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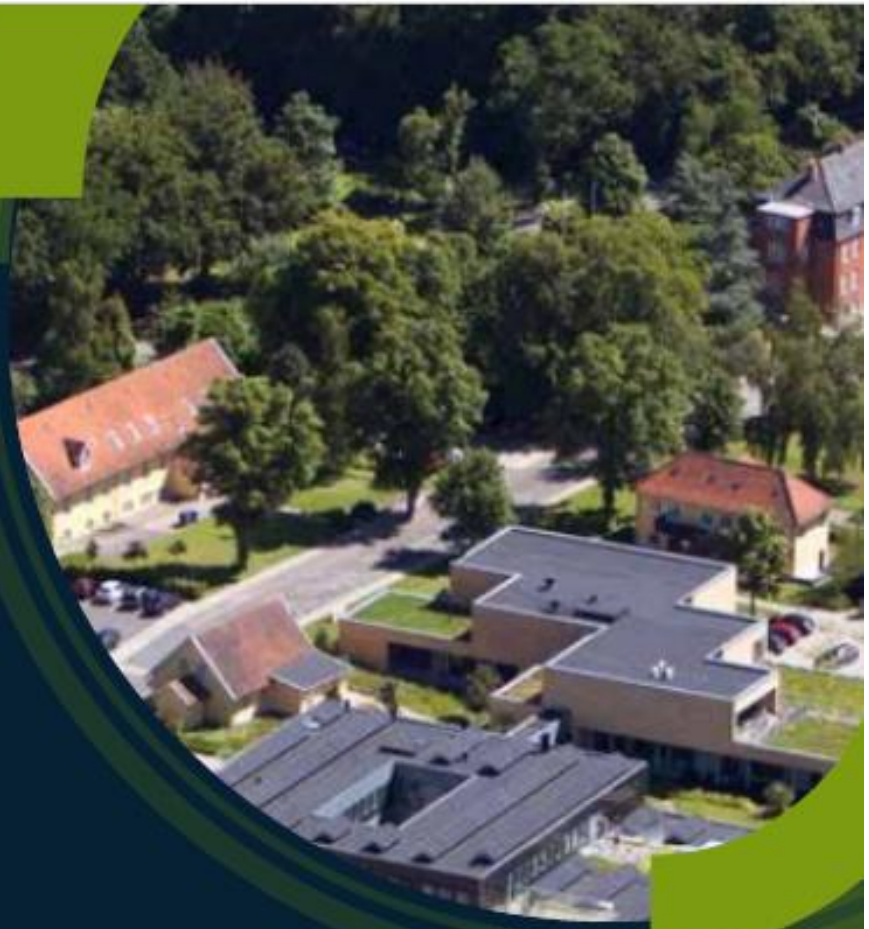

6th Dianalund International Conference on Epilepsy

Overlapping clinical phenotypes in monogenic epilepsies – common molecular pathways?

ABSTRACT BOOK

UNIVERSITY OF COPENHAGEN

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Dear participants

It is our great pleasure to invite you to the 6th Dianalund International Conference on Epilepsy. The topic of the conference is:

"Overlapping clinical phenotypes in monogenic epilepsies – common molecular pathways?"

We hope that you'll be able to join us, and we are looking forward welcoming you to Denmark.

Guido Rubboli

Elena Gardella

Rikke Møller

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ORAL COMMUNICATIONS

OVERLAPPING MOLECULAR PATHWAYS – COMMON CLINICAL PHENOTYPES? STANDARD PROCEDURE FOR COMMON DATA ELEMENTS IN GROUPS OF RARE DISEASES USING THE EXAMPLE OF SYNGAP1 AND CLASSICAL RASOPATHIES

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Purpose Research on rare disease groups, such as rare genetic epilepsies with common molecular pathways, requires structured and consistent phenotype capture to find phenotypic commonalities, differences and a severity classification.
We have standardized the process for selecting common Human Phenotype Ontology (HPO) terms through an approach combining the Delphi method and bioinformatics analysis.
The goal was to establish a domain-specific set of common data elements (DCDEs1) for a European registry for SYNGAP1 and neurodevelopmental RASopathies.

Method In the first step, stakeholders from all involved groups (neuro-paediatricians, human geneticists, psychologists and patient representatives) met weekly to define topics such as seizures, development, behavior, nutrition and malformations. A total of 40 HPO terms were jointly selected for these topics.
In the second step, related HPO terms were obtained. These included terms associated with the affected diseases genes, more specific child terms and comorbid terms, calculated using Cohort Analyzer2 and an approach for comorbid phenotypes3.
In the third step, the participating stakeholders reached a consensus of 65 terms from a selection of the defined and calculated terms, through a three-step Delphi process.

Results As a result, we obtained 65 common HPO terms as DCDEs, covering the desired topics and being suitable for the subsequent phenotyping and grouping into cohorts and phenotyping of patients.

Conclusion This study highlights the benefits of integrating bioinformatics methods to optimize the selection of HPO terms to describe groups of diseases. The protocol is also suitable for other groups of rare diseases and epilepsies and will be made available.

CAN PARALOGOUS EPILEPSY-ASSOCIATED GABAA RECEPTOR VARIANTS BE USED

AS INDICATORS FOR CLINICAL OUTCOMES?

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Background. In our pursuit of precision medicine, accurately predicting whether novel genetic variants are pathogenic is of utmost importance. For this purpose, in silico prediction tools (SIFT/PolyPhen/AlphaMissense) are commonly used. Furthermore, it was recently proposed that information from homologous variants in paralogues genes may be a powerful method to predict pathogenicity under the American College of Medical Genetics (ACMG) guidelines. GABAA receptors, however, have a pseudo-symmetrical assembly of five subunits, and in some cases diverse functional outcomes have been reported for paralogous variants in different subunits.

Methods. We assembled a cohort of eleven individuals harbouring seven paralogous M1 proline missense variants in GABRA1, GABRB2, GABRB3 and GABRG2, and conducted an extensive electrophysiological examination of their functional effects.

Results. All individuals were diagnosed with developmental and epileptic encephalopathy and exhibited a range of neurodevelopmental symptoms, such as developmental delay, intellectual disability, and movement disorders. By comparing the clinical and functional data of this cohort with those for other GABAA receptor variants, we established that all M1 proline variants led to overall gain-of-function. However, the underlying functional outcomes were complex with some variants displaying mixed gain- and loss-of-function traits.

Implications. Our findings corroborate the idea that paralogous variants in fully conserved residues across GABAA receptor subunits can serve as predictive indicators of pathogenicity for novel variants. Our results also highlight the challenges in functionally characterising heteromeric receptors, as the number of variant subunits may influence outcomes. In such cases, integrative approaches that merge clinical and functional data are essential for drawing conclusions.

ENHANCED INHIBITION IN HIPPOCAMPAL PYRAMIDAL NEURONS IN A GAIN-OF-FUNCTION GABRB3 MOUSE MODEL OF EPILEPSY

Chaseley McKenzie, Khaing Phyu Aung, Lauren Bleakley, Susan Lin, Vivian Liao, Ming S Soh, Nathan L Absalom, Rikke S Møller, Mary Chebib, Philip Ahring, Christopher Reid

Pathogenic variants in GABAA receptor subunits are a prominent cause of genetic epilepsies. Recent biophysical analysis of pathogenic GABRB3 variants reveals that both loss- and gain-of-function can cause epilepsy. To date, research has only focused on loss-of-function variants. Here, we engineer a mouse model based on the human de novo gain-of-function GABRB3 p.E77K variant associated with a developmental and epileptic encephalopathy with epileptic spasms, myoclonic seizures, hypotonia, severe developmental delay and autism spectrum disorder. Gabrb3E77K mice exhibited non-Mendelian ratios at birth. Surviving mice continued to die prematurely. Although spontaneous seizures were not observed, Gabrb3E77K mice were more susceptible to a pro-convulsant challenge. ECoG findings showed alterations to brain activity with a clear increase in overall power spectrum. Behavioural assays indicated Gabrb3E77K mice had decreased locomotion and decreased anxiety. Voltage clamp recordings from Gabrb3E77K CA1 hippocampal pyramidal neurons revealed larger spontaneous inhibitory post-synaptic currents consistent with gain-of-function findings from recordings in Xenopus oocytes. The Gabrb3E77K mouse confirms that gain-of-function GABRB3 variants can enhance inhibitory synaptic transmission and lead to epilepsy, providing an opportunity to understand neuronal network mechanisms. The mouse also provides a valuable pre-clinical model for developing targeted interventions for individuals affected by this disease.

UNDERSTANDING AND TREATING STXBP1 NEURODEVELOPMENTAL ENCEPHALOPATHY

Perrier JF, Dos Santos AB, Larsen SD, Martinez CD, Palmqvist J, Larsen V, Andersen S, Sørensen JB, Petersen AM

De novo mutations in STXBP1 are among the most prevalent causes of neurodevelopmental disorders, and lead to haploinsufficiency, cortical hyperexcitability, epilepsy and other symptoms in patients. Given that Munc18-1, the protein encoded by STXBP1, is essential for excitatory and inhibitory synaptic transmission, it is currently not understood why mutations cause hyperexcitability. We discover that overall inhibition in canonical feedforward microcircuits is defective in mouse model for Stxbp1 haploinsufficiency. Unexpectedly, we find that inhibitory synapses formed by parvalbumin-positive interneurons are largely unaffected. Instead, excitatory synapses fail to recruit inhibitory interneurons. Positive allosteric modulators that enhance the responses of glutamate receptors, restores interneuron recruitment, and pyramidal cell hyperexcitability. These molecules also decrease the frequency of spike and wave discharges observed in freely moving animals. These findings establish deficits in excitatory synapses in microcircuits as a key underlying mechanism for cortical hyperexcitability in a mouse model of Stxbp1 disorder and identify compounds enhancing excitation as a direction for therapy.

MODELING AND CHARACTERIZING WOREE SYNDROME: FROM BASIC SCIENCE TO TRANSLATIONAL MEDICINE

Rami I. Aqeilan

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Gene products of common fragile sites (CFSs) have been well-documented in cancer. In recent years, emerging evidence links these products, such as WW domain-containing oxidoreductase (WWOX), with neurological diseases including epilepsy, multiple sclerosis (MS) and Alzheimer's disease. Patients harboring pathogenic germline bi-allelic *WWOX* variants have been described with rare devastating autosomal recessive spinocerebellar ataxia 12 (SCAR12) and WWOX-related epileptic encephalopathy (WOREE syndrome). Individuals with these syndromes present with a highly heterogenous clinical spectrum, the most common being severe epileptic encephalopathy, profound global developmental delay and premature lethality. Using mouse genetics, we modelled WWOX loss of function and revealed that specific neuronal *Wwox* ablation leads to brain hyperexcitability, intractable epilepsy, ataxia, profound hypomyelination and postnatal lethality. Moreover, we recently established brain organoids from CRISPR-engineered human embryonic stem cells (hESCs) and from patient-derived induced pluripotent stem cells (iPSCs). Using these human cell systems, we discovered dramatic cellular and molecular CNS abnormalities, including neural population changes, cortical differentiation malfunctions, and Wnt-signaling and DNA damage response impairments. Prompted by these results, we next designed and provided a proof-of-concept that ectopic WWOX expression, using an adeno-associated viral vector (AAV9) harboring human *WWOX* cDNA and driven by the human neuronal Synapsin I promoter (*AAV9-SynI-WWOX*) could rescue WOREE syndrome phenotypes. Our findings underscore the utility of modeling childhood epileptic encephalopathies using genetically engineered mouse models and brain organoids and their use as a unique platform to test possible therapeutic intervention strategies.

POSTERS

P.001

CAREGIVER-REPORTED NON-SEIZURE OUTCOMES FOLLOWING USE OF CANNABIDIOL (CBD) IN PATIENTS WITH TUBEROUS SCLEROSIS COMPLEX (TSC): INTERIM RESULTS FROM THE BECOME-TSC SURVEY

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Objective: To report preliminary non-seizure findings from the ongoing cross-sectional BECOME-TSC caregiver survey designed to quantify the real-world impact of CBD on seizure and non-seizure outcomes in patients with TSC.

Design/Methods: Caregivers of patients with TSC receiving plant-derived highly purified CBD (Epidiolex®, 100 mg/kg oral solution) for ≥6 months completed an online survey, based on the TSC-associated neuropsychiatric disorders (TAND) questionnaire, other validated measures, and previous caregiver reports. CBD-associated AEs were not assessed.

Results: At time of analysis, 12 caregivers had completed the survey. Mean (SD) patient age was 16 (8) years; 58% were female. Mean (SD) age at seizure onset was 17 (33) months; 50% had a history of infantile spasms. Median CBD dose was 17 mg/kg/day. Most common concomitant ASMs were everolimus (42%) and clonazepam (33%). Co-occurring symptoms included developmental delay (92%), autism spectrum disorder (83%), anxiety disorder (42%), attention deficit hyperactivity disorder (33%), and obsessive-compulsive disorder (33%). Severe-profound intellectual disability (ID) was reported in 75% of patients and mild-moderate ID in 17%; 8% had fluent verbal language. Compared with the pre-CBD initiation period, most respondents reported definite improvements in a patient's ability to be happy (67%), shake head for yes/no answers (63%), accomplish visuospatial tasks (63%), and recall past events (60%). Definite worsening was reported by ≤2 respondents in domains including using repetitive words/phrases, repetitive behaviours, impulsivity, and overactivity/hyperactivity.

Conclusions: These preliminary results show that a substantial proportion of caregivers of people with TSC reported improvement in TAND-related outcomes since initiating CBD.

Funding: Jazz Pharmaceuticals Inc

P.002

IRF2BPL DEVELOPMENTAL AND EPILEPTIC ENCEPHALOPATHY

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Introduction *IRF2BPL* has been associated to neurodevelopmental disorder[1,2,3], Progressive Myoclonic Epilepsy (PME)[4] and Movement Disorders[5], in patients with normal or mildly delayed early development. We report 5 novel patients with developmental encephalopathy (DE) with epilepsy harboring *IRF2BPL* pathogenic variants, along with a review of the patients with severe early developmental delay (DD) published so far[1,2,3].

Novel cases We report five patients aged between two months and 31 years, diagnosed with severe early DD and epilepsy. Two of them were diagnosed with Infantile Spasms (IS), two with early onset myoclonic epilepsy. One patient presented with tonic and focal seizures, at the age of 7 years, followed by drop attacks and regression at 24 years.

Review of the literature Nineteen *IRF2BPL* patients (including this case series) with DE have been described so far, most of them diagnosed with IS. Main neurological findings were intellectual disability, speech and cerebellar impairment, abnormal eye movements, pyramidal signs, and dysphagia; a neurological regression could occur later in life. Dysmorphisms and autistic features could be observed as distinctive feature of this group of patients (p*0.001, p*0.019 respectively), in comorbidity with psychiatric, gastro-intestinal, osteo-articular, and endocrinological disorders. *IRF2BPL* variants were mainly frameshift, clustered in the second part of the protein, downstream if compared to variants associated with movement disorders and PME.

Conclusion *IRF2BPL* pathogenic variants can be causative of different phenotypes, including DE with early onset epilepsy, and later onset PME. Variants associated to PME and DE are distinguishable due to their different localization in the protein.

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P.003

CLINICAL SPECTRUM OF A NOVEL, FAMILIAL SCN8A PATHOGENIC VARIANT

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SCN8A variants are associated with a large spectrum of neurodevelopmental disorders and epilepsies.
We describe a large Italian pedigree with six patients (5F) over three generations carrying a heterozygous SCN8A variant (c4771 G>A; p.Val1591Met); the variant, located in the transmembrane domain, is not described in Human Gene Mutation Database. The bioinformatics tools suggest a damaging impact on gene product.
Five patients (4F) suffered from infantile seizures with onset in the first year of life, treated with antiseizure medications; one patient is unaffected.
The youngest patient is 10 years old, she presented with infantile onset seizures persisting to childhood and has been seizure free since age 6. Last EEG showed multifocal sharp waves during wakefulness, increased in frequency during drowsiness and sleep and epileptiform activity during photic stimulation. Valproic Acid is well-tolerated. Brain MRI was normal.
The family includes two additional male family members with febrile seizures (FS) who could not be tested.
All patients appear to have a normal neurological developmental milestones, and described themselves as hyperactive and curious people. Movement disorders have not been reported.
The family members we describe suffered from self-limited infantile epilepsy with good prognosis in both adolescence and adult life, yet the same variant causes infantile self-limited epilepsy in some family members and infantile epilepsy extending to childhood and adulthood in others.
This study expands the genotypic spectrum of SCN8A-related disorders and underscores the importance of genetic testing to guide therapeutic interventions and management of epilepsies with onset in the first year of life.

P.004

GENERALIZED EPILEPSY, INTELLECTUAL DISABILITY, BEHAVIORAL DISOREDER AND FAMILIAR TREMOR DUE TO COMPOUND HETEROZIGOSITY OF THE CAD GENE.

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Biallelic variants of the CAD gene are associated with severe epileptic encephalopathy due to pyrimidine biosynthesis deficiency.
We present a male patient with drug resistant epilepsy, cognitive regression and anemia responsive to oral uridine monophosphate supplementation.

From the age of 4 years, monthly generalized tonic-clonic during daytime and sleep occurred; seizures were resistant to phenobarbital and Valproic Acid and improved from the age of 14 years.
Around this age myoclonia also appeared which could precede tonic-clonic seizures.
In addition to epilepsy since school age, he presented severe behavioral disorders, intentional tremor and progressive cognitive slowing. A sister with same gene variant showed a similar but milder clinical phenotype.
Overtime pharmacological therapy was modified by introducing Carbamazepine, Clonazepam, Diazepam. EEG showed focal bilateral epileptiform activity in centro temporal regions. Brain MRI (1.5 T) was normal.

NGS gene panel analysis showed compound heterozygosity (frameshift and missense variants) of CAD gene, inherited from parents.
Skin biopsy with functional study on fibroblasts confirmed that the missense variant modifies the structure and function of the CAD protein.

With the introduction of low doses uridine monophosphate, he showed a progressive improvement in verbal fluency, gait, planning abilities and environmental participation; it was observed also reduction of sedation, tremor and less repetitive thinking.

This case report extends the phenotypical spectrum of CAD deficiency, including a generalized epilepsy with infantile onset associated with behavioral problems, which underlies a treatable metabolic epilepsy. This highlights the importance of early genetic diagnosis to reveal monogenic epilepsies for which exists target therapy.

P.005

PRECISION MEDICINE APPROACHES FOR SEVERE EPILEPSIES CAUSED BY VARIANTS IN KCNB1 POTASSIUM CHANNELS

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BACKGROUND AND AIMS: Mutations in KCNB1 are associated with Developmental and Epileptic Encephalopathies (DEE1), severe epilepsies mainly characterized by drug-resistant seizures. These mutations induce loss-of-function (LoF) effects, except for one case where gain-of-function (GoF) effects have been observed, therefore requiring distinct therapeutic approaches. Nowadays, no selective modulators of KCNB1 are known. Thus, in this study we propose to check for the presence of KCNB1 variants in DEE-affected patients, and characterize functional and pharmacological properties of mutant channels.

METHODS: Genetic analysis has been performed using next-generation sequencing approaches in DEE-affected patients; KCNB1 variants have been expressed in CHO cells and characterized using patch-clamp recordings.
RESULTS: Nine variants in KCNB1, three novel (p.L35P, p.E180G, p.I204Tfs*38) and six (p.R306C, p.R312H, p.R312C, p.V349F, p.W369*, p.Y380C) previously reported, have been identified in 11 patients with DEE. Functional studies revealed that these variants induce LoF (L35P, I204Tfs*38, R306C, R312C, R312H, V349F, W369*, Y380C) or GoF (E180G) effects. Exposure to 50 □M of the antidepressant drug fluoxetine2 almost completely inhibits currents expressed by both wt channels and those carrying the GoF variant E180G; by contrast, 10 □M of the antidiabetic drug metformin3 activates currents expressed by both wt channels and those carrying the LoF variant R312H.

CONCLUSIONS: fluoxetine and metformin are effective modulators of KCNB1 currents and could therefore potentially be used in precision medicine approaches to reverse specific KCNB1 variant-induced effects in DEE-affected patients.

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P.006

FUNCTIONAL CHARACTERIZATION OF THE P.(ALA1507SER) VARIANT IN *CACNA1A* CAUSING DEVELOPMENTAL EPILEPTIC ENCEPHALOPATHY

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$Ca_v2.1$ is a calcium channel with an important role in the communication between the neurons of the brain and at the nerve-muscle junctions. Loss- or gain-of-function variants of *CACNA1A*, which encodes the pore-forming subunit of $Ca_v2.1$, lead to a broad clinical spectrum including episodic ataxia, spinocerebellar ataxia, hemiplegic migraine, and developmental epileptic encephalopathy. The *CACNA1A* variant p.(Ala1507Ser) was previously identified in an individual with developmental epileptic encephalopathy. Using a cell culture model of the variant, we carry out whole-cell patch clamp experiments to determine the effect of the variant on channel function. We show that the p.(Ala1507Ser) variant leads to loss-of-function, as the current density was significantly smaller in mutant cells than in wild type cells (p<0.01). In addition, the voltage of half-maximal activation (V½) was significantly more negative than in wild type cells (p=0.007), indicating that the mutant channel $Ca_v2.1^{A1507S}$ activates at more negative potentials. A significantly slower $Ca_v2.1^{A1507S}$ current decay was also observed (p=0.002), which suggests prolonged inactivation. As the general practice of seizure treatment is a trial-and-error approach with a great burden for the affected individuals and their families, functional knowledge of variants will facilitate clinicians' choice of the most relevant drugs, avoiding ineffective or even disease-aggravating treatments and adverse drug reactions. Our findings thereby contribute to the translation of the genetic diagnosis to treatment in line with the current paradigm of precision medicine.

P.007

THE "DOUBLE TROUBLE" OF RARE METABOLIC DISORDERS: COMPLEX PHENOTYPE IN A FAMILY WITH GLUT1 & VERY-LONG-CHAIN FATTY ACID DEHYDROGENASE (VLCAD) DEFICIENCIES

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Abstract

GLUT1 Deficiency Syndrome (GLUT1-DS) is a rare neurometabolic condition, characterized by reduced glucose transport into the brain, leading to seizure, cognitive disability or movement disorders. GLUT1-DS exhibits wide phenotypic intra- and inter-familial variability. Very-long-chain acyl-CoA dehydrogenase (VLCAD) deficiency is an autosomal recessive disorder of long-chain fatty acid oxidation (FAO) with variable age of onset and clinical presentation, including metabolic myopathy. We report a 15-year-old adolescent who displayed drug-resistant epilepsy since the age of 2, with daily seizures mainly appearing before meals. Additionally, he had psychomotor delay and exercise-induced chorea-athetoid movements. His 40-year-old father exhibited mild mental retardation, sporadic choreo-athetoid movements and recurrent rhabdomyolysis triggered by exercise or fasting since his twenties. The clinical and laboratory results of the son indicated a diagnosis of GLUT1-DS. Genetic analysis confirmed a heterozygous mutation c.997C>T (p.R333W) in the *SCL2A1* gene, which was also identified in the father. Furthermore, the occurrence of recurrent exercise-induced rhabdomyolysis with elevated plasma C14:1-carnitine levels, a feature never associated with GLUT1-DS, suggested the presence of another metabolic disorder. The detection of two heterozygous mutations (c.553G>A; p.G185S; c.1153C>T, p.R385W) in *ACADVL* unequivocally confirmed the additional diagnosis of VLCAD deficiency in the father. Both father and son refused the ketogenic diet.

In conclusion, the coexistence of these two rare metabolic disorders resulted in a distinct clinical phenotype within this family. The emerging "double trouble" cases of genetic origin emphasize the importance of extensive investigation when atypical features complicate a known phenotype.

P.008

UNRAVELLING GAIN-OF-FUNCTION VS LOSS-OF-FUNCTION *GABRB2* DISEASE.

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Individuals with genetic variants in the *GABRB2* gene, encoding the β2 subunit of the $GABA_A$ receptor, present with a wide range of disease from febrile seizures through neurodevelopmental delay to severe epileptic encephalopathies. Currently, it is unclear which underlying mechanisms determine the risk of developing severe forms of epilepsy and associated co-morbidities. Here, we present a cohort of 42 individuals harbouring 26 missense *GABRB2* variants. Functional assessment of receptor function revealed that 25/26 variants caused significant alterations in receptor sensitivity to GABA. Of these, 17 resulted in gain-of-function (GOF) while eight resulted in loss-of-function (LOF). Genotype-phenotype correlation analysis uncovered that the severe forms of disease were associated with GOF variants. These individuals suffered from severe intellectual disability (80%), movement disorders such as dystonia and dyskinesia (59%), microcephaly (50%) and high risk of early mortality (26%). In contrast, overall milder forms of disease were associated with LOF variants. These individuals typically presented with seizures triggered by fever (92%), mild intellectual disability (54%) and were ambulant (85%). No severe movement disorders or microcephaly were reported for LOF individuals. These findings highlight that GOF *GABRB2* variants overall leads to more severe forms of disease and pave the way for future precision medicine approaches.

P.009

ORAL MAGNESIUM SUPPLEMENTATION PREVENTS SEIZURE CLUSTERS DURING THE MENSTRUAL PERIOD IN A YOUNG ITALIAN WOMAN AFFECTED BY DEVELOPMENTAL AND EPILEPTIC ENCEPHALOPATHY ASSOCIATED WITH A DE NOVO P.GLY544ARG VARIANT OF THE CLCN4 GENE

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Introduction Pathogenic variants in the CLCN4 gene cause loss-of-function (LOF) of the encoded chloride/proton exchanger ClC-4 and result in a neurodevelopmental disorder (also called Raynaud-Claes syndrome) inherited in an X-linked pattern. There are no reported cases of females with variant p.Gly544Arg and there is no targeted treatment for this syndrome.

Case presentation We present a 30-year-old Italian woman with early-onset drug-resistant epilepsy, developmental and epileptic encephalopathy, absence of verbal language development and behavioral impairment. The interictal EEG showed slight inconstant slowing of the background rhythm and generalized spike and polyspike wave discharges. The whole exome sequencing (WES) revealed a de novo heterozygous CLCN4 gene variant (p.Gly544Arg). The introduction of carbamazepine up to 1200 mg/day and the increase of clonazepam up to 4.5 mg/day led to a decrease in seizure frequency to monthly clusters, often with a catamenial pattern. During clusters, the patient presented sudden awakenings from nocturnal sleep, with abnormal behaviors and confusion, as well as frequent diurnal atypical absences and seizures with head and eye deviation to the right, and eyelid and right hemiface myoclonic seizures. From December 2022, oral magnesium supplementation 2.4 g/day was added during the catamenial period, with benefit on cluster duration and intensity.

Discussion and conclusion Our patient is the first case of a de novo p.Gly544Arg variant of the CLCN4 gene in a female proband. The good response to oral magnesium supplementation during the menstrual period might suggest its role as a targeted therapy for seizure clusters in patients with Raynaud-Claes syndrome.

P.010

LONG-TERM EFFICACY AND SAFETY OF GENE THERAPY WITH AN AADC-EXPRESSING AAV VECTOR IN A GIRL WITH AADC DEFICIENCY.

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Introduction: Several studies demonstrated beneficial effects of gene therapy for patients with AADC deficiency using an AADC-expressing AAV vector. Here we present the 6-year long follow up after gene therapy with an AACD-expressing AAV vector of a female patient with AADC deficiency.

Materials and methods: We performed clinical evaluation and retrospective medical records analysis of the thirteen-year-old girl with AADC deficiency.

Results: The diagnosis of AACD deficiency was established at the age of 2 years. The DNA sequencing revealed two variants in DDC gene: c.260C>T (p.P87L) и c.799T>C (p.W267R), which form a compound heterozygote.

At the time of gene therapy she had oculogyric crises that lasted for several hours, limb dystonia, autonomic dysfunction, was completely immobilized.

In March 2018 she received bilateral intraputaminal infusions of a recombinant adeno-associated virus type 2 vector containing the human aromatic L-amino acid decarboxylase gene by stereotactic surgery. Rapid improvements in motor and cognitive function occurred within 6 months after gene therapy and were sustained during follow-up for 6 years. She can walk with a walker, understands the speech, can talk with the help of the letters and study at the 4th class according to the general education program. Oculogyric crises are rare and short, limb dystonia is mild.

Conclusions: Our study confirm that gene therapy with an AADC-expressing AAV vector of aromatic L-amino acid decarboxylase deficiency provides durable and meaningful benefits with a favorable safety profile.

P.011

EVEROLIMUS PRECISION THERAPY IN A PATIENT WITH NPRL3-RELATED EPILEPSY

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Introduction The mTOR, a key regulator of cell growth and metabolism, is inhibited by the GATOR1 complex. Hyperactivation of mTORC1 has been observed in patients with loss-of-function variants in genes encoding components of the GATOR1 complex, including *DEPDC5* and *NPRL3* genes. Everolimus, a synthetic mTOR inhibitor, showed eEicacy in DEPDC5-epilepsy, but contrasting eEects have been reported in NPRL3-epilepsy.

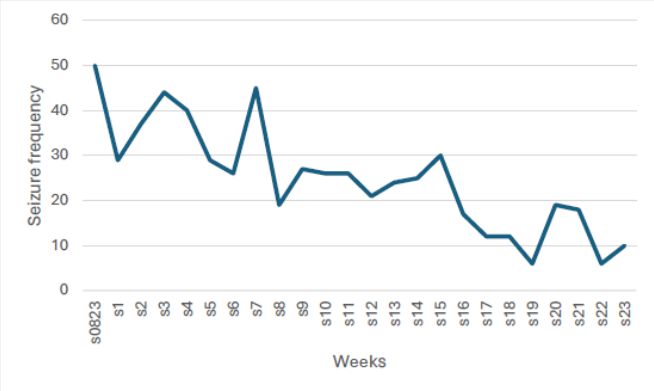
Methods A compassionate use of everolimus as an add-on treatment was granted for a patient with drug resistant *NPRL3*-epilepsy and frequent episodes of status epilepticus. A 60-day baseline seizure frequency and monthly seizure frequency during treatment were assessed. Primary outcome was set as 50% seizure reduction.

Results A 27-year-old intellectually normal patient presented with a long-standing, neonatal onset, MRInegative, intractable epilepsy characterized by focal tonic seizures arising from both hemispheres, associated with a pathogenic frameshift *de novo* variant, p.(C343Sfs*17), in *NPRL3* gene. His baseline seizure frequency was 70 seizures per week. Thirteen anti-seizure medications had been ineEective. Transient seizure reduction was observed with carbamazepine and vigabatrin. Everolimus was started up to 7.5 mg/day with trough levels between 6.2 and 8.5 ng/mL. At the 6-month follow-up, he had a-80% decrease in seizure frequency. He has 13 seizures per week, experiencing up to 4 seizure-free days per week.

Conclusions Although limited to one patient, our data suggest that everolimus may be an eEective precision therapy for drug-resistant *NPRL3*-related epilepsy. Further studies are necessary to validate and support this finding.

Disclosures Patient(s) have been provided product via Managed Access. Novartis has conducted a scientific accuracy and intellectual property review. Novartis has not influenced the content of the publication.

Figure1. Weekly seizure frequency after initiation of everolimus.



P.012

DEVELOPMENT OF THE FIRST GABAAR Δ-SUBUNIT NAM

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δ-containing γ-aminobutyric acid type A receptors (GABAARs) represent promising drug targets in the treatment of several neurological disorders. Compared to synaptic counterparts, these receptor subtypes are located extracellularly and implicated in persistent tonic inhibition. Recently, several gain-of-function (GOF) variants of the α4- and δ-subunit have been identified in patients suffering from neurodevelopmental disorders and generalized epilepsy (1-3), urging the need for ligands that can reduce GABA tonic currents, e.g. negative allosteric modulators (NAMs). Based on rational medicinal chemistry and computational modelling of recently published cryo-EM structures of GABAARs, we designed a series of potential allosteric modulators for δ-containing GABAARs.
From a screening campaign using whole-cell patch-clamp electrophysiology, we identified a NAM, NNJ95, active at recombinant α4β1δ-GABAARs expressed in HEK293 cells. When coapplied with GABA, NNJ95 significantly accelerated the rate of current fade compared to the GABA control current ($p=0.0027$) at α4β1δ -GABAARs. With pre-application, NNJ95 reduced the current amplitude of the subsequent GABA current in a concentration-dependent manner ($IC50=8.87$ μM, Hill slope= -1.57 ($n=6$)). Intriguingly, NNJ95 (1-10 μM) did not reduce the current amplitude at α4β1γ2-, α4β2γ2-, and α1β2γ2-GABAARs, indicating its functional preference for the δ-subunit. To explore the clinical potential of NNJ95, two GOF patient variants, α4(T300I) and δ(L260V), were incorporated into functional α4β1δ receptors expressed in HEK293 cell lines. As for the α4β1δ WT receptors, NNJ95 reduced the current amplitude of the subsequent GABA trace in a concentration-dependent manner compared to the GABA control current. This underlines the clinical potential of NNJ95 – which to our knowledge is the first reported GABAAR δ-subunit NAM.

Disclosures: NNJ95 is part of an invention currently being patent protected by the University of Copenhagen.

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P.013

IF A VARIANT IS PREDICTED TO BE "BENIGN/TOLERATED" BY SILICO TOOLS AND SEEN IN GNOMAD – IS IT BENIGN?

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With increasing use of genetic screening, a growing number of missense variants have been recorded in public databases such as ClinVar and gnomAD. These missense variants can either be benign or pathogenic. Two widely used bioinformatics tools for predicting if a variant is deleterious to the protein function are SIFT and PolyPhen2. Additionally, in some cases, patient variants occur at the same amino acid positions as existing gnomAD entries. Here we sought to investigate whether occurrence in gnomAD and labels of "tolerated/benign" by *in silico* tools, give a good prediction for *GABRG2* variants.

Method: Seven pairs of *GABRG2* missense variants, from patients and gnomAD, were analysed for pathogenicity using SIFT and PolyPhen2. Variants were then evaluated for changes in GABA sensitivity and maximum current amplitudes using two-electrode voltage clamp electrophysiology.

Result: The A106T/A106P pair represents a good example of the findings. Both A106P (3 gnomAD entries) and A106T were deemed "tolerated/benign" by SIFT and PolyPhen2. *In vitro* tests, however, revealed that the A106T variant enhanced GABA sensitivity (gain-of-function). Among 12 patients harbouring A106T, 8 have epilepsy (average seizure age-of-onset at 4 months).

Conclusion: "Tolerated/benign" classification of the same positional missense gnomAD variants by *in silico* tools does not indicate the variant is benign, as the A106P/A106T pair would be predicted benign. All tested gnomAD variants, except A106P and R177Q, were deemed "affect/probably damaging" by both tools. This highlights the importance of integrating functional data to assess variant impact (Figure 1).

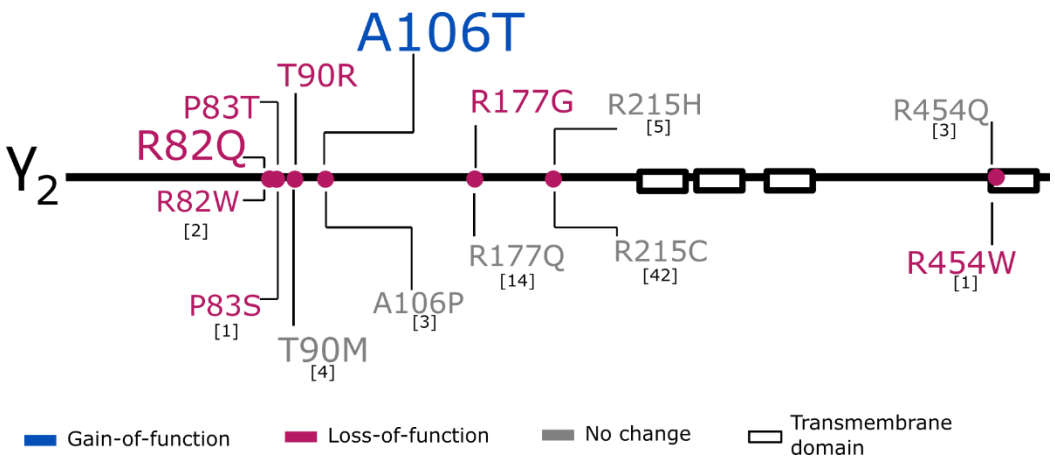

Figure 1: 2D *GABRG2* diagram with variant location (dots). Font size indicates the number of patients, number in the bracket denotes gnomAD frequency.

P.014

KCNA2-GOF AND GOF-LOF DEVELOPMENTAL EPILEPTIC ENCEPHALOPATHY: AN UPDATE ON TREATMENT RESPONSE OF 4-AMINOPYRIDINE

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Background: *KCNA2*-developmental epileptic encephalopathy (DEE) consists of clinically distinguishable groups, based on the electrophysiological dysfunction caused by the variants on the *KCNA2* channel subunits. Gain-of-function (GOF) variants cause a severe phenotype characterised by drug-resistant, generalized seizures, severe developmental delay and ataxia. Variants with GOF-LOF effects are associated with a similar, although more severe, phenotype. 4-Aminopyridine (4-AP) has been described as a promising treatment, improving seizure frequency, ataxia and global functioning, in patients featuring GOF and GOF-LOF variants.

Methods: We performed a retrospective, multicenter study of 26 individuals with GOF and GOF-LOF *KCNA2* variants treated with 4-AP. Data on changes in seizure frequency, EEG and concomitant anti-seizure medication were collected. In addition, the Clinical Global Impression Scale was used to obtain clinicians opinion on seizure burden, ataxia, everyday functioning, language abilities, alertness.

Results: Data were collected on 11 individuals (5 with GOF-LOF, 6 with GOF variants). Seven stopped treatment: 4 because of increase in seizure burden (minimally worse to very much worse), 2 because of decline in motor abilities (much worse and very much worse) and 1 because of gastro-intestinal side-effects. Four individuals continued treatment, reporting improvement in different domains, mainly in seizure burden and ataxia. No clear association between treatment response and specific variants ahs ben observed .

Conclusion: 4-AP is a promising treatment for individuals with GOF-LOF and GOF variants in KCNA2, improving functioning in different domains, however the specific subset of individuals deriving optimal benefits from this treatment, as well as those who may not, remains uncertain.

P.015

TWO NEW CASES OF KCNQ2 MUTATION CHARACTERIZED BY NEONATAL SEIZURES, SUBSEQUENT SELF-LIMITED EPILEPSY AND POSITIVE OUTCOME: A 20-YEAR FOLLOW-UP.

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In the literature, the most common phenotypes described in KCNQ2 mutations are:
- Self-limited familial neonatal epilepsy (SLFNE)
- Neonatal-onset developmental and epileptic encephalopathy.

We report two patients with KCNQ2 mutation presenting with neonatal seizures followed by self-limited epilepsy.

Patient 1. Male (09.24.2005). At five days he showed neonatal seizures and phenobarbital was started on. Seizure-free from day 10. He stopped phenobarbital after 3 years. At 10 years, focal seizures with secondary generalization appeared, he started oxcarbazepine reaching seizure control. Seizure-free since 2016, no medication from 2024. Genetic testing: de novo heterozygous KCNQ2 mutation. No family history of epilepsy. Neuroimaging: negative for cortical dysplasias.

Patient 2. Female (08.19.2005). At two days she showed seizures and phenobarbital was started on. Seizure-free from day 6. Phenobarbital was discontinued after 2 months. At 13 years, focal seizures with secondary generalization appeared and she started oxcarbazepine, reaching seizure control. Seizure-free since 2018, no medication from 2022. Genetic testing: heterozygous KCNQ2 mutation (absent in the mother, the father wasn't tested). In the family history, a father's cousin had neonatal convulsions and then self-limited epilepsy (uncertain information); no other cases of epilepsy. Neuroimaging: normal.

These cases are relevant because KCNQ2 mutations, although rare, are among the most frequently involved in genetic epilepsies. Patient 2 presents a phenotype described in 30% of patients with SLFNE. Patient 1 shows a different phenotype characterized by neonatal convulsions (non-familial), subsequent self-limited epilepsy (different from that described in literature) and positive outcome. Thus, it could represent a new KCNQ2-mutation phenotype.

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P.016

CLINICAL AND EPILEPTOLOGICAL PROFILES OF SYNAPTOPATHIES: PRESENTATION OF 69 PAEDIATRIC CASES.

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Introduction The term "synaptopathies" is used to identify a class of disorders caused by mutations in genes encoding synaptic proteins that are essential for the nervous system development 1. They are considered as the leading causes of genetically determined epileptic and developmental encephalopathies 2.
We present an observational retrospective study based on the longitudinal analysis of 69 patients with synaptopathy who were admitted at Epilepsy Regional Center of Brescia between 2002 and 2023.

Methods The sample consists of 45 patients with pre-synaptic synaptopathy (gene CLTC, CNTNAP2, NRXN1, NRXN3, PRRT2, SLC6A1, STXBP1, STX1B, SYN1, VAMP2), 23 with post-synaptic synaptopathy (gene CLCN4, DLG4, GABRA1, GABRG2, GRIN2A, GRIN2B, GRIN2D, SHANK1, SHANK3, SLC12A5, SYNGAP1) and 1 patient with extra-synaptic synaptopathy (gene LGI1).

Results The diagnosis of epilepsy is made in 68% of cases (15% drug-resistant). 4% of cases presents with isolated seizures. A neurodevelopmental disorder is associated in 63% of total sample (62.3% language disorder, 40.6% intellectual disability, 34.8% movement disorder, 10% ADHD, and 4% autism spectrum disorder).
Pre-synaptic synaptopathies start with seizures more frequently (57,8% versus 47,8%), and earlier (18 months versus 4 years) than post-synaptic ones.

Conclusions Epileptic seizures are the main clinical symptom at onset and during follow-up in synaptopathies, with neurodevelopmental disorder in comorbidity. This analysis represents the first known attempt to phenotypically characterize synaptopathies as nosographic entities. Therefore, it can be hypothesized that patients with epilepsy and neurodevelopmental disorder are affected by synaptopathy.

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P.017

CLINICAL FEATURES AND GENOTYPE-PHENOTYPE CORRELATIONS IN EPILEPSY PATIENTS WITH *DE NOVO DYNC1H1* VARIANTS

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Background: *DYNC1H1* variants are involved in a disease spectrum from neuromuscular disorders to concomitant neurodevelopmental disorders. *DYNC1H1*-related epilepsy has been recently reported in small cohorts. We dissect the electro-clinical features of 34 patients harboring *de novo DYNC1H1* pathogenic variants, identify subphenotypes within the *DYNC1H1*-related epilepsy spectrum and compare the genotype-phenotype correlations observed in our cohort with the literature.

Methods: Patients harboring *de novo DYNC1H1* pathogenic variants identified through massively-parallel sequencing were recruited through international collaborations. Clinical data were retrospectively collected. Latent class analysis was performed to identify subphenotypes, and multivariable binary logistic regression analysis was applied to investigate the association with DYNC1H1 protein domains.

Results: *DYNC1H1*-related epilepsy presented with Infantile Epileptic Spasms syndrome (IESS) in seventeen subjects in our cohort (17/34, 50%) and in the 25% of these individuals the epileptic phenotype evolved into Lennox-Gastaut syndrome (LGS). In twelve patients (35%) focal-onset epilepsy was defined. In two patients the epileptic phenotype consisted of generalized myoclonic epilepsy, with a progressive phenotype in one individual harboring a frameshift variant (p.Arg2610GlyfsTer23). In about 60% of our cohort, seizures were drug-resistant. Malformations of Cortical Development were noticed in 76% of our patients, mostly in the lissencephaly/pachygyria spectrum, particularly with posterior predominance in a half of them. Midline and infratentorial abnormalities were additionally reported in 44% and 26% of subjects respectively. We have identified three main classes of subphenotypes within *DYNC1H1*-related epilepsy spectrum.

Conclusions: We propose a classification in which pathogenic *de novo DYNC1H1* variants features drug-resistant IESS in about half of cases with a potential evolution to LGS (Class 1), Developmental and Epileptic Encephalopathy other than IESS and LGS (Class 2), and less severe focal epilepsy or genetic generalized epilepsy including a progressive phenotype (Class 3). We obderved an association between stalk domain variants and Class 1 phenotypes. The variants p.Arg309His and p.Arg1962His are common variants and are associated with Class 1 subphenotype in our cohort. These findings may aid genetic counseling of patients with *DYNC1H1*-related epilepsy and their families.

P.018

PHENOTYPIC SPECTRUM OF SYNGAP1-RELATED DEVELOPMENTAL AND EPILEPTIC ENCEPHALOPATHY

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Purpose: SYNGAP1-related developmental and epileptic encephalopathy (DEE) features psychomotor delay, autism spectrum disorder, epilepsy and behavioral problems. We collected a large cohort of patients with SYNGAP1-related DEE aiming to further refine the phenotype.

Method: Patients with (likely) pathogenic SYNGAP1 variants were recruited through an international network of epilepsy/genetics centers and the Italian SYNGAP1 family association. Data on clinical, genetic and neurophysiologic features were obtained.

Results: 83 patients (48 males; median age 11 years, range: 19 months-48 years) harboring a presumed pathogenic variant in SYNGAP1 (97% de novo and 67% protein-truncating) were included.
Sixty-nine (83%) patients developed epilepsy with focal, generalized tonic-clonic, typical/atypical absences, myoclonic absences, tonic, atonic and myoclonic seizures; 7% experienced seizures during sleep, while 20% reflex seizures triggered by chewing/eating. Mean age at epilepsy onset: 2 years. EEG showed generalized, multifocal, and focal abnormalities. Twenty (30%) patients were seizure free. Valproate was the most effective antiseizure medication, showing >50% seizure reduction in 75% patients (29% as monotherapy). Lamotrigine and Ethosuximide were also beneficial. Sleep disturbances were reported in 54% of cases; the most common sleep disorder was insomnia (53%). Melatonin was administered in 92% of cases, showing efficacy in 70%. Other clinical features: intellectual disability (96%) or borderline IQ (4%), language delay (91%), aggressive behavior (58%), autistic features (56%), neurological symptoms such as ataxia (47%). No clear genotype-phenotype correlations were found.

Conclusion: Our study further outlines the phenotype of SYNGAP1-related DEE, showing also that non-seizure comorbidities, particularly psychiatric and sleep disorders, represent common features requiring proper management.

P.019

FUNCTIONAL, GENOTYPE AND PHENOTYPE CORRELATIONS OF GABRA1 VARIANTS-ASSOCIATED EPILEPSIES.

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Patients with GABRA1 variants exhibit diverse epilepsy phenotypes ranging from mild JME, febrile seizures to severe IESS and Dravet syndrome. To understand the underlying mechanisms responsible for the observed variabilities in the epilepsy phenotypes, a comprehensive evaluation of individuals with GABRA1 variants was performed.
Published (97) and unreported (44) clinical data of 141 individuals with GABRA1 variants were collected, including 76 missense and 6 null variants. To assess pathogenicity, functional characterisation of 76 missense variants was performed using our novel receptor concatenation method on the major GABAA receptor subtype, (α1)2(β3)2Ɣ2. To functionally recapitulate the dominant receptor population found in these individuals, we constructed pentameric GABAA receptors containing 1 variant and 1 wild-type α1 subunit, reflecting the heterozygous genotype.
Out of the 141 individuals, 91 (64.5%) have loss-of-function (LOF) variants, including 6 individuals with 5 null variants and 85 with 35 missense variants. In addition, 38 individuals (27%) have gain-of-function (GOF) variants (30), with one individual harbouring 2 missense GOF variants. The remaining 12 individuals (8.5%) carry 11 variants that do not alter receptor function.
Distinctive traits exist between LOF and GOF patient groups. Patients in the GOF group are likely to have epileptic spasms, more severe epilepsy and intellectual disability, with an earlier onset of seizures. De novo variants and microcephaly are exclusive to the GOF group. LOF patients are more likely to have fever-related seizures, with syndromes like GEFS+ and Dravet being exclusive to this group. Additionally, Individuals with normal intellect is also exclusively found in this group.

P.020

THE USE OF KETOGENIC DIET AS TARGET THERAPY IN A COHORT OF ITALIAN PATIENTS AFFECTED BY GLUT1 DEFICIENCY SYNDROME

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INTRODUCTION Glucose transporter type 1 deficiency syndrome (GLUT1-DS) is a neurometabolic disorder due to pathogenic variants in the SLC2A1 gene, which determine altered glucose entrance into the central nervous system, leading to drug-resistant epilepsy, developmental delay, acquired microcephaly, and movement disorders. Ketogenic diet (KD) is known to be the target therapy for this condition. In this study we report on a cohort of GLUT1-DS patients treated with KD.

METHODS From our database of 44 patients with GLUT1-DS followed at Buzzi Children's Hospital in Milan, we selected those who were treated with KD. We collected demographic, genetic, clinical data.

RESULTS Thirty-five patients were included, with a median age of 13.5 years (range:4months-42years). The median age at clinical onset was 22.5 months. All were started on KD, but six interrupted the treatment because of poor family compliance and difficulty with KD management.
Among those who underwent the KD, the median follow-up time was 4.25 years.
Epilepsy was reported in 28/29 (96.5%) and 18/29 (62%) reached seizure freedom after the KD start, 4/29 (13.8%) had >50% seizure reduction.
Fourteen patients (48%) presented a movement disorder (encompassing paroxysmal exercise-induced dyskinesia, choreoathetosis, abnormal eyes movements); 4/29 (13.8%) presented ataxia. After KD treatment, 6/29 (20.7%) no more presented paroxysmal movement disorders.
Fourteen/29 (48.3%) had intellectual disability, severe (14.3%), moderate (35.7%), mild (50%), one had borderline intellectual functioning.
No severe adverse events were reported.

CONCLUSION We report detailed follow-up of a cohort of GLUT1-DS patients, emphasizing its safety and effectiveness in controlling epileptic seizures and movement disorders.

P.021

CASE REPORT: DEPDC5-RELATED EPILEPSY AND THERAPEUTIC CONSIDERATIONS

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Background DEPDC5 gene pathogenic variants have been associated with autosomal dominant familial and sporadic focal epilepsy, linked to enhanced mTORC1 pathway upregulation (resulting in increased neuronal excitability).

Case presentation We report a case of DEPCD5-related epilepsy with variable foci involving a 3,5-year-old female with maternally inherited heterozygous mutation (c.4185dupA), confirmed by NGS. Nocturnal seizures begun when she was 10 months old; they were characterized by autonomic onset and secondary generalization. She had normal cognitive and motor developmental. Carbamazepine achieved the complete seizure control. The brain MRI was normal. Her mother didn't present neurological sign or symptoms.

Discussion DEPDC5-related epilepsy includes a range of epilepsy syndromes, often characterized by focal seizures, with or without cortical malformation, psychomotor developmental delay, or autism spectrum disorder. Furthermore, individuals from a single family can show variable phenotypic presentation. Seizures are frequently sleep-related and drug-resistant. Because of this variable clinical manifestation, we could not exclude a delayed drug-resistant epilepsy in our patient.

Conclusions FDA approved mTOR inhibitors for the treatment of intractable seizures in TSC patients. These drugs can decrease seizure frequency, but they could be potential precision treatments for this monogenic epilepsy.
The challenge for future research could be to assess the possibility of precision therapeutics using mTOR inhibitors in non-TSC epileptic patients, despite the presence of pharmacoresistance.

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P.022

FEBRILE SEIZURES AND EPILEPSY PLUS: DRAVET SYNDROME-MIMICS AND BEYOND.

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Background: HCN1 variants cause a wide phenotypic spectrum ranging from mild phenotypes to catastrophic neonatal/infantile epileptic encephalopathies.1 HCN1 variants have been linked to Dravet syndrome-mimics.2

Methods: We report two cases with HCN1 variants showing clinical features of the two opposites of the spectrum.

Case report 1: A 2-year-old male experienced his first complex febrile seizure (2 generalized tonic-clonic seizures in the same febrile episode following vaccination) aged 5 months. EEG and neurologic examination were normal. Epilepsy onset was at 17 months with 1) tonic-vibratory seizures with cyanosis and eye deviation, 2) psychomotor arrest with eye deviation and oral automatisms, 3) myoclonic-atonic seizures, both spontaneous and reflex (triggered by tactile/auditory stimuli). At this time he showed psychomotor delay, with further regression and appearance of autistic features after seizure onset.
Brain MRI, array-CGH and metabolic investigations were normal.
EEGs evidenced sharp delta-rhythms and sequences of theta activity at 4,5 Hz on the F-C regions at vertex, interictal generalized spike-waves during sleep and myoclonic-atonic seizures characterized by generalized discharges of irregular spike-waves.
A targeted NGS panel documented a de novo HCN1: NM_021072.4: c.1297G>T (p.Asp433Tyr) variant.
The father showed febrile seizures. Two cousins experienced pediatric epilepsy.

Case report 2: A 13-year-old boy had 15 febrile seizures between 14 months and 4 years, 8months. Aged 9 years, an infection-triggered seizure occurred and three months later a first non-provoked seizure (tonic vibratory with oral automatisms). He had normal developmental milestones, mild motor hindrance and specific learning disorder.
He had normal awake EEGs until 5 years, when occipital epileptiform discharges increasing in slow-wave sleep were documented, evolving to F-C-T discharges with CSWS pattern. No overt regression occurred. Brain MRI was normal.
A targeted NGS panel documented the HCN1: NM_021072.4: c.1232A>G (p.Tyr411Cys) variant, inherited from his father, on nebivolol for rhythmic tachycardic palpitations and with normal sleep EEG. Paternal grandfather had infantile febrile/afebrile seizures. Patient’s sister has dyslexia.

Conclusions: We further highlight how different variants on a same gene may result in distinct clinical pictures, that can instead overlap with phenotypes resulting from variants on different genes.

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P.023

PRECISION MEDICINE APPROACHES IN GRIN-RELATED DISORDERS – WHAT DO WE KNOW AND WHATS NEXT?

Ilona Krey

GRIN-related disorders are rare genetic disorders, associated with a spectrum of mild to severe developmental delay/intellectual disability, epilepsy, muscular hypotonia, spasticity, brain abnormalities and many others symptoms. As a channelopathies, the mechanism of gain or loss-of-function of the NMDAR provides an ideal precision medicine approach theoretically. In reality, however, the therapeutic options aswell as the published knowledge of clinical trials is limited. In the following i would like to give an overview what we know about precision medicine approaches in GRIN-related disorders and discuss the next steps in this field.

What do we know?

- For gain-of-function we know about single case reports with variable benefits of memantine
- RAD-GRIN-101, a phase 1B open-label trial, started in 02/2023, assess safety, tolerability, PK, and potential efficacy of radiprodil for the treatment of GRIN-related disorder in children with a Gain-of-Function variant. First results are expected by the end of 2024
- For Loss-of-function variants, several retrospective cases treated with L-serine are known, showing slight improvements in behavior, development als seizure frequency
- Only one case report with LoF is know with benefits from treatment with spermidine
- Recently the first non-randomized, open-label, single-arm trial (NCT04646447) designed to evaluate tolerability and efficacy of L-serine in 24 children with loss-of-function variants was published. The trial provides evidence that L-serine is a safe treatment for children with GRIN-related disorder due to a loss-of-function variant.

What is next?

- Retrospective case series of GoF treated with memantive (n=20)
- Clinical trials with memantine – necessary?
- Larger trials with l-serine in LoF cases – differences in missense LoF and nonsense variants?
- Difference between memantine and radiprodil?
- New approaches? What about gene therapies? ASO (antisense oligonucleotides)?

P.024

COMPREHENSIVE EVALUATION OF THE IMPACT OF SINGLE NUCLEOTIDE VARIANTS ON *SCN1A* SPLICING

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Purpose: To develop a minigene-based splicing assay capable of evaluating the impact of single nucleotide variants across all protein-coding exons of the *SCN1A* gene.

Methods: Bioinformatic analysis of all reported SCN1A variants was performed using SpliceAI. Mini and midigenes covering all 26 protein-coding exons of the *SCN1A* gene were created using Gibson cloning. Variants were introduced by site-directed mutagenesis. Splicing analysis was performed by RT-PCR 48 hours post-transfection of plasmids into HEK293T cells.

Results: To create a minigene system that correctly reproduce the splicing pattern of *SCN1A* mRNA several novel approached were used including decreasing plasmid promoter strength, mutagenesis of plasmid introns for U12 introns, mutagenesis of cryptic splicing sites, and modulation of cis-regulatory sequences. Ultimately, the minigene system contains 18 splicing vectors covering all 26 protein-coding exons of the *SCN1A*. Overall, 112 variants were tested, including 68 intronic variants, 21 exonic variants and 17 deep-intronic variants. Genotype-phenotype correlation demonstrated that variants affecting splicing are associated with a more severe *SCN1A*-related phenotype than missense variants, regardless of the nature of the splicing change. Additionally, splicing reporters were created to evaluate the impact of deep-intronic variants on the splicing of three *SCN1A* "poison" exons in introns 4, 23, and 25. The inclusion of all "poison" exons was successfully blocked using modified U7-snRNA.

Conclusion: The minigene system developed here can be used for comprehensive analysis of almost all possible variants in the *SCN1A* gene, both already described and those that are yet to be found in patients.

P.025

UNVEILING DEVELOPMENTAL DELAY AND HYPERKINETIC MOVEMENT DISORDER: TWO NOVEL BI-ALLELIC LOSS-OF-FUNCTION *CACNA1B* VARIANTS.

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Objective: This case unveils two unprecedented loss-of-function variants within the *CACNA1B* gene, broadening the understanding of its genotype.

Methods: Case report, whole exome sequencing and literature search on PubMed using the search terms: *CACNA1B*, developmental delay.

Results: A 15 month old girl presented with severe neurodevelopmental delay, head circumference SDS -1.8, and dyskinesia/chorea-like movements. Although at presentation clinical seizures had not been observed, a short EEG track showed continuous epileptic activity in the frontal regions.
Genetic analysis revealed two compound heterozygous variants in *CACNA1B*: c.1812_1820del, p.(Asn604_Met606del), categorized as a variant of unknown significance, and c.5737C>T, p.(Arg1913Ter), identified as a likely pathogenic variant. Both variants were inherited from the parents.
CACNA1B encodes the pore-forming subunit of presynaptic neuronal voltage-gated calcium channels, pivotal in regulating neurotransmitter release.
Gorman et al. (2019) reported six children across three families displaying bi-allelic loss-of-function mutations in *CACNA1B*, resulting in an epilepsy-dyskinesia syndrome characterized by developmental and epileptic encephalopathy, postnatal microcephaly, and hyperkinetic movement disorder—a triad mirrored in our patient.
Bi-allelic loss-of-function mutations disrupt Ca2+ influx, impairing synaptic neurotransmission, and potentially causing involuntary movements and epilepsy, underscoring *CACNA1B*'s significance in human neurodevelopment.

Conclusion: Both variants in our patient affect crucial loci—between domain I and II, and in the C terminal—involved in channel regulation and protein interaction, reinforcing their pathogenic character.

P.026

A RARE KCNQ2 MUTATION VARIANT: A CASE REPORT

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Background: Mutations in the KCNQ2 gene, encoding the voltage-gated potassium channel subunit Kv7.2, are a common genetic cause of neonatal seizures and epilepsy syndromes. These mutations disrupt the normal function of the potassium channels, leading to neuronal hyperexcitability and increased susceptibility to seizures. Although KCNQ2-related disorders typically have a severe early onset and can be associated with developmental delay and intellectual disability, there is considerable phenotypic variability. Some individuals may experience spontaneous resolution of seizures or have a relatively benign course with normal neurodevelopmental outcomes. Early diagnosis through genetic testing is crucial for appropriate management, including the initiation of antiepileptic therapy and genetic counseling for affected families. To date, more than 100 families with KCNQ2- BFNE and at least 100 individuals with KCNQ2-NEE from many nationalities have been described in literature. Currently there are 421 distinct KCNQ2 mutation variants reported as disease-causing. We will discuss a 17-days old female infant with a rare mutation of KCNQ2. To the best of our knowledge this variant has not been reported in the medical literature or on disease-related variation databases.

Case report: We present the case of a 17-day-old female neonate evaluated in our pediatric neurology clinic for the onset of orofacial clonic movements and winking spells lasting less than a minute, occurring 3-4 times daily for the past three days. The initial EEG showed multifocal abnormalities with a normal background rhythm, prompting initiation of phenobarbital therapy which successfully controlled the seizures. Magnetic resonance imaging demonstrated hyperintensity on T2-weighted images in the white matter and thinning of the corpus callosum, consistent with delayed physiological myelination. Genetic testing performed on a salivary sample using a panel analyzing 705 genes revealed a heterozygous mutation in KCNQ2: c.331del(Val111Cysfs*22). This novel mutation results in a premature stop codon and loss of protein function. Follow-up EEG performed after one month showed no significant abnormalities, and clinical assessments up to 8 months of age revealed satisfactory neuromotor development. At 14 months, the child still doesn't walk independently but she speaks few words, no more seizures were detected. This case highlights the importance of recognizing KCNQ2-related disorders in the differential diagnosis of neonatal seizures and underscores the significance of genetic testing in guiding therapeutic interventions and prognostic counseling. Additionally, the successful management and favorable outcome observed in this case contribute to the growing understanding of the clinical spectrum and management of KCNQ2-related epilepsies.

P.027

PHARMACOLOGICAL MANAGEMENT OF SEIZURES IN A RARE SCN8A MUTATION VARIANT: A CASE REPORT

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The term epileptic encephalopathy (EE) is used to describe a clinical condition in which the epileptiform activity interferes with neurological development resulting in cognitive slowing and sometimes regression. The term developmental epileptic encephalopathy (DEE) was created to describe patients with cognitive delays associated with pharmacoresistant seizures. Some SCN8A mutations fall within this group and are associated with different DEE phenotypes. The SCN8A gene encodes a member of the sodium channel alpha subunit that forms the ion pore region of the voltage-gated sodium channel (NaV 1.1–1.9) that plays an important role in the membrane depolarization during the formation of the action potential in neurons. Following the original discovery in 2012 more than 400 individuals with SCN8A related disorders have been identified. We will discuss an 8-month-old infant with early-onset SCN8A pathogenic variant referred to our pediatric neurology unit for a second opinion regarding a suspected case of early-onset seizures. The patient was born full-term through spontaneous delivery. No perinatal distress was reported. The proband's parents were not consanguineous. A paternal uncle was affected by Down syndrome. Regarding neurodevelopment, the patient was unable to maintain a seated position, he could fix and track and had started to vocalize. He also had no problem in maintaining an upright head posture.
At six months of age, the patient was hospitalized due to the onset of an afebrile seizures and subsequently underwent a wakefulness and sleep Long-Term Monitoring study, which resulted in nonspecific findings without any clinical correlation. The transfontanellar brain ultrasound was normal and a brain MRI revealed hyperintensity in T2 sequences in the left centrum semiovale/corona radiata, with no restriction of diffusivity. A small hyposignal region was detected in SWI, indicating a possible hemosiderin component. Following the evaluations, the patient was discharged with a follow-up program without any specific therapy. At 7 months of age, the patient was brought to emergency healthcare for another afebrile seizure, characterized by fixed gaze, oro-buccal automatism, facial clonic jerking, and tonic stiffening of the bilateral upper extremities, lasting almost two minutes with spontaneous resolution. Parents did not report any recent infection. During hospitalization he experienced two new seizures. Subsequently, he underwent an unrevealing video EEG and started prophylactic Valproic Acid (VPA). The patient came to our observation after about one month of therapy because he showed recurrence of epileptic episodes with the same characteristics as before. Considering the ineffectiveness of pharmacological therapy, we decided to perform a limited saliva panel which examines approximately 705 genes, a significant portion of which play a substantial role in the development of various forms of epilepsy. After approximately one month we obtained the following result: heterozygous mutation in the SCN8A gene c.4427G>A, p.(Gly1476Asp). To our knowledge, this case represents the first European report detailing this particular variant. Due to treatment failure with Depakote we decided to shift and initiate a therapy with Carbamazepine (CBZ). After starting Carbamazepine, the patient showed no further seizures. After one month a new EEG was performed which did not show any epileptiform activity or pathological patterns. Furthermore the patient showed an improvement in neuromotor development and achieved developmental milestones according to the scale. At ten month of age the patient was able to sit independently and exhibited typical interaction with the surrounding environment.

P.028

GRIN1-related disorders: genotype-, phenotype- and functional-correlation

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Background: Alterations of the N-methyl-d-aspartate receptor (NMDAR) subunit GluN1, encoded by GRIN1, have been associated with developmental delay/intellectual disability (DD/ID), epilepsy, muscular hypotonia, spasticity and malformation of cortical development (MCD).

Methods: First, we collected phenotypic data of 165 unreported individuals (93 variants) from our GRIN Registry (https://grin-portal.broadinstitute.org). Applying the criteria of the ACMG to all published variants (51 variants, 83 cases) yielded 132 additional cases with pathogenic variants in GRIN1, resulting in a total of 215 individuals (135 variants). Second, 64% (86/135 variants, 120 individuals) of these variants were functionally tested. Finally, we performed genotype-phenotype and functional correlations for all 120 individuals with GRIN1-related disorder (86 variants).

Results: We found that pathogenic missense variants located in the pore were associated with severe developmental phenotypes, including DD/ID with epilepsy and MCD due to a gain-of-function effect. Non-pore lining missense variants led more often to ID/DD without seizure or MCD due to a loss-of function or complex effect in our functional analysis. Specifically, higher fold potency changes of Glutamate and Glycine correlated with epilepsy and MCD.

Conclusion: We demonstrate that non-pore lining variants in GRIN1 do not only share overlapping clinical spectrum (i.e. ID/DD), but also result in electrophysiological consequences (loss-of-function or complex) opposing those of pore lining variants (more often associated with severe phenotypes e.g. ID/DD, seizure and MCD; predominantly gain-of-function). This new understanding of the underlaying pathomechanism will ultimately help in predicting phenotype severity as well as eligibility for potential precision medicine approaches in GRIN1-related disorders.

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P.029

AFG2A-RELATED ENCEPHALOPATHY: CLINICAL PHENOTYPE AND KETOGENIC DIET EFFECT (IN VIVO AND IN VITRO EFFECT)

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Objective: Describe the clinical and epileptic phenotype in a series of AFG2A-related encephalopathy (AFG2A-RE). Analyze a possible genotype-epileptic phenotype correlation. Evaluate the clinical response to KD in AFG2A-RE patients with drug-resistant epilepsy. Describe the mitochondrial effect of KD.

Methods: Observational, retrospective, and descriptive study (bibliographic review and patient's registry) and a multicentric prospective experimental study (in vivo and in vitro). Clinical, EEG and genetic data were assessed. KD treatment response (% seizure reduction). Patients' fibroblasts grown in a KD-mimicking medium.

Results: 51 AFG2A-RE patients were included (40 previously published, 11 new patients). Main clinical mptoms were: intellectual disability(97.92%), hearing loss(93.62%), microcephaly(85.71%), visual impairment(79.49%), epilepsy(74.71%), hypotonia(71.74%), and spasticity(60.87%). %). Most patients presented refractory epilepsy(82.35%). The most frequent variants were: c.2081G>A (13.73%), c.989_991del(11.76%), and c.251G>A(9.8%). Four patients with refractory epilepsy and uncountable daily seizures received KD. Seizure reduction was 0, 30, 70 and 100%. Motor(n=1) and social interaction and awareness(n=2) improvement was objectivated. In vitro studies revealed that AFG2A-deficiency has a characteristic mitochondrial phenotype affecting mitochondrial shape, dynamics and bioenergetics, with less ATP and higher ROS levels. Dynamics and bioenergetics were recovered with KD mimicking medium.

Conclusions: Epilepsy is a frequent feature in AFG2A-RE, and refractory epilepsy is common. Infantile spasms are the predominant seizure type at onset. Treatment with KD improves seizure control, social interaction, awareness and motor symptoms. AFG2A-deficiency has a characteristic mitochondrial phenotype affecting mitochondrial shape, dynamics and bioenergetics, less ATP and were exposed to higher ROS levels. Dynamics and bioenergetics recovered with KD mimicking medium. KD seems to be a good therapeutic option for patients with epilepsy, especially if KD is started early. Prospective studies including larger sample size are needed.

P.030

LESSONS LEARNED FROM A GENOTYPE-FIRST ANALYSIS OF VOLTAGE-GATED ION CHANNELS

Nathan Routledge

Background/Objectives: Voltage-gated ion channels (VGCs), comprising over a hundred genes, regulate neuronal action potential and trigger multiple ion-dependent processes. While some VGCs are linked to channelopathies like epilepsy and developmental epileptic encephalopathy, many remain unassociated. The study of ion channels is a promising avenue for successful treatment development, as understanding their functional and malfunctioning processes can lead to novel corrective treatments. The highly conserved and analogous structure of ion channel alpha units across channels enhances the value of variant observations in one channel for understanding others.

Methods: Using a genotype-first strategy, we screened the Queen's Square Institute of Neurology genomic database, housing 30,000 exomes from diverse backgrounds, focusing on Sodium VGC genes. This approach aimed to assess the genetic epidemiology and prevalence of channelopathies, exploring the clinical and genetic spectrum within a substantial dataset.

Results: Analysing 3,947 variants indicated a prevalence of higher-than-variant-of-uncertain-significance (VUS) classifications in voltage-gated and pore-forming domains across nine channels. SCN8A exhibited a unique pattern, with a concentration of higher-than-VUS variants in the C-terminal. Surprisingly, likely pathogenic variants were found in unexpected contexts, including healthy individuals. While novel homozygous cases were identified in SCN1A and SCN3B.

Conclusion: The study's outcomes highlight the voltage-sensor and pore-forming domains as hotspots in Sodium VGCs, validating existing knowledge while proposing a distinct hotspot in SCN8A. Adopting a genotype-first diagnostic approach may uncover new causal genes and inheritance patterns. However, the robustness of variant classifications warrants scrutiny. This analysis underscores the dynamic nature of channelopathies and emphasizes the need for ongoing evaluation in variant classification methodologies.

P.031

ATP6V0C VARIANTS IN EPILEPSY: UNRAVELING PHENOTYPIC PATTERNS AND GENOTYPE CORRELATIONS.

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Introduction: Variants in ATP6V0C have been associated with neurodevelopmental disorders and seizures. In the present study we aim to delineate the ATP6V0C epilepsy phenotypes.

Methods: We retrospectively analyzed 33 cases from 27 families with ATP6V0C variants. Cases were identified by an international network of epileptologists/geneticists. SPSS was used for statistical analysis.

Results: ATP6V0C variants were predominantly missense (88%), often de novo (73%), and exhibited an average seizure onset at 19 months, with febrile seizures in 77% of cases. Generalized tonic-clonic seizures were the predominant type (82%), with other types such as myoclonic, absences, tonic, and focal seizures occurring in approximately 25-35% of patients. Unspecified developmental and epileptic encephalopathy (n=11) and genetic epilepsy with febrile seizure plus (GEFS+) (n=9) were commonly observed. Developmental delay affected 76% of patients (evenly distributed between severe, moderate, and mild categories), with half experiencing drug-resistant epilepsy (DRE). Valproate showed >50% seizure reduction in 10 of 15 patients but was ineffective in those with DRE. MRI abnormalities were noted in seven severe cases, showing delayed myelination and corpus callosum agenesis. Thirteen cases with TMR-4 variants displayed a more severe phenotype, including higher rates of developmental delay (n=13), DRE (n=8), and abnormal neurological examination (n=8) with hypotonia and dysmorphic facial features. Logistic regression analysis highlighted the significance of affected amino acid position in predicting moderate or severe developmental delay (OR 1.018, p= 0.034). (Figure 1)

Conclusion: ATP6V0C variants are linked to a spectrum of GEFS+ epilepsy phenotypes, underscoring a robust genotype-phenotype correlation.

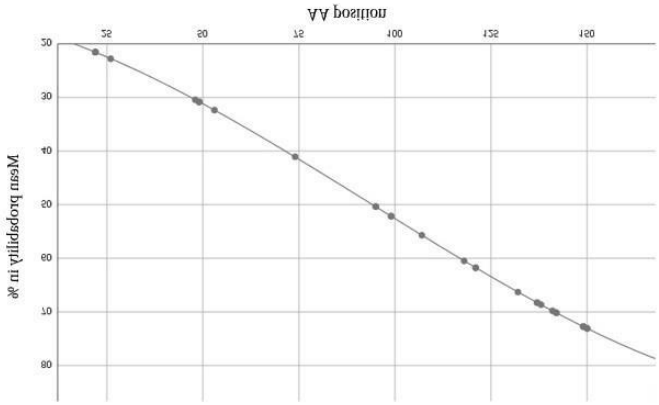

Figure 1 Mean Predicted Probability of Bad Developmental Outcome by AA position

P.032

AN INFANT PRESENTING WITH INFANTILE SPASMS: AHCY DEFICIENCY AND THE EVOLUTION TO PRECISION MEDICINE

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Objective: This case shows the importance of genetic diagnoses, as they will increasingly lead to better understanding of underlying disease mechanisms and the development of targeted therapies.

Methods: Whole exome sequencing and biochemical tests were executed. Literature search was performed on PubMed. Search terms: epilepsy, AHCY, treatment.

Results: Genetic analysis identified two compound heterozygous AHCY gene variants: c.712C>T, p. Arg238Cys, maternally inherited, and c.727G>A, p. Glu243Lys, paternally inherited. Investigations demonstrated elevated S-adenosylmethionine and S-adenosylhomocysteine, confirming S-adenosylhomocysteine hydrolase (AHCY) deficiency.

Conclusion: In our patient, we unraveled two new compound heterozygous AHCY variants, broadening the phenotypic spectrum of AHCY deficiency.
Infantile spasms were promptly controlled when starting anti-seizure medication. Today the patient remains seizure free, but shows a severe neurodevelopmental delay and failure to thrive. Current treatment options for AHCY deficiency are mostly supportive, with only limited improvement of symptoms in most patients, as in ours.
The AHCY gene encodes S-adenosylhomocysteine hydrolase. It is a crucial enzyme for methyltransferase activity, and has a vital role in DNA, RNA, and histone methylation. AHCY activity in the liver has a 4-fold higher AHCY mRNA expression than most other tissues and a 10-fold higher expression than the brain.
Strauss et al. described a case study of a patient with severe AHCY deficiency, where liver transplantation was performed, normalizing biochemical markers and leading to notable improvements in the patient's neurodevelopmental status.
The potential of liver transplantation highlights a paradigm shift in treating AHCY deficiency and opens a window for developing novel therapies.

P.033

SEIZURE AND MOVEMENT DISORDER IN CACNA1E DEVELOPMENTAL AND EPILEPTIC ENCEPHALOPATHY: TWO SIDES OF THE SAME COIN OR SAME SIDE FOR TWO DIFFERENT COINS?

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Pathogenic variants in CACNA1E are associated with early-onset epileptic and developmental encephalopathy. Severe to profound global developmental delay, early onset refractory seizures, severe hypotonia, and macrocephaly are the main clinical features. Patients harbouring the recurrent CACNA1E variant p.(Gly352Arg) typically present with the combination of early-onset developmental and epileptic encephalopathy, dystonia/dyskinesia and contractures.
We describe a 2-year-and-7-months old girl, carrying the p.(Gly352Arg) CACNA1E variant. She has severe developmental and epileptic encephalopathy, very frequent drug-resistant seizures, profound hypotonia, and episodes of dystonia and dyskinesia. Long-term video-EEG-monitoring documented subsequent tonic asymmetric seizures during wakefulness and mild paroxysmal dyskinesias of the trunk out of sleep which were thought to be a movement disorder and instead turned out to be focal hyperkinetic seizures.
Our report highlights a possible overlap between cortical and subcortical phenomena in CACNA1Edevelopmental and epileptic encephalopathy. We also underline how a careful electro-clinical evaluation might be necessary for a correct discernment between the two disorders, playing a fundamental role in the clinical assessment and proper management of children with CACNA1Edevelopmental and epileptic encephalopathy.

P.034

NATURAL HISTORY OF ADULTS WITH KBG SYNDROME: A PHYSICIANS REPORTED EXPERIENCE

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Purpose: KBG syndrome (KBGS) is one of the most common neurodevelopmental syndromes and is caused by haploinsufficiency of *ANKRD11.* The childhood phenotype is extensively reported, but knowledge is limited about adults. We aimed to delineate the clinical features of KBGS in adulthood.

Methods: We collected physician-reported data of adults with molecularly confirmed KBGS through an international collaboration. We next reviewed the literature to determine the scope of previously reported data.

Results: We identified 36 adults with KBGS. Mild cognitive impairment/borderline intellectual disability was reported in 22/36. All were ambulant although 15 had gross and/or fine motor difficulties. Psychiatric and behavioral comorbidities (26/36) included aggression, anxiety, reduced attention span, and autistic features. Cognitive regression during adulthood was reported once. Seizures were diagnosed in 28%, with various seizure types and treatment responses. No adults experienced convulsive status epilepticus, and we observed no cognitive or behavioral regression directly associated with seizures or epileptiform activity on EEG. Infrequent features included dilatation of the ascending aorta (2/36) and autoimmune conditions (4/36). Education, work, and residence varied; some individuals unable to maintain a job, while others held part- or full-time positions. Three adults resided in institutions, 13 lived with their parents, seven lived independently with no or limited support by caregivers, two lived with a partner, and seven had started families. The literature review identified 154 adults reported across the literature, and we will present the features across both datasets.

Conclusion: Our study sheds light on the long-term neurodevelopmental outcomes, seizures, behavioral and psychiatric features, and education, work, and residence situations in adults with KBGS.

P.035

THE PHENOTYPIC AND GENOTYPIC SPECTRUM OF INDIVIDUALS WITH MONO- OR BIALLELIC *ANK3* VARIANTS

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Abstract

ANK3 encodes ankyrin-G, a protein involved in neuronal development and signaling. Alternative splicing gives rise to three ankyrin-G isoforms comprising different domains with distinct expression patterns. Mono- or biallelic *ANK3* variants are associated with non-specific syndromic intellectual disability in 14 individuals (seven with monoallelic and seven with biallelic variants). In this study, we describe the clinical features of 14 additional individuals and review the data on a total of 28 individuals (17 individuals with monoallelic and 11 with biallelic *ANK3* variants) and demonstrate that the phenotype for biallelic variants is more severe. The phenotypic features include language delay (93%), intellectual disability (74%), autism spectrum disorder (71%), hypotonia (62.5%), motor delay (61%), sleep disturbances (45%), and epilepsy (30%). When comparing the phenotypes, a notable observation was the presence of ataxia in patients with biallelic variants. While the majority of the monoallelic variants are predicted to result in a truncated protein, biallelic variants are almost exclusively missense. Moreover, mono- and biallelic variants appear to be localized differently across the three different ankyrin-G isoforms, suggesting isoform-specific pathological mechanisms.

P.036

STRONG CORRELATION TO THE P.(PRO405LEU) KCNA2 PATHOLOGICAL VARIANT AND ENCEPHALOPATHY RELATED TO STATUS EPILEPTICUS DURING SLOW SLEEP (ESES): A CASE REPORT AND REVIEW OF THE LITERATURE

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Background KCNA2 is a gene located on chromosome 1 that encodes a member of the potassium channel voltage-gated, shaker subfamily, called Kv1.2 whose pathological variants are associated with a broad spectrum of neurological disorders1-3. From several studies, that analyzed phenotypic-genotypic correlation, pathological variants of KCNA2 gene are subdivided into three subgroups: loss-of-function variants (LOF) associated to a milder phenotype, gain-of-function (GOF) variants with a moderate-severe phenotype and LOF/GOF linked to the most severe phenotype2-4. Early onset developmental and epileptic encephalopathy (DEE) with focal seizure, mild intellectual disabilities, better outcome to therapy and absences of MRI abnormalities are some of the main features observed in LOF patients2,3. Among all the LOF pathological variants the p.(Pro405Leu) is the most commonly found and strongly related to febrile seizure and Encephalopathy related to Status Epilepticus during slow Sleep (ESES)2.

Cases description In this work we intend to delve deeper into a case of 29-years-old patient with p.(Pro405Leu) variant in the KCNA2 gene already reported in literature, to better describe the relation between the spike and wave index (SWI) during sleep and cognitive status. The patient developed an early onset developmental and epileptic encephalopathy (DEE) characterized by different kinds of febrile and afebrile seizures from the age of 6 months, moderate cognitive impairment (with a total IQ of 40), severe speech issues and ESES from the age of 3 years and 2 months. Several types of antiepileptic drugs (ASMs) have been tried without a significant effect on seizure frequency. Interestingly, a long duration of ESES was observed in our patient until the age of 16 years and a reduction of the SWI up to a minimum of 20% in response to the cortisone treatment.
Moreover, we summarize the clinical features of other 14 patient harboring the same pathological variant and never described before and we compared them to the previously 16 reported cases. Among the all 30 patients we observe a strong association with ESES in 12 patients and other similar clinical features such as: impairing in speech ability with normal understanding and good response to the cortisone therapy.

Conclusion Our report highlight a strong genotype-phenotype correlation between the KCNA2 p.(Pro405Leu) variant in 12 patients and ESES in our patient and in other patients reported in literature. Furthermore, our work aims to speculate on the function of the gene in the human model as this function in the animal model has shown a correlation with disturbances in hypnic physiology that could lead to new hypotheses in the ESES genesis.

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P.037

NEONATAL DEVELOPMENTAL AND EPILEPTIC ENCEPHALOPATHY WITH MOVEMENT DISORDERS AND ARTHROGRYPOSIS (NDEEMA) – NOVEL COMMON PHENOTYPE ACROSS BRAIN-EXPRESSED SODIUM CHANNELOPATHIES

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Purpose: Neonatal developmental and epileptic encephalopathy with movement disorders and arthrogryposis (NDEEMA) is the most severe end of the gain-of-function *SCN1A* disorder spectrum (Brunklaus, 2022). We investigated whether NDEEMA occurs in other brain-expressed sodium channelopathies and characterized its clinical features.

Methods: Patients were identified through a systematic literature search, internal databases or an international network of epileptologists/geneticists, and clinical and genetic information was collected. A systematic literature review was conducted to review studies describing functional effects of these variants.

Results: In addition to 13 individuals already reported, we identified 19 more cases with NDEEMA, bringing the total number of patients to 32. These individuals carried 29 different variants in *SCN1A* (17)*, SCN2A* (6), *SCN3A* (1) or *SCN8A* (8) genes. 17 of 29 variants have at least one paralogous variant that also causes NDEEMA in other sodium channel genes.
Congenital arthrogryposis was present in 32/32 of the individuals, and was associated with additional features of fetal akinesia syndrome in 20/32 patients. 26/28 liveborn individuals developed neonatal epilepsy with tonic seizures and apneas, while 2 died in the first days of life and did not develop seizures. 11/32 patients reported a reduction in seizures with the use of sodium channel blockers, but epilepsy was refractory to treatment in all cases. All patients, for whom information is available, developed movement disorders, with myoclonus, dystonia, and tremor being the most common.
Interestingly, we identified 10 patients with the same variants as the patients with NDEEMA and neonatal-onset epilepsy, but no congenital arthrogryposis. Nevertheless, these individuals presented with severe developmental and epileptic encephalopathy.
A literature review of functional studies revealed that 11 of the 29 variants or their corresponding paralogs in other sodium channel genes had previously been functionally characterized and showed a gain-of-function effect.

Conclusions: We expand the spectrum of gain-of-function *SCN2A*/*SCN3A*/*SCN8A*-related epilepsy phenotypes to include NDEEMA.

P.038

FLUOXETINE AS A PRECISION-MEDICINE APPROACH FOR KCNT1-RELATED EPILEPSY

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Purpose: KCNT1 variants were associated with different epilepsy phenotypes, including EIMFS and focal epilepsy with sleep-related hypermotor seizures. KCNT1 channels are crucial for neuronal firing adaptation and can form heteromeric configurations with KCNT2 subunits. Pre-clinical studies demonstrated that fluoxetine can block currents in both wild-type and mutant KCNT2 channels carrying gain-of-function (GoF) variants. We aimed to assess fluoxetine as a precision medicine approach for patients with KCNT1-related severe epilepsies.

Method: Six unrelated patients aged between 18 months and 20 years with de novo KCNT1 variants were studied; three had EIMFS and three had drug-resistant focal epilepsy. The variants were inserted into plasmids for expression in mammalian cells, and patch-clamp recordings in CHO cells were conducted for functional and pharmacological characterization of the channels. The possible efficacy of fluoxetine to counteract in vitro variant-induced functional effects were tested; when this occurred, fluoxetine was tested as add-on therapy in patients carrying corresponding variants. Neuropsychological assessment, EEG and seizure diary were evaluated at baseline and after three months of treatment. ECG and blood levels of medications were monitored for safety.

Results: Electrophysiological experiments revealed that all variants prompt GoF effects. The in vitro exposure to fluoxetine (10 mM) prompted a significant blockade of currents expressed by either wild-type or mutant KCNT1 channels, thus counteracting variant-induced functional effects. In all patients there was a variable reduction of seizure frequency (25 to 50%), although transient in two patients. Improvement in visual attention and muscle tone was reported. Two patients developed movement disorders. One patient discontinued treatment after 10 months.

Conclusion: Fluoxetine may be an effective precision medicine approach for patients with severe epilepsies caused by GoF variants in KCNT1 channels. Further research is needed to assess long-term efficacy and safety.

P.039

CASE REPORT for the Molecular Therapeutic Board

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Case: 26yo male with refractory epilepsy onset age 5 years and a diagnosis of intellectual disability made by age 2 years. Epilepsy has subsequently been typed as Lennox-Gastaut Syndrome (last assessment in departmental MDT 2022).

Seizure types ever: -generalized tonic-clonic seizures, -tonic seizures, -atypical absences

Current seizures: probably mainly tonic, often clusters with 4-10 seizure days/month (likely underreported). Several emergency admissions in past 12 months

Investigations: Brain MRI (11/2012): right frontal suspected focal cortical dysplasia

Genetics: chromosomal microarray in childhood – normal; Whole exome sequencing + CNV analysis (Whole Exome plus, Blueprint Genetics 2021): heterozygous GABRG2 c.316G>A, p. (Ala106Thr), classified as pathogenic, confirmed de novo (ClinVar entry: VCV000205541.25 - ClinVar - NCBI (nih.gov)). Experimental evidence (Shen et al, Brain 2017; PMID: 27864268) support loss of function, including reduced maximal response to GABA (by 80.85%), reduced GABA current (by 30%), and reduced levels of gamma2-subunits (by 25%).

Previous ASM treatment: carbamazepine, topiramate, levetiracetam, clobazam acetazolamide, phenytoin, oxcarbazepine, rufinamide, prednisolone, VNS

Current ASM treatment: valproate, lamotrigine, zonisamide, clobazam (re-introduced following genetic diagnosis; led to reduction of seizures but causes significant somnolence/fatigue – taking 5mg/day), cannabidiol (introduced very slowly February 2024, effect to be determined – follow- up call this coming week)

P.040

FENFLURAMINE INCREASES SEIZURE-FREE DAYS IN PATIENTS WITH LENNOX-GASTAUT SYNDROME

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Background: Fenfluramine is approved for the treatment of seizures associated with Lennox-Gastaut syndrome (LGS) in patients ≥2 years of age in the US/Europe. Seizures associated with a fall were significantly reduced in fenfluramine-treated LGS patients in a randomized clinical trial (RCT) and its open-label extension (OLE). This post-hoc analysis evaluated percentages of seizure-free days (SFD) in the RCT and OLE.

Methods: Mean percentage SFD during baseline, titration and maintenance (T+M; RCT), and Month 2 to end of study (EOS; OLE) was measured in LGS patients 2-35 years. RCT: 4-week baseline followed by randomization (fenfluramine 0.7 mg/kg/day; fenfluramine 0.2 mg/kg/day; placebo), and 14-week T+M. OLE: 1-month initiation of fenfluramine at 0.2 mg/kg/day, then titration to effectiveness and tolerability. Fenfluramine doses: <0.3 mg/kg/day, 0.3-0.5 mg/kg/day, and >0.5 mg/kg/day.

Results: Mean change in percentage SFD from baseline to T+M in seizures associated with a fall, GTCS, and all countable seizures, respectively: 11.3, 5.3, and 7.5 [fenfluramine 0.7 mg/kg/day; n=87]; 8.2, 4.4, and 8.0 [fenfluramine 0.2 mg/kg/day, n=89]; 4.5, 0.6, and 4.1 [placebo, n=87].

Mean change in percentage SFD from baseline to EOS in seizures associated with a fall, GTCS, and all countable seizures, respectively: 9.7, 1.1, 7.7 [<0.3 mg/kg/day, n=68]; 12.3, 6.1, 9.8 [0.3-0.5 mg/kg/day, n=113]; 15.0, 6.7, 12.5 [>0.5 mg/kg/day, n=60].

Conclusion: Percentage SFD increased for all analyzed seizures. Increased percentage SFD may improve quality of life for patients/caregivers. An increase in GTCS-free days is particularly of interest due to the association between GTCS and sudden unexpected death in epilepsy.

Funding: UCB.

P.041

WHOLE EXOME SEQUENCING OF 135 PATIENTS AT SOUTHERN CHILE: RESULTS OF A 6-YEARS RESEARCH COLLABORATION WITH EMPHASIS IN PATIENTS WITH EPILEPSY.

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Introduction: Whole exome sequencing (WES) is the genetic analysis of first choice in patients with neurodevelopmental and other disorders, with a yield between 20 to 50% of positive cases. However, its commercial cost is still difficult to afford by families in developing countries. Herein we share our clinical experience with WES through a Chile-Japan research collaboration with emphasis in epilepsy cases.

Methods: WES was performed in patients with medical conditions of suspected genetic etiology. Sanger sequencing of the candidate genetic variants was performed in parents for segregation analysis.

Results: Between 2016 and 2022, we performed WES in 135 patients: 83 males, 52 females, with an average age of 7 years (range: 4 months to 23 years). The WES yield in our cohort was 37% (50/135 positive cases). Forty-five different genetic variants were identified, 22 of them novel (not previously reported). Sixteen patients with developmental and epileptic encephalopathies (DEE) and eleven patients with malformations of brain development were analyzed with positive results in 7 and 2 cases, respectively. Pathogenic and likely pathogenic variants were detected in the following genes: ITPA, CAD, SLC6A1 (3 cases), SPTAN1, UNC80 (2 cases), WDR45, MAST1 and MACF1. Of note, the patients with CAD deficiency (a treatable condition) and ITPA-related DEE, were post-mortem diagnoses.

Discussion: the overall yield of WES in our cohort is similar to that reported in other studies. We highlight the high proportion of novel variants and the benefits for our patients and institution of accessing diagnostic genetic analysis through a research collaboration.

P.042

THE NEURODEVELOPMENTAL SPECTRUM OF SYNAPTIC VESICLE CYCLING DISORDERS

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Advances in genomic technologies have enabled identification of many new genetic causes of neurodevelopmental disorders (NDDs). Genes associated with NDDs can be grouped into networks according to their molecular and cellular functions. Synaptic vesicle cycling (SVC) is one functional gene network in which rare, high penetrance variants cause a spectrum of NDDs. Understanding the phenotypic spectrum of SVC disorders could improve diagnostic yield, improve prognostication, and improve care.

In this project, we systematically describe neurodevelopmental phenotypes across 109 individuals with SVC disorders (14 different single gene diagnoses, including SYT1, TRIO, STXBP1 and DNM1) and compare these to 90 individuals with other monogenic NDDs (15 different single gene diagnoses). Quantitative questionnaire measures previously validated in NDD populations and clinical summaries were used to explore characteristics between and within groups.

Individuals within the SVC group were twice as likely to suffer from epilepsies and presented with a much higher prevalence of movement disorders compared to the non-SVC group. We observed a wide range of severity across all behavioural measures within both groups. The SVC group presented, on average, with more severe intellectual disability and visual impairment. The SVC group was not at higher risk of experiencing social-emotional or behavioural difficulties. We are now completing a data-driven analysis to capture the underlying dimensions of our measures. We will then assess associations and interactions between genetic diagnoses, neurological symptoms and behavioural variation. These analyses will help us to determine whether there is network-associated phenotypic homogeneity and predictable within-network variation.

P.043

MAPPING THE TRAJECTORY OF SYT1-ASSOCIATED NEURODEVELOPMENTAL DISORDER

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Synaptotagmin 1 (SYT1) is a presynaptic protein that mediates synaptic vesicle exocytosis and calcium-dependent neurotransmitter release. De novo missense variants in the SYT1 gene manifest as SYT1-associated neurodevelopmental disorder (Baker-Gordon syndrome [BAGOS]), which is characterized by infantile hypotonia, global developmental delay, ophthalmic deficits, early-onset involuntary movement disorders and EEG abnormalities in the absence of overt seizures. As more BAGOS cases have been diagnosed since the first reported case by our lab (Baker et al., 2015), the spectrum of clinical symptomology has expanded in breadth, diversity and temporal heterogeneity (Baker et al, 2018; Melland et al, 2022). However, there is limited information concerning the developmental trajectory of BAGOS, and relationships between clinical symptoms and brain structure / function.

The objectives of this project were to produce a data-driven developmental timeline for BAGOS, and to relate age-related clinical changes to evolution of MRI and EEG abnormalities. Clinical histories and standardised carer-report behavioural questionnaires were collected from 41 individuals diagnosed with BAGOS. Clinical MRI and EEG has also been collated wherever possible. Our main findings are that movement disorders, sleep disorders and autonomic symptoms evolve with time, accompanied by increasingly disturbed EEG. We have early evidence of subtle brain structural change over time which may parallel clinical evolution. Our next steps will be to collect systematic EEG data via a home-based protocol, and work in collaboration with experimental neuroscientists to understand the relationships between specific SYT1 variants, presynaptic dysfunction, patients' electrophysiology and variable symptom trajectories in this condition.

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