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A surrogate for personalized treatment of sodium channelopathies

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Abstract

Sodium channelopathies are a common genetic cause of paroxysmal disorders of the brain, peripheral nervous system, muscle, and heart. The phenotypes produced depend on a combination of the channel affected and its functional consequence; unfortunately, for missense variants, the latter is not clinically available. However we will show that the location of a missense sodium channel variant can be used as a surrogate for functional studies. We present data from epilepsy to illustrate clinical and treatment implications of sodium channel variants, the relationship between function, location and treatment response, then generalize this to other sodium channelopathies.

INTRODUCTION

Voltage-gated sodium channels have been implicated in numerous inherited paroxysmal disorders of the nervous system, muscle, and heart. Our goal is to provide a framework that helps neurologists understand the clinical and treatment implications of sodium channel variants the encounter in clinical practice. This will be accomplished through our objectives of (i) recognizing the relationship between location of a missense sodium channel gene variant and its effect on channel function, and (ii) categorizing clinical phenotype based on functional effect of a variant. The relationship between location, function and treatment response is also discussed. These interactions can be illustrated by the sodium channelopathies seen in people with epilepsy but generalize beyond that disorder.

Multiple mutations in genes that encode α-subunits sodium channels within the central nervous system have been associated with early onset epileptic encephalopathies and certain

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POTENTIAL CONFLICTS OF INTEREST

Nothing to report

autosomal dominant epilepsy syndromes. The α-subunits protein folds into four homologous domains[1]. Each domain consists of six transmembrane segments (termed S1 through S6) connected by small extracellular and intracellular linkers identified by the transmembrane segments they separate. The four domains are linked by large intracellular loops. The transmembrane S4 segment is the main voltage sensor that triggers activation. The adjacent region transmembrane segment S4 and S5 (the S4–5) acts as an electromechanical couple translating depolarization to channel opening. Studies examining the effects of scorpion toxins have shown that the S3–4 segment plays an important role in the movement of S4 during inactivation. The pore through which sodium ions transverse the membrane consists of portions of transmembrane segments S5 and S6 and loop connecting S5–6. The sodium channel α-subunit genes *SCN1A*, *SCN2A*, and *SCN8A* are those most frequently associated with epilepsy.

Kanai noted that missense mutations in the pore of *SCN1A* were associated with more severe epilepsy phenotypes[2]. Using a larger database, Zuberi and colleagues demonstrated that epilepsy-associated missense *SCN1A* variants occurred most commonly in the pore region and voltage sensor than in other locations[3]. Meng and colleagues demonstrated that variants in the pore were more likely to be associated with a severe epilepsy phenotype and noted that these are functionally most often associated with a loss of channel function. In contrast, variants in the voltage sensor region (which they defined as the S3–4, S4 and S4–5 regions) were unlikely to demonstrate a complete loss of function[4].

Although *SCN1A*, *SCN2A*, and *SCN8A* genes are highly conserved, the main epilepsy-associated functional consequences of *SCN1A* variants differ from those of *SCN2A* and *SCN8A*[5]. At the extreme, nonsense and frameshift mutations typically lead to loss of function and are common in epilepsy associated with *SCN1A*, accounting for about half of the cases of *SCN1A*-associated early onset epileptic encephalopathies (EOEE). In addition, many of the missense variants in *SCN1A* that cause EOEE result from complete or partial loss of channel function[4]. In contrast, functional consequences of missense variants in *SCN2A* and *SCN8A* are different, with variants associated with EOEE demonstrating electrophysiological changes that most often result in a gain of function[6–8].

The differing physiological effects can have therapeutic implications. *SCN2A* variants associated with early onset epileptic encephalopathies respond to sodium channel modulating antiepileptic medications. In contrast, when epilepsy is seen with loss-of-function *SCN2A* variants, the seizures have a later onset and tend to respond more poorly to sodium channel agents[8]. Similarly, loss-of-function variants in *SCN1A* respond poorly to sodium channel modulators[9]. It is unknown if these functional differences are reflected in different distribution of epilepsy-associated *SCN2A* and *SCN8A* missense variants as well. To examine this question, we compared the distribution of *SCN1A*, *SCN2A*, and *SCN8A* variants associated with epilepsy and compared the types of neuronal sodium channel gene variants in people with epilepsy and other neurological disorders.

DISTRIBUTION OF VARIANTS IN CENTRAL NERVOUS SYSTEM SODIUM CHANNEL GENES

Pathogenic and potentially pathogenic missense variants in *SCN1A, SCN2A*, and *SCN8A* were identified from the Human Gene Mutation Database (http://www.hgmd.cf.ac.uk; accessed 3/22/16) and from the open access Database of Chromosomal Imbalance and Phenotype in Humans (DECIPHER) v9.14 data (http://decipher.sanger.ac.uk; accessed 4/28/17). Additional pathogenic variants in *SCN8A* were also identified from www.scn8a.net (accessed 2/17/17) and in *SCN8A* and *SCN2A* from recent publications. After review of literature associated with each variant, only variants that could be classified as likely pathogenic or pathogenic for epilepsy based on criteria provided by the American College of Medical Genetics[10] were included in the epilepsy dataset. Some variants were identified in people with neurological disorders other than epilepsy. These were examined separately. If a variant was identified but the presence or absence of seizures could not be determined for the phenotype provided, it was excluded. A comparative dataset of sodium channel variants from 60,706 people free of severe pediatric disease was obtained from the Exome Aggregate Consortium (ExAC; http://exac.broadinstitute.org;[11]).

Domains in the voltage-gated sodium channel amino acid sequence were defined from the SWISS-PROT database (last accessed 12/6/17) and then grouped according to approximate functional domains based on previously published groupings[4]. The pore region was defined as segments S5, S5-S5, S6; the voltage sensor region (VSR) as S4 and its associated linkers (S3–S4 and S4–S5). Other transmembrane segments and their linking regions were grouped (TMO) and the intracellular loops linking domains I–IV were grouped together (Loops). The N-terminus (N) and C-terminus (C) were grouped separately. Thus 6 different regions (VSR, pore, TMO, loops, N, and C) were compared.

Multiple different amino acid substitutions were seen at some locations but the amino acid location was only included once during statistical analysis. Because the number of amino acids differed between each region, the relative frequency of variants was calculated by dividing the number of variants per amino acid in a particular segment by the number of variants per amino acid in the entire protein. This approach permitted a comparison between different channels despite differences in the overall number of variants identified between the channels. This method is similar to that reported elsewhere[3, 5]. A 2 × 6 Fisher's Exact Test was used to compare the distribution of variants across the entire channel. A 2 × 2 Fisher's Exact Test or chi square test (based on the number of variants) was used to compare specific regions within the channels. The p-values listed are based on Fisher's Exact Test unless noted otherwise.

The number of missense, non-sense, frameshift and splice site variants in *SCN1A*, *SCN2A* and *SCN8A* as well as their associated phenotypes are summarized in Table 1. Of the pathogenic or likely pathogenic epilepsy-associated missense variants identified, 386 *SCN1A* variants occurred at unique amino acid locations within NaV1.1, 102 of the 116 epilepsy-associated *SCN2A* variants occurred at unique amino acid locations within NaV1.2, and 42 of the 46 epilepsy-associated *SCN8A* variants occurred at unique amino acid locations within NaV1.6. All nine *SCN1A* variants and all three *SCN8A* variants that

did not have epilepsy as part of their phenotype occurred at unique locations as did nine of 11 *SCN2A* variants. An additional six novel variants in *SCN2A* were identified from exome studies in people with autism[16, 17]; however, detailed information about their phenotypes was not available to ascertain whether participants had concomitant epilepsy. These were excluded from our analysis. In the population-based ExAC database, 372 variants at 328 unique locations in *SCN1A*; 287 variants at 253 unique locations in *SCN2A*, and 249 variants at 219 unique locations in *SCN8A* were identified.

Epilepsy-Associated *SCN1A*, *SCN2A* and *SCN8A* variants

The distribution of epilepsy-associated variants for each gene are shown in Figure 1A. There were no statistical differences between the distribution of epilepsy variants between *SCN2A* and *SCN8A* (p=0.28); therefore, these were combined for subsequent comparisons. The distribution of epilepsy-associated variants in *SCN1A* was significantly different than in *SCN2A/SCN8A* (p<0.0001; Chi-square). The differences were driven by differences in the VSR and pore. Consistent with prior observations, the VSR and pore were the predominant locations in *SCN1A* to harbor variants pathogenic for epilepsy. For *SCN1A*, these regions both had an almost 2-fold enrichment of epilepsy-associated variants compared to the protein as a whole (1.7-fold increase in VSR; 1.8-fold increase in the pore). In contrast, while epilepsy-associated pathogenic variants in the VSR region were common in *SCN2A/8A* (4.0-fold increase; p <0.0001 compared with *SCN1A*), variants in the pore were slightly less likely to occur in *SCN2A/8A* compared to the genes in their entirety (0.97-fold decrease; p <0.001 compared with *SCN1A*).

Overall, there was relative sparing of the intracellular loops for all genes (0.29-fold decrease in *SCN1A* and 0.40-fold decrease in *SCN2A/8A*) and this distribution was not different between the genes (p = 0.26). However, within the intracellular loop that connects domains III and IV there is a region that acts as the inactivation gate[18]. This loop also has been identified as a common region for prolonged QT syndrome-associated variants in *SCN5A*[19]. Comparing the epilepsy and population based databases, this loop has significantly more epilepsy-associated variants than the other intracellular loops for both *SCN1A* (p < 0.01) and *SCN2A/8A* (p<0.001).

The distribution of variants within the segments of the pore and VSR is illustrated in Figure 2. The most notable difference between the pore regions for the sodium channels occurred in the extracellular S5–6 loop; for *SCN1A,* 123 of the 185 (66%) of the epilepsy-associated variants located in the pore occurred in the S5–6 segment. In contrast, only seven of the thirty-four (21%) epilepsy-associated pore variants in *SCN2A/8A* occurred in the S5–6 region (Odds Ratio (OR) 9.1; p<0.0001; Confidence Interval (CI) 4.2–20.2; Chi-Square). The frequency of epilepsy-associated variants in S4–5 was somewhat more common in *SCN2A/8A* compared with *SCN1A* (OR 2.1; 95% CI 1.1–3.9; p =0.02, Chi Square).

Population-Based *SCN1A*, *SCN2A* and *SCN8A* variants

The distribution of variants identified from the ExAC database are shown in Figure 1B. As was the case with the epilepsy-associated variants, there were no statistical differences between the distribution of epilepsy variants between *SCN2A* and *SCN8A* (p=0.27; Fisher's

Exact Test); therefore, these were combined for subsequent comparisons. In contrast to the epilepsy-associated variants, the overall distribution of variants within ExAC did not differ between *SCN1A* and *SCN2A/8A* (p=0.24). With the exception of the pore region, the distribution of variants in ExAC was largely the inverse of that seen with epilepsy associated variants. Regions with relative sparing in the epilepsy group were overrepresented in the population-based dataset and vice versa. For example, in the ExAC database the intracellular loops had a relative variant rate of 1.4 for both *SCN1A* and *SCN2A/8A* and VSR had a relative variant rate of 0.54 for *SCN1A* and 0.30 for *SCN2A/8A* when compared to the proteins as a whole. However, variants in the pore regions were infrequent in both *SCN1A* (0.66-fold decrease) and *SCN2A/8A* (0.58-fold decrease) within the ExAC database.

Non-epilepsy associated pathological *SCN2A* and *SCN8A* missense variants

An additional 11 missense variants (at nine unique locations) in *SCN2A* and three missense variants in *SCN8A* were identified in people with intellectual disabilities who did not have concurrent epilepsy; these were absent from the ExAC database. Although the number of variants is small, the variants seen in people with developmental disabilities without epilepsy tended to be more common in the pore (OR 4.3; CI 1.5–13; p = 0.009) than in other regions when compared to epilepsy-associated variants in *SCN2A/8A*.

FUNCTIONAL EFFECTS OF NON-EPILEPSY ASSOCIATED PATHOLOGICAL MISSENSE VARIANTS IN THE PORE REGION

Frameshift, splice site, and nonsense variants typically result in a loss of function and these have also been reported in *SCN2A* and *SCN8A*. In contrast to *SCN1A* (where all reported frameshift, nonsense, and splice-site variants have epilepsy as part of the phenotype; Table 1), these types of variants in *SCN2A/8A* are not associated with epilepsy in the majority of cases (30 of 45; p <0.0001). For those who did have epilepsy, the onset of seizures tended to occur at over one year of age[8]. The non-epileptic phenotypes associated with frameshift, nonsense, and splice-site variants in *SCN2A* and *SCN8A* included developmental delay, autism, dysmorphic appearance, and schizophrenia. While the effects of missense variants (in *SCN1A, SCN2A*, and *SCN8A*) seen in people with epilepsy have been well studied, less is known about the effects of missense variants seen in people with developmental disorders without epilepsy. To examine the relationship between location, function, gene, and phenotype we selected two *SCN2A* variants (R937C and C1731Y) for *in vitro* functional evaluation. These variants were selected because they were located in the S5–6 region of the pore and were identified in people with developmental delay without epilepsy. These two factors would predict a loss of channel function. The functional studies were done using methods previously described by others (see Acknowledgments)[12–15]. $Na_V1.2$ currents in cells transfected with wild-type or mutant cDNAs were recorded and representative traces are shown in Figure 3. Not all cells that were successfully transfected with the beta subunits (which contained the markers that identified transfected cells) also appeared to be transfected with adequate amounts of cDNA for the alpha subunit (which produces the $Na_V1.2$ current). The percentage of tested cells exhibiting quantifiable sodium currents (defined as peak current ≤−400 pA) was significantly lower for R937C and C1731Y as compared to WT channels (WT, 30%, n = 53; R937C, 0%, n = 45, p < 0.0001; C1731Y, 0%,

n = 40, p <0.0001; Wilcoxon Rank Sum Test). Of the sixteen cells transfected with WT cDNA which had suitable macroscopic currents, the mean current density was −136 ± 33 pA/pF. Similar to R937C and C1731Y, none of the non-transfected cells produced a quantifiable current. Thus, these pore-region *SCN2A* variants did result in the predicted loss of function.

DISCUSSION

Sodium channelopathies are a common cause of early onset epileptic encephalopathies and some self-limited epilepsy syndromes. Here we demonstrated that epilepsy-associated missense variants mutations *SCN2A* and *SCN8A* tend to occur in the main voltage sensor and adjacent linking regions while variants in the pore region are underrepresented in epilepsy. In particular, the S5–6 segment in *SCN2A* and *SCN8A* are largely spared from epilepsy-associated mutations. In contrast, epilepsy-associated *SCN1A* variants occur preferentially from the voltage sensor region and associated linkers and throughout the entire pore. In contrast to *SCN2A* and *SCN8A*, the most common area for *SCN1A*-associated epilepsy is in the S5–6 region of the pore. Few variants in the voltage sensor region or pore are seen in the population-based ExAC database for any of these sodium channels indicating an evolutionary disadvantage for mutations in the pore regardless of the sodium channel gene. This implies that severe childhood onset disorders other than epilepsy may result from *SCN2A* and *SCN8A* variants in the pore region. Severe developmental disability is one such possibility. *SCN2A* is one of the most commonly identified gene in exome sequencing studies examining the genetic contribution to neurodevelopmental disorders[16, 17, 20, 21].

Functional difference may underlie the varied clinical presentations and differences in distribution of the epilepsy-associated sodium channelopathies. The majority of people with *SCN2A* variants predicted to produce loss of channel function (nonsense, frameshift, and splice-site variants) do not have epilepsy[8]. In addition, missense variants in people with *SCN2A* variants seen in non-epileptic individuals with developmental delay result in loss of channel function. Ben-Shalom and colleagues demonstrated that autism-associated *SCN2A* variants in the pore region resulted in loss of channel function[22]. Our findings support their observations. Similarly, two *SCN8A* missense variants seen in people with developmental disabilities without epilepsy have been studied and these variants also produced non-functional channels[23]. These observations support the notion that loss-of-function *SCN2A* and *SCN8A* variants are more commonly associated with conditions other than epilepsy. In contrast, all variants in *SCN1A* which result in complete loss-of-function result in epilepsy[4]. Gain-of-function variants in *SCN1A*, *SCN2A*, and *SCN8A* are also associated with epilepsy[4, 7, 8].

In addition to differing clinical phenotypes, functional consequences of *SCN1A*, *SCN2A*, and *SCN8A* gene variants have important therapeutic implications. Early onset epileptic encephalopathies associated with pathogenic *SCN1A* variants are thought to be poorly responsive to antiepileptic treatments that modulate sodium channel function. In contrast, many patients with *SCN2A* and *SCN8A*-associated early onset epileptic encephalopathies tend to respond better to anticonvulsant medications and the associated missense variants have shown a gain-of-function in *in vitro* functional assessments[6]. Wolff and colleagues[8]

recently showed that there are important phenotype and treatment response differences in people with gain-of-function *SCN2A* variants compared to those with loss-of-function variants. Gain-of-function variants tended to present with earlier onset epilepsy (< 3 months of age) and had a better response to sodium channel modulating anti-epileptic treatments. In contrast, when epilepsy was present in people with loss-of-function variants, the seizures had a later age of onset and were often exacerbated by treatment with sodium channel modulators. Although these functional effects have significant implications for personalization of therapy for sodium-channel epileptic encephalopathies, at present there are numerous obstacles to widespread use of *in vitro* functional studies for missense variants in clinical care[24].

The different distribution of epilepsy-associated variants in *SCN2A/8A* compared to *SCN1A* provides an opportunity to aid in selection of therapies without laborious functional studies. Loss-of-function missense variants often localize to the pore region (especially the S5–6 region); while variants with a gain-of-function (or mixed loss- and gain-of-function) are seen in the voltage sensor (S4) and adjacent linkers. This suggests that certain locations of variants within the resultant protein can be a surrogate when more detailed functional studies are not available. Sodium channel blocking drugs should be avoided in pore-region variants but may be useful for some VSR variants. Even a small subset of people with *SCN1A* mutations could respond favorably to sodium channel modulation, provided the variant is in a region that predicts gain- of-function (e.g. in S4–5). Unfortunately, the functional consequences of variants outside of the pore and S4–5 are less predictable. This approach may also have treatment implications beyond epilepsy. Behavioral disorders can be seen in people with autism and developmental delay and sodium channel modulators such as lamotrigine are potential treatment options. Avoiding these agents in children with *SCN2A* or *SCN8A* variants without epilepsy may be sensible.

The association between functional consequences, clinical phenotype, and location of variant generalizes to sodium channelopathies outside of the central nervous system as well (Table 3)[5]. For example, cardiac conduction disorders associated with *SCN5A* variants depend upon the physiological effect of the variant. Loss of function variants are associated with Brugada Syndrome and gain of function variants are associated with Prolonged QT Syndrome Type 3. The distribution of prolonged QT associated variants shares similarities with the epilepsy-associated gain of function *SCN2A* and *SCN8A* variants, with many variants occurring in the VSR and a there is a relative sparing of the S5–6 region in the pore[25]. In addition, variants in the DIII–DIV are vastly over-represented in prolonged QT syndrome. *SCN5A* variants associated with Brugada Syndrome are more widely distributed within the transmembrane regions; however, analogous to *SCN1A* variants in epilepsy, *SCN5A* variants located within the pore region are associated with a more complete loss of function and a more severe clinical phenotype[26]. Similarly, gain of function variants in *SCN4A* are associated with a number of paroxysmal neuromuscular conditions (such as hyperkalemic periodic paralysis, hypokalemic periodic paralysis, and paramyotonia congenita) and in *SCN9A* are associated with paroxysmal pain disorders (such as paroxysmal extreme pain disorder and inherited erythromelalgia). The majority gain of function variants in *SCN4A* occur within the VSR and pore with relative sparing of S5–6[27]. In addition to the VSR, disorders associated with gain of function variants in *SCN9A* are

also common in the DIII–IV linker. In contrast to the other sodium channels, loss of function variants in *SCN4A* and *SCN9A* are seen primarily when both alleles have a loss of function variant. Taken all together, the clinical phenotype of sodium channelopathies associated with variants in the VSR and DIII–IV linking differ from those seen with variants in the S5–6 portion of the pore.

A limitation to this approach is that the pore and associated electromechanical coupling regions have not been clearly delineated. There is evidence that the intracellular portions of the transmembrane segments S5 and S6 contribute to the coupling of depolarization to channel opening. However, a comprehensive analysis of these regions in neuronal sodium channels is not available. The epilepsy-associated *SCN2A* and *SCN8A* variants in S5 and S6 are more common toward the intracellular part of the these transmembrane regions; however, better definition of this area and characterization of the functional consequences of variants in the region are needed before functional effects can be inferred from the location of a particular variant in the proximal S5 and distal S6 areas.

Symptoms produced by sodium channel variants depend on a combination of channels affected and the functional consequence of the mutation on channel function. This difference is reflected in the distribution of missense variants within the channels. In *SCN2A* and *SCN8A* epilepsy-associated variants are clustered in the voltage sensor region, and spare the pore region; especially the S5–6 segment; and are frequently associated with a gain-of-function; while epilepsy-associated *SCN1A* variants common in both the voltage sensor region and the pore and result from loss-of-function, gain-of-function, or mixed changes. In contrast, loss-of-function variants *SCN2A* and *SCN8A* variants are more often seen with neurological disorders other than epilepsy; as a result, few variants in the pore are seen in people with epilepsy caused by *SCN2A* or *SCN8A* mutations. This distinction has important clinical implications. Epilepsy resulting from variants in the pore region may respond poorly to sodium channel blocking antiepileptic medications while those in the voltage sensor region may respond more favorably to these agents. Location of the variant could also be important for drug studies in people with sodium channelopathies because it provides a simple way of focusing on an even more homogeneous subject population.

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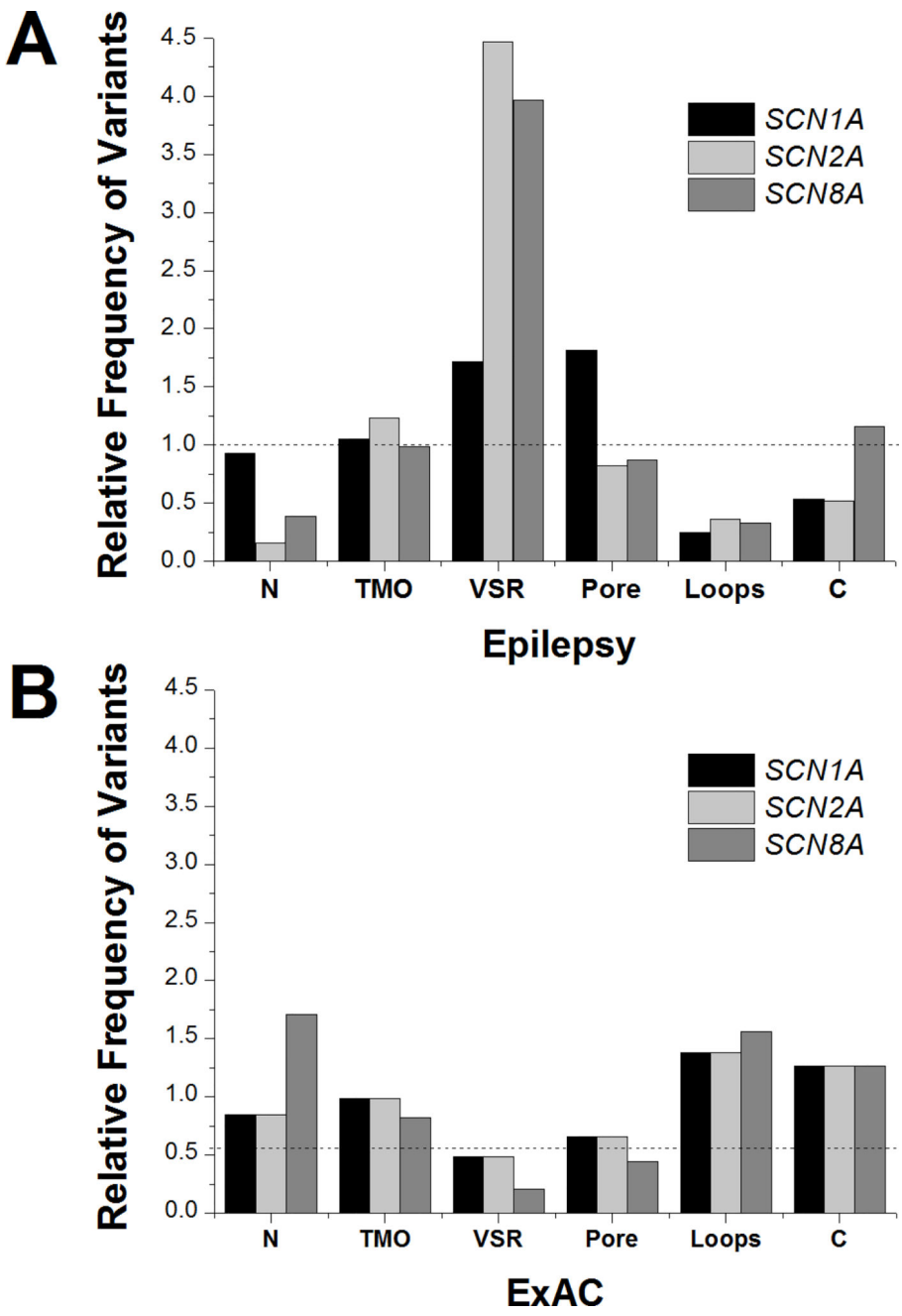

FIGURE 1.

Distribution of (A) epilepsy-associated missense variants and (B) population-based missense variants in *SCN1A*, *SCN2A* and *SCN8A*. The dashed line represents the variant frequency over the entire protein.

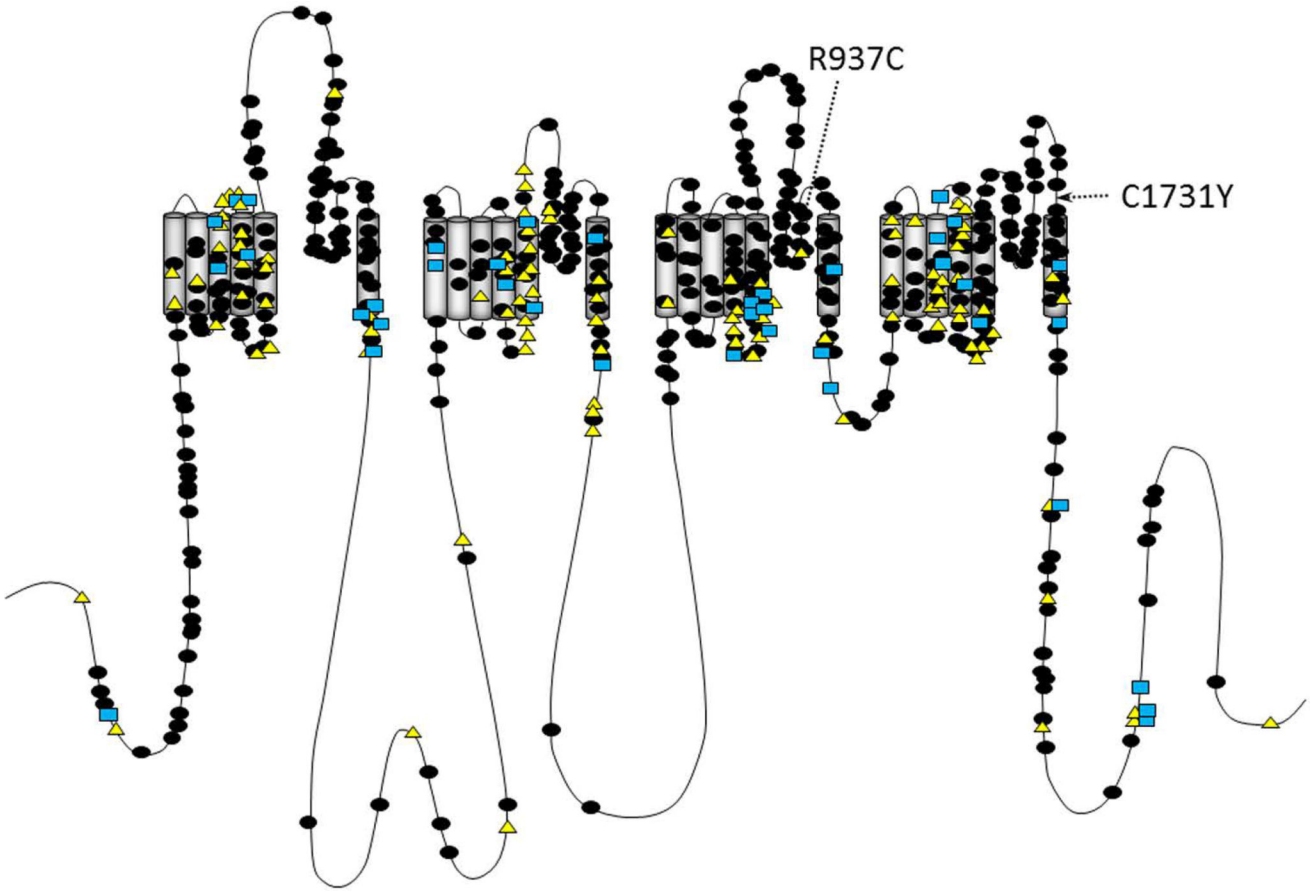

FIGURE 2.

Predicted transmembrane topology of NaV showing the location of epilepsy-associated variants in *SCN1A* (filled circles), *SCN2A* (triangles) and *SCN8A* (squares). Location of variants examined here are identified by the arrows.

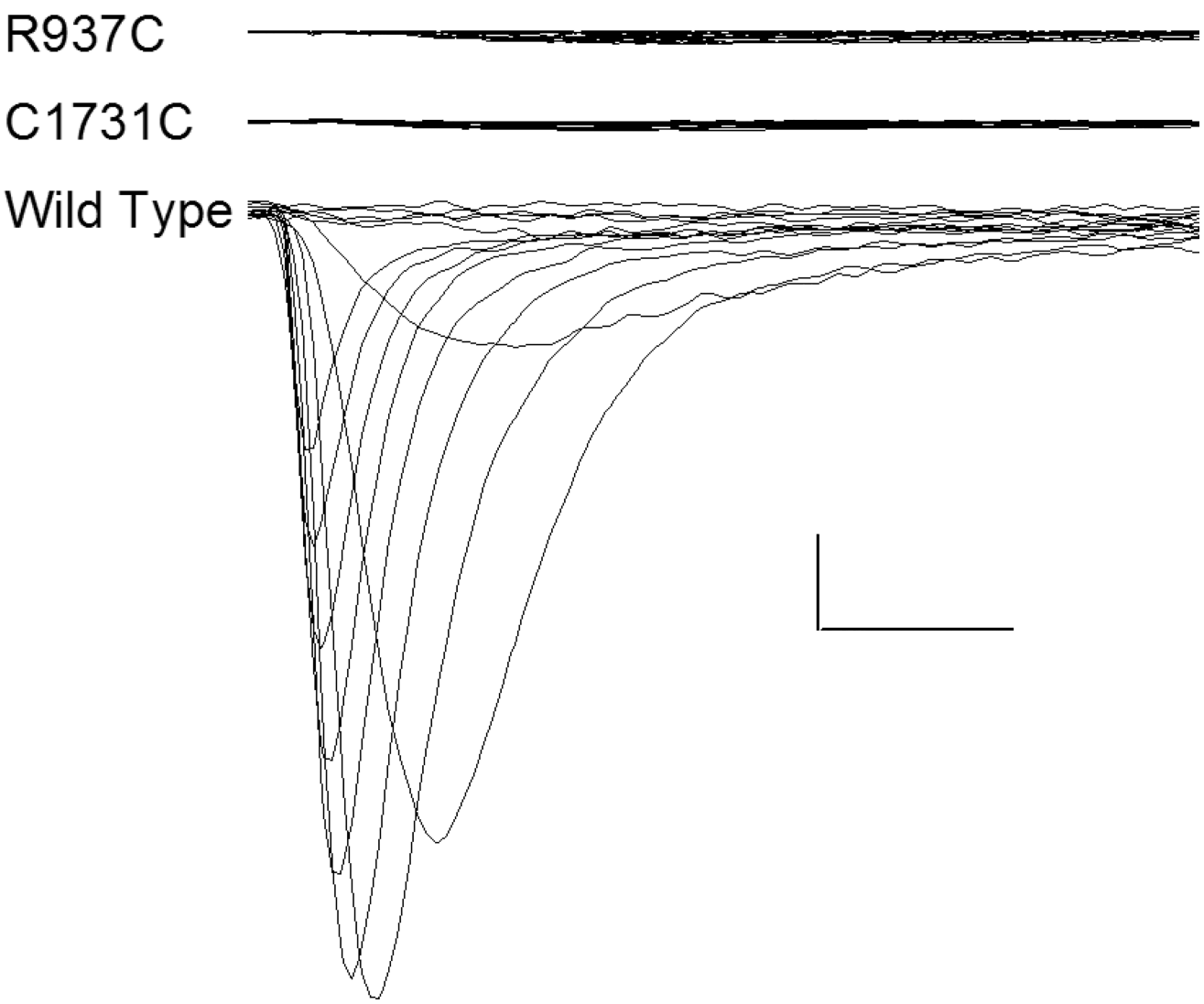

FIGURE 3.

NaV1.2 activation currents in cells transfected with wild-type (WT), R937C, and C1731Y plasmids. The R937C and C1731Y variants have been reported in children without epilepsy. The location of these non-epilepsy associated variants are identified in Figure 2 by the arrows. Currents were activated by voltage steps to between −80 and +60 mV from a holding potential of −120 mV. Calibration bars 200 pA (vertical), 1 ms (horizontal). To ensure that transfection or mutagenesis errors were not the cause of the low currents for R937C and C1731Y, transfections were done with separate preparations of DNA, and transfections were repeated three to four times.

Table 1

Comparisons of Functional Effect, Clinical Phenotype and Variant Type in *SCN1A*, *SCN2A* and *SCN8A* Channelopathies

		PHENOTYPE				
Variant type	**Effect on Channel Function**	**EOEE**	**B(F) NIS (*SCN2A/8A*) or GEFS + (*SCN1A*)**	**Epilepsy (other)**	**Other; No epilepsy**	**Number of variants**
		SCN2A/8A				
Missense	Gain, loss or mixed: dependent on location	65 % (n=113)	15 % (n=26)	12 % (n=22)	8 % (n=14)	175
Nonsense/frameshift/splice site	Complete loss	0	0	33 % (n=15)	67% (n=30)	45
		SCN1A				
Missense	Gain, loss or mixed: dependent on location	83 % (n=434)	9 % (46)	6 % (n=34)	2 % (n=9)	523
Nonsense/frameshift/splice site	Complete loss	93 % (n=476)	1 % (n=7)	6 % (n=28)	0	511

Abbreviations: EOEE = early onset epileptic encephalopathy as associated syndromes; B(F)NIS = Benign (familial) neonatal/infantile seizures; GEFS+ = Genetic Epilepsy with Febrile Seizures Plus; Other; No epilepsy, includes developmental delay, schizophrenia, autism spectrum disorders, ataxia and familial hemiplegic migraine with no history of seizures.

Table 2

Simplified Framework for Functional Effect, Location, and Phenotype of Variants in Sodium Channelopathies*

	SCN1A	SCN2A	SCN4A	SCN5A	SCN8A	SCN9A
Predominant Location of gene	Central nervous system	Central nervous system	Muscle	Heart	Central nervous system	Dorsal Root Ganglion
LOSS OF FUNCTION						
Type of variant	Nonsense and Frameshift more than missense	Nonsense and Frameshift more than missense	Few reports in literature	Missense more than Nonsense	Missense more than Frameshift	Nonsense more than missense
Common location of Missense variants	P > VSR	P	Few reports in literature	Distributed throughout TM	P	P (S5–6)
Prototypic phenotype	EOEE	ASD, DD with or without later onset seizures	Normal (het) Congenital Myopathy (hom / biallelic)	Brugada Syndrome	DD, ataxia	Normal (het) Congenital indifference to pain (hom / biallelic)
GAIN OF FUNCTION**						
Type of variant	Missense	Missense	Missense	Missense	Missense	Missense
Common location of missense variants	VSR	VSR; sparing of S5–6	VSR > P with sparing of S5–6 and S6	DIII–DIV linker, VSR, S6	VSR; sparing of S5–6	DIII–DIV linker, VSR > P with sparing of S5–6
Prototypic phenotype	EOEE > GEFS+	EOEE> B(F)NIS	Paroxysmal neuromuscular disorders	Prolonged QT Syndrome 3	EOEE	Paroxysmal pain disorders

*There is considerable molecular and clinical heterogeneity of the variants. The table lists the most frequent associations but this is not exclusive. This has been compiled from multiple sources[5, 19, 26, 27, 29–32];

**Many missense variants in VSR have complicated functional effects with mixed electrophysiological changes but most exhibit some gain of function properties.

Abbreviations: As in Table 1, in addition, DD = Developmental delay; ASD= Autism spectrum disorders; P = pore (S5, S5–6, S6); VSR= voltage sensor region (S3–4, S4; S4–5); TMO= transmembrane; het= heterozygous, hom=homozygous.