Biological concepts in human sodium channel epilepsies and their relevance in clinical practice

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Summary

Objective: Genetic variants in *SCN1A/2A/3A/8A* have been associated with heterogeneous epilepsy phenotypes and neurodevelopmental disorders (NDD). To better understand the biology of seizure susceptibility in sodium channel (*SCN)*-related epilepsies, our aim was to determine similarities and differences between sodium channel disorders, allowing us to develop a broader perspective on precision treatment than on an individual gene level alone.

Methods: We analysed genotype-phenotype correlations using large *SCN*-patient cohorts and literature datasets and applied variant constraint analysis to identify severe sodium channel disease. We examined temporal patterns of human *SCN*-expression and correlated functional data with the clinical phenotype.

Results: Comparing 865 epilepsy patients (504 *SCN1A*, 140 *SCN2A*, 171 *SCN8A*, 4 *SCN3A*, 46 copy number variation/CNV cases) and analysis of 114 functional studies allowed us to identify common patterns of presentation. All four epilepsy-associated *SCN*-genes demonstrated significant contstraint in both protein truncating (PTV) and missense-variation when compared to other *SCN*-genes. We observed that clinical characteristics associated with different *SCN* channels such as age at seizure onset are related to the corresponding *SCN*-gene expression over time. Individuals with GoF *SCN2A/3A/8A* missense variants or CNV duplications share similar characteristics and are most frequently seen in early onset epilepsy (<3 months) with good reponse to sodium channel blockers (SCBs). Direct comparison of corresponding *SCN*-variants across different *SCN*-subtypes illustrates that the functional effects of variants in identical channel locations are similar, however their clinical manifestation differs, depending on the neuronal network in which they are expressed.

Significance: Functional data that are recorded in a specific *SCN*-variant can serve as a surrogate for the function of a corresponding variant at the same position across different *SCN*-subtypes. Taking a broader view on precision treatment suggests that in those patients with a suspected underlying genetic epilepsy presenting with neonatal or early onset seizures (<3 months) SCBs should be considered.

Key words: *SCN1A*, *SCN2A*, *SCN3A*, *SCN8A*, epilepsy, neurodevelopmental disorders

Key points:

- Corresponding variants in *SCN1A/2A/8A* display similar function but result in different phenotypes depending on the neuronal network in which they are expressed.
- Variant function and location can serve as surrogate for variant effects across related sodium channels.
- Age at onset of sodium channel epilepsies correlates with *SCN* gene expression profiles.
- *SCN1/2/3/8A* show significant contstraint when compared to other sodium channel genes not linked to epilepsy.
- *SCN2A/SCN8A* GoF is commonest in early onset epilepsy (<3 months) and SCBs should be considered in affected individuals.

Introduction

Genetic variants in the genes *SCN1A*, *SCN2A*, *SCN3A*, and *SCN8A*, encoding the four neuronal voltage-gated sodium channels $Na_V1.1$, $Na_V1.2$, $Na_V1.3$, and $Na_V1.6$, are responsible for a significant fraction of early onset genetic epilepsies and neurodevelopmental disorders (NDDs)[1]. Modern sequencing techniques have revolutionized the way we diagnose the genetic causes for these disorders, opening the door to precision medicine. However, it is often difficult to predict the impact of a variant without prior functional characterization. Different variants within the same gene may cause distinct clinical disorders (pleiotropy) with different drug responses, while variants in different channel genes may result in similar phenotypes (genetic heterogeneity). This complexity is well established for the epilepsy related sodium channel genes and is challenging for the development of medical therapies.

The clinical phenotypes associated with different sodium channel (*SCN*) disorders have characteristic presentations. Dravet Syndrome (DS), a severe developmental and epileptic encephalopathy, is caused by *SCN1A* missense and protein truncation variants as well as deletions[2,3]. Missense variants in *SCN1A* also account for approximately 10% of generalized epilepsy with febrile seizure plus ($GEFS^+$) cases[4]. Moreover, small copy number variations (CNVs) including microdeletions within *SCN1A*, as well as large CNVs that include the nearby genes *SCN2A* and *SCN3A* on chromosome 2, are found in a small percentage of DS patient[5–8]. In *SCN1A*, both loss-of-function (LoF) missense and protein truncating variants (PTVs) lead to reduced sodium current in GABAergic interneurons resulting in a classical DS phenotype presenting in the first year of life with prolonged, febrile and afebrile, generalised clonic or hemiclonic seizures. The epilepsy is usually resistant to standard anti-epileptic medication and affected individuals develop cognitive, behavioural, and motor impairment[9,10]. A minority of gain-of-function (GoF) *SCN1A* missense variants have been described in familial hemiplegic migraine type 3 (FHM3)[11].

Variants in *SCN2A* have been identified in different forms of infantile epilepsy including benign infantile seizures, developmental and epileptic encephalopathies (DEEs), Ohtahara or West syndrome[12–14]. Recent studies propose that GoF missense variants in *SCN2A* are associated with neonatal or early infantile seizures at less than 3 months of age, whereas LoF missense and PTVs are associated with later onset epilepsy and ASD/NDDs[15–19]. *SCN8A* encephalopathy presents in infancy with multiple seizure types including focal, tonic, clonic, myoclonic absence seizures, and epileptic spasms[20–23]. The developmental outcome is poor and many patients have motor manifestations including hypotonia and movement disorders. A small number of patients have also been reported with milder phenotypes such as benign infantile seizures, paroxysmal dyskinesia, and isolated intellectual disability (ID)[24–26]. GoF missense variants appear to be associated with epileptic encephalopathy, whereas LoF variants are seen in NDDs without epilepsy[27,28]. *SCN3A*-associated epilepsies are clinically heterogeneous presenting with mainly GoF missense variants, early-onset seizures, epileptic encephalopathy, polymicrogyria and developmental impairment[29–32].

In order to better understand the biology of seizure susceptibility in *SCN*-related epilepsies our aim was to determine similarities and differences between sodium channel disorders and apply variant constraint analysis to identify severe sodium channel disease. This approach allowed us to develop a broader perspective on precision treatment than on an individual gene or variant level and to recognise common patterns among *SCN*-related disorders informing clinical practice.

Methods

Ethics statement

Retrospective review of anonymized clinical referral data and variant findings were approved by the relevant institutional review boards.

Study design and participants

We identified epilepsy patients carrying single nucleotide variants affecting *SCN1A/2A/8A* from two sites: the Danish Epilepsy Centre Filadelfia (Dianalund, Denmark) including case series by Møller et al.[33], Wolff et al.[16], Gardella et al.[34] (in print) and unpublished cases (supplementary table 5) and the Royal Hospital for Children (Glasgow, UK) including case series by Zuberi et al.[2] and unpublished cases (supplementary table 5). Diagnostic criteria have been published previously [2,16,35]. Additional *SCN1A* patients were included from the published case series by Depienne et al.[36]. In order to identify *SCN3A* variants, we performed a PubMed search (up to October 2019) using the terms "epilepsy" and "*SCN3A*". To enrich for high confidence disease-associated variants with large effect, we excluded *SCN* variants present in individuals from the general population. Specifically, we removed patients with variants observed in the Genome Aggregation Database (gnomAD, http://gnomad.broadinstitute.org).

We identified patients carrying copy number variants (CNVs) covering *SCN1A/2A/3A/8A* from three sites: The Boston Children's Hospital (Boston, USA); the Danish Epilepsy Centre Filadelfia (Dianalund, Denmark), and University Hospital Antwerp (Belgium). All local ethics boards approved the enrollment. We performed a literature review (using PubMed up to October 2019) and a DECIPHER database (v9.14)[37] search for individuals carrying a CNV covering *SCN1A/2A/3A/8A*. The following search terms were used: "CNV" in combination with one of the target *SCN*-genes ("*SCN1A*", "*SCN2A*", "*SCN3A*" or "*SCN8A*). Only patients with *SCN*-CNVs (<15 Mb) were included. The clinical phenotype information, including seizure onset and medication response, was collected (supplementary table 1).

Review of *SCN* functional missense variants

To collect functionally tested missense variants, we performed a PubMed screen (up to October 2019) with the terms "clamp" and "*SCN1A*", "*SCN2A*", "*SCN3A*" or "*SCN8A*" using R package RISmed 2.17. We included missense variants of the classic isoforms of *SCNs* from patients presenting with epilepsy and/or neurodevelopmental disorders, which have been functionally tested by whole-cell patch-clamp experiments. Variants observed in the general population, thus present in the Genome Aggregation Database (GnomAD, http://gnomad.broadinstitute.org), were removed from the analyses. We only included variants characterized in mammalian cell lines to improve biophysical comparisons. Variants were categorized either as gain-of-function (GoF), loss-of-function (LoF) or 'mixed' function regarding their biophysical properties. We define any biophysical change entailing an increase in the Na+ permeability as GoF, and the opposite for LoF. A few cases showed a paradoxical change i.e. decrease in the peak current and increase in the persistent current. Where one effect was not clearly dominant, these cases were classified as 'mixed' effect on function. Key electrophysiological features and patient phenotypes are detailed in supplementary table 2.

Variant constraint classification

Genes that have statistically fewer variants than expected are considered to be under evolutionary selection and thus associated with disease when mutated. The missense and PTV constraint scores were derived from the Exome Aggregation Consortium (ExAC). We considered *SCN*-genes with missense Z scores (intolerance to missense variation) ≥ 3.09 or the probability of being loss-of-function intolerant (pLI) scores ≥ 0.9 as intolerant of missense or PTV variants[38].

Statistical analysis

Non-normally distributed data, such as age at seizure onset, are given as median with semi-interquartile ranges (semi-IQR) and the Mann-Whitney U test was used to compute differences in age distribution by variant type and between genes. Variant enrichment and sodium channel blocker response was calculated using Fisher's exact test. Significance was tested at the 5% level and analysis performed using SPSS version 24.0.

Results

Phenotypes vs. *SCN* variant types analysis

We ascertained a total of 865 epilepsy patients that fulfilled the study criteria (supplementary table 3). These consisted of 504 *SCN1A* patients (Glasgow: 261, Denmark: 44, Depienne: 199), 140 *SCN2A* patients (Denmark), 171 *SCN8A* patients (Denmark), four *SCN3A* patients (literature) and 46 CNVs (Boston/Denmark/Belgium/literature).

SCN1A: Among the 504 patients with *SCN1A* variants 490 had DS and 14 GEFS+. Nearly all PTV carriers (99.6%) had DS, compared to 94% of missense carriers. Moreover, we observed a higher proportion of PTVs in *SCN1A* (53%) compared to *SCN2A* (9%, $p<0.001$) and *SCN8A* (4%, $p<0.001$).

SCN2A: Of patients presenting with *SCN2A* variants 50% (70/140) had developmental and epileptic encephalopathies (DEEs, including EOEE, EIMFS, OS, WS, LGS), 19% (26/140) benign epilepsies, 14% (20/140) other unclassified epilepsies and 17% (24/140) primary ASD features with later occurrence of epilepsy. A significantly higher proportion of PTV carriers had autistic features (9 out of 13; 69%) compared to the *SCN2A* missense variant carriers (15 out of 127; 12%; $p<0.001$).

SCN3A: Literature review identified a total of 14 patients with *SCN3A* variants. Six of these were found in gnomAD, three had no detailed age at onset data available and one was inherited from an unaffected father. Of the remaining four patients, three were *de novo*, all presenting within the first days of life with an epileptic encephalopathy and various features including focal seizures, microcephaly, polymicrogyria and developmental delay. The fourth patient presented much later at five years of age with a GEFS+ phenotype.

SCN8A: Among the 171 patients with *SCN8A* variants, 64% (110/171) had DEEs, 25% (42/171) intermediate phenotypes, 6% (11/171) benign epilepsies and 5% (8/171) other unclassified epilepsies.

CNVs: We identified 46 patients with seizures carrying *SCN*-CNVs (10 reported for the first time in this study and 36 from the literature and DECIPHER database[37]). The most commonly observed CNVs affected three genes, *SCN1-2-3A*, due to their clustered genomic locations within 1.4 Mb on chromosome 2q24.3 (supplementary table 1). Apart from *SCN1A* deletions associated with DS phenotypes, all other CNV cases exhibited a heterogeneous epilepsy phenotype with mild to severe neurological disorders such as ID, developmental delay (DD), dysmorphism, and coordination problems. We noted a difference in the reported response to sodium channel blockers depending on CNV type. Of the 13 patients with documented SCB use, a *"positive response"* to SCBs was exclusively seen in those with CNV duplications (9/13), whereas *"no response"* to SCBs was only seen in patients with CNV deletions (4/13, $p=0.006$, supplementary table 1).

Seizure onset vs. *SCN* variant types

Among *SCN*-missense variant carriers, we observed a significant pattern in the emergence of seizures over time: *SCN2A* carriers were the earliest to present with seizures (median 13 days), followed by *SCN8A* carriers (median 4 months; $p_{SCN2A \text{ vs. } SCN8A}<0.001$) and finally *SCN1A* missense carriers (median 6 months; $p_{SCN1A \text{ vs. } SCN2A}<0.001$; figure 1 and supplementary table 3). All three *de novo SCN3A* variants included in this report presented in the first days of life in keeping with the prenatal/neonatal *SCN3A* gene expression profile.

In *SCN2A* patients, missense variant carriers showed a significantly earlier age of onset (median 13 days) compared to PTV carriers (median 36 months; $p<0.001$), with two distinct peaks occurring in the neonatal and later infantile period. A similar pattern was observed between *SCN8A* PTV (median 11 months) and missense carriers (median 4 months; $p=0.04$). There was no difference in age of seizure onset among *SCN1A* missense and PTV carriers.

Patients carrying *SCN*-CNV duplications presented with seizures as early as *SCN2/3/8A* missense variant carriers (medians 3-17 days) and significantly earlier compared to those carrying CNV deletions whose seizure onset occurred much later (medians 3-10 months, $p_{del \text{ vs. } dup}<0.001$), similar to *SCN1/2/8A* PTV carriers (figure 1).

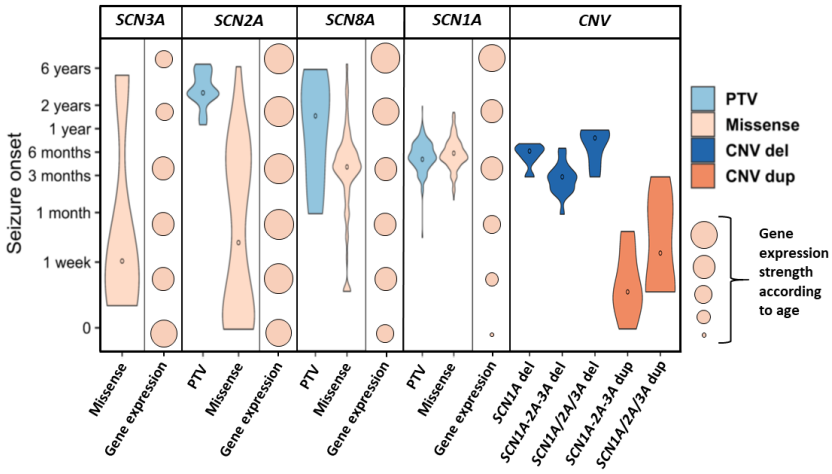

Figure 1 | Age at seizure onset of *SCN*-variant carriers and associated gene expression strength.

Phenotypes vs. functional *SCN* variant effects

We reviewed functional properties of 114 *SCN*-variants fulfilling our inclusion criteria. We identified 53 electrophysiologically tested *SCN1A* variants, 31 *SCN2A*, five *SCN3A* and 25 *SCN8A* variants. The majority of *SCN1A* epilepsy variants (75%) show a LoF of the $Na_V1.1$ channel and a minority show mixed effects (25%). In contrast, the majority of functionally tested epilepsy variants in *SCN2A/3A/8A* exhibit GoF features, 67%, 75% , and 76% respectively, suggesting that increased channel function is a common biophysical defect in *SCN2A/3A/8A*-associated epilepsy (figure 2A, supplementary tables 2 & 4).

Investigating the seizure onset of patients carrying different types of functional variants in the same gene, we observed no difference in seizure onset between *SCN1A* LoF and mixed variants (figure 2B). By contrast, all *SCN2A* GoF missense variants (N=16) were identified in early-onset epilepsy-ascertained patients (median 17 days), and 14 of those (88%) presented at <3 months of age, whereas *SCN2A* LoF variants (N=5) were identified in patients with later onset childhood seizures and NDDs (median 11 months, p<0.001). A similar trend not reaching significance was noticed in the *SCN8A* cohort, where GoF missense variants (N=13) were associated with early-onset epilepsy (median 3 months) compared to LoF (N=3, median 18 months, p=0.07). All seven *SCN8A* variants presenting at <3 months were GoF. The size of the *SCN3A* cohort was very small, however three out of four (75%) were GoF presenting with early onset epilepsy.

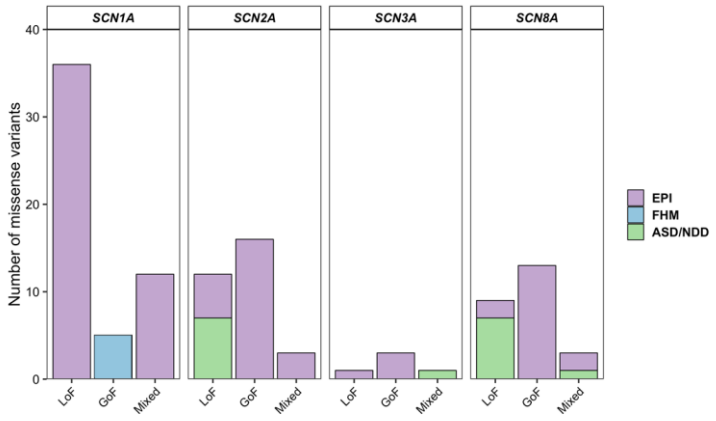

Figure 2A | Frequency of phenotypes according to *SCN* variants and function.

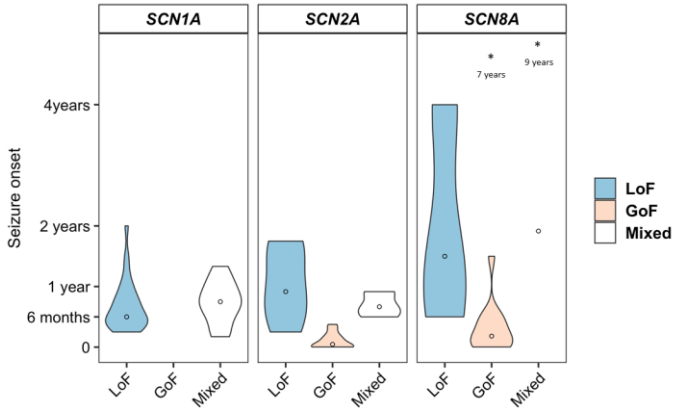

Figure 2B | Differential age at seizure onset according to *SCN* variants and function

Comparison of missense variants across *SCN1A*, *SCN2A* and *SCN8A*

We detected 8 pairs of missense variants with an identical/corresponding variant in a different *SCN*-gene: three *SCN1A/2A* pairs, four *SCN1A/8A* pairs and one *SCN2A/8A* pair (table 1; figure 3). The missense variants in each of those pairs appear to have similar functional consequences (3 GoF and 5 LoF effects). *SCN1A* LoF is associated with DS/GEFS+, while GoF variants are seen in FHM3. However, the corresponding LoF *SCN2A* and *SCN8A* variants lead to primary neurodevelopmental disorders/ASD whereas GoF variants result in severe early onset epilepsy (DEE).

To illustrate the distribution of missense variants and their function between the three different channel subtypes, we plotted the position of 138 *SCN1A* variants (Depienne 85/functional studies 53), 158 *SCN2A* variants (Denmark 127/functional studies 31) and 189 *SCN8A* variants (Denmark 164/functional studies 25) across the SCN-protein, showing that variants are mainly clustered in homologous domains (figure 3). Whilst *SCN1A* missense variants are distributed across the entire homologous domain, only very few *SCN2A/8A* variants are found in the S5-6 pore loop regions. Variants that occurred in the S5-6 pore loop regions appeared to be predominantly LoF, regardless of the channel subtype (89%, 16 out of 18), whereas variants that occurred for example in the voltage sensing S3-4, S4 and S4-5 regions harboured a mixture of GoF (17%), mixed (29%) and LoF (54%) effects (figure 3; supplementary table 2).

Table 1: Corresponding variants, phenotypes and function across different brain sodium channels

Pair	Gene/Variant	Function	Phenotype	Reference	Corresponding Gene/Variant	Function	Phenotype	Reference
1	***SCN1A***; L263V; D1 S5	GoF; WCC: Y, $\uparrow I_{NaP}$, $\leftarrow V_{1/2\ Act.}$, $\rightarrow V_{1/2\ FI}$	FHM3	Kahlig (2008)	***SCN8A***; L267V; D1 S5	Likely* GoF (Phenotype suggestive of GoF)	DEE; Sz onset 2.5 months, Sz reduction with SCBs	Denis (2019)
2	***SCN1A***; R946C; D2 S5-6	LoF; WCC: None	Dravet syndrome	Volkers (2011)	***SCN2A***; R937C; D2 S5-6	LoF; WCC: None	ASD	Begemann (2019)
3	***SCN1A***; R946H; D2 S5-6	LoF; WCC: None	Dravet syndrome	Volkers (2011)	***SCN2A***; R937H; D2 S5-6	LoF; WCC: None	ASD	Ben-Shalom (2017)
4	***SCN1A***; G979R; D2 S6	LoF; WCC: None	Dravet syndrome	Rhodes (2005)	***SCN8A***; G964R; D2 S6	LoF; WCC: None	NDD without epilepsy	Wagnon (2017)
5	***SCN1A***; Q1489K; D3-4 linker	GoF; WCC: Y, $\uparrow I_{NaP}$, $\leftarrow V_{1/2\ Act.,\ no\ change} V_{1/2\ FI}$	FHM3	Kahlig (2008) Cestèle (2013)	***SCN8A***; Q1470K; D3-4 linker	Likely* GoF (Phenotype suggestive of GoF)	DEE, Sz onset 1 day, Sz free with SCBs	Denis (2019)
6	***SCN1A***; P1632S; D4 S3-4	LoF; WCC: Y, $\leftarrow V_{1/2\ Act.}$, $\leftarrow V_{1/2\ FI}$,	Dravet syndrome	Rhodes (2005)	***SCN2A***; P1622S; D4 S3-4	LoF; WCC: Y, $\leftarrow V_{1/2\ FI}$,	ASD and Sz onset 21 months	Wolff (2017)
7	***SCN1A***; R1657C; D4 S4-5	LoF; WCC: Y, $\downarrow$CD, $\downarrow I_{NaP}$, $\rightarrow V_{1/2\ Act.}$, $\leftarrow V_{1/2\ FI}$	GEFS+	Lossin (2003)	***SCN8A***; R1638C; D4 S4-5	LoF; WCC: Y, $\rightarrow V_{1/2\ Act.,\ no\ change} V_{1/2\ FI}$	NDD without epilepsy	Wengert (2019)
8	***SCN2A***; R1882Q; C-Term	GoF; WCC: Y, $\uparrow$CD, $\uparrow I_{NaP}$, $\leftarrow V_{1/2\ Act.}$, $\rightarrow V_{1/2\ FI}$	DEE, Sz onset 1 day	Wolff (2017)	***SCN8A***; R1872Q; C-Term	GoF; WCC: Y, $\uparrow$CD, $\leftarrow V_{1/2\ Act.}$, $\rightarrow V_{1/2\ FI}$	DEE, Sz onset 4 months	Wagnon (2015)

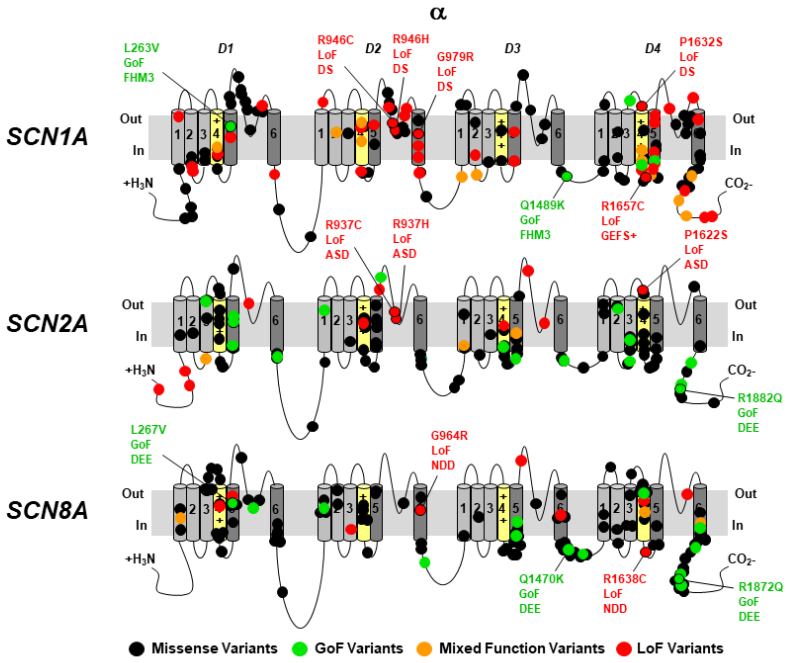

Figure 3 | Comparison of missense variants and function effects across *SCN1A/2A/8A*.

Phenotype vs. *SCNs* variant intolerance

Using constraint analysis we aimed to determine if there were common features between epilepsy-associated sodium channel genes and non-epilepsy-associated sodium channel genes. The *SCN*-family (*SCN1-11A*, 10 genes) shows a high degree of protein sequence conservation, especially in the transmembrane domains[39]. To understand why *SCN1A/2A/3A/8A* are particularly associated with severe early-onset *de novo* epilepsies and NDDs, we first evaluated variant intolerance of each *SCN*-gene. Among 60,000 individuals from the general population annotated in the ExAC database, *SCN1A*, *SCN2A*, *SCN3A* and *SCN8A* all show strong depletion for PTV (pLI score >0.9) and missense variants (missense Z-score >3.09; figure 4). This suggests strong evolutionary constraints on epilepsy associated *SCN*-genes in contrast to variants in *SCN4/9/10/11A* that are tolerated for both truncating and missense variants and mainly associated with familial (less severe) *SCN*-disease.

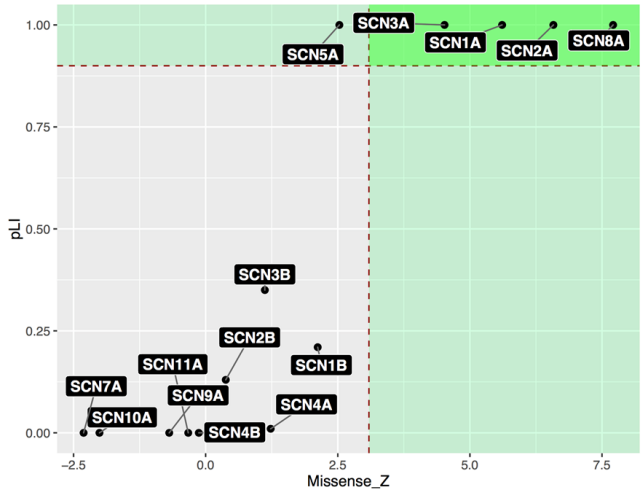

Figure 4 | Variant constraints of *SCNs*.

Discussion

Genotype-phenotype correlations across the four brain-expressed *SCNs* reveal distinct patterns of functional effects. The majority of *SCN1A*-related epilepsies are caused by LoF missense variants, full gene deletions, and PTVs. The clinical features of DS patients are consistent, presenting at similar ages regardless of variant type. GEFS+ patients tend to present later and carry mainly missense variants[2,40]. Only a small minority of *SCN1A* variants present with an epilepsy phenotype different from the GEFS+/DS spectrum. The variant T226M was recently reported in patients presenting with a more severe early infantile epileptic encephalopathy than typical *SCN1A* Dravet syndrome[41]. This variant has been shown to have some gain-of-function effects, resulting in cells that are no longer able to fire action potentials due to accumulation of channels in inactivated states. Subsequently a mixed effect is observed where in some conditions the currents can be larger, however ultimately leading to a loss of neuronal activity[42,43].

By contrast, the majority of *SCN2A/3A/8A*-associated early-onset epilepsies including benign epilepsies and epileptic encephalopathies are caused by GoF missense variants and full gene duplications. The PTVs in *SCN2A/3A/8A* do not lead to a clinically defined epilepsy syndrome but to heterogeneous NDDs including autism with or without later onset seizures[16,18,23,28,44]. Moreover, in the *SCNs* CNV cohort, we observed that patients with duplications presented with significantly earlier seizure onset and responded better to sodium channel blockers compared to patients with deletions. This early seizure onset is likely caused by duplication of the *SCN2A/SCN3A* genes, which are the earliest *SCNs* expressed during development, resulting in GoF effects due to SCN2A/SCN3A protein overexpression[45].

Variant effects across different channel subtypes

Our direct comparison of corresponding *SCN*-variants across different sodium channel subtypes illustrates that the functional effects of variants at identical channel locations are similar, however their clinical manifestation differs, depending on the neuronal network they are expressed in. The same functional effect, for example LoF due to an *SCN1A* variant at a specific location will lead to DS in an inhibitory network. However, a variant in *SCN2A* at the same location, displaying the same LoF function effect, will lead to NDD/ASD while expressed in an excitatory network. Only very few GoF variants are seen in *SCN1A* presenting with milder FHM3 phenotypes[11] suggesting that GoF may be better tolerated in inhibitory networks compared to excitatory networks, where they lead to severe DEE. Our findings suggest that functional measurements that are recorded in a specific *SCN*-variant may serve as a surrogate for the function of a corresponding variant at the same position across different *SCN*-subtypes.

Comparing the distribution of missense variants among the different *SCN*-subtypes revealed that whilst variants are mainly clustered in homologous domains (particularly the voltage sensing and pore regions), there is a difference in distribution between *SCN1A* and *SCN2A/8A*. *SCN1A* variants are frequently seen in the S5-6 intervening pore loop that is vital for channel function, whereas only very few *SCN2/8A* variants are observed in this region. Voltage gated sodium channels have a central pore surrounded by four pore-forming modules composed of S5 and S6 segments and an intervening S5-6 pore loop. This loop forms a large extracellular funnel with an ion selectivity filter vital to control ion selectivity[46]. Almost all variants reported in this region lead to LoF, underscoring its functional significance. Previously we were able to show that missense variants in the S5-6 pore loop region are enriched in *SCN1A*[2] in keeping with LoF being the key mechanism in *SCN1A* variants. This is different for *SCN2A* and *SCN8A* variants, who frequently present with both GoF and LoF properties. This split between GoF and LoF effects is also seen in the cardiac sodium channel *SCN5A* where GoF variants cause LQT3 and LoF variants Brugada syndrome. Loss-of-function Brugada syndrome variants are mainly observed in the S5-6 pore loop, whilst no pore loop variants are seen in gain-of-function LQT3 carriers[47]. We observe the same effect in *SCN2A/8A*, where variants in the S5-6 pore loop region appear to be mainly LoF, implying that variants in this region often lead to LoF across different SCN[48] .Sodium channel blockers are unlikely to be effective in patients with LoF variants in this region. Contrary to previous work, we observe that variants in the S4 region are not associated with one predominant effect, but a range of LoF, mixed and GoF effects, suggesting that function is determined by the individual variant change, rather than a particular S4 region effect[48].

Age-specific expression of sodium channels

In human fetal brains, *SCN1A* is expressed at a lower level compared to *SCN2A/3A/8A*, and steadily increases throughout childhood into adult life[49,50]. This differential gene expression profile is mirrored in the phenotypical seizure presentation, as the earliest seizure onset is observed in patients carrying variants in *SCN2A* (and *SCN3A*), followed by *SCN8A* and *SCN1A* respectively (figure 1). *SCN1A* is predominantly expressed in inhibitory neurons, whereas, *SCN2A/3A/8A* are predominantly expressed in excitatory neurons. However, iPSC-work has shown that increased excitability of principal neurons equally contributes to network hyperexcitability in DS[51]. The distinct developmental- and neuronal type-specific expression of *SCN1A* may explain the phenotypic differences and variations in drug response with exacerbation of seizures in DS patients due to SCB therapy[9,16,19,52].

Epilepsy patients with distinct types of *SCN2A* variants display a significantly differential seizure manifestation: GoF missense carriers usually present within the first two months after birth, whereas LoF missense carriers present on average nine months later, while PTV carriers exhibit seizures typically after 3 years[16,17]. Furthermore, CNV duplications covering *SCN2A* are associated with neonatal onset seizures. This mirrors Allen Brain Atlas data illustrating that *SCN2A* is highly expressed in the prenatal stage, in particular at mid/late fetal-neonatal stage. We observed 2 distinct peaks of presentation among *SCN2A* missense variants: those presenting early-on (<3 months) with GoF and those presenting later with LoF. Contributing to the different ages of onset and clinical symptoms may be the two different developmental expression patterns of $Na_V1.2$ channels in myelinated and unmyelinated nerve fibers[16,53,54]. Recent work showed that early infantile epilepsy patients carrying *SCN2A* GoF missense variants responded well to SCBs, compared to late-onset patients carrying LoF variants[16,19]. Thus, taken together, the association between *SCN2A* and early seizure onset can be mostly explained by the early developmental expression of *SCN2A* and elevated channel function due to GoF variants and duplications.

SCN2A/8A expression correlations

Patients with *SCN8A* missense variants have later onset seizures compared to *SCN2A* carriers in keeping with work by Liao *et al.* demonstrating that $Na_V1.2$ is expressed early in axon initial segments of excitatory neurons while $Na_V1.6$ is not expressed early on but becomes the predominant excitatory channel during development[53]. Moreover, an *in vivo* study identified that $Na_V1.2$ channels could replace missing $Na_V1.6$ channels at nodes of Ranvier and axon initial segments of neurons in *SCN8A* knockout mice[55]. This *SCN2A/8A* co-expression might offer a reciprocal rescue mechanism for both, *SCN2A* and *SCN8A* variants and is clinically reflected in the good response of both epilepsies to SCBs, particularly for those presenting with early onset GoF[9]. Taken together, the correlated expression profiles and phenotypic similarities suggest that $Na_V1.2$ and $Na_V1.6$ appear to compensate partially upon the disruption in either *SCN2A* or *SCN8A* function.

SCN constraint analysis aids variant interpretation

Our results show that the marked evolutionary constraint among *SCN*-genes suggests variants identified in *SCN1A/2A/3A/8A* are intolerant of both truncating and missense variants and more likely to be associated with dominant early-onset *de novo* disorders such as severe epilepsy and NDDs. *SCN5A* are intolerant of LoF variants seen in life threatening Brugada syndrome[47]. By comparison, variants in familial *SCN* disease such as *SCN4A* periodic paralysis/myotonia or *SCN9/10/11A* related pain disorders are better tolerated for both truncating and missense variants (figure 4)[47]. Our analysis further supports the emerging evidence that *SCN3A*, which shows strong depletion for PTV and missense variants, is a good candidate gene for epilepsy even though only a few patients have been reported to date[29–32,56].

Additionally, the variant constraint results indicate that, besides *SCN1A/2A/3A/8A*, other members of the *SCN*-gene family are unlikely to cause severe epilepsy/NDDs. For example, *SCN9A* has an established role in familial pain disorders[47], however, its pathogenicity in severe forms of epilepsy has never been confirmed. Using variants in >60,000 individuals from the general population, we observed that *SCN9A* variant numbers were similar to variant numbers expected by chance. This suggests variants in *SCN9A* are less likely to contribute to severe

epilepsy compared to variants in *SCN1A/2A/3A/8A.* Therefore, in clinical practice, constraint analysis could aid interpretation of *SCN*-variants in diseases, which are under negative natural selection.

Clinical relevance and implications for precision medicine

We observe common patterns across different *SCN*-related disorders allowing us to take a broader view on precision treatment beyond single genes or variants, supporting specific recommendations. Patients with *SCN1A*-positive DS whose epilepsy usually starts with febrile seizures after 3 months, is caused by loss of inhibitory neuronal function and responds well to benzodiazepines but worsens with SCBs[9,57,58]. Among *SCN2A* variant carriers the responsivess to medication appears to be more complex and directly linked to variant function. Those with early onset seizures (<3 months) due to GoF effects appear to respond well to SCBs whereas those with later onset epilepsy and NDDs due to LoF variants often remain treatment resistant[16–19,44]. There are only limited reports on pathogenic *SCN3A* variants, however most of these present within the first days of life due to GoF effects and there is evidence to show that mutant channels may respond to SCBs[32]. Recent case series of patients with *SCN8A* variants clearly demonstrate how variants associated with NDDs showed LoF effects, whereas those associated with epilepsy showed GoF effects with good response to SCBs[20,24,26,28,59].

This study presents clinical and experimental evidence that GoF *SCN2/3/8A* variants and copy number duplications respond well to sodium channel blockage. We can show that the likelihood of an *SCN2A* or *SCN8A* variant being GoF is particularly high in very young children <3 months of age (88% and 100% respectively) and SCB treatment is recommended in infants where an *SCN2A* or *SCN8A* variant has been confirmed.

We would argue that once emergency AED management and imaging/metabolic tests have been completed in a young child presenting with seizures in the first 3 months of life, and a genetic diagnosis seems likely, there is a rationale to consider SCB treatment. At this early stage genetic testing results are often not available and may take weeks and months to conclude. However, there is robust population and cohort based evidence showing that the genetic epilepsies commonly presenting at this early age (<3 months) are *KCNQ2*, *KCNQ3*, *CDKL5*, *SCN2A* and *STXBP1*, but not *SCN1A*[60,61]. These young infants will in the majority of cases respond to SCBs without the expectation for seizures to worsen when SCBs are given. The theoretical risk of seizure exacerbation due to SCBs is comparatively low, given that *SCN1A* variants are unlikely to present at this young age. We suggest that in those patients with a suspected underlying genetic cause presenting with neonatal or very early onset seizures (<3 months) SCBs should be considered, whereas in later onset epilepsy SCBs appear mainly effective in *SCN8A* related disease and are contraindicated in Dravet syndrome.

Abbreviations

DS: Dravet Syndrome

GoF: Gain-of-function

LoF: Loss-of-function

CNV: Copy Number Variation

NDDs: Neurodevelopmental Disorders

PTVs: Protein Truncating Variants

SCBs: Sodium Channel Blockers

SCN/Nav: Sodium Channel

EOEE: Early onset epileptic encephalopathy

EIMFS: Epilepsy of infancy with migrating focal seizures

OS: Ohtahara syndrome

WS: West syndrome

LGS: Lennox-Gastaut syndrome

IS: Infantile spasms

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Confilicts of Interest

Nothing to report.

Ethical Publication Statement

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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Figure Legends

Figure 1 | Age at seizure onset of *SCN*-variant carriers and associated gene expression strength.

Legend: Seizure onset age scale (y-axis is log10 transformed), PTV = protein truncating variant carriers, Missense = missense variant carriers, CNV del = copy number variant deletion carriers, CNV dup = copy number variant duplication carriers, Number of patients: *SCN1A* = 504, *SCN2A* = 140, *SCN3A* = 4, *SCN8A* = 171, CNV = 46. Gene expression strength shown by age (timepoints: Preterm, 0-4 months, 4 months-1year, 2-3 years, 4-8 years, >8 years). The larger the circle the stronger the gene expression (Epilepsy-associated *SCNs* exhibit specific development-dependent gene expression patterns; RNA-seq expression data obtained from Allen Brain Atlas; http://www.brainspan.org/static/download.html).

Figures 2A & B | Summary of electrophysiologically tested *SCN1A/2A/3A/8A* variants in the literature.

Figure 2A | Frequency of phenotypes according to *SCN* variants and function.

Legend: EPI = epilepsy, FHM = familial hemiplegic migraine, ASD = autism spectrum disorder, NDD = neurodevelopmental disorder, LoF = loss-of-function, GoF = gain-of-function, Mixed = mixed function (Supplementary table 2 and 4).

Figure 2B | Differential age at seizure onset according to *SCN* variants and function

Legend: Seizure onset age scale (y-axis is log10 transformed), LoF = loss-of-function, GoF = gain-of-function, Mixed = mixed function. Number of patients: *SCN1A* = 40, *SCN2A* = 24, *SCN8A* = 18 (Supplementary table 2 and 4).

Figure 3 | Comparison of missense variants and function effects across *SCN1A/2A/8A*.

Legend: Identical/corresponding variant pairs across different SCNs are highlighted (as per table 1; the sequence between *SCN1A/2A/8A* variants differs slightly which is why the corresponding numbers are not the same), LoF = loss-of-function, GoF = gain-of-function, DS = Dravet syndrome, FHM3 = familial hemiplegic migraine type 3, GEFS+ = genetic epilepsy with febrile seizures plus, DEE = developmental and epileptic encephalopathy, ASD = autism spectrum disorder, NDD = neurodevelopmental disorder.

Figure 4 | Variant constraints of *SCNs*.
Constraint missense Z-scores and pLI scores for *SCN* genes in the general population (60, 000 individuals in ExAC database). High missense Z-scores (>3.09, x-axis) suggest that genes are intolerant of missense variants. High pLI scores (> 0.9, y-axis) suggest that genes are intolerant for protein-truncating variants. The missense and PTV constrained group contains four epilepsy-associated genes, *SCN1A/2A/3A/8A*.