Phenotypic heterogeneity in SCN2A-Developmental & Epileptic Encephalopathy: A Function of Function

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Introduction

SCN2A-associated developmental & epileptic encephalopathy (DEE) is a phenotypically heterogeneous disorder due to variants that cause functional changes in the voltage-gated sodium channel $Na_V1.2$. The functional effects of variants may induce a gain or loss in channel function with some variants appearing to have a mixed impact on function.[1] A hypothesis derived from observations in 6 patients[2] suggests that gain of function (GoF) variants are associated with neonatal-onset epilepsy (NeoSz), mixed function (Mixed) variants with later-onset epilepsy (LateSz), often presenting with infantile spasms, and loss of function (LoF) variants are associated primarily with an autism spectrum disorder (ASD) presentation with or without seizures.

Objectives

- Phenotypic characterization of SCN2A-DEE-affected patients
- Determining the impact of variants on $Na_V1.2$ function
- Correlate the clinical phenotype with $Na_V1.2$ dysfunction

Methods

Two overlapping studies, the SCN2A Clinical Trial Readiness Study (CTRS, a longitudinal study) and the Global Survey (a one-time survey) were launched in April 2022. Parents were recruited on-line through the FamilieSCN2A Foundation

Eligibility

- Available genetic test report demonstrating variant pathogenicity
- English reading competency of parent/caregiver

- **Clinical phenotype from on-line surveys**
  - Epilepsy, seizure types, age at seizure onset
  - Functional abilities
    a. Mobility: Functional Motor Scale at 5 yards (FMS5),
    b. Communication: Communication Functional Classification System (CFCS),
    c. Hand use: Purposeful hand grasp (palmer or pincer) .
    d. Eating: Use of feeding tube (partial or exclusive), independence for feeding
  - Medical history: parent-reported diagnoses of
    a. Cortical Visual Impairment (CVI) or other non-ocular concerns about vision function and behavior,
    b. Movement and tone disorders (hypotonia, spasticity, dystonia)
    c. Scoliosis
    d. Dysautonomic features
- **Characterization of functional impact on $Na_V1.2$ function:**
  - *SCN2A* variants were transfected into HEK293T cells based on the adult isoform of the *SCN2A* gene.
  - Functional studies performed with automated patch clamp recording.
    - Primary criteria: Type of variant and, for missense variants, current density relative to wild-type (WT).
      - Severe Loss of Function (SvLOS): Whole gene deletions and truncating mutations or current density <0.25 relative to WT
      - Loss of Function (LOS): current density 0.25 to 0.79 relative to WT
      - Wild-type like (WTL): current density 0.80 to 0.19 relative to WT
      - Gain of Function (GOF): current density ≥ 1.2 relative to WT
    - Secondary criteria: For LOS and WTL variants, shifts in voltage-dependence of activation and inactivation, time constant of inactivation measured at 0 mV, and persistent sodium current were used to classify further as LOF, GOF, WTL, or Mixed impact.

Results

Of 81 SCN2A-affected individuals, 76 submitted a total of 63 unique variants that have been studied functionally.

CLINICAL PHENOTYPING

Demographics (N=76 subjects)

- Median age at enrolment: 6 years (IQR 3.5 to 9.7)
- Sex: 33 (43%) female
- Origin: 71% North American:

Seizure and Epilepsy Phenotypes

- 65 had a history of epilepsy
- Age of seizure onset was skewed to early life: Median 5.5 months (IQR 0 days – 16 months, max 108 months).

Primary/Composite phenotype

Neonatal onset epilepsy: 24 (32%)
Later onset epilepsy: 41 (54%)
Autism without epilepsy: 11 (14%)

Other medical, functional, and neurological morbidities

- Cortical Visual Impairment (CVI): 32 (42%)
- Dystonia: 12 (17%)
- Spasticity: 14 (20%)
- Scoliosis: 16 (21%)
- No purposeful hand grasp: 29 (40%)
- Exclusive G-tube feeding: 15 (20%).

In children ≥2 years-old (N=63),

- 48 (79%) were noncommunicative with close, familiar people
- 25 (40%)required a wheeled mobility device for household distances.

Poor communication and hypotonia were common regardless of the primary phenotype group. Other features and disorders were most common in the neonatal seizure-onset and least in the autism only group (Figure 1).

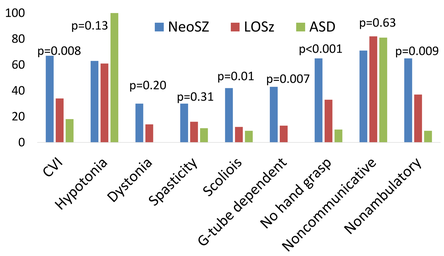


Figure 2. Medical, functional, and neurological morbidities across the three primary phenotype groups

FUNCTIONAL STUDIES OF VARIANTS

In the 76 SCN2A-affected individuals, there were 63 unique variants, including one full gene deletion, 11 frameshift, 6 nonsense, and 45 missense variants. There were nine recurrent variants six of which were found in two patients each, two in three patients each, and one was found in four different patients.

PRIMARY CRITERIA –variant type and current density

- All 11 frameshift, 6 nonsense, and 1 whole gene deletion were classified as SvLOF.

The 45 unique missense variants were expressed in HEK293T cells for automated patch-clamp analysis. Based upon current density alone variants were classified as.

GOF (>1.2) : N=1
LOF (0.25 – 0.79): N=16
SvLOF (<0.25) : N=31
WTL (0.80 – 1.19): N=15

SECONDARY CRITERIA – Additional properties of LOF and WTL variants:

- The 16 LOF and 15 WTL variants were further classified based on other functional parameters resulting in the reclassification of 2 LOF and 10 WTL variants (Figure 1).

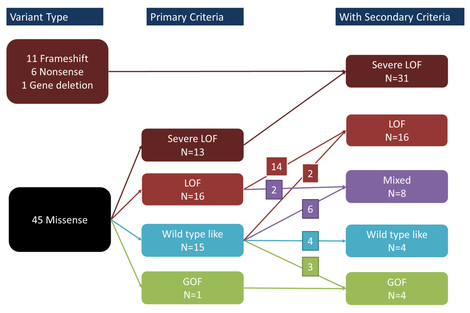


Figure 2. Impact on $Na_V1.2$ function based on primary and, for LOF and WTL missense variants, secondary criteria.

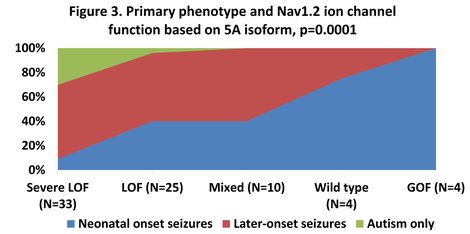
- Primary patient phenotype varies substantially with classification of the functional impact of variants on the $Na_V1.2$ channel. Any associations between channel classification and other phenotypic features (in Figure 1) were mostly due to the primary phenotype.

Table: Associations between primary phenotype and $Na_V1.2$ function

	Effect of variant on $Na_V1.2$ function (N, %)					
Primary Phenotype	GOF (N=4)	LOF (N=25)	SvLOF (N=33)	Mixed (N=10)	WTL (N=4)	p-value
Neonatal-onset seizures	4 (100%)	10 (40%)	3 (9%)	4 (40%)	3 (75%)	0.0001 8 d.f.
Later-onset seizures	0	14 (56%)	20 (61%)	6 (60%)	1 (25%)	
Autism only	0	1 (4%)	10 (30%)	0	0	
Infantile spasms						
Absent	4 (100%)	12 (48%)	28 (85%)	6 (60%)	3 (75%)	0.02 4 d.f.
Present	0	13 (52%)	5 (15%)	4 (40%)	1 (25%)	

- Ordering of phenotypic findings by approximate impact on $Na_V1.2$ channel function (Figure 3), emphasizes the divergence in clinical phenotype by functional impact of the *SCN2A* variant.

Figure 3. Primary phenotype and Nav1.2 ion channel function based on 5A isoform, p=0.0001



Discussion

- SCN2A-DEE is associated with substantial functional disability, which varies to a degree with occurrence of epilepsy and the age at onset of initial seizures.
- Functional impact of *SCN2A* variants is substantially associated with the occurrence of any seizures (versus ASD only), and age at seizure onset, as well as a history of infantile spasms.
- These findings roughly correspond (figure 3) to expectations from those of Begemann et al.[2] regarding GOF, LOF, and Mixed function variants.
- The Nav1.2 protein is present in two distinct isoforms during development, the neonatal (5N) and adult (5A). The HEK293T modeling was performed with the 5A isoform, which shows a depolarized voltage-dependence of activation compared to the adult isoform, which would result in reduced neuronal excitability. Repeat functional modeling with the 5N isoform may shift the classification toward GOF for some of the WTL and milder LOF variants and better reflect how they are expressed during early brain development.
- Implications for trials of $Na_V1.2$ modulating therapies include the need for functional characterization of variants of patients in such trials and the importance of accurate phenotyping so as to identify appropriate clinical outcomes and trial endpoints.

Citations

1. Sanders et al. Cell Press Rev, 2018
2. Begemann et al., Molec Med 2019.

Acknowledgment

Funded by the FamileSCN2A Foundation (all clinical data) and NINDS grant U54-NS108874 (all functional studies)