Mutations in sodium-channel gene SCN9A cause a spectrum of human genetic pain disorders

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The voltage-gated sodium-channel type IX α subunit, known as Na⁺,v1.7 and encoded by the gene SCN9A, is located in peripheral neurons and plays an important role in action potential production in these cells. Recent genetic studies have identified Na⁺,v1.7 dysfunction in three different human pain disorders. Gain-of-function missense mutations in Na⁺,v1.7 have been shown to cause primary erythermalgia and paroxysmal extreme pain disorder, while nonsense mutations in Na⁺,v1.7 result in loss of Na⁺,v1.7 function and a condition known as channelopathy-associated insensitivity to pain, a rare disorder in which affected individuals are unable to feel physical pain. This review highlights these recent developments and discusses the critical role of Na⁺,v1.7 in pain sensation in humans.

Pain is one of the most pervasive symptoms in clinical medicine; it occurs in a multitude of clinical conditions and is encountered by clinicians in every subspecialty. Yet treatment of chronic or recurrent pain remains challenging, in part because the therapeutic armamentarium is incomplete. Hopefully, this will change as a result of increased understanding of the molecular basis of pain. Over the past several years, elucidation of the genetic defects underlying three monogenic pain disorders has provided important insights about human pain and its molecular substrates. Here, we briefly review these recent advances.

A genetic basis for pain

The unraveling of the human genome may allow us to compare variations at the genetic level with interindividual differences in pain thresholds and pain perception. Most studies in the past have focused on genetic polymorphisms that might be responsible for interindividual differences in pain perception. For example, a common functional SNP (V58M) in the catechol-O-methyltransferase (COMT) gene modifies pain sensitivity (1). COMT has broad biological functions, including the metabolism of catecholamines, such as neurotransmitters, that modulate neuronal cell signaling. Individuals homozygous for the Val genotype are less sensitive to pain compared with those with Met homozygosity (1); however, the differences in pain sensitivity between groups are relatively subtle. A more dramatic set of observations has been reported in studies of rare Mendelian disorders. Over a decade ago, mutations in the voltage-dependent calcium channel, P/Q type 1A α subunit (CACNL1A4) were identified in families with familial hemiplegic migraine, a subtype of migraine with aura and paralysis (2). This finding indicated that channel dysfunction could lead to human disorders in which pain is a prominent symptom. More recent genetic studies have identified the voltage-gated sodium-channel type IX α subunit (SCN9A, referred to herein as Na⁺,v1.7) as a key player in three conditions in which recurrent pain or the inability to sense pain is a prominent symptom (3–8). These disorders — primary erythermalgia (PE), paroxysmal extreme pain disorder (PEPD), and channelopathy-associated insensitivity to pain (CIP) — are typified by very different pain phenotypes. Remarkably, recent work has shown that different types of channelopathies (diseases caused by disturbed function of ion channel subunits or the proteins that regulate them), all involving the same Na⁺,v1.7 sodium channel, underlie all three of these disorders (3–8). These discoveries allow better understanding not only of the molecular pathogenesis of these particular disorders but also of the molecular pathophysiology of pain (9).

Sodium channels

Voltage-gated sodium channels play a critical role in the generation and conduction of action potentials and are thus important for electrical signaling by most excitable cells (10, 11). Sodium channels are integral membrane proteins and are comprised of a large α subunit, which forms the voltage-sensitive and ion-selective pore, and smaller auxiliary β subunit(s) that can modulate the kinetics and voltage dependence of channel gating (12). To date, we know of 9 isoforms of the sodium-channel α subunit (Na⁺,v1.1–Na⁺,v1.9), each with a unique central and peripheral nervous system distribution (10). Four closely related sodium channels (Na⁺,v1.1, -1.2, -1.3, and -1.7) are encoded by a set of 4 genes (SCN1A, SCN2A, SCN3A, and SCN9A, respectively) located within a cluster on chromosome 2q24.3. Mutations in the genes encoding Nav1.1, -1.2, and -1.3 are responsible for a group of epilepsy syndromes with overlapping clinical characteristics but divergent clinical severity (13, 14). Here, we focus on one of the α subunits, Na⁺,v1.7, because of its critical role in pain sensation.

Na⁺,v1.7 is encoded by SCN9A, a 113.5-kb gene comprising 26 exons (OMIM 603415) (Figure 1A). The encoded sodium channel is composed of 1977 amino acids organized into 4 domains, each with 6 transmembrane segments (15), and is predominantly expressed in the dorsal root ganglion (DRG) neurons and sympathetic ganglion neurons (16) (Figure 1B). Immunohistochemical studies show that Na⁺,v1.7 is present at the distal ends of the wire-like projections...
of neurons known as neurites, close to the impulse trigger zone where neuronal firing is initiated (16) (Figure 2). Interestingly, the large majority of DRG neurons that express Na\(_v\)1.7 are pain sensing (nociceptive), suggesting a role for this sodium channel in the pathogenesis of pain (17). In addition to Na\(_v\)1.7, Na\(_v\)1.8 and Na\(_v\)1.9 are also predominantly present in small nociceptive sensory neurons and the nerve fibers emanating from them (18, 19).

**Physiology of Na\(_v\)1.7**

In sensory neurons, multiple voltage-dependent sodium currents can be differentiated by their gating kinetics and voltage dependence and can also be defined by their sensitivity to the voltage-gated sodium-channel blocker tetrodotoxin (12). The Na\(_v\)1.7 channel produces a rapidly activating and inactivating current that is sensitive to submicromolar levels of tetrodotoxin. This is in contrast with Na\(_v\)1.8, which is also present within DRG neurons but is fairly resistant to tetrodotoxin. Na\(_v\)1.7 appears to be important in early phases of neuronal electogenesis. Na\(_v\)1.7 is characterized by slow transition of the channel into an inactive state when it is depolarized, even to a minor degree, a property that allows these channels to remain available for activation with small or slowly developing depolarizations, usually mimicked by electrophysiologists as ramp-like stimuli (20). Thus, Na\(_v\)1.7 acts as a “threshold channel” that amplifies small, subtle depolarizations such as generator potentials, thereby bringing neurons to voltages that stimulate Na\(_v\)1.8, which has a more depolarized activation threshold and

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**Figure 1**

Mutations in the sodium-channel subunit Na\(_v\)1.7 that are associated with the genetic pain disorders PE, PEPD, and CIP. (A) Na\(_v\)1.7 is encoded by the 113.5-kb gene SCN9A, comprising 26 coding exons. The identity and location of known patient mutations in Na\(_v\)1.7 that have been linked to PE (*), PEPD (^), and CIP (#) are shown. Note that the mutations are spread over the entire gene sequence; however, mutations linked to PEPD tend to be located closer to the 3′ end of the gene. (B) A schematic of the Na\(_v\)1.7 sodium-channel subunit showing the 4 domains (D1–D4), each with 6 transmembrane segments. Locations of known mutations associated with genetic pain disorders PE, PEPD, and CIP are shown. COOH indicates the C-terminus of the peptide chain. HN indicates the N-terminus of the peptide chain.
which produces most of the transmembrane current responsible for the depolarizing phase of action potentials (21). In this regard, Na\(_{\text{v}}\)1.7 is poised as a molecular gatekeeper of pain detection at peripheral nociceptors.

**Inflammatory mediators and pain**

A number of (inflammatory) mediators, such as prostaglandin (22), adenosine (23), and serotonin (24), affect the electrophysiological properties of voltage-gated sodium channels. These mediators increase the magnitude of the current, lead to activation of the channel at more hyperpolarized potentials, and enhance the rates of channel activation and inactivation. As a consequence, inflammation can sensitize nociceptive neurons. In an experimental model of inflammatory pain in which an irritant was injected into the hind paw in rats, Na\(_{\text{v}}\)1.7 protein expression was upregulated within DRG neurons that project their axons to the inflamed area (25), a change that should increase excitability of these cells. Collectively, these data suggest that Na\(_{\text{v}}\)1.7 contributes, at least in part, to pain associated with inflammation.

**Animal studies of Nav1.7**

To obtain insight into the physiological role of Na\(_{\text{v}}\)1.7, Nassar et al. generated targeted knockout mice that lack Na\(_{\text{v}}\)1.7 within nociceptive DRG neurons (26). Selective deletion of Na\(_{\text{v}}\)1.7 in nociceptors from mice produces a phenotype in which heat-induced pain thresholds are minimally altered, there is no change in punctate mechanical pain threshold, and cold-evoked channel activity is unchanged. In contrast, there is a general failure to develop pain or hypersensitivity in response to inflammatory stimuli, while neuropathic pain (chronic pain resulting from injury to the nervous system) remains intact. These results are consistent with an important role of Na\(_{\text{v}}\)1.7 in setting the inflammatory pain threshold. To assess the role of Na\(_{\text{v}}\)1.7 further, especially in relation to other sodium channels expressed in peripheral sensory neurons, the same researchers created mice deficient in both Na\(_{\text{v}}\)1.7 and Na\(_{\text{v}}\)1.8 (27). Mice deficient in Na\(_{\text{v}}\)1.8 had deficits in sensing inflammatory pain (initiated by tissue damage/inflammation) and visceral pain (initiated by damage or injury to internal organs) but not neuropathic pain (28). The thermal pain threshold in mice deficient in both Na\(_{\text{v}}\)1.7 and Na\(_{\text{v}}\)1.8 mice was twice that of mice lacking only Na\(_{\text{v}}\)1.7. There was no effect on induced neuropathic pain in the double knockouts, and the effect of the loss of Na\(_{\text{v}}\)1.7 in raising the threshold for inflammatory pain was so overwhelming that no additional effect of Na\(_{\text{v}}\)1.8 deletion was seen. Collectively, these results clearly implicate Na\(_{\text{v}}\)1.7 as a major sodium channel in peripheral nociception and suggest a functional link to Na\(_{\text{v}}\)1.8. Although insightful, these data should be interpreted with caution, as direct evaluation of pain in mice is not possible. Instead, researchers rely on behavioral changes of animals such as signs of paw guarding, lifting, and limping. As a consequence, the relevance of the observed changes to human pain remains to be determined.

**Primary erythermalgia**

Primary or idiopathic erythermalgia (OMIM 133020) is an autosomal dominant, inherited disorder. Clinically, PE is characterized by attacks or episodes of symmetrical burning pain of the feet, lower legs, and sometimes hands, elevated skin temperature of affected areas, and reddened extremities (Figure 3) (29–32). PE is sometimes termed erythromelalgia, although some authorities reserve the latter term for a condition that is caused by arteriolar inflammation as a result of platelet-rich thrombi in the end-arterial microvasculature, in which the platelet count is invariably elevated (> 400 \(\times\) 10\(^9\) cells/l) and a short course of aspirin brings swift relief (33). Platelet counts in PE are invariably normal, and aspirin is ineffective. Patients with PE usually develop symptoms within the first decade of life. As the disease progresses, the erythema can extend to the upper legs, nose tip, earlobes, and chin. In the early years of the disease, the erythema is intermittent, but at later ages, the feet and hands may be constantly red and edematous. Complaints are provoked by...
exercise, prolonged standing, or exposure to warmth, which usually compels patients not to wear socks or closed shoes, even during the winter. Patients typically sleep with uncovered feet, often cooled by a fan. Cold alleviates these complaints, and some patients search for relief by immersion of feet in ice-cold water. The greatest threat is that these actions can lead to trench foot with subsequent skin infections and even to limb amputations (34).

A genome-wide linkage study in a large kindred of individuals with PE detected strong evidence for linkage with polymorphic markers on chromosome 2q (35). Haplotype analysis in four additional families confirmed the locus, and recombinant events defined the critical interval to 7.94 cM. Subsequent analysis of another family allowed narrowing of the region to 5.98 cM (3). This interval contains five genes encoding sodium-channel subunits. After confirming the presence of this genetic interval in two affected families, two candidate genes, including SCN9A, were tested (3). A missense mutation (L858H) in SCN9A was identified that segregated with the disease in a three-generation Chinese family while an I848T mutation was present in a single sporadic case. Both mutations affected conserved residues in the pore-forming α subunit of the Na\textsubscript{v1.7} channel, and multiple alignment indicated that the affected amino acids are conserved in sodium channels. Subsequent independent studies confirmed these findings and identified missense mutations (mutations in which one amino acid is replaced by another) in individuals from all of the families that had been examined in the original linkage study (4). To date, nearly a dozen SCN9A mutations in multiple families have been identified as causing PE (5, 6, 36–41). Most of these mutations have been found in families from The Netherlands, the United States, Belgium, France, Canada, and China, with a clear autosomal dominant inheritance pattern, although a few represent de novo founder mutations (a mutation that arose in the DNA of an individual several generations earlier and whom is considered to be a founder of a distinct population) (5, 6).

All of the PE mutations detected to date are missense mutations that change important and highly conserved amino acid residues of the Na\textsubscript{v1.7} protein. The majority of mutations that cause PE are located in cytoplasmic linkers of the Na\textsubscript{v1.7} channel, but some mutations (e.g., F216S and N395K) are located in transmembrane domains of the channel (Figure 1B). The PE mutations cause a hyperpolarizing shift in the voltage dependence of channel activation, which allows the channel to be activated by smaller than normal depolarizations, thereby likely enhancing the activity of Na\textsubscript{v1.7}. Most of the PE mutations also slow deactivation, thus keeping the channel open longer once it is activated (Figure 4). In addition, in response to a slow, depolarizing stimulus, most mutant channels will generate a larger than normal inward sodi

![Figure 4](http://www.jci.org) Four clinical situations in which Na\textsubscript{v1.7} channel activity is altered. PE and PEPD are autosomal dominant (AD) conditions, while CIP is inherited via an autosomal recessive (AR) trait. The mutations of the Na\textsubscript{v1.7} channel grossly dictate that there is a gain of function (increased channel activity) in PE and PEPD, while Na\textsubscript{v1.8} channel function is lost (absent) in CIP. The resultant phenotype is reflected in the lower row. In humans, the Na\textsubscript{v1.7} mutations result in pain in the feet and hands (in PE) or ocular, mandibular, and/or rectal pain (in PEPD). In CIP, there is a loss of Na\textsubscript{v1.7} channel function, resulting in an inability to register pain.
hyperexcitability of nociceptors together with sympathetic dysfunction (flushing/erythema) that is at least in large part due to hyperexcitability of sympathetic ganglion neurons (43).

Paroxysmal extreme pain disorder
The condition first described in 1959 as rectal, ocular, and submaxillary pain (46) has recently been renamed PEPD (OMIM 167400) (47). PEPD is an autosomal dominant disorder characterized by paroxysmal episodes (of sudden onset and increased intensity upon recurrence) of pain at different body sites, accompanied by skin flushing. There are four well-defined types of painful episodes. The first occurs at birth with an archetypical red flush spread over the buttocks and down the backs of the legs to the soles of the feet (48). A second pattern involves rectal pain that is most evident in childhood and typically occurs at defecation, as a sudden (short-lived) onset of burning pain that moves down to the lower extremities (47). The pain is followed by red discoloration of the skin of the pubic area, scrotum, perineum, buttocks, and the backs of both legs and soles of the feet, lasting for about an hour. The ocular pattern of pain is described as an intense burning sensation, lasting 30–60 seconds, followed by conjunctival injection (nonuniform redness of the conjunctiva) and erythema of the eyelids and of the skin in the temporal region, lasting a few minutes (49, 50). Attacks may be precipitated by yawning and crying but also occur spontaneously. Last, there is paroxysmal pain in the mandibular region, which the two alleles are identical by descent), and a genome-wide search led to the identification of a 20-cM homozygous region on chromosome 2q24.3 with a maximum 2-point lod score of 3.2 (a lod score of 3 or more is generally taken to indicate that 2 gene loci are close to each other on the chromosomes; a lod score of 3 means the odds are a thousand to one in favor of genetic linkage). Further refinement of the region to 11.7 Mb was facilitated by addition of a third family. A bioinformatics approach suggested SCN9A as the best candidate disease gene. Sequencing led to the identification of 87994 mutations in 8 families. All mutations were private, i.e., each family possessed a unique mutation. Interestingly, one individual was compound heterozygous for R996C and a de novo mutation (V1298D). This individual was more severely affected than his father. In another family in whom R996C was the only mutation identified, there was a less severe phenotype. In five families with typical PEPD, there were no SCN9A mutations found (7). These findings are consistent with the genetic heterogeneity of PE and suggest locus heterogeneity in PEPD.

Functional analysis of three mutations ([I1461T, T1464I, and M1627K]) that are attributed to PEPD was carried out in transfection experiments using a cell-based assay in which a mutant sodium channel was introduced into cells that normally do not express sodium channels (7). On the basis of these experiments, these mutations were reported to impair inactivation of the α subunit of the Na,1.7 channel (Figure 4). Steady-state inactivation was only partial in mutant channels and had shifted toward higher voltages in the context of near-normal activation. These changes are predicted to promote prolonged action potentials and repetitive neuron firing in response to provoking stimuli, such as stretching and exposure to cold temperatures (7). The different effects of PE mutations (which enhance channel activation) and PEPD mutations (which impair channel inactivation) might contribute in part to the different symptomatology in these two disorders. In either case, these results are in keeping with the notion that Na,1.7 plays a critical role in modulation of the pain threshold.

Channelopathy-associated insensitivity to pain
In contrast with PE and PEPD, CIP (OMIM 243000) is an autosomal recessive disorder (8, 53). Individuals with congenital indifference to pain have painless injuries beginning in infancy but otherwise normal sensory responses upon examination. Perception of passive movement, joint position, and vibration is normal, as are tactile thresholds and light touch perception. There is intact ability to distinguish between sharp and dull stimuli and to detect differences in temperature. The insensitivity to pain does not appear to be due to axonal degeneration, as the nerves appear to be normal on gross examination (8). The complications of the disease follow the inability to feel pain, and most individuals will have injuries to lip or tongue caused by biting themselves in the first 4 years of life. Patients have frequent bruises and cuts, usually have a history of fractures that go unnoticed, and are often only diagnosed because of limping or lack of use of a limb. The literature contains very colorful descriptions of patients with congenital inability to perceive any form of pain. Individuals have been reported to walk over burning coals and to place knives through their arms and drive spikes through a hand as part of crucifixion reenactment (8).

Cox et al. described 6 patients stemming from three consanguineous families of northern Pakistani origin (8). The highly inbred population allowed for autozygosity mapping (homozgyosity in which the two alleles are identical by descent), and a genome-wide search led to the identification of a 20-cM homozygous region on chromosome 2q24.3 with a maximum 2-point lod score of 3.2 (a lod score of 3 or more is generally taken to indicate that 2 gene loci are close to each other on the chromosomes; a lod score of 3 means the odds are a thousand to one in favor of genetic linkage). Further refinement of the region to 11.7 Mb was facilitated by addition of a third family. A bioinformatics approach suggested SCN9A as the best candidate disease gene. Sequencing led to the identification of different homozygous mutations of SCN9A, and each family possessed a unique mutation. The mutations were identified in exon 10 (S459X), exon 13 (I767X), and exon 15 (W897X) (8). All mutations are nonsense mutations, that is, they change a codon that codes for one amino acid into a codon that does not specify any amino acid. These results were confirmed by two studies: one study in 9 western European and North and South American families (54) and another in a large Canadian family (55). Both studies used linkage analysis, searched for homozygous haplotypes, identified the same gene, and detected 10 truncating SCN9A mutations. The majority of affected patients were homozygous for SCN9A mutations, but 2 patients were compound heterozygous for different SCN9A mutations (54). Functional studies show that CIP-associated mutations cause loss of function of Na,1.7 (8, 55) (Figure 4). This is in contrast with the genetic basis of PE and PEPD, in which the disorders result from gain-of-function mutations. In DRG neurons expressing mutant Na,1.7, the firing of action potentials was greatly impaired and comparable to background (8).
Implications and questions
Collectively, the data from recent studies indicate that Na\textsubscript{1.7} function is an essential and nonredundant requirement for nociception in humans. However, the genetic findings do not fully explain the clinical presentations described. Given the widespread expression of Na\textsubscript{1.7} throughout the sensory nervous system, it is remarkable that PE and PEPD have such different tissue distributions of pain. Moreover, the variability in age of clinical onset remains unexplained. Also, although physiological studies have revealed a temperature-dependent shift that brings the activation threshold of PE mutant channels close to that of wild-type Na\textsubscript{1.7} channels, possibly contributing to the alleviation of pain by cooling in PE (56), the paroxysmal nature of the painful attacks in PE and PEPD is not fully understood. These observations argue that there may be factors other than the mutated Na\textsubscript{1.7} channel, such as still-to-be-identified binding partners (other protein molecules that the channel interacts with, such as fibroblast growth factor homologous factors, which are known to modulate the physiological properties of the sodium-channel isoforms) that are important in determining the topographic and temporal pattern of these symptoms (57, 58).

The important role of mutations that change the sequence of SCN9A in various pain disorders suggests that SCN9A polymorphisms might contribute to intersubject variability in the sensation of pain in humans. This should encourage researchers to look for SCN9A polymorphisms that are associated with chronic pain disorders other than PE and PEPD. It might also be expected that some SCN9A polymorphisms might confer protection against pain.

Implications for new therapeutic approaches to pain
Neuropathic pain in PE is therapeutically challenging (59). Indeed, Na\textsubscript{1.7} represents a target that might be inhibited by small molecules in a subtype-specific or state-dependent manner during ectopic discharge, producing pain relief while sparing other neuronal functions. The development of subtype selectivity of potentially therapeutically useful molecules has proven to be a challenge. Several classes of drugs, including local anesthetics (e.g., lidocaine), systemic antiarrhythmics (e.g., mexiletine), and antiepileptic drugs such as phenytoin or carbamazepine, target sodium channels and act as channel blockers, although they do not show a high degree of channel subtype specificity and thus inhibit many types of sodium channels rather than selectively blocking Na\textsubscript{1.7} (52, 58). These agents, which act primarily through use-dependent blocks of sodium channels indeed are part of the armamentarium for the treatment of many types of chronic pain, including some forms of neuropathic pain. Several of these drugs have shown a degree of efficacy in patients with pain due to mutations in Na\textsubscript{1.7}. Some PE patients have responded to oral mexiletine (600 mg daily) (60). Interestingly, some PE mutations attenuate the inhibitory effect on sodium channels of the sodium-channel blocker lidocaine, while other PE mutations do not, suggesting that the response to treatment with sodium-channel blockers in PE may depend on the specific genotype (61). Carbamazepine is effective in some patients with PEPD, as it stabilizes the inactivated state of sodium channels, meaning that fewer of these channels are available to open, making brain cells less excitable (7). In contrast, preliminary results in PE indicate low or absent effectiveness of this drug (62). Moreover, in those cases in which lidocaine or mexiletine are helpful in PE, the efficacy of these agents is only partial or transient (63).

In conclusion, these observations, while drawn from a small number of patients, suggest that blockade of voltage-gated sodium channels is a promising therapeutic option for the treatment of pain but emphasize the need for the design of more highly focused, Na\textsubscript{1.7}-specific blockers or genetically tailored pharmacological options for future testing. There will undoubtedly be progress along these lines in the future.

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