



Sodium channel mutations in epilepsy and other neurological disorders

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Since the first mutations of the neuronal sodium channel *SCN1A* were identified 5 years ago, more than 150 mutations have been described in patients with epilepsy. Many are sporadic mutations and cause loss of function, which demonstrates haploinsufficiency of *SCN1A*. Mutations resulting in persistent sodium current are also common. Coding variants of *SCN2A*, *SCN8A*, and *SCN9A* have also been identified in patients with seizures, ataxia, and sensitivity to pain, respectively. The rapid pace of discoveries suggests that sodium channel mutations are significant factors in the etiology of neurological disease and may contribute to psychiatric disorders as well.

Introduction

Voltage-gated sodium channels are essential for the initiation and propagation of action potentials in neurons. The sodium channel α subunits are large, transmembrane proteins with approximately 2,000 amino acid residues, composed of 4 homologous domains containing well-characterized voltage sensor and pore regions (Figure 1). The transmembrane segments are highly conserved through evolution. The 4 domains associate within the membrane to form a sodium-permeable pore, through which sodium ions flow down a concentration gradient during propagation of an action potential. The transmembrane sodium gradient is subsequently restored by the activity of the ATP-dependent sodium/potassium pump. The 3-dimensional structures of related bacterial potassium channels have recently been elucidated (1, 2).

Each sodium channel α subunit is associated with 1 or more β subunits, $\beta 1$ to $\beta 4$, that are transmembrane proteins with a single extracellular IgG loop and a short intracellular C terminus (Figure 1). Association with β subunits influences the level of cell surface expression, voltage dependence, and kinetics of the α subunit, as well as association with other signaling and cytoskeletal molecules (3, 4).

Duplication of the α subunit genes during evolution generated 9 mammalian genes encoding active channels that differ in tissue specificity and biophysical properties (Table 1) (5, 6). Many disease mutations have been characterized in the skeletal muscle and cardiac channels, but exploration of the role of the 7 neuronal sodium channels in disease is in an early stage.

$\beta 1$ Subunit mutations and GEFS+

Generalized epilepsy with febrile seizures plus (GEFS+) (OMIM 604233) is a mild, dominantly inherited epilepsy characterized by febrile seizures in childhood progressing to generalized epilepsy in adults (7, 8). The first connection between sodium channels and epilepsy was the discovery of a $\beta 1$ subunit mutation in a large Australian family with GEFS+ (9). Affected family members are heterozygous for the missense mutation C121W in the extracellular Ig domain of the $\beta 1$ subunit. The mutant channel promotes cell surface expression of the α subunit but exhibits

impaired modulation of sodium channel function and cell adhesion (10). A 5-amino acid deletion in the extracellular domain of $\beta 1$ was subsequently found in a family with febrile seizures and early-onset absence epilepsy (11). Impaired inactivation of sodium channel α subunits is the likely mechanism relating $\beta 1$ mutations to neuronal hyperexcitability in epilepsy.

Inherited and de novo mutations of *SCN1A* in GEFS+ severe myoclonic epilepsy of infancy

In 1999, linkage analysis in 2 large families localized a second GEFS+ locus to an interval of chromosome 2q24 that includes a sodium channel gene cluster (12, 13). Sequencing of *SCN1A* demonstrated that affected individuals are heterozygous for missense mutations in highly evolutionarily conserved amino acid residues, T875M in 1 family and R1648H in the other (14). Since the initial report, 11 additional *SCN1A* missense mutations have been reported in GEFS+ families (Figure 2A), approximately 10% of cases tested (14–25).

In 2001, Peter De Jonghe and colleagues discovered mutations of *SCN1A* in 7 patients with severe myoclonic epilepsy of infancy (SMEI) (26). This disorder is characterized by early onset, usually within the first 6 months of life, followed by progressive worsening of seizures often accompanied by mental deterioration (OMIM 182389). More than 150 mutations have been identified in children with this disorder (Table 2), approximately 50% of SMEI patients tested. As in GEFS+, the SMEI patients are heterozygous for the mutant alleles. Among 75 cases in which both parents have been tested, in 69 cases, or 90%, the mutations arose de novo in the affected children.

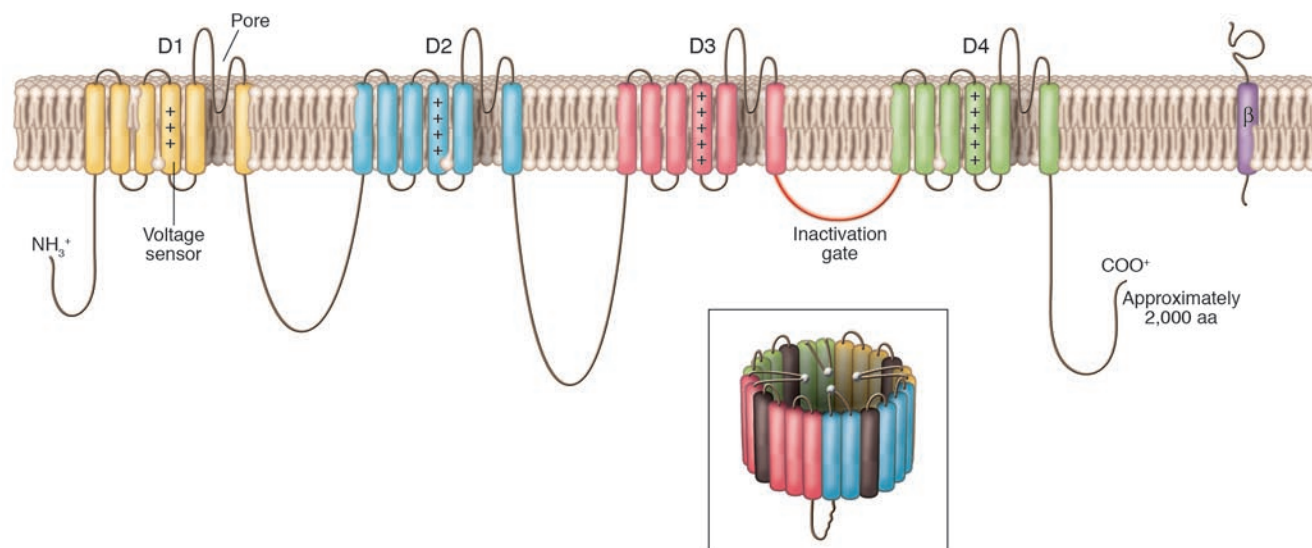
The mutation spectrum in SMEI differs from that in GEFS+. Approximately half of the SMEI mutations are nonsense or frameshift mutations that result in protein truncation (Figure 2B). The truncation mutations are randomly distributed across the *SCN1A* protein, including the N-terminal domain, transmembrane segments, cytoplasmic loops, and C terminus. The remaining SMEI mutations are missense mutations that, as in GEFS+, are concentrated within the transmembrane segments of the protein (Figure 2C). Within the large cytoplasmic loops of the α subunits, the only missense mutations are those located very close to the adjacent transmembrane segments.

The large number of de novo *SCN1A* mutations in children with SMEI demonstrate the importance of considering mutation in the etiology of neurological disease, even in the absence of a positive family history.

Nonstandard abbreviations used: GEFS+, generalized epilepsy with febrile seizures plus; SMEI, severe myoclonic epilepsy of infancy.

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**Figure 1**

The sodium channel α and β subunits are transmembrane proteins. The 4 homologous domains of the α subunit are represented in different colors. The transmembrane segments associate in the membrane to form an Na^+ -permeable pore lined by the re-entrant S5–S6 pore-loop segments (inset).

Recurrent mutations in *SCN1A*

Approximately 25% of reported mutations in *SCN1A* have occurred independently more than once (27, 28). Examination of the altered nucleotides at the sites of recurrent mutation revealed 2 frequent mechanisms. Fifteen mutations resulted from deamination of m^{CpG} dinucleotides, including 5 CpGs located in an arginine codon of the CGA class. Deamination converts this codon to UGA, a nonsense codon, resulting in an R-to-X mutation at the amino acid level. Two tetranucleotide direct repeats in *SCN1A* have been the sites of frameshift mutations due to deletion of 1 copy of the repeat. The likely mechanism is slipped-strand mispairing. The frameshift mutations are at amino acid residues K1846 and L1670.

Rare mutations of *SCN2A*

Although *SCN1A* and *SCN2A* are closely related genes, similar in size and exon organization, only a few epilepsy mutations have been detected in *SCN2A* (Figure 2D) (29–32). The difference may be partly accounted for by the likelihood that many more patients have been screened for *SCN1A* mutations. Most missense mutations of *SCN2A* were found in patients with benign familial neonatal-infantile seizures (OMIM 607745), mild syndromes that present within the first year of life but do not progress to adult epilepsy (Table 2). One truncation mutation of *SCN2A* has been identified in a patient with intractable epilepsy resembling SMEI (30).

Haploinsufficiency of *SCN1A*

A small proportion of human genes exhibit haploinsufficiency, meaning that abnormal function results from quantitative reduction of gene expression to 50% of normal levels. Among the epilepsy mutations, the most severe phenotypes are found in individuals who are heterozygous for loss-of-function mutations of *SCN1A*. This is indicated by the high proportion of truncation mutations among SMEI patients, and the lack of truncation mutations in patients with GEFS+, which is milder (Figure 2, A and B). In SMEI, the clinical features of early onset, seizure severity, and

progressive mental deterioration do not differ between patients with truncations located near the N terminus or the C terminus, which indicates that loss of function is the common cause. Rhodes et al. examined the functional effect of 5 missense mutations from patients with severe SMEI and found that 2 of those also result in complete loss of channel activity (33).

Unlike *SCN1A*, *SCN2A* appears not to exhibit haploinsufficiency. In contrast to the many null mutations of *SCN1A*, only a single truncation mutation has been identified in *SCN2A*, in a patient with a severe form of epilepsy resembling SMEI (30) (Figure 2D). In this case the truncated protein was reported to have a dominant-negative effect, rather than loss of function (30).

Analysis of mice with knockout alleles supports the conclusion that *SCN1A* exhibits haploinsufficiency while *SCN2A* does not. Heterozygotes for the *SCN1A* knockout allele exhibit spontaneous seizures and reduced lifespan (34), while heterozygotes for the *SCN2A*-null allele are viable without visible abnormalities (35). *SCN8A*-heterozygous-null mice lack visible abnormalities, but

Table 1

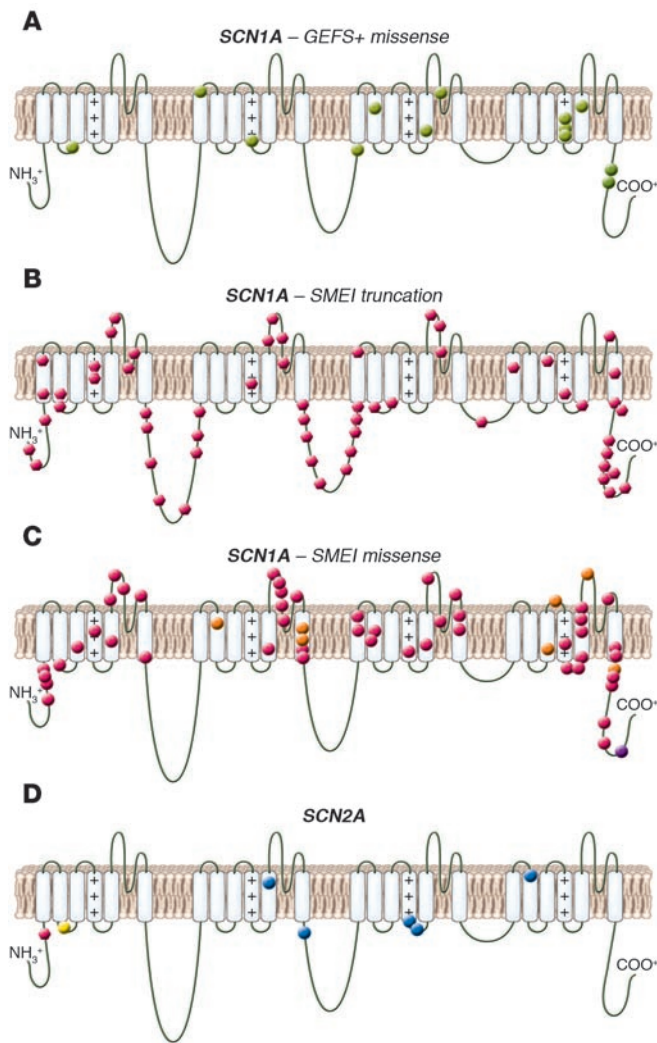
Mammalian voltage-gated sodium channel genes

Gene	Protein	Human chromosome	Major expression	Minor expression
<i>SCN1A</i>	$\text{Na}_v1.1$	2q24	CNS, PNS	Cardiac muscle
<i>SCN2A</i>	$\text{Na}_v1.2$	2q24	CNS, PNS	
<i>SCN3A</i>	$\text{Na}_v1.3$	2q24	CNS, PNS	
<i>SCN4A</i>	$\text{Na}_v1.4$	17q23	Skeletal muscle	
<i>SCN5A</i>	$\text{Na}_v1.5$	3p21	Cardiac muscle	Skeletal muscle, brain
<i>SCN8A</i>	$\text{Na}_v1.6$	12q13	CNS, PNS	Cardiac muscle
<i>SCN9A</i>	$\text{Na}_v1.7$	2q24	PNS	
<i>SCN10A</i>	$\text{Na}_v1.8$	3p21	PNS	
<i>SCN11A</i>	$\text{Na}_v1.9$	3p21	PNS	

PNS, peripheral nervous system.



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**Figure 2**

More than 150 mutations in the sodium channel protein have been identified in patients with GEFS+ and SMEI. **(A)** Missense mutations of *SCN1A* identified in families with GEFS+ (14, 16, 17, 19, 21–25). **(B)** Truncation mutations of *SCN1A* identified in SMEI patients (18, 26, 27, 44, 47, 50, 53, 54, 79–84). **(C)** Missense mutations of *SCN1A* in patients with SMEI (red), intractable childhood epilepsy with generalized tonic-clonic seizures (ICEGTC) (orange), and infantile spasms (purple) (18, 26, 27, 44, 47, 50, 53, 79–84). **(D)** Mutations of *SCN2A* in patients with benign familial neonatal-infantile seizures (BFNIS) (blue), GEFS+ (yellow), and SMEI (red) (29–32).

ron, this persistent current is thought to reduce the depolarization threshold required for firing, resulting directly in hyperexcitability. Another common mechanism is demonstrated by the GEFS+ mutation D188V, which spends less time in the inactivated state than the wild-type channel (Figure 3B). The result is greater availability of channels for opening in response to depolarization, another route to hyperexcitability. The altered biophysical properties of representative mutant channels are summarized in Table 3.

A unique biochemical mechanism was observed for the missense mutation D1866Y, located in the C-terminal domain of *SCN1A*, in a family with GEFS+ (23). In the *Xenopus* oocyte system, the mutant channel exhibited a depolarized shift in voltage dependence of fast inactivation; the effect was tenfold greater in the presence of the $\beta 1$ subunit (Figure 3C). Modeling with the program NEURON (<http://www.neuron.yale.edu/neuron/>) indicated that this shift is sufficient to produce neuronal hyperexcitability. Because the difference between the wild-type and mutant channels was increased by the presence of the $\beta 1$ subunit, the effect of the mutation on subunit interaction was tested. Yeast 2-hybrid screen and co-immunoprecipitation demonstrated direct interaction between the C-terminal cytoplasmic domains of the α and β subunits, which was impaired by the D1866Y mutation (23). The D1866Y mutation thus defines an intracellular interaction domain that appears to be required, in combination with the extracellular interaction domain (23), to form the stable α/β complex. Since mutations in either *SCN1A* or $\beta 1$ can result in GEFS+, it is not surprising that impaired interaction between the 2 subunits could also cause the disease.

The GEFS+ mutation R1648H has been examined in 3 expression systems with different outcomes. When the mutation was introduced into the rat *SCN1A* cDNA and examined in the *Xenopus*

subtle deficiencies have been detected in tests of learning and anxiety (36). The $\beta 1$ and $\beta 2$ genes do not exhibit haploinsufficiency in mice heterozygous for targeted knockout alleles (37, 38).

Functional effects of *SCN1A* missense mutations

Twenty *SCN1A* missense mutations have been evaluated in functional assays (23, 24, 33, 39–46). Functional analysis is complicated by the difficulty of cloning neuronal sodium channel cDNAs, which are uniquely unstable during propagation in bacterial cultures (unlike the muscle sodium channel cDNAs or the calcium and potassium channel cDNAs). Functional studies in the *Xenopus* oocyte system and in transfected mammalian cells do not always agree, and there is little experimental basis for extrapolation to in vivo effects. Nonetheless, some interesting patterns have emerged relating altered channel function to the neuronal hyperexcitability that is thought to underlie seizure disorders.

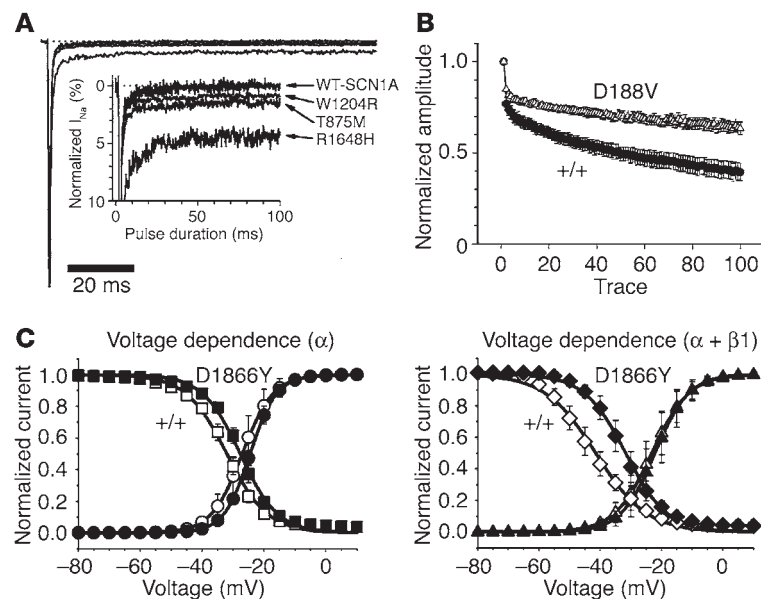
One common functional abnormality is impaired channel inactivation leading to increased persistent current. Normally, the voltage-gated sodium channels open rapidly in response to altered membrane potential and inactivate rapidly, declining to 1% of maximal current within a few milliseconds (Figure 3A). In 3 different GEFS+ mutations (14, 19), persistent current was increased to 2–5% of peak current (39) (Figure 3A). In the context of the neu-

Table 2

Sodium channel mutations in patients with various types of epilepsy

	<i>SCN1A</i> $Na_v1.1$	<i>SCN2A</i> $Na_v1.2$	<i>SCN1B</i> $\beta 1$
SMEI (at least 90% sporadic)	150	1	-
GEFS+	13	1	2
ICEGTC	7	-	-
Infantile spasms	1	-	-
Benign familial neonatal-infantile	-	6	-

ICEGTC, intractable childhood epilepsy with generalized tonic-clonic seizures.

**Figure 3**

The effects of GEFS+ mutations on *SCN1A* channel properties have been studied in the *Xenopus* oocyte system and in transfected mammalian cells. **(A)** Whole-cell recordings from HEK tsA201 cells transfected with the indicated mutant *SCN1A* cDNAs demonstrate increased persistent current from the mutant channels (39). **(B)** Mean normalized amplitudes of sodium currents elicited by 80-Hz pulse trains in HEK cells expressing *SCN2A* cDNA containing the GEFS+ mutation D188V demonstrate reduced cumulative inactivation of the mutant channel during high-frequency trains of channel activation (40). **(C)** Voltage-dependent gating of *SCN1A* cDNA constructs in *Xenopus* oocytes expressed in the absence (left) or presence (right) of the $\beta 1$ cDNA (85). See text for discussion.

oocyte expression system, accelerated recovery from inactivation was observed (45). In the human cDNA in transfected mammalian cells, persistent current was the major effect (Figure 3A) (39). Alekov et al. introduced R1648H into the *SCN4A* cDNA and expressed the clone in mammalian HEK tsA201 cells. In this context, they observed slowed inactivation and accelerated recovery from inactivation, leading to increased channel availability, but no persistent current (42). A second substitution at the same residue, R1648C, was identified in a patient with SMEI (47). In transfected cells, persistent current was generated by R1648C at a level indistinguishable from that of the mutation R1648H that causes GEFS+ (33). Thus, the heterologous expression systems are not able to distinguish between missense mutations that lead to mild disease in vivo and those that lead to severe disease.

The data indicate that seizures can result from increased *SCN1A* channel activity, as in the missense mutations described above, and from reduced activity, as in the truncation mutations. Neuronal firing patterns appear to be extremely sensitive to subtle changes in sodium channel function. In the future, the most physiologically relevant data are likely to be obtained from measurements of neuronal currents in knock-in mouse models carrying human mutations.

We investigated the in vivo effect of an *SCN2A* mutation with impaired inactivation in the Q54 transgenic mouse (48). Analysis of the mutation *SCN2A*^{GAL879–881QQQ} in the *Xenopus* oocyte system revealed an increase in persistent current and in the percentage of current that inactivated with a slow time constant (Figure 4, B and C) (49). When the mutant cDNA was expressed in transgenic mice under the control of the neuron-specific enolase promoter, the mice exhibited progressive seizures of hippocampal origin accompanied by loss of neurons called hippocampal sclerosis (Figure 4, A and E). Persistent sodium current was detected in recordings from CA1 neurons of the transgenic mice (Figure 4D), demonstrating agreement between the *Xenopus* assay and the in vivo effect. The phenotype of the Q54 mice most closely resembles human mesial temporal lobe epilepsy.

Toward mutation-specific therapy

Selecting the appropriate antiepileptic drug for newly diagnosed epilepsy patients is a difficult process, and many drugs have adverse side effects. An important goal of mutation characterization is the development of individualized treatments tailored to each patient mutation. It is already clear that sodium channel blockers are con-

Table 3

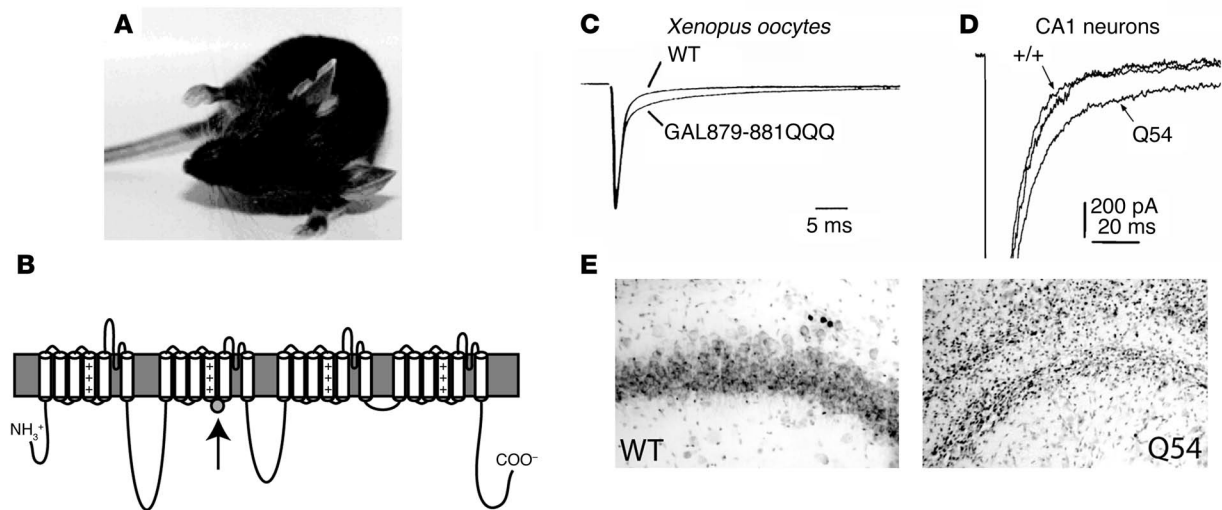
A variety of functional abnormalities in mutant alleles of *SCN1A* encoding the sodium channel Na_v1.1

Mutation	Major biophysical abnormalities observed in indicated experimental system
D188V	Decreased entry into and increased rate of recovery from slow inactivated state (<i>m</i>) (40)
T875M	Enhanced entry into slow inactivated state (<i>X</i>) (45); enhanced entry and hyperpolarizing shift into steady-state slow inactivation (<i>m</i>) (39, 42); increased persistent current (<i>m</i>) (39, 42)
W1204R	Hyperpolarized shift in voltage dependence of activation and inactivation (<i>X</i>) (46); increased persistent current (<i>m</i>) (39)
R1648H	Increased rate of recovery from fast inactivation (<i>X</i>) (45); increased persistent current (<i>m</i>) (39); slowed inactivation and increased recovery from inactivation (<i>m</i>) (41)
R1648C	Increased persistent current (<i>m</i>) (33)
I1656M	Depolarized shift in voltage dependence of activation (<i>m</i>) (43)
G1674R	No measurable sodium current (<i>m</i>) (33)
D1866Y	Depolarized shift in voltage dependence of fast inactivation (<i>X</i>) due to reduced association with $\beta 1$ subunit (<i>m</i>) (23)

The most prominent effects observed in each study are indicated; most mutations altered several biophysical properties of the channel. *m*, transfected mammalian cells; *X*, *Xenopus* oocyte system.



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**Figure 4**

An *SCN2A* mutation with persistent current causes seizures in the Q54 transgenic mouse. (A) Focal motor seizure in a Q54 mouse. (B) The GAL879–881QQQ mutation is located in the D2S4–S5 linker. (C) The mutant channel generates persistent current in *Xenopus* oocytes. (D) Whole-cell sodium currents recorded from CA1 hippocampal neurons from presymptomatic Q54 mice demonstrate increased persistent current. (E) Nissl-stained sections of hippocampus area CA3 reveal extensive neuronal cell loss in a Q54 mouse compared with a wild-type littermate. Adapted with permission from *Neuroscience* (48).

traindicated for patients with SMEI who are heterozygous for null alleles of *SCN1A*, since further reduction of the level of functional channel worsens their condition. In the case of mutations causing persistent current, open-state blockers may be appropriate. Pharmacological analysis of mutant *SCN5A* channels in LQT-3 provides a model for development of mutation-targeted therapy for a sodium channel defect (50, 51). Genetic variation in drug-metabolizing enzymes may also be predictive of pharmacological effectiveness of antiepileptic drugs (52).

Variable expressivity and incomplete penetrance

Variable expressivity, differences in clinical severity among individuals carrying the same mutation, is a striking feature of both GEFS+ and SMEI. For example, in the GEFS+ family carrying the mutation T875M, approximately half of the heterozygotes experienced febrile seizures in childhood only, while the other half had a variety of seizure types as adults (14). As another example, although most SMEI mutations arise de novo in affected individuals, a few patients are known to have inherited the *SCN1A* mutation from a mildly affected parent (53, 54). Several factors may be considered as contributing to differences between individuals carrying the same sodium channel mutation. There is *intrinsic stochastic variability* in developmental processes even between inbred animals homozygous throughout the genome, as demonstrated by the variable phenotypes of inbred mice with certain mutant genotypes. These processes may be especially important during development of neuronal connectivity in the CNS. Second, it has been suggested that the *accumulation of somatic mutations* during the lifetime of an individual may contribute to the development of neurological disease, and especially focal disorders such as epilepsy (55). Third, *environmental insults* such as trauma may exacerbate the phenotype of individuals carrying a mild mutation. Fourth, multiple differences in genetic background, or *modifier genes*, are segregating in families,

and the combined effect of multiple susceptibility factors may result in greater clinical severity in some individuals.

The influence of modifier genes is amenable to analysis using genetic differences between inbred strains of mice as models. Modifier genes that influence susceptibility to spontaneous seizures (56), kainate-induced seizures (57), electroconvulsive shock (58), and pentylenetetrazol-induced seizures (59) have been mapped to specific mouse chromosomes. Recently, we mapped 2 modifier loci, *Moe1* (*modifier of epilepsy*) and *Moe2*, that influence clinical severity of epilepsy due to a sodium channel mutation in *Scn2a*^{Q54} mice (60). With current genomic technology, it is feasible to clone modifier genes and evaluate their role in human disease. For example, a gene that modifies the severity of an *SCN8A*-induced movement disorder was recently cloned and identified as a putative RNA splicing factor (61).

SCN8A and movement disorders

Mutations of *Scn8a* in the mouse result in movement disorders such as ataxia, dystonia, and tremor (62). Conditional inactivation of *Scn8a* in cerebellar neurons results in a milder ataxia (63, 64). Screening of 150 patients with sporadic or inherited ataxia identified 1 protein truncation mutation that is likely to cause loss of function of *SCN8A* (36). Further screening will be worthwhile to determine the prevalence of *SCN8A* mutations in patients with inherited and sporadic movement disorders.

SCN9A and inherited pain

SCN9A encodes the sodium channel Na_v1.7, which is expressed in peripheral sensory and sympathetic neurons and is localized in the nerve terminals of sensory neurons (65). The missense mutation L858H, in a transmembrane segment, was identified in a Chinese kindred with autosomal dominantly inherited primary erythralgia (OMIM 113020), a disorder characterized by intermittent pain, redness, heat, and swelling in the extremities (66). A second mutation



Table 4
Mouse models of neurological disorders with sodium channel mutations

Gene	Channel	Mutation	Disorder	Reference
<i>SCN1A</i>	Na _v 1.1	Targeted null	Seizures in heterozygote; homozygous lethal at P16	(34)
<i>SCN2A</i>	Na _v 1.2	Targeted null	No abnormalities in heterozygote; homozygous lethal at P1	(35)
		Q54 transgene (GAL → QQQ)	Dominantly inherited temporal lobe epilepsy	(48)
<i>SCN8A</i>	Na _v 1.6	Spontaneous null	Paralysis, lethal	(62)
		Null heterozygote	Learning deficits	(36)
		A1071T	Ataxia, tremor	(86)
		Hypomorph (medJ)	Dystonia	(87)
<i>SCN9A</i>	Na _v 1.7	Conditional null	CRE-dependent lethal	(64)
		Targeted null	Lethal	(70)
<i>SCN10A</i>	Na _v 1.8	Conditional null	Pain resistant	(70)
		Targeted null	Pain resistant	(88)
<i>SCN1B</i>	β1	Targeted null	Seizures in heterozygote; lethal seizures in homozygote	(38)
<i>SCN2B</i>	β2	Targeted null	Heterozygote normal; homozygote susceptible to seizures	(37)

in a nearby residue, I848T, was found in a sporadic case of erythralgia. Functional analysis detected a hyperpolarizing shift in activation and slowed deactivation in both mutants, as well as increased current amplitude in response to slow, small depolarizations, consistent with predicted hyperactivity at the cellular level (67). A third mutation, F1449V, close to D3S6, lowers the current threshold required for action potential generation and repetitive firing (68).

The syndrome familial rectal pain (OMIM 167400) is characterized by brief episodes of intense pain of the submandibular, ocular, and rectal areas with flushing of the surrounding skin. The pain is responsive to the sodium channel blocker carbamazepine. Missense mutations in *SCN9A* have recently been identified in several families with this disorder (69).

Conditional inactivation of *Scn9a* in sensory neurons of the mouse resulted in increased threshold for mechanical, thermal, and inflammatory pain (70). The association of *SCN9A* mutations with inherited pain syndromes, and the pain resistance of the knockout mouse, indicate that specific inhibitors of this sodium channel would be valuable for the treatment of chronic pain. Thus far, the evolutionary conservation of the mammalian α subunit genes has made it difficult to develop inhibitors with specificity for a single channel.

Sodium channels and psychiatric disease?

There is a biological continuum between neurological diseases such as epilepsy and ataxia, and disorders that have been traditionally classified as psychiatric. Because of the essential role of sodium channels in propagating neuronal signals throughout the CNS, and the sensitivity of neuronal firing patterns to subtle mutations in sodium channels, it seems that sodium channel mutations could directly affect emotional and cognitive function. To test this possibility, we screened for mutations in *SCN1A*, *SCN2A*, and *SCN3A* in multiplex families with autism, a psychiatric disorder with high heritability (71). One splice branchpoint and 5 missense mutations were observed among 117 families; these may be con-

sidered candidates for a causal role in autism. The missense mutation R1902C, located within a calmodulin-binding domain of *SCN2A*, was subsequently found to confer a calcium-dependent conformational change on the complex of calmodulin with the C terminus of Na_v1.2 (72). In this context it is interesting that mutations of the voltage-gated calcium channel Ca_v1.2 were recently identified in families with Timothy syndrome, a multiorgan disorder with autism as a feature (73).

Preliminary indication that sodium channel variants may influence cognitive function comes from heterozygotes for a null allele of *SCN8A*, who exhibit a variety of cognitive defects (36). Impaired learning was also observed in heterozygous-null mice (36). An influence of *SCN8A* on attempted suicide was indicated by preferential transmission of 1 allele of a single-nucleotide polymorphism

in intron 21 (74). Autistic features have also been reported in patients with SMEI. Clearly, much work will be required to follow up on these intriguing observations regarding the potential influence of sodium channel and other neuronal ion channel variants on cognitive and psychiatric traits.

Mice with neuronal sodium channel mutations

The development of mouse models is keeping pace with the identification of human mutations, and several lines are available for analysis of disease mechanisms. The features and availability are summarized in Table 4. Four lines with various types of seizures are already available, the Q54 transgenic line with a missense mutation of *SCN2A* and the knockout lines for *SCN1A*, *SCN1B* (β1), and *SCN2B* (β2). Generation of tissue-specific and inducible knock-in mice carrying specific epilepsy-related sodium channel mutations is in progress in several laboratories. Electrophysiological recordings of neurons from knock-in mice are likely to provide the most reliable information for understanding the relationship between specific mutations and the severity of disease. Because of the effort involved in generating these models, only a small number of selected mutations can be analyzed in vivo. Recordings from mice with *SCN8A* mutations have revealed different effects on sodium currents in different types of neurons (75–77). In Purkinje cells, for example, *SCN8A* deficiency reduces bursting activity and resurgent current (77), while in cortical pyramidal neurons the major effect is reduced persistent sodium current (76). Analysis of *SCN1A*-null homozygotes revealed reduced current density in interneurons but not in excitatory neurons of the hippocampus (34). In addition to elucidating the pathophysiology of epileptogenesis, mouse models will permit evaluation of therapies directed toward specific classes of sodium channel mutations.

Persistent issues: sodium channels and disease

The identification of nearly 200 sodium channel mutations in patients with epilepsy raises several issues for future research. The relatively subtle functional effects of some of the epilepsy mutations



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suggest that the nervous system is intolerant of even minor variation in the properties of these channel proteins, which have a direct role in neuronal firing. The large number of de novo mutations in the sporadic disorder SMEI suggests that mutation should be considered as a possible cause of neurological disease even in the absence of family history. A de novo mutation of *SCN9A* was identified in a sporadic case of erythralgia. Development of genotyping technology has made it easier to screen for mutations, and a commercial sequencing test for *SCN1A* mutations was recently introduced. However, the cost of several thousand dollars per test, as well as ethical and practical concerns about the implications of mutation detection, may slow the application of genetic testing for neurological disease.

Evaluation of coding variants detected in genetic screens poses significant challenges. Which missense mutations cause disease and which are neutral? Large numbers of unaffected controls (approximately 1,000) must be tested to provide statistically significant evidence that a variant discovered in a patient is not also found in unaffected individuals. Functional assays can be helpful in recognizing disease mutations, but the difficulty of generating expression constructs and interpreting assays in exogenous expression systems makes these tests problematic for clinical applications.

Does quantitative variation in sodium channel abundance due to mutation in noncoding regulatory sequences contribute to neurological disease? The haploinsufficiency of *SCN1A* revealed by the loss-of-function coding mutations suggests that reduced

expression itself may be sufficient to cause disease. Screening for variation in expression requires access to neuronal RNA samples. Recent studies using cDNA microarray hybridization or allele-specific primer extension reactions suggest that as many as 20% of human genes may vary in expression levels among individuals (78). This interesting area remains essentially unexplored.

If we could identify variants in modifier genes underlying the variable expressivity of sodium channel mutations, perhaps using animal models carrying human mutations, new targets for treatment intervention might result. The growing role of sodium channel mutations in neurological disease provides increased incentive for developing new and more specific sodium channel inhibitors. We anticipate that large-scale sequencing of ion channel genes from patients with epilepsy and other neurological disorders will be carried out within the next few years, with major impact on our understanding of the issues discussed here.

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