

# Painful and painless channelopathies

David L H Bennett, C Geoffrey Woods



The discovery of genetic variants that substantially alter an individual's perception of pain has led to a step-change in our understanding of molecular events underlying the detection and transmission of noxious stimuli by the peripheral nervous system. For example, the voltage-gated sodium ion channel Na<sub>v</sub>1.7 is expressed selectively in sensory and autonomic neurons; inactivating mutations in *SCN9A*, which encodes Na<sub>v</sub>1.7, result in congenital insensitivity to pain, whereas gain-of-function mutations in this gene produce distinct pain syndromes such as inherited erythromelalgia, paroxysmal extreme pain disorder, and small-fibre neuropathy. Heterozygous mutations in *TRPA1*, which encodes the transient receptor potential cation channel, can cause familial episodic pain syndromes, and variants of genes coding for the voltage-gated sodium channels Na<sub>v</sub>1.8 (*SCN10A*) and Na<sub>v</sub>1.9 (*SCN11A*) lead to small-fibre neuropathy and congenital insensitivity to pain, respectively. Furthermore, other genetic polymorphisms have been identified that contribute to risk or severity of more complex pain phenotypes. Novel models of sensory disorders are in development—eg, using human sensory neurons differentiated from human induced pluripotent stem cells. Understanding rare heritable pain disorders not only improves diagnosis and treatment of patients but may also reveal new targets for analgesic drug development.

## Introduction

Rapid growth is taking place in the discovery of rare genetic variants associated with mendelian disorders of pain perception. Progress has been made because of enhanced ascertainment of patients with pain disorders, greatly improved phenotyping, and new sequencing and bioinformatics technologies. In some individuals, gene mutations lead to insensitivity to pain, whereas in others, mutations lead to increased pain perception. Many variants reside in genes encoding ion channels, which play an important part in determining the excitability and function of nociceptors. Restricted expression of these molecules in sensory neurons, and their pivotal role in chronic and acute pain states, means that these genetic findings are now being translated into analgesic drug discovery programmes.

Chronic pain represents a substantial health burden, affecting one in five people in Europe; for 40% of these individuals, treatment is inadequate.<sup>1</sup> Common acquired chronic pain states are associated with altered expression and dysfunction of ion channels. Application of modern genomics to persistent pain states is challenging because of the complexity of phenotyping and the large cohort sizes needed; early findings suggest, however, that variants in ion channels modulate the risk, severity, and persistence of pain after injury. In this Review, we discuss recent advances in the understanding of the molecular basis of nociception, describe the clinical features and genetics of mendelian disorders of pain perception, and highlight the role of ion channels in acquired pain syndromes.

## Ion-channel function in nociceptors

The term nociceptor was originally coined to describe sensory neurons that detect high-threshold stimuli causing—or with the potential to cause—tissue injury.<sup>2</sup> Such stimuli include extremes of temperature, mechanical force, or chemicals (eg, acid or prostaglandins).<sup>3,4</sup> The soma of nociceptors resides in

dorsal root or trigeminal ganglia, and the neurons have unmyelinated or small-diameter myelinated axons. Nociceptors have a pseudo-unipolar morphology; a peripheral terminal innervates a target organ, such as skin, and a central terminal provides connectivity to the dorsal horn of the spinal cord (figure 1A–1C). During the past two decades, rapid advances have been made in our understanding of how nociceptors detect signals and transmit them to the CNS, leading to the perception of pain. Such nociceptor input from the periphery is subject to extensive processing and modulation within the CNS, such that perceived pain is dependent on environmental context, emotional factors, attentional mechanisms, and past experience.<sup>9</sup> Here, we will focus on the role of nociceptors, rather than central processing.

Both ligand-gated and voltage-gated ion channels have a pivotal role in the processes of detection and transmission of high-threshold stimuli by nociceptors (figure 1D–1F). An example is the transient receptor potential (TRP) family of ion channels.<sup>10</sup> TRPV1 was the first to be linked to nociception and is a non-selective cation channel activated by noxious heat, capsaicin (the active ingredient in chilli peppers), and low pH.<sup>11,12</sup> Several TRP channels were shown subsequently to be expressed by sensory neurons. Every TRP channel is attuned to detect specific physical and chemical stimuli, ranging from innocuous warming (TRPV3) to high temperatures (TRPV2). TRPA1 is activated by noxious cold and various environmental irritants—eg, mustard, cinnamon, wasabi, and acrolein (the active component of tear gas).<sup>13–16</sup> Although TRPA1 is not the noxious mechanotransducer (the identity of which remains a mystery), it amplifies the response to high-threshold mechanical stimuli.<sup>17</sup> Subpopulations of nociceptors express ion channels in complex patterns, either in combination or in a mutually exclusive fashion, and these differences in expression ultimately determine the physiological heterogeneity of nociceptors. For example, some nociceptors respond to noxious thermal, mechanical, and chemical stimuli (polymodal nociceptor)

*Lancet Neurol* 2014

Published Online

May 7, 2014

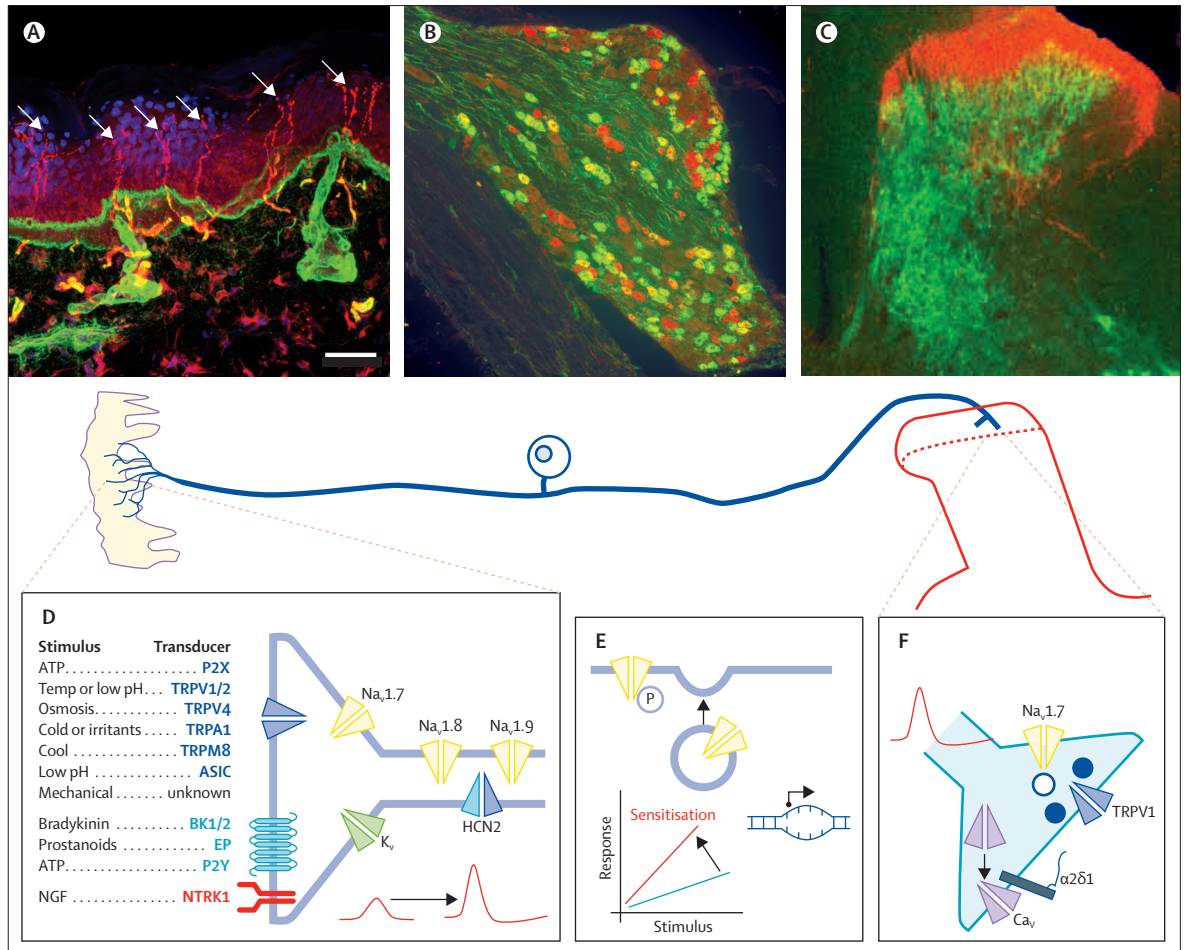
[http://dx.doi.org/10.1016/S1474-4422\(14\)70024-9](http://dx.doi.org/10.1016/S1474-4422(14)70024-9)

See Online for an audio interview with David Bennett

Nuffield Department of Clinical Neurosciences, University of Oxford, Oxford, UK (D L H Bennett FRCP); and Department of Medical Genetics, The Clinical Medical School, University of Cambridge, Cambridge, UK (Prof C G Woods FRCP)

Correspondence to:

Dr David L H Bennett, Nuffield Department of Clinical Neurosciences, University of Oxford, Oxford OX3 9DU, UK  
[David.bennett@ndcn.ox.ac.uk](mailto:David.bennett@ndcn.ox.ac.uk)



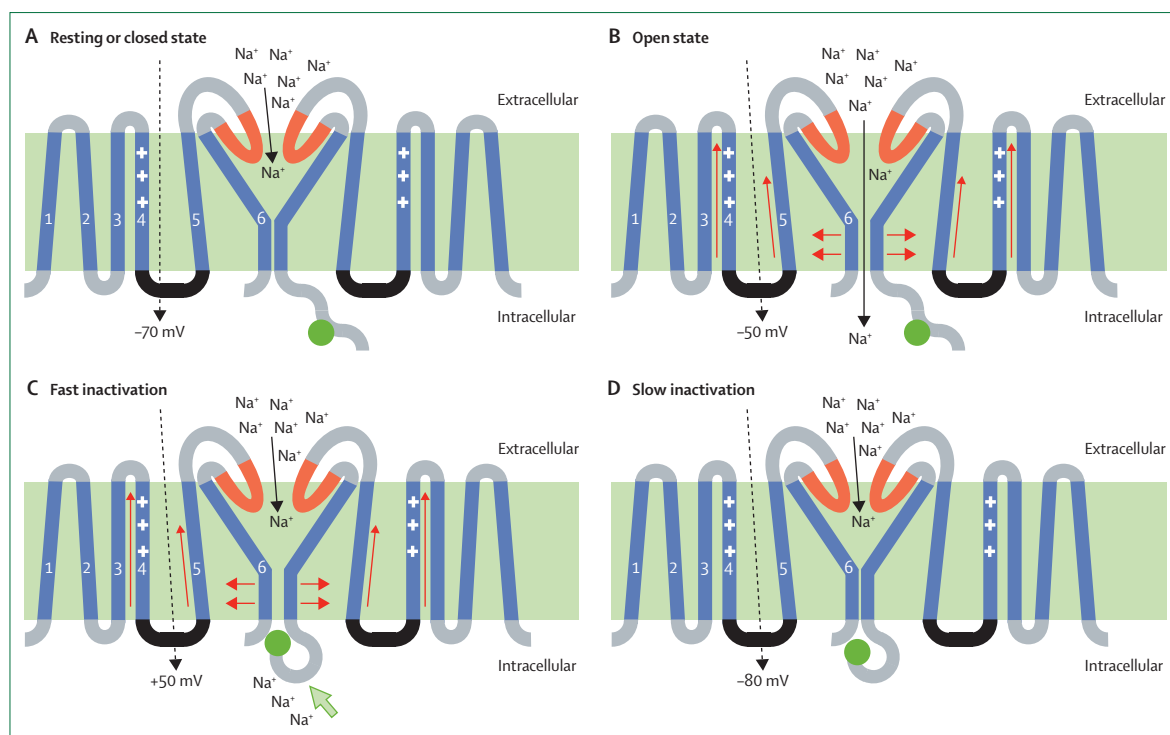
**Figure 1: Structure and functional components of a nociceptor**

Nociceptors provide connectivity between peripheral targets (in this case, skin) and the dorsal horn of the spinal cord. (A) Peripheral nociceptor terminals (arrows) are seen as free nerve endings (immunolabelled with PGP9.5, red) crossing the basement membrane (collagen IV, green) into human epidermis. (B) Cell bodies of distinct populations of nociceptors reside within dorsal root ganglia and differ by histochemical characteristics, ion-channel expression, connectivity, and functional properties. Nociceptors can be divided into two roughly equal populations: those that express neuropeptides (immunolabelled with CGRP, red); and those that bind isolectin B4 (green).<sup>5</sup> (C) Nociceptors project to superficial laminae of the dorsal horn (immunolabelled with CGRP, red), by contrast with low-threshold myelinated afferent fibres that project to deeper laminae (labelled with cholera toxin, green). (D) A stylised view of a nociceptor terminal, showing just some of the ligand-gated ion channels (blue), G-protein-coupled and tyrosine-kinase receptors (red) by which these neurons transduce extremes of temperature (Temp), low pH, and various chemical stimuli. The voltage-gated sodium channels ( $\text{Na}_v$ ; yellow) have a key role in responding to small depolarisations and action-potential generation. Voltage-gated potassium channels ( $\text{K}_v$ ; green) are important breaks on excitability.<sup>6</sup> The hyperpolarising activated cation channel HCN2 acts as a so-called pacemaker, modulating ectopic activity after nerve injury. (E) Altered ion-channel activity contributes to peripheral sensitisation, and mechanisms include stimulation of signalling pathways downstream of G-protein-coupled and tyrosine-kinase receptors and phosphorylation (P) events (upper left), altered trafficking to the membrane (centre), and, over longer periods, changes in transcription (lower right). (F) Central terminals are also important in nociceptive processing.  $\text{Na}_v1.7$  might also have a role in determining ingress of action potentials to central terminals and neurotransmitter release. Transient receptor potential (TRP) channels are also expressed centrally and can modulate presynaptic activity.<sup>7</sup> The  $\alpha2\delta1$  subunit is part of the voltage-gated calcium-channel complex ( $\text{Ca}_v$ ; purple) and has an important role in trafficking of voltage-gated calcium channels to presynaptic terminals.<sup>8</sup> GABA<sub>A</sub> pentonoids bind to  $\alpha2\delta1$  and impair this trafficking.

whereas others are insensitive to mechanical and thermal stimuli unless sensitised in the context of inflammation.<sup>18</sup> Transduction agents are important because they allow nociceptors to become sensitised after injury and inflammation through altered expression,<sup>19</sup> trafficking,<sup>20</sup> phosphorylation events,<sup>21</sup> or interaction with G-protein-coupled receptors,<sup>13</sup> sometimes leading to abnormal hypersensitivity and, ultimately, chronic pain states.

Voltage-gated sodium channels ( $\text{Na}_v$ ) are key determinants of nociceptor excitability (figure 2). The

channels  $\text{Na}_v1.7$ ,<sup>22</sup>  $\text{Na}_v1.8$ ,<sup>23</sup> and  $\text{Na}_v1.9$ <sup>24</sup> are all expressed preferentially in peripheral neurons but have different kinetics and show subtly distinct patterns of expression; all have been linked to pain.  $\text{Na}_v1.7$  channels are expressed in the peripheral terminals of sensory neurons, along their axons, at the node of Ranvier of thin myelinated sensory neurons, within the neuronal soma of dorsal root ganglia, and at the central terminals of nociceptors in the superficial laminae of the spinal cord.<sup>25,26</sup>  $\text{Na}_v1.7$  channels can produce a substantial ramp current in



**Figure 2: Schematic showing structural and functional features of voltage-gated sodium channels**

The protein molecule (eg, Na<sub>v</sub>1.7, Na<sub>v</sub>1.8, or Na<sub>v</sub>1.9) forms a pore inserted through the cell membrane (light green). The sodium channel is composed of four sets (termed domains) of six transmembrane segments arranged as a cross, with the sodium channel in the centre. Only the first and third set of six transmembrane segments is shown here. Transmembrane segments 1–4 function as a block (light blue; domain 4 indicated with white +). Transmembrane segment 4 responds to potential difference across the cell membrane, and this function is controlled by three positively charged arginine residues present at the location of the + signs. The link between transmembrane segments 4 and 5 is rigid (black). A fold in the protein is the selectivity filter (orange), which allows only Na<sup>+</sup> to enter the centre of the protein. Transmembrane segment 6 forms the wall of the central pore. When the pore opens it allows Na<sup>+</sup> to pass into the interior of the cell. An intracellular inactivation motif is formed of three hydrophobic aminoacids— isoleucine, phenylalanine, and methionine (designated the IFM motif; dark green circle)—and can move into the intracellular opening of the pore and block it. (A) The cell membrane is at a resting potential of -70 mV, the IFM domain is in its resting intracellular conformation, and the pore is closed. (B) Depolarisation to -50 mV causes the voltage-sensing transmembrane segment 4 to move outwards (long red arrow) and the pore to open (short red arrows). Na<sup>+</sup> flow causes a localised increase in positive charge. (C) The change in potential difference after pore opening causes the IFM domain to block the open pore (green arrow), inhibiting further Na<sup>+</sup> flow into the cell. (D) Activity of other ion channels produces a hyperpolarised membrane potential (about -80 mV), causing transmembrane segment 4 to return to its resting position and closure of the pore.

response to small slow depolarisation<sup>27</sup> and, therefore, might be important in amplifying small subthreshold stimuli, hence acting as an important determinant of threshold in nociceptor terminals. Furthermore, similar to the olfactory system,<sup>28</sup> Na<sub>v</sub>1.7 channels might regulate neurotransmitter release at the central terminals of nociceptors.<sup>29</sup> Na<sub>v</sub>1.8 channels are crucial for transmission of nociceptive information because they carry most of the current underlying the depolarising phase of the action potential in neurons of dorsal root ganglia<sup>30,31</sup> and are important for repetitive firing in these neurons. Na<sub>v</sub>1.9 sodium channels have slow-gating kinetics<sup>32</sup> and can generate persistent currents at near-resting membrane potential, meaning that these channels are an important regulator of membrane excitability.<sup>33–35</sup>

### Congenital insensitivity to pain

Hereditary sensory and autonomic neuropathies (HSANs) are a clinical group of inherited disorders in which sensory and autonomic neurons either fail to

develop or degenerate.<sup>36</sup> They are classified according to the neuronal population implicated and the associated clinical features. Involvement of nociceptors results in insensitivity to noxious stimuli. For example, NGF is a key neurotrophic factor needed for target-derived survival of nociceptors.<sup>37</sup> Mutations in *NGF*<sup>38</sup> (which cause HSAN type V) or *NTRK1*<sup>39</sup> (which encodes the NGF high-affinity receptor and cause HSAN type IV) result in cell death and axonal degeneration of small-diameter sensory neurons, leading to congenital insensitivity to pain, anhidrosis, and learning impairment. HSAN type IV and HSAN type V are sometimes known as congenital insensitivity to pain with anhidrosis. Some patients with congenital insensitivity to pain have a structurally normal peripheral nervous system, with free nerve endings noted in the epidermis and normal intelligence,<sup>40</sup> which indicates strongly that gene variants might be causing the disorder because of altered function rather than impaired structural integrity of nociceptors.

	Mutated gene (protein)	Type and effect of mutation	Main phenotype	Additional features
Congenital insensitivity to pain	SCN9A (Na <sub>v</sub> 1.7)	Biallelic, null	No pain; other somatosensory modalities normal	Anosmia
Hereditary sensory and autonomic neuropathy type IID	SCN9A (Na <sub>v</sub> 1.7)	Biallelic, null	No pain; autonomic features include hypohidrosis; nerve-conduction studies show abnormalities	Anosmia (variable)
Congenital insensitivity to pain	SCN11A (Na <sub>v</sub> 1.9)	Heterozygous, activating	No pain	Hyperhidrosis; muscular weakness; infant gut dysmotility
Inherited erythromelalgia	SCN9A (Na <sub>v</sub> 1.7)	Heterozygous, activating	Onset by age 20 years; episodic pain triggered by warmth; feet affected more frequently than hands	Erythema of feet
Paroxysmal extreme pain disorder	SCN9A (Na <sub>v</sub> 1.7)	Heterozygous, activating	Onset at birth; episodic pain; sacral region is affected most frequently, face is affected more often than the limbs; physical triggers include defecation	Erythema of the sacrum; tonic attacks
Small-fibre neuropathy	SCN9A (Na <sub>v</sub> 1.7)	Heterozygous, activating	Onset at any age but more common in early adulthood; persistent burning pain; feet affected more frequently than hands	Could be autonomic features
Small-fibre neuropathy	SCN10A (Na <sub>v</sub> 1.8)	Heterozygous, activating	Persistent burning pain	Could be autonomic features
Familial episodic pain syndrome type I	TRPA1 (TRPA1)	Heterozygous, activating	Onset at birth or in infancy; episodic chest or arm pain; triggers are hunger and cold	..
Familial episodic pain syndrome type III	SCN11A (Na <sub>v</sub> 1.9)	Heterozygous, activating	Onset in first decade; episodic hand and foot pain; triggers are intercurrent illness or exercise	..

Na<sub>v</sub>=sodium ion channel.

**Table 1:** Clinical features of human disorders caused by mutations in ion-channel genes that lead to altered pain perception and are inherited in a mendelian manner

### Congenital insensitivity to pain caused by inactivating SCN9A mutations

In 2006, a cause of the congenital insensitivity to pain phenotype was discovered—biallelic null mutations in the gene *SCN9A* (table 1).<sup>41–43</sup> *SCN9A* encodes the voltage-gated sodium channel Na<sub>v</sub>1.7. Congenital insensitivity to pain caused by inactivating mutations in *SCN9A* was initially referred to as channelopathy-associated insensitivity to pain. However, with identification of a related pain disorder caused by mutations in *SCN11A*, the term is now obsolete.

The typical phenotype of congenital insensitivity to pain caused by inactivating mutations in *SCN9A* comprises congenital insensitivity to pain, congenital anosmia, normal intelligence, and no other detectable anomalies in the peripheral nervous system or the CNS. No nociceptive pain is felt anywhere in the body from birth. Initial observation is usually made by parents, retrospectively, when the child makes no response to immunisations and injuries. Over the first year of life, bruises and cuts accumulate from injuries, but signs of a pain disorder escalate with the eruption of teeth; the lips, anterior tongue, and fingers are chewed, which can result in permanent damage. The tip of the tongue can be lost, but it may heal well and speech is not affected; lip injuries can be repaired, but usually a scar remains; and fingertips can be lost as wounds from bites frequently become infected. The first decade of a child's life is marred by self-induced injuries including bruises, burns, and scalds; historically, up to a third of families are accused of physical child abuse. Many children are at first thought to be impaired mentally because they are clumsy and cannot use pain feedback to train themselves to use their bodies optimally. As children approach age 10 years they learn to mimic pain behaviour: they realise that their actions and

injuries cause concern and, therefore, begin to learn pain empathy. In the second decade of life, the damage becomes more severe because of the child's increasing height and weight. Boys in particular engage in harmful behaviours—eg, picking fights or excessive risk-taking at play or in sports. Injuries received at this stage of life can result in progressive accumulation of orthopaedic fractures and breaks, which although painless might eventually be crippling. Very few men are reported (CGW, unpublished observation) as having congenital insensitivity to pain caused by inactivating mutations in *SCN9A* because their excessive risk-taking and resultant painless physical injuries lead to higher mortality in early life. Cognitive and motor development is normal in both affected males and females. Nociceptive stimuli, such as pin-prick and extremes of pressure or temperature, do not elicit a pain response; the individual can detect the stimulus but would—eg, on a temperature ramp—comment on increasing warmth rather than report any pain, even at high temperatures such as 50°C, a temperature that unaffected people would clearly find painful. Other sensory modalities such as vibration and light touch are normal, as is motor function. The one other consistent finding on examination is anosmia. No features of autonomic dysfunction are noted.

The diagnosis of congenital insensitivity to pain caused by inactivating mutations in *SCN9A* can be confirmed by identification of biallelic null mutations in the gene, leading to translation of no functional Na<sub>v</sub>1.7 protein (figure 2A). Most pathogenic mutations are easy to identify as nonsense, small out-of-frame deletions or duplications, and splice-site mutations. Functional studies might be needed to ascertain if missense mutations are null or activating; for activating mutations, the associated pathogenicity must also be noted



(eg, inherited erythromelalgia, paroxysmal extreme pain disorder, or small-fibre neuropathy).

Na<sub>v</sub>1.7 sodium channels have an important role in determining the threshold excitability of peripheral nociceptor terminals and might also play a part in neurotransmitter release from central terminals. Although nociceptors express other voltage-gated sodium channels (eg, Na<sub>v</sub>1.8 and Na<sub>v</sub>1.9), these cannot compensate for a paucity of Na<sub>v</sub>1.7 channels. Therefore, the absence of pain perception in people with inactivating mutations in *SCN9A* is attributable to the unique electrophysiological responsiveness and subcellular localisation of Na<sub>v</sub>1.7 channels. For instance, compared with Na<sub>v</sub>1.7 channels, the voltage dependence of activation of Na<sub>v</sub>1.8 is much more depolarised. Therefore, Na<sub>v</sub>1.8 makes an important contribution to the upstroke of the action potential; however, unlike Na<sub>v</sub>1.7, it will not respond to the small changes in membrane potential that are needed for initial transduction events in sensory terminals and Na<sub>v</sub>1.8, therefore, cannot compensate for the absence of Na<sub>v</sub>1.7.

Although Na<sub>v</sub>1.7 channels have a clear role in sensory neurons, their functional relevance to nociceptive signalling might not be restricted to these neurons. The behaviour of mice in which Na<sub>v</sub>1.7 protein has been ablated in different populations of peripheral neurons has been compared to the human phenotype for congenital insensitivity to pain caused by inactivating mutations in *SCN9A*. When Na<sub>v</sub>1.7 channels were removed from sensory neurons,<sup>29</sup> a strikingly reduced behavioural response to noxious mechanical stimuli was recorded; however, to see a reduction in response to suprathreshold thermal stimuli in a hot-plate test, knockout of Na<sub>v</sub>1.7 from both sensory and sympathetic neurons was needed.

#### HSAN type IID due to inactivating *SCN9A* mutations

Although sensory neurons are typically structurally normal, as shown by findings of nerve<sup>41,42,44,45</sup> and skin biopsy,<sup>46</sup> two groups of researchers have reported that morphological abnormalities can be present. Nilsen and colleagues<sup>47</sup> described a patient with congenital insensitivity to pain due to a compound heterozygote nonsense mutation in *SCN9A* in whom intraepidermal nerve fibre density was reduced significantly. Furthermore, Yuan and coworkers<sup>48</sup> reported two Japanese kindreds with a nonsense mutation in *SCN9A* and either congenital-onset or adolescent-onset loss of pain and temperature sensation and autonomic dysfunction (anhidrosis). In these people, findings of nerve-conduction studies indicated a definite reduction in sensory compound action potentials, and, in one individual, pathological analysis confirmed loss of large myelinated fibres. This distinct phenotype has been denoted HSAN type IID (table 1). These two Japanese families show that further studies are needed to account for why different phenotypes can be caused by nonsense *SCN9A* mutations.

#### Congenital insensitivity to pain caused by activating *SCN11A* mutations

Two unrelated children have been described with congenital insensitivity to pain secondary to a gain-of-function mutation in *SCN11A* (table 1), which encodes the voltage-gated sodium channel Na<sub>v</sub>1.9.<sup>49</sup> We too have a patient with the same phenotype and genotype (CGW, unpublished observation).

No nociceptive pain was felt anywhere in the body from birth in all three patients, resulting in multiple burns, cuts, and bruises, self-induced injuries, fractures, and slow or poor wound healing. All individuals sweat excessively (hyperhidrosis) without the usual precipitants of exercise or raised temperature; no evidence suggests cardiovascular autonomic dysfunction. The sense of smell is intact. Affected individuals have mild muscular weakness, which led to an early diagnosis of developmental delay; however, cognitive development and intelligence are normal. Gastrointestinal dysfunction was a relevant problem in the first year of life, with episodes of diarrhoea and constipation; in some children, parenteral nutrition was needed. Findings of sural nerve biopsy are normal. In two individuals, motor and sensory nerve-conduction velocities are slightly reduced, but with normal amplitude. Findings of muscle and intestinal biopsies, electromyography, brain MRI, and malabsorption studies are normal. Laparotomy in one patient showed a morphologically inconspicuous small intestine with reduced peristaltic waves and a grossly enlarged colon. The phenotype of congenital insensitivity to pain caused by an activating mutation in *SCN11A* is unique; the features shared with congenital insensitivity to pain caused by inactivating mutations in *SCN9A* are congenital insensitivity to pain and normal intelligence.

All three affected individuals have the same heterozygous mutation in *SCN11A*, 2432T→C. This variation causes an aminoacid change (Leu811Pro) in the Na<sub>v</sub>1.9 sodium channel. All three children are the only affected member of their families, no parent had the *SCN11A* 2432T→C mutation. Therefore, congenital insensitivity to pain caused by an activating mutation in *SCN11A* seems to be caused by a recurrent de-novo mutation.

The leucine at position 811 of the Na<sub>v</sub>1.9 protein is conserved in all ten human voltage-gated sodium channels. It occupies a very specific location, being the last aminoacid of one of the four transmembrane segments that form the pore of the Na<sub>v</sub>1.9 sodium channel. Leipold and colleagues<sup>49</sup> investigated the electrophysiological behaviour of Na<sub>v</sub>1.9 in man and mouse. Substantial discordance was noted between species: mice heterozygous for the corresponding Na<sub>v</sub>1.9 mutation had normal thresholds to noxious thermal and mechanical stimuli. Although mouse genetics has provided a powerful method for investigation of pain neurobiology, this finding emphasises that caution is needed when interpreting the outcomes of pain-related behaviour in mouse models. The heterozygous Leu811Pro mutation causes a gain of function in man, with a leftward

shift in channel activation and deactivation kinetics and slowed channel inactivation, consistent with the hypothesis that the altered residue interferes with voltage-dependent gate closure and channel inactivation (figure 2B). The result of this gain-of-function mutation in the Na<sub>v</sub>1.9 protein is sustained depolarisation of the cell membrane (reducing the negative membrane potential), which is suggested to lead to inactivation of the other voltage-gated sodium channels (ie, Na<sub>v</sub>1.7 and Na<sub>v</sub>1.8) that form the main constituent of the action potential and, hence, conduction block in nociceptors.

### Gain-of-function gene variants associated with heritable pain states

#### Inherited erythromelalgia

Erythromelalgia is a symptom complex of pain and erythema of the hands and feet.<sup>50</sup> The disorder usually starts in the feet but can affect the hands and, occasionally, the nose and ears. Pain is initially episodic, with attacks precipitated by warmth (such that most patients are unable to wear enclosed shoes), exercise, prolonged standing, and sometimes alcohol; the pain is relieved by cooling.<sup>51,52</sup> In some patients, pain becomes constant, with fluctuations. Pain is severe and disabling, and use of ice-cold water to provide relief (figure 3A) can cause skin ulceration. Erythromelalgia can be secondary to blood dyscrasias, connective-tissue disorders, and drug reactions; however, inherited erythromelalgia is usually associated with onset in the first two decades of life. Yang and colleagues<sup>54</sup> reported a Chinese kindred with an autosomal-dominant pattern of inheritance. With linkage analysis and candidate-gene sequencing, they identified a point mutation in the Na<sub>v</sub>1.7 sodium channel (Leu858His); sequencing of further probands showed another mutation—Leu858Ile. Subsequently, multiple missense mutations in *SCN9A*, which encodes the Na<sub>v</sub>1.7 channel, have been associated with erythromelalgia (table 1, figure 3B).<sup>55</sup> There are hotspots for mutations—eg, in the linker between transmembrane segments 4 and 5. Structural studies in several ion channels suggest that this region might connect the voltage sensor (segments 1–4) to the channel pore (segments 5 and 6; figure 2),<sup>56</sup> thus, mutations in this region could have an effect on channel activation.

Electrophysiological analysis of Na<sub>v</sub>1.7 mutants has indicated several pathophysiological mechanisms leading to enhanced excitability. A hyperpolarising shift in the voltage dependence of activation is a constant feature of inherited erythromelalgia (figure 3C and 3D). Other effects of mutations include slowed deactivation and increased current response to slow depolarisation (ramp currents; figure 2D).<sup>55</sup> When mutant ion channels are expressed in cells of dorsal root ganglia, a lowered threshold is observed for the generation of action potentials and a higher frequency of repetitive firing.<sup>57</sup> Microneurographic recordings from sensory axons in patients with erythromelalgia<sup>58</sup> show augmented activity-

dependent slowing and mechanically insensitive afferents with pathological spontaneous activity (several cases were described as hereditary in this series, which was undertaken before availability of mutation screening). A relation exists between biophysical abnormalities of the mutant channel and clinical severity: the greater the hyperpolarising shift in the voltage dependence of activation, the younger the age of onset.<sup>53,59</sup> A correlation is also present with temperature sensitivity: the degree of dysfunction of mutant Na<sub>v</sub>1.7 versus wild-type channels is greater at higher temperature.<sup>60</sup> Extensive genetic screening indicates that inherited erythromelalgia cannot be distinguished from acquired erythromelalgia merely on the basis of age of onset and the presence of family history: several cases have been associated with de-novo mutations.<sup>61</sup> Furthermore, a patient with an age of onset in her late 50s and no family history had a Trp1538Arg Na<sub>v</sub>1.7 mutation with gain of function on electrophysiological analysis.<sup>53</sup>

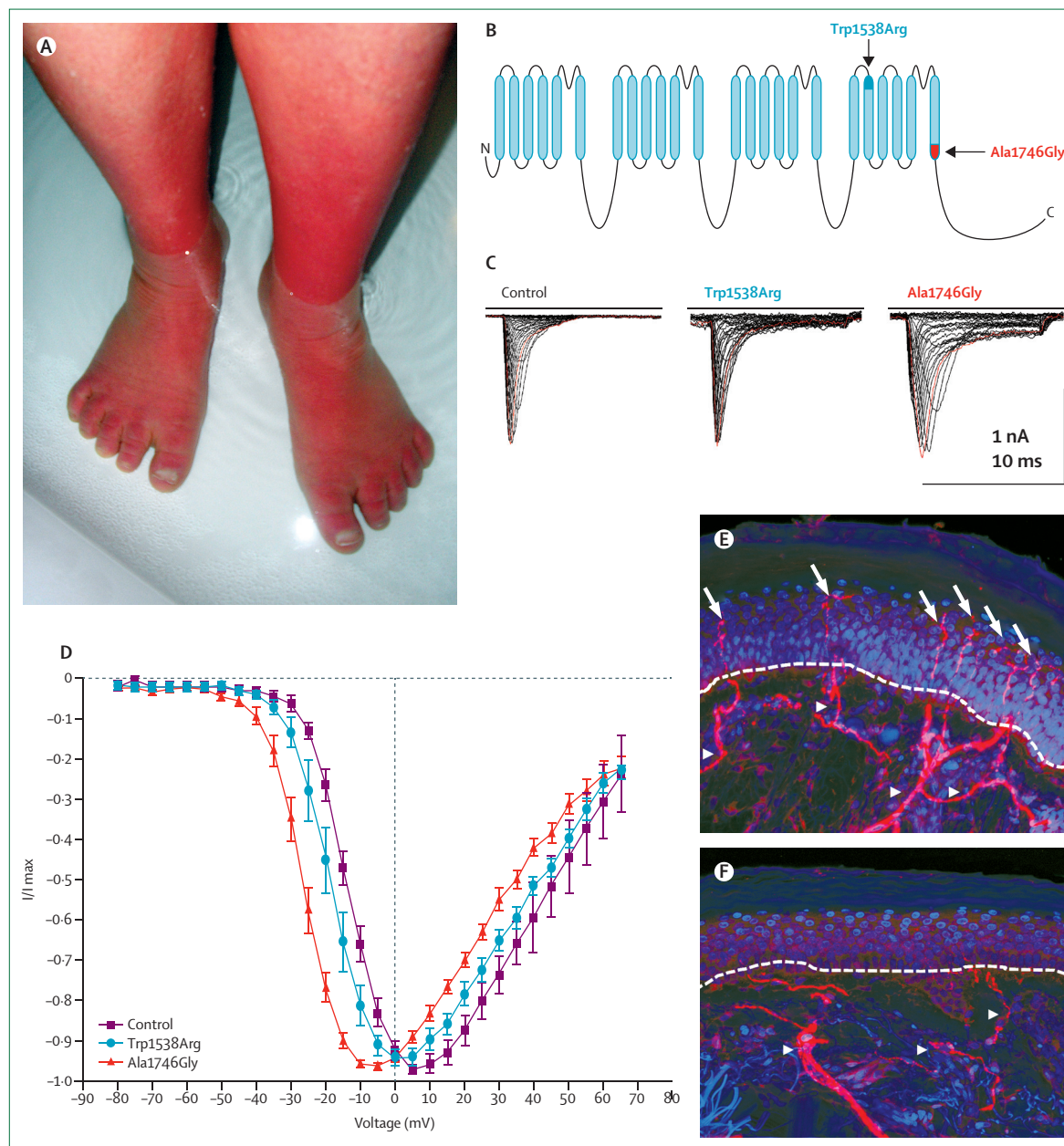
Treatment of primary erythromelalgia is challenging and based essentially on small case series. Use of drugs such as anticonvulsants and local anaesthetics with known broad activity against voltage-gated sodium channels seems logical. The therapeutic window is narrow because of cardiac and CNS side-effects. Success—both on a clinical level and when tested in vitro—is dependent on the exact mutation. Carbamazepine and mexiletine both show efficacy in the context of specific mutations, and structural modelling predicts pharmacological responsiveness.<sup>62,63</sup> Topical lidocaine plasters can be applied to the most affected areas (eg, the soles of the feet) and have the great advantage of few side-effects. Development of selective blockers of Na<sub>v</sub>1.7 sodium channels is ongoing, and inherited erythromelalgia is a substrate for proof-of-concept trials.<sup>64</sup> Occasionally, patients devise their own physical measures to keep their feet cool; for example, perfusing cooled water through a plastic sock enclosing the feet (used to cool limbs during orthopaedic surgery) can be very helpful.<sup>52</sup>

#### Paroxysmal extreme pain disorder

Paroxysmal extreme pain disorder (formerly known as familial rectal pain) is inherited in an autosomal-dominant fashion and characterised by paroxysmal episodes of severe perineal and rectal, ocular, and mandibular pain. Pain is associated with autonomic features that can include flushing (which sometimes results in harlequin colour change), lacrimation, rhinorrhoea, and tonic attacks with apnoea and bradycardia.<sup>65–67</sup> Tonic attacks might be confused with epilepsy; however, findings of electroencephalography are normal. Important stimuli are physical factors—eg, defecation or eating—but strong emotion can also be a trigger. Between episodes, physical examination is normal. Most patients present at birth or in infancy. The frequency of rectal pain episodes typically falls with increasing age, although ocular and mandibular

pain can become more common. Linkage analysis followed by candidate-gene sequencing identified missense mutations in *SCN9A*, which encodes Na<sub>v</sub>1.7 sodium channels (table 1), segregating with this disorder. These

mutations lead to sensory neuronal hyperexcitability<sup>68</sup> via a mechanism that is distinct from that seen in inherited erythromelalgia. In the case of paroxysmal extreme pain disorder, impaired fast inactivation of the Na<sub>v</sub>1.7 channel



(figure 2C) leads to a persistent sodium current<sup>69</sup> and more rapid recovery from fast inactivation.<sup>68</sup> A further feature of these mutations is the generation of resurgent currents.<sup>70</sup>

Carbamazepine is effective for reduction of the frequency and severity of attacks in most patients. Furthermore, it lowers the persistent sodium current generated by the mutant Na<sub>v</sub>1.7 channel that is associated with paroxysmal extreme pain disorder.<sup>69</sup> By contrast to inherited erythromelalgia, paroxysmal extreme pain disorder is characterised by a much more proximal distribution of pain, is triggered by mechanical factors rather than increased temperature, and the associated mutations impair inactivation rather than promote activation. Although, in general, inherited erythromelalgia and paroxysmal extreme pain disorder are clinically distinct, a patient has been described who has a mutation in the Na<sub>v</sub>1.7 channel and clinical features and biophysical characteristics common to both disorders.<sup>71</sup>

#### Small-fibre neuropathy

Small-fibre neuropathy refers to selective injury to unmyelinated and thin myelinated axons, which is usually length-dependent.<sup>72</sup> The typical presenting symptom is burning pain of the feet. Small-fibre neuropathy is distinct from inherited erythromelalgia in that symptoms are not clearly exacerbated by warming and relieved by cooling (a virtually invariant feature of inherited erythromelalgia). Although erythema is present in some patients with small-fibre neuropathy, it is relatively mild. Large fibres are not affected and, as such, deep-tendon reflexes and large-fibre sensory function are both normal. Autonomic signs are recorded commonly in people with small-fibre neuropathy; autonomic-function tests are needed to prove autonomic involvement, but these tests are not widely available. The gold-standard diagnostic test is a reduction of intraepidermal nerve-fibre density, assessed by skin biopsy (figure 3E and 3F).<sup>73</sup> Moreover, quantitative sensory testing might show raised thermal thresholds. Small-fibre neuropathy might be acquired secondary to diabetes mellitus, for example; however, the condition in a substantial proportion of patients (up to 50%) has been described as idiopathic.

Gain-of-function missense variants in Na<sub>v</sub>1.7 channels have been reported in 30% of individuals with small-fibre neuropathy in whom no underlying cause of the disorder could be identified (table 1).<sup>74</sup> These changes are best described as variants rather than mutations because some of them arise with a minor allele frequency of up to 3.7% (eg, Met932Leu [rs12478318]). The penetrance of these variants is not yet known; of eight patients in the initial report, three recalled similar symptoms in family members. Future work, in which segregation of these variants is followed up through pedigrees, will establish if variants are fully penetrant or important risk factors interacting with other genetic or environmental factors.

All reported variants result in substitution of highly conserved aminoacids within Na<sub>v</sub>1.7 channels; these

variants are, in general, distinct to those causing inherited erythromelalgia. Specific variants, such as Ile228Met (rs71428908), show phenotypic heterogeneity and can result in either small-fibre neuropathy or inherited erythromelalgia, with varying levels of facial pain, even in members of the same family.<sup>75</sup> Functional analysis shows impaired slow inactivation, depolarised slow and fast inactivation, and increased resurgent currents (figure 2D); however, the hyperpolarising shift in voltage dependence of activation reported with inherited erythromelalgia is not seen.<sup>74,76</sup> These biophysical changes lead to dorsal root ganglia cell hyperexcitability, including a depolarising shift in resting membrane potential, reduced current threshold, enhanced firing frequency, and spontaneous activity. How do these variants lead to loss of structural integrity of sensory axons? One variant, Ile228Met, significantly diminishes neurite outgrowth in cells of dorsal root ganglia,<sup>77</sup> which could be ameliorated by carbamazepine—a use-dependent blocker of voltage-gated sodium channels. Enhanced activity of Na<sub>v</sub>1.7 channels is predicted to augment sodium load, reverse the operation of the sodium-calcium exchanger, and increase intra-axonal calcium concentrations, which could lead to axonal degeneration. Indeed, inhibition of reverse operation of the sodium-calcium exchanger could enhance neurite outgrowth of dorsal root ganglia cells transfected with Ile228Met, thus suggesting a novel target for neural repair in people with small-fibre neuropathy.

Analysis of *SCN10A*, which encodes the voltage-gated sodium channel Na<sub>v</sub>1.8, showed that nine of 104 patients with predominantly small-fibre neuropathy symptoms (and negative for mutations in the Na<sub>v</sub>1.7 channel) had missense Na<sub>v</sub>1.8 variants (table 1).<sup>78</sup> Predictive algorithms suggested three variants could be pathogenic and these underwent functional profiling, of which two showed a gain of function at channel level (including enhanced ramp responses and repriming) and enhanced activation. These mutations led to hyperexcitability of small neurons in dorsal root ganglia, assessed by current clamp, including reduced current threshold, increased firing frequency, and spontaneous activity.

#### Familial episodic pain syndrome due to *TRPA1* mutation

Familial episodic pain syndrome type I is a rare, inherited, painful channelopathy. In a large Colombian pedigree, this disorder showed an autosomal-dominant pattern of inheritance.<sup>79</sup> Patients report severe episodes of pain, mainly affecting the thorax and arms but occasionally radiating to the abdomen and legs. These pain attacks start in infancy, are triggered by cold and hunger, last about 60–90 min, and respond poorly to conventional analgesia. Between attacks, neurological examination is normal, as are findings of nerve-conduction studies, and intraepidermal nerve-fibre density is unchanged (figure 3E). Linkage analysis and candidate-gene sequencing indicated a gain-of-function missense mutation in *TRPA1*, which encodes the TRP channel



TRPA1 (Asn855Ser; table 1). Patients with familial episodic pain syndrome showed hypersensitivity to mustard oil, an agonist at this channel, and this effect correlated with a gain of function of the mutant channel, when assessed electrophysiologically. In particular, the mutant channel passed more current after activation at normal resting potentials. The effects of the mutation were ameliorated by a small-molecule blocker of TRPA1, indicating that there may ultimately be therapeutic options for these patients.

#### Familial episodic pain syndrome due to SCN11A mutation

Two large Chinese kindreds with familial episodic pain inherited in an autosomal dominant manner have been reported (which is termed type III).<sup>80</sup> The clinical presentation of familial episodic pain syndrome type III is distinct to the type I disorder (caused by *TRPA1* mutations) in that pain has a distal (affecting hands and feet) rather than a proximal (chest and arms) distribution. Pain episodes usually happen near the end of the day and arise in clusters; triggers include intercurrent illness and fatigue after exercise. Pain is relieved by non-steroidal anti-inflammatory drugs. Neurological examination is normal and, in particular, no evidence is seen of sensory loss. A combination of linkage analysis and whole-exome sequencing in both Chinese pedigrees has revealed missense mutations (Arg225Cys and Ala808Gly) in *SCN11A*, which encodes the Na<sub>v</sub>1.9 sodium channel (table 1).<sup>80</sup> Findings of electrophysiological analyses show that these mutations cause hyperexcitability of cells of dorsal root ganglia, with increased peak current densities and enhanced action potential firing after current injection. By contrast with the Leu811Pro mutation in the Na<sub>v</sub>1.9 ion channel, which is associated with congenital insensitivity to pain, these mutations do not result in a depolarising shift in the resting membrane potential. Therefore, different mutations in Na<sub>v</sub>1.9—all resulting in gain of function but with distinct biophysical

characteristics—have opposite effects on dorsal root ganglia cell excitability and the clinical phenotype.

#### Gene variants and risk of developing chronic acquired pain syndromes

Polymorphisms in the genes encoding ion channels might affect an individual's susceptibility to, and severity of, chronic pain after tissue inflammation or neural injury (table 2).<sup>81–91</sup> Testing this hypothesis has been challenging not only because detailed pain phenotyping is needed but also in view of the number of patients required to power such studies adequately. Chronic pain is not a homogeneous occurrence, many pathophysiological mechanisms can coexist in one individual,<sup>92</sup> and experimental pain models in man suggest that pain evoked by different stimuli has a distinct genetic basis.<sup>93</sup> Indeed, evidence shows that specific polymorphisms in *TRPV1* and *TRPA1* do not alter neuropathic pain severity but do alter the pattern of somatosensory dysfunction on sensory testing.<sup>94</sup>

A single nucleotide polymorphism (SNP) in *SCN9A* results in an aminoacid substitution of arginine to tryptophan (Arg1150Trp; rs6746030) in the Na<sub>v</sub>1.7 sodium channel, which enhances cell excitability of dorsal root ganglia.<sup>81,82</sup> Reimann and colleagues<sup>82</sup> reported that the minor allele was associated with increased pain in patients with osteoarthritis, sciatica, and phantom limb syndrome. This finding was not replicated in a larger cohort with osteoarthritis<sup>83</sup> nor in patients with chronic widespread pain.<sup>84</sup>

*CACNA2D3* encodes the  $\alpha 2\delta 3$  subunit of the voltage-dependent calcium-channel complex. This gene has been implicated in thermal pain-related behaviour in flies and mice.<sup>84</sup> In healthy volunteers, the rare allele of an SNP in *CACNA2D3* (rs6777055, located within an intron) was associated with reduced acute thermal pain and diminished chronic pain after lumbar discectomy (no replication studies have been done to date).

SNP	Protein mutation or coding	Functional assessment	Clinical cohorts (patients [n])	Effect on pain perception	
SCN9A	rs6746030	Arg1150Trp	Enhanced dorsal-root ganglion excitability <sup>81,82</sup>	Osteoarthritis (578)*, <sup>82</sup> osteoarthritis (4295), <sup>83</sup> sciatica (195)*, <sup>82</sup> lumbar root (179)*, <sup>82</sup> phantom limb (100)*, <sup>82</sup> pancreatitis (205)*, <sup>82</sup> chronic widespread pain (1071 case, 3212 control), <sup>84</sup> Parkinson's disease pain (229)* <sup>85</sup>	Increased
SCN9A	rs7958311	Val1104Leu	Not tested	Postoperative pain (200)* <sup>86</sup>	Decreased
CACNA2D3	rs6777055	Intronic	Not applicable	Lumbar discectomy (169)* <sup>87</sup>	Decreased
KCNS1	rs734784	Ile488Val	Not tested	Lumbar discectomy (151)*, <sup>88</sup> phantom limb pain (199)*, <sup>88</sup> phantom limb pain (100)*, <sup>88</sup> sciatica (195)*, <sup>88</sup> post mastectomy (529), <sup>88</sup> HIV neuropathy (342) <sup>89</sup> †	Increased
CACNG2	rs4820242; rs2284015; rs2284017	Intronic	Not applicable	Post mastectomy (549)* <sup>90</sup>	Increased (in haplotype block)
P2RX7	rs208294	His155Tyr	Hyperfunctional; pore formation in macrophage	Post mastectomy (534)* <sup>91</sup>	Increased
P2RX7	rs7958311	Arg270His	Hypofunctional; pore formation in macrophage	Post mastectomy (534)* <sup>91</sup>	Decreased

SNP=single nucleotide polymorphism. \*Significant association. †In this study, although rs734784 was not associated with pain intensity in the context of HIV neuropathy in a black South African population, several haplotypes of population-specific tag SNPs did correlate with pain intensity.

**Table 2: Studies examining the association between SNPs in candidate ion-channel genes and altered pain perception in chronic pain syndromes**

*KCNS1* encodes the voltage-gated potassium channel subunit K<sub>v</sub>9.1, which is electrically silent but can modify the function of other functional  $\alpha$  subunits. Reduced expression of K<sub>v</sub>9.1 results in neuronal hyperexcitability in rodent models.<sup>95</sup> In a cohort of human experimental pain models (ie, healthy controls) and patients with neuropathic pain,<sup>88</sup> an SNP in *KCNS1* (rs734784), which resulted in substitution of isoleucine for valine (Ile488Val), was associated with a substantial increase in acute pain in controls and in patients with neuropathic pain after radiculopathy or post amputation, but the variant had no effect on post-mastectomy pain.<sup>88</sup> These study findings suggest that the premorbid pain threshold or sensitivity to pain correlates positively with risk of chronic neuropathic pain and that *KCNS1* plays a part in this process. However, this hypothesis has yet to be proven in a prospective cohort. In a black South African population with HIV-associated sensory neuropathy,<sup>89</sup> the SNP rs734784 was not associated with pain intensity; however, several haplotypes of population-specific SNPs did correlate significantly with pain intensity.

*CACNG2* encodes a protein that is included among the  $\gamma$  subunit of voltage-dependent calcium channel family. However, this name is a misnomer; the functional role of *CACNG2* is as a type 1 transmembrane AMPA receptor regulatory peptide. This protein regulates trafficking of this key receptor (for the excitatory neurotransmitter glutamate) to the synapse. *CACNG2* affects susceptibility to chronic pain after nerve injury in mice; in patients, an association was noted between increased susceptibility to pain after mastectomy and the haplotype of three intronic SNPs (rs4820242, rs2284015, and rs2284017).<sup>90</sup> No replication studies have been done to date.

*P2RX7* is an ATP-gated ionotropic receptor that is highly expressed on cells of the myeloid lineage and has a role in cytokine release.<sup>96</sup> Activation leads to the opening of a channel for small cations, and then the opening of a larger non-selective pore. Mouse strains with a *P2RX7* gene variant that impairs pore formation showed reduced mechanical hypersensitivity after nerve injury.<sup>91</sup> In human cohorts of osteoarthritis and post-mastectomy pain, the SNP rs7958311, which causes a substitution of arginine for histidine (Arg270His), is also linked to impaired pore formation and was associated with a significant reduction in chronic pain.<sup>91</sup>

In summary, findings of several studies show an association between the risk or severity of pain and polymorphisms in ion channels and associated proteins. However, these initial findings must be regarded as preliminary because either they are not supported by replication studies or such studies are awaited. Such replication in large, distinct, and well-defined chronic pain states (eg, neuropathic pain vs osteoarthritis-related pain) will be needed to establish whether polymorphisms either confer risk or severity of chronic pain across a range of disorders or are specific to particular causes of disease. Most studies so far have used fairly rudimentary phenotyping, such as a global rating of ongoing pain. Careful analysis of symptom descriptors and quantitative sensory testing could reveal genetic polymorphisms modulating specific pathophysiological aspects of persistent pain. As with many other human traits, the effect size of individual common genetic polymorphisms on pain severity is likely to be small.<sup>97</sup> For example, a haplotype of *P2RX7* that is associated significantly with post-mastectomy pain accounted for 4.5% of variance in the trait. However, such an association provides biological insight that a particular gene or pathway may be a potential therapeutic target.

### Treatments for pain that target ion channels

New analgesics effective for the treatment of chronic pain are needed urgently. Current agents that have activity directed against ion channels (eg, GABA<sub>A</sub> pentinoids, carbamazepine, and mexiletine) were not developed as analgesics but as antiepileptic or antiarrhythmic drugs. None are highly efficacious, and we cannot predict efficacy in individual patients.<sup>98</sup> Most agents have side-effects that can limit their usefulness in practice. Ion-channel genes are good candidates for novel analgesic targets: the effect on pain, and the side-effects, can be predicted from the human phenotype (eg, anosmia with Na<sub>v</sub>1.7 antagonists, gut dysmotility with Na<sub>v</sub>1.9 antagonists);<sup>99</sup> ion channels are cell-membrane proteins and, hence, easily accessible to drugs within the bloodstream.

The process of discovering chemical and antibody antagonists (eg, against Na<sub>v</sub>1.7 and Na<sub>v</sub>1.8) or agonists (eg, of Na<sub>v</sub>1.9) has been enhanced greatly by construction of large molecule libraries, by computer modelling of proteins, and by combinatorial chemistry. Na<sub>v</sub>1.7 antagonists are expected to ameliorate acute and inflammatory pain, but whether they have a role in neuropathic pain remains unknown. Human trials of Na<sub>v</sub>1.7 antagonists are in progress,<sup>64</sup> but the extent to which the ion channel will need to be blocked is unknown. People who are haploinsufficient for Na<sub>v</sub>1.7 have normal pain perception. The aim would be to block hypersensitivity by reduction of primary afferent drive rather than achieve complete insensitivity to noxious stimuli, which could lead to self-injury. Na<sub>v</sub>1.8 antagonists have shown efficacy against neuropathic pain in rodents, but human trials are awaited.<sup>100</sup> However, concern has

#### Search strategy and selection criteria

We searched PubMed between January, 1990, and Nov 1, 2013, for papers published in English, using combinations of terms including: "SCN9a", "SCN10a", "SCN11a", "Na<sub>v</sub>1.7", "Na<sub>v</sub>1.8", "Na<sub>v</sub>1.9", "TRPA1", "erythromelalgia", "paroxysmal extreme pain disorder", "small fibre neuropathy", and "familial episodic pain disorder". The final reference list was generated on the basis of relevance to the topics covered in this Review. Our primary sources stem from our own clinical and research practices, which have possibly influenced our choice of the topics to include and exclude from this Review.

been raised that cardiac arrhythmias might arise after blockade.<sup>101</sup> In a mouse model of nerve injury, loss of the pacemaker potassium channel HCN2 within sensory neurons blocked neuropathic pain.<sup>102</sup> However, loss of HCN2 in mice within the CNS and the heart leads to absence epilepsy and cardiac sinus dysrhythmia, respectively.<sup>103</sup> Ivabradine is an antagonist that has proven efficacy for treatment of angina<sup>104</sup> and blocks all four hyperpolarisation activated cyclic nucleotide-gated potassium (HCN) channels. A known side-effect of ivabradine is bradycardia (presumably due to antagonism of either HCN2 or HCN4, or both) but we do not know if this effect will limit the usefulness of ivabradine as an analgesic. A further interesting idea is to exploit selective expression of TRPV1 in nociceptors: coapplication of a TRPV1 agonist and QX-314 (a polar-membrane-impermeant lidocaine derivative) enables entry of this molecule into axons and produces pain-specific local anaesthesia in rodents.<sup>105</sup>

### Conclusions and future directions

Understanding the genetic basis of rare heritable human pain disorders has improved our knowledge of pain pathophysiology and, perhaps unsurprisingly, highlighted the key functions of ion channels expressed in nociceptors. Perhaps what was unexpected is the primacy of the three voltage-gated sodium channels—Na<sub>v</sub>1.7, Na<sub>v</sub>1.8, and Na<sub>v</sub>1.9—in acute pain sensing. Polymorphisms in mendelian pain genes might also establish the risk and severity of acquired pain states, such as painful diabetic neuropathy or osteoarthritis. Application of new sequencing technologies enabling whole exome or genome sequencing<sup>106</sup> and better clinical phenotyping of pain disorders<sup>92</sup> is likely to enhance our understanding of how genetic variants in ion channels are linked to human pain perception. Development of transgenic mouse models of human disease, and studying the genetic basis of sensory function in rodents, will generate important complementary data for human studies.<sup>107</sup> Such large datasets need integration and consolidation, and publicly accessible databases have now been established.<sup>108,109</sup> Sensory neuronal differentiation protocols for human inducible pluripotent stem cells provide an opportunity to study ion-channel dysfunction in human sensory neurons<sup>110</sup> and should be a potent technique for drug discovery. We are optimistic that this knowledge, combined with improved human experimental pain models and clinical trial design, will ultimately translate to better treatment for both inherited and acquired chronic pain syndromes.

#### Contributors

DLHB and CGW contributed equally to the design, literature search, and writing of the Review.

#### Declaration of interests

DLHB has consulted for and received honoraria from Astellas and Acorda Therapeutics and has received grant support from Pfizer in the form of a joint Medical Research Council (MRC) CASE PhD studentship; he is a senior Wellcome clinical scientist and member of the Wellcome Trust-funded London Pain Consortium; he is a member

of the European collaboration, which has received support from the Innovative Medicines Initiative Joint Undertaking (grant agreement 115007), resources of which are composed of financial contributions from the European Union's seventh framework programme (FP7/2007–2013) and the European Federation of Pharmaceutical Industries and Association's companies' in-kind contribution; and he is a member of the Innovative Medicines Initiative StemBANCC. CGW has received grant support from Pfizer in the form of a joint MRC MICA award and joint MRC CASE PhD studentship; his primary research focus is mendelian disorders of painlessness and he holds research funding from the MRC (Industrial Collaborative Award with partners in the University of Cambridge and Neusentis) and the National Institute for Health Research Cambridge Biomedical Research Centre; and he is funded by the University of Cambridge through the National Health Service-funded academic reserve.

#### Acknowledgments

We thank Juan Ramirez Rozo and Margarita Calvo for help with figure 1.

#### References

- Breivik H, Collett B, Ventafridda V, Cohen R, Gallacher D. Survey of chronic pain in Europe: prevalence, impact on daily life, and treatment. *Eur J Pain* 2006; **10**: 287–333.
- Sherrington CS. Qualitative difference of spinal reflex corresponding with qualitative difference of cutaneous stimulus. *J Physiol* 1903; **30**: 39–46.
- Reichling DB, Green PG, Levine JD. The fundamental unit of pain is the cell. *Pain* 2013; **154** (suppl 1): S2–9.
- Dubin AE, Patapoutian A. Nociceptors: the sensors of the pain pathway. *J Clin Invest* 2010; **120**: 3760–72.
- Snider WD, McMahon SB. Tackling pain at the source: new ideas about nociceptors. *Neuron* 1998; **20**: 629–32.
- Hao J, Padilla F, Dandonneau M, et al. Kv1.1 channels act as mechanical brake in the senses of touch and pain. *Neuron* 2013; **77**: 899–914.
- Kim YS, Chu Y, Han L, et al. Central terminal sensitization of TRPV1 by descending serotonergic facilitation modulates chronic pain. *Neuron* 2014; **81**: 873–87.
- Dolphin AC. Calcium channel auxiliary  $\alpha 2\delta$  and  $\beta$  subunits: trafficking and one step beyond. *Nat Rev Neurosci* 2012; **13**: 542–55.
- Tracey I, Mantyh PW. The cerebral signature for pain perception and its modulation. *Neuron* 2007; **55**: 377–91.
- Patapoutian A, Tate S, Woolf CJ. Transient receptor potential channels: targeting pain at the source. *Nat Rev Drug Discov* 2009; **8**: 55–68.
- Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D. The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature* 1997; **389**: 816–24.
- Tominaga M, Caterina MJ, Malmberg AB, et al. The cloned capsaicin receptor integrates multiple pain-producing stimuli. *Neuron* 1998; **21**: 531–43.
- Bandell M, Story GM, Hwang SW, et al. Noxious cold ion channel TRPA1 is activated by pungent compounds and bradykinin. *Neuron* 2004; **41**: 849–57.
- Jordt SE, Bautista DM, Chuang HH, et al. Mustard oils and cannabinoids excite sensory nerve fibres through the TRP channel ANKTM1. *Nature* 2004; **427**: 260–65.
- Peier AM, Moqrich A, Hergarden AC, et al. A TRP channel that senses cold stimuli and menthol. *Cell* 2002; **108**: 705–15.
- Macpherson LJ, Xiao B, Kwan KY, et al. An ion channel essential for sensing chemical damage. *J Neurosci* 2007; **27**: 11412–15.
- Kwan KY, Glazer JM, Corey DP, Rice FL, Stucky CL. TRPA1 modulates mechanotransduction in cutaneous sensory neurons. *J Neurosci* 2009; **29**: 4808–19.
- McMahon S, Koltzenburg M. The changing role of primary afferent neurones in pain. *Pain* 1990; **43**: 269–72.
- Ji RR, Samad TA, Jin SX, Schmall R, Woolf CJ. p38 MAPK activation by NGF in primary sensory neurons after inflammation increases TRPV1 levels and maintains heat hyperalgesia. *Neuron* 2002; **36**: 57–68.
- Schmidt M, Dubin AE, Petrus MJ, Earley TJ, Patapoutian A. Nociceptive signals induce trafficking of TRPA1 to the plasma membrane. *Neuron* 2009; **64**: 498–509.

- 21 Yao X, Kwan HY, Huang Y. Regulation of TRP channels by phosphorylation. *Neurosignals* 2005; **14**: 273–80.
- 22 Toledo-Aral JJ, Moss BL, He ZJ, et al. Identification of PN1, a predominant voltage-dependent sodium channel expressed principally in peripheral neurons. *Proc Natl Acad Sci USA* 1997; **94**: 1527–32.
- 23 Akopian AN, Sivilotti L, Wood JN. A tetrodotoxin-resistant voltage-gated sodium channel expressed by sensory neurons. *Nature* 1996; **379**: 257–62.
- 24 Dib-Hajj SD, Tyrrell L, Black JA, Waxman SG. NaN, a novel voltage-gated Na channel, is expressed preferentially in peripheral sensory neurons and down-regulated after axotomy. *Proc Natl Acad Sci USA* 1998; **95**: 8963–68.
- 25 Djouhri L, Newton R, Levinson SR, Berry CM, Carruthers B, Lawson SN. Sensory and electrophysiological properties of guinea-pig sensory neurones expressing Nav 1.7 (PN1) Na<sup>+</sup> channel alpha subunit protein. *J Physiol* 2003; **546**: 565–76.
- 26 Black JA, Frézel N, Dib-Hajj SD, Waxman SG. Expression of Nav1.7 in DRG neurons extends from peripheral terminals in the skin to central preterminal branches and terminals in the dorsal horn. *Mol Pain* 2012; **8**: 82.
- 27 Cummins TR, Howe JR, Waxman SG. Slow closed-state inactivation: a novel mechanism underlying ramp currents in cells expressing the hNE/PN1 sodium channel. *J Neurosci* 1998; **18**: 9607–19.
- 28 Weiss J, Pyrski M, Jacobi E, et al. Loss-of-function mutations in sodium channel Nav1.7 cause anosmia. *Nature* 2011; **472**: 186–90.
- 29 Minett MS, Nassar MA, Clark AK, et al. Distinct Nav1.7-dependent pain sensations require different sets of sensory and sympathetic neurons. *Nat Commun* 2012; **3**: 791.
- 30 Blair NT, Bean BP. Roles of tetrodotoxin (TTX)-sensitive Na<sup>+</sup> current, TTX-resistant Na<sup>+</sup> current, and Ca<sup>2+</sup> current in the action potentials of nociceptive sensory neurons. *J Neurosci* 2002; **22**: 10277–90.
- 31 Renganathan M, Cummins TR, Waxman SG. Contribution of Na(v)1.8 sodium channels to action potential electrogenesis in DRG neurons. *J Neurophysiol* 2001; **86**: 629–40.
- 32 Dib-Hajj S, Black JA, Cummins TR, Waxman SG. NaN/Nav1.9: a sodium channel with unique properties. *Trends Neurosci* 2002; **25**: 253–59.
- 33 Herzog RI, Cummins TR, Waxman SG. Persistent TTX-resistant Na<sup>+</sup> current affects resting potential and response to depolarization in simulated spinal sensory neurons. *J Neurophysiol* 2001; **86**: 1351–64.
- 34 Priest BT, Murphy BA, Lindia JA, et al. Contribution of the tetrodotoxin-resistant voltage-gated sodium channel Nav1.9 to sensory transmission and nociceptive behavior. *Proc Natl Acad Sci USA* 2005; **102**: 9382–87.
- 35 Ostman JA, Nassar MA, Wood JN, Baker MD. GTP up-regulated persistent Na<sup>+</sup> current and enhanced nociceptor excitability require Nav1.9. *J Physiol* 2008; **586**: 1077–87.
- 36 Rothier A, Baets J, Timmerman V, Janssens K. Mechanisms of disease in hereditary sensory and autonomic neuropathies. *Nat Rev Neurol* 2012; **8**: 73–85.
- 37 Crowley C, Spencer SD, Nishimura MC, et al. Mice lacking nerve growth factor display perinatal loss of sensory and sympathetic neurons yet develop basal forebrain cholinergic neurons. *Cell* 1994; **76**: 1001–11.
- 38 Einarsdottir E, Carlsson A, Minde J, et al. A mutation in the nerve growth factor beta gene (*NGFB*) causes loss of pain perception. *Hum Mol Genet* 2004; **13**: 799–805.
- 39 Indo Y, Tsuruta M, Hayashida Y, et al. Mutations in the *TRKA/NGF* receptor gene in patients with congenital insensitivity to pain with anhidrosis. *Nat Genet* 1996; **13**: 485–88.
- 40 Baxter DW, Olszewski J. Congenital universal insensitivity to pain. *Brain* 1960; **83**: 381–93.
- 41 Cox JJ, Reimann F, Nicholas AK, et al. An *SCN9A* channelopathy causes congenital inability to experience pain. *Nature* 2006; **444**: 894–98.
- 42 Goldberg YP, MacFarlane J, MacDonald ML, et al. Loss-of-function mutations in the Nav1.7 gene underlie congenital indifference to pain in multiple human populations. *Clin Genet* 2007; **71**: 311–19.
- 43 Ahmad S, Dahllund L, Eriksson AB, et al. A stop codon mutation in *SCN9A* causes lack of pain sensation. *Hum Mol Genet* 2007; **16**: 2114–21.
- 44 Cox JJ, Sheynin J, Shorer Z, et al. Congenital insensitivity to pain: novel *SCN9A* missense and in-frame deletion mutations. *Hum Mutat* 2010; **31**: E1670–86.
- 45 Staud R, Price DD, Janicke D, et al. Two novel mutations of *SCN9A* (Nav1.7) are associated with partial congenital insensitivity to pain. *Eur J Pain* 2011; **15**: 223–30.
- 46 Klein CJ, Wu Y, Kilfoyle DH, et al. Infrequent *SCN9A* mutations in congenital insensitivity to pain and erythromelalgia. *J Neurol Neurosurg Psychiatry* 2013; **84**: 386–91.
- 47 Nilsen KB, Nicholas AK, Woods CG, Mellgren SI, Nebuchennykh M, Aasly J. Two novel *SCN9A* mutations causing insensitivity to pain. *Pain* 2009; **143**: 155–58.
- 48 Yuan J, Matsuura E, Higuchi Y, et al. Hereditary sensory and autonomic neuropathy type IID caused by an *SCN9A* mutation. *Neurology* 2013; **80**: 1641–49.
- 49 Leipold E, Liebmann L, Korenke GC, et al. A *de novo* gain-of-function mutation in *SCN11A* causes loss of pain perception. *Nat Genet* 2013; **45**: 1399–404.
- 50 Mitchell SW. On a rare vaso-motor neurosis of the extremities, and on the maladies with which it may be confounded. *Am J Med Sci* 1878; **151**: 17–36.
- 51 Drenth JP, Waxman SG. Mutations in sodium-channel gene *SCN9A* cause a spectrum of human genetic pain disorders. *J Clin Invest* 2007; **117**: 3603–09.
- 52 Segerdahl AR, Xie J, Paterson K, Ramirez JD, Tracey I, Bennett DL. Imaging the neural correlates of neuropathic pain and pleasurable relief associated with inherited erythromelalgia in a single subject with quantitative arterial spin labelling. *Pain* 2012; **153**: 1122–27.
- 53 Cregg R, Laguda B, Werdehausen R, et al. Novel mutations mapping to the fourth sodium channel domain of Nav1.7 result in variable clinical manifestations of primary erythromelalgia. *Neuromolecular Med* 2013; **15**: 265–78.
- 54 Yang Y, Wang Y, Li S, et al. Mutations in *SCN9A*, encoding a sodium channel alpha subunit, in patients with primary erythromelalgia. *J Med Genet* 2004; **41**: 171–74.
- 55 Fischer TZ, Waxman SG. Familial pain syndromes from mutations of the Nav1.7 sodium channel. *Ann NY Acad Sci* 2010; **1184**: 196–207.
- 56 Yarov-Yarovsky V, Baker D, Catterall WA. Voltage sensor conformations in the open and closed states in ROSETTA structural models of K(+) channels. *Proc Natl Acad Sci USA* 2006; **103**: 7292–97.
- 57 Dib-Hajj SD, Rush AM, Cummins TR, et al. Gain-of-function mutation in Nav1.7 in familial erythromelalgia induces bursting of sensory neurons. *Brain* 2005; **128**: 1847–54.
- 58 Orstavik K, Weidner C, Schmidt R, et al. Pathological C-fibres in patients with a chronic painful condition. *Brain* 2003; **126**: 567–78.
- 59 Han C, Dib-Hajj SD, Lin Z, et al. Early- and late-onset inherited erythromelalgia: genotype-phenotype correlation. *Brain* 2009; **132**: 1711–22.
- 60 Han C, Lampert A, Rush AM, et al. Temperature dependence of erythromelalgia mutation L858F in sodium channel Nav1.7. *Mol Pain* 2007; **3**: 3.
- 61 Han C, Rush AM, Dib-Hajj SD, et al. Sporadic onset of erythromelalgia: a gain-of-function mutation in Nav1.7. *Ann Neurol* 2006; **59**: 553–58.
- 62 Choi JS, Zhang L, Dib-Hajj SD, et al. Mexiletine-responsive erythromelalgia due to a new Na(v)1.7 mutation showing use-dependent current fall-off. *Exp Neurol* 2009; **216**: 383–89.
- 63 Yang Y, Dib-Hajj SD, Zhang J, et al. Structural modelling and mutant cycle analysis predict pharmacoresponsiveness of a Na(V)1.7 mutant channel. *Nat Commun* 2012; **3**: 1186.
- 64 Goldberg YP, Price N, Namdari R, et al. Treatment of Na(v)1.7-mediated pain in inherited erythromelalgia using a novel sodium channel blocker. *Pain* 2012; **153**: 80–85.
- 65 Hayden R, Grossman M. Rectal, ocular, and submaxillary pain; a familial autonomic disorder related to proctalgia fugax: report of a family. *AMA J Dis Child* 1959; **97**: 479–82.
- 66 Fertleman CR, Ferrie CD, Aicardi J, et al. Paroxysmal extreme pain disorder (previously familial rectal pain syndrome). *Neurology* 2007; **69**: 586–95.
- 67 Choi JS, Boralevi F, Brissaud O, et al. Paroxysmal extreme pain disorder: a molecular lesion of peripheral neurons. *Nat Rev Neurol* 2011; **7**: 51–55.



- 68 Dib-Hajj SD, Estacion M, Jarecki BW, et al. Paroxysmal extreme pain disorder M1627K mutation in human Nav1.7 renders DRG neurons hyperexcitable. *Mol Pain* 2008; 4: 37.
- 69 Fertleman CR, Baker MD, Parker KA, et al. SCN9A mutations in paroxysmal extreme pain disorder: allelic variants underlie distinct channel defects and phenotypes. *Neuron* 2006; 52: 767–74.
- 70 Jarecki BW, Piekarz AD, Jackson JO 2nd, Cummins TR. Human voltage-gated sodium channel mutations that cause inherited neuronal and muscle channelopathies increase resurgent sodium currents. *J Clin Invest* 2010; 120: 369–78.
- 71 Estacion M, Dib-Hajj SD, Benke PJ, et al. Nav1.7 gain-of-function mutations as a continuum: A1632E displays physiological changes associated with erythromalgia and paroxysmal extreme pain disorder mutations and produces symptoms of both disorders. *J Neurosci* 2008; 28: 11079–88.
- 72 Hoeijmakers JG, Faber CG, Lauria G, Merkies IS, Waxman SG. Small-fibre neuropathies: advances in diagnosis, pathophysiology and management. *Nat Rev Neurol* 2012; 8: 369–79.
- 73 Devigili G, Tugnoli V, Penza P, et al. The diagnostic criteria for small fibre neuropathy: from symptoms to neuropathology. *Brain* 2008; 131: 1912–25.
- 74 Faber CG, Hoeijmakers JG, Ahn HS, et al. Gain of function Nav1.7 mutations in idiopathic small fiber neuropathy. *Ann Neurol* 2012; 71: 26–39.
- 75 Estacion M, Han C, Choi JS, et al. Intra- and interfamilial phenotypic diversity in pain syndromes associated with a gain-of-function variant of Nav1.7. *Mol Pain* 2011; 7: 92.
- 76 Han C, Hoeijmakers JG, Ahn HS, et al. Nav1.7-related small fiber neuropathy: impaired slow-inactivation and DRG neuron hyperexcitability. *Neurology* 2012; 78: 1635–43.
- 77 Persson AK, Liu S, Faber CG, Merkies IS, Black JA, Waxman SG. Neuropathy-associated Nav1.7 variant I228M impairs integrity of dorsal root ganglion neuron axons. *Ann Neurol* 2013; 73: 140–45.
- 78 Faber CG, Lauria G, Merkies IS, et al. Gain-of-function Nav1.8 mutations in painful neuropathy. *Proc Natl Acad Sci USA* 2012; 109: 19444–49.
- 79 Kremeyer B, Lopera F, Cox JJ, et al. A gain-of-function mutation in TRPA1 causes familial episodic pain syndrome. *Neuron* 2010; 66: 671–80.
- 80 Zhang XY, Wen J, Yang W, et al. Gain-of-function mutations in SCN11A cause familial episodic pain. *Am J Hum Genet* 2013; 93: 957–66.
- 81 Estacion M, Harty TP, Choi JS, Tyrrell L, Dib-Hajj SD, Waxman SG. A sodium channel gene SCN9A polymorphism that increases nociceptor excitability. *Ann Neurol* 2009; 66: 862–66.
- 82 Reimann F, Cox JJ, Belfer I, et al. Pain perception is altered by a nucleotide polymorphism in SCN9A. *Proc Natl Acad Sci USA* 2010; 107: 5148–53.
- 83 Valdes AM, Arden NK, Vaughn FL, et al. Role of the Nav1.7 R1150W amino acid change in susceptibility to symptomatic knee osteoarthritis and multiple regional pain. *Arthritis Care Res (Hoboken)* 2011; 63: 440–44.
- 84 Holliday KL, Thomson W, Neogi T, et al. The non-synonymous SNP, R1150W, in SCN9A is not associated with chronic widespread pain susceptibility. *Mol Pain* 2012; 8: 72.
- 85 Greenbaum L, Tegeder I, Barhum Y, Melamed E, Roditi Y, Djaldetti R. Contribution of genetic variants to pain susceptibility in Parkinson disease. *Eur J Pain* 2012; 16: 1243–50.
- 86 Duan G, Xiang G, Zhang X, Yuan R, Zhan H, Qi D. A single-nucleotide polymorphism in SCN9A may decrease postoperative pain sensitivity in the general population. *Anesthesiology* 2013; 118: 436–42.
- 87 Neely GG, Hess A, Costigan M, et al. A genome-wide Drosophila screen for heat nociception identifies  $\alpha 2\delta 3$  as an evolutionarily conserved pain gene. *Cell* 2010; 143: 628–38.
- 88 Costigan M, Belfer I, Griffin RS, et al. Multiple chronic pain states are associated with a common amino acid-changing allele in KCNS1. *Brain* 2010; 133: 2519–27.
- 89 Hendry L, Lombard Z, Wadley A, Kamberman P. KCNS1, but not GCH1, is associated with pain intensity in a black southern African population with HIV-associated sensory neuropathy: a genetic association study. *J Acquir Immune Defic Syndr* 2013; 63: 27–30.
- 90 Nissenbaum J, Devor M, Seltzer Z, et al. Susceptibility to chronic pain following nerve injury is genetically affected by CACNG2. *Genome Res* 2010; 20: 1180–90.
- 91 Sorge RE, Trang T, Dorfman R, et al. Genetically determined P2X7 receptor pore formation regulates variability in chronic pain sensitivity. *Nat Med* 2012; 18: 595–99.
- 92 von Hehn CA, Baron R, Woolf CJ. Deconstructing the neuropathic pain phenotype to reveal neural mechanisms. *Neuron* 2012; 73: 638–52.
- 93 Norbury TA, MacGregor AJ, Urwin J, Spector TD, McMahon SB. Heritability of responses to painful stimuli in women: a classical twin study. *Brain* 2007; 130: 3041–49.
- 94 Binder A, May D, Baron R, et al. Transient receptor potential channel polymorphisms are associated with the somatosensory function in neuropathic pain patients. *PLoS One* 2011; 6: e17387.
- 95 Tsantoulas C, Zhu L, Shaifita Y, et al. Sensory neuron downregulation of the Kv9.1 potassium channel subunit mediates neuropathic pain following nerve injury. *J Neurosci* 2012; 32: 17502–13.
- 96 Volonté C, Apolloni S, Skaper SD, Burnstock G. P2X7 receptors: channels, pores and more. *CNS Neurol Disord Drug Targets* 2012; 11: 705–21.
- 97 Goldstein DB. Common genetic variation and human traits. *N Engl J Med* 2009; 360: 1696–98.
- 98 Moore A, Derry S, Eccleston C, Kalso E. Expect analgesic failure; pursue analgesic success. *BMJ* 2013; 346: f2690.
- 99 Copel C, Clerc N, Osorio N, Delmas P, Mazet B. The Nav1.9 channel regulates colonic motility in mice. *Front Neurosci* 2013; 7: 58.
- 100 Scanio MJ, Shi L, Drizin I, et al. Discovery and biological evaluation of potent, selective, orally bioavailable, pyrazine-based blockers of the Na(v)1.8 sodium channel with efficacy in a model of neuropathic pain. *Bioorg Med Chem* 2010; 18: 7816–25.
- 101 Bezzina CR, Barc J, Mizusawa Y, et al. Common variants at SCN5A-SCN10A and HEY2 are associated with Brugada syndrome, a rare disease with high risk of sudden cardiac death. *Nat Genet* 2013; 45: 1044–49.
- 102 Emery EC, Young GT, Berrocoso EM, Chen L, McNaughton PA. HCN2 ion channels play a central role in inflammatory and neuropathic pain. *Science* 2011; 333: 1462–66.
- 103 Ludwig A, Budde T, Stieber J, et al. Absence epilepsy and sinus dysrhythmia in mice lacking the pacemaker channel HCN2. *EMBO J* 2003; 22: 216–24.
- 104 Borer JS, Fox K, Jaillon P, Lerebours G, and the Ivabradine Investigators Group. Antianginal and antiischemic effects of ivabradine, an I(f) inhibitor, in stable angina: a randomized, double-blind, multicentered, placebo-controlled trial. *Circulation* 2003; 107: 817–23.
- 105 Binshtok AM, Bean BP, Woolf CJ. Inhibition of nociceptors by TRPV1-mediated entry of impermeant sodium channel blockers. *Nature* 2007; 449: 607–10.
- 106 Foo JN, Liu JJ, Tan EK. Whole-genome and whole-exome sequencing in neurological diseases. *Nat Rev Neurol* 2012; 8: 508–17.
- 107 Mogil JS. Pain genetics: past, present and future. *Trends Genet* 2012; 28: 258–66.
- 108 Lacroix-Fralish ML, Ledoux JB, Mogil JS. The Pain Genes Database: an interactive web browser of pain-related transgenic knockout studies. *Pain* 2007; 131: 3.e1–4.
- 109 Perkins JR, Lees J, Antunes-Martins A, et al. PainNetworks: a web-based resource for the visualisation of pain-related genes in the context of their network associations. *Pain* 2013; 154: e1–12.
- 110 Chambers SM, Qi Y, Mica Y, et al. Combined small-molecule inhibition accelerates developmental timing and converts human pluripotent stem cells into nociceptors. *Nat Biotechnol* 2012; 30: 715–20.