocacy Group Partnership: ZTTK SON-Shine Foundation

For more information about the work of ZTTK SON-Shine Foundation and its partners, please visit https://zttksonshinefoundation.org/.

26 **Mansi Gupta, MS**
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Revolutionizing Neurology: Harnessing AI to Enhance Diagnosis and Treatment

Since neurological disorders account for most deaths and causes of disability combined, creative, scalable, and long-lasting remedies are needed. With the adoption of the Inter Sectoral Global Action Plan by the World Health Organization in 2022, brain health has gained international attention. In the discipline of neurology, artificial intelligence (AI) has become a potent instrument that has a big influence on the diagnosis and treatment of neurological illnesses. AI and neuroscience have a long history of working together. With its potential to improve brain disease detection and treatment, artificial intelligence (AI) holds great promise for revolutionizing neurology. AI is now used in systems for precise brain mapping, surgical planning, and epilepsy diagnosis. It may help design individualized treatment programs based on unique neurological profiles and help discover innovative therapies for neurological diseases like Parkinson's and Alzheimer's. The ability of AI to identify intricate patterns in data opens the door to ground-breaking improvements in the identification and management of brain disorders. These days, AI can interpret electroencephalograms (EEG) to prognosticate coma, detect seizures well before ictus, predict Alzheimer's disease, which converts mild cognitive impairment to dementia, diagnose stroke from CT/MRI scans, identify papilledema and diabetic retinopathy from retinal scans, and categorize neurodegenerative diseases based on gait and handwriting. In conclusion, the application of AI in neurology has transformed research, diagnosis, and therapy. With the development of AI technology, neurological illnesses will become increasingly understandable, improving patient care and quality of life. The fusion of artificial intelligence and neurology provides a window into a future where compassion and creativity come together to completely transform neurological treatment. An overview of the application of AI in neurology and its potential for revolution is given in this abstract.

27 **Mustafa Qadir Hameed, MD**
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Positive allosteric modulation of Kv3.1 potassium channels protects against SUDEP and seizures in a mouse Dravet syndrome model

Dravet syndrome (DS) is caused by pathogenic SCN1A gene variants, leading to Nav1.1 voltage-gated sodium channel haploinsufficiency. Reduced parvalbumin-positive interneuron (PVI) firing resultant from Nav1.1 deficiency is a major DS pathophysiology. The Kv3.1 voltage-gated potassium channel is preferentially expressed in PVIs, is critical for their fast-spiking properties, and its expression is also depressed in a mouse DS (Scn1a+/-) model. Since conventional anti-seizure drugs are largely ineffective in DS, a novel anti-seizure DS therapeutic target is highly desirable. Given Kv3.1's role in PVI function, we tested whether Kv3.1 positive allosteric modulation (PAM) suppresses seizures in Scn1a+/- mice.

P17-19 Scn1a+/- mice received vehicle (VEH) or AUT1 (Kv3.1 PAM) 30 min prior to induction of febrile seizures (FS). Another cohort of juvenile Scn1a+/- mice were administered either AUT1 or VEH twice daily for 2 weeks starting at P17. Lastly, one cohort of adult Scn1a+/- mice underwent baseline video-EEG monitoring for generalized tonic-clonic seizures (GTCS) for 7 days. Mice with GTCS were randomly assigned to receive daily VEH or AUT1 during additional 1-week video-EEG monitoring.

AUT1 delayed FS onset in juvenile Scn1a+/- mice (p=0.017) and increased mean FS temperature threshold (increase from baseline; VEH: +4.5 ± 0.8C; AUT1: +5.3 ± 1.2C, p=0.046). 10% of AUT1 mice developed FS at the VEH group median threshold of +4.3C (p=0.041). Long-term AUT1 treatment also reduced SUDEP incidence in juvenile Scn1a+/- mice (p=0.011). 100% of VEH controls died by P32 compared to 42% of AUT1 treated mice (p=0.038). AUT1 reduced seizure frequency compared to VEH (AUT1: 53.5 ± 31.7%; VEH: 255.5 ± 202.8%; p = 0.026) in seizing adult Scn1a+/- mice.

Our findings indicate that acute Kv3.1 potentiation may protect against early mortality and both febrile and spontaneous seizures in DS. These results provide a basis for continued work on developing novel DS therapies targeting Kv3.1 channel biology.

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29

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Patient Advocacy Group Partnership: SSADH Association and Galibra Neuroscience

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30

Brad Hoffman, MBA
SSADH Association

25 Years of Paving the Path to a Clinical Trial Curing SSADH through Gene Therapy

Succinic semialdehyde dehydrogenase deficiency (SSADHD) is a rare inborn metabolic disorder caused by loss-of-function mutations in the ALDH5A1 gene. In SSADHD, ALDH5A1 malfunction leads to pathologic accumulation of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) and its metabolite gamma-hydroxybutyrate (GHB), resulting in broad-spectrum encephalopathy including developmental delay, epilepsy, and a risk of sudden unexpected death in epilepsy (SUDEP). As of 2024, there are under 500 documented SSADHD cases (though more patients are likely undiagnosed). To date, available treatments for SSADHD are symptomatic and ineffective. A major unmet medical need for SSADHD is treatment directly addressing the underlying enzyme deficiency such as enzyme replacement therapy (ERT) and gene therapy. The SSADH Association is a major leading patient advocacy group for patients with SSADHD, aimed to raise the awareness of this rare disorder and bring effective treatment to this under-represented patient population. The SSADH Association has funded and participated in projects ranging from scientific tool development including mouse and patient induced pluripotent stem cells (iPSC) models, newborn screening, biomarker development, to natural history study critical for future clinical trial design. Here, we highlight our progress on the development of gene therapy, which targets the underlying genetic cause and potentially provides a curative effect on SSADHD. Proof-of-concept of gene therapy in SSADH-deficient mice already demonstrated phenotype reversibility. Upon treatment, we found significantly enhanced survival and lifespan extension in mutant mice. The status of gene therapy development currently focuses on AAV9 efficacy and toxicology studies. In anticipating clinical translation, we are preparing meeting with the FDA, as well as continuing fund-raising efforts through a recent start-up, Galibra Neuroscience, which aims to develop gene therapy for SSADHD as well as other GABA-related neurodevelopmental disorders.

31 **Minqing Jiang, PhD**
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F1 generation SHANK3 heterozygous mutant Macaques exhibit stereotypy, cognitive and sensory deficits

Phelan-McDermid syndrome (PMS) is a neurodevelopmental disorder with a terminal deletion in 22q13 that results in the loss of function of the SHANK3 gene. Loss of SHANK3 has been identified in gene-linkage studies to be strongly associated with ASD and intellectual disabilities. To gain further insights into SHANK3 mutation and associated behavioral, physiological and cognitive changes reflecting autism, we derived F1 generation mutants from our earlier developed founder SHANK3 macaques. Through a systematic phenotypic characterization, we found a consistent but heterogeneous distribution of behavioral signs that can be mapped onto human autism spectrum disorder. Auditory EEG/ ERP results suggest an early sensory processing deficit. We also found significant differences in some tests of learning and cognition between mutants and controls. Resting state functional connectivity indicated a global hypoconnectivity but local hyper-connectivity, especially in visual sensory circuits. Our results from multiple tests suggest that the SHANK3 mutant phenotype in macaques can characterized in an unbiased manner.

32 **Jiahe Jin, BS**
Neurobiology
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Building human inner ear and retinal dual-organoid models to develop novel antisense therapy for USH2A deafblind patients

Usher syndrome (USH) is the most common inherited genetic disease leading to combined deafness and blindness. Mutations in USH2A account for half of USH cases. However, the large size of the USH2A gene (~15.6kb) hampers traditional gene therapy approaches. Alternatively, antisense oligonucleotides (ASOs) can be engineered to target and bypass deleterious mutations. In this work, we focused on mutations in exon 19-20 of USH2A. We developed a library of ASOs to skip these 2 exons, restore protein expression and prevent hearing and vision loss in USH2A patients. To assess ASO efficacy and safety before clinical translation, we developed human induced pluripotent stem cell (hiPSC)-derived inner ear organoids (IEOs) and retinal organoids (ROs). Usherin, a protein crucial for the integrity of the ankle links in inner ear hair cells and the connecting cilium in photoreceptor cells, is the product of the USH2A gene. Using patient derived and control wild type IEO and RO models, we assessed and validated several ASOs that led to in-frame dual exon skip in hair cells and photoreceptor cells. This work justifies the use of IEO and RO for drug screening and therapeutic development.

33 **Brooke Keough, BA**
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Alterations in resting EEG power in children with Phelan-McDermid syndrome

Phelan-McDermid syndrome (PMS) is a rare genetic condition caused by a mutation or deletion of the SHANK3 gene at chromosome 22q13, which regulates the expression of a scaffolding protein in the postsynaptic densities of excitatory synapses. PMS is one of the most common monogenic forms of autism spectrum disorder (ASD) and accounts for approximately 1% of the individuals with ASD. Prior works have shown increased neuronal excitability and decreased synaptic transmission in SHANK3-mutant human neurons. However, alterations in brain activities among humans with PMS and how these alterations are associated with their behavioral phenotypes have not been characterized. Utilizing resting electroencephalography (EEG) data collected from children (2 – 11 years) with PMS (n=41), idiopathic ASD (iASD; n=40), or typical development (TD; n=42), we calculated and parameterized power measures and compared them among groups while accounting for age differences across individuals. Posterior aperiodic exponent is significantly lower in the PMS group

than the TD group (FDR-corrected p=0.030), suggesting an increase in the neuronal excitation to inhibition ratio. Aperiodic-adjusted alpha band (6 – 11 Hz) activity in the posterior region is significantly lower in children with PMS than children with TD (FDR-correct p<0.001) or iASD (FDR-correct p=0.039). The same EEG measure was also found to be positively associated with nonverbal developmental quotient (p=0.027) across individuals with PMS. These findings suggest alterations in neuronal excitability and neural synchronization in humans with PMS, offering opportunities for back translation of findings into animal models and potential brain-based biomarkers to target in clinical trials.

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Clinical and Molecular Outcomes from the 5-Year Natural History Study of Succinic Semialdehyde Dehydrogenase Deficiency

Background: Succinic semialdehyde dehydrogenase deficiency (SSADHD) is a rare inherited metabolic disorder characterized by impaired catabolism of γ-aminobutyrate (GABA). Boston Children's Hospital is the main clinical site for its 5-year and ongoing NIH-sponsored SSADHD Natural History Study.

Methods: SSADHD subjects underwent clinical and neuropsychological assessments, biochemical quantification of GABA, EEG (standard and high density), MEG, TMS, MRI and MRS, and genetic tests. This was parallel to molecular investigations of in vitro GABAergic neurons derived from induced human pluripotent stem cells (hiPSCs) of SSADHD subjects and biochemical analyses performed on a versatile murine model that uses an inducible and reversible rescue strategy allowing on-demand and cell-specific gene therapy.

Results: In the 62 SSADHD study participants, increased severity coincided with older age (R= -0.302, p = 0.03), as well as age-adjusted lower values of plasma GABA (R = 0.337, p = 0.02). GHB analyses in mice indicated that HOM ALDH5A1lox-STOP mice had 812.7±165.3 µM GHB blood content, compared to 8.2±0.8 µM in WT littermates, an ~100x increase in GHB blood level at this young age, suggesting that 50% SSADH is sufficient to regulate blood GHB content, but the total absence of SSADH leads to significant blood GHB accumulation. In our work using SSADHD-derived hiPSC, we developed an in vitro model of SSADHD from hiPSC, which differentiated into GABAergic neurons, constituting the basis of the SSADHD in vitro model and allowing for drug screening and molecular and biochemical dissection of pathological processes.

Conclusions: The convergence of findings from the SSADHD Natural History Study with iPSC and animal model work deepens our knowledge of the pathophysiology and longitudinal clinical course of SSADHD. This further enables the identification of biomarkers and changes throughout development that will be essential for upcoming targeted trials of enzyme replacement and gene therapy for this complex neurodevelopmental disorder.

35 **Henry Lee, MPhil, PhD**
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Phenotypic Rescue in a Mouse Model of Succinic Semialdehyde Dehydrogenase Deficiency (SSADHD) upon Gene Therapy

Succinic Semialdehyde Dehydrogenase Deficiency (SSADHD) is a rare genetic metabolic disorder caused by ALDH5A1 mutations. ALDH5A1 encodes SSADH, essential for the catabolism of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA). In SSADHD, pathologic accumulation of GABA and metabolite gamma-hydroxybutyrate (GHB) leads to broad spectrum encephalopathy. Paradoxically, despite heightened ambient GABA, patients with SSADHD are susceptible to seizures and sudden unexpected death in epilepsy (SUDEP), highlighting the significance of compensatory down-regulation of GABA receptors over pathologic GABA build-up. A major unmet medical need for SSADHD is treatment directly addressing the underlying genetic cause in the form of gene therapy. However, it was not known whether SSADHD phenotypes are reversible. To address this question, we constructed an inducible SSADH mouse model, Aldh5a1lox-STOP, allowing Cre-dependent 'on-demand' SSADH restoration. Proof-of-concept Aldh5a1 restoration via adeno-associated virus (AAV) increased

Aldh5a1lox-STOP mice survival and reversed SSADHD-relevant phenotypes. AAV-Cre-mediated brain-wide rescue led to over 80% survival at P100 (n=15), compared to 0% survival in non-rescued mice at that age (n=15). Surviving mutant mice expressed 68.6±3.7% WT SSADH protein level, and their body weight returned to that of WT control. As a step toward the clinical translation of SSADH-targeted gene replacement, we designed an AAV vector encompassing a human ALDH5A1 full-length native promoter (FLnP) driving a functional ALDH5A1 gene, namely AAV-FLnP-hALDH5A1. We found that AAV-FLnP-hALDH5A1 (packaged into a blood-brain-barrier penetrable capsid AAV-PHP.eB) treatment at P14 resulted in weight gain and 50% survival at P100 (n=20) compared to 0% in untreated mice (n=25). Notably, surviving mice expressed SSADH protein at 70-95% WT levels in the brain and ~50% WT levels in the heart, suggesting the importance of brain and heart SSADH restoration for successful gene therapy. Finally, AAV9-FLnP-ALDH5A1 treatment resulted in 45% survival (n=11), indicating a translatable path toward AAV9-mediated SSADH gene therapy.

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Patient Advocacy Group Partnership: CureSHANK

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37 **Amal Mathew, MS**
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Computationally Characterizing Communicative and Affective Movement Patterns in Individuals with Profound Neurodevelopmental Disorders Using Computer Vision.

This study quantifies body dynamics of individuals with global neurodevelopmental differences (NDDs) and minimal-to-no speech using computer vision techniques. Utilizing pre-collected data from in-home recordings and caregiver interviews, we identified a subset of communicative and affective states: "rejection," "request," "happiness," and "frustration." The YOLOv8 pose estimation model tracked 17 key body landmarks, and movements were quantified by calculating the Euclidean distance between key points in consecutive frames, with a noise threshold of 500 pixels.

Our analysis revealed distinct movement patterns for each state. "Rejection" involved substantial movements in the left wrist (mean movement=79.75 +/- 3.94 pixels, 0.67% of frames), left hip (62.09 +/- 8.74 pixels, 2.01%), and right wrist (6.02 +/- 2.52 pixels, 1.34%), emphasizing left-dominated turning and hand motions. "Requests" involved the left elbow (74.20 +/- 19.54 pixels, 2.45%), left wrist (57.25 +/- 24.28 pixels, 2.33%), and right wrist (18.14 +/- 21.09 pixels, 0.59%), suggesting broader arm-based movements. "Happiness" was dominated by quick expressive movements of the left elbow (81.94 +/- 28.65 pixels, 1.65%), left wrist (77.74 +/- 51.08 pixels, 1.82%), and left hip (59.50 +/- 19.37 pixels, 0.91%). "Frustration" was characterized by longer, more intense engagement of the left wrist (94.07 +/- 41.95 pixels, 3.36%), left elbow (89.73 +/- 32.29 pixels, 3.08%), and left ear (5.75 +/- 7.07 pixels, 0.51%), indicating head rotation.

These findings support the hypothesis that non-speaking individuals with NDDs use specific body movements to

convey information, identifiable through machine learning and caregiver insight. Incorporating facial expressions and vocalizations across more participants will further advance these findings. This work showcases the potential of computer vision to quantify communicative expressions, reducing manual labeling time and facilitating new augmentative communication technologies.

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Post-Movement Beta Rebound on EEG is Reduced in Pediatric Epilepsy Patients Compared to Age-Matched Healthy Controls

Rationale: Brain dynamics in patients with epilepsy may be distinct from those in healthy persons and distinguishable on electroencephalography (EEG) even when EEG is without visually-apparent anomalies. Neurophysiological studies often find motor system differences between epilepsy patients and healthy controls. Simple motor tasks robustly drive beta frequency (14-28Hz) EEG power and may provide biomarkers for neuropsychiatric conditions. Pharmacologic evidence suggests GABAergic inputs to an area are responsible for local beta activity, and modeling indicates inhibitory signaling drives beta dynamics during motor events. We test whether beta "rebound," an increase in power above baseline after suppression during a motor event, differs between healthy control participants and epilepsy patients.

Methods: Patients with epilepsy (n=35, ages 11-24) and healthy controls (n=12, ages 12-23) were exposed to 2-second tones and pushed a button after tone offset, where participants completed 45-716 trials (mean of 188 trials per participant). Parasagittal and central EEG channels were transformed into time-frequency space by complex g wavelet and averaged. Beta rebound was quantified as the 80th percentile power after button press.

Results: Epilepsy patients had significantly lower beta rebound power than healthy controls (means 0.06 vs. 0.45dB above baseline; U=65.0, p=4.4*10-4). We replicated others' finding of a correlation between beta rebound power and age among all participants (R=0.36, p=8.6*10-3). In the epilepsy cohort alone, however, there was no significant correlation with age (R=0.14, p=0.381).

Conclusions: Patients with epilepsy have lower beta rebound power than healthy controls. The lack of a correlation with age within the epilepsy cohort suggests an altered maturational trajectory of the beta rebound. Ongoing recruitment will power tests of whether beta rebound identifies epilepsy patients with different morbidities or responses to therapy.

39 Madeline Moore, BS
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Genetic and epigenetic factors that regulate KCC2 function and expression

Neurodevelopmental disorders are a pressing issue in the United States, impacting approximately 1 in 6 children, with many of these cases lacking a successful drug treatment. Disruptions in GABAergic inhibition, which causes imbalances in excitation and inhibition in neural circuits, contribute critically to the pathogenesis of neurodevelopmental disorders. The potassium chloride cotransporter 2 (KCC2), which extrudes chloride from the neuron to maintain proper intracellular chloride concentration, plays a pivotal role in regulating the polarity and efficacy of GABAergic inhibition. Deficiencies in KCC2 expression or function are associated with many neurodevelopmental disorders.

We assessed the impacts of 14 clinically identified pathogenic human KCC2 variants in mouse neurons with a knockout/replacement assay, revealing a common feature of reduced cytosolic abundance and reduced dendritic localization of mutant KCC2 protein in neurons. We further created knock-in human stem cell-derived models of several of the KCC2 variants and found neuronal function deficits in GABA reversal potential and excitatory synapse development which corroborates with the quantitative immunofluorescence results.

Our previous work discovered that small molecule inhibitors of the g-like tyrosine kinase 3 (Flt3) pathway, such as KW-2449, stimulates the expression of KCC2 in neurons, promotes synapse maturation, and reduces inflammation. Through bioinformatic analysis of KW-2449 transcriptomic changes, we nominated over 50 candidate transcription factors (TF) for a

curated CRISPR-based knockdown screening assay. Through quantitative immunostaining of KCC2 in neurons with different TF knockdown, our work reveals the transcription factors downstream of FLT3 signaling that regulate KCC2 expression in neurons.

Taken together, our work in understanding the pathogenicity of KCC2 variants at the molecular level and in unraveling the gene regulatory logic of KCC2 advances the understanding of this important transporter in the healthy and diseased brain and has wide implications in guiding the development of novel brain disease therapeutics.

40 **Jack, O'Keeffe Donohue, MB, BCH, BAO**
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Longitudinal Clinical and Neurodevelopmental Characteristics in CHD2-Related Disorders: A single-center retrospective study

Rationale: Pathogenic CHD2 variants cause developmental and epileptic encephalopathy, intellectual disability (ID), and autism spectrum disorder (ASD). Large cohort studies have characterized clinical features, but we lack an understanding of disease course over time, which is essential for clinical trial readiness. Here we looked at detailed phenotyping of patients with CHD2-related disorders at a single institution.

Methods: Patients with CHD2 variants from 2013 to 2023 were identified using internal databases. Data encompassed genetic reports, clinical notes, and EEG and MRI findings.

Results: Twenty-one patients with CHD2 variants were identified; 16 patients had either pathogenic (n=10) or likely pathogenic variants (n=7) and were included in the analysis. Mean age at genetic diagnosis was 8.2 years (±5.3 years) and at last follow-up was 12.3 years (±6.2 years).

Sixteen patients had seizures. Mean age at first seizure was 3.8 years (±2.96 years), with 13 with drug-resistant epilepsy and 10 with status epilepticus. Common seizure types included generalized tonic-clonic (13) and myoclonic (12), and common triggers were fatigue (7), light (7), and eye closure (3). All had abnormal EEGs, and 15 patients trialed three or more anti-seizure medications (ASMs). At last follow-up, 12 took two or more ASMs, most often VPA (10). Five trialed Ketogenic and two Modified Atkins Diets; none remained on dietary therapy at last follow-up. 11 patients had stably refractory epilepsy over time, with one worsening and four improving.

Eight patients were diagnosed with ID, eight with ASD, and three with both. Neuropsychology testing was available for nine patients, and six patients had multiple tests over time. There was no significant change in neuropsychology testing with age or time.

Conclusions: We find that epilepsy and neuropsychology phenotypes in patients with CHD2-related disorders are largely stable over time. This work highlights the need for larger prospective natural history studies.

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Phenotypic and Genotypic Expansion of KMT2C-Related Disorder from a Boston Children's Hospital Clinical Cohort

Background: KMT2C-related disorder is a rare genetic condition caused by loss of function of KMT2C. It is characterized by global developmental delay (GDD), intellectual disability (ID), hypotonia, dysmorphic facial features, poor growth, skeletal differences, and vision impairment. Neurological features may include seizures, attention-deficit/hyperactivity disorder (ADHD), autism spectrum disorder (ASD), and psychiatric conditions. The disorder is genetically and clinically distinct from EHMT1-related Kleefstra syndrome (KS).

Objective: Due to the rarity of this condition, we seek to contribute phenotypic trends to the expanding understanding of KMT2C. We also aim to further differentiate this condition from EHMT1-related KS.

Methods: We performed a retrospective analysis of 10 individuals with KMT2C-related disorder, identified by pathogenic or likely pathogenic variants in KMT2C. Individuals were clinically evaluated at the Boston Children's Hospital (BCH) KS Clinic. We analyzed demographic and clinical data obtained by clinicians in the BCH KS Clinic.

Results: All 10 individuals (5 female, 5 male) were evaluated prior to the age of 20 and harbored intragenic KMT2C variants. Variant types included missense (2/10, 20%), frameshift (1/10, 10%), nonsense (5/10, 50%), and splice site (2/10, 20%).

The most prevalent phenotypes included dysmorphic facial features (6/10, 60%), language delay or communication disorder (6/10, 60%), ASD (4/10, 40%), ADHD (4/10, 40%), anxiety (4/10, 40%), short stature and/or growth hormone deficiency (5/10, 50%). Additional phenotypes included visual impairments (6/10, 60%), seizures (1/10, 10%), and scoliosis (1/10, 10%). Novel phenotypes included ataxia (1/10, 10%) and early childhood-onset profound sensorineural hearing loss (1/10, 10%). Only one individual was diagnosed with ID, but not all individuals within the cohort had formal IQ testing.

Conclusion: Our analysis provides insights into the phenotypic and genotypic spectrum of the KMT2C-related disorder patient population, which is distinct from EHMT1-related KS. Our results support existing literature and highlight novel phenotypes seen within our patient cohort.

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Patient Advocacy Group Partnership: Koolen-de Vries Syndrome Foundation

For more information about the work of the Koolen-de Vries Syndrome Foundation and its partners, please visit https://kdvsfoundation.org/.

43 **Marija Pranjić, PhD**
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Movement-related cortical potentials underlying motor preparation and execution in children with attention-deficit/hyperactivity disorder

Background: Approximately 50% of children with attention-deficit/hyperactivity disorder (ADHD) also display difficulties with motor control that are consistent with developmental coordination disorder (DCD). Although coexisting ADHD+DCD symptomatology is associated with greater functional impairment than either disorder alone, it is not well understood whether ADHD and DCD have shared or separate etiologies. In particular, it remains unknown whether motor difficulties in children with ADHD occur during movement preparation, execution, or both.

Methods: Sixty-six right-handed children with ADHD and 30 typically developing (TD) children (ages 7–11 years) completed event-related potential (ERP) recordings and neuropsychological testing, including a visual-motor integration (Beery VMI) test. Caregivers reported on ADHD symptoms. Movement-related cortical potentials (MRCPs) were extracted during two ERP tasks. We tested (1) whether children with ADHD have atypical MRCPs during movement preparation and/or execution compared to TDs, and (2) whether MRCPs related to VMI performance and ADHD diagnosis are separate or shared.

Results: In both ERP tasks, children with ADHD were significantly less accurate and displayed attenuated correct trial MRCP amplitudes at a fronto-midline electrode during movement preparation but not execution. Greater ADHD symptom severity was associated with reduced VMI scores, over and above age and IQ. ADHD diagnosis and reduced error trial MRCP amplitudes each explained unique variance in VMI performance. In contrast, attenuated correct trial MRCP amplitudes were associated with ADHD diagnosis.

Conclusions: Compared to TDs, children with ADHD display atypical MRCPs during movement preparation but not execution. Additionally, distinct cortical processes are linked with VMI performance and ADHD diagnosis, providing support for the separate etiology hypothesis. Comorbidity between ADHD and DCD is likely due to a combination of behavioral and neurobiological vulnerabilities.

44 **Danilo Predes PhD**
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Unraveling the Molecular Basis of ZNF711-Associated Intellectual Disability

Intellectual Disability (ID) has its roots in neurodevelopmental defects and affects 1-3% of the general population. Recent findings have highlighted key genes that modulate Wnt signaling during neurodevelopment to affect cognition. More specifically, loss-of-function (LOF) mutations of KDM5C, a histone demethylase, causes ID in humans through hyperactivation of Wnt/β-catenin signaling during neurodevelopment, hindering neuronal differentiation and leading to abnormal behavior. Of note, modulation of Wnt signaling during neurodevelopment is sufficient to induce abnormal behavior in mice or to correct KDM5C LOF-driven defect, highlighting the critical role of Wnt signaling in this process.

Seeking to identify novel Wnt signaling regulators crucially linked to neurodevelopment, we performed an unbiased gene reporter-based overexpression screening, which identified ZNF711. This X-chromosome gene encodes a nuclear protein of unknown function that harbors 11 C2H2 zinc fingers (ZFs). Notably, ZNF711 mutations, including frameshifts, are linked to non-syndromic X-linked ID (XLID) and autism, however, the function of ZNF711 in neurodevelopment is still unknown. Here, we define the mechanism of action of ZNF711 that cooperates with the transcription factor TCF4 (encoded by TCF7L2) to activate Wnt signaling. We hypothesized that ZNF711 LOF mutations causes neurodevelopmental defects and ID by suppressing the Wnt signaling pathway. To tackle this, we generated for the first time the ZNF711 knockout (KO) mouse. Interestingly, the KO mice display reduced exploratory behavior during the Open Field test, suggesting anxiety. Next, we aim to explore the KO mouse phenotype further and generate knockout lines of human-induced Pluripotent Stem (iPS) cells to evaluate whether ZNF711 LOF can lead to abnormal neuronal differentiation. Finally, we will determine the therapeutic value of Wnt signaling modulation. In sum, we aim to define the etiology of the ZNF711-linked XLID and propose a therapeutical intervention targeting Wnt signaling.

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Patient Advocacy Group Partnership: IDefine

For more information about the work of IDefine and its partners, please visit https://www.idefine.org/.

46 **Peter Riley**
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Retinal examination outcomes in SMA patients taking risdiplam (Evrysdi)

Evrysdi (risdiplam) is a SMN2 splicing modifier taken orally for treatment of Spinal Muscular Atrophy (SMA), a rare genetic progressive neuromuscular disorder that causes the death of motor neurons and muscle weakness. Preclinical studies in animal models showed retinal toxicity associated with very high-dose risdiplam, but no retinal toxicity has been noted in all risdiplam clinical trials conducted in humans thus far.

We performed a chart review, and identified 48 patients who have taken Evrysdi through the SMA program at Boston Children's Hospital. Duration of Evrysdi treatment ranged from 2 to 50 months. Retinal assessments for patients on Evrysdi included ophthalmoscopy, OCT of maculas, and full field ERG. No clinically significant abnormalities have been detected to date.

As the retinal toxicity risk of long-term Evrysdi treatment remains unknown, we advocate for periodic exams to monitor retinal structure and function. OCT and ERG are optimum for retinal monitoring, but at a minimum dilated eye exams with visualization of the retina are recommended.

47 **Tsambika Rizas**
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Northeastern University

Operational Categorization of Communicative Expressions from Non-Speaking Individuals with Profound Neurodevelopmental Disorders using In-Depth Caregiver Interviews

Communication from non- and minimally-speaking individuals with profound neurodevelopmental disorders (NDDs) is poorly understood. These individuals use a range of means to express themselves, including nonverbal vocalizations, canonical and idiosyncratic gestures, body movements, facial expressions, and augmentative and alternative communication (AAC) devices. The aim of this research was to develop a comprehensive framework of non-verbal communication that thoroughly described the range of communicative functions used by these individuals while minimizing the number of categories.

This exploratory work analyzed 6 recorded caregiver-child interactions from a novel ~15-minute remotely-administered natural communication sampling paradigm (ROSCO; ~96 minutes of audio/video). Each caregiver rewatched the recorded ROSCO session with an examiner and was prompted to describe the meaning and social directedness of each of their child's communicative acts using their own words. These caregiver descriptions (~353 minutes of interview data) were then transcribed using automated speech recognition software (WhisperAI) and the description of each communicative act was manually isolated and analyzed.

Initially, 34 different communicative functions were identified. Frequency analysis quantified the usage of each type of communication across sessions, and the framework was updated to resolve similarities and ambiguities of overlapping states by caregivers. For example, "frustration" and "annoyance," were merged, while "disengaged" was separated from "distracted" as these signified distinct states to most caregivers.

Ultimately, 6 primary functional communication categories were determined – Requesting, Protesting/Rejecting, Commenting, Responding, Expressing Emotions, and Self-Directed Behaviors (e.g., stimming) – with additional subcategories highlighting common specific communicative intents, such as requesting help or expressing frustration, for a total of 24 subcategories. This framework is currently being evaluated for construct and content validity with new ROSCO data. It provides a structured approach for analysis of communication from non-speaking individuals with NDDs, acknowledging the nuance of non-spoken communication actions and behaviors and enabling more systematic monitoring of these individuals' communicative abilities over time.

48 **Wardiya, Afshar-Saber, PhD**
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Modeling neurodevelopmental disorders using stem cell-derived neurons: our progress on SSADH Deficiency

Succinic semialdehyde dehydrogenase (SSADH) deficiency is an autosomal-recessive neurometabolic disorder caused by bi-allelic mutations in the ALDH5A1 gene. It is the most prevalent inherited disorder of GABA metabolism and is characterized by accumulation of two neuromodulators, gamma-aminobutyric acid (GABA) and gamma-hydroxybutyric acid (GHB), in the CNS. In this study, we used fourteen iPSC lines: three patient lines and sex matched parental controls and CRISPR corrected lines each transduced with hNGN2 and hDLX2-hASCL1 to generate excitatory neurons and GABAergic neurons. We show that hiPSCs can differentiate into excitatory neurons and GABAergic neurons regardless of the allelic dosage of ALDH5A1. We found that hiPSC-derived excitatory neurons display altered neurite outgrowth and synaptic development which leads to hyperactivity of the developing excitatory neuronal network. Moreover, we showed that the CRISPR correction ALDH5A1corr/corr shows similar network activity to the parental control ALDH5A1+/- suggesting that hiPSC-derived excitatory neurons network's hyperactivation is linked to the ALDH5A1 mutation. Additionally, we identified neuron subtype-specific metabolic and gene expression changes linked to SSADH deficiency and we showed that similarly to clinical presentation, SSADHD

results in increased GABA and GHB levels in hiPSC-derived GABAergic neurons. Furthermore, we developed an imaging platform based on calcium imaging and optogenetics to manipulate the network of neurons formed by hiPSC-derived GABAergic and excitatory neurons in vitro in a high-throughput fashion. Finally, we demonstrated we rescued these phenotypes using ALDH5A1 mRNA demonstrating the potential of the mRNA-based therapeutics in SSADH deficiency.

49 **Katharina Sarnow, PhD**
Neurobiology
Boston Children's Hospital

Investigating neuroimmune/tumor interactions with human brain organoids

Diffuse intrinsic pontine glioma (DIPG) is a highly aggressive pediatric brain tumor with a poor prognosis (<12 months survival). There is no effective treatment for DIPG. Due to its invasive growth pattern within the pontine area, complete surgical resection of DIPG is difficult. Up to a quarter of the cells in a DIPG tumor originate from the immune lineage. To understand the interactions between tumor cells and microglia - the brain's primary immune cells - in a human 3D context, we developed a neuroimmune-competent brain organoid/DIPG fusion model system. The microglia-containing brain organoids (MiCBOs) were generated through self-assembly and co-development of human embryonic stem cell-derived neuronal progenitor and GFP-labeled myeloid precursor cells. Extensive testing of various growth conditions established two optimal settings that resulted in substantial amounts of GFP+ microglia in organoids over two months. We applied confocal and two-photon live microscopy to assess the distribution and motility of the microglia in the MiCBOs and found viable, highly motile, and widely distributed microglia that show a high resemblance to those found in the human brain. Tumor cell invasion and microglia migration were evaluated by confocal live cell imaging and immunofluorescence stainings. Different primary tumor cell lines show distinguishable invasion patterns and grades of diffusion into the brain organoids. We further utilized single-cell RNA-Sequencing to reveal distinct clustering of microglia cells because of tumor confrontation and the emergence of a new molecular subgroup of tumor cells due to microglia exposure. This suggests signaling interactions between these two cell types in the organoids.

Our robust, reproducible, and scalable MiCBO model contains viable and motile microglia. It can be easily expanded for screening candidate therapeutics that stimulate the immune response to suppress tumor progression. The MiCBO/DIPG fusion organoid provides a tractable and modular system for studying human tumor-brain interactions in a 3D setting.

50 **Luca Schierbaum, MD**
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Title: Additional Diagnostic Yield of Short-Read Whole Genome Sequencing in Childhood-Onset Movement Disorders: A One-Year Follow-Up Study

Objective: Hereditary movement disorders comprise a broad clinical spectrum, with over 500 disease-causing genes. Standard clinical genetic testing has limitations. Here, we assess the additional diagnostic yield of short-read whole genome sequencing (srWGS) in children with early-onset movement disorders.

Methods: This study included 42 children with movement disorders of unclear etiology evaluated at an academic tertiary care movement disorders program, between March 2023-2024. SrWGS was conducted using the Illumina NovaSeq platform. Data was processed through DRAGEN. Variants were prioritized according to frequency, deleteriousness, associated phenotypes and reviewed by an interdisciplinary team.

Results: SrWGS data from 42 patients (and parents were available) was analyzed. Spasticity was the predominant phenotype in 71.4% (30/42) followed by ataxia (26/42, 61.9%) and dystonia (23/42, 54.8%). A genetic diagnosis could be established in 35.7% (14/42) and candidate genes were identified in 38.1% (16/42). 85.7% (36/42) had previously undergone clinical genetic testing, with five solved cases showing variants already identified upon prior testing (33.33%). Among solved cases, analysis on the genomic level revealed a diagnosis in 7.14% (3/42) through identification of a repeat expansion in HTT, a duplication

in MECP2 and an ALU-insertion in ATM. In 4.8% (2/42) candidate copy number variations were identified at the genome level. SrWGS provided additional diagnostic yield in 11.3% (5/42).

Conclusions: The additional diagnostic yield of srWGS in a cohort of childhood-onset movement disorders is limited with most cases solved at the exome level. To leverage the additional potential of srWGS further development of analysis pipelines is warranted.

51 **Siddhant Bikram Shah, BTech**
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Acoustic analysis of nonverbal vocalizations from non-speaking individuals with complex neurodevelopmental disorders

Quantitative analysis of nonverbal vocalizations from non-speaking individuals could elucidate potential objective outcome measures for clinical trials and interventions, as well as facilitate novel augmentative communication technology. This study assessed acoustic similarities between 6551 affective and communicative vocalizations (frustration, delight, request, self-talk, etc.) from 8 non-speaking individuals with profound autism and neurodevelopmental disorders (NDDs) using the open-access ReCANVo dataset. We augmented the audio with noise, pitch shifts, and tempo changes, and extracted features using pre-trained Wav2Vec2 model representations, mel-spectrograms, and low-level descriptors to train a classification network (dim 24; 85/15 train/val). We calculated a mean vector (dim 24) for each class and computed their cosine similarities. Across 8 individuals and 7 vocalization classes, 'Request' vocalizations were most globally similar (AvgCS=0.944), suggesting that these sounds may encompass multiple other communicative states, while 'Delighted' sounds were the most distinct (AvgCS=0.878). Grouping all the positive and negative affective classes into two classes resulted in a highly separable latent structure (Acc=0.845, MacroF1=0.825), supporting the hypothesis that affective vocalizations are discernable via acoustical properties alone. However, the multi-class model (Acc=0.589, MacroF1=0.542) was sensitive to speaker gender, age, and class imbalances even after augmentation. Further examining the participant with the most samples (P01; n=1687; Acc=0.676; MacroF1=0.695), 'Request' was most similar to 'Social' (CS=0.978), suggesting shared underlying acoustics between these communal sounds. 'Delighted' and 'Frustrated' were the least similar (CS=0.715), again supporting affective differentiation via acoustical features alone. Increased sample sizes and better real-world labeling techniques are needed to improve model performance. However, these preliminary results help advance our understanding of communication in non-speaking individuals with profound NDDs, generate more sensitive clinical measures, and spur the development of more holistic interactive augmentative communication technologies.

52 **Sara Kornfeld Simpson, PhD**
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Altered physiology of primary visual cortex in Fragile X Syndrome revealed by field potential recordings

One prominent feature of electroencephalogram (EEG) recordings measured in typically-developing adults is a narrow-band oscillatory peak that is strongest over occipital cortex and centered around 10.5 Hz. In Fragile X Syndrome (FXS), this "alpha rhythm" is altered, but the lack of an apparent correlate of this phenotype in the Fmr1 KO mouse has limited study of this potential biomarker. Here, we identify a comparable phenotype in the mouse model by using field potential recordings to generate a reverse-translational pipeline from human to mouse and compare altered physiology of occipital cortex across species. Consistent with previous reports, we find that the center frequency of this narrow-band peak is shifted down to around 7.5 Hz in EEG recordings of adults with FXS. A comparable narrow-band peak centered around 5.5 Hz is identifiable in resting-state data of wild-type mice measured with an electrode over the cortical surface of primary visual cortex (V1), and this peak is shifted down to around 4.5 Hz in Fmr1 KO mice. To further characterize these oscillations in mice, we recorded the local field potential in layer 4 of V1 in awake, head-fixed mice sitting in either total darkness or in front of a static, illuminated gray screen. The addition of luminance induced robust, state-dependent narrow-band oscillations centered

around 5 Hz in wild-type mice that were substantially reduced in power and had weaker, phase-shifted coupling to 15-30 Hz oscillations in both Fmr1 KO mice and in mice where Fmr1 was conditionally knocked out of excitatory neurons and astroglia in cortex. We observe a similar weakening of periodic alpha activity in occipital cortex EEG of children, but not adults, with FXS. Thus, comparable alterations to "alpha rhythms" in FXS across development can be identified through either surface or depth field potential recordings in V1 of the Fmr1 KO mouse.

53 **Aditi Singh, PhD**
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Fmr1-/y and Syngap+/- mouse models exhibit divergent translation environments and deficits of synaptic plasticity

Dysregulated protein synthesis leading to altered synaptic plasticity is central to the pathogenic mechanism in the Syngap+/- mouse model of SYNGAP1 haploinsufficiency and the Fmr1-/y mouse model of Fragile X Syndrome (FXS).

Here, we use Translating Ribosome Affinity Purification and RNA-seq (TRAP-seq) to test the hypothesis that a shared population of translating mRNAs is dysregulated in hippocampal CA1 pyramidal neurons of Syngap+/- and Fmr1-/y mouse models.

Our results identify marginal overlap in translation profiles of CA1 pyramidal neurons between Syngap+/- and Fmr1-/y. A number of shared functional pathways are alternatively expressed in both models, however the dysregulation appears to be in opposite directions. Moreover, when we characterized these two mutant models on synaptic plasticity spectrum, we find that CA1-TRAP from Syngap+/- hippocampus shows upregulation in longer-length mRNAs that are similar to those seen in long-term synaptic potentiation (LTP) while opposite to those in the Fmr1-/y CA1-TRAP and mGluR1/5-induced long-term synaptic depression (mGluR-LTD).

Together, these results highlight that Syngap+/- and Fmr1-/y exhibit distinct translational profiles leading to discrete deficits of synaptic plasticity despite shared cognitive impairments. These findings emphasize the importance of understanding the differential impact of genetic mutations on synaptic functions including molecular mechanisms underlying altered synaptic plasticity.

54 **Anna Stewart, BS**
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Characterization of Communication Profiles of Toddlers with Down Syndrome and Autism

Background: Children with autism spectrum disorder (ASD) and Down syndrome (DS) exhibit diverse developmental trajectories, especially in expressive language abilities, which range from fluent speech to lifelong non-verbal communication. Characterizing similarities and differences in language development in these two distinct, but overlapping, neurodevelopmental disorders can inform our understanding of language impairment and improve intervention. Current standardized assessments often fail to adequately capture the skills and developmental gains of children with limited expressive communication, hindering our ability to monitor language development in children with neurodevelopmental disorders (NDDs). Natural Language Sampling (NLS) provides a naturalistic measure of expressive language, capturing elements often excluded from standardized assessments. Combine standardized and NLS measures could provide a consistent methodology for characterizing language development in NDDs.

Objective: Leveraging standardized language assessments (Preschool Language Scale) and NLS we aimed to characterize similarities and differences in communication profiles of toddlers with DS (n = 13) and ASD with language impairments (ASD-LI) (N = 18).

Methods: NLS were collected using the Eliciting Language Samples for Analysis (ELSA-T) - a play-based activity created to spontaneously elicit expressive communication in children with NDDs. Samples were transcribed using Systematic Analysis of Language Transcripts (SALT) notation.

Results: Preliminary results indicate that DS preschoolers score higher in expressive (p = 0.001) and receptive (p = 0.002) language measures, and exhibit greater intelligibility (p = 0.042) and longer utterances (p = 0.019) compared to ASD-LI children. NLS measures of Mean Length of Utterance in Words (MLU) and Percent Intelligibility were highly correlated with standard expressive and receptive language measures (Pearson R range 0.73-0.84). Both groups had limited intelligibility (DS = 23%, ASD=10%).

Summary: Both standard language measures and NLS measures showed significant differences between toddlers with DS and ASD-LI. Future directions will characterize unintelligible vocalizations and determine how characteristics of unintelligible vocalizations relate to language gains.

55 **Abigail Sveden, MS, CGC**
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Disparities in VUS Among Ethnoracial Groups in the National Brain Gene Registry

Barriers to genomic testing for historically marginalized ethnoracial populations are well-documented. Such groups also face higher variants of uncertain significance (VUS) burden compared to non-Hispanic/Latine White (White) patients. Here, we describe revisions to ethnoracial descriptors for an intellectual and developmental disability (IDD) registry, the Brain Gene Registry (BGR), and assess disparities relating to VUS among ethnoracial groups.

The BGR pairs genotypic and phenotypic data from individuals with IDD gene variants. Following a review of recent guidelines and group discussion, BGR ethnoracial descriptors were revised for inclusivity, and participant identities were remapped to these descriptors. Alongside ethnoracial descriptors, we analyzed the proportion of variants classified as VUS and, for VUS, test type and inheritance.

BGR ethnoracial descriptors were revised to use inclusive language, add Middle Eastern/North African, and add subcategories for "Asian." Revisions also allowed participants to select multiple ethnoracial descriptors.

With these revised descriptors, BGR participants were described as 6% Asian, 5% Black/African American/African, 10% Hispanic/Latine, 1% Middle Eastern/North African, 8% Multiracial/multiethnic, 70% White, and 5% Unknown. Overall, VUS accounted for 225/585 (38%) variants. There was a higher proportion of VUS among non-White groups (45%, 74/164) than White counterparts (32%, 125/389, p = 0.004). The Black/African American/African group had the highest proportion of VUS (16/30, 53%). Within the VUS cohort, 20% (15/74) of VUS in non-White groups were identified through trio testing versus 37% (46/125) in the White group (p = 0.011). Only one non-White individual harbored a de novo VUS, compared to 17% (21/125) of White individuals (p < 0.001).

Ethnoracial descriptors in the BGR were revised to align with recent recommendations. The higher proportion of VUS in non-White groups is likely multifactorial, with barriers to optimal test selection and parental testing as significant contributors. Moving forward, additional efforts for inclusivity and resolving VUS for non-White groups are critical.

56 **Amy Tam, BS**
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De novo versus inherited SPAST-related hereditary spastic paraplegia (SPG4) presents distinct outcomes

Objective: SPAST-associated hereditary spastic paraplegia (HSP), also known as SPG4, is the most common subtype of HSP. Children with SPG4 have been observed to have greater occurrence of de novo variants. Patients with de novo SPG4 are more severely affected than patients with inherited SPG4, even when comparing amongst individuals sharing the same SPAST variant. Here, we systematically document disease severity and health-related quality of life (HrQoL) for children and young adults with SPG4.

Methods: We evaluated HrQoL using the Caregiver Priorities and Child Health Index of Life with Disabilities (CPCHILD) and assessed clinical impairment using the Spastic Paraplegia Rating Scale (SPRS), SPATAX-EUROSPA Disability Stage scale

(SPATAX), and modified Ashworth scale (MAS). Our cohort included 49 patients with de novo SPG4, 28 patients with inherited SPG4, and 12 patients with untested parents, recruited from the Registry and Natural History Study for Early Onset Hereditary Spastic Paraplegia (NCT04712812).

Results: Patients with de novo SPG4 typically reported impaired HrQoL in all domains, and on average, reported greater impairment compared to patients with inherited SPG4 or untested parents. Mean CPCHILD scores for these groups were: de novo SPG4: 64±11 (SD, n=32); inherited SPG4: 88±9 (SD, n=11); SPG4 of unknown inheritance: 82±22 (SD, n=8). CPCHILD scores correlated inversely with SPRS scores (R^2 = 0.67, p_adj = 8.7e-5), indicating that greater motor impairment correlated with lower HrQoL. Additionally, our survey highlighted areas important to caregivers, such as emotions, overall health, communication, and moving about indoors.

Interpretations: These results outline areas of unmet need for children and young adults with SPG4. The insights into clinical impairment and HrQoL can inform future interventional trials.

57 **Sneham Tiwari, PhD**
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Early death in DEPDC5 loss of function mosaic Zebrafish models

The DEPDC5 (DEP domain-containing protein 5) gene, encoding a repressor of the mTORC1 signaling pathway, has an important role in regulation of mTORC1. Germline, germline mosaic, or brain somatic variants in DEPDC5 are associated with Focal cortical dysplasia (FCD) and other focal brain malformations associated with focal epilepsy. Disease-associated loss-of-function variants in DEPDC5 lead to mTORC1 activation in dysmorphic neurons. To study the effect of Depdc5 loss of function, we have generated a mosaic Depdc5 loss-of-function zebrafish model. We designed a construct based on a GAL4-VP16/UAS plasmid and inserted it in the competent cells. DNA extracted from positive colonies after transfection was used in the microinjection. Wildtype male and female Casper fish were crossed, and eggs were harvested post-fertilization and prepared for microinjections. DEPDC5 construct along with CRISPR/Cas9mRNA, depdc5 gRNA, and universal sgRNA were injected into one-cell stage embryos (2nl/per embryo). At 24hpf, embryos, with red fluorescence were indicated as successful insertion of the Depdc5 plasmid and cutting. Positive larvae were observed for survival until 14dpf. We observed that the introduction of the Depdc5 construct resulted in the early death of larvae starting by 1dpf. We studied the mosaic mutant larvae, for their morphological and behavioral patterns and observed significant difference in their morphology and observed abnormal behavioral patterns and neurological hyper-excitability in the mutants when compared to the wild-type controls. Our developed DEPDC5 loss of function mosaic models are crucial as they recapitulate the premature death related to DEPDC5-related epilepsy.

58 **Shreya Tripathy, BA**
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Perinatal Strokes Associated with Autism Spectrum Disorder Impact Distinct Brain Networks Compared to Non-Autistic Peers

Recent research from our institution suggested that children with venous, rather than arterial, perinatal stroke are three times likelier to develop autism spectrum disorder (ASD). Here, we analyze the same patient cohort to identify whether this finding is explained by differences in affected brain networks.

Data from 40 patients with perinatal stroke were identified from a retrospective registry; 20 of whom had an ASD diagnosis. Groups were matched on sex, stroke subtype, and lesion size. Lesions were manually segmented and registered to a standard template. We performed a voxel-wise lesion-symptom mapping (VLSM) analysis to identify relationships between lesion locations and ASD diagnosis. Next, we performed a lesion network mapping (LNM) analysis, generating functional connectivity maps for each lesion using resting state data from 1000 healthy nine-year-olds. We then statistically compared

lesion connectivity patterns between groups, controlling for stroke subtype and lesion size. Lastly, we conducted a logistic regression to assess the ability of connectivity to identified ROIs to predict ASD diagnosis.

VLSM analysis identified a significant association between ASD diagnosis and lesions in the left temporoparietal junction (ITPJ), implicated in theory of mind, when controlling for stroke subtype (FDR, $p<0.05$). LNM analysis identified distinct functional connectivity in ASD in this same region, extending through the middle temporal gyrus to the anterior temporal lobe, in the cerebellum, and in the ventromedial prefrontal and posterior cingulate cortex, regions associated with the default mode network and social cognition (FDR, $p<0.05$). Our logistic regression model explained 28.3% of the variance in diagnosis, with ASD-associated lesions displaying stronger connectivity to the ITPJ ($p=0.007$). When controlling for ASD, LNM analysis by stroke subtype found a significant difference in connectivity to the left superior temporal lobe, part of the middle cerebral artery territory (FDR, $p<0.05$).

59 **Lukas Vogelsang, PhD**
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On the potential clinical significance of initially degraded sensory experience during early development

Human perceptual development unfolds in a stereotypical temporal manner. Sensory inputs are initially strongly degraded and improve in quality over the first months or years of life. These initially degraded inputs have traditionally been considered to represent 'limitations' imposed by constraints of the developing neural system. We propose, however, that they may, in fact, be adaptive and facilitate, rather than hinder, the acquisition of important representations and processing strategies that subserve robust perception later in life.

This proposal is supported by joint experimental and computational findings that have recently been presented – notably in the domains of visual acuity (Vogelsang et al., PNAS, 2018) and color vision (Vogelsang et al., Science, 2024). Experimental evidence primarily derives from reports of children born blind and treated for their blindness later in life. In stark contrast to neonates, these children commence their visual experience with a remarkably mature visual system and, therefore, effectively skip the initial period of degraded vision. Examinations of their perceptual profiles have revealed significant recognition deficits in generalization to color-reduced or color-shifted images, as well as poor performance on tasks relying on extended spatial integration. Results of computational simulations strongly suggest that these deficits can be accounted for by the children's lack of initially degraded inputs.

These results have important implications for understanding normal perceptual development and help account for some of the deficits reported in individuals who underwent atypical developmental trajectories. This work also has potential implications for designing clinical interventions for atypically-developed individuals. Specifically, late-sighted children could potentially benefit from an initial period of degraded visual experience immediately post-surgically. Similarly, computational results on the significance of low-pass-filtered sounds as part of prenatal hearing (Vogelsang et al., Developmental Science, 2023) have potential implications for optimizing the auditory experience of prematurely-born babies in neonatal ICUs.

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CAGS: From Diagnosis to Leading a Foundation

For more information about the work of cureCAGS and its partners, please visit www.cureCAGS.org.

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A high-throughput in vitro platform for 2D & 3D image-based comparative phenotypic profiling of hiPSC-derived neural cells from diverse NDD patients

Human induced pluripotent stem cells (hiPSCs) have provided an alternative model system to study diverse mechanisms in normal human development and patient-specific disease processes. Unlike traditional animal model organisms, these in vitro cell culture systems allow for a direct and scalable approach for the generation of specific cell types to interrogate the key cellular, molecular, and biochemical signatures underlying species-specific regulation of cell identity acquisition and pathological manifestations of disease states. Human pluripotent stem cell-derived neural progenitors, cortical neurons, and cerebral organoids have been widely utilized for investigating typical brain development, and they have also been used for modeling neurodevelopmental and neurodegenerative disorders. While many directed differentiation protocols have been developed to control neural cell fate specification with high precision and reproducibility, a great number of these established protocols involve a population-based cell culture format with low throughput potential. We have established a streamlined approach for the high-throughput in vitro generation of hiPSC-based neural derivatives in both 2D & 3D cell culture settings, and we have applied this platform to cell-based phenotyping assays using fluorescent dyes for cell imaging characterization. This has allowed us to generate multiple 2D & 3D neural derivatives from a broad spectrum of patient hiPSC lines representing diverse genetic neurodevelopmental disorders (NDDs). This approach also allows for direct and effective comparative phenotypic profiling across many different NDD patient hiPSCs lines in parallel, which is further amenable to therapeutic applications like small molecule compound screening studies for target discovery.

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The KCNQ2 variant human iPSC-derived glutamatergic neurons display hyperactive bursting phenotypes due to an imbalance in their synaptic marker expressions associated with the development of neonatal seizures and autism

KCNQ2 variants are associated with neonatal seizures and epileptic encephalopathy, which can lead to developmental and cognitive disabilities, and autism. KCNQ2 codes for a voltage-gated potassium channel and regulates neuronal excitability and action potential properties. Previous studies have found that mutations in different regions of the KCNQ2 protein led to distinct molecular mechanisms, resulting in varied electrophysiological profiles. Currently, there are no comprehensive studies investigating the disease phenotypes using human induced pluripotent stem cell (iPSC)-derived neurons with different KCNQ2 variants.

To characterize disease phenotypes in different KCNQ2 deficient neurons we used CRISPR-Cas9 to correct the KCNQ2 variants in the patient-derived iPSCs and generated glutamatergic neurons from the iPSCs. We then compared the patient-derived cells with their isogenic control lines through morphological measurements, functional neural network analysis, synaptic marker expression, and transcriptional profiling.

In two patient cell lines (p.T274M and p.G256W), we observed longer neurite outgrowth during early network formation at day 7 and day 9. In the p.G256W line we found longer neurite outgrowth at day 13 and day 30 post-differentiation. Functional analysis revealed increased burst duration across all patient cell lines. In two patient cell lines (p. L292_L293delinsPF and p.T274M), we detected an increased number of spikes per burst, number of network bursts, and network bursting frequency. This functional finding can be explained by the increase in SYN1 and PSD95 synaptic puncta in these two patient cell lines compared to their isogenic control lines. Transcriptional profiling revealed overlapping genes associated with synaptic transmission, cell adhesion, and GTPase signal transduction, as well as unique differentially expressed genes among each KCNQ2 variant line.

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Machine learning-based approach for quantitative imaging in iPSC-derived neuronal models of Neurodevelopmental Disorders

Neurodevelopmental disorders (NDDs) are a group of disorders that affect a child's brain development and cause neurological dysfunction that can lead to lifelong disability or even early mortality. Human-induced pluripotent stem cells (hiPSCs) have become a popular model for studying NDD disease mechanisms in vitro. Calcium imaging is a functional assay that characterizes neuronal activity and network connectivity at a single-cell level and in a non-invasive manner for chronic live-cell imaging. We developed a high-throughput platform that combines calcium imaging and machine learning to investigate the neuronal activity of iPSC-derived 2-dimensional (2D) culture. To overcome variability in the expression of the calcium indicator, we generated an iPSC line stably expressing GCaMP6s and differentiated the iPSCs into excitatory neurons using the NGN2 differentiation method. The automated image acquisition was accomplished through the Perfect Focus System in the Nikon Eclipse Ti-2 microscope and a program that automatically selects multiple fields of view. The cell bodies in the recordings were segmented using a machine learning-based approach, where we trained and compared the performance of two neural network models. A Python-based program then quantified the neuronal activities of each labeled cell in different parameters (e.g., calcium transient amplitude, event frequency, and global connectivity) based on the calcium traces converted from the ΔF/F signal. Future work includes connecting the automated acquisition and analysis systems smoothly to establish a high-throughput, objective, and rapid imaging-analysis pipeline. We hope to use this platform to investigate the disease mechanisms of NDDs and perform drug screening for potential therapeutic candidates.

64 **Randee Young, PhD**
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Pathogenic PPP2R5D variants cause neurite outgrowth defects that are rescued by targeted allele-specific knockdown in patient-derived neurons

A significant barrier to treating neurodevelopmental disorders is a limited understanding of disease mechanisms. Heterozygous missense mutations in PPP2R5D cause the rare PPP2R5D-related neurodevelopmental disorder, also known as Jordan's Syndrome, which is associated with megalencephaly, developmental delay, intellectual disability, seizures, autism spectrum disorder, and early onset Parkinson's disease. This study aimed to determine the impact of the pathogenic PPP2R5D variants E198K and E420K in developing neurons and provide proof of principle that targeted reduction of the E198K variant is a potential therapeutic strategy for the treatment of Jordan's Syndrome. To this end, we differentiated induced pluripotent stem cells from patients with the E198K and E420K PPP2R5D variants along with CRISPR-corrected isogenic controls into neural progenitors and cortical neurons. To address whether allele-specific knockdown of PPP2R5D pathogenic transcripts can rescue neuronal dysfunction in patient-derived neurons, we developed antisense oligonucleotides (ASOs) which selectively and preferentially knockdown the pathogenic allele for E198K, the most common missense variant. Neural progenitors harboring PPP2R5D pathogenic variants are over-proliferative compared to controls, and neurite outgrowth is significantly increased in patient-derived neurons. The ASOs that most efficiently knockdown the E198K allele reversed the cell autonomous neurite outgrowth defects we observed in patient-derived neurons. Our results uncover molecular mechanisms underlying Jordan's Syndrome and demonstrate an allele-specific ASO therapeutic strategy for the treatment of patients with Jordan's Syndrome. This study provides an important framework for the treatment of other neurogenetic disorders.

65 **Matthew Zimon, BS**
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Resting EEG Biomarkers for Stimulant Response in Pediatric ADHD: A Pilot Study

Background: Improved understanding of the neurobiological mechanisms driving individual differences in ADHD medication response is critical to the development of precision medicine guidelines for ADHD. Electroencephalography (EEG) is a cost-effective solution for measuring the unique cortical effects of stimulant medications. Prior brain imaging studies suggest that methylphenidate (MPH) increases regional activation in ventral prefrontal cortices, which can be measured with EEG as frontal theta power. Mixed amphetamine salts (AMP) appear to increase functional network segregation, corresponding to reduced central theta-beta ratio (TBR).

Objective: We conducted a pilot study to test our hypothesis that EEG is sensitive to differential effects of stimulant medications on attentional networks in children with ADHD. Secondarily, we predicted that EEG markers would be associated with symptom improvement.

Methods: Sixteen medication-naïve, 7-11-year-old children with confirmed ADHD completed resting EEG at baseline and on optimal treatment with MPH (n=15) and/or AMP (n=11). Symptom improvement was measured with parent ratings on the NICHQ Vanderbilt Assessment Scale from baseline to medicated follow-up. Resting EEG outcomes of interest were frontal theta and central TBR.

Results: Compared to baseline, participants exhibited greater average frontal theta power on MPH. A decrease in central TBR was associated with AMP treatment, and to a lesser extent, MPH treatment. Greater symptom improvement was associated with reduced frontal theta power on AMP and modestly associated with reduced central TBR on both medications. Finally, greater frontal theta at baseline predicted symptom improvement on both AMP and MPH.

Conclusions: MPH and AMP had distinct effects on resting EEG features, consistent with prior literature. Changes in cortical activity were uniquely associated with symptom improvement for each medication. Baseline frontal theta is a candidate biomarker for response to both stimulant classes. Results support the potential for EEG as a precision medicine tool for ADHD.

66 **Umar Zubair**
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Delineating the Molecular and Clinical Spectrum of Epilepsy-Dyskinesia Syndromes (The Epilepsy-Dyskinesia Spectrum Study)

Objectives: To understand the spectrum and association of epilepsy-dyskinesia syndromes on a clinical and molecular level.

Methods: A cross-sectional multicenter study administered through the Movement Disorders Society Pediatric Movement Disorders Special Interest Group. A multistep consensus review defined 102 genes associated with childhood-onset movement disorders and epilepsy. A standardized survey was used to collect clinical, molecular and neuroimaging data.

Results: A total of 450 cases from 19 countries were included. Mean age at last follow-up was 12.4±9.3 (SD) years. The most prevalent genes were MECP2 (16.9%), PRRT2 (8.2%), ATP1A3 and GNAO1 (7.6%), and SLC2A1 (7.3%). Most patients were diagnosed in early childhood (1-3 years, 48.2%). Epilepsy was present in 67.1%, with peak onset in infancy (24.0%). The most common movement disorders were dystonia (36.2%), stereotypies (21.8%), ataxia (17.3%), and chorea (10.8%). 50.9% of cases presented with more than one phenomenology, with dystonia/chorea (21.2%) being the most common combination. 33.8% presented with a Global Motor Function Classification Score of IV or V, indicating significant motor impairment. Global developmental delay or intellectual disability was observed in 91.8%. Neuroimaging abnormalities were observed in 45.5%, with white matter signal abnormalities observed on brain MR imaging being most common (15.8%).

Conclusions: Our findings confirm the phenotypic pleiotropy and clinical heterogeneity of epilepsy- dyskinesia spectrum disorders, reflecting the crucial role of many genes in brain development. This has implications for counseling, treatment and research aimed at creating clinical trial readiness.

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How Does Dual Therapy Affect Treatment Outcomes of SMA in Symptomatic Children?

Spinal muscular atrophy (SMA) is a recessively inherited genetic neuropathy causing motor neuron loss and progressive muscle weakness. This condition was fatal until the advent of therapies targeting both the causative gene SMN1 and the modifying gene SMN2. Patients now have the option to receive one of three approved therapies: Zolgensma (gene therapy), Spinraza (antisense oligonucleotide), or Evrysdi (oral small molecule). While most patients use one therapy to treat SMA, gene therapy may work synergistically with either of the splicing modulator therapies. There has been no documentation suggesting a contraindication to using dual treatments, but there is little published data on the benefits.

A retrospective chart review was performed to identify patients with SMA who are followed at Boston Children's Hospital and have received gene therapy (Zolgensma) followed by a second treatment of Spinraza or Evrysdi. A cohort of 12 patients met these criteria and have available follow-up motor outcome data. Motor ability outcomes measured by CHOP-INTEND and HFMSE were collected from the International Spinal Muscular Atrophy Consortium registry and clinical notes. CHOP-INTEND and HFMSE scores pre-Zolgensma, post-Zolgensma, and after addition of a second treatment will be compared and analyzed looking for trends.

Treatment outcomes are closely linked with symptomatic state, with pre-symptomatic patients having the most robust outcomes. Children with symptomatic SMA at birth can have significant improvement with early treatment but may have persistent symptoms. Similarly, later treatment can cause suboptimal outcomes. In these cases, dual therapy may be beneficial. As this was a retrospective chart review, not all variables can be controlled. There were no clinically significant side effects attributed to the addition of a second therapy. This study may be influential in making healthcare decisions about adding on a second treatment although more is needed to better inform the standard of care for SMA.