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\(_{1.2}\). 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.\(^\text{1}\) A hypothesis derived from observations in 6 patients\(^\text{2}\) suggests that gain of function (Gof) variants are associated with neonatal-onset epilepsy (Neos1), mixed function (Mixed) variants with later-onset epilepsy (Late2s), 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\(_{1.2}\) function
- Correlate the clinical phenotype with Na\(_{1.2}\) 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 FamiliesSCN2A Foundation.

### Flexibility
- 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 years (FMS5)
  - b. Communication: Communication Functional Classification System (IFCS)
  - 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\(_{1.2}\) 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).
- Secondary criteria: Function of WT 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, WT, 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**
- 69 had a history of epilepsy
- Age of seizure onset was skewed to early life: Median 5.5 months (IQR 9 days – 16 months, max 108 months).

**Primary/Composite phenotype**

<table>
<thead>
<tr>
<th>Type</th>
<th>Count</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonatal on-set epilepsy</td>
<td>24 (32%)</td>
<td></td>
</tr>
<tr>
<td>Later on-set epilepsy</td>
<td>41 (54%)</td>
<td></td>
</tr>
<tr>
<td>Autism without epilepsy</td>
<td>11 (14%)</td>
<td></td>
</tr>
</tbody>
</table>

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).

### Functional Studies of Variants

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 (N=12):**
- N=1
  - LOF (0.25 – 0.79): N=16
  - svLOF (<0.25): N=31
  - WTL (0.80 – 1.19): N=15

**LOF (N=1):**
- N=1
  - svLOF (0.25 – 0.79): N=16
  - WTL (0.80 – 1.19): N=15

**WTL (N=1):**
- N=1

**Mixed (N=2):**
- N=1

#### 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).

### 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.\(^\text{3}\) regarding Gof, LoF, and Mixed function variants.
- The Navi.2 protein is present in two distinct isoforms during development, the neonatal (5N) and adult (5A). The HEK293T modeling was performed with the SA 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 WT and milder LOF variants and better reflect how they are expressed during early brain development.
- Implications for trials of Navi.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

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