Trends in Neurosciences

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Review

Progress in Understanding and Treating *SCN2A*-Mediated Disorders

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Advances in gene discovery for neurodevelopmental disorders have identified *SCN2A* dysfunction as a leading cause of infantile seizures, autism spectrum disorder, and intellectual disability. *SCN2A* encodes the neuronal sodium channel $Na_V1.2$. Functional assays demonstrate strong correlation between genotype and phenotype. This insight can help guide therapeutic decisions and raises the possibility that ligands that selectively enhance or diminish channel function may improve symptoms. The well-defined function of sodium channels makes *SCN2A* an important test case for investigating the neurobiology of neurodevelopmental disorders more generally. Here, we discuss the progress made, through the concerted efforts of a diverse group of academic and industry scientists as well as policy advocates, in understanding and treating *SCN2A*-related disorders.

Highlights

Dysfunction in *SCN2A* has been recently recognized as a major cause of neurodevelopmental disorders (NDDs), including epilepsy, intellectual disability (ID), and autism spectrum disorder (ASD).

SCN2A encodes a neuronal sodium channel, $Na_V1.2$. In contrast to many other NDD-linked genes, there is a well-established body of literature on sodium channel function and dysfunction.

Loss of $Na_V1.2$ function contributes to ASD and ID, whereas gain of function contributes to early onset epilepsy. Such strong and bidirectional genotype–phenotype correlation is rare in brain disorders.

Sodium channel function can be enhanced or suppressed using pharmacology. This may allow for future, targeted treatment of SCN2A-associated disorders.

The neuropathophysiology revealed by investigating SCN2A may provide insight into the underlying biology of NDDs more generally.

The Role of *SCN2A* in Neurodevelopmental Disorders

Our understanding of the genetic causes of neurodevelopmental disorders has progressed dramatically in the past decade, raising hope for better treatments by illuminating the biological mechanisms of disease. In that time, disruption of the **gene** *SCN2A* (see Glossary) has been identified as a prominent cause of a wide range of neurodevelopmental disorders, including autism spectrum disorder (ASD), intellectual disability (ID), and infantile-onset seizures (before the first year of life) of varying severity. Moreover, and notably, the consequences of disease-associated variants on protein function can predict the nature and severity of the resulting phenotype.

SCN2A encodes the neuronal voltage-gated sodium channel $Na_V1.2$, which is involved in the initiation and propagation of **action potentials** in a range of neuron classes. Critically, from a therapeutic perspective, ion channels such as $Na_V1.2$ are 'druggable' targets, and small-molecule compounds can be developed to directly and selectively increase or decrease channel function as the basis for potential treatments. Thus, the well-defined biology describing $Na_V1.2$ function, the strong genotype–phenotype correlations found within a genetically well-defined patient population, and the channel's potential as a target for therapeutics all make *SCN2A* a leading candidate for focused efforts to understanding and treating neurodevelopmental disorders. Following an *SCN2A*-dedicated workshop organized by the Simons Foundation Autism Research Initiative (SFARI) that convened academic scientists, representatives from biotechnology and pharmaceutical companies, as well as funders and family advocacy organizations, we summarize the current state of research, future directions, and consider potential opportunities and challenges in the $Na_V1.2$ field.

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The *SCN2A* Gene

The sodium channel, voltage-gated, type II, alpha (*SCN2A*) gene is located on the positive strand of chromosome 2 (2q24.3) in humans, between the sodium channel gene *SCN3A* and the nuclear protein gene *CSRNP3*. The sodium channel genes *SCN1A* and *SCN9A* are within 1000 kbp downstream. The *SCN2A* mRNA transcript contains 27 **exons** that encode a 2005-amino acid protein called $Na_V1.2$. Because of the large last coding exon, only stop codons in the first 1591 amino acids would be expected to induce **nonsense-mediated decay** (Figure 1A) [1]. There are two major developmentally regulated splice **isoforms** of $Na_V1.2$ that use mutually exclusive copies of the fifth coding exon and differ by one amino acid at position 209: Asparagine (Asn/N; termed 5N) versus aspartic acid (Asp/D; 5A) [2,3] (Figure 1A).

The Structure and Function of *SCN2A*/$Na_V1.2$

SCN2A encodes the neuronal sodium channel $Na_V1.2$, which is one of several sodium channels involved in initiation and propagation of action potentials in neurons. $Na_V1.2$ is one of four sodium channel paralogs expressed throughout the central nervous system, along with $Na_V1.1$ (*SCN1A*), $Na_V1.3$ (*SCN3A*), and $Na_V1.6$ (*SCN8A*). Sodium channels are pseudo-heterotetrameric protein structures consisting of four highly similar domains, termed I, II, III, and IV (Figure 1). Each domain contains six membrane-spanning segments S1–S6 (Figure 1) [4] with S1–S4v forming the voltage-sensing domains and S5–S6 forming the pore loops and DEKA-selectivity filter. The positively charged fourth segment, called the voltage sensor or S4v, is sensitive to charge differences between the intracellular and extracellular sides of the membrane. When the intracellular electrical potential becomes less negative, the four S4v segments initiate conformational changes in the channel that lead to channel activation. Activation opens the central ion-selectivity pore, permitting electrical conduction by way of sodium flux into the cell (Figure 1D). The inactivation gate then physically occludes the pore on the intracellular side, a shift in conformation termed 'fast inactivation'. From here, the membrane must **hyperpolarize** to release the inactivation gate and recover from inactivation. While exceptions have been noted, sodium channels typically must go through all three steps to be available for subsequent activation [5].

These steps of activation, conductance, inactivation, and recovery from inactivation vary across sodium channel isoforms, in both kinetics and membrane voltage sensitivity. This is due to small differences in the amino acid sequence of the channels. Similarly, **missense** variants can conceivably alter the biophysical properties of the channel, especially if such variants are in parts of the protein that are important for sensing voltage (e.g., within the voltage-sensing domain) or permitting ion flux (e.g., within the pore domain; Figure 1). Furthermore, variants at essential sites for interactions with the beta subunit (e.g., *SCN1B*), other auxiliary proteins (e.g., calmodulin, fibroblast growth factor homologous factors), modulatory systems, or scaffolding proteins can all impact $Na_V1.2$ activity [6]. How these variants can affect different aspects of channel function is described in detail in the following sections.

Developmental and Cell Type-Specific Patterns of $Na_V1.2$ Expression across Brain Regions

$Na_V1.2$ is widely expressed throughout the human central nervous system, but not in peripheral tissues [7,8]. In cortical structures, $Na_V1.2$ is co-expressed with $Na_V1.6$ predominately in excitatory, glutamatergic neurons. Inhibitory interneurons largely express $Na_V1.1$ instead of $Na_V1.2$ [9,10], though $Na_V1.2$ channels have been identified in some multipolar and bouquet-class interneurons in mouse [11]. $Na_V1.2$ may co-express with $Na_V1.1$ in the somatostatin-expressing interneuron subclass, though this observation is not consistent between human and mouse tissue [11,12]. Similar distributions across excitatory and inhibitory cells are found in the hippocampus [10,11,13].

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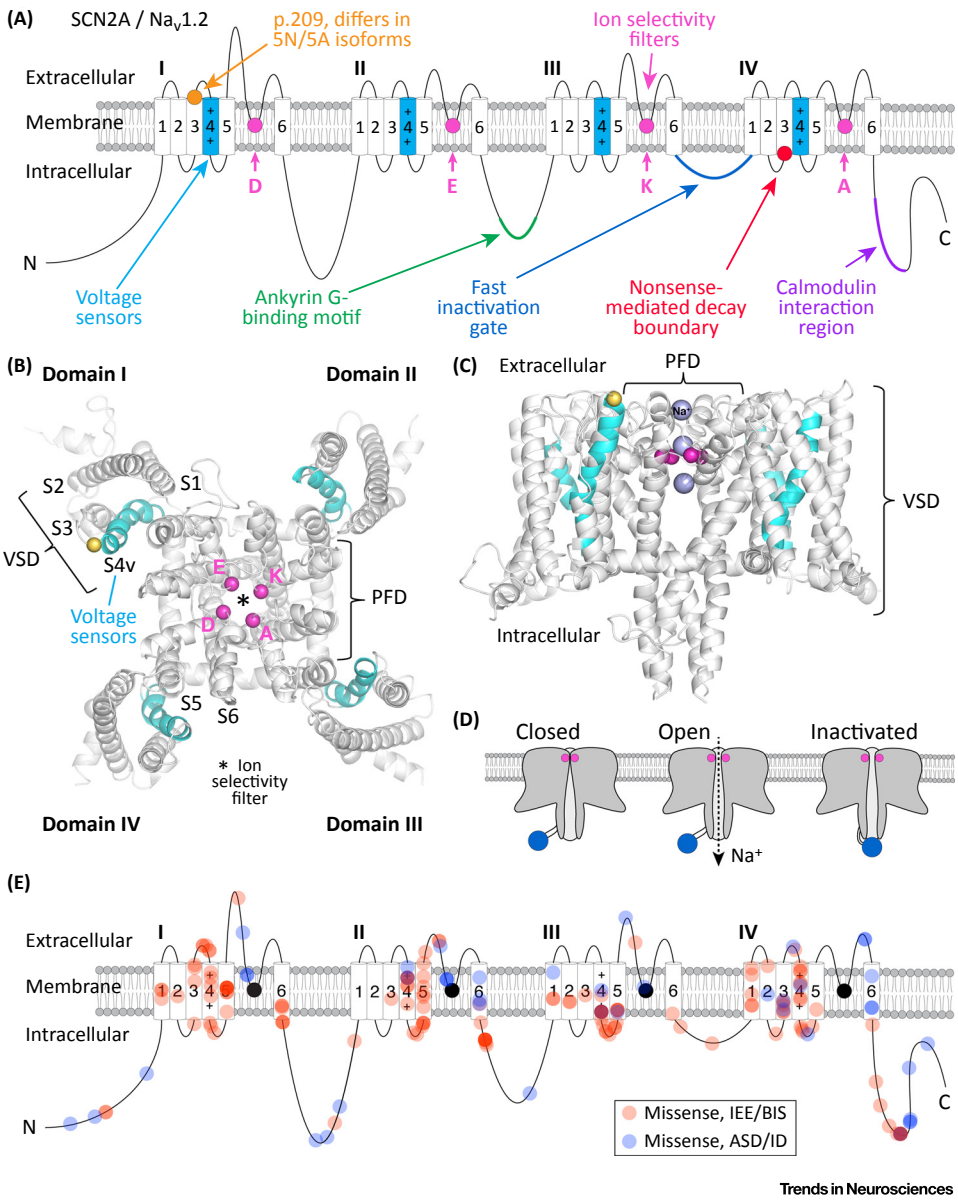

Figure 1. Overview of *SCN2A*/$Na_V1.2$. (A) $Na_V1.2$ resides in the cell membrane and is a pseudo-heterotetramer composed of four domains, called I–IV. Each domain contains a voltage sensor (cyan) and a pore loop with a ring of amino acids (DEKA) making up the ion-selectivity filter (pink). The cytoplasmic loop between Domains III and IV is the fast inactivation gate (dark blue, see D). Two isoforms are known, differing by one amino acid at p.209 (orange) [2]. The channel binds to Ankyrin G (*ANK3*), which anchors it to the membrane (green) [79], and interacts with calmodulin, which likely has a regulatory role (purple) [80]. Stop codons before the nonsense-mediated decay boundary (red) are predicted to prevent protein translation, while those after this boundary may not. (B) $Na_V1.2$ homology model of the transmembrane region, top view. Each domain is labeled. For Domain I, transmembrane segments 1–6 are labeled S1–S6. S1–S4v represents the voltage-sensing domain (VSD). The voltage sensor is labeled S4v and is colored cyan. S5–S6 represents the pore-forming domain (PFD). The Cα carbons from each residue in the DEKA motif are represented as pink spheres. The Cα carbon of the residue that differs between the neonatal and adult isoforms is represented as a yellow sphere. The selectivity filter is represented by an asterisk. Model [81] is based on the $Na_V1.7$ chimeric crystallographic structure [61]. (C) $Na_V1.2$ homology model of the transmembrane region, side view. Purple spheres represent sodium passing through the selectivity filter of $Na_V1.2$. (D) $Na_V1.2$ is closed at resting potential. Depolarization opens the channel, allowing sodium flux, after which the channel is blocked by the fast inactivation gate (blue). As the membrane voltage returns to rest, the channel resets

(See figure legend on the bottom of the next page.)

Glossary

Action potential: an electrical signal used by neurons to transmit information from one neuron to the next. Action potentials are mediated largely by the opening of channels that allow positive sodium ions into the cell, 'depolarizing' the 'membrane potential', then the opening of potassium channels that allow positive ions to leave the cell, 'hyperpolarizing' the membrane potential. Variants in sodium channels that mediate action potentials can affect their initiation or propagation.

Action potential threshold: the voltage at which a neuron will generate an action potential. This value is dynamic and depends on the sodium channel isoforms expressed within the neuron and the recent electrical activity of the neuron. Threshold can also be affected by the number of channels expressed on the neuron membrane.

Brugada syndrome: a heart rhythm disorder that can lead to ventricular arrhythmias and sudden death. It is frequently caused by *SCN5A* mutations.

Depolarize/Hyperpolarize/Repolarize: the opening of channels that allow positive ions into the cell, such as sodium channels, is said to 'depolarize' the membrane potential, as they bring the membrane potential closer to 0 mV, or past 0 mV into the positive range. Conversely, ion channels that allow positive ions to leave the cell (e.g., potassium channels) 'hyperpolarize' the membrane potential away from 0 mV and back toward resting membrane potential. This is often called 'repolarization', especially in the context of action potential generation.

Dravet syndrome: an epileptic encephalopathy that presents with prolonged seizures, often alternating hemiconvulsions provoked by fever, in the first year of life. The interictal EEG and development are normal at onset, but signs of regression appear in the second year of life and are often accompanied by convulsive status epilepticus, myoclonic seizures, and EEG abnormalities. The majority of cases are caused by loss-of-function *SCN1A* mutations, which encodes $Na_V1.1$, a sodium channel expressed in inhibitory interneurons.

The 5N and 5A splice isoforms of *SCN2A* are developmentally regulated, with 5N co-expressed with 5A in the fetal brain, eventually transitioning to neurons predominantly expressing 5A after the second postnatal week in mouse [2,14]. These isoforms influence channel voltage dependence; results indicate that the adult 5A encodes a channel that activates at more hyperpolarized voltages, resulting in increased neuronal activity [3,15].

In addition to alternative splicing, the subcellular distribution of $Na_V1.2$ changes markedly over development in excitatory pyramidal cells. Early on, $Na_V1.2$ is the only sodium channel isoform expressed in the axon initial segment (AIS) [3,16,17], an axonal subcompartment located proximal to the soma that is the site of action potential initiation [18]. In humans, this early developmental period spans from the late second trimester, when the cortex is beginning to form, to 1–2 years of age. At this time, $Na_V1.2$ in the distal AIS, as well as the rest of the axon, is largely replaced by $Na_V1.6$ (*SCN8A*), which has a lower voltage threshold for activation. Consequently, the distal AIS becomes the site of action potential initiation in mature neurons [19,20]. $Na_V1.2$ is at this point restricted to the region of AIS most proximal to the soma, where it functions as a reserve pool of channels, right next to the cell body. Once action potentials are initiated in the AIS by $Na_V1.6$, $Na_V1.2$ is thought to help action potentials backpropagate into the somatodendritic compartment [21]. These backpropagating action potentials could then influence many functions, including activity-dependent gene transcription, synaptic integration, and synaptic plasticity.

Another prominent site of $Na_V1.2$ **expression** is the cerebellar cortex [22]. Here, $Na_V1.2$ channels are coexpressed with $Na_V1.6$ in unmyelinated axons of excitatory granule cells that form parallel fiber synapses on Purkinje neurons. In contrast to the developmental loss of $Na_V1.2$ in cortical myelinated axons, $Na_V1.2$ expression persists throughout development in the cerebellum, with an apparent peak in density within the second and third postnatal weeks in mice [22,23]. This suggests that $Na_V1.2$ serves different roles in mature neurons in the neocortex and cerebellum.

SCN2A-Mediated Disorders

Variants in *SCN2A* are primarily associated with three distinct disorders [24,25]: (i) infantile epileptic encephalopathy (IEE), characterized by infantile-onset seizures, before 12 months of age, followed by neurodevelopmental delay; (ii) benign (familial) infantile seizures (BISs), characterized by infantile-onset seizures, before 12 months of age, that resolve by 2 years of age without overt long-term neuropsychiatric sequelae; and (iii) ASD/ID, characterized by global developmental delay, particularly of social and language milestones. Up to a third of children in the ASD/ID category identified to date also develop childhood-onset seizures ($\geq$12 months).

While most children with *SCN2A* variants fit into one of these three categories, there are some exceptions, including epileptic encephalopathy with choreoathetoid movements [26,27], BISs with late-onset episodic ataxia [28–31], childhood-onset epileptic encephalopathy [32], and schizophrenia [33,34]. Further work is required to understand how these disorders relate to the other three distinct categories and the role of cerebellar $Na_V1.2$ in ataxia, hypotonia, and other symptoms.

through hyperpolarization. (E) Location of missense variants observed in individuals with infantile epileptic encephalopathy (IEE) and benign (familial) infantile seizures (BISs; red) and autism spectrum disorder/intellectual disability (ASD/ID; blue). Increased color intensity represents multiple variants in proximity. Panels A and E are based on previously published illustrations [26].

EEG: recording of cortical electrical activity by a net of electrodes placed on the scalp.
Exon: a segment of a gene that is not removed during splicing; these often contain the information to determine the order of amino acids in a protein.
Expression: the amount of RNA produced from a gene in a given tissue or cell.
Gain of function: when a genetic variant in an ion channel makes the channel more excitable. One example is when a variant hyperpolarizes the voltage required for channel opening.
Gene: a region of DNA that is transcribed into RNA (e.g., *SCN2A*). For protein coding genes, this RNA is spliced, keeping exons and removing introns, then translated to determine the order of amino acids in a protein (e.g., $Na_V1.2$) by a ribosome.
Generalized epilepsy with febrile seizures plus (GEFS+): the definition of this syndrome, previously known as 'generalized' epilepsy with febrile seizures plus, has changed over the past 10 years since its initial description. Currently, this is considered a childhood-onset familial syndrome that encompasses several phenotypes, characterized by a spectrum of severity, that include combinations of febrile seizures, focal seizures, and generalized seizures.
Isoform: versions of the RNA from a gene, often due to the inclusion of different exons; also called alternative splicing.
Lennox–Gastaut syndrome: this severe epileptic encephalopathy has typical onset in early childhood (3–5 years old) and is characterized by multiple seizure types (nocturnal tonic seizures and atonic seizures are typical) refractory to treatments, diffuse 'slow' spike-and-wave and paroxysmal fast activity (EEG pattern), and intellectual disability.
Loss of function: when a genetic variant in an ion channel lowers ion channel excitability, or destroys channel function altogether. Examples of this include 'nonsense-mediated decay', a block of the pore, or changes in voltage dependence that make it harder for a channel to open.
Membrane potential: the voltage differential between the inside and outside of a neuron's membrane.

The 276 known *SCN2A* cases identified to date (see Table S1 in the Supplemental Information online) probably represent only a fraction of *SCN2A* cases, as more than 400 are predicted to be born each year in the USA alone (Table 1). Widespread adoption of genetic testing in IEE, ASD, and ID is expected to increase the frequency of known *SCN2A* cases to hundreds per year, similar to the incidence of *SCN1A*-mediated **Dravet syndrome** at 6.4 per 100 000 births [35]. We would also expect the incidence of **loss-of-function** cases to be approximately fivefold higher than **gain-of-function** cases (Table 1). At present, the number of known *SCN2A* cases is roughly equal between IEE and ASD/ID, likely reflecting differences in the adoption of genetic testing.

Children with *SCN2A*-Associated Infantile Epileptic Encephalopathy

While epileptic encephalopathy frequently presents with seizures in the neonatal period, with some families retrospectively reporting rhythmic movements *in utero*, it is likely that seizure onset after 3 months occurs in 20–40% of cases [24,25]. Of note, age of onset is often similar in individuals with the same genetic variant. Seizure type varies widely between individuals, with most experiencing more than one type of seizure. Some of the children with the earliest age of seizure onset are classified as having **Ohtahara syndrome**, based on tonic seizures and **electroencephalography (EEG)** burst suppression, or epilepsy of infancy with migrating focal seizures. By contrast, onset after 3 months often meets criteria for **West syndrome**, with infantile spasms and a hypsarrhythmia pattern on EEG, evolving to **Lennox–Gastaut syndrome** with multiple seizure types as the child gets older [25]. Development varies from moderate to profound global developmental delay, often with comorbid hypotonia, microcephaly, cerebral and/or cerebellar atrophy, and cortical visual impairment. Some children also have movement disorders, including dystonia, chorea, or episodic ataxia.

Children with *SCN2A*-Associated Autism Spectrum Disorder/Intellectual Disability

In children with ASD/ID, early development is generally unremarkable up to about 6 months of age, followed by moderate motor delay and pronounced verbal delay. About a third of documented cases develop seizures, often between the age of 18 months and 4 years. These seizures are associated with more severe developmental delay and sometimes regression, particularly if the seizures are severe. Importantly, seizure prevalence is likely overestimated in the currently identified population since genetic screening is more common in the presence of syndromic features, such as seizures.

Neurons that are not actively generating action potentials typically have a 'resting membrane potential' between −60 and −90 mV (millivolts).
Missense: a DNA variant that alters a single amino acid in a protein. This can have no impact on function, impair function, enhance function, or create new effects.
Nonsense-mediated decay: a process that destroys RNA if there is a premature stop codon, preventing a protein being made.
Ohtahara syndrome: also known as early infantile epileptic encephalopathy (EIEE), this severe epileptic encephalopathy has typical onset at age ≤3 months and is characterized by intractable tonic seizures and burst suppression on EEG. Patients with EIEE may evolve into either West or Lennox–Gastaut syndromes with age.
Protein truncating variant: a DNA variant that results in a premature stop codon, disrupting that copy of the gene. This includes nonsense, canonical splice site, and frameshift variants.
West syndrome: also known as infantile spasms (ISs), this severe epileptic encephalopathy has typical onset between 3 and 12 months of age and is characterized by the classic triad of infantile spasms (seizure type), hypsarrhythmia (EEG pattern), and developmental regression. Patients with IS may evolve into Lennox–Gastaut syndrome with age.

Table 1. Frequency of *SCN2A*-Related Disorders in the General Population and Literature[a]

	IEE	BIS	ASD/ID	Total
Cases per 100 000 births	120 [71,72]	20 [71,72]	2500 [73]	N/A
Population frequency	0.12%	0.02%	2.5%	N/A
Percent of cases with *SCN2A* variant	1.2% [74]	11% [75]	0.3% [76]	N/A
Estimate of *SCN2A*-related cases per 100 000 births	1.2% of 120 = 1.4	11% of 20 = 2.2	0.3% of 2500 = 7.5	11.1
Expected *SCN2A*-mediated disorders children born each year in the USA	56 cases	88 cases	298 cases	442 cases from IEE, BIS, and ASD/ID
Year *SCN2A* association documented	2009 [56]	2002 [77]	2012 [78]	N/A
Published variants	107 cases	35 families	89 cases	276 cases from all phenotypes

[a]Abbreviation: N/A, not applicable.

While children with *SCN2A*-associated ASD/ID do not exhibit a distinctive syndrome, several behavioral characteristics seem to be shared (unpublished observations, scn2a.org family group). Children are often described as placid and contented and enjoy physical contact with caregivers. They tend to engage in repetitive actions, including chewing objects, hand gestures, and roaming. Children rarely initiate social interactions and the response to social cues from others is slow. Eye contact is rarely made, and hand contact with people or novel objects is avoided. Motor skills are usually delayed to a lesser degree than social skills. Some children have signs of cerebellar involvement, including an unsteady or ataxic gait, clumsiness, and hypotonia. A case report described a muted response to pain and other aversive stimuli [36], which fits with parents' experiences, though some report increased sensitivity to heat. Comorbidities include cortical visual impairment, disrupted sleep, gastrointestinal disturbances, and uncoordinated oral movements.

Toward a Model of *SCN2A* Pathophysiology

An integrated analysis of genetic and electrophysiological data led to a model to account for the three disorders associated with *SCN2A* (Figure 2) [24]. Variants associated with greater $Na_V1.2$ channel activity (i.e., gain of function) lead to IEE and BIS, while those associated with diminished channel activity (i.e., loss of function) lead to ASD/ID (Figure 2). Further, the degree to which the gain of function variants potentiate $Na_V1.2$ activity distinguishes IEE from BIS, with severe variants leading to IEE and milder variants leading to BIS. This model is supported by several strands of evidence, including (i) *in vitro* electrophysiology; (ii) the restriction of **protein truncating variants**, resulting in loss of function, to ASD/ID cases; (iii) shared symptoms between individuals with recurrent missense variants (see Table S1 in the Supplemental Information online); and (iv) the clustering of IEE/BIS variants around the voltage sensor domain of the channel while the ASD/ID missense variants cluster near the pore loop regions (Figure 1E). The subsequent publication of 71 novel *SCN2A* patients from Europe [25] further support the model proposed by Ben-Shalom *et al*. [24].

As with any model, there are likely to be exceptions. At least one has come to light: the variant K1422E alters the third residue in the DEKA ion-selectivity filter (Figure 1) and is predicted to produce both loss-of-function and gain-of-function effects [37,38]. A child with this variant developed seizures at 13 months with developmental regression and features of ASD [39].

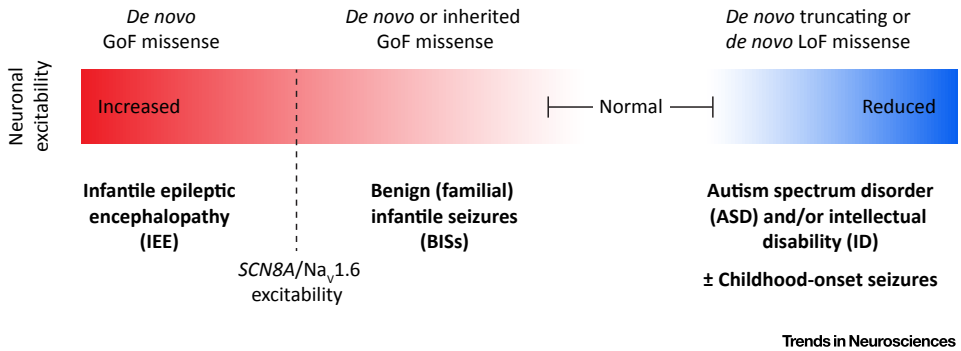

Figure 2. Current Model of *SCN2A* Pathophysiology. Gain-of-function (GoF) variants (left) potentiate glutamatergic neuronal excitability, leading to infantile-onset seizure phenotypes, whereas loss-of-function (LoF) variants (right) impair excitability, leading to autism spectrum disorder (ASD) and/or intellectual disability (ID) [24]. Seizure severity is correlated with the degree of GoF, with variants that cause the most excitability leading to infantile epileptic encephalopathy (IEE), while milder variants lead to benign (familial) infantile seizures (BISs) that resolve around 1–2 years of age without apparent neurological sequelae. The threshold that distinguishes IEE and BIS variants may be related to the degree of compensation of neuronal excitability by $Na_V1.6$, which replaces $Na_V1.2$ in generating action potentials and has a lower threshold for activation than wild-type $Na_V1.2$.

In summary, genotype and phenotype appear to be strongly correlated in disorders associated with variations of *SCN2A*. Identification of additional variants, their functional characterization, and consistent phenotyping should refine this model further, provide additional insights into *SCN2A* pathology and $Na_V1.2$ function, and point to new potential therapeutic interventions [40].

Linking Disorders to Changes in Channel Function

Several hotspots within the $Na_V1.2$ protein are enriched with disorder-associated variants, including S4v–S5 in the voltage-sensing domain, the intracellular N- and C-terminal domains, and the pore loops around the ion-selectivity filter (Figure 1E). To date, only about 20 of the hundreds of disorder-associated variants have been electrophysiologically assessed (summarized in [24,25]). Nevertheless, some patterns are beginning to emerge, with variants within particular subdomains often evoking similar changes to channel biophysical properties (Table 2).

While each aspect of $Na_V1.2$ function is listed separately in Table 2, it is common for one point variant to alter multiple channel properties. In some cases, the concerted changes to voltage dependence and kinetics are consistent, permitting easy interpretation of whether the variant enhances or decreases channel activity (i.e., gain of function and loss of function, respectively). In other cases, a mix of what appear to be gain- and loss-of-function effects is observed, and it is more difficult to understand how neuronal excitability is affected. Compartmental modeling can provide insight into the net effect on neuronal excitability in such conditions [24]. Even with these efforts, it can be difficult to place certain variants into the 'gain' or 'loss' categories. For example, some IEE-associated variants hyperpolarize the voltage dependence of activation to such a degree that neurons may end up being less excitable over time, as sodium channels may be activated at rest, but the neuron never hyperpolarizes to allow the same channels to recover from inactivation. This variant is characterized as a 'gain-of-function' variant when describing channel biophysics, but this description may not hold at the level of overall neuronal excitability. Similarly, a variant at the ion-selectivity filter (e.g., p.K1422E) can have mixed effects on excitability. This variant converts the channel into a nonselective cation channel with increased persistent, Ca^{2+}-dominated current and reduced, nonselective conductance when activated [37,38]. Moreover, some variants can lead to an 'omega current', which stems from ion permeation through the S4v region when the central pore of the channel is closed [41]. Developing models that capture net neuronal excitability effects in such cases is difficult, especially considering potential downstream effects on Ca^{2+}-mediated cell signaling.

SCN2A Homology in Animal Models and Other Channels

Voltage-gated sodium channels (Na_V) are derived from a calcium channel (Ca_V3) early in the evolution of animals [42–44] with the DEKA ion-selectivity filter in Na_V1 being derived from the ancestral DEEA filter (Figure 3, Table 3). Of note, the variant p.K1422E, described earlier (Table 2), reverts the $Na_V1.2$ filter to the ancestral state. The ten voltage-gated sodium channels in humans are derived from this Na_V1 channel (Figure 3) [45–47] with different model organisms reflecting stages in this differentiation (Table 3). $Na_V1.2$ retains a high degree of homology to both $Na_V1.1$ and $Na_V1.3$ and moderate homology to $Na_V1.4$, $Na_V1.5$, $Na_V1.6$, and $Na_V1.7$ (Figure 3B). Loss-of-function variants of $Na_V1.1$ cause Dravet syndrome [48], **generalized epilepsy with febrile seizures plus** [49], and have also been associated with ASD [50,51]. $Na_V1.5$ is expressed in the heart, and its disruption can lead to cardiac arrhythmias, including heart block, long QT, **Brugada syndrome**, and cardiomyopathy [52]. Therapies aimed at modifying *SCN2A*/$Na_V1.2$ function will need to be selective for $Na_V1.2$ to avoid off-target activity with these other sodium channels within both the brain and the periphery to prevent potential side effects.

Table 2. Electrophysiology and Biophysics of *SCN2A* Variants[a]

Effect on $Na_V1.2$ channel	Mutation type	Common location in channel (if multiple cases observed)	Gain of function (GoF)	Example	PMID [Refs.]	Loss of function (LoF)	Example	PMID [Refs.]
Protein truncation	Nonsense, canonical splice sites, frameshift insertion/ deletions, and large deletions	Anywhere in the first 1591 amino acids or 4772 nucleotides	N/A	N/A	N/A	Mutations that prevent translation of one copy of the channel	C959X	22495306 [78]; 28256214 [24]
Gene duplication	Large duplication	Entire channel	Additional copies of channel translated	chr2:165798270–166304847 duplication	27153334 [82]	N/A	N/A	N/A
Nonconducting	Missense	Often clustered in the pore loop	N/A	N/A	N/A	Prevents sodium flux through channel	R379H	25363760 [83]; 28256214 [24]
Activation (voltage dependence)	Missense	Often clustered near the voltage sensor	Hyperpolarizing shifts (i.e., channel opens more readily at voltages near Vrest)	I1473M	19786696 [56]	Depolarizing shifts (more difficult to open channels for any given voltage)	D82G	28256214 [24]
Inactivation (voltage dependence)	Missense	Often clustered near the base of the channel, especially in the intracellular region that links transmembrane domains 4 and 5	Depolarizing shifts (i.e., more channels available for activation at resting **membrane potential**)	L1330F	17021166 [84]	Hyperpolarizing shifts (fewer channels available for activation)	P1622S	28379373 [25]
Activation (kinetics)	Missense	Similar to voltage dependence	Faster channel activation	L1563V	17021166 [84]	Slower channel activation	R1319Q	17021166 [84]
Fast inactivation (kinetics)	Missense	Typically interacting with the 'ball and chain' inactivation gate between Subdomains III and IV, but can also be associated with voltage-sensing components	Slower channel inactivation	F1597L	28379373 [25]	Faster channel inactivation	D12N	28256214 [24]
Slow inactivation (kinetics)	Missense	Similar to voltage dependence	Faster recovery from inactivation	V261M	20371507 [23]	Slower recovery from inactivation	Unknown	N/A
Persistent current	Missense	Usually related to shifts in activation and inactivation that increase the size of the window current	Excess sodium flux at rest	M252V	20956790 [28]	Unknown	N/A	N/A

Table 2. (continued)

Effect on $Na_V1.2$ channel	Mutation type	Common location in channel (if multiple cases observed)	Gain of function (GoF)	Example	PMID [Refs.]	Loss of function (LoF)	Example	PMID [Refs.]
Omega current	Missense	Arginine residues lining S4 transmembrane domain, may result in a mix of GoF and LoF aspects	Unknown	N/A	N/A	Unknown	N/A	N/A
Ion selectivity	Missense	Ion-selectivity filter – formed by the apposition of D, E, K, and A residues, one from each repeat	Channel seems to have gained a persistent calcium current when at rest	K1422E	1313551 [37]; 9035373 [38]	Overall conductance is reduced when active and nonselective for cations	K1422E	1313551 [37]; 9035373 [38]
Trafficking	Missense	Unknown, but Ankyrin G-binding domain is likely	Unknown	N/A	N/A	Unknown	N/A	N/A
Channel regulation	Missense	C-terminus calmodulin regulatory domain	Unknown	N/A	N/A	Disrupted Ca-dependent regulation of voltage dependence	R1902C	15316014 [85]

[a]Abbreviation: N/A, not applicable.

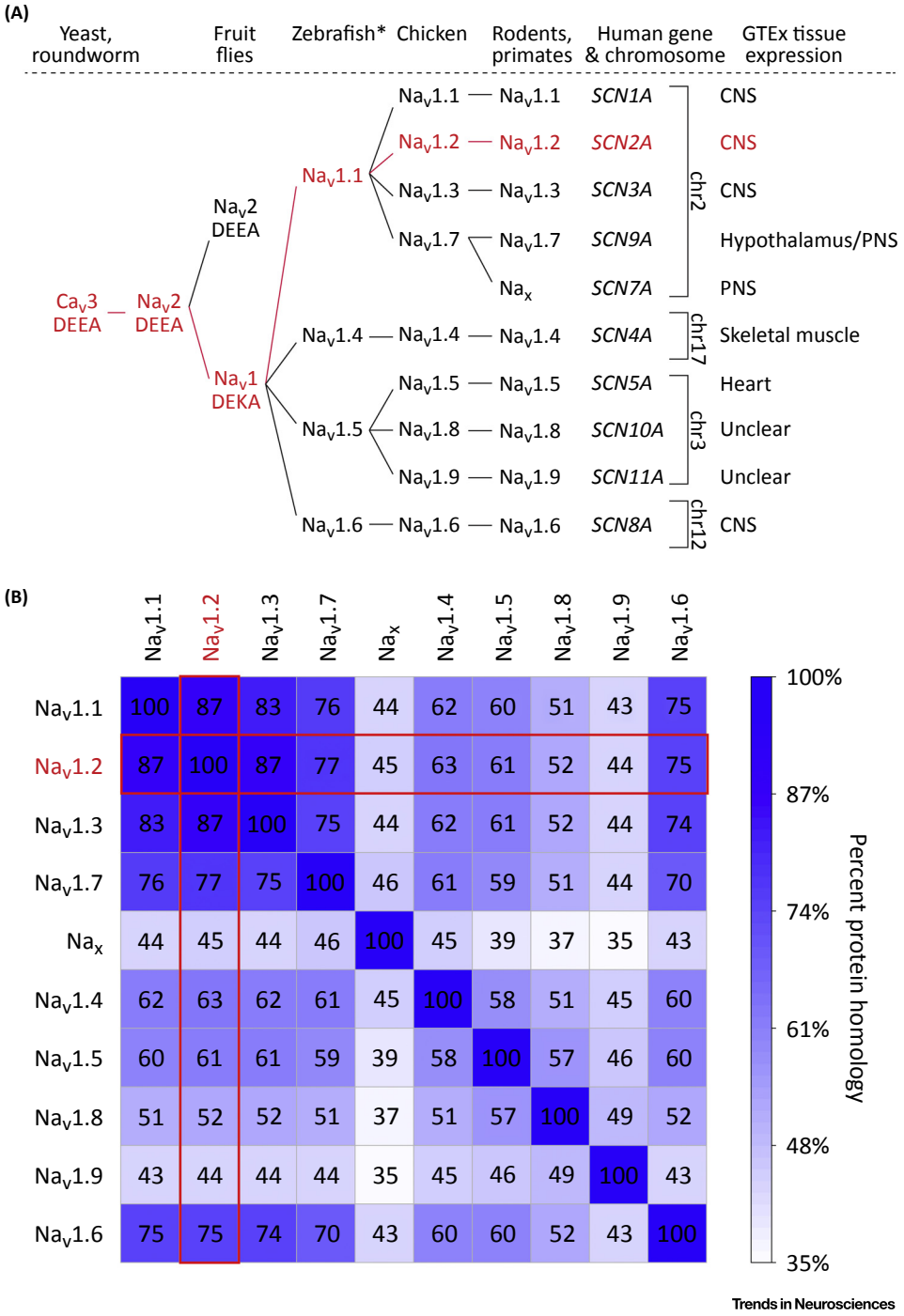

Figure 3. Homology of *SCN2A* across Model Organisms and Voltage-Gated Sodium Channels. (A) Voltage-gated sodium channels (Na_V) are derived from T-type voltage-gated calcium channels (Ca_V3) [42,43]. In fruit flies the ancestral Na_V2 channel, with the ion-selectivity filter composed of the four amino acids DEEA, coexists with the Na_V1 channel with the DEKA filter. All Na_V channels in humans are derived from this Na_V1 DEKA filter, while the Na_V2 filter has been lost [45,46]. A series of gene duplication and differentiation events give rise to the ten channels seen in humans and reflected in the proximity of similar channels in the genome [45,46]. (B) Homology between the 10 Na_V channels in humans. A more intense shade of blue indicates higher homology. *Zebrafish have two copies of each of these four Na_V channels due to relatively recent genome duplication. CNS, central nervous system; GTEx, Genotype-Tissue Expression Project (www.gtexportal.org); PNS, peripheral nervous system.

Table 3. *SCN2A* Homologs in Model Organisms

Species	Number of Na_V channel isoforms	Closest human $Na_V1.2$ homolog	Percent homology to human $Na_V1.2$
Yeast (*Saccharomyces cerevisiae*)	0	N/A	N/A
Worm (*Caenorhabditis elegans*)	0	N/A	N/A
Fruit Fly (*Drosophila melanogaster*)	2	*Para*/Para	70%
Zebrafish (*Danio rerio*)	4 × 2[a]	*Scn1lab*/$Na_V1.1$Lb	86%
Frog (*Xenopus tropicalis*)	6	*Scn2a*/$Na_V1.2$	85%
Chicken (*Gallus gallus*)	9	*SCN2A*/$Na_V1.2$	93%
Mouse (*Mus musculus*)	10	*Scn2a*/$Na_V1.2$	98%
Rat (*Rattus norvegicus*)	10	*Scn2a*/$Na_V1.2$	98%
Rhesus (*Macaca mulatta*)	10	*Scn2a*/$Na_V1.2$	99.9%
Human (*Homo sapiens*)	10	*SCN2A*/$Na_V1.2$	100%

[a]Zebrafish have two copies of each of these four Na_V channels due to relatively recent genome duplication.

Existing Treatments

Seizures associated with *SCN2A* variants often cannot be controlled even with the use of multiple antiepileptic drugs (AEDs). However, for infants with postnatal-onset seizures (≤3 months), nonselective sodium channel blockers, such as phenytoin and carbamazepine, are more effective [25], as would be expected based on a gain-of-function variant [24]. Of note, this is the opposite of current guidelines for empiric neonatal seizure treatment [53] and is a compelling reason for rapid genetic testing for this condition [54,55]. For children with ASD/ID and childhood-onset seizures (≥12 months), the opposite is true, with nonsodium channel inhibiting AEDs offering the best options, including levetiracetam, benzodiazepines, and valproate. For seizures starting between 3 months and 12 months there are limited *SCN2A*-specific data, so standard AED guidelines are recommended [53]. However, the gain-of-function nature of these variants suggest sodium channel inhibitors would be more efficacious. Some reports have described clinical improvement with less mainstream medications, including lidocaine in children with IEE [56,57], ethosuximide for late-onset seizures [25], and acetazolamide for episodic ataxia [28,29], though data on the generalizability of these effects are limited. This effort to think broadly about medications parallels new and emerging therapies that provide benefit in Dravet syndrome, such as bromides, fenfluramine, and cannabidiol [58]. Whether such medications would provide benefit with *SCN2A*-associated late-onset seizure remains unknown. When considering such treatments, it is critical to remember that, at least in cortical regions, $Na_V1.1$ channels affected in Dravet are more commonly expressed in inhibitory neurons [59], and that loss of function with $Na_V1.1$ may have opposing effects on brain networks compared with $Na_V1.2$ loss of function in excitatory neurons.

At present, no *SCN2A*-specific recommendations can be made for the treatment of other symptoms. Standard management for children with global developmental delay, ASD, and comorbid disorders should be followed.

Future Treatments

There is a clear unmet medical need for improved treatment options for *SCN2A*-associated disorders. Based on the current model of *SCN2A* pathology (see Figure 2), restoring $Na_V1.2$ channel function to the 'normal' range may provide therapeutic benefit for individuals with both classes of disorders (gain and loss of function). Since there is precedent for small-molecule

compounds that either enhance or diminish ion channel activity [60–62], it is conceivable to develop drugs for modulating $Na_V1.2$ activity to treat IEE and ASD/ID. However, this approach has potential associated challenges. First, the high degree of conservation (Figure 3B) between *SCN2A*/$Na_V1.2$ and other voltage-gated sodium channels, including *SCN5A*/$Na_V1.5$ that can induce cardiac arrhythmias if disrupted, demonstrates that high selectivity for $Na_V1.2$ will be essential to reduce side effects. Second, the *SCN2A* pathology will likely require normalizing the $Na_V1.2$ channel current while avoiding overly enhancing or decreasing $Na_V1.2$ channel activity that could result in IEE or ASD/ID symptoms, respectively. In addition, biophysical characterization of unknown patient-specific variants may be necessary to confirm the appropriate therapeutic intervention, especially in cases where mixes of gain-of-function and loss-of-function characteristics are present (Table 2).

An additional consideration is the reversibility of the symptoms. *SCN2A* expression begins early in gestation [8], and channel dysfunction may alter brain development. In keeping with this concern, computational modeling of loss-of-function variants suggests that neuronal excitability is restored in the mature brain as $Na_V1.6$ replaces $Na_V1.2$ as the critical channel underlying action potential generation at 2 years of age [24]. Despite this proposed functional compensation in action potential initiation, symptoms of ASD/ID continue beyond this age. It will therefore be critical to understand the physiological changes that persist within the brain in individuals with loss-of-function variants and whether these changes can be rescued by restoring $Na_V1.2$ function. Similarly, the encephalopathy in gain-of-function variants may be secondary to seizures and tractable to treatment; alternatively, seizures may simply be one feature of a more complex neurodevelopmental disorder that begins *in utero*.

To address these questions, and to understand where and when therapeutic interventions can occur, more advanced experimental tools must be applied. Transgenic rodent models, such as mice, rats, and voles, provide the opportunity to assay neurons in the context of the brain to capture nonautonomous, circuit-level, and behavioral effects. Understanding how alterations in $Na_V1.2$ function affect developing and mature neuronal integration, and then understanding how this affects network function, is key to enable effective interventions. Further, experiments equivalent to the studies of symptom reversibility in *MECP2* knockout mice [63] are critical for both loss-of-function and gain-of-function phenotypes.

Beyond rodent models, additional models and approaches can be brought to bear. Patient-derived induced pluripotent stem cell-based approaches including neuronal cultures [64,65] and organoids [66,67] hold great promise in this regard, as they would allow researchers to probe for the functional effects of *SCN2A* genetic variants on many levels including neuronal excitability, developmental progression, and network behavior. Importantly, this can be done in human neurons, under physiological expression levels of the channel and under each patient's unique genetic background. Further, studies in non-human primates may provide a better understanding of how therapeutics that prove beneficial in rodent models translate to more complex systems [68].

Toward Personalized Therapeutics

To develop treatments for *SCN2A*-mediated symptoms beyond epilepsy it is critical to advance the understanding of both gain-of-function and loss-of-function phenotypes. More specifically, observational studies are needed to characterize the natural course of the diseases and to identify endpoints for future interventional trials. Analysis of data from publications and clinical cohorts has already provided insights into genotype–phenotype relationships and appropriate AEDs [24,25]. Further progress will require collaboration between scientists and families, including the participation of families in the FamilieSCN2A Foundation (www.scn2a.org) and

the associated *SCN2A* patient registry (https://simonsvipconnect.org). There is room for further development of the tools for collecting and analyzing *SCN2A* patient data including greater data consistency, longitudinal, rather than cross-sectional, phenotypes [69], and detailed records of the medications used and their efficacy and side effects. Furthermore, biomarkers that directly reflect the disease pathophysiology and could serve as surrogate marker for efficacious treatments – optimally translating to preclinical animal models – would be of great utility. To this end, EEG, which directly measures electrophysiological activity, may well reflect $Na_V1.2$ dysfunction. Alongside these phenotype data, it will be necessary to perform functional analyses and develop a database to record these data and outcomes (Table 2), possibly in concert with homologous sodium channels (Figure 3). The implementation of methods for performing high-throughput analysis of the impact of variants on channel function would accelerate this process considerably.

Concluding Remarks

SCN2A variants can lead to at least two severe disorders, with gain of function leading to infantile-onset seizures and encephalopathy, and loss of function leading to ASD and/or ID. Normalization of $Na_V1.2$ function has great potential to yield therapeutic benefit in both conditions, though the issues of therapeutic window and selectivity to other sodium channels must be addressed. Concurrent development of such therapies, alongside basic science to understand the neurological impact and reversibility of these variants, is essential (see Outstanding Questions). The results of these endeavors will have direct implications for families affected by *SCN2A* variants. In line with the principle that rare genetic disorders may inform therapeutic strategies for more common disorders [70], we hope that insights gleaned from *SCN2A* may also have wider implications for epileptic encephalopathy, ASD, and ID.

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A.J.C. has consulted for Praxis Precision Medicines. A.L.G. is a member of a CNS scientific advisory board for Otsuka Pharmaceuticals. At the time of the workshop, T.S.O. was an employee of F. Hoffmann-La Roche, Ltd. D.M., J.F.H., and O.K. are full-time employees of F. Hoffmann-La Roche, Ltd. J.R.E. is an employee and shareholder of Xenon Pharmaceuticals. S.P. is a shareholder of RogCon Biotechnology and Praxis Precision Medicines.

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Outstanding Questions

What is the contribution of $Na_V1.2$ to neuronal processing across brain regions?

In which cell classes, and in which cellular compartments, are $Na_V1.2$ channels expressed? How is their expression developmentally regulated across cell classes and within compartments?

How do alterations in $Na_V1.2$ function produce particular phenotypes within these cells and brain regions?

Do changes in $Na_V1.2$ exert cell-autonomous effects, or is the resulting dysfunction more of a circuit-level phenomenon stemming from interactions between cells?

To what extent does the etiology of *SCN2A*-associated disorders overlap with other neurodevelopmental disorders?

What is the full range of symptomatology in *SCN2A*-associated disorders? And do patients with loss-of-function mutations truly have an increased rate of seizures, or is this an ascertainment bias in the current data?

The current SCN2A genotype–phenotype model does not explain choreoathetoid movements, episodic ataxia, or late-onset epileptic encephalopathy, and provides only limited insight into the variation in symptom severity or treatment response. Consistent genetic, functional, and phenotype data for individuals with *SCN2A*-associated disorders would help refine this model.

Why do sodium channel blockers improve seizure control only in some of the individuals with *SCN2A*-associated early onset seizures? Why do not sodium channel blockers improve seizure control in *SCN2A*-associated late-onset seizures?

What are the therapeutic windows for treating *SCN2A*-associated disorders? Are there critical periods of neuronal development in which intervention must occur?

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