The $Na_V1.7$ sodium channel: from molecule to man

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Abstract | The voltage-gated sodium channel $Na_V1.7$ is preferentially expressed in peripheral somatic and visceral sensory neurons, olfactory sensory neurons and sympathetic ganglion neurons. $Na_V1.7$ accumulates at nerve fibre endings and amplifies small subthreshold depolarizations, poising it to act as a threshold channel that regulates excitability. Genetic and functional studies have added to the evidence that $Na_V1.7$ is a major contributor to pain signalling in humans, and homology modelling based on crystal structures of ion channels suggests an atomic-level structural basis for the altered gating of mutant $Na_V1.7$ that causes pain.

Neuropathic pain
Pain resulting from lesions or diseases of the somatosensory system.

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Voltage-gated sodium channels are essential for electrogenesis in excitable cells. Nine pore-forming α-subunits of such channels (referred to as channels hereinafter), $Na_V1.1$–$Na_V1.9$, have been identified in mammals[1]. These isoforms share a common overall structural motif (FIG. 1). They are each composed of a long polypeptide (1,700–2,000 amino acids) that folds into four homologous domains (DI–DIV) that are linked by three loops (L1–L3), with each domain having six transmembrane segments (S1–S6)[2]. The recent determination of the crystal structure of the homotetrameric bacterial sodium channel[3] has provided insights into the atomic structure of mammalian sodium channels and the interactions between the voltage-sensing domain (VSD; encompassing S1–S4) and the pore module (PM; S5–S6) within each of the four homologous domains. Genetic, structural and functional studies have shown that $Na_V1.7$ regulates sensory neuron excitability and is a major contributor to several sensory modalities, and have established the contribution of this sodium channel isoform to human pain disorders (FIG. 1).

The nine sodium channel isoforms display different kinetics and voltage-dependent properties[1]. Their differential deployment in different types of neurons endows these cells with distinct firing properties. Sodium channels associate with multiple protein partners that regulate channel trafficking and gating[4–7], allowing sodium channel properties to be modulated in a cell-type-specific manner (for examples, see REFS 8–10), highlighting the need to study these channels within their native neuronal background whenever practicable. For example, the pathogenic G616R variant of $Na_V1.7$ displays gating abnormalities within dorsal root ganglion (DRG) neurons that are not seen when these channels are expressed in HEK 293 cells[11]. Methods are now available that allow the expression and functional profiling of sodium channels in peripheral neurons, which more closely mimic the *in vivo* environment of such channels[12].

In humans, gain-of-function mutations in *SCN9A*, which encodes $Na_V1.7$, lead to severe neuropathic pain, whereas loss-of-function mutations in this gene lead to an indifference to pain[13]. Studies involving animal injury models and functional studies of neuronal excitability following expression of human mutant $Na_V1.7$ have provided mechanistic insights into the role of this channel in the pathophysiology of pain. Additional studies have linked $Na_V1.7$ to other sensory modalities, including olfaction[14,15], the afferent limb of the cough reflex[16] and acid sensing[17].

In this Review, we discuss functional and modelling studies of $Na_V1.7$ that have yielded new insights into the structure–function relationship of gating mechanisms in this channel and its contribution to neuronal responses under normal and pathological conditions. We also explore strategies for targeting $Na_V1.7$ in the treatment of pain and, finally, identify unanswered questions regarding the role of $Na_V1.7$ in pain signalling.

Cellular and subcellular distribution

Three sodium channels — $Na_V1.7$, $Na_V1.8$ and $Na_V1.9$ — are preferentially expressed in peripheral neurons. $Na_V1.7$ expression was first detected in somatosensory and sympathetic ganglion neurons[18], and has since been reported in myenteric neurons[19], olfactory sensory neurons (OSNs)[14,15], visceral sensory neurons[16,20] and smooth myocytes[21–23]. $Na_V1.7$ is expressed in both large and small diameter DRG neurons (FIG. 2), including

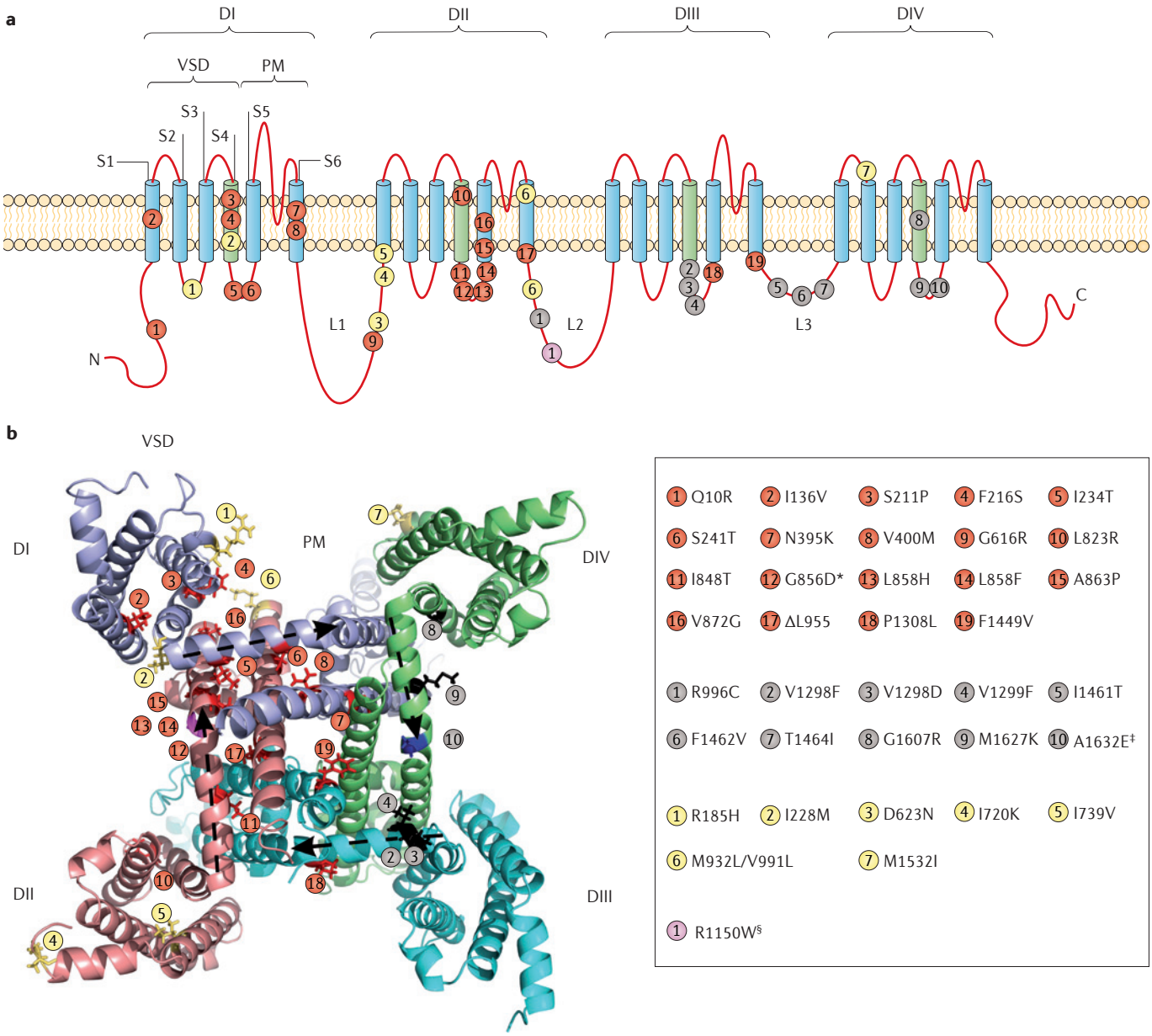

Figure 1 | **Domain structure of $Na_v1.7$ and locations of characterized mutations in $Na_v1.7$-related pain disorders. a** | The sodium channel α-subunit is a long polypeptide that folds into four homologous domains (DI–DIV), each of which consists of six transmembrane segments (S1–S6). The four domains are joined by three loops (L1-L3). Within each domain, S1–S4 comprise the voltage-sensing domain (VSD; S4, depicted in green, characteristically contains positively charged arginine and lysine residues), and S5–S6 and their extracellular linker comprise the pore module (PM). The linear schematic of the full-length channel shows the locations of amino acids affected by the gain-of-function *SCN9A* mutations that are linked to inherited erythromelalgia (IEM; red symbols), paroxysmal extreme pain disorder (PEPD; grey symbols), and small-fibre neuropathy (SFN; yellow symbols). **b** | View of the folded $Na_v1.7$ from the intracellular side of the membrane based on the recently determined crystal structure of a bacterial sodium channel[3]. The structure shows the central ion-conducting PM and four peripheral VSDs. Conformational changes in the VSDs in response to membrane depolarization are transmitted to the PMs through the S4–S5 linkers (identified by arrows through the helices). Mutations that seem distant from each other on the linear model can in fact be in close proximity to each other in the more biologically relevant folded structure. *The patient with this mutation showed symptoms common to IEM and SFN. ‡The patient carrying this mutation showed symptoms and channel properties common to both IEM and PEPD. §This substitution is encoded by a polymorphism that was present in approximately 30% of ethnically matched Caucasian individuals of European descent in a control population[81]. Part **a** is modified, with permission, from REF. 37 © (2007) Elsevier Science.

Box 1 | **$Na_V1.7$ contributes most of the sodium current in OSNs**

$Na_V1.7$ is the predominant sodium channel in olfactory sensory neurons (OSNs)[14,15]. Although an elaborate Ca^{2+}-based and Cl^--based signalling amplification system in the OSN cilia can boost odorant receptor potential[133,134], the abundant expression of $Na_V1.7$ in these cells[14,15] and the ability of $Na_V1.7$ to boost weak depolarizations, support a role for this sodium channel in the initiation of action potential firing along the peripheral olfactory neuraxis. Mouse and rat OSNs produce a tetrodotoxin (TTX)-sensitive current[14,15,135] that is consistent with the predominant expression of $Na_V1.7$ in these cells. Interestingly, the hyperpolarized activation and inactivation properties of this TTX-sensitive current are different from those recorded from $Na_V1.7$ expressed in HEK 293 cells[33,136] or dorsal root ganglion (DRG) neurons[11,35], and those in native rat DRG neurons[137–139]. Ahn *et al.*[14] reported identical sequences of the $Na_V1.7$ cDNA in mouse OSN and DRG neuron samples. Together, these data suggest that post-translational modulation of $Na_V1.7$ or interaction with OSN-specific channel partners may lead to altered gating properties of $Na_V1.7$ in OSNs compared with DRG neurons.

functionally identified Aβ-fibres and C-fibres[24]. $Na_V1.7$ is also the predominant sodium channel isoform present in OSNs[14,15] (BOX 1) and in nodose ganglion neurons[16]. Measurable $Na_V1.7$ levels have not been detected in the CNS[18,25] (but see the discussion below on the purported role of $Na_V1.7$ in epilepsy). $Na_V1.7$ expression has also been detected within some non-excitable cells, including prostate and breast tumour cells[26,27], human erythroid progenitor cells[28] and immune cells[29]. $Na_V1.7$ and $Na_V1.8$ are both expressed at relatively high levels within functionally identified nociceptive neurons (nociceptors)[24,30], in which their co-expression has important functional implications[10]. Last, $Na_V1.7$ is present peripherally within free nerve endings in the epidermis[31] and centrally within superficial lamina of the dorsal horn in the spinal cord[32]. The presence of $Na_V1.7$ at nerve endings (FIG. 2) is consistent with its proposed role in amplifying weak stimuli[33].

Biophysical properties

$Na_V1.7$ produces a rapidly activating and inactivating, but slowly repriming (slow recovery from inactivation), current that is blocked by nanomolar concentrations of tetrodotoxin (TTX)[34]. The slow repriming nature of $Na_V1.7$ makes it well-suited for low-frequency firing in C-fibres, but less well-suited to neurons that fire at a high frequency[33,35]. Importantly, $Na_V1.7$ is characterized by slow closed-state inactivation, allowing the channel to produce a substantial ramp current in response to small, slow depolarizations[33,35]. The ability of $Na_V1.7$ to boost subthreshold stimuli increases the probability of neurons reaching their threshold for firing action potentials. Thus, $Na_V1.7$ is considered to be a threshold channel[36,37]. $Na_V1.7$ produces resurgent currents in DRG neurons[38,39], which are triggered by repolarization following a strong depolarization. Resurgent currents support burst firing in, for example, cerebellar Purkinje neurons[40,41]. Production of a resurgent current by a given sodium channel isoform crucially depends on cell background; the same sodium channel that produces a robust resurgent current in one neuronal type may not generate such a current in a different neuronal type[40–42]. Thus, it is not surprising that $Na_V1.7$ produces a resurgent current only in a subset of DRG neurons.

Nociceptors
Pain-sensing or damage-sensing neurons.

Repriming
Refolding of a channel after opening and inactivating to restore a closed, but available channel. The channel is refractory to additional stimulations during repriming.

Ramp current
Inward current due to transient channel activation in response to the small, slow depolarization of cell membranes.

Roles in multiple sensory modalities

Pain. As stated above, $Na_V1.7$ is expressed in both large and small diameter DRG neurons[13], including 85% of functionally identified nociceptors[24]. These observations, together with its properties as a threshold channel, suggested that $Na_V1.7$ contributes to pain signalling. The recent discovery of gain-of-function *SCN9A* mutations in human pain disorders solidified the status of $Na_V1.7$ as having a central role in pain signalling[13], and its involvement in pathological pain signalling is discussed below.

Olfaction. The initial discovery that global knockout of *Scn9a* in mice is neonatally lethal, probably because the newborn mice do not feed[43], and the subsequent discovery that humans with homozygous *SCN9A*-null mutations are anosmic[44,45] suggested that $Na_V1.7$ is important in olfaction. Nassar *et al.*[43] noted the absence of milk in the stomach of *Scn9a*$^{-/-}$ pups. As no hand-feeding was attempted to rescue these mice, the most parsimonious explanation for the observed neonatal lethality is anosmia, leading to a loss in the ability to suckle. In agreement with this observation, $Na_V1.7$ is the predominant sodium channel isoform present in presynaptic OSNs in rodents[14,15]. Knockout of *Scn9a* in OSNs in mice blocks odorant-induced synaptic transmission to mitral cells in the olfactory glomeruli and leads to weight loss in these mice[15], providing compelling evidence that $Na_V1.7$ has a central role in the sense of smell.

Cough reflex. Two types of coughs involve the vagus nerve: aspiration-induced cough and irritating, itchy urge-to-cough. Aspiration-induced cough is mediated by the stimulation of touch-sensitive Aδ-fibres and occurs even in unconscious subjects, whereas irritating, itchy urge-to-cough is mediated by C-fibre stimulants, including acidic compounds, and occurs only in conscious animals[46]. Nodose ganglion neurons produce both TTX-sensitive and TTX-resistant currents[47], but action potentials in the vagus nerves of rats or guinea pigs are completely blocked with 1 μM TTX[16,48], suggesting a crucial role for TTX-sensitive channels in the cough reflex. Recent data suggest that $Na_V1.7$ produces almost all of the TTX-sensitive current in the majority of nodose ganglion neurons in guinea pigs, and adeno-associated virus (AAV)-mediated short hairpin RNA (shRNA) knockdown of $Na_V1.7$ expression in these neurons markedly increases the rheobase and attenuates the firing of both Aδ-fibres and C-fibres[16]. In agreement with this finding, selective knockdown of $Na_V1.7$ expression in nodose ganglion neurons suppresses citric acid-induced coughing in guinea pigs, without having any effect on the rate of respiration[16]. Whether knocking down $Na_V1.7$ expression has a similar effect on aspiration-induced cough remains untested.

Acid sensing. Naked mole rats do not develop pain-related behaviours when they are exposed to acid or capsaicin, despite the presence of transient receptor potential vanilloid subfamily member 1 (TRPV1) channels in their nociceptors[49]. This mystery has recently been resolved by the identification of a variant amino

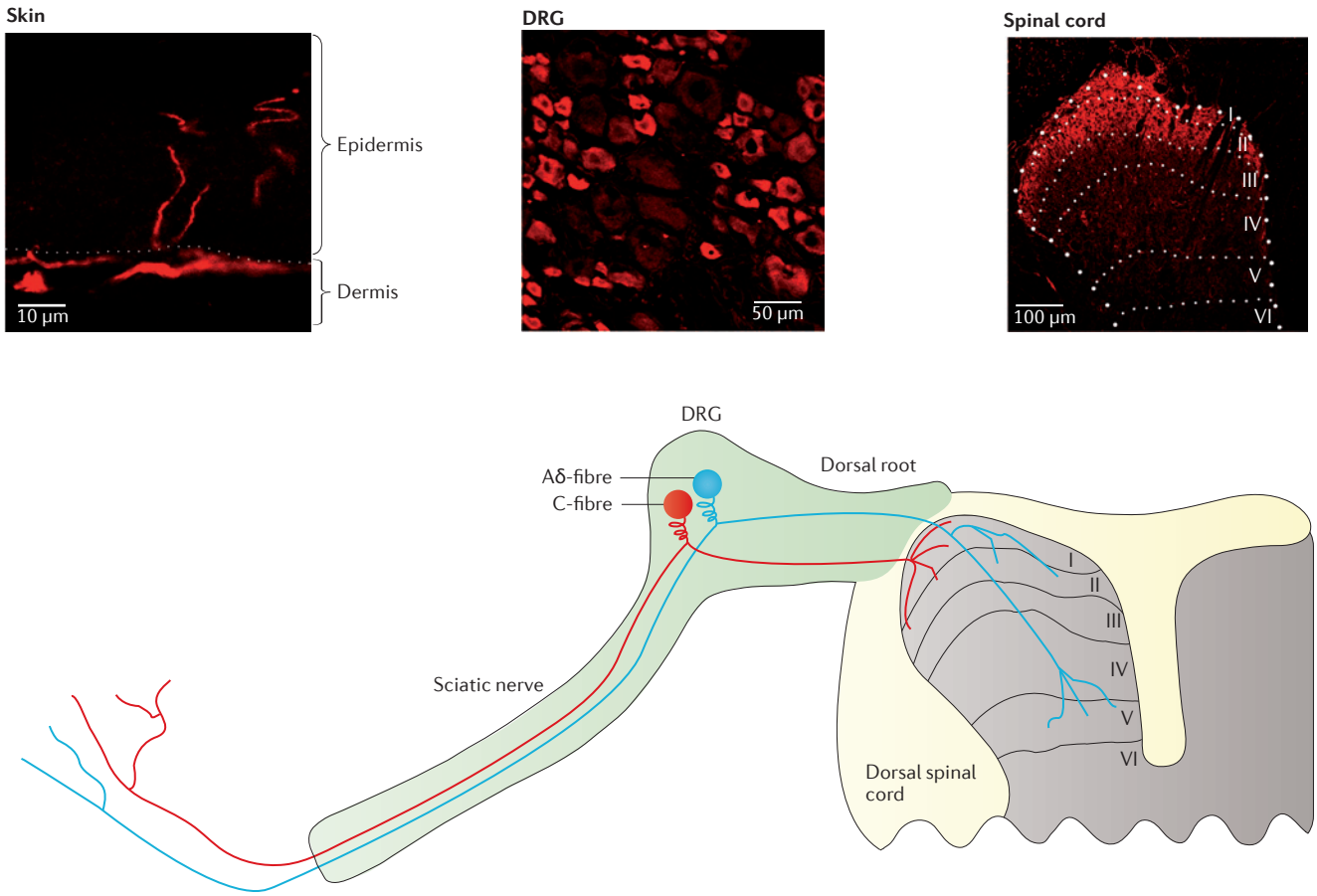

Figure 2 | **Pain signal transmission from peripheral terminals of DRG neurons that form synapses onto second-order neurons within the spinal cord.** Dorsal root ganglion (DRG) neurons can be broadly classified into three types based on their soma size and the state of myelination of their axons: large diameter with heavily myelinated and rapidly conducting axons (Aβ-fibres; not shown here for simplicity); medium diameter with thinly myelinated and intermediate conducting axons (Aδ-fibres; cyan); and small diameter with unmyelinated and slowly conducting axons (C-fibres; red). Five voltage-gated sodium channels are reported to be expressed in DRG neurons[13], with $Na_V1.7$ expressed in the majority of small unmyelinated neurons and in a notable population of medium and large diameter myelinated neurons (see middle panel; $Na_V1.7$ expression is shown in red in this and other panels). Signals originating from the periphery are initiated by external stimuli (for example, thermal, mechanical or chemical stimuli) or injury- and inflammation-induced mediators (for example, cytokines or trophic factors), and are transduced by specific G protein-coupled receptors or acid- and ligand-gated ion channels at peripheral termini. Membrane depolarizations evoked by the graded receptor potential are integrated by voltage-gated sodium channels; when a threshold is reached, an action potential is initiated at these terminals and centrally propagated. $Na_V1.7$ extends to the peripheral ends of these terminals (left panel) where it amplifies small depolarizing inputs. Although $Na_V1.7$ is considered a peripheral sodium channel because it is expressed in peripheral neurons, it is present in central axonal projections of DRG neurons and their presynaptic terminals within the dorsal horn (right panel) where it may facilitate impulse invasion or evoked release of neurotransmitters that may include substance P, calcitonin-gene related peptide and glutamate.

acid sequence in the outer vestibule of their $Na_V1.7$ channels[17]. In almost all mammalian orthologues of $Na_V1.7$, the extracellular linker between S5 and S6 in DIV includes a KKV tripeptide sequence. This tripeptide sequence is replaced by EKE in the naked mole rat and by EKD in the microbat, which also lacks acid-induced pain-related behaviours. Interestingly, these two species live in large colonies in which high concentrations of CO_2 can be generated. Such high levels of CO_2 can cause tissue acidification and acid-induced pain in other animals. The EKE substitution in human $Na_V1.7$ enhances acid-induced blockade of this channel, consistent with a failure to induce firing of action potentials in naked mole rat nociceptors[17]. The corresponding tripeptide sequence in human $Na_V1.6$, the other TTX-sensitive channel in adult nociceptors, is DKE, suggesting that it might be more sensitive to acidic conditions than $Na_V1.7$.

Putative role in epilepsy

One study reported the presence of *SCN9A* variants in patients with seizures, including those with Dravet syndrome (Online Mendelian Inheritance in Man (OMIM)

Neuroma
A collection of demyelinated and dysmyelinated axon sprouts and connective tissue that result from abortive regeneration of transected axons.

Inherited sodium channelopathies
Pathologies linked to mutations or functional variants in sodium channels that can be transmitted to progeny.

database #607208); these variants were present in the control population used in the study at >1% allele frequency[50]. However, the function of $Na_V1.7$ in CNS neurons and its role, if any, in the pathophysiology of seizures has not been established, although a knock-in mouse expressing one of these variants was reported to exhibit seizures. Importantly, neither patients with small-fibre neuropathy (SFN)[39] carrying the same $Na_V1.7$ variants reported by Singh *et al.*[50], nor patients with other gain-of-function *SCN9A* mutations associated with inherited erythromelalgia (IEM; also known as familial erythromelalgia and primary erythermalgia; OMIM #133020)[13,51] have reported seizures. Thus, the contribution of *SCN9A* mutations, if any, to epilepsy remains incompletely understood.

Roles in pain states

$Na_V1.7$ in acquired pain conditions. $Na_V1.7$ has an important role in pain signalling[13,51]. Axotomy of peripheral axons can produce a neuroma in which ectopic impulses arise, causing spontaneous pain[52]. Application of TTX at concentrations that block only TTX-sensitive channels ameliorates neuropathic pain behaviour in a rat axotomy model[53], suggesting that these channels contribute to spontaneous pain. Although the TTX-sensitive sodium channel $Na_V1.3$ has been implicated in ectopic firing and spontaneous pain[13], $Na_V1.7$ accumulates at nerve endings within neuromas together with activated mitogen-activated protein kinase 1 (MAPK1; also known as ERK2) and MAPK3 (also known as ERK1) in humans[54] and in rats[31]. MAPK1 and MAPK3 phosphorylate $Na_V1.7$ at four sites within L1, producing a graded hyperpolarizing shift of channel activation. The extent of graded hyperpolaroizing shift in $Na_V1.7$ activation depends on the number of phosphorylated residues[55]. Together with the finding that MAPK1 and MAPK3 exert a pro-excitatory effect on DRG neurons[55], these data suggest that $Na_V1.7$ can contribute to injury-mediated DRG neuron excitability.

$Na_V1.7$ expression levels and TTX-sensitive current density are increased in DRG neurons in response to inflammation[56]. The increase in $Na_V1.7$ expression levels is more robust than that of $Na_V1.3$ — the other TTX-sensitive channel that is upregulated under these conditions[56,57]. $Na_V1.7$ levels in DRG neurons are also increased in diabetic rats[58,59], a change that is predicted to contribute to hyperexcitability associated with pain. A direct contribution of $Na_V1.7$ to pathological DRG neuronal hyperexcitability is further supported by knockdown and knockout studies in rodents. Knockdown of $Na_V1.7$ attenuates complete Freund's adjuvant-induced thermal hyperalgesia[60] and diabetic pain[61]. Conditional knockout of $Na_V1.7$ expression in mouse DRG neurons, where $Na_V1.8$ is expressed, abrogates inflammation-induced and burn injury-induced thermal hyperalgesia, but does not impair mechanical allodynia or hyperalgesia (neuropathic pain)[43,62,63]. However, a recent report[62] provided evidence suggesting that knocking out $Na_V1.7$ expression in both DRG and sympathetic neurons abrogated neuropathic pain (BOX 2).

$Na_V1.7$ in inherited pain disorders. The co-segregation of a familial mutation and disease symptoms in more than one generation provides a compelling case for a direct link between a target gene and a disease. Recently, mutations in *SCN9A* that alter the functional properties of $Na_V1.7$ in a pro-excitatory manner have been shown to produce familial pain disorders that follow a Mendelian inheritance pattern (inherited sodium channelopathies). These findings provided a causative link in these pain disorders and confirmed that $Na_V1.7$ has a central role in pain signalling in humans. Dominantly inherited gain-of-function missense mutations in *SCN9A* are found in individuals with IEM[64] and paroxysmal extreme pain disorder (PEPD; previously known as familial rectal pain; OMIM #167400)[65]. By contrast, recessively inherited loss-of-function mutations in *SCN9A* are linked to complete insensitivity (indifference) to pain (CIP; OMIM #243000)[66]. Functional characterization of these gain-of-function mutations has elucidated the pathophysiological basis for DRG neuron excitability in these disorders, establishing a mechanistic link to pain.

Pain in IEM is localized to the feet and hands, and symptoms of this condition usually appear in early childhood[13,51]. Multiple families with IEM carry mutations in *SCN9A* that segregate with disease in affected individuals, providing strong genetic evidence for the pathogenicity of these mutations (FIG. 1). The familial IEM mutations in *SCN9A* that have been characterized to date all shift the voltage-dependence of $Na_V1.7$ activation in a hyperpolarized direction (FIG. 3a), increase ramp current (FIG. 3b) and slow deactivation. IEM-linked *SCN9A* mutations can impair slow inactivation (FIG. 3c), thus enhancing DRG neuron hyperexcitability[67], whereas other IEM mutations enhance slow inactivation and therefore attenuate DRG neuron excitability[68].

Another distinct set of mutations in *SCN9A* underlies PEPD, in which severe perirectal pain typically starts in infancy[65]. The rectal pain is accompanied with skin flushing of the lower or upper body or face and can present in a harlequin pattern[69], which can alternate between the left and right sides of the body during different pain episodes[70]. PEPD-linked *SCN9A* mutations produce different effects on $Na_V1.7$ gating compared with IEM-associated mutations[13,65]. PEPD-linked *SCN9A*

Box 2 | $Na_V1.7$ in sympathetic neurons and pain signalling

The contribution of $Na_V1.7$ to electrogenesis in sympathetic neurons and the contribution of these neurons to pain are not well understood. Although $Na_V1.7$ is normally expressed in sympathetic neurons[18], individuals with $Na_V1.7$-related complete insensitivity to pain (CIP) do not report sympathetic deficits[66], suggesting that the role of $Na_V1.7$ in these neurons might be redundant. Gain-of-function mutant $Na_V1.7$ in patients with severe pain can depolarize the resting membrane potential of dorsal root ganglion (DRG) neurons and sympathetic neurons. The resulting effect renders DRG neurons hyperexcitable and sympathetic neurons hypoexcitable[10], suggesting that severe pain may still occur even when sympathetic neuron excitability is reduced. However, studies in mice suggest that functional features of both sensory and sympathetic neurons, which are dependent on $Na_V1.7$, contribute to the manifestation of pain symptoms[43,62]. Minett *et al.*[62] reported that knocking out *Scn9a* (the gene encoding $Na_V1.7$) in DRG neurons alone does not cause a total loss of pain, whereas knocking out the expression of this channel in both sensory and sympathetic neurons recapitulates features of human CIP. Future studies are needed to investigate the role of $Na_V1.7$ in sympathetic neurons and pain signalling.

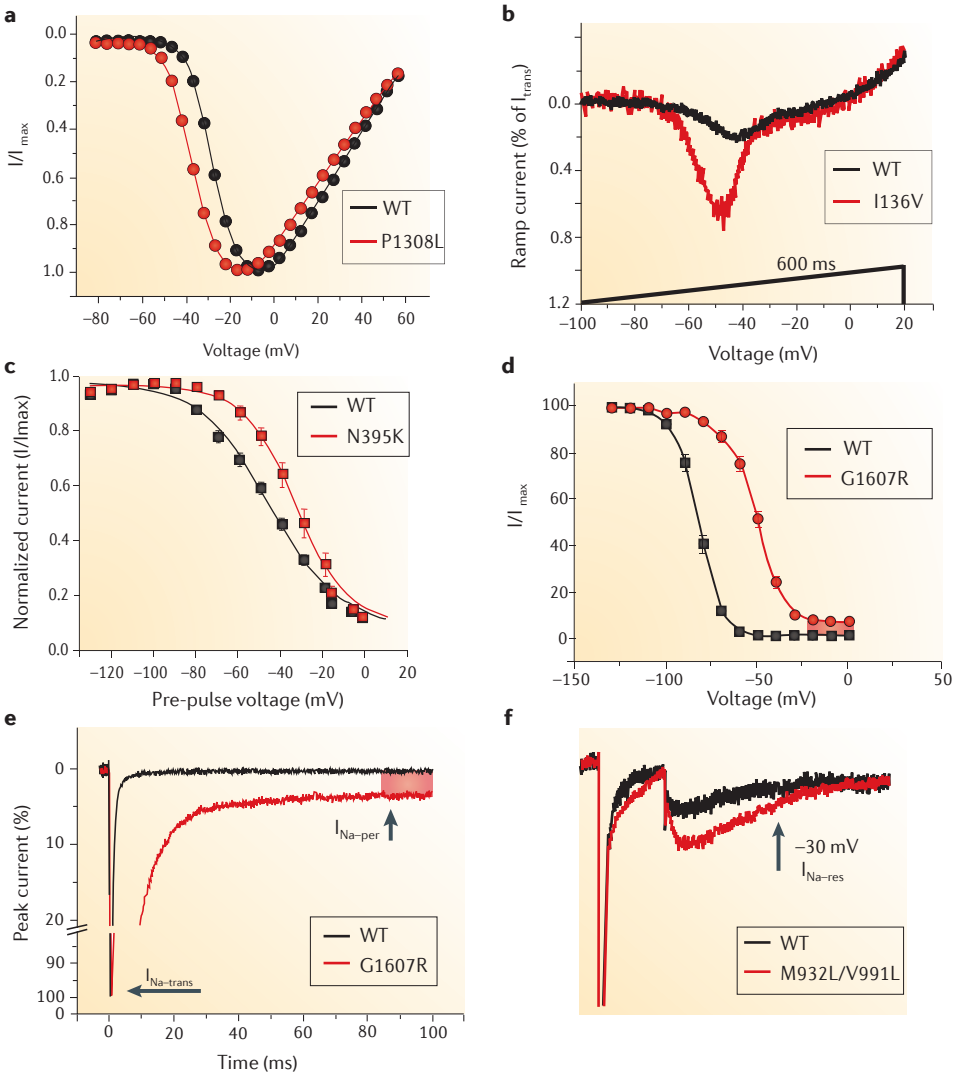

Figure 3 | **Biophysical properties of wild-type and mutant $Na_V1.7$ channels. a** | Inherited erythromelalgia (IEM)-related *SCN9A* mutations shift the activation of $Na_V1.7$ in a hyperpolarized direction, allowing the mutant channels to open in response to a weaker depolarization than open wild-type (WT) channels. A comparison of the activation of WT and P1308L mutant $Na_V1.7$ channels[73] shows that the latter exhibits a hyperpolarizing shift (–9.6 mV) in activation. **b** | Activation of $Na_V1.7$ boosts small, slow depolarizations, producing ramp currents. The ramp currents produced by the IEM I136V mutant $Na_V1.7$ channel[140], normalized to maximal peak currents elicited by step depolarizations, are markedly increased compared with the ramp currents for WT $Na_V1.7$ channels. **c** | The slow-inactivated state of $Na_V1.7$ makes these channels unavailable for further opening after they have been activated by sustained (>10 s) membrane depolarization. Mutations in *SCN9A* that impair slow inactivation (such as N395K and I739V) increase the firing rate of dorsal root ganglion (DRG) neurons[67,141]. Error bars represent standard error of the mean. **d** | Fast inactivation is a process that transiently makes $Na_V1.7$ unavailable for further opening after it has been activated by relatively short (100–500 ms) depolarizations. A hallmark of paroxysmal extreme pain disorder (PEPD)-related *SCN9A* mutations is that they cause a depolarizing shift in fast inactivation that results in fewer inactivated channels at any given potential, and resistance of a subpopulation of channels to inactivation. The PEPD G1607R mutant $Na_V1.7$ channel[70] shows a –30 mV depolarizing shift in fast inactivation, and the presence of a subpopulation of channels that resist inactivation (represented by orange shading in the graph). Error bars represent standard error of the mean. **e** | Normalized current traces for WT and G1607R $Na_V1.7$ evoked by a depolarizing pulse to 0 mV show the transient current ($I_{Na\text{-}trans}$) and that the mutant channels retain a persistent current ($I_{Na\text{-}per}$) at the end of a 100 ms depolarizing pulse (represented by orange shading in the graph). **f** | Resurgent currents ($I_{Na\text{-}res}$) are triggered by repolarization following a strong depolarization, and support burst firing. Note the increase in resurgent current recorded from DRG neurons expressing the M932L/V991L $Na_V1.7$ variant from a patient with small-fibre neuropathy[39]. Impaired fast- and slow-inactivation and resurgent currents are manifested by PEPD and SFN channel variants, as indicated in the main text, and the panels in this figure should be regarded as examples of these changes. Part **a** is modified from REF. 73. Part **b** is modified from REF. 140. Part **c** is modified, with permission, from REF. 67 © (2007) The Physiological Society. Parts **d** and **e** are modified, with permission, from REF. 70 © (2011) Macmillan Publishers Limited. All rights reserved. Part **f** is modified, with permission, from REF. 39 © (2012) American Neurological Association.

Fast inactivation
Inactivation (within milliseconds) of sodium channels occurs by blocking the cytoplasmic vestibule of the channel by a tetrapeptide (inactivation gate) within the linker joining domains III and IV, and terminating the inflow of sodium ions.

Haploinsufficiency
When one functional copy of a gene is not enough to prevent a deficit.

mutations shift the voltage-dependence of steady-state fast inactivation towards a depolarizing direction (FIG. 3d) and, depending upon the specific mutation, make channel inactivation incomplete, which results in a persistent current (FIG. 3d,e). PEPD, but not IEM, mutant $Na_V1.7$ manifests increased resurgent currents[38] (FIG. 3f).

The IEM-linked *SCN9A* mutations studied to date[13] lower the threshold for single action potentials (FIG. 4a–c) and increase the frequency of firing in DRG neurons (FIG. 4d–f), with many IEM-linked *SCN9A* mutations causing a depolarizing shift in resting potential[13]. At the cellular level, PEPD mutant $Na_V1.7$ lowers the threshold for single action potentials and increases the frequency of firing in DRG neurons, without altering the resting potential[71–73]. Importantly, these functional profiles have been obtained by recordings from the somas of DRG neurons. It will be important, in the future, to assess the properties of these mutant channels and their effect on excitability near nerve terminals where $Na_V1.7$ is thought to exert its influence as a threshold channel. A recent study from our group[74] has begun to address this point, demonstrating a resting potential for DRG neurites close to −60 mV, a potential at which $Na_V1.7$ channels are not strongly inactivated and are available for activation in fine diameter axons of DRG neurons. This study also demonstrated that action potential electrogenesis in DRG neurites in culture is driven by the sequential activation of TTX-sensitive and then TTX-resistant sodium currents.

De novo mutations in *SCN9A* in individuals with IEM, but without a family history of this disorder, produce similar functional changes in mutant $Na_V1.7$ to those produced by familial mutations and render DRG neurons hyperexcitable, which is consistent with the pathogenicity of these mutant variants[75,76]. However, the molecular genetic basis of delayed onset of pain in adult-onset IEM is not yet understood. As in IEM, *de novo* mutations in *SCN9A* in individuals with PEPD and no family history of this disorder have been identified; the effects of these *de novo* mutations on $Na_V1.7$ gating is similar to those in familial PEPD, which is consistent with the pathogenicity of these mutations[70].

The distinct and focal patterns of pain in IEM and PEPD are remarkable, considering that $Na_V1.7$ is expressed in most DRG neurons (FIG. 2) and trigeminal neurons. An individual with a mixed phenotype that included symptoms of IEM and PEPD symptoms was found to carry the *SCN9A* mutation A1632E, which hyperpolarizes activation and depolarizes steady-state fast inactivation[71]. Thus, $Na_V1.7$-associated IEM and PEPD might be considered to be part of a clinical and physiological continuum that can produce IEM, PEPD and disorders that have characteristics of both of these conditions.

Recessively inherited *SCN9A* nonsense or splicing-defective mutations have been linked to $Na_V1.7$-related CIP[66]. Heterozygous parent carriers of these mutations are asymptomatic, indicating that the loss of one *SCN9A* allele does not lead to clinically manifested haploinsufficiency. Truncated $Na_V1.7$ CIP fragments do not assemble into functional channels[66,77] and do not act as dominant negative proteins[77], which reflects the normal pain experienced in the heterozygous carrier parents of patients with CIP. Although the first cases of $Na_V1.7$-related CIP were from consanguineous families[66], later cases were identified in non-consanguineous marriages[44,45], indicating that there is a higher incidence of carriers of non-functional *SCN9A* alleles in the general population than was predicted from the initial reports. However, neither homozygous nonsense mutations nor compound heterozygous null mutations have been reported in healthy individuals. Patients with $Na_V1.7$-related CIP do not experience any form of pain. Notably, they do not display motor, cognitive, sympathetic or gastrointestinal deficits, and have intact sensory modalities[66,77]. An exception to this is that several patients have reported that they have an impaired sense of smell[44,45], although a recent study has described several members of a family with a non-sense *SCN9A* mutation, CIP and normal sense of smell[78].

Although the expression of wild-type $Na_V1.7$ at 50% of the normal protein level (that is, there is one functional allele) is sufficient for a normal pain phenotype (that is, there is no haploinsufficiency), the minimal level of functional $Na_V1.7$ required to maintain the capacity to experience normal pain is not known. Interestingly, an individual with incomplete CIP (the patient retained some pain sensation) was found to carry compound heterozygous mutations in *SCN9A*, including a missense mutation (C1719R) affecting the S5–S6 extracellular linker in DIV, and a one base-pair deletion in the 5′ splice donor site of exon 17 of *SCN9A*[79]. Impaired splice donor sites, like most splice-site mutations, may cause exclusion of exon 17 and therefore lead to non-functional channels, which is consistent with the phenotype of impaired pain sensing. The reporting of some pain experience in this individual suggests that successful but inefficient exon 17 inclusion and production of functional $Na_V1.7$ have occurred, but at levels that do not support full manifestation of pain.

Positive symptoms (pain) or negative symptoms (loss of pain sensing and anosmia) of patients with *SCN9A*-linked conditions can be explained by the effects of *SCN9A* gain-of-function and loss-of-function mutations, respectively, on nociceptors. The lack of an effect of *SCN9A* mutations on other sensory modalities is, however, not well understood. Although $Na_V1.7$ is expressed in more than 50% of Aβ low-threshold mechanoreceptors[24], individuals with CIP have normal nerve conduction, tactile sense and vibration sense[66,77], suggesting that $Na_V1.7$ function is redundant in these neurons. By contrast, normal proprioception in patients with CIP is consistent with the absence of $Na_V1.7$ in muscle afferents[24]. It is not fully understood why *SCN9A* gain-of-function mutations do not cause positive symptoms in carriers; for example, causing them to become 'hyper-smellers'.

Functional variants as risk factors. In agreement with the 'common disease, common variant' hypothesis[80], the R1150W variant of $Na_V1.7$ has been associated with enhanced pain sensation[81,82]. Estacion *et al.*[81] demonstrated that the W1150 minor allele was present in 30% of people in an ethnically matched control population of Caucasian individuals of European descent. The W1150

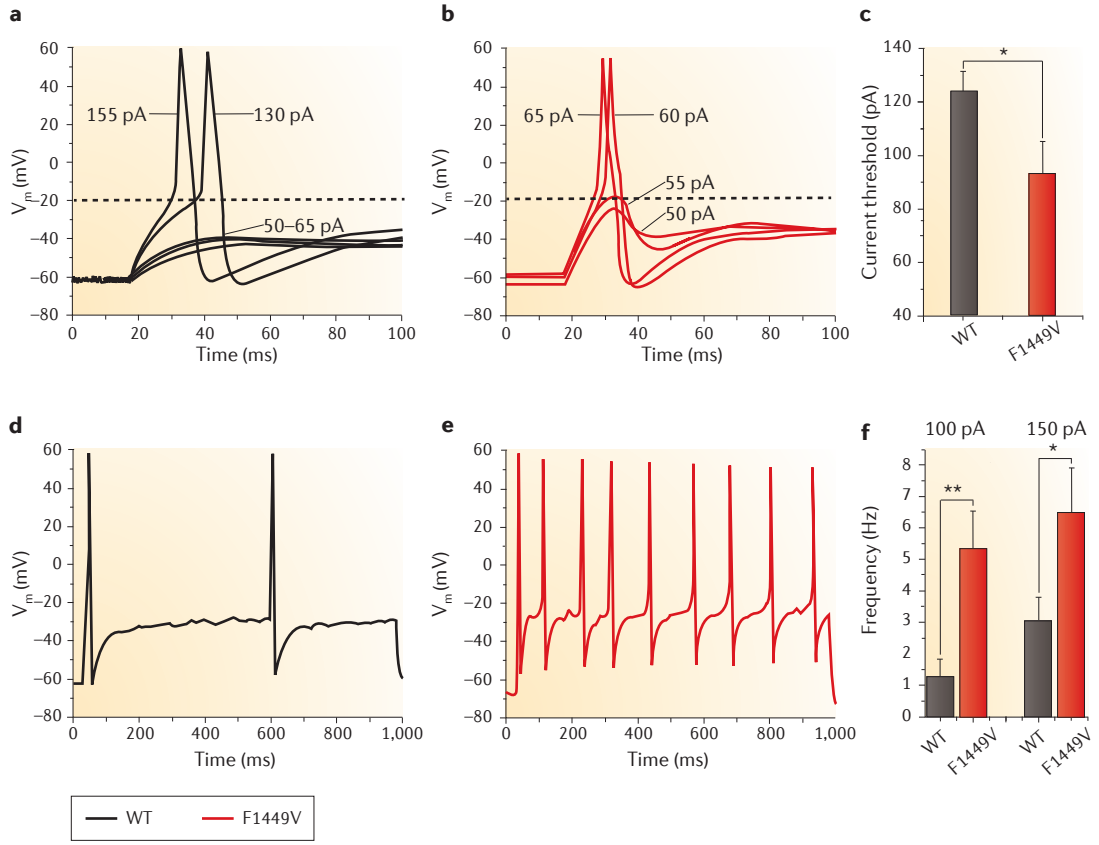

Figure 4 | **The F1449V mutation in $Na_V1.7$ makes DRG neurons hyperexcitable. a,b** | Representative traces from small (<30 µm) dorsal root ganglion (DRG) neurons expressing wild-type (WT) $Na_V1.7$ or $Na_V1.7$ with the F1449V mutation (the variant linked to inherited erythromelalgia). These traces show that neurons expressing the mutant channel have a lower current threshold for action potential generation. **c** | The average current threshold is notably reduced in cells expressing F1449V compared with cells expressing WT channels (**P*<0.05). **d,e** | A neuron expressing WT $Na_V1.7$ responds to a 950 ms stimulation of 150 pA with a lower number of action potentials than does the neuron expressing the F1449V mutant (same cells as in panels **a** and **b**). **f** | There is a sizeable increase in the frequency of firing of action potentials in response to 100 pA and 150 pA stimuli (950 ms) with expression of F1449V versus expression of WT $Na_V1.7$ (**P*<0.05; ***P*<0.01). Figure is reproduced, with permission, from REF. 94 © (2005) Oxford University Press.

variant of $Na_V1.7$ induces hyperexcitability of DRG neurons, suggesting that carriers of this polymorphism might be predisposed to hyperalgesia. Indeed, a genome-wide association study found that the R1150W polymorphism is associated with an increased pain perception in patients with osteoarthritis, phantom limb pain or lumbar root pain, and that the effect is most strongly associated with C-fibre activation[82].

About 30% of individuals with idiopathic SFN express functional mutant $Na_V1.7$ channels arising from gain-of-function *SCN9A* missense variants[39], which may not be fully penetrant when found in families[83]. People carrying these gain-of-function $Na_V1.7$ variants are hypersensitive to pain, which reflects the expression of this channel in DRG neurons. These individuals also manifest profound autonomic dysfunction, which reflects the expression of $Na_V1.7$ in sympathetic neurons[10,18]. Gain-of-function attributes of $Na_V1.7$ variants in SFN include depolarized fast inactivation (FIG. 3d) and/or slow inactivation (FIG. 3c), or an increase the fraction of cells that produce resurgent currents (FIG. 3f). Surprisingly, however, individuals with *SCN9A*-null mutations do not manifest autonomic system deficits[66], suggesting that there is a redundant function for this channel in sympathetic neurons.

Does $Na_V1.7$ play a role in the dorsal horn? Based on studies in HEK 293 cells and DRG neuron somata, and on computer simulations, $Na_V1.7$ is thought to act as a threshold channel that activates at relatively hyperpolarized potentials, thus amplifying small, slow depolarizations at potentials negative to an action potential threshold[36,84]. This role, however, does not explain the total lack of pain sensation in patients with $Na_V1.7$-related CIP even in response to the most intense stimulation, such as dental work or child-bearing labour. One possible theory is that $Na_V1.7$ at central termini of primary afferents (FIG. 2) may play a part in synaptic transmission of pain signals.

Consistent with this hypothesis, Minett *et al.*[62] showed that evoked release of substance P into the spinal cord in response to sciatic nerve stimulation, and synaptic potentiation of wide dynamic range neurons receiving

input from primary afferents are attenuated in mice that had $Na_V1.7$ knocked out in DRG neurons. $Na_V1.7$ may have a role in facilitating the invasion of incoming action potentials from peripheral nociceptors into central preterminal exon branches or into terminals within the spinal cord. Alternatively, $Na_V1.7$ may be involved within the terminals in the process of neurotransmitter release onto second-order dorsal horn neurons. Thus, we speculate that $Na_V1.7$, deployed near presynaptic terminals in the dorsal horn[32], is important for release of neurotransmitters such as substance P. If this speculation is correct, then $Na_V1.7$ inhibitors that act on both peripheral and central compartments might be needed for clinical efficacy.

Structural features of $Na_V1.7$

Our ability to understand the mechanistic bases of pathogenic *SCN9A* mutations and to develop rationally designed small-molecule inhibitors for the treatment of hyperexcitability disorders is limited by the lack of a high-resolution crystal structure of a eukaryotic sodium channel. High-resolution crystal structures of ion channels are necessary for a comprehensive understanding of the links between voltage-sensing and channel activation and inactivation, ion selectivity, and drug interactions. Our current understanding of these channel properties was derived from comparative sequence analysis, and from functional assays that measured ion conductance or fluorescence emission of tagged channels[2]. Atomic structural modelling following the determination of high-resolution crystal structures of potassium channels[85–87] and, more recently, a bacterial sodium channel[3] has advanced our understanding of the structure–function relationship of human *SCN9A* mutations, which is discussed below.

Lessons learnt from potassium and bacterial sodium channels. Crystallographic studies of potassium channels provided the first direct evidence for the structural basis for ion selectivity, pore gating and coupling of a voltage sensor to the pore components[85–87]. These studies also yielded valuable insights into kinetics and sequence determinants of different gating mechanisms. Identification of the homotetramer bacterial voltage-gated sodium channel[88], with the monomer possessing the six transmembrane segment architecture of the homologous domains in the eukaryotic channels, facilitated the production of sufficient channel protein for crystallization and high-resolution structural studies. Intriguingly, bacterial voltage-gated sodium channels are most similar to DIII of human sodium channels[89]. The first high-resolution crystal structure (resolved at 2.7 Å) of a pre-open conformation of the voltage-gated sodium channel from the bacterium *Arcobacter butzleri* (Na_VAb)[3] suggested that the S4 segments are in the activated position, but that the activation gate at the cytoplasmic end of the pore domain is closed. This study provided structural evidence for several of the gating steps of sodium channels and demonstrated a possible route for access of small hydrophobic pore-blocking molecules.

Because of the nature of eukaryotic sodium channels as four-domain polypeptides, which are linked by cytoplasmic loops with divergent lengths and sequences in the different members of the sodium channel family, there may be subtle yet important structural differences between these channels and the bacterial homotetrameric channels. Thus, caution is warranted in extrapolating from high-resolution crystal structures of a symmetrical homotetrameric bacterial sodium channel to eukaryotic single polypeptide multi-domain sodium channel isoforms. Moreover, individual channel mutations should optimally be assessed in their native isoform. For example, the S241L mutation within the DI S4–S5 linker of $Na_V1.7$ produces a marked hyperpolarizing shift in its activation, steady-state fast and slow inactivation, compared with wild-type channels[90]. By contrast, substitution of the corresponding residue in $Na_V1.4$, S246L, hyperpolarizes steady-state fast and slow inactivation of the channel but, unlike S241L in $Na_V1.7$, S246L had no effect on $Na_V1.4$ activation[91], thus providing an example of an isoform-specific effect of conserved residues.

Atomic structural modelling of the putative activation gate. From a homology model of the $Na_V1.7$ pore components, based on the crystal structure of the *Streptomyces lividans* potassium channel KcsA[85], we were able to identify a putative activation gate[92]. This modelling approach identified an aromatic residue within the cytoplasm-proximal portion of each of the pore-lining S6 helices (DI Y405, DII F960, DIII F1449 and DIV F1752) that were predicted to form a hydrophobic ring at the cytoplasmic end of the pore that stabilizes the channel's pre-open state. These aromatic residues in the four S6 helices form an energetically stable assembly due to extensive van der Waals bonds between their side chains, which is further strengthened by additional edge-face interaction with the adjacent aromatic residues[93]. The hydrophobic ring is predicted to raise the energy barrier for the movement of S6, which is necessary to open the channel's pore, thus stabilizing the closed or pre-open state of the channel. Although the activation gate at the narrow cytoplasmic vestibule of the channel in the Na_VAb crystal structure consists of four methionine 221 residues (one from each monomer)[3], modelling of $Na_V1.7$ based on the Na_VAb crystal structure recapitulates the activation gate that was previously identified based on the KcsA structure (FIG. 5).

Evidence for the formation of this hydrophobic block is provided by functional studies of the F1449V mutation in $Na_V1.7$ that is found in patients with IEM. This mutation lowers the threshold for $Na_V1.7$ activation[94]. The F1449V substitution is predicted to destabilize interactions with the adjacent aromatic residues, thus reducing the energetic barrier for DIII S6 helix movement and facilitating bending motions associated with pore opening. The increased propensity of the DIII S6 helix to move would be expected to hasten channel activation. Support for this model of activation comes from studies of inwardly rectifying potassium (Kir) channels, which form a similar quadruple phenylalanine hydrophobic ring[95]. Substitution of F168 in Kir6.2 (which is analogous to F1449 in $Na_V1.7$) with smaller residues favours the channel's open-state,

Atomic structural modelling
Construction of a model of a folded protein based on the atom coordinates of a related member of the family whose high-resolution crystal structure is determined and additional constraints derived from studies of distant members of the superfamily.

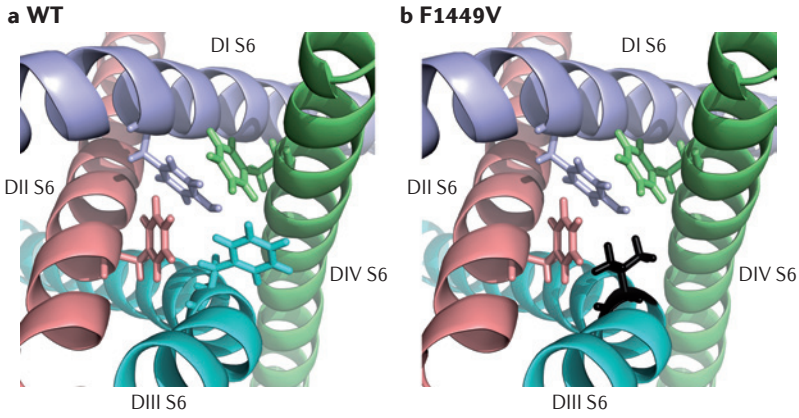

Figure 5 | **A model of the putative activation gate of $Na_V1.7$.** The folded structure of the two S6 transmembrane segments presented here were based on the crystal structure of a bacterial sodium channel[3]. The carboxy-terminal aromatic residue of each S6 is shown in stick representation for wild-type (WT; **a**) $Na_V1.7$ and $Na_V1.7$ with the F1449V mutation (**b**). The assembly of aromatic residues at the cytoplasmic C terminus of each of the S6 segments forms the putative activation gate of $Na_V1.7$. The F1449V mutation in the homologous domain III (DIII) disrupts the hydrophobic ring and destabilizes the pre-open state of the channel.

whereas substitution of F168 with the aromatic amino acid tryptophan retains wild-type-like function[95].

There are limitations on the use of the crystal structure of a homotetrameric voltage-gated ion channels (bacterial sodium channel and various potassium channels) for modelling the multi-domain mammalian $Na_V1.7$, and our functional studies of the effect of F1449V on the activation gate provide an instructive example of such a limitation. Although the model suggested that phenylalanine or tyrosine residues at the carboxyl termini of the S6 segments in $Na_V1.7$ stabilize the channel's closed or pre-open state, functional analysis showed that these residues have different effects. Specifically, DII F960V and DIII F1449V markedly hyperpolarize channel activation, whereas DI Y405V and DIV F1752V do not alter channel activation[92]. This may reflect the functional specialization of the four homologous, yet not identical, domains of eukaryotic sodium channels.

Dependence on neuronal background

Gating of wild-type or mutant sodium channels can be modulated in a cell-type-dependent manner, and this phenomenon can have important clinical implications. For example, resurgent sodium currents can be recorded from only a subset of small diameter DRG neurons transfected with $Na_V1.6$ (REF. 42) or $Na_V1.7$ (REF. 38), and $Na_V1.8$ channels exhibit slow-inactivation properties that are differentially regulated in different subpopulations of (peptidergic and non-peptidergic) small diameter DRG neurons[9]. For example, a single mutation in *SCN9A*, leading to L858H[10] or I739V[96], renders DRG neurons hyperexcitable but superior cervical ganglion (SCG) neurons hypoexcitable. The latter phenomenon is related to the depolarization of the resting potential in both DRG and SCG neurons by the mutant $Na_V1.7$, which leads to resting inactivation of all of the sodium channel isoforms in SCG neurons and hypoexcitability. The presence of $Na_V1.8$, which is relatively resistant to inactivation by depolarization[97–99], in DRG neurons renders these neurons hyperexcitable in response to depolarization[10]. These data demonstrate that sodium channel mutations can have a range of cell-background-dependent effects in different types of neurons.

Targeting $Na_V1.7$ for pain treatment

The clear involvement of $Na_V1.7$ in human pain, and the lack of serious cognitive, cardiac and adverse motor effects with a total loss of $Na_V1.7$, as demonstrated in individuals with CIP, have fuelled intense efforts to develop $Na_V1.7$-specific inhibitors or modulators for the treatment of pain. Despite these intensive efforts, progress has been slow[100]. Nonetheless, the occasional reports of patients with IEM who respond to monotherapy using pan-sodium channel blockers[101,102], and the responsiveness of patients with PEPD to carbamazepine, suggest that small molecules may be developed to either inhibit or modulate $Na_V1.7$ in a manner that can reduce excitability of DRG neurons and provide pain relief. Using the IEM $Na_V1.7$ V400M carbamazepine-responsive mutation[102] as a 'seed' for an atomic-level modelling and thermodynamic analysis, Yang *et al.*[103] were able to predict carbamazepine-responsiveness of a second IEM mutation, $Na_V1.7$ S241T, suggesting that, in the future, pharmacogenomic guided therapy may be possible. Alternative strategies may include the development of isoform-specific blockers or modulators of gating states of sodium channels that are differentially altered under pathological conditions; the development of compounds that weakly cross the blood–brain barrier to minimize CNS-related adverse effects; and gene therapy.

Small-molecule blockers. Several purportedly selective small-molecule inhibitors of $Na_V1.7$ have been described and have shown efficacy in animal models of pain[104–107]. These reports, however, lack documentation for selectivity against human sodium channel isoforms in a native neuronal environment. Reports detailing the efficacies of these compounds in animal models of pain should therefore be interpreted with caution, as these results could be due to inhibition of any of the neuronal sodium channel isoforms. A small-molecule blocker with robust selectivity for human $Na_V1.7$ was recently developed[108]. This orally bioavailable compound bound preferentially to the slow-inactivated state of the channel, and showed notable selectivity for $Na_V1.7$ over other voltage-gated sodium channel isoforms (by 10-fold to 900-fold). The compound also showed 1,000-fold selectivity for $Na_V1.7$ over potassium and calcium channels. These favourable properties suggest that this small-molecule blocker holds promise for future clinical studies.

State-dependent blockers. Local anaesthetics, anticonvulsants and tricyclic compounds block sodium channels, mostly in a use-dependent fashion, and are among the first-line treatments that are currently available for

neuropathic pain[109,110]. However, these agents are not isoform-specific and only provide partial pain relief due in part to their limited therapeutic window that results from CNS-related adverse effects, such as dizziness or sedation[111,112]. Despite these limitations, lidocaine derivatives and carbamazepine are effective in patients carrying certain *SCN9A* mutations that render $Na_V1.7$ pharmacoresponsive[65,101,102], suggesting that personalized, genomically based therapeutics for pain is possible.

Patients with PEPD harbouring *SCN9A* mutations respond favourably to treatment with carbamazepine, which acts to counterbalance impaired fast inactivation of the mutant channel and hence reduces the persistent current caused by these mutations[65]. Although most patients with $Na_V1.7$-linked IEM do not respond to pharmacotherapy, a few have reported control of pain symptoms with lidocaine, mexiletine or carbamazepine. Successful lidocaine or mexiletine monotherapy was reported in a patient carrying the $Na_V1.7$ V872G mutation, possibly resulting from enhanced lidocaine use-dependent block of the mutant channels[101]. Three members of a family with IEM, carrying the mutation $Na_V1.7$ V400M, reported control of their pain symptoms with carbamazepine[102]. Preincubation of V400M channels with clinically relevant concentrations of carbamazepine induced a depolarizing shift in activation, which returned to wild-type voltages[102]. This normalization of activation suggests that carbamazepine acts in an allosteric manner on the mutant $Na_V1.7$ channel and induces a wild-type-like pre-open state.

Computer simulation studies[67] and functional characterization of the $Na_V1.7$ delL955 mutation[68] suggest that enhancing the slow inactivation of $Na_V1.7$ may allow an alternative approach to the treatment of pain. Lacosamide, a functionalized amino acid with sodium channel-blocking activity, showed beneficial effects in animal studies and clinical trials of epilepsy, in animal models of acute, inflammatory and neuropathic pain[113–116], and in initial clinical trials for diabetic neuropathic pain[117,118]. Lacosamide's blocking activity is unusual in that it involves selective enhancement of the slow inactivation of voltage-gated sodium channels, including $Na_V1.3$, $Na_V1.7$ and $Na_V1.8$ (REF. 119). Interestingly, lacosamide induces substantially greater inhibition of $Na_V1.3$, $Na_V1.7$ and $Na_V1.8$ when these channels are in an inactivated state[119]. This feature of lacosamide might mean it would exhibit a better safety profile and greater tolerability than state-independent voltage-gated sodium channel blockers, as it might preferentially target injured depolarized neurons with hyperactive sodium channels[120]. Although lacosamide has not been approved for the treatment of human neuropathic pain[121], targeting of the $Na_V1.7$ slow-inactivated state might provide a viable drug-development option.

Natural toxins. Natural peptide toxins might provide a source of isoform-specific inhibitors of sodium channels, because binding of these toxins to channels is regulated by multiple contact points, and minor sequence changes in the channel could have a profound effect on the affinity of the channel–toxin interaction. Venoms of a variety of snails are reservoirs of peptide toxins, and some of these have demonstrated sodium channel isoform selectivity[122–124]. However, $Na_V1.7$ is among the channels that are only weakly blocked by the conotoxins identified to date[122,123]. By contrast, peptide toxins from tarantulas manifest preferential effect on $Na_V1.7$. For example, ProTx-II is ~50-fold more selective for $Na_V1.7$ than $Na_V1.5$ (REFS125,126). Huwentoxin-I and huwentoxin-IV are potent inhibitors of $Na_V1.7$ and other neuronal TTX-sensitive channels, but are not effective against $Na_V1.4$ (REFS127,128). The exchange of two residues in the DII S3–S4 linker of $Na_V1.7$ and $Na_V1.4$ reverses the affinity of huwentoxins to these channels[128]. Additionally, a charge-conserving substitution in KIIIA, a member of the μ-conotoxin subfamily, enhances the selectivity for $Na_V1.7$ over $Na_V1.2$ and $Na_V1.4$ (REF. 129). It may therefore be possible to engineer peptide toxins with the desirable $Na_V1.7$ isoform specificity.

Peptide toxins, however, have poor oral bioavailability and it is difficult to deliver them to nerve endings, implying that their use as therapeutic agents remains limited. However, modification of conotoxins by cyclization can enhance their stability *in vivo* without compromising their biological activity[130], and it may be possible to develop cyclized $Na_V1.7$-specific peptide toxins when such molecules become available.

Gene therapy. Advances in virus-mediated gene therapy have led to the initiation of Phase I trials for pain involving a herpes simplex virus (HSV) platform to transfer human preproencephalin (*PENK*) to DRG neurons[131]. Local delivery of a gene product within the projection zone of an injured or diseased nerve associated with a focal pain syndrome (as in post-herpetic neuralgia or peripheral nerve injury) could be used to treat pain in a topologically defined manner, reducing systemic adverse effects. Animal studies have provided the proof-of-principle for this approach, showing that anti-$Na_V1.7$ antisense constructs, delivered by a HSV virion, can attenuate pain behaviour in mice following peripheral inflammation[60] and in diabetic rats[61]. We have recently succeeded in targeting another sodium channel, $Na_V1.3$, in DRG neurons using RNA interference molecules (shRNA for gene knockdown) delivered using the non-virulent AAV platform[132], which is less immunogenic than other viral delivery platforms, suggesting that a similar strategy for targeting $Na_V1.7$ using AAV-mediated delivery of shRNA may be successful.

Summary and future directions

$Na_V1.7$ has proven to be a key player at the organismal level in human pain, at the cellular level as a major regulator of neuronal excitability and at the molecular level as a platform for discovering the contribution of specific residues to gating mechanisms. Studies of the rare monogenic disorders IEM, PEPD and CIP definitively show that $Na_V1.7$ is critically important for human pain, and studies on SFN demonstrate a role for this channel in more common pain disorders. In addition to insights into the pathophysiology of pain gleaned

from studying mutant $Na_V1.7$ in its native neuron, modelling of mutant channels, based upon the crystal structures of the bacterial sodium channel and other ion channels, has led to identification of the putative activation gate of $Na_V1.7$, and allows predictions of the dynamic interaction of the voltage-sensor and pore segments within the same domain and between different domains. Assessment of naturally occurring mutations in these studies could be especially informative, as they are already known to have large effects on gating properties of the channel. Finally, the relatively restricted expression pattern of $Na_V1.7$, its central role in pain signalling in humans, and the minimal cognitive, cardiac, motor and sensory deficits in people totally lacking $Na_V1.7$ have shown that this channel is a valid and indeed attractive target for drug development, and support the view that single target engagement for pain treatment might have therapeutic potential.

Nevertheless, despite progress in our understanding of $Na_V1.7$ and its contribution to diverse sensory modalities, crucial questions remain unanswered. For example, why do patients with IEM or PEPD mutations manifest different pain topography despite the ubiquitous expression of $Na_V1.7$ in sensory neurons? Why does skin flushing in some patients with PEPD alternate from side to side of the body? Why does the age-of-onset of IEM symptoms vary from infancy to adulthood? Why is there no evidence for compensatory changes that rescue nociception in CIP? In addition to missense or nonsense substitutions or loss-of-function mutations of splice sites in *SCN9A*, do synonymous or intronic insertions–deletions affect splicing efficiency or RNA stability and cause disease? What is the relative contribution of $Na_V1.7$ to signal integration and transmission at peripheral and central termini of sensory and sympathetic neurons? Finally, why are the gating properties of the TTX-sensitive current in OSNs, which are mostly carried by $Na_V1.7$, markedly different from those in HEK 293 cells and DRG neurons? These questions, and other related questions, will undoubtedly be answered in the near future.

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Competing interests statement
The authors declare competing financial interests. See Web version for details.

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