## Genetic basis of cardiac ion channel diseases

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Chapter 2

# Voltage-gated sodium channels: Action players with many faces

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## Abstract

Voltage-gated sodium channels are responsible for the upstroke of the action potential and thereby play an important role in propagation of the electrical impulse in excitable tissues like muscle, nerve and the heart. Duplication of the sodium channels encoding genes during evolution generated the sodium channel gene family with the different isoforms differing in biophysical properties and tissue distribution. In this review article, mutations in these genes leading to various inherited disorders are discussed.

## Keywords

Arrhythmias, electrophysiology, epilepsy, genetics, heart, ion channels, muscle, nerve, periodic paralysis, sodium

# 2.1 Introduction

Voltage-gated sodium ($Na^+$) channels are responsible for the upstroke of the action potential and thereby play an important role in propagation of the electrical excitable impulse in tissues including muscle, nerve and the heart. The channels are composed of pore-forming α-subunits of ~260 kDa associated with one or two β-subunits of 30–40 kDa that alter the properties of the channel. The α-subunit gene family consists of nine genes (and one additional sodium channel-like gene, see below), that are highly conserved across species. The channels are characterized by differential sensitivities to the sodium channel blocker tetrodotoxin (TTX) and inactivation kinetics: highly TTX-sensitive α-subunits (encoded by *SCN1A*, *SCN2A*, *SCN3A*, *SCN4A*, *SCN8A*, *SCN9A*) have faster inactivation kinetics compared to α-subunits that are less sensitive to TTX (encoded by *SCN5A*, *SCN10A*, *SCN11A*). Mutations in these genes cause various inherited disorders, which will be discussed in this review article.

# 2.2 The sodium channel genes

Duplication of genes encoding the α-subunits during evolution generated the sodium channel gene family with the different genes differing in their biophysical properties and tissue distribution (Table 1).[1] *SCN9A*, *SCN10A* and *SCN11A* are expressed in the peripheral nervous system. *SCN1A*, *SCN2A*, *SCN3A* and *SCN8A* are also expressed in the peripheral nervous system, but are more abundant in the brain and the rest of the central nervous system. *SCN4A* and *SCN5A* are highly expressed in muscle: *SCN4A* is expressed in adult skeletal muscle and *SCN5A* in embryonic and denervated skeletal muscle and heart muscle. *SCN6A*/*SCN7A* (probably referring to the same gene) is expressed in a diversity of tissues, including peripheral nervous system, heart, uterus and skeletal muscle.[2,3] Since *SCN6A*/*SCN7A* is the only sodium channel encoding gene that has not been expressed in an exogenous system and consensus amino acid sequences essential for voltage sensitivity and channel inactivation are not well conserved,[4] it was debated whether this gene encodes a functional voltage-gated $Na^+$ channel. On the other hand, *SCN6A*/*SCN7A* has been implicated in regulation of salt-intake behavior in a knockout mouse model.[5,6]

Alternative RNA splicing has been described for several α-subunits (Table 2): *SCN1A* (extended exon 11), *SCN3A* (3 different variants of exon 12), *SCN5A* (exon 18, difference of one amino acid), *SCN8A* (extended exon 12), *SCN9A* (extended exon 11) and *SCN11A* (lacking exon 16).[7-13] Additionaly, developmentally regulated splice variants of *SCN2A* (exon 5N and 5A), *SCN3A* (exon 5N and 5A), *SCN8A* (exon 5N and 5A) and *SCN9A* (exon 5N and 5A) that differ in a few amino acids and are either predominantly expressed neonatally (N) or in adults (A) have been described.[10,14-16] In the case of *SCN8A*, another splice variant (exon 18N and 18A) contains an in-frame stop-codon in the neonatally expressed variant which encodes a truncated two-domain protein the function of which is unknown.[8] In addition, four alternatively spliced non-coding exons which generate alternative 5'UTRs for transcripts of this gene have been reported.[17]

Electrophysiological studies on splice variants of *SCN2A* did not show any functional differences,[18] while splice variants of *SCN3A*, *SCN5A* and *SCN8A* resulted in channels that have altered kinetics.[7,12,19] The functional significance of the other alternative splicing events is still unknown.

The four β-subunit isoforms can be divided into 2 groups. β1 (*SCN1B*; localized in brain, skeletal and cardiac muscle[20]) and β3 (*SCN3B*; localized primarily in neuronal tissue,[21,22] but also detected in the heart[22-24]) are most similar in amino acid sequence and are noncovalently associated with α-subunits.[22,25] β2 (*SCN2B*; localized in the central nervous system and cardiac muscle) and β4 (*SCN4B*; localized in many tissues, including brain, heart, and skeletal muscle[26]) subunits are also closely related in amino acid sequence to one another but as opposed to β1 and β3 are disulfide-linked to the α-subunits.[21,26] Alternative RNA splicing has also been described for β-subunits: *SCN1Ba* and *SCN1Bb* differ beyond the immunoglobulin (Ig)-loop region, resulting in a distinct C-terminal cytoplasmic domain and a longer *SCN1Bb* transcript.[27,28]

**Table 1**: The voltage-gated sodium channel gene family: Tissue specificity and clinical association of mutations in these genes

| *Channel* | *Gene* | *Location* | *Primary tissues where channels are expressed* | *Clinical association* |
|---|---|---|---|---|
| $Na_v1.1$ | *SCN1A* | 2q24 | CNS; brain | **Brain Sodium Channelopathies:** generalized epilepsy with febrile seizures plus; severe myoclonic epilepsy of infancy; familial febrile convulsions-3; intractable childhood epilepsies and frequent generalized tonic-clonic seizures; familial hemiplegic migraine; familial autism? |
| $Na_v1.2$ | *SCN2A* | 2q23-q24.3 | CNS; brain | **Brain Sodium Channelopathies:** familial neonatal-infantile seizures; severe myoclonic epilepsy of infancy; familial autism? |
| $Na_v1.3$ | *SCN3A* | 2q24 | CNS; brain | **Brain Sodium Channelopathies*** |
| $Na_v1.4$ | *SCN4A* | 17q23.1-q25.3 | adult skeletal muscle | **Muscle Sodium Channelopathies:** hyperkalemic periodic paralysis; hypokalemic periodic paralysis; paramyotonia congenita; potassium-aggravated myotonia |
| $Na_v1.5$ | *SCN5A* | 3p21 | heart muscle; skeletal muscle | **Cardiac Sodium Channelopathies:** long QT syndrome; Brugada syndrome; conduction defects; sick sinus syndrome, dilated cardiomlyopathy, sudden infant death syndrome, atrial standstill |
| $Na_x$ | *SCN6A/ SCN7A* | 2q21-q23 | PNS; heart muscle; skeletal muscle; uterus | |
| $Na_v1.6$ | *SCN8A* | 12q13 | CNS; brain | **Brain Sodium Channelopathies:** cerebellar atrophy; ataxia; mental retardation; suicide attempt |
| $Na_v1.7$ | *SCN9A* | 2q24 | PNS; | **Peripheral Nerve Sodium Channelopathies:** erythermalgia; familial rectal pain |
| $Na_v1.8$ | *SCN10A* | 3p24.2-p22 | PNS | **Peripheral Nerve Sodium Channelopathies:** pain sensitization* |
| $Na_v1.9$ | *SCN11A* | 3p24-p21 | PNS | **Peripheral Nerve Sodium Channelopathies:** pain sensitization* |
| $Na_v\beta.1$ | *SCN1B* | 19q13.1 | brain; heart muscle; skeletal muscle | **Brain Sodium Channelopathies:** generalized epilepsy with febrile seizures plus |
| $Na_v\beta.4$ | *SCN4B* | 11q23 | brain; PNS; heart muscle; skeletal muscle | **Cardiac Sodium Channelopathies:** long QT syndrome |

CNS: central nervous system; PNS: peripheral nervous system;

* Speculated clinical association derived from animal models

**Table 2**: Different splice variants of voltage-gated sodium channels

| *Gene name* | *Splice variants* | *GenBank accession number* | *Comments* | *Tissue expression data* |
|---|---|---|---|---|
| *SCN1A* | variant 1<br>variant 2 | AB093548<br>NM_006920 | variant 1 contains extended exon 11 | ND |
| *SCN2A* | neonatal isoform<br>adult isoform | | exon 5N<br>exon 5A | Auld et al [18], Schaller et al [11] |
| *SCN3A* | neonatal isoform<br>adult isoform<br>splice variant | AF035685<br>AF035686<br>NM_006922 | exon 5N<br>exon 5A<br>this variant contains extended exon 12 | Thimmapaya et al [12] |
| *SCN5A* | variant 1<br>variant 2 | NM_198056<br>NM_000335 | variant 1 contains 1 extra amino acid in exon 18 | Makielski et al [7] |
| *SCN8A* | neonatal isoform<br>adult isoform<br>neonatal isoform<br>adult isoform<br>splice variant | AY682082<br>AY682081<br>AF050730<br>AF050730<br>AY682083 | exon 5N<br>exon 5A<br>exon 18N<br>exon 18A<br>this variant contains extended exon 12 | Raymond et al [10]; Plummer et al [8] |
| *SCN9A* | neonatal isoform<br>adult isoform<br>splice variant | AY682085<br>AY682084<br>AY682086 | exon 5N<br>exon 5A<br>this variant contains extended exon 11 | Raymond et al [10] |
| *SCN11A* | splice variant | AY686224 | Δ exon 16 | Raymond et al [10] |
| *SCN1B* | variant a<br>variant b | NM_001037<br>NM_199037 | variant b encodes longer transcript | Qin et al [28] |

N: neonatally; A: adult; ND: not determined

# 2.3 Structure and function of sodium channel subunits

***α-Subunits***

The pore-forming α-subunits are large transmembrane proteins that contain four structurally homologous domains (DI-DIV), each composed of six helical transmembrane segments (S1-S6) (see Catterall et al. for review,[29] see figure). The S5 and S6 segments and the P-loop between them from each domain line the channel pore. The pore contains the selectivity filter also referred to as the DEKA ring (consisting of aspartic acid, glutamate, lysine, alanine; one of these amino acids per P-loop), which attracts positive $Na^+$ ions and excludes negatively charged ions.[30] The lysine residue in the P-loop of DIII is important for discrimination for $Na^+$ over $Ca^{2+}$.[31,32]

Depending on the membrane potential, voltage-gated $Na^+$ channels can switch between three functional states: resting (closed), activated (open), and inactivated (closed). The highly conserved S4 region in each domain has a positive amino acid at every third position, and is considered the voltage sensor. The transition from the resting state to the activated state occurs when a change in transmembrane voltage moves S4 from within the pore towards the extracellular side of the cell, activating the channel which becomes permeable to ions.[33] Inactivation is mediated mainly by the inactivation gate (DIII-DIV linker), which blocks the inside of the channel shortly after it has been activated, and the C-terminal cytoplasmic domain.[34-36] During an action potential the channel normally remains open for only a few milliseconds after depolarization before it is being inactivated. When the membrane potential becomes negative after the repolarization phase of the action potential, the channels return to their resting state and can be activated again during the next action potential.

***β-Subunits***

β-Subunits consist of one transmembrane segment, an intracellular domain and a glycosylated extracellular domain. The structure of the extracellular domain resembles the structure of the V-like family of Ig-fold proteins, containing domains similar to the variable regions of antibodies and including motifs as found in cell adhesion molecules.[37]

The multifunctional β-subunits control channel gating, regulate the level of expression of the α-subunit at the plasma membrane[38,39] and are involved in cell adhesion through interaction with the cytoskeleton, extracellular matrix, and other cell adhesion molecules that regulate cell migration and aggregation.[40]

# 2.4 Channelopathies

## 2.4.1 Cardiac Sodium Channelopathies

*SCN5A (Figure 1C)*
Mutations in the cardiac voltage-gated $Na^+$ channel α-subunit gene *SCN5A* result in multiple arrhythmia syndromes.[41] Mutations leading to loss of $Na_V1.5$ channel function can result in Brugada syndrome (BrS; MIM 601144),[42] (progressive) cardiac conduction defect (PCCD; MIM 113900),[43] sick sinus syndrome (SSS; MIM 608567),[44] sudden infant death syndrome (SIDS; MIM 272120)[45] and dilated cardiomyopathy associated with conduction defects and arrhythmias (CMD1E; MIM 601154).[46] In combination with modifier genes, a loss-of-function defect causes atrial standstill.[47] Mutations leading to a gain-of-function of the channel cause long QT syndrome type 3 (LQT3; MIM 603830).[48] Some mutations in this gene lead to more than one disease phenotype, referred to as overlap syndromes of cardiac $Na^+$ channelopathy, which are usually only recognized in large families.[49,50]

*Loss-of-function mutations: Brugada syndrome and conduction defects*
The Brugada syndrome, with an estimated 5–50 cases per 10,000 individuals (with a higher incidence in Asia than in the United States and Europe[51]), is an autosomal dominant disorder characterized by sudden cardiac death from ventricular tachyarrhythmias, in combination with a typical ECG pattern of ST segment elevation in leads V1-V3. It is believed to cause 4 to 12% of all sudden cardiac deaths and ~20% of deaths in patients without structural abnormalities.[52] To date, 80 mutations in *SCN5A* (of which 14 % are nonsense or frameshift mutations, leading to truncation of the protein) have been described in BrS patients or in BrS patients with a mixed (overlap) phenotype (Inherited Arrhythmias Database: http://www.fsm.it/cardmoc/). These loss-of-function mutations are associated with dysfunctional channels or with a reduction of membrane expression of the channel due to a trafficking defect. Loss of $Na^+$ channel function reduces the upstroke of the action potential and may slow down action potential propagation. Thus, not surprisingly, patients with BrS often present with (progressive) conduction defects.[53,54] Loss-of-function mutations in *SCN5A* can also cause isolated cardiac conduction disease, i.e. without ECG features of BrS. Recently, a haplotype in the promoter region of *SCN5A* that occurs frequently in Asians was found to be associated with slower cardiac conduction,[55] suggesting that decreased expression of *SCN5A* transcripts may contribute to differences in BrS prevalence as a function of ethnicity.

*Gain-of-function mutations: Long QT syndrome*
Multiple genes have been associated with LQTS, an inherited cardiac arrhythmia associated with syncope and sudden death from *torsades de pointes* polymorphic ventricular tachycardia, estimated to affect 1 per 5000 individuals. One subtype of this syndrome is associated with mutations in *SCN5A* (LQT3). Gain-of-function mutations in $Na_V1.5$ result in an increase in the late component of the $Na^+$ current by slowing of inactivation or an increase in the reversibility of inactivation, resulting in a slow and constant entry of $Na^+$ in the plateau phase of the action potential, leading to a prolonged QT interval on the surface electrocardiogram (ECG).[56]

Because in *SCN5A*-related LQTS QT-prolongation is most pronounced at lower heart rates, bradycardia presents an important factor in developing lethal arrhythmias in LQTS families with mutations in *SCN5A*.[57] Thus far, 62 LQTS-causing missense mutations and small (in frame) insertions and deletions have been identified in *SCN5A* (Inherited Arrhythmias Database: http://www.fsm.it/cardmoc/).

*SCN4B*

Very recently, the first mutation in the *SCN4B* gene encoding $Na_V\beta.4$ was presented.[58] This missense mutation functionally disturbs $Na_V1.5$ in a LQTS patient and therefore *SCN4B* very likely is a new LQTS-susceptibility gene.

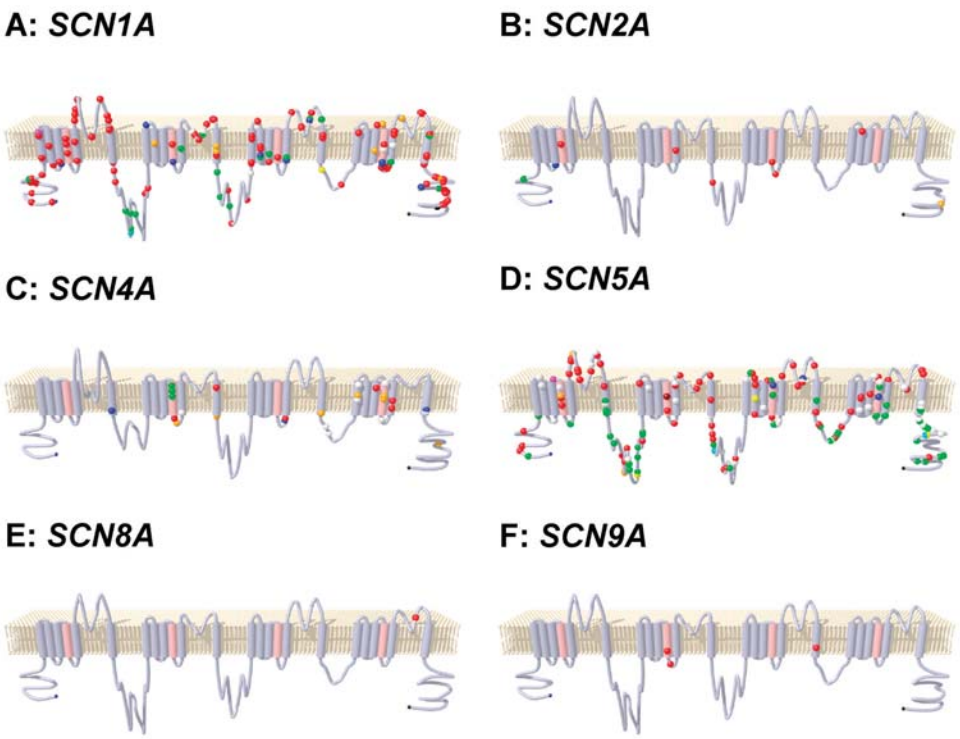


**Figure 1:** Diagrammatic representation of voltage-gated sodium channels showing locations of mutations causing channelopathies. A: *SCN1A*; Generalized epilepsy with febrile seizures plus (blue), Severe myoclonic epilepsy of infancy (red), Intractable childhood epilepsy and frequent generalized tonic-clonic seizures (orange), Borderline severe myoclonic epilepsy of infancy (green), Familial febrile convulsions-3 (purple), Familial autism (light blue), Familial hemiplegic migraine (yellow) and mixed phenotypes (white). B: *SCN2A*; Benign familial neonatal-infantile seizures (red), Severe myoclonic epilepsy of infancy (green), Febrile and afebrile seizures (blue), and Familial autism (orange). C: *SCN4A*; Hyperkalemic periodic paralysis (red), Hypokalemic periodic paralysis (green), Paramyotonia congenita (orange), Potassium-aggravated myotonia (blue) and mixed phenotypes (white). D: *SCN5A*; Brugada syndrome (red), Long QT syndrome type 3 (green), (Progressive) cardiac conduction defects (orange), Sick sinus syndrome (blue), Atrial standstill (purple), Sudden infant death syndrome (light blue), Drug-induced torsade des pointes (yellow), Dilated cardiomyopathy (brown) and mixed phenotype (white). E: *SCN8A*; Ataxia. F: *SCN9A*; Primary erythermalgia.

## 2.4.2 Neuronal Channelopathies

### *Brain Sodium Channelopathies*

*SCN1A (Figure 1A)*

*SCN1A*, the neuronal voltage-gated $Na^+$ channel α-subunit gene encoding $Na_V1.1$, is part of the *SCN1A-SCN2A-SCN3A* gene cluster on chromosome 2q24. Missense mutations have been identified in patients with generalized epilepsy with febrile seizures plus (GEFS+; MIM 604233),[59] an autosomal dominant epilepsy characterized by febrile seizures in children, and afebrile seizures in adults.[60,61] A large number of mutations of *SCN1A* have been identified in patients with severe myoclonic epilepsy of infancy (SMEI; MIM 607208),[62] a rare disorder characterized by various types of generalized and partial seizures, including myoclonic seizures. Many of the mutations characterized in children with SMEI are *de novo* (69 out of 75 cases).[63] Also, many of the observed mutations in SMEI patients are nonsense or frameshift mutations that cause protein truncation,[64] with deletion of the C-terminal cytoplasmic domain resulting in disease of similar severity to deletion of the N-terminal cytoplasmic domain.[63] No nonsense or frameshift mutations in *SCN1A* have been described so far in patients with GEFS+. One report suggests that in SMEI, pore region missense mutations are associated with a more-severe phenotype.[65]

*SCN1A* mutations have also been associated with intractable childhood epilepsies and frequent generalized tonic-clonic seizures (ICEGTC),[66] familial febrile convulsions-3 (FEB3; MIM: 604403),[67] and borderline SMEI (SMEB, when not all the SMEI criteria are fulfilled).[68]

In 2005, Dichgans et al.[69] described a mutation in *SCN1A* leading to familial hemiplegic migraine (FHM; MIM 141500), an autosomal dominant severe subtype of migraine with aura. This gain-of-function missense mutation in the inactivation gate of the channel was present in three families with the same disorder. This finding underlines the molecular links between migraine and epilepsy, two common paroxysmal disorders.

The association of *SCN1A* mutations with familial autism is also being investigated.[70]

*SCN2A (Figure 1B)*

Despite the fact that *SCN1A* and *SCN2A* are closely related genes, only a few epilepsy mutations have been identified in *SCN2A* encoding $Na_V1.2$.[14,71,72] Loss-of-function missense mutations in *SCN2A* were found in patients with benign familial neonatal-infantile seizures (BFNIS; MIM 607745), a mild autosomal dominant syndrome in which afebrile seizures occur in clusters during the first year of life but does not progress to adult epilepsy. Interestingly, one mutation (located in the conserved transmembrane segment S4 of DIII) was identified in affected members of 3 families, which occurred independently according to haplotype analysis.[73]

Unlike *SCN1A*, *SCN2A* does not show evidence of haploinsufficiency. Only one (*de novo*) truncation mutation has been identified in *SCN2A* in a patient with intractable epilepsy and mental decline, a severe form of epilepsy resembling SMEI.[71] In this case the truncated protein had a dominant-negative effect possibly arising from direct or indirect cytoskeletal interactions of the mutant protein.

One mutation in *SCN2A* has been described that might play a role in autism, but needs to be further analyzed.[70]

*SCN3A*

Studies in rats have indicated that seizure activity induces alterations in the developmental splicing of neonatal and adult *SCN2A* and *SCN3A* transcripts,[74] whose genes are located side by side on the chromosome: seizures were found to re-activate the neonatal splicing event, causing an increase in the presence of the neonatal *SCN2A* and *SCN3A* transcripts in localized regions of the adult rat brain. In contrast to *SCN2A*, *SCN3A* mRNA was found to be expressed at significantly higher levels in CA4 hilar cells in the epileptic hippocampus when compared with control, and therefore possibly contributes to the pathophysiology of epilepsy.[75] However, no mutations in this gene have been reported.

*SCN8A (Figure 1E)*

Mutations in the mouse ortholog of *SCN8A* cause ataxia and other movement disorders.[76] So far, only one protein truncation mutation has been described in human, causing cerebellar atrophy, ataxia and mental retardation.[77] This loss-of-function frameshift mutation was located in the pore loop of domain IV, resulting in truncation of the C-terminal cytoplasmic domain. Interestingly, Wasserman et al. described a single nucleotide polymorphism in *SCN8A* that may contribute to risk for suicide attempt, possibly through alterations in neuronal conduction which hypothetically could lead to disturbed analysis of incoming information in periods of emotional and physical stress.[78]

*SCN1B*

Mutations in the $Na_V\beta.1$ encoding *SCN1B* gene are associated with GEFS+.[79] The loss-of-function mutation C121W has been described in two families with GEFS+.[80] In another family with febrile seizures plus and early-onset absence epilepsy, a mutation in the splice acceptor site of *SCN1B* that predicts a deletion of five amino acids in the extracellular Ig-loop region was identified that could lead to loss-of-function.[81] Both mutations are expected to disrupt proper folding of the protein and therefore can inhibit interaction with $\alpha$-subunits or impair subcellular distribution, which will reduce the inactivation rate of $Na^+$ channels and results in neuronal hyperexcitability.[81,82]

To study the loss-of-function effects of *SCN1B in vivo*, knockout mice were generated, which appear ataxic and reveal spontaneous seizures, growth retardation, and premature death.[83] These phenotypes were the result of slowing of neuronal action potential conduction, reduced number of mature nodes of Ranvier, alterations in nodal architecture, loss of $Na^+$ channel-contactin interactions, and abnormalities in the expression of *SCN1A* and *SCN3A*. From this, it was clear that *SCN1B* regulates $Na^+$ channel density and localization, is involved in axo-glial communication at nodes of Ranvier, and is required for normal action potential conduction and control of excitability *in vivo*.

## *Peripheral Nerve Sodium Channelopathies*

### *SCN9A (Figure 1F)*

Mutations in the *SCN9A* gene encoding the $Na_V1.7$ channel cause primary erythermalgia (MIM 133020), a rare autosomal dominant disorder characterized by sporadic intense burning pain with redness and heat in the extremities.[84] These gain-of-function mutations modify thresholds of activation and are therefore likely to contribute to increased excitability of spinal sensory neurons that express the channels and may cause the abnormal pain sensations in patients suffering from this disorder.[85,86] Missense mutations in *SCN9A* have also been associated with familial rectal pain (FRP; MIM 167400), a disorder characterized by brief episodes of excruciating pain of the submandibular, ocular, and rectal areas with flushing of the surrounding skin.[87] Additionally, conditional inactivation of *SCN9A* in sensory neurons of the mouse resulted in increased threshold for mechanical, thermal, and inflammatory pain.[88] The association of *SCN9A* mutations with pain syndromes shows that this $Na^+$ channel could be a target for local anesthetics.

### *SCN10A*

*SCN10A* encodes $Na_V1.8$, a channel that is restricted to the peripheral sensory nervous system.[89] The down-regulation of *SCN10A* expression in rat can (I) prevent thermal hyperalgesia (hypersensitivity to noxious stimuli) and allodynia (pain response to non-noxious stimuli) in a rat model of neuropathic pain[90] and can (II) suppress responses caused by pain in a rat model of visceral pain.[91] No mutations in humans have been described thus far, but *SCN10A* could be a potential target for analgesic drugs.

### *SCN11A*

The $Na_V1.9$ channel encoded by *SCN11A* is expected to contribute to setting the resting membrane potential and modulating sub-threshold electrogenesis in nociceptive neurons.[92] Its expression is adjusted in response to axotomy[92] and inflammation.[93] Priest et al.[94] observed no differences in passive membrane properties and action potential characteristics between acutely dissociated peripheral sensory neurons in the dorsal root ganglia between wildtype and $Na_V1.9$ knockout mice. However, expression of *SCN11A* contributes to the persistent thermal hypersensitivity and spontaneous pain behavior after peripheral administration of inflammatory agents.[94] Although no mutations in *SCN11A* in humans have been described to date, this gene can possibly act as a target for analgesic drugs.

## 2.4.3 Muscle Sodium Channelopathies

*SCN4A (Figure 1C)*

Mutations in the muscle voltage-gated $Na^+$ channel $Na_V1.4$ encoding gene *SCN4A* have been identified in a group of related muscular disorders, including hyperkalemic periodic paralysis (HYPP; MIM 170500)[95] and hypokalemic periodic paralysis (HOKPP; MIM 170400),[96] paramyotonia congenita (PMC; MIM 168300),[97] and a group of disorders classified as potassium-aggravated myotonia (MIM 608390).[98] The gain-of-function mutations in *SCN4A* associated with HYPP, PMC and myotonia cause a disruption of fast inactivation,[99-101] which results in channel re-opening and intracellular $Na^+$ accumulation. In that case, muscle cells depolarize and generate recurrent action potentials. This can lead to enduring hyperexcitability which causes myotonia,[102] or it can lead to general opening of the channel which can cause paralysis.[103] In contrast, HOKPP is associated with loss-of-function mutations leading to hypoexcitability of the fiber membrane resulting in muscle weakness.[104-106] However, the mechanism leading to this decreased excitability is still poorly understood.

## 2.5 Discussion

In the last decades, much research has been done to identify new phenotype-linked mutations in voltage-gated $Na^+$ channels. Despite the fact that the relationship between mutations, altered $Na^+$ channel function, and disease phenotypes has become clearer, many questions still remain. Our understanding of the sodium channelopathies is complicated by several factors, among which is the complexity of the clinical phenotypes (pleiotropy), the allelic heterogeneity involved, the diverse impact of the different mutations on channel function, and the contribution of other (yet-unknown) genetic and environmental factors to the final form or severity of the disease.[107,108] Gain-of-function and loss-of-function mutations in the same gene lead to different diseases. In contrast to the obvious association between truncation mutations and loss of channel function, no relationships have been described so far between the locations within the channel protein of amino-acid changing mutations and these mechanisms, which appear to be random. In rare instances, a given mutation may even harbor biophysical defects associated with both gain and loss of channel function.[63]

Genetic factors other than the causal mutation itself that play a role in modulation of disease severity are starting to be uncovered. For example, the combined effect of a mutation in *SCN5A* and polymorphisms in the atrial-specific gap junction protein connexin40 gene has been reported to cause familial atrial standstill.[47] In mice, genetic variation in a putative RNA splicing factor (SCNM1) has been shown to modulate movement disorder severity in *SCN8A* mutant mice.[109] In another mouse study, an interaction between two mild mutations, one in *SCN2A* and the other in the potassium channel gene *KCNQ2*, has been described to result in severe epilepsy.[110] Furthermore, two modifier loci affecting epilepsy severity caused by a *SCN2A* mutation have been reported.[111] The genetic factors modulating disease severity may also reside in the gene affected itself[55] and even on the same allele. In *SCN5A* for example, a common polymorphism that attenuates the biophysical defect of a mutation on the same allele has been described.[112]

Not all voltage-gated $Na^+$ channels have been linked to human disease. Although no mutations have been described in *SCN3A*, *SCN10A* and *SCN11A*, *in vivo* animal studies indicate that other $Na^+$ channelopathies probably exist. Furthermore, the different splice variants of the $Na^+$ channel genes could play a role in the pathogenesis of the sodium channelopathies.

Since voltage-gated $Na^+$ channels are action players with many faces, our understanding of how mutations cause disease has lagged somewhat behind. In the next years, our increasing knowledge may lead to better targeted treatment of patients suffering from these disorders.

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