Voltage-gated sodium channel mutations and painful neuropathy: Na\textsubscript{v}1.9 joins the family

In its simplest terms neuropathic pain arises as a consequence of hyperexcitability within the somatosensory nervous system. It is not surprising therefore that voltage-gated sodium channels (VGSCs), with their key role in regulating neuronal excitability, have come to the fore as pathophysiological factors in human neuropathic pain states. Not only can mutations in these ion channels lead to altered impulse generation/conduction in sensory neurons but they may also lead to degeneration of axon terminals. The VGSC family of proteins has nine members, of which Na\textsubscript{v}1.7, Na\textsubscript{v}1.8 and Na\textsubscript{v}1.9 (encoded by the genes SCN9A, SCN10A and SCN11A, respectively) are preferentially expressed in peripheral neurons (Eijkelkamp et al., 2012). While gain of function variants of Na\textsubscript{v}1.7 and Na\textsubscript{v}1.8 have previously been reported in patients with painful peripheral neuropathy, in this issue of Brain, Huang et al. (2014) present the first evidence for a causative role of missense mutations in Na\textsubscript{v}1.9 in painful neuropathy.

The biophysical properties and distribution of Na\textsubscript{v}1.7, Na\textsubscript{v}1.8 and Na\textsubscript{v}1.9 regulate key aspects of nociceptor function. Na\textsubscript{v}1.7 is likely to be an important determinant of threshold for excitation in nociceptor terminals and may also regulate neurotransmitter release at the central terminals of nociceptors. Homozygous, loss of function mutations in Na\textsubscript{v}1.7 result in congenital inability to experience pain and anosmia in man, whereas heterozygous gain of function mutations have been linked to the distinct clinical pain syndromes of inherited erythromelalgia (IEM: pain and erythema exacerbated by warming), paroxysmal extreme pain disorder (PEPD: proximal pain and autonomic features in ocular/mandibular and sacral regions), and small fibre neuropathy (SFN: degeneration of small diameter sensory and autonomic axons usually presenting with severe burning pain in extremities (Persson et al., 2013)). Na\textsubscript{v}1.8 carries most of the current underlying the depolarizing phase of the action potential in dorsal root ganglion neurons and so is critical for transmission of action potentials and repetitive firing. Heterozygous gain of function mutations in Na\textsubscript{v}1.8 have recently been linked to small fibre neuropathy (Faber et al., 2012).

So what of Na\textsubscript{v}1.9 (encoded by the gene SCN11A), which is the focus of the report by Huang et al. in this issue of Brain? Na\textsubscript{v}1.9 is expressed particularly by small diameter dorsal root ganglion cells, the majority of which will be nociceptors (Dib-Hajj et al., 1998; Tate et al., 1998). It is also expressed within the autonomic system by nodose ganglion neurons and in visceral afferent neurons innervating the intestine. Na\textsubscript{v}1.9 has slow gating kinetics and generates persistent currents near resting membrane potential; it is therefore an important regulator of membrane excitability (Baker et al., 2003).

The starting point for Huang et al. was to study a cohort of almost 400 patients with painful neuropathy. The majority had pure small fibre neuropathy, but a small subgroup (n = 24) were classified as having predominantly small fibre neuropathy because of additional evidence (on clinical and neurophysiological grounds) of large fibre involvement. In this cohort, mutations in SCN9A and SCN10A were found in 9% and 4% of subjects, respectively. In the remaining 345 patients (and one additional case in which an SCN9A mutation was present, but did not cause hyperexcitability) the authors went on to sequence the SCN11A gene. They found eight heterozygous SCN11A variants in 12 patients. Two of these variants, I381T (located in domain I, transmembrane segment 6-D1/S6, present in two patients) and L1158P (DIII/S4, present in two patients), were selected for a detailed and elegant functional analysis.

To summarize the clinical features: in all four cases the symptoms were of late onset (after the age of 50). Pain was an important feature and additional symptoms included numbness, paraesthesia and autonomic symptoms including vascular changes in skin, orthostatic dizziness, hyperhidrosis and gastrointestinal disturbance. These autonomic features are likely to be relevant given that Na\textsubscript{v}1.9 is known to be expressed in the autonomic nervous system and has a role in gastrointestinal motility (Copel et al., 2013). Symptoms and signs were not restricted to small fibre modalities and two patients were diagnosed with predominantly small fibre, rather than pure small fibre, neuropathy. A family history of pain was reported in two of the cases although segregation of mutations within the pedigrees was not determined. We do not yet know how penetrant these mutations are. It is entirely possible that these mutations are not always fully penetrant but act as important risk factors for painful neuropathy interacting with other (for instance environmental) determinants.
Clarification of this point will be important for providing genetic counselling to families. Another important point raised by the authors and which is a further challenge in clinical practice is that not all variants/mutations in VGSCs cause hyperexcitability. An illustration is given in this paper in which one subject was found to have a variant in the SCN9A gene, which did not lead to hyperexcitability, whereas subsequent sequencing of SCN11A revealed a variant that did cause hyperexcitability. The issue of ascribing pathogenicity is likely to become increasingly pertinent in this era of exome sequencing. In silico predictions of mutation effects can obviously be helpful but ultimately functional assessment (as performed here) is required.

To look at the impact of mutations the authors expressed human wild-type or mutant Na\(_{\alpha1.9}\) in dorsal root ganglion neurons obtained from Na\(_{\alpha1.9}\) knockout mice. The advantage of this approach is that this is a much more ‘natural’ environment for testing the functional impact of mutations in the presence of normal accessory proteins and other VGSCs within dorsal root ganglia. Both the L1158P and I381T Na\(_{\alpha1.9}\) mutations led to gain-of-function with channel gating properties altered in a direction that is likely to lead to increased excitability. Current clamp recordings were used to assess the effect of mutations on dorsal root ganglion neuron excitability. Both mutations resulted in a depolarization of the resting membrane potential. The amount of current injection required to generate an action potential was reduced and firing frequency to supra-threshold stimuli increased. The I381T mutation increased spontaneous firing of dorsal root ganglion neurons. Dynamic clamp recordings were then performed to deal with the confounding factor of supranormal levels of channel expression following dorsal root ganglion neuron transfection. In this paradigm a computer model is used to simulate ion channel conductances and generate a current that is injected back into the neuron as simultaneous electrophysiological recordings are made. This was used to add back either 100% wild-type or a mixture of 50% wild-type and 50% I381T mutant current (as these are heterozygous mutations) in a carefully titrated manner. These dynamic clamp recordings demonstrate that, relative to control conditions, the addition of 50% I381T mutant current leads to depolarization of the resting membrane potential, a reduced threshold for action potential firing following current injection, and increased firing frequency. In summary both of these mutations lead to gain of function attributes of Na\(_{\alpha1.9}\), depolarization of the resting membrane potential and hyperexcitability; a finding that in the case of the I381T mutation has also been replicated using dynamic clamp recordings.

These findings should be discussed in the light of two other recent papers drawing attention to the role of Na\(_{\alpha1.9}\) in regulating human pain sensibility. The first describes gain of function mutations in Na\(_{\alpha1.9}\) (two mutations: R225C or A808G in two independent pedigrees) causing a distinct pain syndrome (Zhang et al., 2013). This is not a neuropathy (no sensory loss), but subjects report episodic pain triggered by intercurrent illness, fatigue and exercise and relieved by non-steroidal anti-inflammatory drugs. These mutations were shown to cause hyperexcitability of dorsal root ganglion cells with increased peak current densities and enhanced action potential firing following current injection (the resting membrane potential did not change). The second paper describes the finding of a heterozygous mutation in Na\(_{\alpha1.9}\) (L811P) in two unrelated individuals suffering from congenital inability to experience pain (CIP) (Leipold et al., 2013). Intriguingly therefore different gain-of-function mutations in Na\(_{\alpha1.9}\) are resulting in opposite clinical outcomes: either insensitivity to pain or chronic neuropathic pain. The proposed mechanism by which L811P causes CIP is altered channel gating (a $-29 \text{ mV}$ shift in voltage dependence of activation) and such a large depolarizing shift in the resting membrane potential, that this results in resting inactivation of other VGSC. On theoretical grounds this would be difficult to achieve in dorsal root ganglion cells given that the voltage dependency of steady-state inactivation of Na\(_{\alpha1.8}\) is depolarized by 20–40 mV compared to other VGSCs. Furthermore the findings presented here by Huang et al. refute this explanation: they show that a depolarized resting membrane potential (either through heterologous expression of mutant channels or direct current injection using dynamic clamp) leads to increased dorsal root ganglion cell excitability. As the authors carefully discuss, it is not easy to reconcile these differing findings/interpretations. It is possible that insensitivity to pain due to the L811P mutation does not relate to an altered resting membrane potential, but to the marked shift in channel gating that although causing hyperexcitability, ultimately leads to fatigue of these neurons.

Notwithstanding some of these challenges in ascribing clinical phenotype to channel biophysics it is clear that gain of function mutations in Na\(_{\alpha1.9}\) can cause neuropathic pain in humans. We do not yet know whether these mutations are fully penetrant and whether [as has been shown for Na\(_{\alpha1.7}\) (Persson et al., 2013)] these mutations have an active role in promoting axon degeneration. Mutations in Na\(_{\alpha1.7}\), 1.8 and 1.9 have now all been associated with painful neuropathy as a consequence of dorsal root ganglion cell hyperexcitability and as such are targets for the development of novel analgesics, which may have the added advantage of also being disease-modifying.

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**References**


