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Sodium channelopathies in neurodevelopmental disorders

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Abstract

The voltage-gated sodium channel α subunit genes comprise a highly conserved gene family. Mutations of three of these genes, *SCN1A*, *SCN2A* and *SCN8A,* are responsible for a significant burden of neurological disease. Recent progress in identification and functional characterization of patient mutations is generating new insights and novel approaches to therapy for these devastating disorders. In this paper we review the basic elements of sodium channel function that are used to characterize patient mutations. We summarize a large body of work using global and conditional mouse mutants to characterize the *in vivo* roles of these channels. We provide an overview of the neurological disorders associated with mutations of each of these genes and examples of the effects of patient mutations on channel function. Finally, we highlight therapeutic interventions that are emerging from new insights into mechanisms of sodium channelopathies.

The sodium channel gene family

The voltage-gated sodium channel α subunit gene family is comprised of ten genes in the human genome (Figure 1A). The three sodium channel genes expressed at a high level in neurons of the central nervous system are shown in red. The gene family was generated by whole genome duplication events during early chordate evolution generating four sodium channel loci, followed by tandem gene duplications within the loci on chromosomes 2 and 3 later in vertebrate evolution[1,2]. The channels are key players in the initiation and propagation of action potentials, the unit of electrophysiological activity in neurons. Sodium channels are among the most highly evolutionarily conserved genes in the human genome, and retain regions of significant sequence identity to invertebrate and prokaryotic sodium channels. Deviations from normal channel function have major clinical consequences that include seizures, intellectual disability, behavioral abnormalities and movement disorders. The genes *SCN1A* (Nav1.1), *SCN2A* (Nav1.2) and *SCN8A* (Nav1.6) together account for >95% of brain sodium channel transcripts and are responsible for most of the known neurological sodium channelopathies. In one survey of 8,565 individuals with epilepsy and neurodevelopmental disorders, 5% carried mutations in one of these three genes [3].

The structure of the sodium channel protein is represented in Figure 1B. The protein includes four homologous domains each containing six transmembrane segments with high sequence conservation, two large cytoplasmic loops with lower sequence conservation, a highly conserved inactivation gate, and cytoplasmic N-terminal and C-terminal domains. Consistent with their more recent divergence, *SCN1A* and *SCN2A* are more closely related to each other than to *SCN8A* (Figure 1C). The 24 transmembrane (TM) segments exhibit

93% amino acid sequence identity between *SCN1A* and *SCN2A* but only 85% and 90% identity to *SCN8A*. An example of an invariant TM segment is shown in Figure 1D. In the more divergent N-terminus, there is 88% sequence identity between *SCN1A* and *SCN2A* but only 66% identity to *SCN8A*. In spite of extensive sequence conservation, the channels have diverged in function and regulation, and each of these genes is essential in the mammalian genome. In this review, we describe the functions of these closely related channels and examine the clinical consequences of genetic variation. Evolutionary conservation offers a clue to functional impact, and variation at residues that are identical in all three channels (e.g. Figure 1D) tend to be more deleterious than variants at residues that have diverged. Clinical exome sequencing has revealed a major role for rare sodium channel variants in neurodevelopmental disorders (Table 1). Current research is focused on distinguishing between neutral and deleterious variants and understanding the relationship between altered channel function and clinical outcome. Recent progress in physiological, molecular and clinical studies is generating novel therapeutic approaches.

Sodium channel α subunits are associated in the neuronal cell membrane with single-transmembrane β subunits encoded by the genes *SCNB1* to *SCNB4*[4]. The β subunits influence trafficking and electrophysiological properties of the α subunits but do not themselves have channel activity. Their clinical roles have been recently reviewed[5] and will not be discussed. *SCN3A* encodes a sodium channel that is expressed at a high level early in the development of the CNS and at a low level in the adult CNS. A small but growing number of mutations of *SCN3A* have been identified in patients with epileptic encephalopathy [6] and cortical malformations including polymicrogyria and microcephaly [7,8]. These patients were recently reviewed[8] and are not included here.

Physiology of $Na_v1.1$, $Na_v1.2$ and $Na_v1.6$.

The voltage-gated sodium channel α subunit is a large protein of 2,000 amino acids with a complex mode of action. It has been selected through evolution to open transiently in response to depolarization of the neuronal membrane and to close within milliseconds, generating a brief inward flow of sodium ions. Single amino acid substitutions frequently change multiple components of channel function, making it difficult to systematically classify mutations. We are still at the stage of identifying rare variants, cataloging functional effects on a few parameters, and looking for correlations with clinical outcomes. We describe some basic features of the human sodium channels for the non-expert reader, as a starting point for the discussion of pathogenic consequences of patient mutations.

Electrophysiology

Sodium channels are located throughout the neuronal cell membrane on axons, dendrites and soma. In response to a depolarizing shift in electrical potential across the membrane, conformational changes in the positively-charged transmembrane segments initiate the transition from closed to open channel state, permitting an influx of sodium ions and initiation of the action potential. Fast inactivation follows within milliseconds: the channel pore is blocked by the inactivation gate and the channel enters the inactive conformation. The influx of sodium ions is thus limited to a brief interval. Recovery from

inactivation returns the inactivation gate to its resting position and restores the stable closed conformation. Changes in this progression underlie the pathogenesis of sodium channel mutations.

Electrophysiological measurements used to assess the functional effects of patient mutations are shown in Figure 2. Peak or transient current refers to the maximal inward flow of sodium ions at the beginning of the action potential. The small remaining current at 100 msec after the peak is defined as 'persistent current' (Figure 2A). The voltage-dependence of channel activation describes channel opening (Figure 2B), and the voltage dependence of inactivation describes channel closing (Figure 2C); these are altered by many pathogenic mutations. 'Resurgent current' is generated when channels open during repolarization after an action potentialm and contributes to repetitive neuronal firing (Figure 2D).

Partial or complete loss of function mutations are recognized by reduction of peak current. Gain-of-function mutations result in qualitative changes of other parameters. Increased persistent current, due to impaired stability of the closed channel conformation, is frequently associated with seizures. $Na^v1.6$ generates a higher proportion of persistent and resurgent current than $Na_v1.1$ or $Na_v1.2$ in many types of neurons [9,10]. A shift the voltage-dependence of activation towards more negative values, a hyperpolarizing shift, leads to premature channel opening and excess neuronal firing. Conversely, depolarizing variants shift the voltage dependence of activation towards more positive potentials and reduce channel activity. The more hyperpolarized voltage dependence of activation of Nav1.6 underlies its role in initiation of action potentials. Delayed channel inactivation can also contribute to excess neuronal activity.

Experimental measurements are influenced by the choice of cells in studies of transfected channels. Cells commonly used include kidney-derived HEK cells, neuroblastoma-derived ND7/23 cells, and cultured primary neurons. In one recent study of $Na_v1.6$ variants, combined analysis in neuroblastoma and primary neuronal cultures was more consistent than either alone in predicting clinical consequences [11]. Reprogrammed neurons from patient-derived iPSCs make it possible to assess the function of mutant channels in the context of the patient's individual genetic background [12]. Biophysical properties are influenced by interactions with β-subunits, calmodulin, and other proteins present in different types of neurons[13–15] and many pathogenic mutations alter more than one biophysical parameter. These variables make it difficult to classify the functional effects of deleterious mutations assayed in different laboratories. Animal models provide access to the effects of mutations in different types of neurons, but *in vivo* models are limited to a small number of mutations.

Subcellular localization

The sodium channels are localized in the neuronal cell membrane, with concentration at the axon initial segment (AIS) and nodes of Ranvier of myelinated neurons. Action potentials are initiated at the AIS and propagated in two directions. Forward propagation from the *distal* AIS (further from the cell body) initiates conduction down the axon to the nerve terminus. $Na_v1.6$ is the major channel in the distal AIS of adult neurons[16–23]. A recent study employing photoactivation localization microscopy estimated that the concentration of $Na_v1.6$ is 40-fold higher at the AIS than in the soma and proximal dendrites[24].

The hyperpolarized voltage dependence of Nav1.6 contributes to the initiation of action potentials at the distal AIS; in the absence of Nav1.6 the threshold for initiation of action potentials is increased[9,19,25–30].

The *proximal* AIS (closer to the cell body) is occupied by $Na_v1.1$ or $Na_v1.2$, depending on cell type and stage of development[17,20,21]. Back propagation from the proximal AIS to the soma and dendrites modulates synaptic strength and mediates learning and memory. Within the hippocampus, $Na_v1.1$ is found at the AIS of interneurons but not excitatory neurons[21,22,31]. Nav1.2 is localized to the soma and dendrites of pyramidal neurons[32].

The concentration of sodium channels at the nodes of Ranvier mediates saltatory conduction in myelinated neurons. $Na_v1.2$ is the major nodal channel during early development, and replaced by $Na_v1.6$ during postnatal development[33,34]. $Na_v1.1$ is also expressed in some nodes of Ranvier[31]. In mice lacking $Na_v1.6$, there is reduced transmission at the neuromuscular junction and hind limb paralysis[35]. In adult unmyelinated neurons, Nav1.2 is localized along the length of the axon[16,34,36].

Channel localization is mediated by interaction with protein complexes that include the structural proteins Ankyrin G and MAP1B. Ankyrin G binds a conserved 9-residue motif in intracellular loop 2 of the voltage gated channels (Figure 1B)[37–39]. Ankyrin G binding is sufficient to localize proteins to the AIS and nodes of Ranvier[38,39] and mutation of the ankyrin binding motif prevents localization[23,39]. The cytoplasmic N-terminus of Nav1.6 contains a binding site for the microtubule-binding protein MAP1B[40]. Interaction with MAP1B stabilizes Nav1.6 at the AIS by preventing rapid endocytosis[41]. MAP1B does not interact with Nav1.1 and may play a role in preferential localization of Nav1.6 to the distal AIS[40]. Neither Ankyrin G nor MAP1B is required for somatodendritic localization of Nav1.6[24,39,41]. The missense mutation p.Ser21Pro in the N-terminus of mouse *Scn8a* prevents correct localization by trapping the mutant channel in the Golgi[42].

Alternative splicing of sodium channel transcripts.

SCN1A, *SCN2A* and *SCN8A* undergo two types of alternative splicing. Each gene contains two copies of the 5th exon encoding transmembrane segments 3 and 4 of domain 1. The choice between inclusion of exon 5N (neonatal) versus exon 5A (adult) is developmentally regulated. The peptides encoded by exons 5A and 5N differ by three amino acids in *SCN1A*[43], and by a single amino acid in *SCN2A* and *SCN8A*[44]. In $Na_v1.2$, the voltage dependence of activation of channels expressing exon 5N is more depolarized than channels with exon 5A, resulting in delayed channel opening[45]. Three pathogenic mutations of *SCN2A* were reported to have a more severe effect on the neonatal protein than the adult form[46]. These differences may explain the clinical improvement in some sodium channelopathies after the switch from neonatal to adult splice form.

The gene region encoding domain III contains another set of alternatively spliced exons that are designated “poison exons” because they contain in-frame stop codons that truncate the channel protein. The structure of these exons is similar but not identical in the three genes. In *SCN8A*, the pair of mutually exclusive exons 18A and 18N encode segments 3 and 4 of domain III, corresponding to exons 5A and 5N in domain I, and indicating a

shared evolutionary origin[47]. Exon 18N contains an in-frame stop codon, and is expressed at a low level in non-neuronal tissues including glia[47,48]. Transcripts containing 18N are subject to nonsense-mediated decay. Inclusion of exon 18A appears to be restricted to neurons, and is mediated by neuron-specific splice factors including RbFox1[48-50]. Cultured astrocytes and oligodendrocytes express only exon 18N, preventing expression of full-length Nav1.6 protein[48]. Poison exons may represent a fail-safe mechanism to prevent damage to non-neuronal cells that could result from expression of voltage-gated sodium channels[44].

In *SCN1A* and *SCN2A,* individual poison exons are also located in regions encoding domain III[51,52]. These poison exons are potential targets for manipulating gene expression. For example, blocking the inclusion of exon 20N in transcripts of *SCN1A* increases the expression of Nav1.1 *in vivo*[53]; the therapeutic application is discussed later.

Sodium channel function in the mouse.

The evolutionary conservation of the sodium channel gene family in mouse and human has led to a large body of experimental data on the *in vivo* function of specific sodium channel genes. We discuss below the effects of global knockout of each gene (Table 2A) and the use of conditional knockouts to dissect the pathogenic effects of sodium channel mutations in different classes of neurons (Table 2B and 2C).

Global knock-out mice.

The distinct *in vivo* functions of Nav1.1, Nav1.2 and Nav1.6 are evident from comparison of knock-out mice with (null) mutations of each gene. Complete. (homozygous) loss of each gene is lethal, but the phenotypic effects of inactivation differ (Table 2A). Global inactivation of *Scn1a* results in a seizure disorder with onset at 3 weeks[31,54]. Global inactivation of *Scn2a* results in neonatal death due to respiratory failure[55]. Global inactivation of *Scn8a* causes failure of the neuromuscular junction and hind limb paralysis [34,56].

Heterozygous null mice with 50% of normal channel gene expression have less severe abnormalities. Haploinsufficiency for *Scn1a* results in spontaneous convulsive seizures, like the homozygote, with later onset than in homozygotes, 50% lethality, and impaired social interaction and poor spatial learning[57,58]. Haploinsufficiency for *Scn2a* causes absence seizures (brief periods of immobility) and behavioral abnormalities with normal survival[59,60]. Haploinsufficiency for *Scn8a* results in absence seizures[61] and anxiety-like behavior[62] with normal life span [35,56].

Genetic modifiers and gene interactions in the mouse.

Genetic divergence between inbred strains of mice has been used to identify modifier genes that influence disease severity. For example haploinsufficiency of *Scn1a*, a model of Dravet Syndrome, results in spontaneous seizures in strain C57BL/6J but not in strain 129[63]. This difference was traced to a previously unrecognized splice site variant in the *Gabra2* gene in strain C57BL/6J that causes a three-fold reduction in expression of the α2 subunit of the $GABA_A$ receptor[63,64]. This *Gabra2* variant also accelerates seizure onset in mice with an epileptogenic mutation of *Scn8a*[65].

Strain C57BL/6J also carries an exonic splice site variant in the gene encoding the splice factor *Scnm1,* resulting in that exacerbated dystonia in the partial loss-of-function medJ mutant of *Scn8a*[66–68]. Variants in the human orthologs of these modifier genes may contribute to observed differences among patients with identical sodium channel mutations[69].

Interactions between multiple ion channel variants have also been demonstrated by combining mutants in the mouse. For example, heterozygous loss-of-function of *Scn8a* is protective against seizures in *Scn1a* haploinsufficient mice[70,71]. Variation in the potassium channel *Kcnv2* modifies the severity of seizures caused by a gain-of-function variant of *Scn2a* [72]. Conversely, haploinsufficiency of *Scn2a* mitigates seizures in the Kcn1a$^{-/-}$ mice [73]. These observations predict potential gene interactions in patients. In a study of patients with monogenic epilepsy due to cation channel variants, the frequency of secondary deleterious variants in other ion channels was higher than in controls, suggesting exacerbation of the primary pathogenic mutation[74].

Sodium channel mutations in human disease.

The past few years has seen a tremendous increase in the association of sodium channelopathies with neurodevelopmental disorders. Not surprisingly in view of their evolutionary and functional similarities, there is considerable overlap among clinical conditions caused by mutations of *SCN1A*, *SCN2A* and *SCN8A* (Table 1). To date, the highest number of sodium channel mutations have been identified in patients with developmental and epileptic encephalopathy (DEE), complex disorders characterized by onset of intractable seizures within the first year of life, intellectual disabilities, movement disorders and elevated risk of sudden unexpected death in epilepsy (SUDEP). Most DEE mutations arise *de novo* in the patient; a few are inherited from a unaffected mosaic parent[75]. In addition to DEE, *SCN1A*, *SCN2A* and *SCN8A* are associated with mild seizure disorders and are high confidence genes for autism spectrum disorder (Gene.SFARI.org 2020).

Pathogenic mutations identified by exome or genome sequencing in patients are classified by electrophysiological assay as either ‘loss-of-function’ (LOF), including protein truncation and inactivating missense mutations, or ‘gain-of-function’ (GOF), amino acid substitutions that alter biophysical properties like voltage-dependence, resurgent current, and persistent current (Figure 2). What appear to be minor changes in these parameters in assays often have major impact *in vivo*. It is medically important to distinguish between GOF and LOF mutations because of their different implication for treatment. Patients with GOF mutations often benefit from sodium channel blockers, while LOF mutations are exacerbated by further reduction in sodium channel activity. Thousands of sodium channel variants have been identified in patients, but only a few hundred have been subjected to functional studies, and most newly described missense variants must still be classified as Variants of Unknown Significance.

In this section we summarize current knowledge regarding the clinical consequences of mutations in the three major sodium channel genes, pointing out overlaps and differences, followed by discussion of new therapies and questions for the future.

Databases are available with compiled information about patient variants of *SCN1A* (www.gzneurosci.com/scn1adatabase/), *SCN2A* and *SCN8A* (SCN8A.net).

SCN1A.

Developmental and epileptic encephalopathy (DEE).—Dravet Syndrome is the most common of the DEEs, with an incidence of 1/20,900 in the US population [76]. Eighty to 90% of patients with Dravet Syndrome have *de novo* mutations of *SCN1A*, and more than 1250 unique mutations have been reported[77,78]. The average age of seizure onset is 6 months. The first seizure is often triggered by fever or other elevated body temperature[79,80]. Development is often normal during the first year, but most patients develop cognitive, intellectual and motor co-morbidities during the second year of life[81]. Ataxia is a comorbidity in 60% of patients[79].

The major molecular mechanism underlying Dravet Syndrome is haploinsufficiency of *SCN1A*[80]. Most mutations are located in coding sequences, and more than half result in protein truncation by frameshift, nonsense or splice site mechanisms[82]. Missense mutations in Dravet Syndrome also result in loss of channel function, as shown for the patient mutation p.Ser259Arg (Figure 3A)[83,84]. To explain the 5 to10% of Dravet Syndrome patients lacking mutations in coding exons, attention has been directed to the noncoding sequences of *SCN1A*. Recently, noncoding variants in intron 20 were demonstrated to reduce *SCN1A* expression by increasing the inclusion of the ‘poison exon’ 20N, containing an in-frame stop codon, leading to protein truncation[85].

Mouse models of Dravet Syndrome with haploinsufficiency of *Scn1a* reproduce clinical phenotypes including early onset spontaneous tonic-clonic seizures, susceptibility to elevated temperature, and behavioral abnormalities. To identify the neurons contributing to seizures, conditional (floxed) alleles of mouse *Scn1a* have been combined with neuron-specific CRE recombinase. Seizures can result from loss of *Scn1a* expression in inhibitory neurons[86,87], more specifically in parvalbumin-positive fast-spiking inhibitory neuron [87]. Inactivation of *Scn1a* in the hippocampus by Cre injection causes learning deficits and elevated sensitivity to thermally-induced seizures[88]. Studies in global haploinsufficient mice suggest that reduced excitability of Purkinje neurons may contribute to ataxia[54,89].

The lethal seizure phenotype in the Dravet mouse models exhibits incomplete penetrance, and as many as 50% of $Scn1a^{+/-}$ mice are unaffected. This may be explained by a compensatory up-regulation of other sodium channels around one month of age, with sufficient variability to completely protect some individuals [90,91].

SUDEP is the leading cause of mortality in Dravet Syndrome, accounting for up 20% of deaths[92]. *Scn1a* haploinsufficient mice exhibit sudden, early death accompanied by impaired cardiac and respiratory function[93–95]. Neuronal sodium channels are expressed in cardiomyocytes at approximately 1% of their level in neurons, and expression of *Scn1a* in cardiomyocytes could mediate a direct effect of pathogenic mutations on cardiac function[93]. However, in *Scn1a* haploinsufficient mice, apnea and respiratory failure precede the cessation of cardiac function[96].

Other *SCN1A* seizure disorders.—Mutations of *SCN1A* were originally identified in patients with GEFS+ (genetic epilepsy with febrile seizures plus)[97], which manifests as childhood febrile seizures with afebrile seizures sometimes continuing beyond the age of 6 years[80]. The majority of *SCN1A* mutations in GEFS+ are missense mutations, with effects on channel function that range from partial loss of function to gain of function[82,98]. The p.Arg1648His mutation is a gain-of-function variant identified in GEFS+ that exhibits increased persistent current (Figure 3B)[99]. Other rare syndromes include epilepsy of infancy with migrating focal seizures, and myoclonic-atonic epilepsy[80]. The same mutation can generate a range of severity within the same family[97,100], suggesting the influence of unidentified genetic modifiers.

Familial hemiplegic migraine.—*SCN1A* is one of three genes implicated in familial hemiplegic migraine. This rare autosomal dominant disorder is characterized by severe migraine with aura accompanied by transient hemiplegia (unilateral paralysis)[101]. Functional analysis of ten *SCN1A* mutations associated with familial hemiplegic migraine demonstrated GOF effects[101–103].

SCN2A.

Developmental and epileptic encephalopathy (DEE).—*SCN2A*-associated DEE is characterized by severe seizures, intellectual disability, and movement disorders including dystonia and chorea[104]. Many patients exhibit autistic behaviors[104]. A distinction has been made between early and late onset forms of the disorder. Early seizure onset, prior to 3 months of age, is associated with GOF mutations of *SCN2A* including increased persistent and peak currents, delayed channel inactivation, and hyperpolarized voltage dependence of activation[104]. The variants p.Phe1597Leu and p.Ile1473Met both cause a hyperpolarizing shift in voltage dependence of channel activation that results in premature channel opening [104,105]. The variant *SCN2A*- p.Leu1432Pro causes a hyperpolarizing shift in the voltage dependence of activation and inactivation as well as altered channel kinetics, leading to early onset DEE[106]. Patients with GOF mutations respond to treatment with sodium channel blockers[104]. In a transgenic model of a GOF mutation in *Scn2a*, spontaneous seizures are accompanied by elevated activity of hippocampal CA1 and CA3 neurons[107,108].

In contrast to the early onset cases, DEE with seizure onset after 3 months of age is associated with partial or complete loss of function of *SCN2A*, including missense, frameshift, nonsense, and splice-site mutations[104,105,109–111]. The missense mutation p.Pro1622Ser results in a hyperpolarizing shift of fast inactivation (Figure 3C)[104]. The protein truncation mutation p.Arg102Stop was identified in a child with onset of intractable seizures at 19 months of age, severe mental decline and autistic behavior[109]. In patients with partial or complete loss of function of *SCN2A*, symptoms are exacerbated by sodium channel blockers[104].

Benign Familial Neonatal-Infantile Seizures (BFNIS).—Gain of function mutations of *SCN2A* are also responsible for BFNIS, a transient disorder characterized by seizure onset before 8 months of age, seizure clusters during the first few years of life, and resolution after 2 years of age[104,112]. Missense mutations in BFNIS are clustered in

transmembrane segments S4 and S5[113]. The p.Leu1653Val variant causes accelerated channel opening (Figure 3D)[114]. Other observed changes include hyperpolarized voltage dependence of activation and depolarized voltage dependence of inactivation[114,115]. The GOF variants can be managed with sodium channel blockers and usually resolve with age[111]. Most BFNIS variants are inherited and less deleterious than the *de novo* mutations in DEE[104,111].

Autism Syndrome Disorder (ASD) and Intellectual Disability (ID).—Mutation of *SCN2A* is strongly associated with ASD[116] and is estimated to account for 7.5 cases of ASD/ID per 100,000 births[109,117]. These heterozygous mutations result in partial or complete loss of channel function. Protein truncating mutations are common[113,116]. Missense mutations cluster around the ion selectivity filter of the pore loop; for example, the LOF mutation p.Arg937His causes complete loss of sodium current (Figure 3E)[116].

It is not clear why some LOF mutations in *SCN2A* result in DEE while others lead to autism and intellectual disability. The phenotypes of mice with haploinsufficiency of *Scn2a* include behavioral abnormalities and absence epilepsy (brief periods of immobility and staring) but no spontaneous convulsive seizures[118]. Conditional deletion of one copy of *Scn2a* in excitatory neurons also results in absence seizures and abnormal behavior[59,118] (Table 2B). Loss of *Scn2a* reduces backpropagation of action potentials to the soma and dendrites of excitatory neurons, resulting in synaptic impairment that may contribute to ASD and ID[32].

Episodic ataxia.—Another condition caused by gain-of-function mutations of *SCN2A* is episodic ataxia[119,120]. Aaxic episodes begin after 10 months of age, last for minutes to hours, and occur on a weekly to monthly basis[119]. Most affected individuals also experience BFNIS-like seizures by 3 months of age[119]. The later onset of ataxia compared with seizures may reflect the later initiation of *SCN2A* expression in cerebellum compared with forebrain[120]. Many episodic ataxia mutations are located in DIVS4 or the adjacent intracellular linker. The recurrent mutation p.Ala263Val[119] causes elevated persistent current and slowed channel inactivation (Figure 3F)[120]. Homozygous knock-in of p.Ala263Val in the mouse results in seizures and increased mortality[121].

SCN8A.

Developmental and epileptic encephalopathy (DEE).—The major class of *SCN8A* mutations in DEE are *de novo* GOF mutations causing elevated channel activity with major effects in excitatory neurons. This is more similar to pathogenesis of *SCN2A* mutations than the haploinsufficiency of *SCN1A* in inhibitory neurons. DEE mutations in *SCN8A* are *de novo* missense mutations[122]. The average age of onset is 4 months, with multiple seizure types, developmental delay, cognitive impairment, movement disorders and elevated risk of lethality[122–124].

SCN8A mutations have been identified in more than 300 patients[125]. Patient mutations are localized in transmembrane segments, the inactivation gate and the C-terminus of Nav1.6. Electrophysiological consequences of patient mutations include premature channel opening (Figure 3G), impaired channel inactivation (Figure 3H), and elevated resurgent current (Figure 3I), all leading to elevated neuronal activity (Figure 4). The increase of neuronal

firing caused by p.Asn1768Asp was demonstrated in transfected hippocampal neurons[126] (Figure 4A). In the mouse knock-in model of p.Asn1768Asp, there is spontaneous firing of hippocampal CA1 neurons (Figure 4B)[127] and burst firing of neurons of the entorhinal cortex[128] (Figure 4C). Neurons in cortical layer 2/3 do not exhibit either of these abnormalities (Figure 4B).

p.Arg1872Trp is a recurrent *de novo* mutation of *SCN8A* that has been observed in 8 unrelated individuals with DEE[129]. This mutation causes premature channel opening and impaired inactivation (Figure 3H). Substitution of Arg1872 with leucine and glutamine is also recurrent, with more than 20 independent patient mutations reported[129]. These mutations are predicted to weaken the ionic interaction between the positively charged arginine residue 1872 in the cytoplasmic C-terminus and negatively charged residues in the inactivation gate[130] resulting in destabilization of the closed conformation and excess channel activity.

In a conditional mouse model of Scn8a-p.Arg1872Trp, CRE mediated activation of the mutant channel in excitatory neurons of the forebrain is sufficient to initiate spontaneous convulsive seizures and death (Table 2C)[131]. When the mutant channel was activated in adult mice, lethal seizures began within weeks, demonstrating a likely requirement for life-long treatment.

Movement disorders.—Ataxia may occur alone or in combination with epilepsy in patients with *SCN8A* mutations[132]. Movement disorders without seizures have been described in patients with partial or complete loss-of-function mutations[133]. In the mouse, loss of function of *Scn8a* may be accompanied by ataxia, dystonia or hind limb paralysis (Table 2B)[42,134–137]. The ataxia observed with GOF mutations of human *SCN8A* may result from use-dependent block of firing that mimics LOF in motor pathways.

Autism spectrum disorders and intellectual disability.—Autistic-like behaviors and intellectual disability are common co-morbidities in DEE due to GOF mutations of *SCN8A*. LOF mutations of *SCN8A* can cause autism or intellectual disability without seizures[124,138,139]. Liu et al compared *SCN8A* variants from patients with seizures and patients with ASD/ID[11]. When tested in transfected neurons, GOF was associated with seizures and LOF was associated with ASD. Intellectual disability unaccompanied by seizures is seen in patients with LOF mutations of *SCN8A*[140–142].

In a conditional mouse model, inactivation of *Scn8a* in inhibitory neurons resulted in absence seizures[143]. RNAi mediated knockdown of *Scn8a* in the reticular thalamic nucleus also induced absence seizures (Table 2B). Deletion of *Scn8a* in thalamic reticular neurons was thought to lead to seizures by reducing inhibitory input into the thalamus [143]. Inactivation of *Scn8a* in forebrain excitatory neurons resulted in replacement of $Na_v1.6$ by $Na_v1.2$ at the AIS, and reduced persistent current, but movement was unaffected[28].

Protein truncation mutations of *SCN8A* are under-represented in control and patient populations studied to date. The deficit of protein truncation mutations in the gnomAD database indicates that haploinsufficiency is not tolerated in a neurologically

normal population (pLI=1.0, OE=0.07) (gnomad.broadinstitute.org)[144]. The missing haploinsufficiency may be associate with movement disorders that have not been subjected to large scale sequencing, such as isolated ataxia, dystonia and tremor. It is possible that haploinsufficiency of human *SCN8A* leads to prenatal or early postnatal lethality, although this is not the case in the mouse.

Overall, there is considerable overlap in clinical consequences of mutations in *SCN1A*, *SCN2A* and *SCN8A*. The interesting differences in molecular mechanisms reflect divergence in aspects of subcellular function and distribution among neuronal circuits that we are just beginning to understand. Mutations in all three genes can result in seizure disorders, autism and intellectual disability. The effects of mutations on individual neurons have been characterized by electrophysiological methods, but the relationship between single-cell function and circuit and network consequences remain to be established. Understanding these processes will have important implications for therapeutic interventions.

New Therapies for Sodium Channelopathies.

Many patients with gain-of-function mutations respond to the classical sodium channel blockers, but most continue to experience some seizures and undesirable side effects. It has been difficult to develop drugs that distinguish among the closely related sodium channels. Genetic therapies can achieve target specificity based on DNA sequence differences among the channels, but their delivery across the blood brain barrier still requires invasive procedures. Advances in diagnosing sodium channel mutations has stimulated increased efforts to develop better treatment for both gain-of-function and loss-of-function disorders, using pharmacology as well as new genetic technologies, briefly reviewed below.

Pharmacology.

Channel-specific activators and inhibitors are predicted to have fewer side effects than the currently-available non-specific sodium channel blockers. The Nav1.6-specific channel blocker NBI-921352 (XEN901), in development by Xenon Pharmaceuticals, is scheduled to begin Phase 2 clinical trials in the United States in 2020. The persistent-current blocker PRAX330 reduces neuronal excitability *in vitro* and has shown promise in mouse models of *SCN1A* and *SCN8A* epilepsy[145–148]. The discovery that reduced *Gabra2* exacerbates *SCN1A* and *SCN8A* epilepsies suggests that that positive allosteric modulators of α2 subunit-containing GABA channels could be effective in these disorders[63–65]. Supporting this prediction, the $GABA_A$ activator clobazam reduces susceptibility to febrile seizures in *Scn1a*$^{+/-}$ mice[149]. Intraventricular infusion of a spider venom peptide that specifically activates *SCN1A* was shown to ameliorate seizures in the Dravet mouse [150].

Antisense Oligonucleotides (ASOs).

Allele-specific oligonucleotides (ASOs) targeting specific DNA sequences have been approved for treatment of spinal muscular atrophy[151] and Batten's disease[152], and show promise for several types of epilepsy. The *SCN1A* gene contains an alternatively-spliced "poison exon" with an in-frame stop codon. Approximately 50% of transcripts in young

wildtype mice contain the poison exon[53]. ASOs complementary to the "poison exon" block its inclusion by steric hindrance and increase the abundance of full length transcript[53]. This ASO rescued seizures in a mouse model of Dravet Syndrome [153] and is currently in clinical trial.

Since *SCN8A* encephalopathy results from gain-of-function mutations and elevated neuronal activity, appropriate treatment would decrease transcript levels. Intracerebroventricular administration of an antisense ASO reduced *Scn8a* transcripts by 50%, delayed seizure onset and extended lifespan[154]. Repeated ASO administration prolonged the effect, suggesting that chronic treatment would be effective[154]. The *Scn8a* ASO also rescued the mouse model of Dravet syndrome caused by haploinsufficiency of *Scn1a*, suggesting that reducing neuronal excitability by reduction of *SCN8A* could be a general approach to seizures of various etiologies[154].

CRISPR-based genetic therapy.

A general approach to treatment of haploinsufficient disorders is to increase the expression of the wildtype gene in the affected heterozygotes. CRISPR-activation technology can be applied for this purpose by fusion of transcriptional activation domains to the dCas9 protein and using an sgRNA to direct the protein to the promoter of the wildtype gene. This approach was tested for treatment of Dravet Syndrome in two mouse models. Intracerebroventricular injection of AAV carrying a transcriptional activator directed to the promoter of the *Scn1a* gene resulted in elevated activity of inhibitory neurons *in vivo* and resistance to thermally induced seizures[155]. Conditional upregulation of the wildtype *Scn1a* specifically in inhibitory neurons also reduced seizure susceptibility, with a modest effect on prolonging survival[156]. These examples provide proof of-principle that upregulation of *Scn1a* could be therapeutic for Dravet Syndrome, when administration of CRISPR to the CNS becomes feasible.

Outstanding issues.

The complexities of sodium channel function and the heterogeneity of channel levels in different types of neurons leave many questions for future investigation. The effects of loss of function in these channels is better understood. For missense mutations, it is difficult to predict the effects on biophysical properties of the channel. When GOF has been shown, it is still difficult to predict clinical clinical prognosis. There is a pressing need for functional analysis of the backlog of patient variants of unknown significance (VUS). The advent of high-throughput electrophysiology for variant analysis will contribute to the solution of this bottleneck[157,158]. Another large-scale approach is the application of saturation mutagenesis to generate libraries containing every possible missense variant in a gene, followed by pooled functional characterization. The goal of this approach is to generate a complete catalog of functional consequences for each gene that can be consulted after the identification of a novel patient mutation. An effort has been initiated for the cardiac channel *SCN5A*[159]. Analysis of reprogrammed neurons from patient-derived iPSC cells is another new development for analysis of patient mutations. In one recent study, seven mutations of *SCN8A* were studied in reprogrammed neurons; the correction of abnormal currents by

riluzole in the cultured cells predicted the therapeutic response subsequently observed in 3 patients [12].

The impact of genetic variants in other genes in the patient genome, along with stochastic events during development, have impacts that are beyond current experimental access. Variation in genetic background may contribute to the surprising observation that, in rare cases, LOF mutations of Nav1.6 may generate seizures [160,161]. One approach is to examine variants present in exome sequences of family members with divergent severity, as recently demonstrated for a family with pathogenic mutation of *SCN9A* [162].

Another challenge for the future is development of better methods for intracellular localization of specific channels in different types of neurons. In addition to filling gaps in basic knowledge, these methods could detect altered localization caused by patient mutations *In vivo* expression of molecularly tagged channels, such as those developed to study transport in cultured neurons [41], could increase sensitivity and eliminate dependence on immunostaining.

Ultimately, when faced with a newly diagnosed patient with a novel sodium channel mutation, we would like to be able to predict the biophysical effects, clinical course and effective therapy. As we move towards clinical trials for new therapies, family foundations focused on sodium channelopathies are making important contributions towards educating newly diagnosed families, compiling information about natural history for clinical trials, and supporting targeted research. These include the Dravet Syndrome Foundation (www.dravetfoundation.org), FamilieSCN2A Foundation (www.scn2a.org), Wishes for Elliott (www.wishesforelliott.com), and The Cute Syndrome Foundation (TCSF) (thecutesyndrome.com).

In summary, the discovery of monogenic causes underlying complex neurodevelopmantal disorders has clarified etiologies and accelerated efforts to develop targeted treatments. The depth of basic knowledge about the sodium channels have made these disorders early targets for precision medicine efforts. The monogenic epilepsies are particularly amenable to tests of clinical efficacy, because seizure frequency and severity can be acurately quantitated. We are seeing dramatically enhanced prospects for treatment of the sodium channelopathies, approaching the goal of improved quality of life for individuals living with these debilitating disorders.

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BOX 1.

Definitions

Ortholog: Evolutionarily corresponding gene in two species, e.g. mouse *Scn1a* and human *SCN1A*.

Sodium channel modifier gene: An unrelated gene whose expression can modify the severity of a sodium channel disorder.

Haploinsufficiency: a gene for which 50% of normal expression is insufficient and results in disease.

Gain of Function Variant (GOF): A missense variant with altered amino acid sequence that results in abnormal channel function.

Loss of Function Variant (LOF): A variant that abolishes channel function.

Partial Loss of Function Variant: A variant that retains a reduced level of normal function.

Poison Exon: an alternatively spliced exon that results in protein truncation, for example due to the presence of an in-frame stop codon.

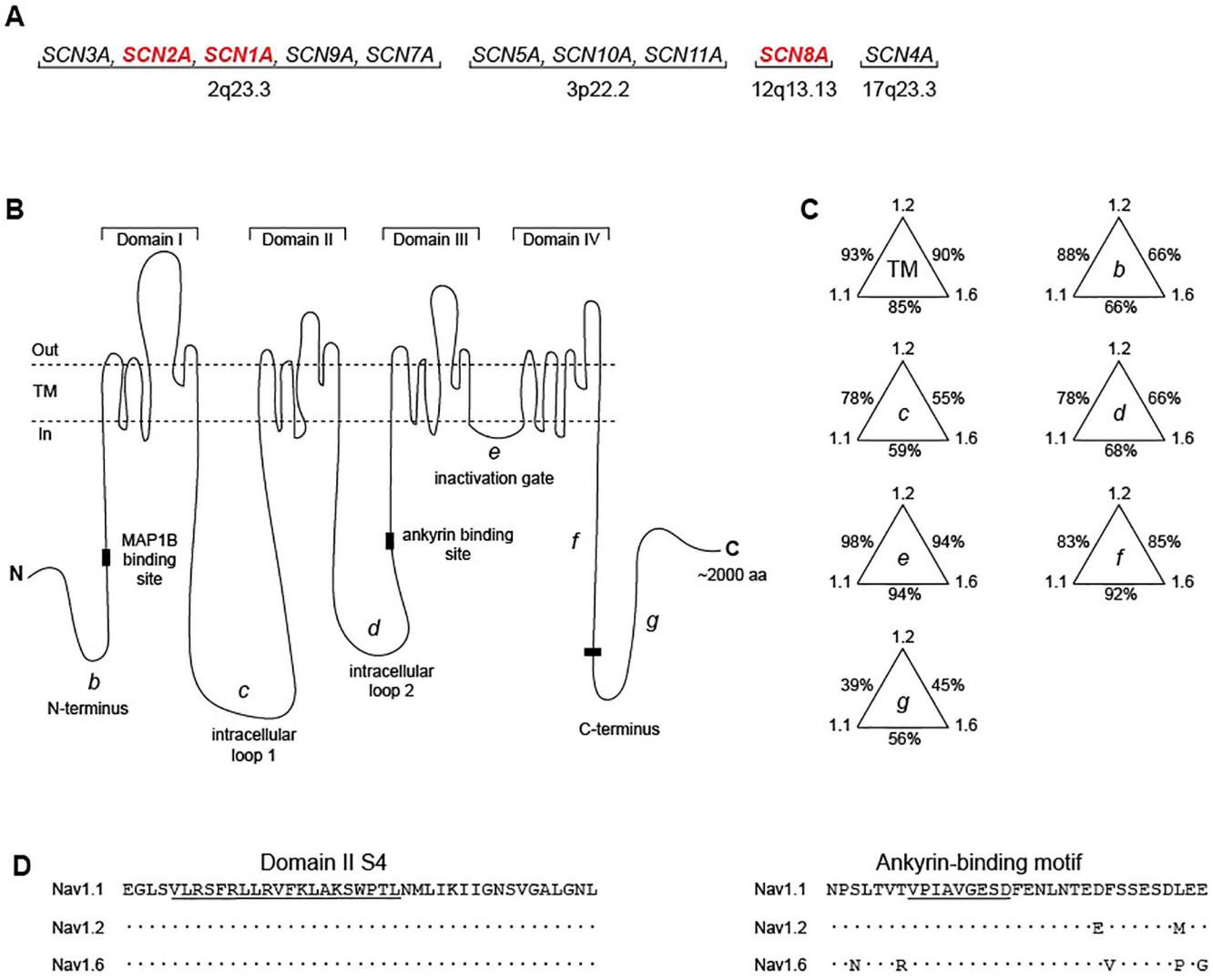

Figure 1. Evolutionary conservation of human sodium channel genes.

(A) Chromosomal locations of human voltage-gated sodium channel genes. The channels with high expression in the adult CNS (red) are covered in this review. (B) The voltage-gated sodium channel α subunit is composed of four transmembrane domains separated by intracellular loops. *TM*, transmembrane segments; *b*, N-terminus; *c*, cytoplasmic loop 1; *d*, cytoplasmic loop 2; *e*, inactivation gate; *f*, proximal half of C-terminus; *g*, distal half of C-terminus. (C) Percent conservation of amino acid sequence in the protein domains of *SCN1A* (Nav1.1), *SCN2A* (Nav1.2), and *SCN8A* (Nav1.6). Labels refer to domains in panel B. (D). Examples of regions of high sequence conservation in transmembrane segment DIS4 (left) and around the 9 residue ankyrin binding motif (right) [38]. Dots represent amino acid identity.

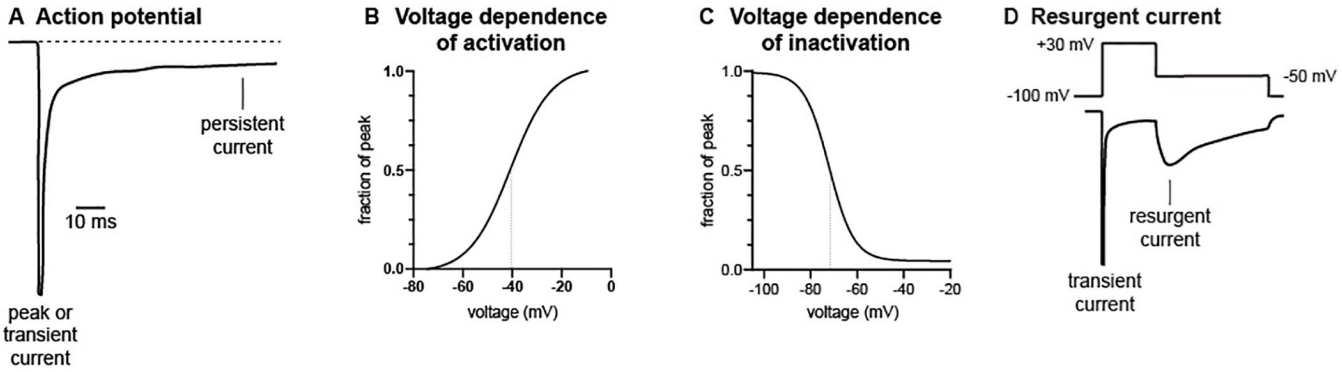

Figure 2. Channel properties frequently used to characterize patient mutations.

A, peak and persistent current. B, voltage dependence of channel activation. C, voltage dependence of channel inactivation. D, resurgent current. Vertical lines in B and C mark the voltage at which 50% of channels are active.

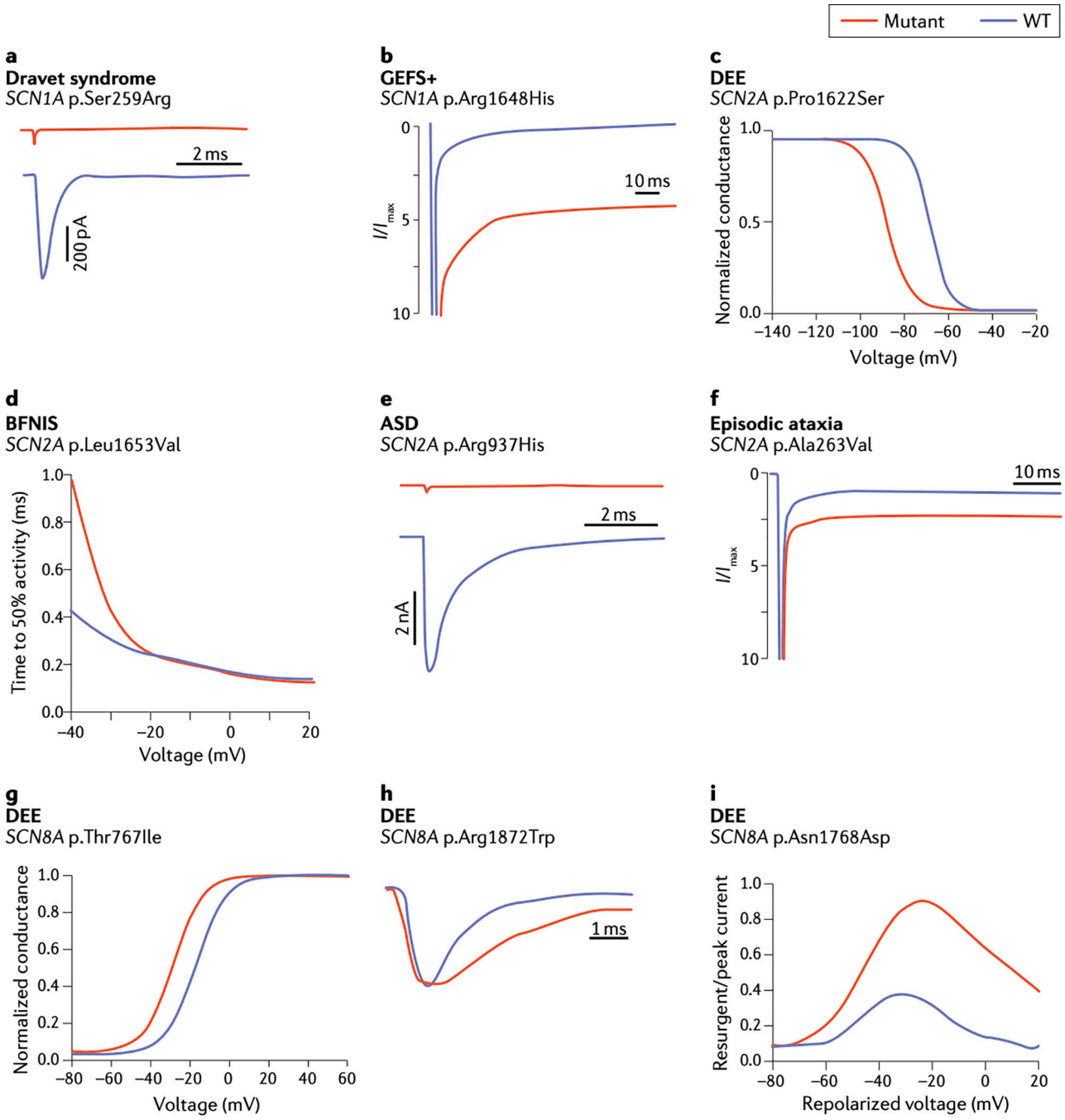

Figure 3. Functional effects of patient mutations in *SCN1A*, *SCN2A* and *SCN3A*.

Representative examples adapted from the indicated publications, which contain experimental details. **A**. The Dravet Syndrome mutation p.S259R in *SCN1A* causes complete loss of channel function [83]. **B**. The inherited variant p.R1648H in *SCN1A* in a family with GEFS+ causes increased persistent current [99]. **C**. p.P1622S in *SCN2A* in a patient with late-onset DEE causes a hyperpolarizing shift in the voltage dependence of inactivation [104]. **D**. p.L1653V in *SCN2A* in a family with benign familial neonatal-infantile seizures (BFNIS) causes rapid channel activation [114]. **E**. p.R937H in *SCN2A* in a patient

with autism spectrum disorder (ASD) causes loss of channel function [116]. **F**. p.A263V in *SCN2A* in a patient with episodic ataxia causes increased persistent current [120]. **G**. The mutation p.T767I in *SCN8A* in a patient with DEE causes premature channel activation [122]. **H**. *De novo* mutation p.R1872W in *SCN8A* in a patient with DEE causes delayed channel inactivation [163]. **I**. *De novo* mutation p.N1768D in *SCN8A* in a patient with DEE causes elevated resurgent current [164].

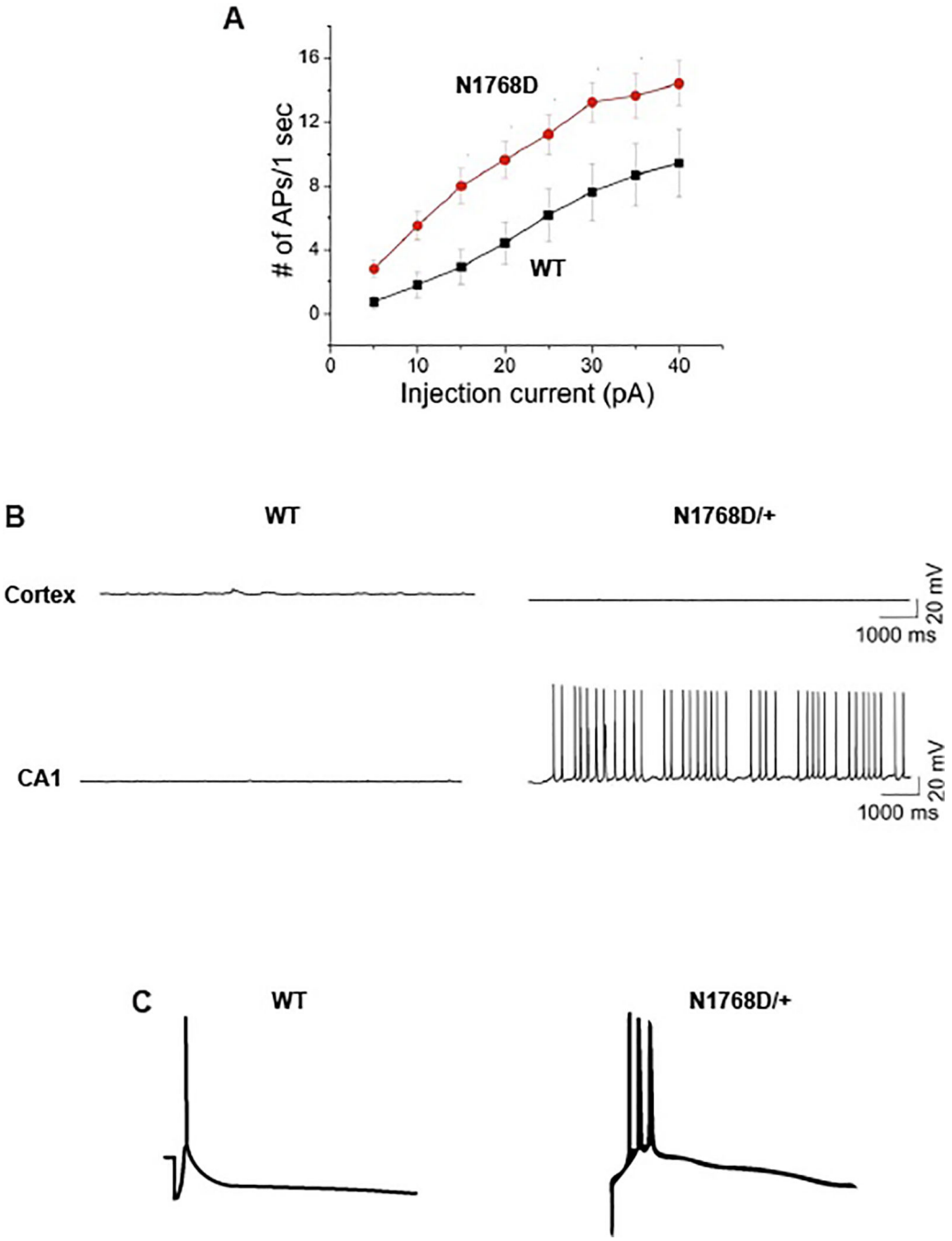

Figure 4. Electrophysiological and cellular mechanisms underlying seizures in an in vivo mouse model of human sodium channelopathy.

The *de novo* mutation *SCN8A*-p.Arg1768Asp was identified in a child with DEE. Functional effects include impaired inactivation, elevated persistent current [165] and elevated resurgent current [164]. The altered biophysical properties of *SCN8A* result in elevated neuronal activity at the cellular level. **A**. In response to electrical stimulation, cultured hippocampal neurons transfected with the mutant channel generate more action potentials than cells transfected with wildtype channel [126]. **B**. Slice recordings from

Scn8a$^{N1768D/+}$ knock-in mice demonstrate spontaneous firing of hippocampal CA1 neurons [127]. Spontaneous firing is not seen in layer 2/3 cortical neurons from the same mice. **C**. Enterorhinal cortex neurons from the knock-in mouse exhibit burst firing after synaptic stimulation [128].

Table 1.

Clinical disorders associated with mutations of *SCN1A*, *SCN2A* and *SCN8A*.

Gene	Protein	Type of mutation	Common clinical diagnoses	OMIM #
SCN1A	$Na_v1.1$	Gain of function	GEFS+	604403
			Familial hemiplegic migraine	609634
		Loss of function	DEE (Dravet syndrome)	607208
SCN2A	$Na_v1.2$	Gain of function	DEE	613721
			BFNIS	607745
			Episodic ataxia	618924
		Loss of function	Autism spectrum disorder	-----
			Intellectual disability	-----
			DEE	613721
SCN8A	$Na_v1.6$	Gain of function	DEE	614558
		Loss of function	Intellectual disability	614306
			Movement disorder	618364
			Autism spectrum disorder	-----

DEE, Developmental and Epileptic Encephalopathy; GEFS+, Generalized Epilepsy with Febrile Seizures Plus; BFNIS, Benign Familial Neonatal and Infantile Seizures; OMIM, On-line Mendelian Inheritance in Man (omim.org).

Table 2.

Global and regional knock-out of sodium channel genes in the mouse CNS.

A. Global knock-out		**CRE**	**Specificity**	**Phenotypes**	**Ref.**
Scn1a	+/−	na	Global	Convulsive seizures, strain dependent reduced survival, autistic-behaviors, deficits in spatial learning and memory, hyperactivity	31,54,57,58,63,166
	−/−	na	Global	Convulsive seizures, lethal @ P14	31,54
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Scn2a	+/−	na	Global	Absence seizures	59
				Behaviors seen in models of autism and schizophrenia	118
	−/−	na	Global	Lethal @ P2, hypoxia, neuronal cell death	55,59
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Scn8a	+/−	na	Global	Anxiety-like behaviors;	62
				Protection from induced and genetic seizures	71,167
	−/−	na	Global	Lethal @ P21, hind limb paralysis, muscle atrophy	56
B. Regional knock-out					
Scn1a ^{null}	F/+	Vgat	GABAergic inhibitory neurons	Convulsive seizures, sudden death	87
	F/+	Dlx1/2-I12b	Forebrain GABAergic interneurons	Convulsive seizures, sudden death	86
				Autistic-like behaviors, deficits in spatial learning and memory	57
	F/+	PV	Parvalbumin-expressing neurons	Some convulsive seizures and sudden death, susceptibility to thermally-induced seizures	87,168
				Autistic-like behaviors, lack of social novelty preference, impaired spatial memory	60,168
	F/+	SST	Somatostatin -expressing neurons	Hyperactivity without autistic-like behaviors, no spontaneous convulsive seizures, susceptibility to thermally-induced seizures	60,168
	F/+	PV+SST	PV and SST-expressing neurons	Deficits in long-term spatial memory, susceptibility to	168

A. Global knock-out		CRE	Specificity	Phenotypes	Ref.
				thermally-induced seizures	
	F/F	PV	Parvalbumin-expressing neurons	Convulsive seizures, sudden death, ataxia	87
	F/+	EmxI	Forebrain excitatory neurons	No seizures	87
	F/F	AAV-Cre	Hippocampal injection	Spontaneous seizures, nonlethal, susceptibility to thermally-induced seizures, deficits in spatial learning and memory	88,169
	F/F	AAV-Cre	Cortical injection	Spontaneous seizures, nonlethal, susceptibility to thermally-induced seizures	169
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Scn2a ^{null}	F/+	EmxI	Forebrain excitatory neurons	Absence seizures	59
				Anxiety-like behaviors, increased vertical activity	118
	F/F	EmxI	Forebrain excitatory neurons	Lethal @ P2	59
	F/+	Vgat	GABAergic inhibitory neurons	No seizures, 30% sudden death	59
				Anxiety-like behaviors	118
	F/F	Vgat	GABAergic inhibitory neurons	Lethal P2	59
	F/F	Trpc4	Cortical layer 5 pyramidal neurons	Absence seizures	170
	F/F	Ntsr1	Cortical layer 6 pyramidal neurons	No seizures	170
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Scn8a ^{null}	F/F	Pcp2	Purkinje cell	Ataxia, impaired motor functions, reduced firing of Purkinje neurons	137
				Impaired delay conditioning and Morris water maze	171
	F/F	α6	Cerebellar granule cell	Reduced learning in rotarod test	137
	F/+	EmxI	Forebrain excitatory neurons	Protection from induced seizures, flurothyl	143
	F/+	Dlx5/6	Inhibitory neurons	Absence seizures	143
	F/F	NEX	Forebrain excitatory neurons	No abnormality ***in vivo***, reduced persistent Na^+ current in AIS of layer 5 pyramidal neurons	28
	F/F	Lenti-Cre	Hippocampal injection	Protection from induced seizures	167

A. Global knock-out		**CRE**	**Specificity**	**Phenotypes**	**Ref.**
	+/+	RNAi	Hippocampal injection	Prevents seizure in mouse model of mesial temporal lobe epilepsy (MTLE)	172
	+/+	RNAi	Injection of thalamic reticular nucleus cells	Absence seizures	143
C. Knock-in of gain of function mutation					
Scn8a ^{R1872W}	R1872W/+	EIIa	Global	Lethal convulsive seizures @ 2 weeks	131
		EmxI	Forebrain excitatory neurons	Lethal convulsive seizures @ 3 weeks	131
		Dlx5/6	Inhibitory neurons	No abnormality	131
		CAG-ER	Inducible, global	Lethal convulsive seizures	131

KO, knockout; F/+, floxed heterozygote; F/F, floxed homozygote; lenti, lentivirus injection.