$Na_V1.9$: a sodium channel linked to human pain

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Abstract | The voltage-gated sodium channel $Na_V1.9$ is preferentially expressed in nociceptors and has been shown in rodent models to have a major role in inflammatory and neuropathic pain. These studies suggest that by selectively targeting $Na_V1.9$, it might be possible to ameliorate pain without inducing adverse CNS side effects such as sedation, confusion and addictive potential. Three recent studies in humans — two genetic and functional studies in rare genetic disorders, and a third study showing a role for $Na_V1.9$ in painful peripheral neuropathy — have demonstrated that $Na_V1.9$ plays an important part both in regulating sensory neuron excitability and in pain signalling. With this human validation, attention is turning to this channel as a potential therapeutic target for pain.

Nine genes (*SCN1A*–*SCN5A* and *SCN8A*–*SCN11A*) in mammals encode the pore-forming α-subunits of sodium channels (referred to throughout as channels), $Na_V1.1$–$Na_V1.9$, which have distinct biophysical and pharmacological properties and tissue distribution patterns[1]. Each of these channels has a conserved motif of 24 transmembrane segments that are organized into four homologous domains (DI–DIV), each with six transmembrane segments (S1–S6) that are linked by cytoplasmic loops, and cytoplasmic amino and carboxyl termini. The S1–S4 segments of each domain form the voltage-sensing domain, whereas S5 and S6, and the extracellular linker that joins these two segments, form the pore domain. The S5–S6 linker in each domain contains two membrane re-entrant segments (SS1 and SS2) that contribute to the sodium-selectivity filter, and the DI–SS2 carries a residue that determines susceptibility to blockade by tetrodotoxin (TTX). Whereas the presence of an aromatic residue confers sensitivity to nanomolar concentrations of TTX, a serine or cysteine in this position confers resistance to micromolar concentrations of TTX[1].

Three of these channels — $Na_V1.7$, $Na_V1.8$ and $Na_V1.9$ — are preferentially expressed in peripheral neurons and have been shown to have an important role in preclinical animal models of both normal and pathological pain. $Na_V1.7$ and $Na_V1.8$ channels have garnered intense interest because of evidence for their role in human pain disorders[2–5], whereas there has previously been no such evidence for $Na_V1.9$. Our understanding of $Na_V1.9$ in general has also lagged behind as, unlike $Na_V1.7$ and $Na_V1.8$, it has proved difficult to study $Na_V1.9$ in heterologous expression systems, because it is only expressed at low levels and is difficult to study in isolation in native neurons.

Recent genetic and functional findings that link $Na_V1.9$ to human pain disorders[6–9] have triggered a renewal of interest in $Na_V1.9$ as an important contributor to pain in humans. In this Progress article, we discuss functional studies of $Na_V1.9$ that have yielded new insights into the unique gating characteristics of this channel and its contribution to the response of sensory neurons under normal and pathological conditions. We also highlight studies that have validated $Na_V1.9$ as an important contributor to pain in humans, and identify remaining unanswered questions about the role of $Na_V1.9$ in pain signalling.

$Na_V1.9$ expression

$Na_V1.9$ was first identified in neurons of the dorsal root ganglion (DRG) and trigeminal ganglia from rats[10]. *SCN11A*, the gene encoding $Na_V1.9$, was mapped to human chromosome 3 (3p21–24) within a cluster including *SCN5A* and *SCN10A* (the genes encoding $Na_V1.5$ and $Na_V1.8$, respectively), and to an analogous region on mouse chromosome 9 (REF. 11). The human *SCN11A* open reading frame encodes a protein of 1,765 amino acids that shows the least homology to other members of the Na_V family. Nonetheless, $Na_V1.9$ possesses all of the hallmarks of a Na_V channel that are described above.

$Na_V1.9$ is preferentially expressed in small-diameter (<30 μm diameter) DRG neurons, in trigeminal ganglion neurons and in intrinsic myenteric neurons[12,13]. $Na_V1.9$ is expressed in functionally identified nociceptors[14,15]. $Na_V1.9$ has been localized within DRG neuronal somata and free nerve terminals, and at central terminals within the outer layers of the substantia gelatinosa in the spinal cord[11] (FIG. 1a–d). It is also localized in presynaptic terminals within the dorsal horn[16]; however, our data suggest that, although it is translocated to the dorsal horn, the channel is present at only low levels at this site. Nevertheless, computer simulations show that even at 50% of its normal density in DRG neuron cell bodies, $Na_V1.9$ contributes >75% of its normal depolarizing effect[17], suggesting that the channel could modulate neurotransmitter release in the dorsal horn even when present at a low density.

Among the small-diameter neurons of the DRG, $Na_V1.9$ seems to be preferentially expressed in somatosensory non-peptidergic DRG neurons, which can be identified by binding to the *Griffonia simplicifolia* isolectin IB4 (REF. 12). $IB4^+$ nociceptors transmit signals concerning noxious stimuli from the periphery to limbic and affective regions of the brain through multisynaptic circuits[18], and produce smaller noxious-heat-activated currents than do peptidergic $IB4^-$ neurons[19]. This preferential cellular distribution of $Na_V1.9$ may contribute to the distinct firing properties of $IB4^+$ neurons[15]. However, it has recently been shown that $Na_V1.9$ is predominantly present in $IB4^-$ peptidergic DRG neurons that innervate the colon[20]. Irrespective of the cell type in which it is expressed, the presence of $Na_V1.9$ in the peripheral and central termini of primary afferents suggests that the channel has a role in the integration of receptor potentials and possibly in

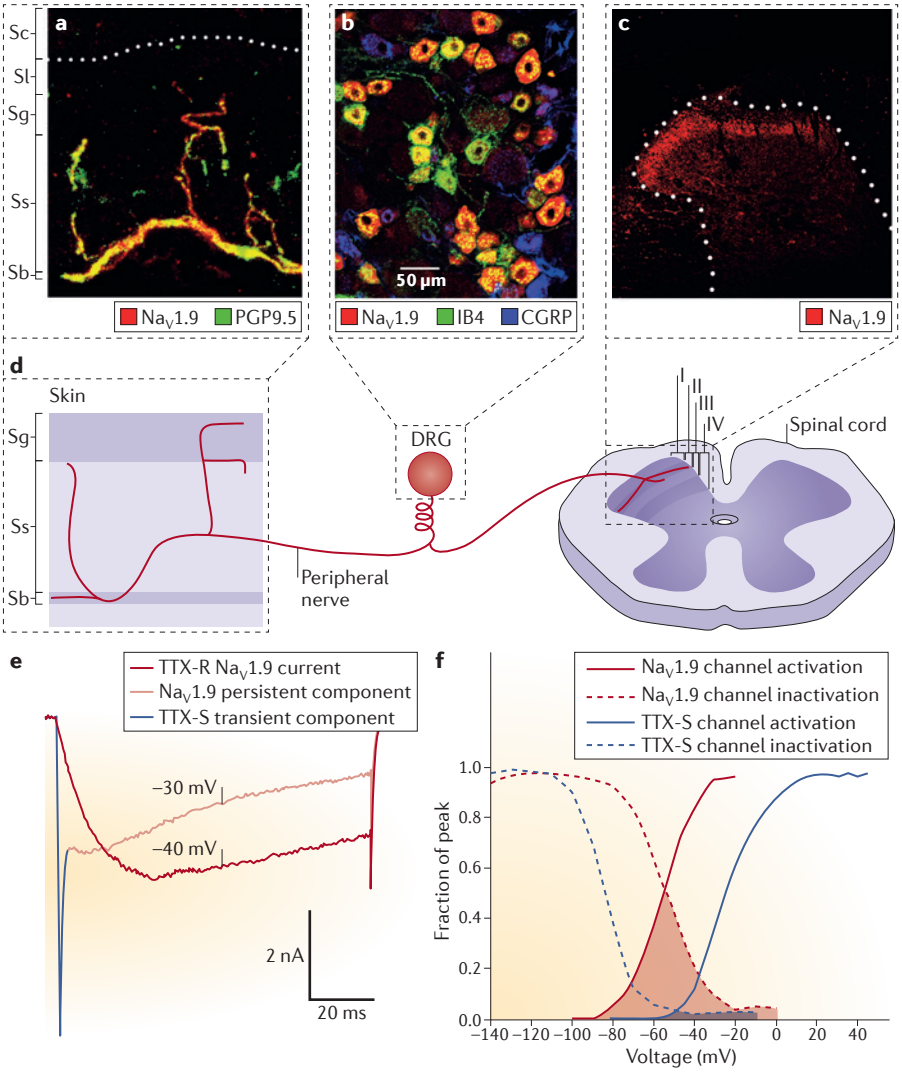
modulating neurotransmitter release in the dorsal horn of the spinal cord at the first synapse of the pain-signalling pathway.

Physiology of $Na_V1.9$ channels

$Na_V1.9$ mediates a TTX-resistant (TTX-R) sodium ion current, which is consistent with the presence of a serine residue at the TTX-susceptibility-determining site in the DI–SS2 pore region of the channel; amino acid residues with an aromatic ring (for example, tyrosine in $Na_V1.6$ and $Na_V1.7$) at this position in TTX-S channels engage TTX in a cation–π interaction that increases the affinity of the toxin for the binding pocket within the outer vestibule of the channel. By contrast, the presence of serine (as in $Na_V1.8$ and $Na_V1.9$) or cysteine (as in $Na_V1.5$) markedly reduces the affinity of the TTX–channel interaction by several orders of magnitude[21].

Although instability of the $Na_V1.9$ current has been encountered by almost all investigators who have studied this channel, methods and protocols have been designed to minimize this instability and to generate reproducible data[22–25]. The inactivation of $Na_V1.9$ is unusually 'ultra-slow' and results in the persistence of the sodium current after activation (FIG. 1e). $Na_V1.9$ characteristically activates at more hyperpolarized voltages than do other neuronal sodium channels. Thus, there is a large overlap between activation and inactivation, and this overlap is predicted to produce a large 'window current' (that is, a wide range of voltages in which a channel may spontaneously or persistently open), within the physiological voltage domain close to the resting membrane potential of neurons (−70 mV to −40 mV)[22] (FIG. 1f). Importantly, the $Na_V1.9$ current recorded from human native DRG neurons activates at approximately −80 mV, which is 10–20 mV more negative than the voltage at which the $Na_V1.9$ current in rodent DRG neurons is activated — probably owing to species-specific differences in primary protein sequence[26]; a more hyperpolarized activation voltage indicates that human $Na_V1.9$ can open in response to a stimulus weaker than that which can activate rodent $Na_V1.9$. Incontrovertible evidence that $Na_V1.9$ produces the persistent TTX-R current is provided by studies that show that this current is lost in DRG neurons of *Scn11a*-knockout mice[16,27,28] but is restored by the expression of recombinant $Na_V1.9$ channels in these neurons[6,28] and in superior cervical ganglion neurons[9].

The biophysical properties of $Na_V1.9$ — including ultra-slow kinetics and a large window current (FIG. 1f) — suggest that $Na_V1.9$ channels do not contribute much to the amplitude of action potentials, but rather that they may act as threshold channels by contributing a sodium conductance that regulates resting potential and that prolongs the depolarizing response to subthreshold stimuli[17,22], lowering the threshold for single action potentials and increasing repetitive firing[29]. Although the degree of hyperpolarization of the voltage dependence of activation of $Na_V1.9$ is dependent on the ionic composition of the pipette solution during recording[24,25], computer simulations and current-clamp studies using physiological solutions in the recording pipette support the conclusion that this channel acts as a threshold channel[17,29]. Computer simulations show that, even at densities as low as 20% of the density estimated to be present in DRG neurons, $Na_V1.9$ conductance depolarizes the resting potential of the cell[17]. However, the effect of the $Na_V1.9$-induced depolarization is similar to that observed for mutant $Na_V1.7$ channels from patients with inherited erythromelalgia (which causes DRG neuron hyperexcitability); depolarization of the resting potential by such mutant $Na_V1.7$ channels only accounts for a portion of the reduction in action potential threshold, which also results from the increase in sodium conductance[30,31]. Consistent with this finding, one study used guanosine-5-*O*-thiotriphosphate (GTPγS; which potentiates $Na_V1.9$ activity) to directly show that upregulation of the $Na_V1.9$ current lowers the threshold of current that is required to generate action potentials[29] (FIG. 2a,b). The same study also demonstrated that the $Na_V1.9$ current supports repetitive firing (FIG. 2c,d) and can drive spontaneous activity[29] (FIG. 2e,f). Thus, taken together, the computer simulations[17] and empirical evidence[28,32,33] suggest that $Na_V1.9$ acts as a threshold channel.



$Na_V1.9$ in inflammatory pain models

Although acute inflammatory pain and neuropathic pain share common pathophysiological mechanisms that typically result in sensitization of peripheral sensory neurons,

Figure 1 | **Distribution of $Na_V1.9$ in primary afferents.** **a** | The voltage-gated sodium channel $Na_V1.9$ (red) is present in protein gene product 9.5 (PGP9.5; also known as UCHL1)-positive intraepidermal nerve fibres (green), which have endings in the epidermis that extend from the subepidermal nerve bundle. Colocalization of $Na_V1.9$ and PGP9.5 is shown in yellow. The dotted line indicates the demarcation of stratum corneum (Sc; above the line) and stratum lucidum (Sl; below the line) and of the epidermis. The Sc, Sl and the other different layers of the epidermis (namely, the stratum basale (Sb), stratum spinosum (Ss) and stratum granulosum (Sg)) are delineated by the square brackets. **b** | Small-diameter neurons in the dorsal root ganglion (DRG) exhibit robust $Na_V1.9$ immunolabelling (red). DRG neurons display substantial colocalization of $Na_V1.9$ with the *Griffonia simplicifolia* isolectin IB4 (green) but not with calcitonin gene-related peptide (CGRP; blue). **c** | Presynaptic nerve terminals of DRG neurons in the superficial layers of the dorsal horn of the spinal cord display $Na_V1.9$ immunostaining (red). The dorsal horn is outlined by a dotted line. **d** | A schematic representing the localization of $Na_V1.9$ channels at primary afferent peripheral nerve endings in the skin, in DRG somata and in central nerve endings, where they terminate predominantly in the superficial lamina II in the dorsal horn of the spinal cord. **e** | The persistent tetrodotoxin-resistant (TTX-R) sodium currents mediated by $Na_V1.9$ channels and, for comparison, fast-inactivating TTX-sensitive (TTX-S) currents mediated by $Na_V1.6$ and/or $Na_V1.7$ channels recorded from a small-diameter $Na_V1.8$-null mouse DRG neuron that lacks the slow TTX-R current mediated by $Na_V1.8$ channels. Current traces that result from 100 ms depolarizing pulses from a holding potential of −120 mV (to ensure that all Na_V channels are available to open) to the membrane voltages indicated on the traces show that $Na_V1.9$ channels activate at more hyperpolarized potentials than do the fast TTX-S channels. Depolarization to −40 mV activates only $Na_V1.9$ channels (red trace), whereas a depolarizing stimulus at −30 mV shows a compound trace consistent with activation of both a fast-inactivating TTX-S component (blue trace) and the $Na_V1.9$-mediated persistent component (pale red trace). Note the rapid activation and inactivation of the TTX-S current compared with the slow activation and very slow inactivation of $Na_V1.9$. **f** | Fitting the activation data of $Na_V1.9$ currents (solid red curve) and TTX-S currents (solid blue curve) to a Boltzmann function shows that $Na_V1.9$ channels activate at more hyperpolarized potentials (that is, they open more easily at hyperpolarized potentials) than do the fast-activating and fast-inactivating TTX-S sodium channels. The steady-state inactivation curves for TTX-R, $Na_V1.9$-mediated persistent channels (dashed red line) and fast-inactivating TTX-S sodium channels (dashed blue line) show that $Na_V1.9$ channels remain available at more depolarized potentials than do the TTX-S channels. The voltage domain delineated by the overlap between the activation and inactivation curves corresponds to the 'window current' that is produced by the different channels, and is substantially larger for $Na_V1.9$ (red) than for the TTX-S channels (blue). The increased window currents of $Na_V1.9$-expressing DRG neurons are predicted to promote the depolarization of resting membrane potential and thus contribute to boosting weak stimuli. Parts **e**,**f** courtesy of J. Huang, Yale University School of Medicine, Connecticut, USA.

their aetiologies are distinct. Tissue injury leads to cytokine production, as well as to activation of innate and adaptive immune responses, and can also lead to pain — which, depending on the robustness and duration of the immune response, may become persistent. Preclinical studies in rodents support a role for $Na_V1.9$ in inflammatory pain (for a recent review, see REF. 34), and $Na_V1.9$ was also recently implicated in bone cancer pain in rats[35]. Treatment of rat DRG neurons with prostaglandin E_2 (PGE_2), an autocrine and paracrine lipid metabolite of arachidonic acid that acts through cyclooxygenases and that has a major impact on pain signalling[36], increases the amplitude of the $Na_V1.9$ current via a pathway that involves G proteins[37]. Treatment of DRG neurons with other inflammatory mediators, such as interleukin-1β[38] or a mixture of bradykinin, ATP, histamine, PGE_2 and noradrenaline[39], increased the current density (a measure that reflects the number of active channels) of $Na_V1.9$, enhanced the excitability of these neurons by lowering the threshold for action potential generation, and increased the number of action potentials in response to injected depolarizing stimuli. A recent study showed that treatment with a well-defined mixture of inflammatory mediators — bradykinin, ATP, histamine, PGE_2 and serotonin — causes a marked increase in the firing of colon-innervating afferents in wild-type mice but not in *Scn11a*-null mice[20]. This finding supports a role for $Na_V1.9$ in rendering DRG neurons hyperexcitable, thus leading to pain in inflammatory disorders — for example, in osteoarthritis or pathologies in which pro-inflammatory cytokines are produced, such as burn injury — although whether it also has a role in neuropathic pain is less clear.

Genetic validation in humans

Studies in rodents are not necessarily predictive of human therapeutic responses to putative new pain medications[40], and this has highlighted the importance of using genetic and genomic methods to validate sodium channels as therapeutic targets in human pain[41]. Indeed, this approach has confirmed that the $Na_V1.7$ channel is a key player in the generation and/or transmission of the neuronal response to noxious stimuli that leads to pain in humans. Recently, mutations in the gene encoding $Na_V1.9$ (FIG. 3) have been identified in individuals with rare genetic pain diseases and in larger populations of people with painful peripheral neuropathy (TABLE 1), thus validating $Na_V1.9$ as a target in human pain disorders. Of these studies, three reported variants of $Na_V1.9$ that resulted in a gain of function at the channel level and that were associated with increased pain[6,8,9], whereas the fourth study reported a gain of function at the channel level that was associated with an inability to experience pain, which was associated with self-mutilation[7]. Despite the fact that these studies have found opposing effects of different gain-of-function mutations on neuronal firing (discussed below), these studies all link $Na_V1.9$ to human pain.

SCN11A *mutations in familial episodic pain.* Zhang *et al.*[8] described a rare human pain disorder in two large Chinese families with multigenerational autosomal-dominant gain-of-function mutations in *SCN11A*, which they called *SCN11A*-related familial episodic pain. Early in childhood, affected family members reported pain — mainly in the lower extremities — that was exacerbated by fatigue and accompanied by sweating. Painful areas felt extremely cold, and the pain could be relieved by hot compresses. Pain relief could also be achieved by treatment with non-steroidal anti-inflammatory drugs (NSAIDs), consistent with the effects of inflammatory mediators (as discussed above).

Genetic linkage analysis localized the mutation responsible for this pain condition to a region on chromosome 3 that carries a cluster of genes — including *SCN11A* — that encode the three TTX-R Na_V channels. Subsequent whole-exome sequencing followed by Sanger sequencing identified two missense mutations in *SCN11A*, one in each family. Both of these mutations substituted a residue that is invariant in all members of the Na_V family, suggesting that these mutations may alter channel function. One mutation (R225C) altered a residue within the voltage-sensing S4 of DI (DI–S4), and the other mutation (A808G) produced an amino-acid substitution within the pore-lining S6 of DII (DII–S6) (FIG. 3). The two mutations co-segregated with pain in affected individuals, were absent from unaffected individuals and were not found in a control cohort of ethnic Han individuals or in the 1,000 Genomes database, providing strong genetic evidence to support the pathogenicity of these *SCN11A* mutations.

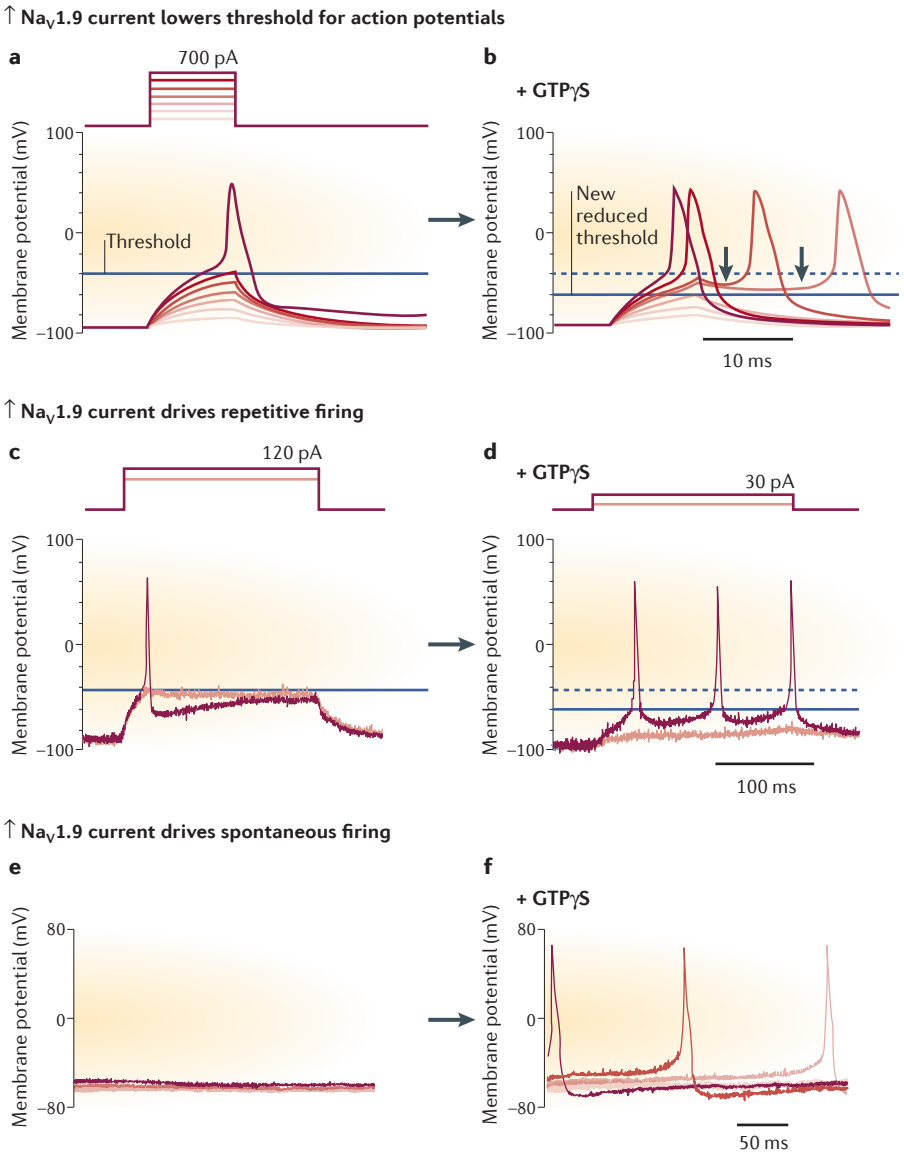


Figure 2 | **$Na_V1.9$ increases the excitability of DRG neurons.** Injecting a stimulus current into a wild-type small-diameter dorsal root ganglion (DRG) neuron (at a holding potential of −90 mV) must depolarize the membrane potential above a certain voltage threshold (indicated by a blue line) before an action potential is fired (part **a**). For the same neuron as shown in part **a**, an increase in $Na_V1.9$ current, after treatment with guanosine 5-*O*-thiotriphosphate (GTPγS), which potentiates $Na_V1.9$ activity as a consequence of activating G protein signalling cascades, markedly reduces the current threshold (known as the rheobase) and voltage threshold at which the first all-or-none action potential is fired (indicated by a solid blue line; the dashed blue line indicates the voltage threshold before enhancement of $Na_V1.9$ currents) (part **b**). The enhanced $Na_V1.9$ current also gives rise to prolongation of previously subthreshold depolarizations (arrows) resulting in the firing of an action potential. Parts **c** and **d** show responses of a wild-type small-diameter DRG neuron to two 200-ms-duration depolarizations (one just subthreshold, and one suprathreshold) from a holding potential of −90 mV, before (part **c**) and after (part **d**) treatment with GTPγS. Increasing the $Na_V1.9$ current lowers the voltage threshold (dashed lines) and the current threshold (30 pA in part **d** versus 120 pA in part **c**) and results in repetitive firing (part **d**). Parts **e** and **f** show current-clamp recordings of 10 sequential responses in a wild-type small-diameter DRG neuron — first at 1 minute after establishing the cell recording configuration (part **e**), and then at 3 minutes later, to allow for GTPγS to activate the G protein signalling cascade (part **f**). There was no firing of action potentials prior to enhancement of the $Na_V1.9$ current (part **e**). However, in neurons in which GTPγS treatment led to an upregulation of $Na_V1.9$ current, action potentials arise in the absence of stimulation, as the membrane potential slowly depolarizes and reaches a potential range that corresponds to the threshold voltage for activating $Na_V1.9$ channels (part **f**), consistent with a role for $Na_V1.9$ in driving spontaneous activity. Adapted with permission from REF. 29, John Wiley & Sons.

Functional testing of these mutant channels in mouse DRG neurons revealed an increase in the $Na_V1.9$ current density that did not reach statistical significance and did not show an effect of the mutations on $Na_V1.9$-gating properties. However, applying a depolarizing current to DRG neurons expressing either of the mutant channels revealed an increased evoked excitability, with a reduced threshold for generating action potentials and a higher firing frequency, without any change in the resting membrane potential. Despite the lack of a clear effect of the mutations on the current density or gating properties of the encoded channel, the genetic evidence, together with the reporting of mutant-channel-induced neuronal hyperexcitability, supports a causal link between $Na_V1.9$ mutations and this rare familial episodic pain.

$Na_V1.9$ and peripheral neuropathy. In another study, Huang *et al.*[6] identified gain-of-function mutations in a cohort of patients with painful peripheral neuropathy, thus providing a link between $Na_V1.9$ and a very common pain disorder that afflicts millions of people around the world. Among 345 individuals with painful peripheral neuropathy, 12 individuals (who did not carry mutations in *SCN9A* or in *SCN10A*) were found to carry one of eight mutations in *SCN11A*. Four of these mutations led to substitutions of highly conserved amino acids in membrane-spanning segments of $Na_V1.9$; three altered amino-acid residues of the cytoplasmic loops or of the C terminus of the channel (FIG. 3); and one affected the 3′ splice acceptor site of intron 24. In addition to experiencing numbness, tingling and pain in the distal extremities, these patients complained of autonomic dysfunction — for example, diarrhoea and a sensation of dry eyes. Intuitively, the pain symptoms could be associated with the effects of mutant $Na_V1.9$ on DRG neuron excitability, whereas dry eyes and diarrhoea might be associated with the presence of the mutant channels in free nerve terminals in the cornea[42] and in visceral afferent neurons[13,43]. The symptom of dry eyes might be explained by corneal nociceptor hyperactivity[44,45]. The fact that the mutant variant of the channel might cause these gastrointestinal symptoms[13,43] is supported by the recent finding that $Na_V1.9$ has a role in the responses to mechanical stimulation of the colon: it contributes to mechanical hypersensitivity of visceral afferents that innervate the colon, and is essential for the response of these neurons to inflammatory mediators such as PGE_2 and ATP, and to

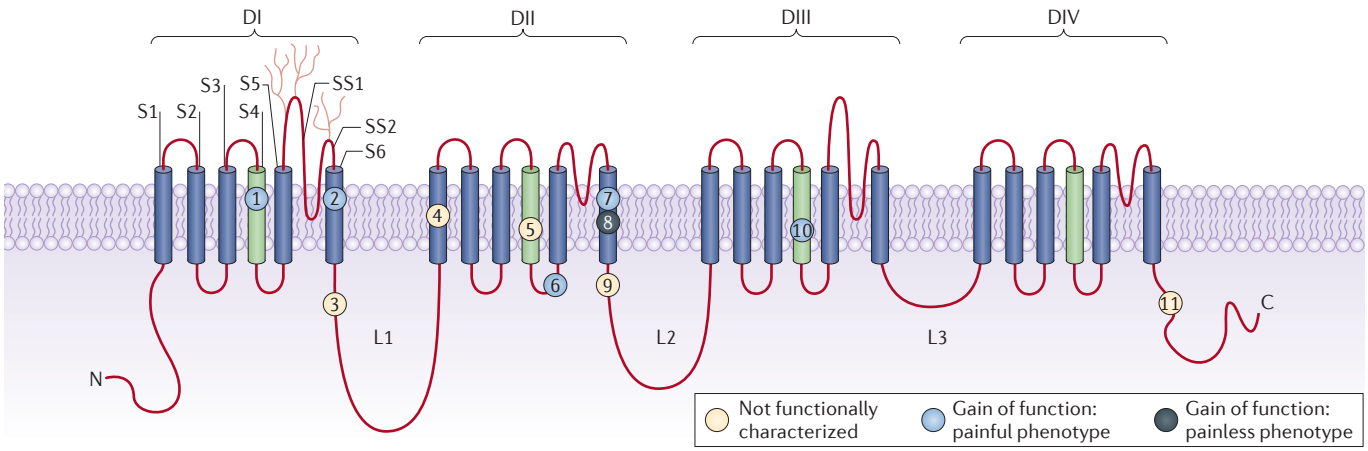


Figure 3 | **Schematic diagram of the $Na_V1.9$ channel and locations of substitutions identified in individuals with pain disorders.** The voltage-gated sodium channel $Na_V1.9$, like other Na_V channels, is composed of 24 transmembrane segments that are organized into four homologous domains (DI–DIV) and cytoplasmic amino and carboxyl termini. The fourth transmembrane segment of each domain (S4; green segments) is an amphiphatic α-helix voltage sensor of the channel and has positively charged lysine or arginine residues that line one face of the helix. This configuration enables the exchange of ion pair partners following membrane depolarization, which facilitates the movement of these residues 'outwards' in the plane of the membrane. The pale red lines represent *N*-linked sugar moieties. Filled circles (numbered 1–11) represent amino-acid substitutions in $Na_V1.9$ from individuals with pain disorders; pale yellow circles denote mutations that have not been tested in functional assays. Mutations 1 (R225C) and 7 (A808G) were each identified in Chinese families with familial episodic pain[8]. Mutations 2 (I381T), 3 (K419N), 4 (A582T), 5 (A681D), 6 (G699R), 9 (A842P), 10 (L1158P) and 11 (F1689L) were identified in individuals with painful peripheral neuropathy (predominantly with small-fibre neuropathy) who had no mutations in the genes encoding the two other peripheral sodium channels $Na_V1.7$ and $Na_V1.8$ (REFS 6,9). The mutation L811P (mutation 8; black) was identified in two individuals with a complex syndrome involving an inability to experience pain and self-mutilation[7]. Thus, unlike mutations 1, 2, 6, 7 and 10 (all blue), which were associated with a gain of function and a painful phenotype, mutation 8 was associated with a painless phenotype despite the gain of channel function.

an inflammatory 'soup' that is derived from the bowel of individuals with inflammatory bowel disorder or Crohn disease[20].

Functional assessment of two of these mutations (I381T in DI–S6 and L1158P in DIII–S4) with whole-cell voltage-clamp recordings in DRG neurons from *Scn11a*-knockout mice showed that these mutations confer gain-of-function attributes on $Na_V1.9$ channels[6]. Current-clamp recordings of rat DRG neurons that expressed the full complement of endogenous sodium channels demonstrated that each of the two mutant channels confers hyperexcitability compared with wild-type $Na_V1.9$ channels, as indicated by a depolarized resting membrane potential, an increased number of neurons firing spontaneously and an increased number of action potentials evoked by a depolarizing current (FIG. 4). A more recent study has identified a new *SCN11A* mutation in an individual with painful neuropathy[9]. The mutation, G699R, is located in the DII–S4–S5 linker (FIG. 3), and functional assessment of the mutant channel demonstrated gain-of-function attributes at the channel level and increased DRG neuron firing (TABLE 1).

Until recently, the functional effects of mutant sodium channels on DRG neuron excitability have been assessed using current-clamp recordings from transfected neurons that express the mutant sodium channels[46]. However, such studies cannot control for the level of expression of the recombinant channel, which adds to the endogenous sodium currents in the transfected neurons. The development of models for wild-type and mutant sodium currents that can be implemented in stimulation protocols in native DRG neurons through the use of dynamic clamp[31] has enabled the study of the effects of precisely calibrated physiological levels of mutant $Na_V1.9$ current on the firing of individual DRG neurons[6]. Using this approach, Huang *et al.*[6] showed that substitution of 50% of the wild-type $Na_V1.9$ current in rat DRG neurons with the corresponding I381T current (to mimic a heterozygous phenotype) causes a substantial depolarization of the resting membrane potential and induces hyperexcitability of DRG neurons, supporting the conclusion that the nociceptors of individuals who carry this mutation can be hyperexcitable — consistent with the pain phenotype.

The two functionally profiled $Na_V1.9$ mutations found by Huang *et al.*[6] (which were each identified in two unrelated individuals with similar symptoms) and the mutation found by Han *et al.*[9] are only present at low frequencies in control populations and in genomic databases. On the basis of these observations, and the functional characterization of the gain-of-function attributes demonstrated by our group, these mutations meet the criteria for 'pathogenic' or 'probably pathogenic' (REF. 47).

SCN11A *mutation and inability to experience pain.* In a third study, Leipold *et al.*[7] described a mutation in *SCN11A* in two unrelated individuals with normal intellectual ability who had a congenital inability to experience pain, a phenotype that was associated with self-mutilation and painless fractures. Additional symptoms included increased sweating, gastrointestinal dysfunction, mild muscular weakness, delayed motor development, and slightly reduced motor and sensory nerve conduction velocities, although with a normal amplitude of compound action potentials (that is, the sum of multiple action potentials in different nerve fibres that occur almost simultaneously). A sural nerve biopsy from one of the affected individuals showed that there was no axonal loss, and MRI did not reveal structural abnormalities in the brain. These findings were interpreted as suggesting that the pain insensitivity was not related to faulty sensory neuron wiring, gross abnormality of brain structure or intellectual disability.

Using whole-exome sequencing on this individual and her unaffected parents, Leipold *et al.*[7] described a *de novo* mutation

Table 1 | **Functionally characterized *SCN11A* mutations in human pain syndromes**

Syndrome	Mutation (location*)	Experiment notes	Effects on channel properties	Effects on DRG neurons	Refs
Familial episodic pain	R225C (DI–S4)	Mutant human $Na_V1.9$ expressed in rodent DRG neurons	• Trend towards increased current density • Although there was a nominal increase in current density, the change was not statistically significant	↑ Excitability	8
	A808G (DII–S6)	Mutant human $Na_V1.9$ expressed in rodent DRG neurons	• Trend towards increased current density • Although there was a nominal increase in current density, the change was not statistically significant	↑ Excitability	8
Painful peripheral neuropathy	I381T (DI–S6)	Mutant human $Na_V1.9$ expressed in rodent DRG neurons	• Hyperpolarized activation (–6.9 mV) • Depolarized inactivation (13.3 mV) • Slower deactivation	↑ Excitability	6
	L1158P (DIII–S4)	Mutant human $Na_V1.9$ expressed in rodent DRG neurons	• Hyperpolarized activation (–6.7 mV) • Slower deactivation	↑ Excitability	6
	G699R (DIII–S4–S5)	Mutant human $Na_V1.9$ expressed in rodent SCG neurons	• Hyperpolarized activation (–10.1 mV) • Depolarized inactivation (6.3 mV) • Slower deactivation	↑ Excitability	9
Inability to experience pain, with self-mutilation	L811P (DII–S6)	Human mutant $Na_V1.9$ expressed in a rodent neuronal cell line for voltage-clamp recordings	• Hyperpolarized activation (–28 mV) • No change in inactivation • Increased current density (by more than twofold) • Slower deactivation • Slower inactivation	↓ Excitability	7
	L811P (DII–S6)	DRG neurons from a knock-in mouse expressing the analogous *Scn11a* mutation	• Hyperpolarized activation (–26 mV) • Hyperpolarized inactivation (–29 mV) • Reduced current density • Slower deactivation	↓ Excitability	7

DRG, dorsal root ganglion; SCG, superior cervical ganglion. *Expressed using domain (D) and transmembrane segment (S).

of *SCN11A* (L811P in DII–S6), and found the same mutation in the other (unrelated) individual who had a similar clinical presentation. The mutation, which was studied using voltage clamp in the ND7/23 rodent neuronal cell line, hyperpolarizes the voltage dependence of human $Na_V1.9$ channel activation by –28 mV, increases $Na_V1.9$ current density at resting membrane potential voltages by threefold and slows $Na_V1.9$ deactivation — all of which are pro-excitatory effects. The authors did not study the effects of the human L811P mutant $Na_V1.9$ on the excitability of DRG neurons; however, they created a knock-in mouse that carried the corresponding mutation in *Scn11a*. Although activation of the $Na_V1.9$ current in DRG neurons from this transgenic mouse was shifted by –26 mV, thus mimicking the hyperpolarizing shift of the voltage dependence of the human mutant channel in the ND7/23 cell line, the analogous mouse mutation also caused an unexpected comparable hyperpolarizing shift in inactivation (–29 mV), thus rendering most of the channels unavailable to open at normal resting membrane potential. Interestingly, only 11% of mice that were heterozygous for the knock-in mutant mouse allele showed severe self-induced tissue lesions. Leipold *et al.*[7] reported that, although the heterozygous transgenic mice displayed no sensory axonal loss, there was impaired transmission of noxious stimuli to the dorsal horn of the spinal cord. This impaired transmission was reflected by a reduced frequency of miniature excitatory postsynaptic currents recorded in acute spinal cord slices from heterozygous knock-in mice, which the authors interpreted as suggesting impairment of neurotransmitter release at the dorsal horn.

Interpretations of $Na_V1.9$ mutation studies. The mechanistic basis for the loss of sensitivity to pain in the patients with the L811P-mutant $Na_V1.9$ channels is puzzling when considered alongside the three other studies of gain-of-function mutations in $Na_V1.9$ that lead to pain disorders. Leipold *et al.*[7] observed a depolarization of approximately 6.7 mV in the resting membrane potential of DRG neurons from a transgenic knock-in mouse that expressed the analogous mouse *Scn11a* mutation, and suggested that this depolarization enhances the inactivation of most Na_V channels at resting potential in these neurons, thereby impairing signal transmission at the first synapse of the nociceptive pathway, in the dorsal horn. However, whether a depolarization of 6–7 mV can, in itself, produce insensitivity to pain is debatable, as previous studies (for example, see REFS 3,48–50) have shown that 5–6-mV depolarizations of the resting potential in DRG neurons that expressed mutant $Na_V1.7$ or $Na_V1.8$ caused hyperexcitability that was associated with severe pain. Indeed, as discussed above, Huang *et al.*[6] described mutations in *SCN11A* in patients with painful peripheral neuropathy that produce an approximately 6 mV depolarization of the resting membrane potential and make these DRG neurons hyperexcitable. Moreover, mimicking a 5 mV depolarization in resting potential by direct injection of current into rat DRG neurons (in the absence of mutant channels) produces hyperexcitability[6,30]. An increase in the excitability of small-diameter DRG neurons, which include nociceptors, when their resting potential is depolarized by 5–6 mV is not unexpected, as the vast majority of such neurons contain $Na_V1.8$. Compared with the other Na_V channels, $Na_V1.8$ is less sensitive to inactivation by depolarizations of up to 20–30 mV from the resting membrane potential[1]. By contrast, depolarization of the resting membrane potential of rat superior cervical ganglion neurons that lack $Na_V1.8$ renders these neurons hypoexcitable[49,50], with excitability rescued by the expression of $Na_V1.8$ (REF. 50). The hypoexcitability of DRG neurons from the L811P knock-in mouse and the lack of pain in people with the L811P *SCN11A* mutation reported by Leipold *et al.*[7] could therefore be explained by an absence of $Na_V1.8$ in the neurons that they studied, a possibility that the authors did

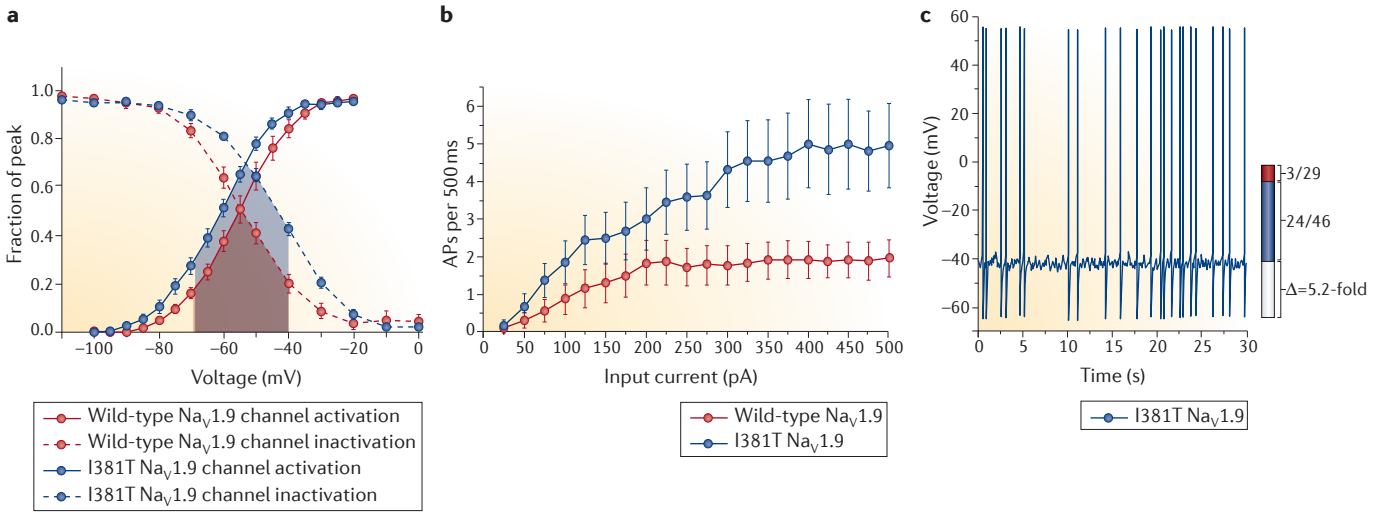


Figure 4 | **Voltage-clamp and current-clamp analyses of Na$_V$1.9 I381T mutant channels.** **a** | The effect of the I381T mutation on the gating properties of the voltage-gated sodium channel Na$_V$1.9 was studied in Na$_V$1.9-null mouse dorsal root ganglion (DRG) neurons that were transfected with wild-type or mutant channel constructs. The curves for the voltage dependence of activation (solid line) and steady-state, fast inactivation (dashed line) for wild-type Na$_V$1.9 (red) and I381T channels (blue) show that the mutation hyperpolarizes activation (thus making the channel easier to open) and depolarizes inactivation (thus increasing the availability of the channel) of the mutant Na$_V$1.9, markedly increasing the window current (shaded area) around the physiological range of the resting potential of DRG neurons. **b** | The effects of the I381T Na$_V$1.9 channels on the firing properties of primary afferents were studied in rat DRG neurons transfected with wild-type or mutant channel constructs. The average total number of action potentials (APs) elicited by injection of a depolarizing stimulus for 500 ms was higher in DRG neurons that expressed the I381T-mutant channels than in DRG neurons that expressed wild-type Na$_V$1.9 channels. **c** | Representative recording of spontaneous firing in a DRG neuron expressing I381T mutant channels. A trace was recorded for 30 s without current injection. The bar graph shows that the proportion of I381T-Na$_V$1.9-expressing DRG neurons that fire spontaneously (blue) is increased by over fivefold (difference shown in white) compared with the proportion of wild-type-Na$_V$1.9-expressing DRG neurons that spontaneously fire (red); proportion values are shown on the right-hand side. Thus, in contrast to the I381T-mutant-expressing neuron (from which the recording was taken), wild-type-Na$_V$1.9-expressing DRG neurons are much less likely to fire spontaneously. Adapted with permission from REF. 6, Oxford University Press.

not investigate. This explanation, however, is inconsistent with the finding that Na$_V$1.8 is expressed in >90% of small-diameter wild-type mouse DRG neurons[51].

The discrepancy between the DRG neuron hypoexcitability that was reported by Leipold *et al.*[7] and the DRG neuron hyperexcitability observed in other studies of mutant Na$_V$ channels[6,30,50] might be attributable to differences in recording methods or in the cells that were assessed. As noted above, several different research groups have encountered a degree of instability in Na$_V$1.9 currents that may introduce variability in the published data, depending on the protocols and methods used to study this channel. Moreover, transfected DRG neurons are typically studied a few days after transfection, whereas Leipold *et al.*[7] recorded from DRG neurons from a mouse that had been producing the mutant Na$_V$1.9 since its embryonic stages. Mutant channels in the knock-in mouse would have been expressed since embryonic stages and could therefore have induced, for example, compensatory changes that could not be studied using acute expression of the mutant channel in transfected DRG neurons.

It is well established that small-diameter axons are especially sensitive to changes in sodium channel activity, as a result of their short length constant and high input impedance[52,53]. Stys *et al.*[54] have demonstrated that sustained Na^+ influx through sodium channels can injure small-diameter central axons. Persson *et al.*[55] have shown, using an *in vitro* assay, that the expression of some gain-of-function Na$_V$ mutations can impair the integrity of the axons of DRG neurons by reducing their length, and that sodium channel blockade can protect against this effect. We have suggested that it is possible that the sustained hyperexcitability of Na$_V$1.8-expressing DRG neurons — owing to the −28 mV shift in activation produced by the L811P *SCN11A* mutation, as discussed above — leads to slow, cumulative and selective injury or even loss of some small-diameter DRG neurons in humans that was not detectable in the 5-month-old mouse model studied by Leipold *et al.*[7], thus leaving intact a population of hypoexcitable DRG neurons that express little or no Na$_V$1.8. This explanation, however, is inconsistent with the normal density of small-diameter and large-diameter axons in the sural nerve from the index patient reported by Leipold *et al.*[7].

An alternative explanation for the discrepancy between the observed hypoexcitability and hyperexcitability mediated by mutant channels is that the hyperactive mutant Na$_V$1.9 channels might produce a frequency-related reduction of excitability, as has been demonstrated in other neuronal systems[56]. In this case, such an effect could possibly be attributable to ATP depletion resulting from low-level but persistent Na^+ influx through the mutant channels, but without causing neuronal loss. This explanation will only be valid if Na$_V$1.9 has a much wider distribution in the human DRG than in the rodent DRG, where the channel is expressed in only around 50% of small-diameter neurons[12] (FIG. 1). Thus, the mechanistic link between the L811P-mutant Na$_V$1.9 channels and the inability to experience pain remains elusive.

Prospects and challenges

Despite exciting progress, there are major gaps in our knowledge of Na$_V$1.9 — particularly of the molecular mechanisms involved in the gating of Na$_V$1.9 channels. Na$_V$1.9

is the least conserved member of the Na_v family, even among its mammalian orthologues[10,11], making it difficult to extrapolate information from structural and functional studies on other sodium channels. Species-specific sequence divergence may have contributed to the marked difference in channel inactivation profiles between the human mutant channel that was expressed in ND7/23 cells and the mutant mouse channel that was studied in the knock-in mouse DRG neurons by Leipold *et al.*[7]. Thus, in future studies of $Na_V1.9$ mutations, it will be important to study the effect of the mutation in the genes encoding human channels in the DRG neuron background. Such studies do not lend themselves to high-throughput assays, which could be useful in, for example, the development of drugs that target the channel. The differentiation of sensory neurons from human induced pluripotent stem cells[57,58] or directly from human fibroblasts[59,60] represents a promising approach for more representative high-throughput assays, especially if this approach is coupled with the successful implementation of genome editing, using technologies such as CRISPR (clustered regularly interspaced short palindromic repeats)[61].

As is the case for other Na_V channels, it is currently difficult to assess the effects of most of the intronic mutations in *SCN11A*, because there is a lack of functional assays that are able to determine the effect of single-nucleotide substitutions, insertions or deletions in the large introns of the Na_V-encoding genes. It is possible that gain-of-function mutations that increase the persistent $Na_V1.9$ current can lead to axonal loss, as is commonly observed in painful small-fibre neuropathy. This suggestion is based, at least in part, on the persistent influx of Na^+, which can reverse the local membrane gradient of Na^+ and Ca^{2+}, leading to a reversal of sodium–calcium exchanger activity and subsequent Ca^{2+}-influx-induced axonal injury[54,55]; however, this possibility would need to be tested in further studies. Another fundamental question that is common to all associations between peripheral Na_V mutations and late-onset pain disorders concerns the molecular and cellular bases for the time required for the development of symptoms. It is likely that genetic, epigenetic and environmental factors, either individually or combined, may be required to translate alterations in the biophysical properties of channels into cellular deficits and eventual clinical symptoms, which can take decades to develop.

The human studies that have been discussed in this article clearly support further investigation of $Na_V1.9$ in human pain disorders. First, although a *SCN11A* mutation frequency of approximately 4% in painful peripheral neuropathy was detected in the study by Huang *et al.*[6], not all of these mutations were functionally tested, and thus the clinical importance of some of the resulting substitutions is not yet clear. Studies on larger cohorts of patients with pain disorders of idiopathic origin are needed to build a complete picture of $Na_V1.9$-related pain pathology. Second, whereas the data that link gain-of-function *SCN11A* mutations to pain[6,8,9] indicate that $Na_V1.9$ inhibitors might be appropriate for pain treatment, the study describing the gain-of-function *SCN11A* mutation that is linked to an inability to experience pain suggests that it might also be appropriate to investigate channel openers. For this reason, it is important to confirm the findings reported by Leipold *et al.*[7] and to establish a mechanistic explanation for the paradoxical findings of a gain of function at the channel level and a loss of function at the cellular level. Finally, individuals with mutant-$Na_V1.9$-associated familial episodic pain were reported to respond to treatment with NSAIDs. Such a therapeutic response to these anti-inflammatory agents — if confirmed — would be consistent with data from animal studies that support a role for $Na_V1.9$ in inflammatory pain[16,27,28]. By contrast, individuals with mutant-$Na_V1.9$-associated painful peripheral neuropathy typically do not respond to NSAID treatment, suggesting that different molecular signal-transduction cascades, and perhaps different neural circuitry, mediate pain in these individuals. This possibility might be studied in the future through the development and assessment of induced pluripotent stem cell lines from these patients, through post-mortem examination of patient DRG neurons and through functional brain imaging.

On the basis of the biological plausibility of $Na_V1.9$ as an electrogenic threshold channel in DRG neurons and myenteric neurons[17,28,29,33], and the genetic, genomic and functional evidence that has recently been published[6–8], $Na_V1.9$ can now be considered a validated human therapeutic target. The identification and functional characterization of variants of $Na_V1.9$ from patients with rare pain disorders and common painful peripheral neuropathies provide compelling impetus for studies that will hopefully lead to the development of novel therapeutics for pain. The recent development of small-molecule, isoform-specific inhibitors[62] and a blocking antibody[63] that target other sodium channel subtypes provides evidence that similar strategies could be adopted to target $Na_V1.9$. In this respect, the high divergence of the primary sequence of $Na_V1.9$ from that of the other Na_V channels may be advantageous. Moreover, the preferential distribution of $Na_V1.9$ within nociceptor neurons, rather than in the majority of other peripheral and central tissues, suggests that an isoform-specific agent that targets this channel might have a favourable therapeutic profile, with minimal cognitive and/or cardiac adverse side effects. Nonetheless, the demonstration of $Na_V1.9$ currents in vasopressin- and oxytocin-producing magnocellular neurosecretory neurons in the hypothalamus[64] argues for vigilance in evaluating the possible side effects — including hypothalamic effects — of modulators of this channel.

The role of primary afferents in chronic pain has been under intensive investigation for decades (reviewed in REFS 65,66). The application of next-generation sequencing approaches will expedite the discovery of additional mutations in *SCN11A*, as well as in the genes encoding $Na_V1.7$ and $Na_V1.8$ (the two other peripheral Na_V channels), that are associated with human pain disorders. The development of novel small-molecule inhibitors or other therapeutic approaches, including gene therapy and biologics that target these channels, hold promise for more effective pain treatments that have minimal adverse effects. The genetic and genomic data that link *SCN11A* mutations to pain disorders in humans support the prediction that Na_V channels that are preferentially expressed in the periphery have important and non-redundant roles in the electrogenesis of sensory neurons, and show that the dysfunction of these channels can have severe consequences at the organismal level. The newly found associations between *SCN11A* mutations and human pain disorders will undoubtedly reinvigorate research efforts that aim to better understand the basic principles of how $Na_V1.9$ channels gate and regulate sensory neuron electrogenesis.

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1. Catterall, W. A., Goldin, A. L. & Waxman, S. G. International Union of Pharmacology. XLVII. Nomenclature and structure–function relationships of voltage-gated sodium channels. *Pharmacol. Rev.* **57**, 397–409 (2005).
2. Dib-Hajj, S. D., Yang, Y., Black, J. A. & Waxman, S. G. The $Na_v1.7$ sodium channel: from molecule to man. *Nat. Rev. Neurosci.* **14**, 49–62 (2013).
3. Faber, C. G. *et al.* Gain-of-function $Na_v1.8$ mutations in painful neuropathy. *Proc. Natl Acad. Sci. USA* **109**, 19444–19449 (2012).
4. Huang, J. *et al.* Small-fiber neuropathy $Na_v1.8$ mutation shifts activation to hyperpolarized potentials and increases excitability of dorsal root ganglion neurons. *J. Neurosci.* **33**, 14087–14097 (2013).
5. Han, C. *et al.* The G1662S $Na_v1.8$ mutation in small fibre neuropathy: impaired inactivation underlying DRG neuron hyperexcitability. *J. Neurol. Neurosurg. Psychiatry* **85**, 499–505 (2014).
6. Huang, J. *et al.* Gain-of-function mutations in sodium channel $Na_v1.9$ in painful neuropathy. *Brain* **137**, 1627–1642 (2014).
7. Leipold, E. *et al.* A *de novo* gain-of-function mutation in *SCN11A* causes loss of pain perception. *Nat. Genet.* **45**, 1399–1404 (2013).
8. Zhang, X. Y. *et al.* Gain-of-function mutations in *SCN11A* cause familial episodic pain. *Am. J. Hum. Genet.* **93**, 957–966 (2013).
9. Han, C. *et al.* The domain II S4-S5 linker in Nav1.9: a missense mutation enhances activation, impairs fast inactivation, and produces human painful neuropathy. *Neuromolecular Med.* **17**, 158–169 (2015).
10. Dib-Hajj, S. D., Tyrrell, L., Black, J. A. & Waxman, S. G. NaN, a novel voltage-gated Na channel, is expressed preferentially in peripheral sensory neurons and down-regulated after axotomy. *Proc. Natl Acad. Sci. USA* **95**, 8963–8968 (1998).
11. Dib-Hajj, S. D. *et al.* Coding sequence, genomic organization, and conserved chromosomal localization of the mouse gene *Scn11a* encoding the sodium channel NaN. *Genomics* **59**, 309–318 (1999).
12. Dib-Hajj, S., Black, J. A., Cummins, T. R. & Waxman, S. G. NaN/Nav1.9: a sodium channel with unique properties. *Trends Neurosci.* **25**, 253–259 (2002).
13. Rugiero, F. *et al.* Selective expression of a persistent tetrodotoxin-resistant Na^+ current and $Na_v1.9$ subunit in myenteric sensory neurons. *J. Neurosci.* **23**, 2715–2725 (2003).
14. Fang, X. *et al.* The presence and role of the tetrodotoxin-resistant sodium channel $Na_v1.9$ (NaN) in nociceptive primary afferent neurons. *J. Neurosci.* **22**, 7425–7433 (2002).
15. Fang, X. *et al.* Intense isolectin-B4 binding in rat dorsal root ganglion neurons distinguishes C-fiber nociceptors with broad action potentials and high Nav1.9 expression. *J. Neurosci.* **26**, 7281–7292 (2006).
16. Amaya, F. *et al.* The voltage-gated sodium channel $Na_v1.9$ is an effector of peripheral inflammatory pain hypersensitivity. *J. Neurosci.* **26**, 12852–12860 (2006).
17. Herzog, R. I., Cummins, T. R. & Waxman, S. G. Persistent TTX-resistant Na^+ current affects resting potential and response to depolarization in simulated spinal sensory neurons. *J. Neurophysiol.* **86**, 1351–1364 (2001).
18. Braz, J. M., Nassar, M. A., Wood, J. N. & Basbaum, A. I. Parallel "pain" pathways arise from subpopulations of primary afferent nociceptor. *Neuron* **47**, 787–793 (2005).
19. Stucky, C. L. & Lewin, G. R. Isolectin B_4-positive and -negative nociceptors are functionally distinct. *J. Neurosci.* **19**, 6497–6505 (1999).
20. Hockley, J. R. *et al.* Multiple roles for $Na_v1.9$ in the activation of visceral afferents by noxious inflammatory, mechanical, and human disease-derived stimuli. *Pain* **155**, 1962–1975 (2014).
21. Santarelli, V. P., Eastwood, A. L., Dougherty, D. A., Horn, R. & Ahern, C. A. A cation-π interaction discriminates among sodium channels that are either sensitive or resistant to tetrodotoxin block. *J. Biol. Chem.* **282**, 8044–8051 (2007).
22. Cummins, T. R. *et al.* A novel persistent tetrodotoxin-resistant sodium current in SNS-null and wild-type small primary sensory neurons. *J. Neurosci.* **19**, RC43 (1999).
23. Maruyama, H. *et al.* Electrophysiological characterization of the tetrodotoxin-resistant Na^+ channel, $Na_v1.9$, in mouse dorsal root ganglion neurons. *Pflugers Arch.* **449**, 76–87 (2004).
24. Coste, B., Osorio, N., Padilla, F., Crest, M. & Delmas, P. Gating and modulation of presumptive $Na_v1.9$ channels in enteric and spinal sensory neurons. *Mol. Cell Neurosci.* **26**, 123–134 (2004).
25. Scroggs, R. S. The distribution of low-threshold TTX-resistant Na^+ currents in rat trigeminal ganglion cells. *Neuroscience* **222**, 205–214 (2012).
26. Dib-Hajj, S. D. *et al.* Two tetrodotoxin-resistant sodium channels in human dorsal root ganglion neurons. *FEBS Lett.* **462**, 117–120 (1999).
27. Priest, B. T. *et al.* Contribution of the tetrodotoxin-resistant voltage-gated sodium channel $Na_v1.9$ to sensory transmission and nociceptive behavior. *Proc. Natl Acad. Sci. USA* **102**, 9382–9387 (2005).
28. Ostman, J. A., Nassar, M. A., Wood, J. N. & Baker, M. D. GTP up-regulated persistent Na^+ current and enhanced nociceptor excitability require $Na_v1.9$. *J. Physiol.* **586**, 1077–1087 (2007).
29. Baker, M. D., Chandra, S. Y., Ding, Y., Waxman, S. G. & Wood, J. N. GTP-induced tetrodotoxin-resistant Na^+ current regulates excitability in mouse and rat small diameter sensory neurones. *J. Physiol.* **548**, 373–382 (2003).
30. Harty, T. P. *et al.* $Na_v1.7$ mutant A863P in erythromelalgia: effects of altered activation and steady-state inactivation on excitability of nociceptive dorsal root ganglion neurons. *J. Neurosci.* **26**, 12566–12575 (2006).
31. Vasylyev, D. V., Han, C., Zhao, P., Dib-Hajj, S. & Waxman, S. G. Dynamic-clamp analysis of wild-type $hNa_v1.7$ and erythromelalgia mutant channel L858H. *J. Neurophysiol.* **111**, 1429–1443 (2014).
32. Copel, C. *et al.* Activation of neurokinin 3 receptor increases $Na_v1.9$ current in enteric neurons. *J. Physiol.* **587**, 1461–1479 (2009).
33. Osorio, N., Korogod, S. & Delmas, P. Specialized functions of Nav1.5 and Nav1.9 channels in electrogenesis of myenteric neurons in intact mouse ganglia. *J. Neurosci.* **34**, 5233–5244 (2014).
34. Dib-Hajj, S. D., Cummins, T. R., Black, J. A. & Waxman, S. G. Sodium channels in normal and pathological pain. *Annu. Rev. Neurosci.* **33**, 325–347 (2010).
35. Qiu, F., Jiang, Y., Zhang, H., Liu, Y. & Mi, W. Increased expression of tetrodotoxin-resistant sodium channels Nav1.8 and Nav1.9 within dorsal root ganglia in a rat model of bone cancer pain. *Neurosci. Lett.* **512**, 61–66 (2012).
36. Petho, G. & Reeh, P. W. Sensory and signaling mechanisms of bradykinin, eicosanoids, platelet-activating factor, and nitric oxide in peripheral nociceptors. *Physiol. Rev.* **92**, 1699–1775 (2012).
37. Rush, A. M. & Waxman, S. G. PGE_2 increases the tetrodotoxin-resistant $Na_v1.9$ sodium current in mouse DRG neurons via G-proteins. *Brain Res.* **1023**, 264–271 (2004).
38. Binshtok, A. M. *et al.* Nociceptors are interleukin-1β sensors. *J. Neurosci.* **28**, 14062–14073 (2008).
39. Maingret, F. *et al.* Inflammatory mediators increase Nav1.9 current and excitability in nociceptors through a coincident detection mechanism. *J. Gen. Physiol.* **131**, 211–225 (2008).
40. Mogil, J. S. Animal models of pain: progress and challenges. *Nat. Rev. Neurosci.* **10**, 283–294 (2009).
41. Dib-Hajj, S. D. & Waxman, S. G. Translational pain research: lessons from genetics and genomics. *Sci. Transl Med.* **6**, 249sr244 (2014).
42. Black, J. A. & Waxman, S. G. Molecular identities of two tetrodotoxin-resistant sodium channels in corneal axons. *Exp. Eye Res.* **75**, 193–199 (2002).
43. Padilla, F. *et al.* Expression and localization of the Nav1.9 sodium channel in enteric neurons and in trigeminal sensory endings: Implication for intestinal reflex function and orofacial pain. *Mol. Cell Neurosci.* **35**, 138–152 (2007).
44. Belmonte, C. & Gallar, J. Cold thermoreceptors, unexpected players in tear production and ocular dryness sensations. *Invest. Ophthalmol. Vis. Sci.* **52**, 3888–3892 (2011).
45. Rosenthal, P. & Borsook, D. The corneal pain system. Part I: the missing piece of the dry eye puzzle. *Ocul. Surf.* **10**, 2–14 (2012).
46. Dib-Hajj, S. D. *et al.* Transfection of rat or mouse neurons by biolistics or electroporation. *Nat. Protoc.* **4**, 1118–1126 (2009).
47. Waxman, S. G. *et al.* Sodium channel genes in pain-related disorders: phenotype–genotype associations and recommendations for clinical use. *Lancet Neurol.* **13**, 1152–1160 (2014).
48. Faber, C. G. *et al.* Gain of function $Na_v1.7$ mutations in idiopathic small fiber neuropathy. *Ann. Neurol.* **71**, 26–39 (2012).
49. Han, C. *et al.* Functional profiles of *SCN9A* variants in dorsal root ganglion neurons and superior cervical ganglion neurons correlate with autonomic symptoms in small fibre neuropathy. *Brain* **135**, 2613–2628 (2012).
50. Rush, A. M. *et al.* A single sodium channel mutation produces hyper- or hypoexcitability in different types of neurons. *Proc. Natl Acad. Sci. USA* **103**, 8245–8250 (2006).
51. Shields, S. D. *et al.* $Na_v1.8$ expression is not restricted to nociceptors in mouse peripheral nervous system. *Pain* **153**, 2017–2030 (2012).
52. Waxman, S. G., Black, J. A., Kocsis, J. D. & Ritchie, J. M. Low density of sodium channels supports action potential conduction in axons of neonatal rat optic nerve. *Proc. Natl Acad. Sci. USA* **86**, 1406–1410 (1989).
53. Donnelly, D. F. Spontaneous action potential generation due to persistent sodium channel currents in simulated carotid body afferent fibers. *J. Appl. Physiol.* **104**, 1394–1401 (2008).
54. Stys, P. K., Waxman, S. G. & Ransom, B. R. Ionic mechanisms of anoxic injury in mammalian CNS white matter: role of Na^+ channels and Na^+–Ca^{2+} exchanger. *J. Neurosci.* **12**, 430–439 (1992).
55. Persson, A. K. *et al.* Neuropathy-associated $Na_v1.7$ variant I228M impairs integrity of dorsal root ganglion neuron axons. *Ann. Neurol.* **73**, 140–145 (2012).
56. Swadlow, H. A. & Waxman, S. G. Observations on impulse conduction along central axons. *Proc. Natl Acad. Sci. USA* **72**, 5156–5159 (1975).
57. Chambers, S. M. *et al.* Combined small-molecule inhibition accelerates developmental timing and converts human pluripotent stem cells into nociceptors. *Nat. Biotechnol.* **30**, 715–720 (2012).
58. Young, G. T. *et al.* Characterizing human stem cell-derived sensory neurons at the single-cell level reveals their ion channel expression and utility in pain research. *Mol. Ther.* **22**, 1530–1543 (2014).
59. Blanchard, J. W. *et al.* Selective conversion of fibroblasts into peripheral sensory neurons. *Nat. Neurosci.* **18**, 25–35 (2015).
60. Wainger, B. J. *et al.* Modeling pain *in vitro* using nociceptor neurons reprogrammed from fibroblasts. *Nat. Neurosci.* **18**, 17–24 (2015).
61. Baker, M. Gene editing at CRISPR speed. *Nat. Biotechnol.* **32**, 309–312 (2014).
62. McCormack, K. *et al.* Voltage sensor interaction site for selective small molecule inhibitors of voltage-gated sodium channels. *Proc. Natl Acad. Sci. USA* **110**, E2724–E2732 (2013).
63. Lee, J. H. *et al.* A monoclonal antibody that targets a $Na_v1.7$ channel voltage sensor for pain and itch relief. *Cell* **157**, 1393–1404 (2014).
64. Black, J. A., Vasylyev, D., Dib-Hajj, S. D. & Waxman, S. G. Nav1.9 expression inmagnocellular neurosecretory cells of supraoptic nucleus. *Exp. Neurol.* **253**, 174–179 (2014).
65. Basbaum, A. I., Bautista, D. M., Scherrer, G. & Julius, D. Cellular and molecular mechanisms of pain. *Cell* **139**, 267–284 (2009).
66. Woolf, C. J. & Ma, Q. Nociceptors — noxious stimulus detectors. *Neuron* **55**, 353–364 (2007).

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Competing interests statement

The authors declare no competing interests.