Opposing Effects on NaV1.2 Function Underlie Differences Between SCN2A Variants Observed in Individuals With Autism Spectrum Disorder or Infantile Seizures

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ABSTRACT

BACKGROUND: Variants in the SCN2A gene that disrupt the encoded neuronal sodium channel NaV1.2 are important risk factors for autism spectrum disorder (ASD), developmental delay, and infantile seizures. Variants observed in infantile seizures are predominantly missense, leading to a gain of function and increased neuronal excitability. How variants associated with ASD affect NaV1.2 function and neuronal excitability are unclear.

METHODS: We examined the properties of 11 ASD-associated SCN2A variants in heterologous expression systems using whole-cell voltage-clamp electrophysiology and immunohistochemistry. Resultant data were incorporated into computational models of developing and mature cortical pyramidal cells that express NaV1.2.

RESULTS: In contrast to gain of function variants that contribute to seizure, we found that all ASD-associated variants dampened or eliminated channel function. Incorporating these electrophysiological results into a compartmental model of developing excitatory neurons demonstrated that all ASD variants, regardless of their mechanism of action, resulted in deficits in neuronal excitability. Corresponding analysis of mature neurons predicted minimal change in neuronal excitability.

CONCLUSIONS: This functional characterization thus identifies SCN2A mutation and NaV1.2 dysfunction as the most frequently observed ASD risk factor detectable by exome sequencing and suggests that associated changes in neuronal excitability, particularly in developing neurons, may contribute to ASD etiology.

Keywords: Autism spectrum disorder, Epilepsy, NaV1.2, electrophysiology, SCN2A, Seizure

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but see (26). In contrast, mutations observed in ASD/developmental delay are either PTVs resulting in loss of Naᵥ1,2 function or missense mutations of unknown effect. These observations raise the hypothesis that SCN2A variants exert opposing effects on Naᵥ1,2 function in infantile seizures and ASD.

Here, we used bioinformatics, electrophysiology, and compartmental modeling to test how different SCN2A variants affect the function of Naᵥ1,2 channels. We report that the majority of missense mutations observed in ASD completely blocked channel conductance, while the remainder altered Naᵥ1,2 biophysical properties in ways that dampened channel function. Regardless of mechanism, all ASD-related variants impaired neuronal excitability in computational models of developing pyramidal cells. These impairments largely resolved because Naᵥ1,6 replaced Naᵥ1,2 in the distal AIS of mature models, supporting the notion that ASD is a consequence of disruption to early brain development leading to persistent neurodevelopmental dysfunction (27). Results from these de novo missense mutations in 8 individuals, along with 4 individuals with de novo PTVs, identify SCN2A mutations in 0.3% of ASD cases (12 of 4109 cases with exome data). SCN2A therefore has the strongest evidence of ASD association of any gene discovered by exome sequencing and is the most frequent single gene contributor to ASD, after fragile X mutations.

METHODS AND MATERIALS

Briefly, wild-type (WT) and variants of SCN2A harboring an ASD-associated mutation were cotransfected with β1-β2 subunits in HEK293 cells. Sodium channel function was assessed with voltage-clamp techniques and immunohistochemistry, and then incorporated into a pyramidal cell computational model. Please see Supplemental Methods in Supplement 1 for details.

RESULTS

A comprehensive literature review identified 148 SCN2A variants (117 unique) in 148 families (Table S1 in Supplement 2). Building on the description by Howell et al. (11), variants clustered in four phenotypic groups: 20 variants in families with BIFS; 8 variants in individuals with infantile seizures and mild developmental delay and/or episodic ataxia; 51 variants in individuals with EE characterized by infantile seizures and moderate to severe developmental delay, often accompanied by central hypotonia, cerebral atrophy, and microcephaly; and 39 variants in individuals with a developmental disorder characterized by ASD and/or intellectual disability. This latter group includes a few individuals with comorbid seizures with onset after the age of 1.5 years, a frequent occurrence in both ASD and developmental delay. A further 16 variants lack sufficient phenotypic information to assign them to one of these four groups, while 15 variants do not clearly match these groups (e.g., febrile seizures, herpes encephalitis, schizophrenia).

Of the 20 variants observed in BIFS, 19 (95%) are missense variants and 1 is a 507 kbp duplication; 18 (90%) of the variants are inherited with the variant segregating with phenotype in multiple affected family members. BIFS missense variants cluster in the transmembrane segments and short connecting loops (16 of 19 variants; 2.3-fold over expectation; p = .0004, binomial test; Figure 1A), particularly in and between transmembrane segments 4 and 5 that are proximal to the voltage sensor (11 of 19 variants; 4.7-fold over expectation; p = 4 × 10⁻⁵, binomial test; Figure 1A).

Similarly, variants observed in EE are mainly missense variants (49 of 51 variants, 96%) along with one 2.6 Mb duplication and one PTV; however, unlike BIFS variants, most are de novo (48 of 49 variants with inheritance data, 98%). EE missense variants also cluster in and between the transmembrane segments (39 of 49 variants; twofold enrichment; p = 2 × 10⁻⁶, binomial test; Figure 1A), particularly segments 4 and 5 (24 of 49 variants; fourfold enrichment; p = 4 × 10⁻¹⁰, binomial test; Figure 1A). While the variants in BIFS and EE cluster in similar domains of the Naᵥ1,2 protein, there are no examples of a variant contributing to both disorders, despite multiple recurrent variants in different families being observed in both BIFS (4xR1319Q) and EE (4xR853Q, 3xE999K, 3xL1342P, 3xR1882Q). Seven BIFS and three EE variants have been characterized electrophysiologically (Tables S1 and S2 in Supplement 2), largely revealing gain of function thought to result in neuronal hyperexcitability [(7,21–25); but see (26)].

In contrast to BIFS and EE, over half of the ASD-associated variants are predicted to prevent translation of one SCN2A allele, with 12 PTVs and two deletions (14 of 27 variants, 52%). Exome Aggregation Consortium (28) and Residual Variation Intolerance Score (29), two methods that use large-scale population data to identify constrained genes that are intolerant of deleterious variation, both predict that SCN2A is highly intolerant of such haploinsufficiency, with 30-fold fewer PTVs observed in an adult population than was expected. Consistent with a highly deleterious effect, all PTVs and deletion variants are de novo (13 of 13 variants for which data are available; Table S1 in Supplement 2). The remaining 13 (48%) variants in ASD cases are missense (Table S1 in Supplement 2), and most of these are also de novo (12 of 13 variants, 92%). Unlike the missense variants in BIFS and EE, ASD-missense variants cluster in the pore loop that forms the sodium ion selectivity filter (8 of 13 variants; 3.8-fold enrichment; p = .0004 binomial test; Figure 1B, C), particularly in the five amino acid residues that line the pore upstream of the ion selectivity filter (3) (6 of 13 variants; 33-fold enrichment; p = 1 × 10⁻⁸ binomial test; Figure 1C). Many individuals with ASD have coexisting intellectual disability (Figure 1D) and developmental delay (Table S1 in Supplement 2). Accordingly, the 12 variants in SCN2A in individuals with developmental delay, but no known diagnosis of ASD, are mostly PTVs (8 of 12 variants, 67%) and de novo (11 of 12 variants, 92%).

This raises the hypothesis that SCN2A variants resulting in Naᵥ1,2 gain of function and increased neuronal excitability lead to infantile seizures, whereas SCN2A variants resulting in Naᵥ1,2 loss of function and decreased neuronal excitability lead to ASD and/or developmental delay. If this hypothesis were correct, we would expect the missense variants observed in ASD to reduce Naᵥ1,2 channel function and decrease neuron excitability. We therefore performed functional analyses of all 12 de novo SCN2A mutations observed in the 4109 ASD cases from the Simons Simplex Collection (2508 families with
a single affected child) and Autism Sequencing Consortium (1601 families with one or more affected children), as these cases were identified as representing idiopathic ASD in clearly defined cohorts (4,9,22).

To confirm that PTV SCN2A mutations indeed impair Na\(_\text{V}1.2\) channel formation, and to determine whether missense mutations alter channel function, we made voltage-clamp recordings from HEK293 cells expressing WT or mutated channels. \(\beta_1\) and \(\beta_2\) subunits were coexpressed in all experiments. As predicted, nonsense (p.C959X, p.G1013X) and frameshift (p.S686fs) PTVs lacked Na\(_{\text{V}}\) currents (Figure 2A, B). Constructs recapitulating a fourth PTV that disrupts a canonical splice site in \(\text{SCN2A}\) (c.4821-1A>T) were not studied here, as it was unclear whether the messenger RNA recombines after this splice event. The missense mutations produced a range of results. Peak currents from D12N-mutated channels were comparable in size to WT (WT: –447 ± 64 pA/pF, n = 18; D12N: –515 ± 65 pA/pF, n = 18; p = .5), while currents from D82G and T1420M variants were smaller than those from WT channels (D82G: –166 ± 32 pA/pF, n = 18, p < .0001 vs. WT; T1420M: –177 ± 22 pA/pF, n = 13, p < .0001 vs. WT). Remarkably, all other missense mutations, including pore-localized arginine mutations observed in multiple ASD cases (R379, R937), resulted in a loss of Na\(_{\text{V}}1.2\) function. Small residual currents were observed, but these were likely generated by endogenously expressed nonselective cation channels (30), as they were observed in all PTV cases (where no channel was formed but the plasmid vector still expressed green fluorescent protein) and in untransfected cells (Figure 2B). Furthermore, no current was observed when using a K\(^+\)-based intracellular solution, indicating that these channels are not permeable to K\(^+\). Despite the lack of current, nonconducting missense mutations still allowed for Na\(_{\text{V}}1.2\) channel formation and trafficking, as membrane-associated Na\(_{\text{V}}1.2\) staining was evident using confocal and total internal reflectance fluorescence microscopy (Figure S2 in Supplement 1).
Thus, of the 12 ASD variants tested here, 9 (75%) resulted in a complete loss of NaV1.2 conductance (Figures 1B and 2B).

We next characterized the electrophysiological properties of the three conducting missense variants. Compared with WT channels, D82G was the only variant in which the voltage dependence of channel activation was depolarized (Figure 2C; WT: \(-26.9 \pm 1.3\) mV, \(n = 14\); D12N: \(-30.2 \pm 1.5\) mV, \(n = 13\); D82G: \(-22.0 \pm 0.9\) mV, \(n = 12\), T1420M: \(-25.5 \pm 1.0\) mV, \(n = 9\); \(p = .004\), WT vs. D82G only). The voltage dependence of inactivation was not altered in any variant (Figure 2D; WT: \(-74.8 \pm 2.0\) mV, \(n = 10\), T1420M: \(-77.0 \pm 0.8\) mV, \(n = 9\); \(p > .1\), all variants vs. WT), though both D12N and T1420M currents inactivated more quickly at any given voltage (Figure 2E, e.g., \(\tau = -30\) ms, WT: \(1.2 \pm 0.1\) ms, \(n = 13\); D12N: \(0.7 \pm 0.1\) ms, \(n = 13\), \(p < .001\) vs. WT; D82G: \(1.4 \pm 0.1\) ms, \(n = 10\), \(p = .5\) vs. WT; T1420M: \(0.9 \pm 0.09\) ms, \(n = 9\); \(p < .01\) vs. WT; repeated measures analysis of variance).

Last, we used fluctuation analysis to estimate single channel conductance and functional channel density (Figure 2F, G). Single channel conductance estimates were comparable to those obtained from recordings of single amplitude. Dots are individual time points, curve is parabolic fit for nonstationary fluctuation analysis. (G) Single-channel conductance and the number of channels contributing to the macroscopic current, as determined in panel (F). Data and statistics presented as in panel (D).
channels (WT: 20.8 ± 1.8 pS, n = 9) (31) and were not affected in any variant (D12N: 20.1 ± 1.2 pS, n = 7; D82G: 24.8 ± 1.6 pS, n = 8; T1420M: 26.9 ± 1.9 pS, n = 9; p > .05, across all vs. WT); however, the number of channels contributing to currents was reduced in D82G and T1420M variants (channels/pF: WT: 345 ± 70, n = 8; D12N: 512 ± 91, n = 7, p = .15; D82G: 111 ± 28, n = 9, p = .003; T1420M: 118 ± 18, n = 8, p = .003, all vs. WT). Surface NaV staining, assessed with total internal reflectance fluorescence, was similarly reduced in T1420M variants (Figure S2C, D in Supplement 1; NaV/green fluorescent protein mean intensity, WT: 0.45 ± 0.05, n = 13; T1420M: 0.31 ± 0.03, n = 13; p = .006); however, no reduction was observed for D82G (0.40 ± 0.03, n = 10, p = .3364). This discrepancy between fluctuation analysis and surface expression for D82G may be due in part to the observed shift in voltage dependent activation. Because D82G channels are less effectively activated at voltages used for fluctuation analysis (see Supplement 1), a smaller fraction of surface-localized channels would contribute to the current.

Overall, these results indicate that ASD-associated mutations may interfere with coupling between neighboring functional channels. Therefore, we coexpressed WT with the nonconducting variant R379H. While peak currents were smaller than WT alone (~160 ± 41 pA, n = 9, p = .0006), no change in voltage dependence, kinetics, or conductance were observed (V1/2 activation: ~22 ± 1.1 mV, n = 9, p = .0472; postcorrection p required for significance: <.0167); V1/2 inactivation: ~76.8 ± 1.0 mV, n = 7, p = .227; τ = ~30 mV: 1.4 ± 0.2 ms, n = 6, p = .350; single-channel conductance: 27.2 ± 3.4 pS, n = 5, p = .112; channels/pF: 94 ± 41, n = 5, p = .011, all vs. WT). This indicates that nonconducting variants lack dominant negative effects on WT channels and suggests that NaV1,2 channels do not couple functionally (6).

Multiple lines of evidence suggest that disruption in neocortical circuits can contribute to infantile seizures and ASD (30,32,34). To test how dysfunction in NaV1,2 affects neuronal excitability, we incorporated each type of NaV1,2 mutation observed into an established computational model of a cortical pyramidal cell (35). Because the mutations observed in ASD are heterozygous, we incorporated equal amounts of WT and mutated channels into the model and incorporated changes to the affected allele based on results in Figure 2 (see Supplement 1). To validate that these models accurately predict changes in neuronal excitability, and to compare ASD mutations with other conditions (Figure 1), BIFS and EE variants were also modeled. These include L1563V and L1330F, which are associated with BIFS and produce modest (~5 mV) shifts in steady-state voltage dependence (24), and E1211K and I1473M, which are associated with EE and produce larger (14–22 mV) changes in voltage dependence (23).

As pyramidal neurons are incorporating into cortical networks during early development, they express only NaV1,2 in the AIS (15–17), and it is thought that SCN2A variants associated with BIFS produce seizures because NaV1,2 plays the dominant role in neuronal excitability at this age (7). This time period may also be critical for ASD, as the early development of prefrontal layer 5/6 pyramidal cells has been implicated in the disorder (13). Therefore, we created a developmental model in which NaV1,2 was expressed throughout the AIS and axon and a mature model in which NaV1,2 was replaced by NaV1,6 in the distal AIS and axon. In control conditions, spike threshold was depolarized in the developmental model relative to the mature model, consistent with the differences in voltage-dependent activation between the two isoforms (Figure 3; Figure S3 in Supplement 1). Intermediary developmental time points with different densities or distributions of NaV1,2 versus NaV1,6 were also modeled (Figure S4 in Supplement 1).

In the developmental model, BIFS variants hyperpolarized spike threshold and enhanced in spike rate, consistent with seizure phenotypes at this early age. EE variants were more excitable, firing spikes even at rest. In contrast, ASD variants were far less excitable than WT. Full PTV/nonconducting missense models had a rheobase spike threshold 7 mV more depolarized than WT conditions. Similarly, spike threshold in models of D12N, D82G, and T1420M variants was depolarized to levels comparable to a 40%–50% reduction in overall NaV1,2 conductance. In all cases, spike rate was suppressed, suggesting that even though missense mutations have different effects on NaV1,2 channels, they produce comparable deficits in neuronal excitability. Therefore, all 12 of the observed ASD variants were predicted to reduce the excitability of cortical pyramidal neurons during early development.

In the mature model, BIFS variants had only modest effects on threshold or spike rate, whereas EE variants still resulted in marked hyperexcitability. This is consistent with seizure resolution in BIFS, but not in EE. Interestingly, the ASD variants also resulted in minimal changes in neuronal excitability. Mature excitability developed gradually as more NaV1,6 was incorporated into the AIS (Figure S4 in Supplement 1). Overall, this suggests that the critical period for SCN2A mutations to produce an ASD phenotype through changes in neuronal excitability occurs during early development.

DISCUSSION

Mutations in SCN2A are strongly associated with both ASD and infantile seizures. Here, we characterized the functional impact of all 11 de novo mutations in SCN2A from ASD cases in the Simons Simplex Collection and Autism Sequencing Consortium. All three PTVs (i.e., nonsense and frameshift) and the majority of missense mutations resulted in a complete loss of NaV1,2 function, despite normal trafficking to the membrane (Figure 2; Figure S2 in Supplement 1). Three missense mutations—D12N, D82G, and T1420M—altered channel function in different ways. Remarkably, all 11 alterations in channel function evoked comparable deficits in neuronal excitability in pyramidal cell simulations of the developing brain (Figure 3).
Figure 3. Neuronal excitability in a cortical pyramidal cell model. (A) Spiking generated with 2.2 nA somatic current in autism spectrum disorder (ASD), benign infantile familial seizures (BIFS), and epileptic encephalopathy (EE) variants. Data are modeled in a developing neuron expressing only Na\textsubscript{v}1.2 in the axon initial segment (AIS) and axon. Top: all conducting ASD variants, compared with wild-type (WT) and protein-truncating variant (PTV)/nonconducting missense variants modeled as a 50% reduction in overall Na\textsubscript{v}1.2 conductance. D82G was modeled as either an activation shift alone (D82G Va) or with a reduction in channel density (D82G Va-#). Bottom: two BIFS and two EE variants, each compared with WT. Data are vertically offset in 100-mV increments; all traces begin at –78 mV. Plots detail the number of spikes evoked in 100-ms epochs with varying somatic current injections. PTV is from 50% reduction model. Data are color coded as in legend. (B) Same as panel (A), but in a mature pyramidal cell model, with a mix of Na\textsubscript{v}1.2 and Na\textsubscript{v}1.6 in the AIS, and Na\textsubscript{v}1.6 in the axon (for distributions, see Figure S3 in Supplement 1). (C) Spike threshold with varying levels of Na\textsubscript{v}1.2 in the AIS, from WT (100%) to different levels of compensation in cells heterozygous for PTV/nonconducting missense variants (to 50%) and conducting missense ASD, BIFS, and EE variants. Colored scale bar corresponds to spike threshold of the first spike observed in each trace; dark blue are conditions in which no spikes were generated. Note different scaling for EE conditions, as these variants were excitable at/near rest.
SCN2A/Na$_v$1.2 Mutations Underlying Autism and Seizure

and were consistent with excitability changes observed in recordings from dissociated neurons from heterozygous SCN2A deficient mice (35). Thus, these results indicate that functional haploinsufficiency of SCN2A, generated by a variety of PTV and missense mutations, leads to substantial ASD risk.

The most common result of ASD-related missense mutation was a loss of channel conductance without dominant negative interactions. One of these mutations occurred in the outer vestibule (C1386R), an area important for channel conductance (37). Several more were observed within the pore at conserved arginine residues (R379, R937). These residues are notable, as mutations here are the only instance of two de novo missense mutations occurring at the same residue in two different families among 4,109 cases with exome data (2). Two missense mutations located on the N-terminal domain altered inactivation kinetics (D12N) and the voltage dependence of steady-state activation (D82), demonstrating, to our knowledge, the first examples of pathological N-terminal mutations in Na$_v$1.2 (38), T1420M, which is in close proximity to the pore selectivity filter (Figure 1), also had deleterious effects on channel function, increasing the speed of inactivation and reducing functional (Figure 2G) and physical channel surface density (Figure S2C, D in Supplement 1). Mutation of a homologous residue in rat Na$_v$1.4 to cysteine had modest effects on channel function (39), suggesting that the observed change in pore hydrophobicity is critical at this residue.

Because all SCN2A mutations analyzed led to haploinsufficiency, including all missense variants characterized here, we can revise our estimate of the contribution of SCN2A mutations to ASD risk. Across the 4109 ASD cases, 12 (0.29%) have mutations resulting in SCN2A haploinsufficiency, a figure marginally higher than that for CHD8 (0.24% if equivalent functional analyses validated three non-PTV mutations along with the seven PTV mutations), making SCN2A the gene with the strongest evidence for ASD-association based on exome analysis and second only to fragile X syndrome as a single gene cause of ASD. Therefore, examination of the neurobiology consequent to SCN2A haploinsufficiency, alongside parallel analysis of genes related to chromatin regulation, synaptic structure, and fragile X syndrome, is likely to provide critical insights into the etiology of ASD.

Modeling ASD-associated SCN2A mutations in pyramidal neurons predicted a reduction in neuronal excitability in the developing brain in which Na$_v$1.2 is present throughout the AIS and axon, but minimal changes in neuronal excitability in the mature brain in which Na$_v$1.2 is restricted to the proximal AIS (Figure 3). If neuronal excitability were the main physiological consequence of reduced Na$_v$1.2 function, this would imply that the contribution of SCN2A mutations to the ASD phenotype occurs primarily during early development. Of note, pyramidal cortical neurons during midfetal development have previously been implicated in ASD through coexpression networks (13). Because the ASD phenotype persists in the mature brain, the decrease in neuronal excitability would need to result in a downstream change that persists after neuronal excitability returns to physiological levels. Examples of such a downstream effect include the formation and maturation of cortical circuits and the balance of excitatory and inhibitory inputs within these circuits (40,41).

Alternatively, Na$_v$1.2 dysfunction may continue to affect circuits even after these early developmental time periods in a manner not simulated in our model. APs initiated in the distal AIS propagate forward, toward axonal release sites, and backward, throughout the soma and dendrites (backpropagation). In mature pyramidal cells, Na$_v$1.2 channels in the proximal AIS are thought to provide an important electrical boost to promote effective backpropagation of APs into the somatodendritic compartment (6), and this function may still be impaired in the mature cell. Backpropagating spikes are critical signals for activity-dependent transcription, dendritic integration, and synaptic plasticity (42,43). In this way, mutations in SCN2A may interact with other ASD-associated mutations at the synapse (44,45), or at the transcriptional level. Thus, even though Na$_v$1.2 channels are restricted to the axon, SCN2A haploinsufficiency may result in cellular and circuit dysfunctions that may be common to other causes of ASD.

In contrast to ASD, the SCN2A variants observed in infantile seizures have a gain of function effect on Na$_v$1.2. Computational models showed that variants observed in BIFS result in increased neuronal excitability during early development, but not in the mature brain (Figure 3), consistent with the observed resolution of infantile seizures without further apparent neurological impairment (Table S1 in Supplement 2). The variants characterized in EE are predicted to result in a greater degree of neuronal excitability that persists in the mature brain (Figure 3). This is consistent with the severe seizure phenotype that often persists beyond infancy and is accompanied by profound neurological impairment from an early age (Table S1 in Supplement 2).

Numerous genetic loci associated with neuropsychiatric disorders are observed across a range of diagnoses including developmental delay, ASD, and schizophrenia. Previously, correlation between phenotype and genotype has been described for some large-scale recurrent deletions and duplications (2,46). Here, we show that functional analysis of apparently similar SCN2A missense mutations (Figure S1 in Supplement 1) can distinguish two neuropsychiatric phenotypes—infantile seizures and ASD/developmental delay—with further correlation between functional severity and phenotypic severity for infantile seizures (Figure 3). Thus, the specific functional effect of each SCN2A mutation is directly affecting the observed neuropsychiatric phenotype, presumably with genetic background, environmental influences, and stochastic effects also playing a modifying role. It remains to be seen if further functional characterization can distinguish more closely related phenotypes such as ASD and intellectual disability (Figure 1D).

While we focused on understanding Na$_v$1.2 dysfunction in cortical pyramidal cells, SCN2A expression in other cell types may also contribute to ASD. Though the majority of cortical interneurons express SCN1A/Na$_v$1.1 at the AIS (47,48), with loss of function resulting in Dravet syndrome with EE (49), somatostatin positive interneurons, which play a role in cortical circuit maturation (50,51), express a mix of both Na$_v$1.1 and Na$_v$1.2 in the proximal AIS (9). Further, Na$_v$1.2 channels are expressed throughout cerebellar granule cell axons (8), potentially contributing to episodic ataxia (25). Consequently, further functional studies, including in vivo
models expressing specific SCN2A variants, will be required to fully understand the pathophysiological consequences of deleterious SCN2A variants. Nevertheless, the comprehensive functional assessment of ASD-associated mutations alongside the existing literature on SCN2A variation and electrophysiology (Table S1 in Supplement 2) provides an initial insight into the genotype-phenotype correlations for this gene. The variants associated with infantile seizures lead to gain of function in Na\(_\text{v}1.2\), resulting in increased neuronal excitability and the extent of the effect on neuronal excitability may explain the varying severity of this phenotype. These gain-of-function variants are usually missense variants in proximity to and the extent of the effect on neuronal excitability may explain the varying severity of this phenotype. These gain-of-function variants are usually missense variants in proximity to the voltage sensing fourth transmembrane segment and possibly in the C-terminus (Figure 1A). In contrast, our analysis of function variants are usually missense variants in proximity to the voltage sensing fourth transmembrane segment and possibly in the N-terminus (Figure 1B). These variants lead to loss of function in Na\(_\text{v}1.2\) (Figure 2), resulting in reduced neuronal excitability during development only (Figure 3). We predict that this defect in neuronal excitability would result in a persistent change in neuronal circuitry or activity that would converge with neurobiological changes observed following disruption of ASD-associated chromatin regulating or synaptic genes.

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SCN2A/Na\textsubscript{v}1.2 Mutations Underlying Autism and Seizure


