Sodium channel genes in pain-related disorders: phenotype–genotype associations and recommendations for clinical use


Human studies have firmly implicated voltage-gated sodium channels in human pain disorders, and targeted and massively parallel genomic sequencing is beginning to be used in clinical practice to determine which sodium channel variants are involved. Missense substitutions of SCN9A, the gene encoding sodium channel Na\textsubscript{1.7}, SCN10A, the gene encoding sodium channel Na\textsubscript{1.8}, and SCN11A, the gene encoding sodium channel Na\textsubscript{1.9}, produce gain-of-function changes that contribute to pain in many human painful disorders. Genomic sequencing might help to establish a diagnosis, and in the future might support individualisation of therapeutic approaches. However, in many cases, and especially in sodium channelopathies, the results from genomic sequencing can only be appropriately interpreted in the context of an extensive functional assessment, or family segregation analysis of phenotype and genotype.

Introduction

Genetic and genomic sequencing methods have firmly implicated voltage-gated sodium channels in human pain disorders.\textsuperscript{1–3} Missense substitutions of SCN9A, the gene encoding sodium channel Na\textsubscript{1.7}, SCN10A, the gene encoding sodium channel Na\textsubscript{1.8}, and SCN11A, the gene encoding sodium channel Na\textsubscript{1.9}, produce gain-of-function changes that make pain-signalling dorsal root ganglion (DRG) neurons hyperactive, and thereby contribute to pain in many human painful disorders. Strong evidence for a pathogenic role of mutations and functional variants of Na\textsubscript{1.7}, Na\textsubscript{1.8}, and Na\textsubscript{1.9} in these disorders has triggered substantial interest in these channels, and a growing number of studies has begun to identify additional variants in these disorders and in other diseases.\textsuperscript{4–7} As an outgrowth of these developments, an increasing number of patients and clinicians have become interested in the sequencing of SCN9A, SCN10A, and SCN11A.

Genomic sequencing might be included as part of the clinical investigation to help to establish a diagnosis—e.g., to resolve diagnostic uncertainty or to provide confirmation of a clinical diagnosis. The process of genetic testing and communication of results to patients should address three criteria: is the test accurate and reliable? (analytical validity); is the test result medically meaningful? (clinical validity); does the test improve health care? (clinical usefulness).

In most cases, and especially in sodium channelopathies, the findings from genomic sequencing cannot be appropriately interpreted in isolation from the clinical context and knowledge about the functional effects of the variant. For some variants, segregation with disease in multigenerational families or strong functional evidence will support interpretation as definitely pathogenic or probably pathogenic. However, if genomic sequencing identifies a novel missense variant without a firmly based functional implication and family segregation analysis of phenotype and genotype, then the clinical significance of the variant might not be clear.

As clinicians and investigators who have identified several missense variants of Na\textsubscript{1.7}, Na\textsubscript{1.8}, and Na\textsubscript{1.9}, and carried out functional assessments of variants of these channels, we summarise here the evidence linking variants of these channels to pain in human beings, discuss some caveats that are important in interpretation of the significance of newly discovered variants, and suggest terminology for classification of variants of these and other voltage-gated sodium channels. We also discuss the interpretation of missense variants of SCN9A, SCN10A, and SCN11A, especially in terms of the complexities of genotype–phenotype correlation.

Although we focus on SCN9A, SCN10A, and SCN11A, we believe that lessons learned from our studies might be relevant to many neurological channelopathies.

Sodium channel mutations and pain

SCN9A: sodium channel Na\textsubscript{1.7}

The painful disorder inherited erythromelalgia (also referred to as primary erythermalgia) is a rare disease characterised by exacerbating burning pain in the distal limbs triggered by mild warmth, most often with clinical onset in infancy. Inherited erythromelalgia was shown in 1992, on the basis of a study in a large multigenerational family, to be inherited in a classical mendelian autosomal dominant manner.\textsuperscript{8} In 2004, linkage analysis and candidate gene sequencing for other families with inherited erythromelalgia identified missense mutations in SCN9A.\textsuperscript{9} In the same year, functional analysis with voltage-clamp recordings showed that these mutations alter the gating properties of Na\textsubscript{1.7} channels, shifting their activation in a hyperpolarising direction (a change that makes it easier to activate the channels), slowing their deactivation (so that they are open for longer, once activated), and increasing their response to slow ramp-like stimuli (thereby increasing the sensory gain in nociceptive nerve terminals where these channels are deployed). Subsequent current-clamp experiments showed that the mutant Na\textsubscript{1.7} channels make small DRG neurons—including nociceptors—hyperexcitable, lowering their
threshold and increasing their rate of firing in response to graded suprathreshold stimulation. In the ensuing decade, functional studies of 22 NaV1.7 mutations, identified in patients with inherited erythromelalgia, have shown gain-of-function changes at the channel and neuronal levels.10,11,13,15–20,28,34,35,38–40,44,46,49,59–61

In 2006, a second infantile-onset pain disorder, paroxysmal extreme pain disorder (PEPD), characterised by severe lower-body pain in response to rectal stimulation, was shown to be caused by another group of mutations of NaV1.7,26 and to be often responsive to carbamazepine.27 The NaV1.7 mutations associated with PEPD do not alter channel activation. By contrast with inherited erythromelalgia mutations, PEPD mutations impair NaV1.7 channel inactivation so that more channels are available to activate even when the neuron membrane potential is depolarised.27,34,39,41,113 Current-clamp analysis showed that NaV1.7 mutant channels in PEPD produce hyperexcitability in DRG neurons.10,11

Channelopathy-associated insensitivity to pain, an autosomal recessive disorder in which patients are insensitive to pain and report an absence of pain in many situations, including fractures, burns, dental extractions, and childbirth, is caused by loss-of-function coding or splicing mutations in SCN9A.3,14,17,52 NaV1.7 is preferentially expressed in sympathetic ganglion neurons and DRG neurons,46,52 and thus it was surprising that autonomic dysfunction was not observed in patients initially described with channelopathy-associated insensitivity to pain. Hereditary sensory and autonomic neuropathy type 2D, characterised clinically by congenital or adolescent-onset loss of pain and temperature sensation, and autonomic dysfunction accompanied by hyposmia, hearing loss, hypogeusia, bone dysplasia, and fractures has been reported in Japanese families carrying a loss-of-function frameshift mutation of SCN9A.9 However, the basis for the phenotypical differences between patients from different ethnic backgrounds with channelopathy-associated insensitivity to pain3,5,6,7,10,13,52 is not understood.

In 2012, Faber and colleagues20 assessed a series of 28 patients with biopsy-confirmed painful, idiopathic, small-fibre neuropathy—in which patients have severe pain and autonomic dysfunction, usually with onset in adulthood—and reported gain-of-function missense mutations in about 28% of this cohort of patients. These NaV1.7 mutations produce a range of gain-of-function changes in channel function (eg, impairment of channel slow-inactivation, or smaller reductions in both fast-inactivation and slow-inactivation), and induce hyperexcitability and spontaneous firing in nociceptors, which seems to contribute to the evoked and spontaneous pain reported by these patients.17,16,17,46

SCN10A: sodium channel NaV1.8
Sodium channel NaV1.8 is known to work in tandem with NaV1.7, with NaV1.7 amplifying the response of nociceptive neurons to small stimuli so as to bring membrane potential close to the threshold for activation of NaV1.8, which produces most of the inward transmembrane current underlying the depolarising phase of the action potential during repetitive firing.20,35–38 Noting that NaV1.8 is preferentially expressed within peripheral sensory neurons and their axons, regulates DRG neuron firing, and has been implicated in pain signalling in rodents, Faber and colleagues28 screened for SCN10A mutations in a series of 104 patients with painful, predominantly small-fibre neuropathy who did not carry mutations in NaV1.7, and identified seven NaV1.8 substitutions in nine patients from this series. Three of the NaV1.8 mutations met criteria for potential pathogenicity on the basis of predictive algorithms, and two were functionally tested and shown to enhance the response of the channel to membrane depolarisation, and produced hyperexcitability within DRG neurons. Two additional SCN10A mutations, identified in patients with painful peripheral neuropathy, have been shown to be gain-of-function at the voltage-clamp and current-clamp levels.36–41

SCN11A: sodium channel NaV1.9
The sodium channel NaV1.9, which is preferentially expressed within small DRG neurons that include pain-signalling neurons,62 either very slowly inactivates or is non-inactivating, and displays broad overlap between activation and inactivation centred close to resting potential.63 As expected from these biophysical attributes, expression of NaV1.9 depolarises resting potential and increases excitability of DRG neurons.66–71 Zhang and colleagues72 reported two mutations—Arg225Cys and Ala808Gly—in the NaV1.9 channel, which segregated with an autosomal dominant phenotype of episodic pain in individuals from two multigeneration Chinese families. The investigators showed through voltage-clamp recordings that these mutations cause a small rise in the current density of NaV1.9, and through current-clamp recordings that they cause increased excitability of DRG neurons. Huang and colleagues60 described seven missense variants of NaV1.9 within a cohort of 344 patients with painful, predominantly small-fibre neuropathy who did not carry SCN9A or SCN10A mutations. Four of these variants substituted aminoacids in conserved, membrane-spanning regions of the channel. Thus far, two of these mutations have been shown to be gain-of-function at the channel level, and heighten the excitability of DRG neurons that express the mutant channel. Taken together, these results provide strong evidence that gain-of-function mutations of NaV1.9 can play a pathogenic part in pain syndromes.

A de-novo missense mutation of NaV1.9 (Leu811Pro), reported by Leipold and colleagues46 in two unrelated patients with an unusual syndrome of loss-of-pain sensation and inclination for self-mutilation raises interesting questions about the association between channel function and cellular function. Patch-clamp analysis from this study suggested that the mutation strongly hyperpolarises the voltage dependence of
channel activation by –28 mV (a gain-of-function change), while reducing the excitability of DRG neurons that express the mutant channel (a loss-of-function at the cellular level). The investigators attributed the reduced excitability of DRG neurons to enhanced resting inactivation of most voltage-gated sodium channels due to a depolarisation of resting potential of about 6–7 mV, and suggested that the mutation impairs transmission from presynaptic afferents to postsynaptic cells within the spinal cord, thereby producing pain insensitivity. Whether the 6–7 mV depolarisation reported by Leipold and colleagues\(^a\) can, in itself, produce insensitivity to pain is debatable; many studies have shown depolarisations of resting potential of this magnitude in DRG neurons transfected with mutations of Na\(_{1.7}\), Na\(_{1.8}\), and Na\(_{1.9}\) associated with nociceptor hyperexcitability that produces severe pain.\(^{29,30,36,42,60}\) and importantly, a depolarisation of this magnitude in wild-type DRG neurons by injection of current, in the absence of any mutation, produces hyperexcitability of these cells.\(^{46}\) Irrespective of the underlying mechanistic explanation, the findings of Leipold and colleagues\(^a\) provide another link between Na\(_{1.9}\) mutations and a human pain disorder, and draw attention to the complexity of interpreting the clinical significance of a sodium channel variant on the basis of one test.

**Clinical interpretation of sequence variants**

In clinical practice it is important for patients and their families to understand whether a variant is the cause of, or a contributor to, their disorder. When a specific gene is a plausible candidate, it can be sequenced as part of the diagnostic process. For some variants, segregation with disease in multigenerational families or strong functional evidence will support interpretation as pathogenic or probably pathogenic. Identification of wild-type genes can exclude any altered function of the encoded channel as disease-causing. However, the situation is more complex when variants are detected that do not affect a part of the channel protein with a known function and thus do not immediately seem to be disease-causing; these substitutions could still be pathogenic because of an unexpected change in channel physiology, or because they could contribute to processes such as post-transcriptional or post-translational regulation.

With the implementation of new high-throughput sequencing approaches, the number of new gene variants that are identified in patients will increase. Some of these variants will have unknown clinical significance. In the absence of more detailed phenotypic or functional information, the databases of single nucleotide variants (eg, the Human Gene Mutation Database and single nucleotide polymorphism databases [dbSNP, 1000 Genomes Project, and NHLBI GO Exome Sequencing Project\(^\text{\textregistered}\)] will need to be used with caution. Factors that can help to determine pathogenicity might include assessment of the segregation of genotype versus the disease phenotype, occurrence of de-novo variants concurrent with the incidence of a sporadic disease (although noting that, on average, 0–13 de-novo mutations occur in each human being born), species conservation, in-silico prediction of pathogenic effect, in-silico splice site prediction, RNA studies, and functional studies. It is important to recognise, however, that most of these factors on their own do not prove pathogenicity.

To standardise the interpretation and reporting of genomic sequence data, the Association for Clinical Genetic Science and the Dutch Society of Clinical Genetic Laboratory Specialists have both provided guidelines for the assessment of pathogenicity and reporting of variants in genes known to cause inherited mendelian and acquired diseases, for which molecular genetic testing has proven clinical validity and usefulness.\(^{73,74}\) We offer some caveats that specifically apply to SCN9A, SCN10A, and SCN11A but might be relevant to other sodium channel subtypes, and suggest a classification for variants of these genes.

**Caveats to interpretation of functional significance of sequence variants**

**Predictive algorithms are not infallible**

In-silico predictive algorithms can provide useful information on the basis of the location of the substituted residue within the channel, the nature of the substitution, and whether or not the substituted aminoacid is conserved. A scarcity of information and data about structure or gene homologues, however, compromises the predictive power of these algorithms. Moreover, even when there is a wealth of structural information, diagnostic conclusions should be drawn with caution. For example, a meta-analysis compared the diagnostic accuracy of various detection algorithms for mutations in the small, well characterised gene UGT1A1.\(^7\) The conclusion was that these algorithms have only moderate diagnostic ability to predict the actual pathogenicity. In the case of sodium channels, variants which substitute aminoacids in membrane-spanning segments of the channel are suggestive of pathogenicity, because they have a high likelihood of altering channel function. Nonetheless, in practice, there are exceptions to this generalisation. This cautionary note is supported by reports of charge-altering variants within functionally important transmembrane segments in each of the four domains of Na\(_{1.7}\) in control DNA samples. Especially well studied are the S4 segments; within all sodium channels, including Na\(_{1.7}\), these contain positively charged arginine, or lysine residues that enable these segments to have a crucially important functional role as a voltage sensor.\(^{73,75}\) The table shows six variants of SCN9A that introduce charge-altering aminoacid substitutions in the S4 segments within each of the four domains of Na\(_{1.7}\). These six variants are from more than 300 reported in dbSNP in individuals who are not noted as having a pain phenotype. The absence of a reported...
pain phenotype in the controls depends on the accuracy and resolution of the clinical profiling of the control population, and the reported absence of a phenotype does not always provide reliable evidence for its absence. At this time, the reason for the disparity between the predicted effects of these variants on channel function and the presence of these variants in individuals reported as healthy controls is not understood. Nonetheless, in some cases even the unequivocal predictions of in-silico algorithms might not be borne out by clinical observation. Whether this can be attributed to variable expressivity, incomplete penetrance, or non-pathogenicity might be partly resolved by functional profiling.

Another complexity arises because several mutations in sodium channels that substitute single aminoacids in parts of the channel of unknown function have been associated with pain disorders, and change channel properties and nociceptor firing. For example, the Gln10Arg substitution in the N-terminus of NaV1.7 from a patient with inherited erythromelalgia produces a 5 mV hyperpolarising shift in channel activation.\(^{13}\) As a second example, the Ile720Lys substitution in intracellular loop 1 of NaV1.7 from a patient with small-fibre neuropathy produced a 9 mV depolarising shift in slow-inactivation of the mutant channel.\(^{29}\) Both of these mutations substantially enhance the excitability of pain-signalling DRG neurons but, in view of the present state of knowledge about the substituted residues, the pro-excitatory effects of the mutations could not have been predicted in silico. The challenge here arises, in part, not from the lack of sophistication or power of the computational algorithms, but rather from our poor understanding of the functions of parts of the channel protein molecule outside of the membrane-spanning domains—eg, the N-terminus and C-terminus, and the linkers and loops that connect membrane-spanning segments.

Taken together, the findings described in this section show that in-silico predictive algorithms, although useful, are not infallible.

The rarity of an allele does not imply pathogenesis

Although more than 24 rare variants of SCN9A, SCN10A, and SCN11A, identified in patients with painful disorders, have been shown by functional analysis to be gain-of-function at the ion channel (voltage-clamp) and in some cases at the cellular (current-clamp) levels, the fact that a particular variant is rare does not necessarily mean that it is pathogenic—ie, that it contributes to the clinical picture of the patient. We have seen several variants of SCN9A and SCN10A in patients with pain syndromes reported in databases with minor allele frequencies of less than 0·01, which did not produce demonstrable gain-of-function changes when studied by voltage-clamp or current-clamp (unpublished findings). Thus no functional evidence exists that these variants produce abnormal pain signalling. Whether these variants contribute to disease pathogenesis or pathophysiology is unclear at this point. Large-scale, genotype-phenotype studies could help to resolve this issue in the future. However, it is important to understand that some rare variants might just be rare variants, and are not necessarily pathogenic.

As an example of the care needed in the interpretation of novel variants, we investigated a family with inherited erythromelalgia that affected one parent and two children (unpublished findings). We detected no mutations in SCN9A, so we investigated SCN10A. One of the affected children had a novel heterozygous missense mutation in SCN10A: in silico analysis predicted a functional change in the ion selectivity filter of the channel pore. The same missense mutation was found in an affected sibling. However, the affected parent had normal SCN10A, whereas the unaffected parent did not, showing the mutation to be non-pathogenic.

A common allele can confer risk for disease phenotype

Estacion and colleagues\(^{25}\) described a non-synonymous SNP in SCN9A (rs8746030G>A), which produces the Arg1150Trp substitution in the NaV1.7 sodium channel, with a minor allele frequency of 17·8% in the control white population; they showed that, when studied by current-clamp, this polymorphism produces a moderate rise in firing frequency in nociceptive DRG neurons. Holliday and colleagues\(^{33}\) did not observe any association of this SNP with chronic widespread pain. However, Reimann and colleagues\(^{32}\) reported that the minor allele at this site alters pain threshold and is associated with high pain scores in patients with sciatica, osteoarthritis, and traumatic limb amputations. These findings suggest that some common alleles of sodium channel genes could contribute to increased risk of acquired pain disorders or account for inter-individual variations in pain perception, although they

<table>
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<th>SNP designation</th>
<th>Base substitution</th>
<th>Aminoacid change (ref seq: NP_002968)</th>
<th>Domain-S4</th>
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SNP=single nucleotide polymorphism. NP=nucleotide polymorphisms. S4=transmembrane segment 4.
are not in themselves necessarily causative of clinically manifest disease.

**Molecular findings might not predict cellular response or clinical phenotype**

Voltage-clamp analysis provides the gold standard for assessment of changes in channel function produced by gene variants. Current-clamp profiling provides a functional assessment of the effect of the variant channels on pain-signalling neurons, and abnormalities at both the voltage-clamp and current-clamp levels are usually strong evidence of pathogenicity. In at least one case—the rs746030/Arg150Trp variant of SCN9A: NaV1.7—changes at the voltage-clamp level are very subtle and do not necessarily predict the increase in excitability that the variant produces in DRG neurons. Compounding the challenge, functional characteristics of ion channels can vary, depending on the cell type in which the channel is expressed. As might be expected from this dependence on cell background, we have encountered several NaV1.7 mutations that display gain-of-function changes when expressed within DRG neurons (where NaV1.7 channels are normally expressed), but not within heterologous expression platforms, such as HEK293 cells. Thus, ideally, functional assessment of sodium channel variants identified in patients with pain syndromes should include voltage-clamp and current-clamp analysis within small DRG neurons which include nociceptors.

**The association between functional changes in mutant channels and disease phenotype can be complex**

It is not always possible to unequivocally predict clinical phenotype from functional assessment of a mutant channel. For example, Eberhardt and colleagues have described an aminoacid mutation of NaV1.7, Ala1632Thr, which depolarises voltage-dependence of fast-inactivation (a functional change usually seen in PEPD mutant channels) in a patient with inherited erythromelalgia. Moreover, overlap syndromes have been reported. Estacion and colleagues described a patient with a pain disorder that included clinical features of both inherited erythromelalgia (burning pain in distal extremities triggered by warmth) and PEPD (rectal sensitivity, harlequin erythema, and subsequent episodes of facial, ocular, or jaw pain); the NaV1.7 aminoacid mutation in this case, Ala1632Glu, produced changes in channel function associated with both inherited erythromelalgia (hyperpolarised activation) and PEPD (impaired inactivation). Diagnosis, although supported in some cases by functional profiling, should be based largely on the clinical status of the patient.

**One variant can produce different pain phenotypes in different patients**

The Ile228Met NaV1.7 mutation impairs slow-inactivation of the channel and has been shown to produce a pain syndrome characterised by onset in the territory of short axons (face and scalp) in some patients, but produce pain with onset in the distal extremities in others. The Ile739Val neuropathy NaV1.7 mutation also impairs slow-inactivation, usually causes distal limb pain, and also segregates with disease phenotype in a family with paroxysmal itch over proximal parts of the arms and the trunk. With the exception of PEPD, in which impaired fast-inactivation of the channel is associated with a particular pain phenotype, it is not yet possible to predict a specific clinical pattern of pain on the basis of the variant. In the case of patients with PEPD, who have mutations close to the isoleucine-phenylalanine-methionine inactivation gate in NaV1.7, the mutation is highly likely to be pathogenic. Clinical diagnosis should be made on the basis of clinical presentation supported when possible by genotyping, but not on the basis of the genotyping alone.

**Some sodium channel variants are variably expressed or only partly penetrant**

Many inherited erythromelalgia and PEPD mutations are fully or nearly fully penetrant. For example, the Phe449Val variant is present in all affected family members tested, but absent from unaffected family members, in a large family with inherited erythromelalgia; pain characteristically occurs with this mutation in early childhood. However, some mutations with adult-onset symptoms are variably expressed. The degree of penetrance can be difficult to ascertain with adult-onset symptoms because the absence of symptoms in a carrier might be due to incomplete penetrance, or, alternatively, due to failure of the mutation to cause manifest disease in individuals who have not lived long enough. By contrast with most patients with inherited erythromelalgia and PEPD who have pain beginning in infancy or early childhood, the Gly616Arg mutation was described in a pedigree with adult-onset symptoms and was associated with inherited erythromelalgia in the proband. One family member who carried the mutation did not display symptoms at age 16 years when the family was analysed, although two siblings carrying the mutation manifested symptoms before the age of 10 years. The mutation seems to be pathogenic because it produces a gain-of-function depolarising shift in voltage-dependent inactivation when studied by voltage-clamp, and produces hyperexcitability of DRG neurons when studied by current-clamp. Although the mechanistic basis for time-dependent onset of symptomatology is not completely understood, this family draws attention to the complexity of mutations characterised by variable expressivity or incomplete penetrance.

Adult onset of symptoms is even more common in patients with small-fibre neuropathy who have mutations of NaV1.7, NaV1.8, or NaV1.9, although the mechanistic cascade from mutant channel to disease onset is likely to be complex. One example is provided by the Ile739Val...
and Arg185His Na\(_{\text{i,7}}\) variants in patients with small-fibre neuropathy, both of which were shown as gain-of-function by voltage-clamp and current-clamp. By contrast with rare variants associated with disease, the Ile739Val and Arg185His variants seem to be present in the general population at frequencies that might be equal to or higher than the frequency in patients with small-fibre neuropathy; thus, some unaffected individuals are likely to be heterozygous for these variants. Clinical manifestations might not be apparent until adulthood, and age-of-onset can vary between patients.\(^7\) These results raise the possibility that these variants do not in themselves cause disease in a mendelian manner, but rather provide a genetic substrate for development of small-fibre neuropathy in combination with other genetic or environmental factors. Accurate to this multihit model, variants such as Ile739Val and Arg185His do not cause disease themselves, but act as risk factors.

### Some sodium channel variants might enhance pharmacoresponsiveness to some medications

Patients with PEPD are often responsive to carbamazepine.\(^8\) With only a few exceptions, available sodium channel blockers are, however, of only limited therapeutic value in other Na\(_{\text{i,7}}\)-related pain disorders. Such examples include a child with inherited erythromelalgia (and the variant Val872Gly) who responded to mexiletine, which had an enhanced, use-dependent blocking action on the mutant channel,\(^9\) and a family with carbamazepine-responsive inherited erythromelalgia, and the variant Val400Met, in which the mutant-induced hyperpolarising shift in activation was corrected with carbamazepine.\(^5\) Studies that have used atomic-level structural modelling and thermodynamic analysis as a basis for pharmacological assessment of a small number of channel variants, suggest that the presence of some substitutions within sodium channels, including Na\(_{\text{i,7}}\), Na\(_{\text{i,8}}\), and Na\(_{\text{i,9}}\), can predict responsiveness to specific medications,\(^2\) but this approach has not yet been validated for clinical use. Moreover, although specific blockers of Na\(_{\text{i,7}}\), Na\(_{\text{i,8}}\), and Na\(_{\text{i,9}}\) are in development, they have not yet been used in patients.

### Some variants might be missed by classic Sanger DNA sequencing

Clinicians should be aware of the pitfalls of genetic testing and the limitations of standard tests. Deep intronic variants, or large heterozygous genomic deletions or rearrangements, are usually missed by classic Sanger DNA sequencing. This can be an issue, especially for loss-of-function disorders. Commercial kits for deletion or duplication testing of SCN9A, SCN10A, and SCN11A are not yet available. Mosaicism for a mutation can likewise be difficult to detect, and might be relevant, especially in gain-of-function disorders. Thus, additional testing strategies have to be considered for patients with a clear phenotype and a negative test result. Future use of next-generation sequencing technologies—e.g., whole-genome sequencing, and advanced bioinformatics, will probably identify large insertions, deletions, or copy-number variants. Additionally, with appropriate functional assessment, they might identify functional intrinsic mutations.

### Panel: Classification of variants of SCN9A, SCN10A, and SCN11A

#### Pathogenic

- Multigenerational segregation with the disease in a family spanning more than three generations, and predictive algorithms unequivocally suggesting pathogenicity
- Segregation with the disease in a family spanning more than three generations, and the variant displays gain-of-function changes by voltage-clamp or current-clamp studies, or both
- Segregation with the disease in a nuclear, single-generation family, and predictive algorithms unequivocally suggesting pathogenicity, and the variant displays gain-of-function changes by voltage-clamp and current-clamp studies
- A patient with insensitivity to pain has a homozygous nonsense variant or substitutions that disrupt the consensus splice sites (noting that voltage-gated sodium channel genes also have non-canonically spliced introns), or compound heterozygous mutations of nonsense or splicing-disrupting substitutions, with missense mutations shown as non-functional after voltage-clamp studies

#### Probably pathogenic

- Segregation with the disease in a nuclear, single-generation family and predictive algorithms unequivocally suggesting pathogenicity
- Segregation with the disease in a nuclear, single-generation family and the variant displays gain-of-function changes by voltage-clamp or current clamp studies
- Only one family member and the variant displays gain-of-function changes through voltage-clamp and current-clamp studies

#### Possibly pathogenic

- Predictive algorithms unequivocally suggest pathogenicity, but segregation cannot be tested or is unclear (e.g., sporadic cases, with possible incomplete penetrance) and no functional studies are available
- Only one family member and the variant displays gain-of-function changes through voltage-clamp or current-clamp studies

#### Variants with uncertain clinical significance

- Variants for which predictive algorithms suggest pathogenicity, that do not belong to any of the previous categories should, in our opinion, be listed as having unknown clinical significance

### Suggestions for clinical interpretation of sodium channel variants

Identification of a sodium channel variant could provide a firm pathophysiological or aetiological explanation for a pain disorder; however, this is not always the case, and correct interpretation and reporting of variants are important to patients and their families. Building on existing guidelines,\(^7\) we recommend that variants of SCN9A, SCN10A, and SCN11A should be classified as described in our panel. In this regard, there is no strict definition of a large family; however, cosegregation of a variant in a multigenerational family, or the presence of the variant in several affected siblings would support
The investigation starts with identification and clinical assessment of a symptomatic patient. Some, but not all, treatment plan. Integration of all the information provides a basis for the most appropriate pathogenicity. Functional testing can contribute substantially to understanding the consequences of the genetic segregate within the family with the disease phenotype might be subjected to in-silico testing that could predict testing—eg, by Sanger sequencing of candidate genes or by a whole exome approach. Variants that are rare and individuals will have a family history that is suggestive of an inherited disorder. The next step consists of DNA testing—eg, by Sanger sequencing of candidate genes or by a whole exome approach. Variants that are rare and segregate within the family with the disease phenotype might be subjected to in-silico testing that could predict pathogenicity. Functional testing can contribute substantially to understanding the consequences of the genetic variant. Collectively, these elements all feed into the diagnostic process and to assessment of the functional and clinical importance of the variant. Integration of all the information provides a basis for the most appropriate treatment plan.

### Search strategy and selection criteria

In writing this Personal View, the authors searched PubMed and genomic databases (HGMD; single nucleotide polymorphism databases [dbSNP, 1000 Genomes Project, and the NHLBI GO Exome Sequencing Project]) for relevant information. However, this Personal View, although based on these sources and the authors’ personal experience, does not present all of the gene variants but, rather, discusses relevant examples.

Mutations of SCN9A, SCN10A, and SCN11A, the genes encoding the peripheral sodium channels NaV1.7, NaV1.8, and NaV1.9, can cause pain disorders. Genetics and genomics have contributed immensely to our understanding of the roles of these channels and their variants in human pain disorders, and in some cases sequencing the genes for these channels can provide very helpful information in the clinic. However, we have also learned that genomic sequencing of SCN9A, SCN10A, and SCN11A will show not only disease-causing variants, but also disease-contributing variants and variants of uncertain significance. Some variants are variably expressed or incompletely penetrant. Some rare alleles might not be pathogenic, and some common alleles might impose risk for disease phenotype. Moreover, one variant can produce different phenotypes in different individuals. As shown in this Personal View, the diagnostic capabilities of mutation-prediction programmes are, at best, moderate. At present, newly described gene variants of SCN9A, SCN10A, and SCN11A should be assessed in the context of phenotype, family history, in-silico analysis, and functional assessment of the variant channel (figure), and should be interpreted cautiously, with the caveats discussed in this Personal View in mind. We suggest that newly identified variants should be classified conservatively, with terminology similar to that used in our panel.

The more sequencing that is done, the greater the chance of finding additional, very rare (previously unreported) variants. Thus in one sense the challenge will grow. However, additional variants that have not yet been functionally assessed will be functionally profiled in the future; therefore, the number of variants that can be identified as pathogenic will rise. Furthermore, high-throughput methods for functional assessment of sodium channel mutations are likely to be developed. Genetic and genomic studies of SCN9A, SCN10A, and SCN11A have already taught much about the molecular and genetic basis for human pain. Going forward, together with newer and less-costly methods for gene sequencing than the ones described, further studies of these genes will bring us closer to a comprehensive understanding of the genotype–phenotype relations in pain-related disorders, and provide a basis for the effective use of sequencing of SCN9A, SCN10A, and SCN11A in an increasing number of patients.

### Conclusions and future directions

Mutations of SCN9A, SCN10A, and SCN11A, the genes encoding the peripheral sodium channels NaV1.7, NaV1.8, and NaV1.9, can cause pain disorders. Genetics and genomics have contributed immensely to our understanding of the roles of these channels and their variants in human pain disorders, and in some cases sequencing the genes for these channels can provide very helpful information in the clinic. However, we have also learned that genomic sequencing of SCN9A, SCN10A, and SCN11A will show not only disease-causing variants, but also disease-contributing variants and variants of uncertain significance. Some variants are variably expressed or incompletely penetrant. Some rare alleles might not be pathogenic, and some common alleles might impose risk for disease phenotype. Moreover, one variant can produce different phenotypes in different individuals. As shown in this Personal View, the diagnostic capabilities of mutation-prediction programmes are, at best, moderate. At present, newly described gene variants of SCN9A, SCN10A, and SCN11A should be assessed in the context of phenotype, family history, in-silico analysis, and functional assessment of the variant channel (figure), and should be interpreted cautiously, with the caveats discussed in this Personal View in mind. We suggest that newly identified variants should be classified conservatively, with terminology similar to that used in our panel.

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