

Predicting the impact of sodium channel mutations in human brain disease

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Summary

Genetic alteration of the sodium channel provides a remarkable opportunity to understand how epilepsy and its comorbidities arise from a molecular disease of excitable membranes, and a chance to create a better future for children with epileptic encephalopathy. In a single cell, the channel reliably acts as a voltage-sensitive switch, enabling axon impulse firing, whereas at a network level, it becomes a variable rheostat for regulating dynamic patterns of neuronal oscillations, including those underlying cognitive development, seizures, and even premature lethality. Despite steady progress linking genetic variation of the channels with distinctive clinical syndromes, our understanding of the intervening biologic complexity underlying each of them is only just beginning. More research on the functional contribution of individual channel subunits to specific brain networks and cellular plasticity in the developing brain is needed before we can reliably advance from precision diagnosis to precision treatment of inherited sodium channel disorders.

KEY WORDS

brain, development, gene, mutation, sodium ion channel

1 | INTRODUCTION

The voltage-gated sodium channel occupies a preeminent, yet unanticipated position in epileptology. Even before the first gene for the pore-forming membrane subunit was isolated in mammalian brain in 1986,¹ most neurologists predicted that altering a sodium channel gene would silence neuronal signaling throughout central neural networks, much like the selective antagonists procaine and tetrodotoxin that block the inward sodium current and action potential firing in nerve and muscle. Moreover, based on the crippling phenotype seen in an experimental model available at that time, the *Drosophila* sodium channel mutant “paralyzed,”² few imagined that genetic disruption of this essential membrane protein would

even be compatible with life, much less a life-long episodic hyperexcitability disorder. Nor was it foreseen that sodium channel mutations would create more than simple binary defects in channel behavior, resulting in a still-growing clinical repertoire of neurologic deficits. It took molecular biologists, biophysicists, and human geneticists two more decades to clarify the paradox.

2 | THE FOUR-DIMENSIONAL COMPLEXITY OF BRAIN SODIUM CHANNEL DISEASE

The solution required appreciation of the complexity, redundancy, and dynamic interplay of not one but multiple

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sodium ion channel subtypes expressed by a family of 14 mammalian sodium channel subunit genes throughout brain development. When a genetic variant in a single subunit is identified in an individual, three questions form a starting point for analyzing the potential outcome on brain excitability. *Where* is the mutated channel protein expressed? *What* is wrong with it? *How* does the deviant channel alter cellular firing patterns and network connectivity? The answers help pinpoint a pathogenic circuit and explain how its altered firing behavior produces the clinical disorder. However, epileptic encephalopathies arise early in brain development, and raise a fourth critical dimension to consider, namely, *when* does the defect become functionally apparent, and does the dysfunctional circuit provoke further reactive downstream changes as the brain matures? In other words, do answers to the first three questions change at later time points during disease progression? Understanding the plasticity of genes and neurons in response to an inborn error of a sodium channel reveals unexpected temporal complexity that requires extensive analysis—not once, but at different stages of brain development. A more complete picture of the natural history of the genetic error allows us to predict with increased confidence the most effective remedy for individual patients at any particular stage of their disorder. Remarkable strides in our basic understanding of sodium channel biophysical structure-functional relationships promise to speed the rational design of highly specific compounds that modulate channel behavior disrupted by human epilepsy mutations.^{3,4} Nevertheless, from the disease phenotype perspective, the crystal structure is not a crystal ball. A brief introduction to the four in vivo dimensions of neurogenetic complexity in sodium channel disorders is given below.

Key Points

- Decoding the pathogenicity of variants in sodium channel genes is essential to accurately diagnose and treat infantile epilepsies
- The molecular anatomy of channel subunit expression within this gene family is complex and evolves in the developing brain
- More basic information on sodium channel biology in immature and mature brain circuits will improve our ability to correctly pinpoint and repair the excitability imbalance

3 | NONUNIFORM CHANNEL SUBUNIT ANATOMY: FROM SINGLE NEURON COMPARTMENT TO BRAIN REGION

First, “the” human sodium channel is actually a protein heterotrimer, the final composition of which is drawn from one member of a family of 10 distinct, pore-forming, alpha subunits coassembled with two of four potential regulatory beta subunits.⁵ Epilepsy is firmly linked to human variants in SCN1A,⁶ SCN2A,⁷ SCN3A,⁸ SCN8A,⁹ and SCN1B.¹⁰ Several other subunits, traditionally considered to be expressed at low levels in whole brain assays, have very restricted regional expression, for example, the “cardiac” sodium channel SCN5A within the limbic system,^{11,12} and may ultimately prove to play contributory roles (SCN4A,¹³ SCN5A,¹⁴ SCN7A,¹⁵ SCN9A,¹⁶ SCN10A¹⁷). Although each channel participates in generating an action potential, the alpha subunits populate different neuronal subcompartments,

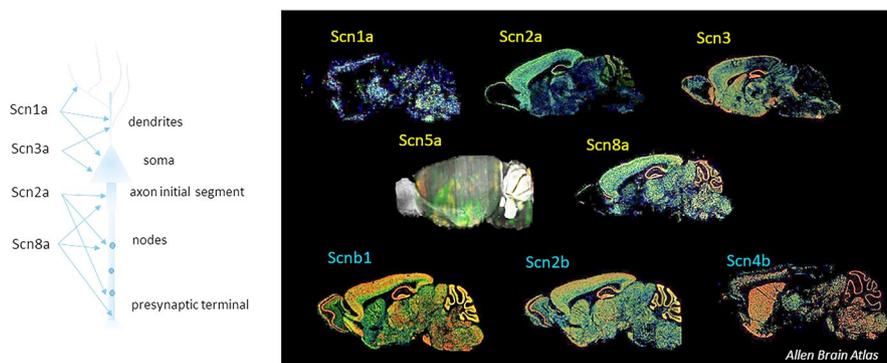


FIGURE 1 Variably overlapping expression of sodium channel subunit genes in subcompartments of a single adult mouse neuron (left) and across adult brain regions (right) contributes to the basis for distinct clinical phenotypes. Heat maps display regional variations in density of mRNAs encoding sodium channel subunits linked to epilepsy in adult mouse brain. The functional expression of sodium current also varies according to age and splice variants found in each cell. Beta subunits (Scnb1-4) regulate the density and behavior of the alpha subunits (Scn1a-8a) and define more complex genetic patterns of circuit dysfunction, because beta subunits assemble with and potentially impair more than one type of alpha subunit. (Courtesy Allen Brain Atlas)

play distinct kinetic roles in membrane electrogenesis, and offer multiple targets for subunit-specific drugs. Channels located in neuronal dendrites and soma influence the behavior of many other types of voltage-sensitive channels and receptors in their membrane vicinity that precisely integrate incoming synaptic inputs. In the axon initial segment, the high density of sodium channels initiates action potentials, and at myelinated nodes of Ranvier, the channels promote rapid impulse conduction to the axon terminal. Sodium channels arising from distinct subunit genes (Figure 1), therefore, actively and independently coregulate the excitability of each compartment. These multiple combinations are the key to understanding selective cellular and circuit vulnerability due to a single gene mutation.

Where is the lesion? The initial electrophysiologic impact of a mutation in a single subunit can be localized to the cerebral circuitry where it is first expressed. However, because members of this channel gene family anatomically overlap with each other in specific spatial patterns (Figure 1), the impact of losing the subunit's specific contribution to the overall sodium current within a circuit is variable. Other available intact family members (or nonfamily members) in a specific neuron may rapidly compensate for the impairment of any one subunit to some degree, and this homeostatic remodeling may explain why firing patterns of some cells that express the mutant channel are not as severely affected as others. However, because cell- and activity-dependent signals that control the expression of membrane ion channels are still poorly understood, the degree of compensation is not a predictable arithmetic function and must be evaluated experimentally. Furthermore, the extent of compensation in every cell may change with age. Thus, what begins as a diagnostic genetic test result returned to the clinician that indicates a well-defined, solitary sequence defect in a single gene, can expand in the developing brain to involve a complex excitability lesion with distinct regional and developmental specificity, dramatically impairing impulse traffic in one network while fine-tuning or even entirely sparing another. This

developmental anatomic complexity allows a mutation of any one channel subunit gene to create complex neurologic syndromes that remain compatible with life and give rise to a striking range of neurologic disorders.

4 | DIVERSITY OF GENETIC VARIATION: MANY SHADES OF SEQUENCE DISRUPTION

Second, functional studies in a single model cell show that diverse types of sequence variants identified in channel subunit genes produce a broad range of biophysical and biologic dysfunction, from almost unmeasurable to severe. Furthermore, nearly everyone, with epilepsy or without, expresses a distinct profile of channel variants, not only among the 14 sodium channel genes, but in hundreds of other voltage-gated membrane ion channels, creating their own personal membrane excitability “channotype.”¹⁸ This new term defines a cluster of functionally interactive ion channel variants whose combined impact on membrane excitability can be assessed in silico by computational models and in vivo with experimental neurogenetic strategies to reveal whether the sum of channel variants enhance or mask the severity of the excitability defect.^{18,19} The channotype extends the relevance of a single mutation “snapshot” into a profile of relevant channel variants that, along with many other loci and epigenomic changes during development, shape the overall clinical severity of a sodium channel mutation in an individual patient and can sharpen the accuracy of genotype-phenotype correlations.

Against this background, more than 1000 human point (single base pair) mutations that lead to single amino acid differences in patients with epilepsy have been mapped onto protein models of the *SCN1A* channel²⁰ (Figure 2). Their phenotypes have been sorted into multiple, partially distinct clinical diagnoses, yet the steadily expanding spectrum²¹ requires that the classification remain fluid.²² Among these *SCN1A*-linked syndromes, clinical severity does not always correspond precisely

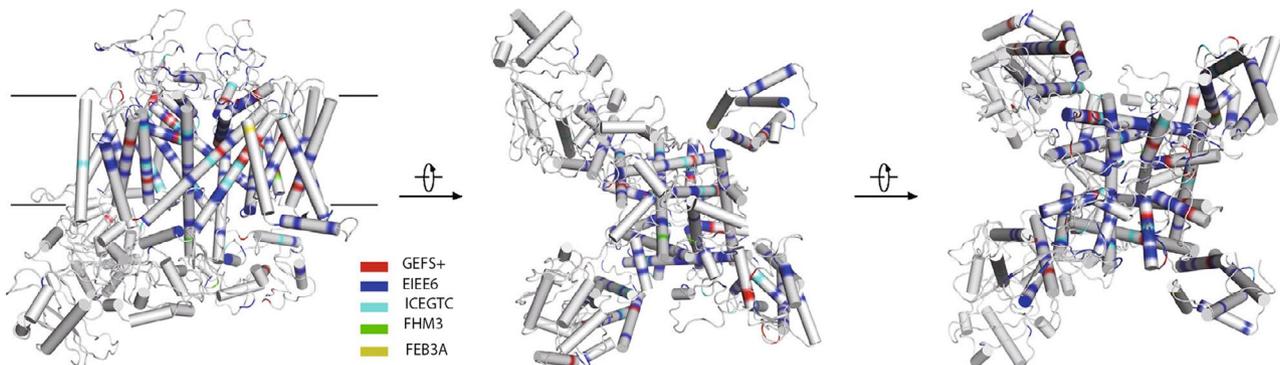


FIGURE 2 Single nucleotide variants in the *Scn1a* sodium channel gene identified in patients with epilepsy. From Huang et al.²⁰ Deleterious sodium channel variants are also found in unaffected individuals (Klassen et al)¹⁸

to the biophysical severity of the mutant channel, despite our ability to measure it in model cells. Various other sodium channel genes fit into the diagnostic phenotype, supporting the idea that the mutation identified by a clinical test is only a starting point to deciphering disease severity and prognosis.

For simplicity, the categorical terms of “gain or loss of function” signify the overall effect of a channel mutation; however, this generally refers only to an electrophysiologic measurement of the amplitude and duration of ion current through a whole cell membrane or even an isolated single pore. Although significant attention is paid to this assay when interpreting single nucleotide variants obtained from clinical exomes for genotype-phenotype correlation with an eye toward “treating the current defect,” a single measurement made in a model cell may not tell the whole story. A source of confusion is that some mutations simultaneously increase current yet diminish the numerical density of channels in the membrane, so that the net alteration in excitability is better determined from analysis of the relevant cell type, preferably derived from the patient. In other cases, nonpore functions mediated by intracellular and extracellular protein interactions may determine the lifetime of the channel in the membrane or the coupling efficiency of the mutant channel to adjacent neuroactive molecules.²³ Furthermore, some variants alter functional channel expression in more profound ways, including how channel transcripts are spliced to form a single protein in different cells. Mutations that produce alternative messenger RNA (mRNA) splicing generate different ratios of protein isoforms in specific cell types,²⁴ giving rise to a potential kaleidoscope of small current changes throughout brain networks. Channel proteins also encode specific functional domains that allow modulation of the sodium current by internal signals. Therefore, the position of the mutation within the channel may have other conditional, and therefore latent, effects on channel function requiring detailed functional characterization.²⁵

4.1 | Compound sodium channel mutations

Infrequently, more than one single nucleotide variant within a single sodium channel gene has been identified in patients, with unforeseen effects.²⁶ This can occur by Mendelian inheritance or, for example, when a *de novo* mutation appears in a gene that already carried a common population variant. Structural variation of various sizes also occurs in DNA, leading not only to potential damage of a gene at the break points but also to changes in whole gene copy number. Small copy number variants within a gene create nucleotide microdeletions or microduplications and may not even be revealed by routine clinical testing. Larger structural variants, although reflecting a single locus, may actually span multiple genes. Remarkably, patients with simultaneous duplication

of three,²⁷ and even five,²⁸ different syntenic sodium channel subunit genes have been reported.

4.2 | Sodium channel mosaics

De novo mutations happen in nongermline proliferating cells during embryonic development and may occur in random daughter cells in the brain, resulting in variable degrees of tissue mosaicism. These somatic mosaics give rise to an alternate phenotypic spectrum according to the size of the mutant subpopulation, which depends on the age and rate of further cell division at the time of mutation. Somatic mutations of both single nucleotides and larger microdeletions that result in *SCN1A* copy number variation have been discovered in cases of Dravet syndrome.^{29,30}

5 | NONPORE FUNCTIONS: A BASIS FOR STRUCTURAL MALFORMATION?

Given the importance of neuronal firing to the strength of synaptic connections, a third intriguing, yet understudied impact of defective sodium channels may involve their role in cell adhesion during cortical development. This has been shown clearly in the case of *SCNB1* regulatory subunits, which project a large immunoglobulin-like hairpin fold into the extracellular space. Of interest, this site corresponds to the mutation site of the first human sodium channel subunit gene linked to epilepsy.¹⁰ *SCNB1* alters the gating kinetics and voltage dependence of all alpha subunits, and might contribute extensively, via nonpore functions of the molecule to ultrastructural synaptic stability, for example, by perturbing ephaptic coupling during early development, as seen in the myocardium.³¹ Cortical malformations associated with sodium channel dysfunction are beginning to be described in experimental models and human patients. Abnormal neural microcircuit patterning has been observed in *Scnb1*³² and *Scn1a* mouse mutants³³ (the orthologous gene in mice is designated by lower case by convention); this altered connectivity due to axon sprouting and synaptogenesis may provoke, as well as reflect, seizure-induced network reorganization. Other reports suggest that *SCN1A* and *SCN2A* subunit sodium channelopathy can coincide with larger scale cortical malformation.^{34–36} Cortical polymicrogyria has been described in cases with mutations in *SCN3A*, perhaps due to its early brain expression.^{37,38}

6 | CELLULAR EXCITABILITY REMODELING DURING MATURATION

The fourth critical dimension is time. To predict the ultimate effects of a mutation in each subunit, we need to know not

only when channels are first expressed in specific cell types and their lifetime in the membrane, but their dynamic potential. Do they remain in their native regional distribution and subcompartmental proportion, and retain their kinetic and even pharmacologic properties once seizures begin? In general, the mechanisms controlling ion channel subunit localization and homeostasis during brain development, along with the microRNAs that likely contribute to their coordinate regulation, are only barely understood.

A key issue is to define the stage when the mutant channel begins to exert a deleterious functional impact on the developing central nervous system (CNS). Not all members of the sodium channel gene family appear in unison or at a specific chronologic age; rather they evolve, replacing fetal with adult isoforms in a cell-specific developmental program that is still only vaguely documented in most brain circuits. A

serial analysis of ion channel mRNAs during the maturation of membrane firing properties in a single cortical cell type, fast-spiking parvalbumin+ interneurons, shows a remarkable shift in the genetic composition of sodium channel subunits (along with others) in the first month of life³⁹ (Figure 3). In mouse brain interneurons, the transcription of some subunits appears early and then subsides (Scn2a, Scn3a, Scnb3), whereas others (Scn1a, Scn8a, Scn9a, Scnb1, Scnb2, Scnb4) appear later and retain high levels of expression throughout adulthood. Other studies reveal that Scn2a appears early in axons and is then replaced in that compartment by Scn8a. Fetal isoforms of Scn2a flux less current compared to adult isoforms, and when mice are engineered to express adult Scn2a isoforms in neonatal brain, there is a dramatic lowering of the seizure threshold.⁴⁰ These data illustrate the variable impact of a mutation in a distinct channel at different

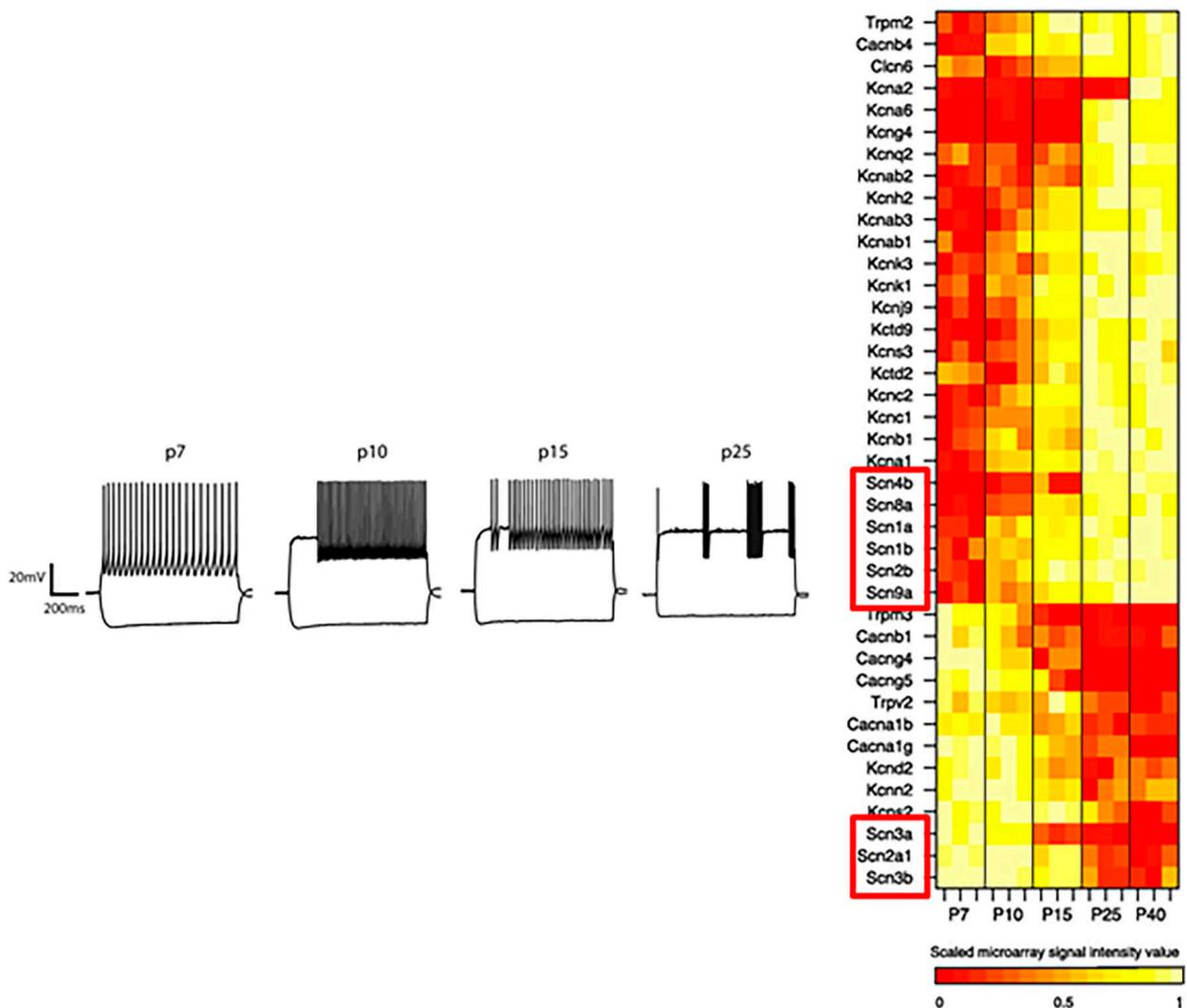


FIGURE 3 Variable developmental impact of channel subunits during brain maturation In neocortical fast-spiking γ -aminobutyric acid (GABA)ergic interneurons, firing properties at 1 week (P7) are dramatically different from those at 1 month (P25), reflecting a clear developmental switch in ion channel expression in the second week. Single-cell transcriptomic study reveals that subunits Scn2a(1), Scn3a, and Scnb3 are expressed early and later decline in density, whereas Scn1a, Scn8a, Scn9a, and Scnb1, Scnb2, and Scnb4 are expressed predominantly at later ages. Modified from Okaty et al³⁹

ages in different circuits, with major implications for pharmacologic treatments targeting a specific channel subunit during disease progression.

6.1 | Acquired changes in sodium currents

To further complicate the endogenous developmental programs of subunit expression, aberrant firing itself may exert a profound effect on homeostatic remodeling, and a subunit mutation may change this reactive profile.^{41,42} Other pathologic insults may trigger reactive sodium channel expression changes, resulting in an “acquired” multi-subunit sodium channelopathy. An interesting example is the finding that overexpression of beta-amyloid peptide, the pathogenic amyloid-forming fragment in Alzheimer disease (AD), drives down *Scn1a* expression in cortical interneurons.⁴³ This secondary *Scn1a* lesion may contribute in part to the hyperexcitability identified in both mouse models and patients with AD dementia.⁴⁴ Recent evidence suggests that not all downstream sodium channel plasticity is malignant. *Scn1a*-deficient interneurons display severely impaired firing patterns in response to depolarization before 1 month of age⁴⁵; however, at least some interneurons in mutant animals fully recover adult firing patterns by 2 months postnatally.^{46,47} The mechanism of this unexpected excitability recovery, as well as its timetable in human is unknown, and will be of certain interest to future repair strategies.

6.1.1 | Sodium channel defects and epilepsy comorbidities

Unsurprisingly, sodium channel mutations lead not only to seizures but also to extensive network imbalance that determines clinically significant epilepsy comorbidity, including cognitive impairment, neurodevelopmental disorders, and premature mortality. These outcomes are of prime importance to the clinician armed with genetic information; however, the basic mechanisms underlying these ancillary phenotypes are even less understood than the seizure mechanisms.

6.2 | Cognitive impairment

Cognitive delay is a defining feature of several sodium channelopathies giving rise to Dravet syndrome, and attempts to dissect the age dependence and critical circuitry are ongoing.^{48,49} A recent RNA-seq profiling study of the hippocampal RNA transcriptome revealed early onset and extensive dysregulation of gene transcription due to age, genetic background, and seizure activity.⁴² A sleep phenotype may contribute to impaired memory consolidation and cognitive performance.⁵⁰ Imaging biomarkers of a metabolic encephalopathy that could favor these deficits appear later in development. Defective 18-fluorodeoxyglucose uptake in Dravet syndrome patients

with *SCN1A* mutations is rare before the age of 3 years despite severe cognitive impairment; however, significant hypometabolism can be detected by 6 years of age.⁵¹

6.3 | SIDS and SUDEP in sodium channel disease

Sudden unexpected death in epilepsy (SUDEP) is now recognized as a major cause of premature mortality in persons with seizure disorders and is dramatically evident in sodium channel disease. Mutations in *SCN1A*, *SCN1B*, *SCN5A*, and *SCN8A* genes are associated with increased risk of early mortality and estimates of SUDEP incidence in Dravet syndrome reach 10% or greater. Of interest, mutation of *SCN1A* has now been associated with sudden infant death syndrome (SIDS) in a small cohort.⁵² Although no epilepsy history or clinical seizures were reported immediately prior to death in these individuals, the pathology of the hippocampus suggests seizure-related synaptic remodeling,⁵³ and subtle clinical events in these infants may have gone unrecognized.

The underlying mechanism of SUDEP centers on impaired cardiorespiratory control in the aftermath of a convulsive seizure. In this regard, sodium channels populate key forebrain and lower brainstem networks and mutations could readily alter the stability of respiratory⁵⁴ and central autonomic reflexes⁵⁵ that govern postictal autoresuscitation. A cardiac basis for arrhythmia has been confirmed in patient cardiac myocytes⁵⁶; however, it remains unclear whether *SCN1A*-linked arrhythmias are themselves lethal.

A recently discovered central autonomic mechanism mediating seizure-linked premature mortality involves the threshold for spreading depolarization (SD) in the lower brainstem. This process, which generates an aberrantly large release of glutamate and potassium in the extracellular space, has been explored in SUDEP mouse models, including *Scn1a* mutations, and can explain why the final seizure is terminal. Following the final seizure and during the period of postictal hypopnea and bradycardia, a prolonged and profound wave of neuronal depolarization silences brainstem cardiorespiratory areas, leading to a sequence of apnea, asystole, bradycardia, and cardiorespiratory arrest in *Scn1a* +/R1407X Dravet syndrome mouse models. This sequence unfolds in the same pattern and time frame documented in human SUDEP.⁵⁷ Slices of medulla from *Scn1a* mutant mice maintained in vitro also show a strikingly lower threshold than wild-type brainstem to trigger SD.⁵⁸ The molecular and cellular explanation for the impact of sodium channel dysfunction on brainstem SD threshold is so far unknown. Of interest, *SCN1A* is one of three genes also responsible for familial hemiplegic migraine (FHM3), a seemingly unrelated syndrome that also includes a spreading depolarization-based aura. In contrast with epilepsy mutations, analyses of these human *FHM3* mutations reveal an overall gain of function associated with this phenotype.⁵⁹ The low

threshold for spreading depolarization, a phenomenon characterized by profound cytotoxic edema,⁶⁰ may also be related to a propensity for severe and fatal cerebral edema reported during status epilepticus in children with Dravet syndrome.⁶¹

7 | SUMMARY

The original formulation of “one gene, one disease” fails to account for the diverse locations and roles of sodium ion channel–related pathophysiology. We now realize that genetic variation alters neuronal sodium currents and human brain circuit activity in complex and highly individual ways, leading to a spectrum of phenotypes that challenge the hope for absolute clinical clairvoyance based on current genetic testing. However, the pace of advances in molecular genetics that will help penetrate this complexity and point to more precise therapies is encouraging. Recently, intronic sequence variants located outside coding regions of the *SCN1A* gene have been detected that cause pathogenic exon skipping, splice-form alterations leading to reduced channel protein, and Dravet syndrome,⁶² opening a door to RNA-directed therapies in certain patients. Genomic profiling of both adult and developmental epilepsies reveals extensive variation not only in ion channel subunits^{18,63} but other nonchannel⁶⁴ proteins known to modulate *SCN1A* by epistasis. Future research advances in phenotype prediction must focus on expanding the list of sodium channel genetic interactors in order to reveal the basis for the rich phenotypic complexity. The mechanism of these epistatic suppressor genes may also point to novel therapeutic approaches. Finally, the relevance of the fourth dimension of sodium channelopathy is also becoming more clear. We now realize that sodium channel dysfunction launches a cascade of downstream molecular remodeling throughout early, and even later, brain development that dramatically sculpts epilepsy syndromes, and potentially complicates a single, life-long management plan for the patient. Some brain cells are critically dependent on a particular sodium channel at a certain age, whereas in others, the contribution of the same sodium channel mutation to network excitability is surprisingly small. This may be due to the capacity of certain neurons to remodel their firing properties. Learning exactly how this homeostatic plasticity is accomplished may provide remarkable insight not only into pathogenesis but also effective age-dependent treatment strategies for pharmacoresistant patients.

CONFLICT OF INTEREST

None of the authors has any conflict of interest to disclose. We confirm that we have read the Journal's position on issues

involved in ethical publication and affirm that this report is consistent with those guidelines.

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