Sodium channel *SCN1A* and epilepsy: mutations and mechanisms

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Summary

Mutations in a number of genes encoding voltage-gated sodium channels cause a variety of epilepsy syndromes in humans, including genetic (generalized) epilepsy with febrile seizures Plus (GEFS+) and Dravet syndrome (DS, severe myoclonic epilepsy of infancy). The vast majority of these mutations are in the *SCN1A* gene, and all are dominantly inherited. Most of the mutations that cause DS result in loss of function, whereas all of the known mutations that cause GEFS+ are missense, presumably altering channel activity. Family members with the same GEFS+ mutation often display a wide range of seizure types and severities, and at least part of this variability likely results from variation in other genes. Many different biophysical effects of *SCN1A*-GEFS+ mutations have been observed in heterologous expression systems, consistent with both gain and loss of channel activity. However, results from mouse models suggest that the primary effect of both GEFS+ and DS mutations is to decrease the activity of GABAergic inhibitory neurons. Decreased activity of the inhibitory circuitry is thus likely to be a major factor contributing to seizure generation in patients with GEFS+ and DS, and may be a general consequence of *SCN1A* mutations.

Keywords

GEFS; Dravet syndrome; Genetics; Knock-in mice; Knockout mice

Introduction

The epilepsies are a diverse collection of neurological disorders involving recurrent and unprovoked seizures that are the manifestation of abnormal electrical activity in the central nervous system (CNS), and they represent one of the most common neurological diseases, with a lifetime incidence of up to 3% in the general population (Hauser et al., 1993). Although many epilepsies are secondary to injury or another disease, approximately 40% are idiopathic, meaning that the underlying cause is unknown (Steinlein, 2002). It is presumed that most of the idiopathic epilepsies result from genetic abnormalities, with the majority likely caused by mutations in multiple currently unidentified genes. Nevertheless, research has uncovered a growing number of single-gene mutations that cause epilepsy (Noebels, 2003). The majority of these genes encode ion channels or receptors, including voltage-gated sodium, potassium, calcium, and chloride channels and receptors for acetylcholine and

γ-amino butyric acid (GABA). Even in these cases, though, the mechanisms by which the defects cause epilepsy are not well understood.

It seems logical that mutations in voltage-gated sodium channels would cause epilepsy, because these channels are in part responsible for controlling electrical excitability. Membrane depolarization activates the channel, causing a voltage-dependent conformational change that increases the permeability to sodium ions, further depolarizing the cell. This is followed by inactivation, in which the channel closes and the permeability to sodium ions decreases, allowing the membrane potential to return to its resting level. The sodium channel protein consists of a highly processed approximately 260-kDa α subunit that comprises four homologous domains termed I–IV (Figure 1). Within each domain, there are six transmembrane segments called S1–S6. A hairpin-like P-loop between S5 and S6 forms part of the channel pore, and the intracellular loop that connects domains III and IV forms the inactivation gate (Catterall, 2000). Channels in the adult CNS are associated with accessory β1, β2, β3, or β4 subunits. The β subunits are expressed in a complementary fashion, so that α subunits in the CNS are associated with either β1 or β3, and β2 or β4 (Isom et al., 1994;Morgan et al., 2000;Yu et al., 2003). Each β subunit consists of a single transmembrane segment, an extracellular IgG-like loop, and an intracellular C-terminus (Figure 1). The β2 or β4 subunit is covalently linked to the α subunit by a disulfide bond (Messner & Catterall, 1985), and the β1 or β3 subunit is noncovalently attached. The voltage dependence, kinetics, and localization of the α subunits are modulated by interactions with the β subunits.

The mammalian voltage-gated sodium channel α subunits are encoded by nine genes, termed *SCN1A* through *SCN11A* (*SCN6A* and *SCN7A* represent the same gene that encodes a non-voltage-gated sodium channel). The genes encode nine distinct isoforms, termed $Na_v1.1$ through $Na_v1.9$ (Goldin et al., 2000; Goldin, 2002). Four isoforms are expressed at high levels in the CNS ($Na_v1.1$, $Na_v1.2$, $Na_v1.3$ and $Na_v1.6$), and four are expressed at high levels in the peripheral nervous system ($Na_v1.6$, $Na_v1.7$, $Na_v1.8$ and $Na_v1.9$). The remaining two isoforms are expressed predominantly in adult skeletal muscle ($Na_v1.4$) and in embryonic skeletal muscle and heart muscle ($Na_v1.5$). The isoforms in the CNS are present at different times in development and in different locations (Trimmer & Rhodes, 2004). $Na_v1.1$ becomes detectable shortly after birth and increases until adulthood (Beckh et al., 1989). $Na_v1.2$ becomes detectable during embryonic development and reaches maximal levels during adulthood (Beckh et al., 1989). $Na_v1.3$ is often considered an embryonic isoform, because its expression peaks at birth and it is usually undetectable in adult rodents (Beckh et al., 1989), but it has been detected at low levels in adulthood in humans (Whitaker et al., 2001). $Na_v1.3$ expression has also been observed in the adult rat nervous system after dorsal root ganglion injury (Black et al., 1999; Hains et al., 2003) and in some epileptic rodent models (Yu et al., 2006; Guo et al., 2008). $Na_v1.6$ is expressed during late embryonic and early postnatal periods (Felts et al., 1997; Schaller & Caldwell, 2000) and is also present at high levels during adulthood (Schaller et al., 1995).

In the adult CNS, $Na_v1.1$ is the predominant channel in the caudal regions and the spinal cord, whereas levels of $Na_v1.2$ are highest in the rostral regions (Gordon et al., 1987; Beckh et al., 1989). $Na_v1.1$ is detected at high levels in the dendrites and cell bodies (Westenbroek et al., 1989; Gong et al., 1999), and it is also present in the axon initial segment of fast-spiking parvalbumin positive neurons (Ogiwara et al., 2007). $Na_v1.2$ is observed at high levels in the dendrites and unmyelinated axons (Gong et al., 1999). $Na_v1.6$ is present in both sensory and motor pathways, and its subcellular distribution includes axons, dendrites, cell bodies, and pre- and post-synaptic sites (Whitaker et al., 1999; Tzoumaka et al., 2000; Caldwell et al., 2000). $Na_v1.6$ is the main sodium channel at the nodes of Ranvier (Krzemien et al., 2000; Caldwell et al., 2000).

Because of their distinct subcellular distribution and functional properties, each sodium channel isoform most likely plays a unique role in the initiation and propagation of action potentials. For example, action potentials are initiated preferentially at the distal end of the axon initial segment compared to the proximal end. This preference may reflect the high density of $Na_v1.6$ channels in the distal end compared to the high density of $Na_v1.2$ channels in the proximal end, as $Na_v1.6$ channels have a lower threshold for activation (Hu et al., 2009). Because $Na_v1.1$ is clustered at the axon initial segments of parvalbumin-positive basket cells (Ogiwara et al., 2007), which project their terminal axonal arbors around the soma and proximal dendrites of excitatory neurons, it may play an important role in modulating excitability of the network.

Epilepsy Syndromes Caused by *SCN1A* Mutations

Mutations in three of the sodium channel α subunit genes that are mainly expressed in the CNS have been shown to cause epilepsy in humans. Mutations in *SCN1A* ($Na_v1.1$) and *SCN2A* ($Na_v1.2$) cause several subtypes of dominant idiopathic generalized epilepsy. Mutations in both genes lead to genetic epilepsy with febrile seizures plus (GEFS+; MIM 604233) (Escayg et al., 2000; Escayg et al., 2001; Sugawara et al., 2001; Wallace et al., 2001b). This syndrome is also caused by mutations in the *SCN1B* gene encoding the sodium channel β1 subunit (Wallace et al., 1998; Wallace et al., 2002) and two GABA$_A$ receptor genes: *GABRG2* encoding the γ2 subunit (Baulac et al., 2001; Wallace et al., 2001a; Harkin et al., 2002) and *GABRD* encoding the δ subunit (Dibbens et al., 2004). *SCN1A* mutations are the main cause of both Dravet syndrome (DS, MIM 607208) and intractable childhood epilepsy with generalized tonic-clonic seizures, also known as severe idiopathic generalized epilepsy of infancy (Mulley et al., 2005; Fujiwara, 2006). *SCN2A* mutations cause benign familial neonatal-infantile seizures (BFNIS; MIM 607745) and some cases of DS (Kamiya et al., 2004; Ogiwara et al., 2009; Shi et al., 2009), and a mutation in *SCN1B* has also been identified in a patient with DS (Patino et al., 2009). One mutation in *SCN3A* ($Na_v1.3$) has been reported in a patient with partial epilepsy (Holland et al., 2008). Interestingly, mutations in *SCN9A*, which is considered to be expressed predominantly in the peripheral nervous system, were recently identified in patients with febrile seizures. *SCN9A* might also serve as a genetic modifier of DS (Singh et al., 2009).

GEFS+ is an autosomal dominant disorder with a complex and heterogeneous clinical presentation (Scheffer & Berkovic, 1997; Singh et al., 1999). The syndrome was originally called generalized epilepsy with febrile seizures plus, which is misleading because the clinical phenotype also includes partial epilepsy. Therefore, the term autosomal dominant epilepsy with febrile seizures plus was proposed by Ito et al. (2002), and more recently Scheffer et al. (2009; Ottman et al., 2010) proposed the alternative nomenclature genetic epilepsy with febrile seizures plus. Febrile seizures occur in approximately 5% of all children under the age of six years and typically are not associated with increased risk of epilepsy in adolescence and adulthood (Nelson & Ellenberg, 1976; Commission on Classification and Terminology of the International League Against Epilepsy, 1989). In contrast, patients with GEFS+ demonstrate febrile seizures that persist beyond six years of age and are associated with generalized or partial epilepsies, such as absence epilepsy, myoclonic seizures, atonic seizures, and myoclonic-astatic epilepsy (Scheffer & Berkovic, 1997; Singh et al., 1999). Affected individuals within GEFS+ families often display a wide variety of epilepsy subtypes with markedly different ages of onset and severity, suggesting the action of genetic and/or environmental modifiers.

DS is a catastrophic early-life epilepsy disorder in which the seizures are usually refractory to treatment and are associated with intellectual disability. This syndrome includes classical DS (severe myoclonic epilepsy of infancy) and borderline DS (severe myoclonic epilepsy of

infancy borderline), in which patients show only a subset of clinical features (Scheffer et al., 2009; Mullen & Scheffer, 2009; Ottman et al., 2010). DS is characterized by febrile hemiclonic seizures or generalized status epilepticus starting at approximately six months of age, with other seizure types including partial, absence, atonic, and myoclonic seizures occurring after one year. In classical DS, development is delayed and patients often experience motor impairment, including spasticity and ataxia (Scheffer & Berkovic, 2003).

Genetics of *SCN1A* Mutations

Mutations in *SCN1A* account for approximately 10% of GEFS+ cases and mutations or deletions of *SCN1A* account for approximately 85% of DS cases (reviewed in Lossin, 2009; Claes et al., 2009). Updated lists of these mutations can be found at http://web.scn1a.info and http://www.molgen.ua.ac.be/SCN1AMutations/. Approximately 30 *SCN1A*-GEFS+ mutations have been identified to date. These have all been missense mutants (amino acid substitutions) that presumably alter but do not abolish *SCN1A* activity. In contrast, approximately half of the more than 600 *SCN1A* mutations in DS patients result from frameshift, nonsense, and splice site mutations (reviewed in Lossin, 2009; Claes et al., 2009). Several of these mutations occur very early in the sequence of *SCN1A*, strongly suggesting that expression of the mutant allele is reduced or that a non-functional protein product is generated from the mutant allele, demonstrating haploinsufficiency of *SCN1A* (Meisler & Kearney, 2005; Catterall et al., 2008).

A large number of *SCN1A* missense mutations have also been identified in DS patients. It is likely that many of these also abolish channel function, possibly by altering the properties of the channel, trafficking or subcellular localization, or interactions with other molecules. Unlike GEFS+ mutations that segregate within affected families, most DS mutations are de novo in the affected child. In a recent study, Heron et al. (2009) demonstrated that these de novo mutations most frequently originate on the paternal chromosome. The greater number of mitoses during spermatogenesis compared with oogenesis and the susceptibility of the methylated DNA of sperm cells to acquire mutations likely account for this observation.

Adding to the complexity of untangling the molecular mechanisms involving *SCN1A* mutations, microdeletions within *SCN1A*, and sometimes extending to include as many as 49 contiguous genes, including *SCN2A*, *SCN3A*, and *SCN9A*, have also been found in approximately 2–3% of DS patients (Suls et al., 2006; Wang et al., 2008; Davidsson et al., 2008; Marini et al., 2009). Although some of these patients display additional dysmorphic features (Davidsson et al., 2008), their clinical presentation is often surprisingly indistinguishable from DS patients in whom only *SCN1A* is affected. This suggests that haploinsufficiency for the adjacent genes may not contribute significantly to the disease presentation. Partial duplication/amplification of *SCN1A* has also been identified in three DS patients (Marini et al., 2009). Because these copy number variations are not detected by standard polymerase chain reaction screening, the percentage of DS cases resulting from *SCN1A* mutations is most likely an underestimate. Marini et al. (2009) estimated a 2–3% frequency of chromosomal abnormalities causing DS, which still leaves approximately 15% of cases in which no abnormality of *SCN1A* has been identified. Only a small number of DS cases have been shown to be due to mutations in *SCN1B*, *SCN2A* or *GABRG2* (Harkin et al., 2002; Kamiya et al., 2004; Ogiwara et al., 2009; Shi et al., 2009; Patino et al., 2009), so the unexplained cases may be due to mutations in additional genes or may have polygenic etiologies (Marini et al., 2009).

Somatic and Germline Mosaicism

While the majority of DS patients carry de novo *SCN1A* mutations, some cases have been described in which the mutation was inherited from an asymptomatic or mildly affected

parent, suggesting that the expression of the mutation in the transmitting parent was modified by environmental and/or genetic factors or was the result of somatic mosaicism. Somatic and germline *SCN1A* mosaicism has been reported in a small number of cases (Gennaro et al., 2006; Morimoto et al., 2006; Depienne et al., 2006; Marini et al., 2006). The possibility of *SCN1A* mosaicism in the parents of children with DS should therefore be considered when performing genetic counseling and determining recurrent risk.

Genetic Modifiers in GEFS+ and DS

One of the characteristics of GEFS+ is that family members with the same *SCN1A* mutation often display a wide range of seizure types and severities, suggesting that additional environmental or genetic factors likely influence clinical presentation. As mentioned earlier, similar factors may also alter the severity of *SCN1A*-DS mutations that are sometimes inherited from an asymptomatic or mildly affected parent. Furthermore, it is likely that a subset of the identified *SCN1A*-DS missense mutations only causes a modest alteration in channel function, and additional inherited or de novo variants at other loci are required to produce the severe clinical presentation.

A number of studies are beginning to provide evidence for the involvement of genetic modifiers in GEFS+ and DS. We previously showed that mice with mutations in *Scn8a* display elevated thresholds to chemically induced seizures when compared with wild-type littermates (Martin et al., 2007). Interestingly, in the presence of an *Scn8a* mutation, normal seizure thresholds and life spans were restored in heterozygous *Scn1a* knockout mice which serve as a model of DS (Martin et al., 2007). In a screen for genetic modifiers of DS, Ohmori et al. (2008) sequenced the coding exons of *SCN1B*, *GABRG2*, and *CACNB4* in 38 *SCN1A*-positive DS patients. The amino acid substitution R468Q in *CACNB4*, the voltage-gated calcium channel β4 subunit, was identified in one individual. Increased calcium currents were seen when R468Q mutant channels were examined in BHK cells. This mutation may therefore result in increased neurotransmitter release from excitatory cells, which when combined with reduced inhibition as a result of the *SCN1A* mutation, may cause a more severe clinical presentation. Recently, Singh et al. (2009) proposed a role for *SCN9A* as a genetic modifier of DS. In that study, six DS patients who harbored missense or splice site mutations in *SCN1A* were also found to carry *SCN9A* variants. This raises the possibility that alterations in *SCN9A* may exacerbate the impact of otherwise less deleterious *SCN1A* mutations on neuronal excitability.

Functional Effects of *SCN1A* Mutations

The biophysical effects of several *SCN1A* GEFS+ and DS mutations have been examined using heterologous expression systems. The locations of the mutations for which functional data are mentioned in this review are shown in Figure 1. We characterized the effects of five GEFS+ mutations (R859C, T875M, W1204R, R1648H and D1866Y) by expression in *Xenopus* oocytes (Spampanato et al., 2001;Spampanato et al., 2003;Spampanato et al., 2004;Barela et al., 2006), and other mutations have been characterized by expression in human embryonic kidney cells (HEK or tsA201) (Alekov et al., 2000;Alekov et al., 2001;Lossin et al., 2002;Lossin et al., 2003;Cossette et al., 2003). These mutations altered sodium channel activity in many different ways, with some of the effects predicted to either increase or decrease sodium channel activity (Table 1). For example, in oocyte expression studies, R1648H dramatically accelerated recovery from inactivation, W1204R shifted the voltage-dependence of activation and inactivation in the negative direction, R859C shifted the voltage-dependence of activation in the positive direction, and T875M enhanced slow inactivation. The alterations due to R1648H and W1204R are predicted to increase sodium

channel function and neuronal excitability, whereas the alterations due to R859C and T875M should decrease channel function and neuronal excitability.

The observed functional effects of *SCN1A* mutations are also influenced by the expression system. For example, the most prominent effect of R1648H, W1204R, and T1875M in tsA201 cells was an increase in the level of persistent current, with a marked increase for R1648H and a slight increase for T875M and W1204R (Lossin et al., 2002). The increased persistent current resulting from the R1648H mutation was due to a higher probability of late reopening and a fraction of channels with prolonged open times (Vanoye et al., 2006). It was previously shown in a transgenic *Scn2a* mouse model that increased persistent current can lead to seizures (Kearney et al., 2001), so a reasonable hypothesis was that this is the physiological change that causes seizures in GEFS+ (Lossin et al., 2002; Cannon, 2002).

Taken together, the data from the heterologous expression systems failed to uncover a consistent mechanism of pathogenesis and suggested that *SCN1A* mutations causing either gain-of-function or loss-of-function in Na$_v$1.1 can predispose the CNS to abnormal excitability. However, this conclusion assumes that the effects of the mutations in heterologous systems are a direct reflection of their effects in native neurons, which may not be the case for a number of reasons (Ragsdale, 2008). First, the specific β subunits that are present in each type of neuron (β1 or β3 and β2 or β4) could alter the properties of the sodium channels, and hence the effects of the mutations. Second, the GEFS+ mutations could alter aspects of channel processing or trafficking that may be unique to neurons, so that the physiologically relevant phenotype would not be seen in another cell type. Third, neurons express multiple sodium channel isoforms, as well as an array of other voltage- and ligand-gated ion channels. The repertoire of channels can have a dramatic effect on the properties of a mutation, as has been shown for an *SCN9A* mutation that causes erythermalgia, and which made sympathetic neurons hypoexcitable but sensory neurons hyperexcitable because of the presence of Na$_v$1.8 (Rush et al., 2006). Finally, studies in heterologous systems cannot reveal whether the mutations primarily affect excitatory or inhibitory neurons. A comparable alteration in sodium channel function in excitatory or inhibitory neurons is likely to have opposite effects on network excitability. For these reasons, it is essential to determine the effects of the mutations in native neurons in which *SCN1A* is normally expressed. This can be done most effectively using genetic mouse models.

Mouse Models of Human *SCN1A* Syndromes

Two different mouse models of DS have been constructed and analyzed (Table 2). Yu et al. (2006) constructed a mouse model of DS by targeted disruption of the mouse *Scn1a* gene. Heterozygous mutants (*Scn1a*$^{+/−}$) with 50% of normal Na$_v$1.1 levels were found to exhibit spontaneous seizures, reduced thresholds to hyperthermia-induced seizures, and a high mortality rate from four weeks after birth (Yu et al., 2006;Oakley et al., 2009). Homozygous mutants, which completely lack Na$_v$1.1, were ataxic and died 15 days after birth. Electrophysiological analysis of hippocampal pyramidal neurons from homozygous *Scn1a* knockout mice revealed normal levels of sodium currents, indicating that Na$_v$1.1 does not contribute significantly to sodium currents in these neurons. In contrast, sodium currents were significantly reduced in hippocampal interneurons that are critical for GABA-mediated neuronal inhibition. In addition, GABAergic cerebellar Purkinje neurons demonstrated significantly decreased peak, persistent and resurgent sodium currents, which is a likely explanation for the severe ataxia in these mice (Kalume et al., 2007). The *Scn1a* knockout mice were also found to have a compensatory increase in Na$_v$1.3 expression. This was an unexpected finding, because Na$_v$1.3 is highly expressed in the developing embryo, but

decreases to almost undetectable levels in the adult mouse. It is not clear what role the increased $Na_v1.3$ expression plays in the pathogenesis of epilepsy in these mice.

The second mouse model of DS was constructed by Ogiwara et al. (2007), who incorporated a premature stop codon (R1407X) that was found in three human patients. Both homozygous and heterozygous mice demonstrated decreased $Na_v1.1$ expression and spontaneous seizures. Wild-type $Na_v1.1$ channels were observed to cluster primarily at the axon initial segments of parvalbumin-positive interneurons, and trains of action potentials in these neurons from the mutant mice demonstrated decreased amplitudes late in the burst. The results from both knockout mouse models are consistent and suggest that a reduction in neuronal inhibition contributes to the severe seizures associated with DS.

To examine the effects of a GEFS+ *SCN1A* mutation on channel function in vivo, we generated mice carrying the R1648H mutation (Table 2), which was previously shown to decrease use-dependent inactivation in *Xenopus* oocytes (Spampanato et al., 2001) and increase persistent current in tsA201 cells (Lossin et al., 2002). To determine how the mutation affected sodium channel function in neurons, we first generated a bacterial artificial chromosome (BAC) transgenic mouse model (Tang et al., 2009). This model had two advantages for these studies. First, the transgene included the entire *Scn1a* gene, so it was likely to be regulated and expressed in the same neurons as the native channels. Second, the transgene was engineered to also include the E954Q substitution that makes the channel resistant to tetrodotoxin (TTX) and saxitoxin (STX) (Kontis & Goldin, 1993). The predominant sodium channels in the mammalian CNS are all blocked by nanomolar concentrations of TTX or STX, so the R1648H mutant channels could be studied in isolation by recording in the presence of either of these toxins.

The effects of the R1648H mutation depended on the neurons in which it was studied. In inhibitory neurons from the BAC transgenic mice, the mutation caused delayed recovery from inactivation and increased use-dependent inactivation, which is the exact opposite of the effects that were observed in oocytes (Spampanato et al., 2001). In addition, there was no detectable increase in persistent current, unlike the findings in tsA201 cells (Lossin et al., 2002). In contrast, mutant sodium channels in excitatory pyramidal neurons showed no alteration in use-dependent inactivation or recovery from inactivation, but they did have a negative shift in the voltage-dependence of inactivation. In addition, the mutation decreased the total sodium current amplitude in bipolar neurons, but it did not affect the current amplitude in pyramidal neurons, further suggesting a specific impairment of inhibitory function. The BAC transgenic mice did not exhibit spontaneous seizures, but they did have a more severe response to the proconvulsant kainic acid compared with mice expressing a control *Scn1a* transgene.

To more accurately model the human disorder, we also introduced the R1648H mutation into the orthologous mouse gene by constructing a knock-in mouse model of GEFS+ (Martin et al., 2010). Mice homozygous for the R1648H mutation ($Scn1a^{RH/RH}$) exhibited spontaneous generalized seizures and premature death between P16 and P26, whereas heterozygous mutants ($Scn1a^{RH/+}$) exhibited infrequent spontaneous generalized seizures, reduced threshold and accelerated propagation of hyperthermia-induced seizures, and decreased threshold to flurothyl-induced seizures. Inhibitory cortical interneurons from P5– P15 $Scn1a^{RH/+}$ and $Scn1a^{RH/RH}$ mice demonstrated slower recovery from inactivation and greater use-dependent inactivation compared with wild-type cells. In contrast, excitatory cortical pyramidal neurons demonstrated slightly faster recovery from inactivation. The R1648H mutation specifically decreased the total sodium current amplitude in bipolar neurons, consistent with the results from the BAC transgenic mice (Tang et al., 2009).

The alteration in sodium channel properties in bipolar neurons from the knock-in mice had a significant effect on the excitability of those cells and would most likely result in decreased inhibition. Bipolar neurons from $Scn1a^{RH/RH}$ mice fired a maximum of 20 action potentials in response to a 2-s injection of 70-pA current, whereas bipolar neurons from wild-type and $Scn1a^{RH/+}$ mice fired approximately 35 action potentials in response to the same stimulus. Pyramidal neurons from all three genotypes fired similar numbers of action potentials over the range of currents injected, with the exception of $Scn1a^{RH/RH}$ mice, which showed reduced numbers when 30–50 pA of current was injected. However, since the number of action potentials was comparable for higher levels of current ($\geq$ 70 pA), it is unclear whether the difference at lower levels of current is physiologically significant. Reduced inhibition is consistent with the rapid, abnormal propagation of experimental febrile seizures in these mice. Most $Scn1a^{RH/RH}$ mutants became immobile (the typical onset of experimental febrile seizure), then immediately progressed to a tonic seizure. This progression was rarely seen in wild-type or $Scn1a^{RH/+}$ mice, and when evident took several minutes to occur.

The results from both the BAC transgenic and knock-in mouse models suggest that the primary effect of the R1648H GEFS+ mutation is to cause cell-specific impairment of interneuron sodium channel activity, leading to decreased inhibition. Given the importance of inhibitory circuits in controlling the excitability of postsynaptic neurons, it seems likely that reduced firing of cortical interneurons, and possibly other GABAergic interneurons, contributes to seizure generation in the $Scn1a^{R1648H}$ mouse models. This model is consistent with the fact that mutations of GABA$_A$ receptor genes *GABRG2*, *GABRD*, *GABRA1* and *GABRB3* cause GEFS+, DS and childhood absence epilepsy by decreasing either the function or number of GABA$_A$ receptors (Kang & Macdonald, 2009).

Conclusions

Mutations in the genes encoding voltage-gated sodium channels cause a variety of epilepsy syndromes in humans, with most of the mutations occurring in *SCN1A*. All of these mutations are dominant, and they can result in either loss of function (most commonly observed in DS) or altered function (most commonly observed in GEFS+). Based on the analysis of mouse models of these two syndromes, decreased activity of the inhibitory circuitry is likely a major factor contributing to seizure generation in the mice, and by extension in GEFS+ and DS patients. Impairment of inhibition could be a general effect of *SCN1A* mutations causing epilepsy, although the analysis of mouse models with other *Scn1a* mutations is needed to determine whether this is a consistent mechanism.

Acknowledgments

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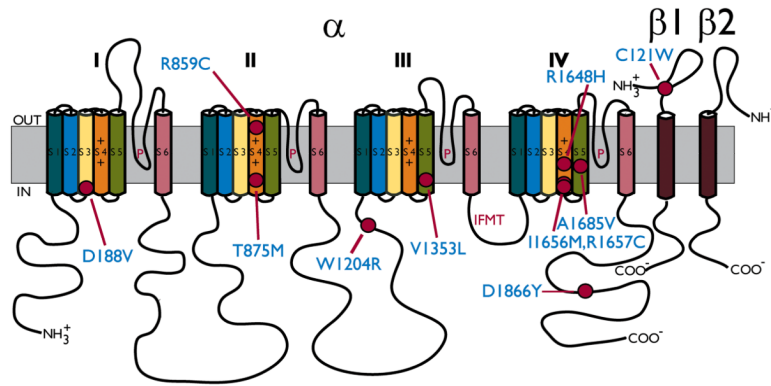
Figure 1. Epilepsy-Causing Mutations in *SCN1A*

Schematic diagram of the $Na_v1.1$ sodium channel α subunit and associated β1 and β2 subunits. The $Na_v1.1$ α subunit is associated non-covalently with β1 or β3, and it is associated via a disulfide linkage with either β2 or β4. The α subunit consists of four homologous domains (I–IV), each containing six transmembrane segments (S1–S6). The voltage-sensing S4 segment has multiple positively charged amino acids (+), and the intracellular loop between domains III and IV functions as the inactivation gate, containing four residues (IFMT) that interact with the inactivation docking site. The GEFS+ mutations that are mentioned in this review are indicated by filled circles. A complete list of the epilepsy-causing mutations in *SCN1A* can be found at http://web.scn1a.info (Lossin, 2009) and http://www.molgen.ua.ac.be/SCN1AMutations/(Claes et al., 2009).

Table 1

Functional Effects of the *SCN1A* Sodium Channel Mutations that Cause GEFS+

Increased Sodium Channel Activity			
Mutation	**Channel**	**Cell Type**	**Effects**
D188V	rNa$_v$1.2	HEK	↓ Use-dependence, Faster recovery from slow inactivation
T875M	rNa$_v$1.1	*Xenopus* oocytes	↑ Persistence
W1204R	rNa$_v$1.1	*Xenopus* oocytes	Negative voltage-dependence
W1204R	hNa$_v$1.1	tsA201	↑ Persistence
R1648H	rNa$_v$1.1	*Xenopus* oocytes	↓ Use-dependence, Faster recovery
R1648H	hNa$_v$1.1	tsA201	↑ Persistence
R1648H	hNa$_v$1.4	tsA201	Faster recovery
R1648C	rNa$_v$1.1	tsA201	↑ Persistence
D1866Y	rNa$_v$1.1	*Xenopus* oocytes	↑ Persistence
Decreased Sodium Channel Activity			
Mutation	**Channel**	**Cell Type**	**Effects**
R859C	rNa$_v$1.1	*Xenopus* oocytes	Positive Voltage-dependence, Slower recovery from slow inactivation, ↓ Current
T875M	rNa$_v$1.1	*Xenopus* oocytes	↑ Slow inactivation
T875M	hNa$_v$1.4	tsA201	↑ Fast and slow inactivation
V1353L	hNa$_v$1.1	tsA201	No sodium current
I1656M	hNa$_v$1.1	tsA201	Positive voltage-dependence
R1657C	hNa$_v$1.1	tsA201	Positive voltage-dependence, ↓ Current
A1685V	hNa$_v$1.1	tsA201	No sodium current

Table 2

Mouse Models of Human Epilepsy Caused by *SCN1A* Mutations

Human Syndrome	Type of Model	Salient Features	References
DS	Knockout	Spontaneous seizures, febrile seizures, ataxia and death at P15 in homozygous mice Spontaneous seizures, febrile seizures and impaired coordination in heterozygous mice Decreased sodium current density in inhibitory interneurons and cerebellar Purkinje neurons, but not in excitatory pyramidal neurons Decreased action potential firing in inhibitory interneurons Upregulation of $Na_v1.3$ in hippocampal interneurons	(Yu et al., 2006; Kalume et al., 2007; Oakley et al., 2009)
DS	Knock-In of R1407X nonsense mutation	Spontaneous seizures, unstable gait and death by P20 in homozygous mice Spontaneous seizures in heterozygous mice Spike amplitude decrements in parvalbumin-positive interneurons from heterozygous mice $Na_v1.1$ was clustered at the axon initial segments of parvalbumin-positive interneurons	(Ogiwara et al., 2007)
GEFS+	BAC transgene with R1648H mutation	Increased seizure susceptibility to kainic acid Decreased sodium current density in inhibitory bipolar neurons Delayed recovery from inactivation and increased use-dependent inactivation of mutant sodium channels in inhibitory bipolar neurons Hyperpolarizing shift in the voltage-dependence of inactivation of mutant sodium channels in excitatory pyramidal neurons	(Tang et al., 2009)
GEFS+	Knock-in of R1648H mutation	Spontaneous seizures and death between P16 and P26 in homozygous mice Infrequent spontaneous seizures, reduced seizure thresholds and accelerated propagation of febrile seizures in heterozygous mice Slower recovery from inactivation, greater use-dependent inactivation, reduced sodium current density and decreased action potential firing in bipolar inhibitory neurons	(Martin et al., 2010)