Familial pain syndromes from mutations of the Na\textsubscript{v}1.7 sodium channel

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The literature currently suggests that voltage-gated sodium channels play a major role in the pathogenesis of neuropathic pain. Alterations in the expression and targeting of specific sodium channels within injured dorsal root ganglia neurons appear to predispose the neurons to abnormal firing properties, allowing for the development of neuropathic pain. Mutations of one particular sodium channel (Na\textsubscript{v}1.7) have been shown to cause inherited dorsal root ganglion pain syndromes, specifically in erythromelalgia and paroxysmal extreme pain disorder. Inherited erythromelalgia is the first human pain syndrome to be understood at a molecular level, having been linked to gain-of-function mutations of Na\textsubscript{v}1.7. Conversely, a loss-of-function of the Na\textsubscript{v}1.7 channel can produce channelopathy-associated insensitivity to pain. Therefore, the Na\textsubscript{v}1.7 channel may provide a unique target for the pharmacotherapy of pain in humans. In this review article we summarize current knowledge regarding several different disease manifestations arising from changes within the Na\textsubscript{v}1.7 channel.

Keywords: neuropathic pain; sodium channels; erythromelalgia; paroxysmal extreme pain disorder; channelopathy-associated insensitivity to pain; dorsal root ganglion; voltage clamp

Introduction

Primary nociceptive or pain-signaling sensory neurons, specifically within dorsal root ganglia (DRG) and trigeminal ganglia, constitute the peripheral entry point of the pain pathway. Usually, these neurons are relatively quiescent. When stimulated, these neurons produce a series of action potentials, allowing information about the external sensory world to be transmitted to the brain. Injury to these neurons causes them to become hyperexcitable, thus giving rise to abnormal, unprovoked spontaneous action potentials or pathological bursting, which results in chronic pain.\textsuperscript{1-4}

In mammalian neurons, voltage-gated sodium channels produce action potentials in response to membrane depolarization. Sodium channels are composed of a single \(\alpha\)-subunit and several auxiliary \(\beta\)-subunits.\textsuperscript{5} The \(\alpha\)-subunit endows the channel with voltage sensitivity and forms the ion-selective pore, while the \(\beta\)-subunits appear to influence channel gating and targeting.\textsuperscript{6-10} Here we focus on sodium channel \(\alpha\)-subunits which, for brevity, we will refer to as sodium channels (Na\textsubscript{v}). Although the literature indicates that there are multiple sodium channels, only seven (Na\textsubscript{v}1.1, Na\textsubscript{v}1.2, Na\textsubscript{v}1.3, Na\textsubscript{v}1.6, Na\textsubscript{v}1.7, Na\textsubscript{v}1.8, and Na\textsubscript{v}1.9) have been found in the nervous system.\textsuperscript{11} These different sodium channels share a common structure but are encoded by different genes and manifest distinct voltage-dependent and kinetic properties. Sodium channels are expressed in a regionally and temporally specific pattern in the nervous system. Interestingly, the majority of neurons express multiple sodium channel isoforms, with different ensembles of sodium channel isoforms endowing different types of neurons with unique functional properties.\textsuperscript{12}

Multiple sodium channels in dorsal root ganglion neurons

It has been well established through early electrophysiologic studies that DRG neurons produce
multiple, distinct sodium currents, which can be distinguished from one another by their differences in voltage dependence, kinetics, and sensitivity to the neurotoxin tetrodotoxin (TTX). Importantly, multiple distinct sodium currents have been recorded from individual neurons, suggesting that multiple types of sodium channels are present within them.

Of the known neuronally expressed sodium channels, Na\(_v\)1.7, Na\(_v\)1.8, and Na\(_v\)1.9 are preferentially expressed in DRG and trigeminal ganglia neurons, most of which are nociceptive. The Na\(_v\)1.7 channel is expressed in almost all DRG neurons at various levels, while the Na\(_v\)1.8 and Na\(_v\)1.9 channels are primarily expressed in small DRG neurons that include nociceptors. The Na\(_v\)1.8 and Na\(_v\)1.9 channels are also distinguished from the Na\(_v\)1.7 channel by their resistance to TTX. Expression of the Na\(_v\)1.8 and Na\(_v\)1.9 channels are both downregulated in DRG neurons after sciatic nerve transection but are elevated under inflammatory conditions. Although Na\(_v\)1.3 channels are not expressed above background levels in adult DRG neurons, the Na\(_v\)1.3 channel has been shown to be upregulated in DRG neurons following injury. Therefore, along with the Na\(_v\)1.8 and Na\(_v\)1.9 channels, Na\(_v\)1.3 has been suggested to play a role in neuropathic pain.

Multiple studies have shown the importance of voltage-gated ion channels in the perception and transmission of pain. For example, a mouse knockout model of the voltage-gated Na\(_v\)1.8 channel results in animals with deficits of thermal pain perception and visceral pain perception but not neuropathic pain. When Na\(_v\)1.7 is selectively knocked out of nociceptive neurons, the mice display markedly reduced inflammatory pain responses and a deficit in heat-induced pain threshold, while the mechanical pain and cold-evoked thresholds appear to be intact. On the other hand, in mice, global knockout of Na\(_v\)1.7 has been found to be lethal, possibly due to a failure to feed. Interestingly, when both Na\(_v\)1.7 and Na\(_v\)1.8 channels were knocked out of mice, alterations only in inflammatory pain were seen.

In humans, point mutations within the SCN9A gene, which codes for the Na\(_v\)1.7 channel, have been associated with two different pain syndromes caused by a “gain-in-function” of the channel: inherited erythromelalgia (IEM) and paroxysmal extreme pain disorder (PEPD). Conversely, several cases have been described in the literature of otherwise healthy patients with a congenital inability to experience pain, arising from nonsense mutations of Na\(_v\)1.7, which produce truncated, nonfunctional proteins. These various studies, both in animals and in humans, have helped to establish ion channels, especially Na\(_v\)1.7, as a major factor of peripheral nociception.

**Inherited erythromelalgia**

IEM, originally described by S. Weir Mitchell in 1878, is characterized by intense episodic burning pain associated with redness and warmth of the affected extremities. In the absence of an underlying cause (such as in myeloproliferative diseases or as a side effect of medication), it is termed primary erythromelalgia and in many instances occurs as an autosomal dominant trait.

Clinical onset of the inherited form of this disease has been reported as early as 1 year of age, often manifesting itself before the end of the first decade of life. Attacks are described by patients as a burning pain (which patients describe as excruciating, “like hot lava poured into my body”) with accompanying redness in the distal extremities (feet, sometimes hands) in response to warm stimuli or moderate exercise. A characteristic feature is relief obtained by immersing the extremities in ice, which can lead to ulceration and gangrene. In the absence of a clear etiology, treatment has been empirical and is partially effective at best. IEM shares a number of clinical features with, and is sometimes confused with, reflex sympathetic dystrophy (RSD) since both are characterized by severe pain and vasomotor disturbances, but in contrast to RSD, erythromelalgia is bilateral and symmetric. Since attacks are episodic and can be counted, IEM appears to provide a human model that is especially tractable for therapeutic trials.

**Mutations of Na\(_v\)1.7: genetic analysis and expression studies**

Importantly, IEM is the first known inherited painful neuropathy to be examined at a molecular level. Until recently the pathogenesis of this disease...
was unknown. Hypotheses encompassing vascular shunting, neuropathic etiologies, microvascular etiologies, and inflammatory etiologies have all been suggested.

In 2004, Yang and coworkers, using linkage analysis, identified two independent point mutations in the gene encoding Na\(_{\text{v}}\)1.7 in two separate families in China with IEM. A single amino-acid substitution (F1449V) was subsequently identified in the Na\(_{\text{v}}\)1.7 channel in another large American family. In each of these kindreds, all affected family members carried this mutation, while it was absent in unaffected family members and in the alleles from an ethnically matched control group. A number of other mutations, all in Na\(_{\text{v}}\)1.7, have subsequently been found in other families with IEM. Thus far, penetrance appears to be close to 100% for IEM mutations.

Consistent with a role in painful neuropathy, Na\(_{\text{v}}\)1.7 channels are preferentially expressed in nociceptive DRG neurons and their nerve endings. In these neurons, Na\(_{\text{v}}\)1.7 produces “threshold currents” close to resting potential, amplifying small depolarizations such as generator potentials, causing a hyperpolarizing shift in activation and slow deactivation. The hyperpolarizing shift in the voltage dependence of activation of the Na\(_{\text{v}}\)1.7 channel (which makes it easier to open the channel) and the slowed deactivation (which keeps the channel open longer once it is activated), in turn, are expected to decrease the threshold for action potential generation in sensory neurons, thus increasing neuronal excitability. The response of Na\(_{\text{v}}\)1.7 channels to slow ramp depolarizations (e.g., 0.2 mV/ms depolarizations from −100 to −20 mV) (Fig. 1D) are also significantly enhanced compared with wild-type (WT) channels. Because the ramp currents are evoked close to the resting potential of DRG neurons, the larger ramp currents in DRG neurons expressing IEM mutations of Na\(_{\text{v}}\)1.7 are poised to amplify the response to small depolarizing inputs. This, in turn, would increase excitability.

Subsequent studies used current-clamp recording to directly show that this mutation renders sensory neurons hyperexcitable. When DRG neurons, a cell type known to express Na\(_{\text{v}}\)1.7 channels, are transfected with WT Na\(_{\text{v}}\)1.7 channels, they produce all-or-none action potentials in response to injections of 135 pA or greater (Fig. 2A). However, in DRG neurons expressing the L858H IEM mutant channel, a much lower current input is required for the generation of an action potential (Fig. 2B). The current threshold is significantly decreased, by more than 40%, in cells expressing IEM mutant channels, such as the L858H Na\(_{\text{v}}\)1.7 mutation, compared with WT Na\(_{\text{v}}\)1.7 channels (Fig. 2C). IEM mutations also enhance repetitive firing in DRG neurons (Fig. 3B) compared with WT cells (Fig. 3A). These experiments show that an IEM mutation of Na\(_{\text{v}}\)1.7 can produce hyperexcitability (decreased threshold and enhanced repetitive firing) within DRG neurons, providing a molecular explanation for the pain experienced in patients with IEM.

Our group has found de novo “founder” mutations that increase DRG neuron excitability in several sporadic cases of erythromelalgia, and thus has indicted Na\(_{\text{v}}\)1.7 mutations in some individuals who do not have a family history of the disorder. In addition, several mutations in the gene for Na\(_{\text{v}}\)1.7 have been found, which appear to modulate the channel’s sensitivity to local anaesthetics. Choi and colleagues identified a new Na\(_{\text{v}}\)1.7 mutation, V872G, in a patient who reported pain relief with mexiletine and observed that the mutation resulted in stronger use-dependent current fall-off when the channels were exposed to mexiletine. Fischer and colleagues reported a family with carbamazepine (CBZ)-responsive IEM and demonstrated that the mutation confers CBZ-sensitivity in the mutant channel.

While missense mutations in Na\(_{\text{v}}\)1.7 channel have been found in many families with IEM, this disease may be genetically heterogeneous because some cases of familial early-onset and adult-onset IEM do not have mutations in the coding exons of SCN9A. To date, mutations within the coding regions of the Na\(_{\text{v}}\)1.8 channel and the Na\(_{\text{v}}\)1.9 channel have not been observed in these cases of IEM, suggesting that other target genes or mutations in the noncoding regions of SCN9A, the gene encoding Na\(_{\text{v}}\)1.7, may result in the IEM phenotype.
Figure 1. The L858H mutation produces a gain-of-function in Na\textsubscript{v}1.7. (A) Schematic of the Na\textsubscript{v}1.7 channel. The L858H mutation substitutes a single amino acid within the domain II/S4-S5 linker. (B) Cells expressing the mutant L858H channel display a hyperpolarizing shift in activation. (C) Representative tail currents of WT and L858H channels, showing that the mutant channel causes slow deactivation. (D) Time constants for tail current deactivation are increased for L858H Na\textsubscript{v}1.7 channels. (E) Representative ramp currents in HEK293 cells expressing WT or L858H channels, showing that cells expressing the mutant channel generate larger currents in response to small, slow depolarizations. This may, in turn, allow for an earlier initiation of the action potential by the mutant channel. (Adapted with permission from Cummins and colleagues.\textsuperscript{43} Panels B–D are from Fig. 1, and Panel E is from Fig. 3 of that article.)

**Paroxysmal extreme pain disorder**

A second autosomal dominant pain disorder, resulting from a different set of gain-of-function mutations that impair inactivation of Na\textsubscript{v}1.7, is PEPD.\textsuperscript{59–61} Severe pain in PEPD patients, accompanied by autonomic manifestations such as skin flushing, can start as early as infancy.\textsuperscript{62} The pain attacks are most severe in the lower part of the body and can be triggered by a bowel movement or probing of the perianal area; they may also be accompanied by tonic nonepileptic seizures, bradycardia, and/or apnea, which appears to be more common in infancy and young children.\textsuperscript{62} The cause for the seizure-like activity and cardiac symptoms is not well understood at this time. As the person ages, the
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Figure 2. L858H renders DRG neurons hyperexcitable. Representative traces from cells expressing WT Na\textsubscript{\textalpha}1.7 (A) and the L858H IEM mutant channel (B) show that action potentials were evoked with smaller depolarizing stimuli in cells housing the mutant channel. (C) The average current threshold for action potential firing of DRG neurons expressing WT Na\textsubscript{\textalpha}1.7 channels is significantly greater than that of neurons expressing L858H mutant channels, indicating that the L858H mutation renders sensory neurons hyperexcitable. (Adapted with permission from Rush and colleagues.\textsuperscript{54} Panels A–C are from Fig. 1 of that article.)

Mutations of Na\textsubscript{\textalpha}1.7: genetic analysis and expression studies

The first described PEPD mutations, located in domain III and IV of Na\textsubscript{\textalpha}1.7, were reported in 2006 to impair fast-inactivation without altering channel activation, leading to a persistent current that is attenuated by CBZ.\textsuperscript{60} Dib-Hajj and collaborators\textsuperscript{59} extended the initial analysis to examine other gating properties of the M1627K mutant Na\textsubscript{\textalpha}1.7 channel from a family with PEPD, showing that the mutant channel recovers from fast-inactivation faster than the WT channel and produces larger currents in response to ramp stimuli. Figure 4 shows whole cell currents from representative human embryonic kidney (HEK) cells expressing WT Na\textsubscript{\textalpha}1.7 (Fig. 4B) and the M1627K mutant channel (Fig. 4C), elicited with a series of depolarizing test pulses. The peak current-voltage relationship between the WT channels and the mutant channels were about the same (Fig. 5A), and the time constants for deactivation were not altered for the M1627K channel (Fig. 5B), indicating that the M1627K mutation does not alter Na\textsubscript{\textalpha}1.7 activation properties. However, the voltage-dependence of steady-state fast-inactivation...
Figure 3. The L858H mutation increases firing frequency in DRG neurons. (A) Representative DRG neuron expressing WT Na\textsubscript{v}1.7 fires a single action potential in response to a current injection. (B) Representative DRG neuron expressing L858H fires five action potentials in response to the same current injection. (C) For the entire population of DRG neurons studied, the firing frequency evoked by 50-pA current stimuli was 0.32 ± 0.13 Hz after transfection with WT channels and 2.06 ± 0.79 Hz after transfection with L858H, and the firing frequency evoked by 100-pA stimuli was 0.89 ± 0.28 Hz after transfection with WT and 3.37 ± 1.13 Hz after transfection with L858H (*, \(P < 0.05\)). (Adapted with permission from Rush and colleagues.\textsuperscript{54} Panels A–C are from Fig. 3 of that article.)

Figure 4. Currents produced by PEPD mutant M1672K decay slower than WT Na\textsubscript{v}1.7 currents. (A) Schematic of the Na\textsubscript{v}1.7 channel. The M1627K substitution is located in the domain IV/S4-S5 linker. Representative WT (B) and M1627K (C) Na\textsubscript{v}1.7 currents are shown. Cells were held at −100 mV and currents were elicited with 50-ms test pulses to potentials ranging from −80 to 40 mV. For better comparison, WT and M1627K currents elicited with −30 mV (D) and +25 mV (E) depolarizations are shown superimposed. Although the rate of activation is not apparently altered, the decay phase is slowed for mutant channels, indicating an abnormality of channel inactivation. (Adapted with permission from Dib-Hajj and colleagues.\textsuperscript{59} Panels B–E are from Fig. 2 of this article.)

was dramatically shifted in the depolarizing direction by the M1627K mutation (Fig. 5C). Similar changes in fast-inactivation have been seen with some\textsuperscript{40,45,46} but not all IEM mutations.\textsuperscript{42,43,47,56} The ramp currents elicited with slow ramp depolarizations in the M1627K PEPD mutation were significantly larger for the mutant channels than for the WT channels (Fig. 5D). Using current clamp, Dib-Hajj and collaborators demonstrated that the M1627K mutation reduces the threshold for generation of single action potentials and increases the number of action potentials in DRG neurons in response to graded stimuli,\textsuperscript{59} thus showing that the mutant channel renders DRG neurons hyperexcitable (Fig. 6). These data provide a link between altered channel properties and the pain symptoms.
Figure 5. The M1627K mutation alters inactivation properties of Nav1.7. (A) Normalized peak current-voltage relationship for WT and M1627K channels, showing that channel activation is not substantially altered by the M1627K mutation. (B) The time constants for deactivation, which reflect the channel's transition from an open to a closed state, were also not significantly altered between cells expressing WT Nav1.7 or M1627K channels. (C) Steady-state fast-inactivation of the M1627K Nav1.7 channels is shifted in a depolarizing direction. (D) The average relative ramp current (ramp current divided by peak transient current amplitude) is larger for M1627K than for WT. (Adapted with permission from Dib-Hajj and colleagues. Panels A–C are from Fig. 3 and Panel D is from Fig. 6 of that article.)

experienced by PEPD patients. Why IEM patients experience pain in the feet and hands, triggered by warmth, while PEPD patients experience pain in the perirectal area, triggered by rectal stimuli and then perimandibular and/or periocular pain, is not currently known.

The A1632E mutation—part of a continuum between IEM and PEPD

In 2008 a new mutation in Na\textsubscript{1.7}, A1632E, was described\textsuperscript{63} in a patient with a unique mixture of symptoms, which included clinical characteristics of both IEM and PEPD. This mutation alters a conserved amino acid within the linker between transmembrane segments S4 and S5 of domain IV, which is close to the PEPD mutation M1627K.\textsuperscript{59,60} The amino acid sequence within this loop is highly conserved among most sodium channels, excluding Na\textsubscript{1.9}. The patient described with this particular mutation had had apnea, bradycardia, and poor feeding since birth. The bradycardia episodes were often precipitated by touching, feeding, urination, or defecation. As the patient grew older, the patient also complained of episodes of pain described as “hot needles” in the feet, hands, and head, often precipitated by warmth and attenuated by cooling.

The A1632E mutation from this patient was shown to have biophysical characteristics common to both IEM and PEPD mutations, altering activation and inactivation (Fig. 7A), deactivation (Fig. 7B), and slow ramp currents (Fig. 7C) of the channel.\textsuperscript{63} Figure 8 shows diagrammatically how this particular mutation exhibits electrophysiological properties commonly seen in patients with either PEPD or IEM characteristics.\textsuperscript{63} The A1632E
mutation and its associated phenotype suggest that IEM and PEPD mutations may be part of a physiological continuum that can produce a continuum of clinical pictures.

Channelopathy-associated insensitivity to pain

Subsequent to the identification of the role of the Na$_v$1.7 sodium channel in IEM, Cox and coworkers$^{33}$ reported several families in which loss-of-function mutations of Na$_v$ 1.7 were associated with profound insensitivity to pain; other sensory modalities were preserved and the remainder of the patients’ central and peripheral nervous systems were intact by report. Affected individuals displayed painless burns, fractures, and injuries of the lips and tongue, and were reported never to have felt pain in any part of the body, in response to any injury or noxious stimulus. The patients did not appear to exhibit autonomic or motor abnormalities, and reportedly had normal tear formation, sweating ability, reflexes, and intelligence. Cox and coworkers$^{33}$ found that in all three of the studied families, distinct, homozygous nonsense mutations of Na$_v$1.7 produced truncated, nonfunctional proteins. Patch-clamping experiments, done in HEK 293 cells co-expressing either WT or mutant channels together with β$_1$ and β$_2$ subunits, showed a loss-of-function in cells containing the mutant
Figure 7. A1632E shifts both activation and fast inactivation of the mutant channel, as previously observed in IEM and PEPD mutations, respectively. (A) Activation and fast inactivation curves for WT and mutant cells are shown. The mutant cells exhibit hyperpolarized activation and impaired fast inactivation when compared with cells expressing the WT channel. (B) The A1632E mutation also slows deactivation, thus causing the channel to close more slowly than the WT channel. This feature has been observed in Na\textsubscript{1.7} mutations that produce IEM.

Figure 8. A comparison of IEM and PEPD mutations. This diagram plots the shifts seen in known IEM mutations (open squares) and PEPD mutations (gray circles). The WT control is plotted as a black diamond at (0,0). The dotted lines through (0,0) demarcate between positive and negative shifts and indicate the outcome for the shifts. The A1632E mutation from a patient with clinical characteristics of both IEM and PEPD is plotted with the star symbol and shows shifts in activation and inactivation common to both IEM and PEPD mutants. The identity of each numbered symbol is as follows: 1, T1464I (Fertleman et al., 2006); 2, V1298F (Jarecki et al., 2008); 3, V1299F (Jarecki et al., 2008); 4, I1461T (Jarecki et al., 2008); 5, M1627K (Fertleman, 2006); 6, I1461T (Fertleman, 2006); 7, I136V (Cheng et al., 2008); 8, S241T (Lampert et al., 2006); 9, F1449V (Dib-Hajj, 2005); 10, A863P (Harty et al., 2006); 11, L858F (Han et al., 2006); 12, F216S (Choi et al., 2006); 13, L858H (Cummins et al., 2004); and 14, I848T (Cummins et al., 2004). (Adapted with permission from Estacion and colleagues. 63 This is Fig. 8 in that article.) Additional families with similar loss-of-function mutations of Na\textsubscript{1.7} and a similar clinical picture were subsequently reported by Ahmad and colleagues\textsuperscript{32} and Goldberg and colleagues.\textsuperscript{34} The example data traces comparing the deactivation during repolarization to -50 mV are shown in the inset. (C) The ramp response is enhanced by the A1632E mutant channels. (Adapted with permission from Estacion and colleagues.\textsuperscript{63} Panels A and B are from Fig. 2 and Panel C is from Fig. 3 of that article.)
remarkably dense loss of pain sensibility in these patients provides strong evidence for a central function of Na\textsubscript{v}1.7 in human pain.

**Conclusion**

It is becoming clear that gain-of-function mutations of Na\textsubscript{v}1.7 cause syndromes characterized by severe pain\textsuperscript{39–47,55,59,60,63–65} while loss-of-function mutations in Na\textsubscript{v}1.7 produce insensitivity to pain.\textsuperscript{32–34} Upregulated expression of Na\textsubscript{v}1.7, together with Na\textsubscript{v}1.8, another sodium channel isoform that works together with Na\textsubscript{v}1.7 to produce high-frequency firing of DRG neurons, occurs within injured axons in human painful neurons.\textsuperscript{66} These observations in humans, together with observations in rodent models that show upregulation of Na\textsubscript{v}1.7 within nociceptive DRG neurons in response to inflammation within their peripheral projection fields,\textsuperscript{67} and in which knockout of Na\textsubscript{v}1.7 in nociceptors is associated with attenuated inflammatory pain responses,\textsuperscript{31} establish Na\textsubscript{v}1.7 as a critically important molecule along the pain pathway, where it appears to set the gain on pain responses.\textsuperscript{27} The recent observations in humans have accelerated the search for Na\textsubscript{v}1.7-specific blockers as potential pain therapeutics. Whether these agents, once identified, will alleviate pain in a clinically useful manner remains to be established.

**Conflicts of interest**

The authors declare no conflicts of interest.

**References**


