A Sodium Channel Gene 
SCN9A Polymorphism That Increases Nociceptor Excitability

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Sodium channel NaV1.7, encoded by the SCN9A gene, is preferentially expressed in nociceptive primary sensory neurons, where it amplifies small depolarizations. In studies on a family with inherited erythromelalgia associated with NaV1.7 gain-of-function mutation A863P, we identified a nonsynonymous single-nucleotide polymorphism within SCN9A in the affected proband and several unaffected family members; this polymorphism (c. 3448C>T, Single Nucleotide Polymorphisms database rs6746030, which produces the amino acid substitution R1150W in human NaV1.7 [hNaV1.7]) is present in 1.1 to 12.7% of control chromosomes, depending on ethnicity. In this study, we examined the effect of the R1150W substitution on function of the hNaV1.7 channel, and on the firing of dorsal root ganglion (DRG) neurons in which this channel is normally expressed. We show that this polymorphism depolarizes activation (7.9–11mV in different assays). Current-clamp analysis shows that the 1150W allele depolarizes (6mV) resting membrane potential and increases (~2-fold) the firing frequency in response to depolarization in DRG neurons in which it is present. Our results suggest that polymorphisms in the NaV1.7 channel may influence susceptibility to pain.


It is now clear that, in the human nervous system, 9 different isoforms of voltage-gated sodium channels function in different ways, collaborating to produce electrical activity within nerve cells. The NaV1.7 sodium channel (encoded by the gene SCN9A), in particular, is preferentially expressed in pain-signaling dorsal root ganglion (DRG) neurons (nociceptors) and has been shown to play a critically important role in these cells, amplifying small depolarizations so as to increase the gain in pain signaling. Gain-of-function mutations of NaV1.7 have been shown to cause the painful disorders inherited erythromelalgia (IEM), and paroxysmal extreme pain disorder, whereas loss-of-function mutations cause congenital insensitivity to pain, underscoring the important role of hNaV1.7 in human pain signaling.

In studies on a family with IEM associated with NaV1.7 gain-of-function mutation A863P, we identified a nonsynonymous single-nucleotide (c. 3448C>T) polymorphism within SCN9A (Single Nucleotide Polymorphisms database [dbSNP] rs6746030) in the affected proband, and in several unaffected family members (father and brother of proband), and reported that this polymorphism is present in 14% of ethnically matched, Caucasian control chromosomes. This polymorphism, in exon 18 of SCN9A, substitutes a nonpolar tryptophan (W) for a positively charged arginine (R) at residue 1150 in the reference NaV1.7 sequence and is located within the C-terminus of L2, the loop that joins domains II and III of the channel. R1150 occurs within a highly conserved sequence of sodium channels and is invariant in all mammalian NaV1.7 channels isolated thus far. Moreover, almost all other sodium channels possess a polar or positively charged residue at this site (arginine in NaV1.2, NaV1.5, NaV1.7, and NaV1.8, lysine in NaV1.3 and NaV1.4, glutamine in NaV1.1 and NaV1.6, and cysteine/histidine in NaV1.9 from different species). Given the conservation of this residue in NaV1.7 channels from different species and the presence of a positively charged residue in most other sodium channels, the substitution by a tryptophan residue suggests a functional effect of the polymorphism on the biophysical properties of the NaV1.7 channel, which is known to play a central role in human pain signaling. We therefore studied the effect of the R1150W substitution on the function of the NaV1.7 sodium channel, and on the firing of pain-signaling DRG neurons in which this channel is normally expressed. Here we show that expression of this polymorphism has important functional implications.

Subjects and Methods
Exon 18 of SCN9A, which carries the c. 3448C>T polymorphism, was amplified from a Caucasian control sample of 39 individuals (182 chromosomes), as previously described. The amplicons were sequenced, and frequency of the C and T alleles, which encode the 1150R and 1150W isoforms, was determined.

The functional properties of hNaV1.71150R and hNaV1.71150W isoforms heterologously expressed in HEK293 cells were assessed using patch-clamp recordings. A tryptophan was substituted for arginine at residue 1150 within...
the tetrodotoxin-resistant version of hNaV1.7 plasmid using QuikChange XL site-directed mutagenesis (Stratagene, La Jolla, CA). Recordings were performed in voltage-clamp mode in both transiently transfected HEK293 cells (including human β1 and β2 subunits) and stably expressing clonal HEK293 cell lines (without β subunits). The effect of the hNaV1.71150W allele on excitability was assessed using current-clamp recording from small (22–28μm)-diameter rat DRG neurons, which are largely nociceptors, following transfection with either the hNaV1.71150R or the hNaV1.71150W channel, together with green fluorescent protein (GFP), using Rat Neuron Nucleofector Solution (Lonza, Walkersville, MD).

### Results

Previously, we reported the c. 3448C>T polymorphism within SCN9A (dbSNP rs6746030) in 14% of ethnically matched Caucasian control chromosomes, with a sample size of 100 chromosomes. We have now determined the allele frequency in a larger sample of 91 Caucasian control subjects (Coriell Institute, Camden, NJ) and found the following distribution: 70.32% (64/91 individuals) homozygous for the C allele, 28.57% (26/91 individuals) heterozygous for the C and T alleles, and 3.29% (3/91 individuals) homozygous for the T allele. Thus, the frequency of the T allele within this Caucasian control population is 17.58% (32/182 chromosomes).

We analyzed the effects of the R1150W polymorphism by transiently expressing hNaV1.71150R and hNaV1.71150W alleles, together with sodium channel β1 and β2 subunits and GFP, within HEK293 cells. This analysis, using patch-clamp voltage-clamp methods, demonstrated a 7.9mV depolarizing shift in the V1/2 (midpoint of voltage-dependence) for activation (hNaV1.71150R V1/2 = −15.9 ± 0.8 mV, n = 33; hNaV1.71150W V1/2 = −8.0 ± 1.1 mV, n = 17; p < 0.001), as shown in Figure 1. The V1/2 for fast inactivation (Fig 2A) was unchanged by the polymorphism (hNaV1.71150R V1/2 = −76.9 ± 0.8 mV, n = 33; hNaV1.71150W V1/2 = −77.9 ± 7.5 mV, n = 17). Although the slope factor was shallower for the hNaV1.71150W allele compared with the hNaV1.71150R allele (hNaV1.71150R k = 6.9 ± 0.1; hNaV1.71150W k = 7.5 ± 0.3; p < 0.05), availability for the 2 alleles was not significantly different at potentials from −130 to −10 mV, suggesting that this does not contribute to differences in excitability. The parameters of slow inactivation (Fig 2B) were not significantly altered by the hNaV1.71150W allele compared with the hNaV1.71150R allele (hNaV1.71150R V1/2 = −80.0 ± 1.4 mV, k = 15.8 ± 0.6, fit minimum = 0.14 ± 0.02 [n = 33]; hNaV1.71150W V1/2 = −76.8 ± 4.8 mV, k = 14.3 ± 0.7, fit minimum = 0.15 ± 0.03 [n = 17]).

We confirmed the depolarizing effect of the R1150 polymorphism on the voltage dependence of activation in a second set of recordings on stably expressing HEK293 cell lines that revealed an 11mV depolarizing shift of activation voltage dependence (hNaV1.71150R V1/2 = −29.6 ± 2.0 mV, n = 7; hNaV1.71150W V1/2 = −19.5 ± 2.0 mV, n = 6; p < 0.01). This analysis of stably transfected cells confirmed the absence of a change in fast inactivation.

To assess the effect of the 1150W allele on excitability, we carried out current-clamp recordings on small rat DRG neurons, which are largely nociceptors, after transfection with hNaV1.71150W or hNaV1.71150R and GFP, recording from cells that displayed GFP signal indicating successful transfection. This analysis revealed a statistically significant 6mV depolarizing shift in resting potential in hNaV1.71150R-transfected neurons compared with hNaV1.71150R-transfected neurons (hNaV1.71150R: V1/2 = −57.4 ± 1.1 mV, n = 48; hNaV1.71150W: V1/2 = −51.5 ± 1.3 mV, n = 30; p < 0.005). Current threshold showed a trend toward a reduction...
in cells expressing hNaV1.71150W (215 ± 32pA, n = 20) compared with hNaV1.71150W (238 ± 25pA, n = 48), although this was not statistically significant (p = 0.57). This analysis also showed that, in response to depolarizing current stimulation, DRG neurons expressing the 1150W allele fired more action potentials compared with similar cells expressing the 1150R allele, with cells expressing hNaV1.71150W generating about twice as many action potentials at stimulus intensities ranging from 50 to 500pA (p < 0.05 at all stimulus intensities >100 pA) (Fig 3).

Discussion

The Na1.7 sodium channel, which is preferentially expressed in nociceptive DRG neurons,1,2 plays an important role in electrogenesis in these cells, where it amplifies small depolarizations so as to increase the gain in pain signaling.3 In our sample of 91 Caucasian control subjects, the single nucleotide polymorphism c.3448C>T (dbSNP rs6746030) is present within 17.58% of ethnically matched control chromosomes.8 Examination of the dbSNP shows that the frequency of the rs6746030 polymorphism in control samples is ethnicity dependent: American Caucasians of European descent, 12.7% (sample size: 118 chromosomes); American Chinese of Han descent, 5.6% (sample size: 90 chromosomes); Americans of Japanese descent, 1.1% (sample size: 90 chromosomes); sub-Saharan African, 13.3% (sample size: 120 chromosomes).

The present observations demonstrate that the 1150W allele of NaV1.7 shifts activation voltage dependence 7.9 to 11mV in a depolarizing direction after expression with (7.9mV) or without (11mV)1 and β2 subunits in a heterologous expression system, and has a strong effect on the function of DRG neurons, depolarizing their resting potential and increasing their firing rate. The mechanism by which the 1150W NaV1.7 allele increases the firing rate of DRG neurons is not yet fully understood, and may involve interactions of the NaV1.7 channel protein with factors that are specifically expressed within DRG neurons. Prior studies indicate that a depolarizing shift in NaV1.7 activation voltage dependence can contribute to a decrease in excitability13,14 if electrogenic pumps are not considered, but this shift will also reduce the window current in nociceptors housing the hNaV1.71150W allele. This latter change would be expected to attenuate the standing influx of Na+ ions required for maintenance of Na+/K+ adenosine triphosphatase activity in DRG neurons in which NaV1.7 is a dominant source of window current, thereby depolarizing these nociceptors as a result of diminishing hyperpolarizing pump current and/or a reduction of ion gradients due to insufficient pump rate.15,16 The present results are consistent with earlier findings, demonstrating that depolarization of nociceptive DRG neurons can make these
cells hyperexcitable as a result of the presence within these cells of NaV1.8 sodium channels, which support repetitive firing and are relatively resistant to inactivation by depolarization. In nociceptive DRG neurons, depolarization by 6mV, that is, of the same magnitude as produced by the hNaV1.7<sup>1150W</sup> allele, has been shown to increase excitability.

Irrespective of the underlying mechanism, our results demonstrate that expression of the 1150W polymorphism in NaV1.7 doubles the firing frequency in small DRG neurons. The firing frequencies we observed in DRG neurons expressing hNaV1.7<sub>1150W</sub> are similar, in fact, to those seen in DRG neurons expressing the Q10R NaV1.7 mutation, found in a patient with IEM with onset of pain at 14 years, much older than in most patients with IEM. Why this patient, who presumably harbored this NaV1.7 mutation throughout life, did not experience pain earlier, and why most humans carrying the 1150W NaV1.7 polymorphism do not develop a chronic pain syndrome such as IEM, is not yet understood. Nonetheless, even in the absence of detailed understanding of biophysical mechanisms, increased firing frequencies in nociceptive DRG neurons would be expected to produce pain or lower pain threshold, a prediction that could be tested by correlating pain phenotype versus genotype in a population of human subjects. Interestingly, although the 1150W NaV1.7 polymorphism is found in ~13.3 to 17.5% of Caucasian and sub-Saharan African control chromosomes (and is present at lower frequencies, 1.1–5.6%, in Asian control chromosomes), Drenth et al described its presence in a patient carrying the diagnosis of sporadic primary erythromelalgia, suggesting that it may cause a pain syndrome with low penetrance (in contrast to most IEM mutations, which exhibit nearly 100% penetrance), or that its phenotypic expression may be modulated by disease-modifier genes, as has been shown for another sodium channel, NaV1.6. Whether the 1150W polymorphism influences the severity of IEM in a patient with an IEM mutation and 1150W is not known, because in that pedigree there were no other subjects with the IEM mutation.

Gain-of-function mutations of NaV1.7 have previously been demonstrated to produce clinical syndromes characterized by severe pain, whereas loss-of-function...
mutations of Na\textsubscript{v}1.7 produce inability to experience pain. The results reported here indicate that a polymorphism in \textit{SCN9A}, the gene encoding the human Na\textsubscript{v}1.7 sodium channel, can influence the excitability of nociceptive DRG neurons. These observations suggest the possibility that polymorphisms of the Na\textsubscript{v}1.7 sodium channel may contribute to alterations in pain sensitivity or susceptibility to chronic pain, and underscore the potential importance of Na\textsubscript{v}1.7 as a molecular target for treatment of pain.

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References