Differential effect of lacosamide on Na_v1.7 variants from responsive and non-responsive patients with small fibre neuropathy

Julie I.R. Labau,1,2,3,4,* Mark Estacion,1,2,3,* Brian S. Tanaka,1,2,3,* Bianca T.A. de Greef,4,6 Janneke G.J. Hoeijmakers,4 Margot Geerts,4 Monique M. Gerrits,7 Hubert J.M. Smeets,5 Catharina G. Faber,4 Ingemar S.J. Merkies,4,8 Giuseppe Lauria,9,10 Sulayman D. Dib-Hajj1,2,3 and Stephen G. Waxman1,2,3

These authors contributed equally to this work.

Small fibre neuropathy is a common pain disorder, which in many cases fails to respond to treatment with existing medications. Gain-of-function mutations of voltage-gated sodium channel Na_v1.7 underlie dorsal root ganglion neuronal hyperexcitability and pain in a subset of patients with small fibre neuropathy. Recent clinical studies have demonstrated that lacosamide, which blocks sodium channels in a use-dependent manner, attenuates pain in some patients with Na_v1.7 mutations; however, only a subgroup of these patients responded to the drug. Here, we used voltage-clamp recordings to evaluate the effects of lacosamide on five Na_v1.7 variants from patients who were responsive or non-responsive to treatment. We show that, at the clinically achievable concentration of 30 μM, lacosamide acts as a potent sodium channel inhibitor of Na_v1.7 variants carried by responsive patients, via a hyperpolarizing shift of voltage-dependence of both fast and slow inactivation and enhancement of use-dependent inhibition. By contrast, the effects of lacosamide on slow inactivation and use-dependence in Na_v1.7 variants from non-responsive patients were less robust. Importantly, we found that lacosamide selectively enhances fast inactivation only in variants from responders. Taken together, these findings begin to unravel biophysical underpinnings that contribute to responsiveness to lacosamide in patients with small fibre neuropathy carrying select Na_v1.7 variants.

1 Department of Neurology, Yale University School of Medicine, New Haven, CT 06510, USA
2 Center for Neuroscience and Regeneration Research, Yale University School of Medicine, New Haven, CT 06510, USA
3 Rehabilitation Research Center, Veterans Affairs Connecticut Healthcare System, West Haven, CT 06516, USA
4 Department of Neurology, School of Mental Health and Neuroscience, Maastricht University Medical Center, Maastricht, The Netherlands
5 Department of Genetics and Cell Biology, Clinical Genomics Unit, Maastricht University, Maastricht, The Netherlands
6 Department of Clinical Epidemiology and Medical Technology Assessment (KEMTA), Maastricht University Medical Centre, Maastricht, The Netherlands
7 Department of Clinical Genetics, Maastricht University Medical Centre+, Maastricht, The Netherlands
8 Department of Neurology, St. Elisabeth Hospital, Willemstad, Curacao
9 Neuroalgology Unit, IRCCS Foundation, “Carlo Besta” Neurological Institute, Milan, Italy
10 Department of Biomedical and Clinical Sciences “Luigi Sacco”, University of Milan, Italy

Correspondence to: Stephen G. Waxman, MD, PhD
The Center for Neuroscience and Regeneration Research
127A, Bldg. 34
VA Connecticut Healthcare System
of-function mutations in Nav1.7 have been identified in forms of small fibre neuropathy (SFN). Conversely, loss-melalgia, paroxysmal extreme pain disorder, and some protein) have been reported in patients with inherited erythrocytoses, often accompanied with autonomic dysfunction, tend excitability. Prominent burning pain in the distal extremities by dorsal root ganglion (DRG) neuronal and axonal hyperexcitability. Sodium channel (VGSC) expression and function in neuropathic pain represents a high priority. Genetic studies have provided compelling evidence regarding the contribution of altered voltage-gated sodium channel (VGSC) expression and function in neuropathic pain. The Na\(_{1.7}\) sodium channel has drawn particular interest because it is preferentially expressed in peripheral sensory and sympathetic neurons and has directly been linked to multiple human pain conditions: gain-of-function missense mutations in SCN9A (encoding the Na\(_{1.7}\) protein) have been reported in patients with inherited erythromelalgia, paroxysmal extreme pain disorder, and some forms of small fibre neuropathy (SFN). Conversely, loss-of-function mutations in Na\(_{1.7}\) have been identified in individuals with congenital complete insensitivity to pain (Dib-Hajj et al., 2013; Bennett et al., 2019; Dib-Hajj and Waxman, 2019).

SFN is morphologically characterized by injury to the small intraepidermal nerve fibres, specifically the unmyelinated C and thinly myelinated A\(_{\delta}\)-fibres, and functionally by dorsal root ganglion (DRG) neuronal and axonal hyperexcitability. Prominent burning pain in the distal extremities, often accompanied with autonomic dysfunction, tend to dominate the clinical picture (Faber et al., 2012; Brouwer et al., 2014; Sopacua et al., 2019). Sodium channel variants have been described in ~15% of patients with SFN, with a higher frequency in missense mutants of SCN9A (de Greef et al., 2018; Eijkenboom et al., 2019). Many of these variants have been functionally profiled and shown to confer gain-of-function attributes on the channel and render DRG neurons hyperexcitable (Dib-Hajj et al., 2013; Bennett et al., 2019; Dib-Hajj and Waxman, 2019). The gain-of-function attributes of these variants increases confidence in their pathogenicity (Waxman et al., 2014), suggesting that carriers of these variants might benefit from treatments using sodium channel blockers.

Lacosamide, an FDA-approved anti-epileptic drug (AED), has been reported to preferentially enhance slow inactivation of VGSC, including Na\(_{1.7}\) (Errington et al., 2006, 2008; Niespodziany et al., 2013). However, a recent study has alternatively suggested that lacosamide binds to the fast-inactivated state of Na\(_{1.7}\), but at a slower rate (Jo and Bean, 2017). There is also evidence that lacosamide may alter the effect of CRMP2 (collapsin-response mediator protein 2) on Na\(_{1.7}\) channel trafficking (Wilson and Khanna, 2015; Moutal et al., 2017). Furthermore, lacosamide has been found to have a higher affinity for tetrodotoxin-sensitive sodium channels over other ion channels and central receptors (Sheets et al., 2008; Moutal et al., 2017) that are targeted by traditional AEDs (Errington et al., 2006), conferring increased selectivity. A retrospective study of patients with seizures who had switched from other AED drugs to lacosamide, because of deleterious effects from their original AED treatment, has shown successful conversion in many of these patients (Kim et al., 2019), suggesting lacosamide to have improved tolerability. Lacosamide has also been tested in small clinical trials as a potential therapy for painful diabetic neuropathy where the majority of patients reported significant pain improvement with <10% reporting adverse effects (Rauck et al., 2007; Shaibani et al., 2009a, b; Wymer et al., 2009). However, until recently, the clinical effects of lacosamide on patients with Na\(_{1.7}\)-related pain disorders had yet to be investigated.

A recent randomized, placebo-controlled, double-blind, crossover trial (de Greef et al., 2019) assessed the effect of lacosamide treatment in 24 patients with SFN carrying multiple Na\(_{1.7}\) variants. Fifteen different mutations were identified in this cohort, of which five were carried by several patients. Different subjects in this trial exhibited a spectrum of responses to treatment, with some patients reporting substantial pain relief while others did not show any improvement (Fig. 1). In a different study, lacosamide has been shown to produce pain relief in one patient with SFN (Namer et al., 2019). However, the factors responsible for variable lacosamide responsiveness in the de Greef et al. (2019) study are not known. In the present study, we report the Na\(_{1.7}\) variants in these patients and assess the
pharmacogenomic correlation between the Na\textsubscript{v}1.7 genetic variants and the response to lacosamide in this series of patients. Using voltage-clamp recordings of HEK293 (human embryonic kidney) cells expressing variants from both responsive and non-responsive patients, we characterized the effect of lacosamide on the biophysical properties of the Na\textsubscript{v}1.7 channel, at a clinically achievable concentration, and identify for the first time a hyperpolarizing effect on fast inactivation, which reduces the fraction of the channels that are available to open, as a distinguishing property of variants that correlates with lacosamide responsiveness in patients with SFN.

**Materials and methods**

**Patients**

Twenty-four participants (18–80 years old) were recruited at the Maastricht University Medical Center+ (UMC+) for the Lacosamide Efficacy-N’-Safety Study (LENSS) (de Greef et al., 2019). Subjects were selected based on diagnosis of pure SFN with no associated conditions (except for diabetes mellitus), with a mutation in the \textit{SCN9A} gene (Na\textsubscript{v}1.7 channel) of class III, IV or V (variants of uncertain pathogenicity, likely to be or clearly pathogenic, respectively), using genetic classification criteria, as described previously (Wallis et al., 2013). The clinical study design and results were published previously (de Greef et al., 2016, 2019). Briefly, the participants received either 200 mg of lacosamide or placebo twice daily for 8 weeks, preceded by a titration period and followed by a tapering period, and a 2-week washout period before receiving the alternative compound. They were asked to rate their pain twice daily using the daily pain intensity numerical rating scale (PI-NRS) ranging from 0 to 10. The patients were considered responsive to treatment when at least a 1-point reduction was reported from the individual baseline pain scores.

**Plasmids and HEK293 cell transfection**

The construct of the human Na\textsubscript{v}1.7 wild-type isoform was rendered tetrodotoxin-resistant and fused to an eGFP-2A linker, as previously described (Yang et al., 2016). The construct produces a green fluorescent protein with the Na\textsubscript{v}1.7 channel as independent proteins from the same transcript, which enables the visual identification of transfected cells based on their fluorescence. Na\textsubscript{v}1.7-related SFN mutations identified in participants in this study (I739V, I228M, L1267V, W719C and W1538R) were introduced into the constructs using QuikChange\textsuperscript{®} XL site-directed mutagenesis.

![Figure 1 Clinical response of patients carrying specific Na\textsubscript{v}1.7 variants.](image-url)
NaCl, 3 KCl, 1 MgCl2, 1 CaCl2, 10 HEPES (pH 7.3 with
EGTA (pH 7.3 with CsOH, adjusted to 320 mOsm with dex-
trose). The extracellular solution contained (in mM): 140
NaCl, 3 KCl, 1 MgCl2, 1 CaCl2, 10 HEPES (pH 7.3 with
NaOH, adjusted to 320 mOsm with dextrose). Voltage-
clamp protocols for the biophysical characterization of
VGSCs were initiated 5 min after establishing whole-cell
configuration on an EPC-10 USB amplifier (HEKA
Electronics) and acquired using PatchMaster software (HEKA
Electronics) at an acquisition rate of 50 kHz with a low-pass
Bessel filter setting of 2.9 kHz. Voltage errors were minimized
with 60–90% series resistance compensation, and only cells
with <4 mV voltage error after compensation were included
for analysis. When appropriate, linear leak currents and cap-
icitance artefacts were corrected by P/6 subtraction.

To examine the current-voltage (I-V) relationship, a series of
step depolarizations from −80 mV to +50 mV in 5-mV incre-
ments were applied from a holding potential of −120 mV at 5-
s interpulse intervals. To evaluate the effect of lacosamide on
steady-state fast inactivation, the membrane potential was held
at conditioning potentials that varied from −140 mV to 10 mV
for 500 ms, and cells were then given a 20-ms test pulse to 0
mV to elicit current from remaining available channels.

For the slow inactivation protocol, the cell was held at condi-
tioning potentials that varied from −130 mV to 10 mV for
30 s; the membrane potential was then pulsed to −120 mV
for 100 ms to allow channels not in the slow inactivated state
to recover from fast inactivation, and then given a 20-ms test
pulse to 0 mV to elicit current from available channels.

To measure the use-dependence of inhibition of lacosamide
at 20 Hz stimulation, series of 20-ms pulses were applied at
−10 mV. Peak inward currents were measured and normalized
to the maximum current amplitude.

Electrophysiology
Whole-cell voltage-clamp recordings were obtained at room
temperature on isolated HEK293 cells showing green fluores-
cence. Recordings were alternatively performed on coverslips
of HEK293 cells expressing wild-type or a Na1.7 variant,
either treated with vehicle (extracellular solution) or laco-
samide. Electrodes were pulled from 1.65-mm outer diameter
borosilicate glass micropipettes (WPI) and had a resistance of
0.8–2.0 MΩ when filled with intracellular pipette solution,
which contained (in mM): 140 CsF, 10 NaCl, 10 HEPES, 1
EGTA (pH 7.3 with CsOH, adjusted to 320 mOsm with dext-
rose). The extracellular solution contained (in mM): 140
NaCl, 3 KCl, 1 MgCl2, 1 CaCl2, 10 HEPES (pH 7.3 with
NaOH, adjusted to 320 mOsm with dextrose). Voltage-
clamp protocols for the biophysical characterization of
VGSCs were initiated 5 min after establishing whole-cell
configuration on an EPC-10 USB amplifier (HEKA
Electronics) and acquired using PatchMaster software (HEKA
Electronics) at an acquisition rate of 50 kHz with a low-pass
Bessel filter setting of 2.9 kHz. Voltage errors were minimized
with 60–90% series resistance compensation, and only cells
with <4 mV voltage error after compensation were included
for analysis. When appropriate, linear leak currents and cap-
icitance artefacts were corrected by P/6 subtraction.

To examine the current-voltage (I-V) relationship, a series of
step depolarizations from −80 mV to +50 mV in 5-mV incre-
ments were applied from a holding potential of −120 mV at 5-
s interpulse intervals. To evaluate the effect of lacosamide on
steady-state fast inactivation, the membrane potential was held
at conditioning potentials that varied from −140 mV to 10 mV
for 500 ms, and cells were then given a 20-ms test pulse to 0
mV to elicit current from remaining available channels.

For the slow inactivation protocol, the cell was held at condi-
tioning potentials that varied from −130 mV to 10 mV for
30 s; the membrane potential was then pulsed to −120 mV
for 100 ms to allow channels not in the slow inactivated state
to recover from fast inactivation, and then given a 20-ms test
pulse to 0 mV to elicit current from available channels.

To measure the use-dependence of inhibition of lacosamide
at 20 Hz stimulation, series of 20-ms pulses were applied at
−10 mV. Peak inward currents were measured and normalized
to the maximum current amplitude.

In vitro pharmacology
A bi-daily 200 mg lacosamide dose results in a maximum plasma
concentration of 30 μM (Cawello, 2015). The patients
assessed in this study received lacosamide at this bi-daily dose
during the trial (de Greef et al., 2019); thus 30 μM of laco-
samide was used for the studies described here.
Lacosamide (Vimpat®, UCB, obtained from the West
Haven VA Medical Center pharmacy), with a concentration
of 10 mg/ml (39.9 mM) in aqueous saline (pH 4), was diluted
in the extracellular bath solution (vehicle; described above) to
achieve the final working concentration of 30 μM. Working
solutions were prepared fresh daily.

The extracellular bath solution was continuously perfused at
a consistent flow rate of 1 ml/min through a 250 μm perfusion
pipette, using a pressure-regulated system (AutoMate
Scientific). Complete bath exchange was enabled via aspiration
from the opposite end of the cell chamber from the perfusion
pipette. To control for time-dependent changes in channel
properties, independent cell cohorts were exposed to only
one test concentration of lacosamide or vehicle at a consistent
time after initiation of whole-cell recording. Specifically,
the solution was exchanged for 2 min, corresponding to an abso-
lute bath exchange of 2 ml, starting 2 min following breakage
of the cell membrane.

Statistical analyses
Whole-cell voltage-clamp data were analysed using Fitmaster
(HEKA Electronics), Excel (Microsoft) and Origin (OriginLab
Corporation, Microcal Software, Northampton, MA). Data
are expressed as means ± standard error of the mean
(SEM). Statistical significance was determined by unpaired
Student’s t-test and was reached when P < 0.05.

Data availability
Data that support the findings of this study are available upon
reasonable request.

Results
Clinical characterization
Whole-exome sequencing of the patients’ genomes identi-
fied 15 different SCN9A mutations, including five recurrent
variants (Supplementary Table 1). Patients carrying differ-
ent Na1.7 variants displayed a range of responses to laco-
samide. We selected variants that were most representative
of the range of clinical responsiveness for biophysical ana-
lysis. Specifically, we selected two variants from responsive
patients (≥1 point on the PI-NRS scale) and three variants
from non-responsive patients (<1 point on the PI-NRS
scale), based on the pain scores plot in the clinical trial
study (de Greef et al., 2019), shown in Fig. 1.

We considered the W719C (2157G>C; p.Trp719Cys) and the I739V (c.2215A>G; p.Ile739Val) variants to be
associated with responders. W719C showed the largest re-
sponse to lacosamide compared to the other variants, with
a 3.65-point decrease from the baseline average score.
I739V, which has been functionally profiled in detail pre-
viously (Fu et al., 2012; Han et al., 2012), was found in
different patients. While four I739V carriers displayed
a 3.65-point decrease from the baseline average score.

By contrast, I228M (c.684C>G; p.Ile228Met), L1267V (c.3799C>G; p.Leu1267Val) and W1538R (c.4612T>C; p.Trp1538Arg) variants were associated with non-responders. L1267V was selected because it was carried by four patients. Three did not respond to lacosamide, while one carrier of this variant responded to both lacosamide and placebo; there was a 3.0-point reduction in the baseline pain score following the lacosamide phase compared to the placebo phase (Fig. 1E). I228M is a well-studied variant with a clearly defined pathogenicity (Estacion et al., 2011; Persson et al., 2013) (Fig. 1B), and W1538R, previously characterized as a gain-of-function variant altering cellular excitability (Cregg et al., 2013), was identified in two carriers with compound mutations in Na\textsubscript{v}1.7, both of whom were non-responders (Fig. 1F). The two patients carrying the W1538R variation were both found to also carry a M932L/V991L (c.2794A>G; p.Met932Leu/Val991Leu) double mutation, with one of them also carrying I739V in addition to the M932L/V991L mutation. Notably, patients who only carry M932L/V991L or I739V variants alone have been responsive to lacosamide treatment (Supplementary Table 1). The pain scores are presented in Fig. 1.

The locations of the variants within the Na\textsubscript{v}1.7 backbone are shown in Fig. 2. Both responder variants are located in the second domain (DII): I739V is located within the first transmembrane segment of the voltage-sensing domain (VSD; DII/S1) (Faber et al., 2012), while W719C is found 20 amino acids upstream, at the end of the first intracellular loop linking DI/S6 to DII/S1 (Linker 1). The non-responder variants are spread across the three other domains within the VSD (S1–S4) of the channel: I228M is located in DI/S4 (Faber et al., 2012), L1267V in DIII/S3 (Brouwer et al., 2014) and W1538R in DIV/S2 (Cregg et al., 2013; Kapetis et al., 2017).

**Lacosamide fails to alter the voltage-dependence of activation in responsive and non-responsive Na\textsubscript{v}1.7 variants**

Representative Na\textsubscript{v}1.7 sodium currents recorded after perfusion with 30 µM lacosamide or vehicle control are shown in Fig. 3. Consistent with previous data (Errington et al., 2006, 2008), the voltage-dependence of activation, represented as the Boltzmann fit of normalized conductance, was unaffected by the clinically achievable concentration of 30 µM lacosamide in both the mutant and wild-type cells (Fig. 3).

**Lacosamide evokes a hyperpolarizing shift in slow inactivation in most of the Na\textsubscript{v}1.7 variants**

Lacosamide has previously been reported to have large effects on sodium channel slow inactivation (Errington et al., 2006, 2008; Niespodziany et al., 2013). To assess the potential impact of the variants on the lacosamide-mediated shift in slow inactivation, we compared the normalized slow inactivation curves from each of the variants examined in the presence of 30 µM lacosamide or vehicle control, as shown in Fig. 4. As expected, lacosamide shifted the voltage-dependence of slow inactivation of the wild-type channel to more hyperpolarized potentials (i.e. enhancing slow inactivation, thus reducing the number of Na\textsubscript{v}1.7 channels that are available to open at physiological membrane potentials; Fig. 4A). The half-inactivation voltage (V\textsubscript{1/2}) of slow-inactivation Boltzmann fits from each variant are shown in Table 1. The variants from patients who were scored as responders showed substantially enhanced slow inactivation in response to lacosamide: W719C demonstrated a –20 mV hyperpolarized shift (Fig. 4C, P = 0.02), while I739V channels showed a –12.2 mV shift to more hyperpolarized potentials (Fig. 4D, P < 0.01). The shifts in voltage-dependence of slow inactivation for the non-responsive variants (L1267V, W1538R, and I228M) were –9.3 mV, +1.4 mV, and –12.1 mV, respectively (Fig. 4B, E and F). Except for W1538R, lacosamide induced a significant hyperpolarizing shift in voltage-dependence of slow inactivation in all variants.

**Lacosamide enhances fast inactivation selectively in responsive variants**

While recent data suggest the potential of lacosamide to affect steady state fast inactivation in Na\textsubscript{v}1.7 wild-type
Figure 3  Lacosamide effects on voltage-dependence of activation for each Na$_v$1.7 variant. (A) Sodium current traces elicited by the activation protocol from different cells exposed to either lacosamide (LCM) or vehicle control. Sodium inward currents were elicited from a holding potential of $-120$ mV to various depolarizing steps ranging from $-80$ to $+40$ mV in 5 mV increments. (B) Normalized current-voltage relationship obtained by measuring the peak inward current elicited as a function of the stimulus voltage. (C) Voltage-dependence of activation for cells treated with lacosamide or vehicle control. The peak inward currents were transformed to normalized conductance from current-voltage plot.
channels (Jo and Bean, 2017), most previous reports have shown little to no effects of lacosamide on fast inactivation. Nonetheless, other compounds that bind to the local anaesthetic site, such as carbamazepine, phenytoin, or lamotrigine, have been shown to enhance fast inactivation (Kuo and Lu, 1997; Mantegazza et al., 2010). We compared the effect of lacosamide on fast inactivation for the five variants and found that lacosamide significantly enhanced fast inactivation exclusively in variants carried by patients who were scored as responsive, while having no significant effect on wild-type or on variants from patients who scored as non-responsive (Fig. 5). I739V displayed a large \(-14.6\) mV hyperpolarizing shift (Fig. 5D, \(P < 0.01\)), and W719C channels exhibited a \(-9.7\) mV shift (Fig. 5C, \(P < 0.01\)). The \(V_{1/2}\) of the fast-inactivation Boltzmann fits from each variant are documented in Table 1.

### Table 1

| Variant   | Slow inactivation (\(V_{1/2}\)) | Fast inactivation (\(V_{1/2}\)) | Use-dependence |
|-----------|---------------------------------|---------------------------------|----------------|
|           | Vehicle Lacosamide              | Vehicle Lacosamide              |                |
| Wild-type | –70.6 ± 1.4 (\(n=16\))          | –86.8 ± 1.4 (\(n=19\))          | 0.82 ± 0.02 (\(n=20\)) |
| Responders W719C | –56.9 ± 4.4 (\(n=8\))          | –88.4 ± 1.3** (\(n=5\))          | 0.96 ± 0.01 (\(n=10\)) |
| I739V    | –67.1 ± 1.6 (\(n=12\))          | –99.8 ± 1.1** (\(n=6\))          | 0.82 ± 0.01 (\(n=16\)) |
| Non-responders L1267V | –74.4 ± 2.1 (\(n=9\))          | –82.5 ± 2.4 (\(n=7\))           | 0.89 ± 0.02 (\(n=12\)) |
| W1538R   | –69.4 ± 4.0 (\(n=10\))         | –79.5 ± 3.0 (\(n=10\))          | 0.91 ± 0.02 (\(n=11\)) |
| I228M    | –66.0 ± 2.9 (\(n=7\))          | –87.1 ± 1.6 (\(n=11\))          | 0.82 ± 0.03 (\(n=6\)) |

Lacosamide significantly enhanced slow inactivation in wild-type and all mutant channels, except for W1538R, while fast inactivation was only affected by lacosamide in W719C and I739V channels. Use-dependence of inhibition at 20 Hz was changed in the two responder variants as well as in wild-type and I228M. Data are presented as mean ± SEM. Significant values are represented by \(*P < 0.05\), \(**P < 0.01\).
Lacosamide produces a variable effect on use-dependent block in non-responders

We also examined the development of use-dependent block at 20 Hz for each of the variants upon exposure to 30 μM lacosamide or vehicle control (Fig. 6). Lacosamide enhanced use-dependent inhibition in wild-type channels (Fig. 6A) and in variants from patients who were scored as responders (Fig. 6C and D). Interestingly, lacosamide did not increase use-dependent blockade in two of the variants identified in non-responsive patients (L1267V and W1538R; Fig. 6E and F), while it did in the non-responsive I228M variant (Fig. 6B and Table 1). The degree of use-dependent block from each Nav1.7 mutation is shown in Table 1.

Discussion

Pharmacotherapy for the treatment of pain in SFN is limited, with many patients reporting inadequate pain relief from currently available medications, including sodium channel blockers. However, while mutations in peripheral sodium channels have been linked to pathological pain, there have only been a few studies documenting treatment outcomes in patients with specific Na\textsubscript{1.7} mutations. We have recently described pharmacogenomically-guided targeting of rare but drug-responsive Na\textsubscript{1.7} mutations with carbamazepine in patients with inherited erythromelalgia (Yang et al., 2012, 2018; Geha et al., 2016; Han et al., 2018). However, thus far there has not been a pharmacogenomic study of patients with more common pain disorders who carry Na\textsubscript{1.7} channel mutations. In this study, we build on the findings of the LENS trial (de Greef et al., 2019), which investigated the efficacy of lacosamide for the treatment of patients with Na\textsubscript{1.7}-related SFN, and provide biophysical data that may explicate why a subset of Na\textsubscript{1.7} variants differentially responded to lacosamide treatment.

Lacosamide has been shown to operate via different mechanisms of action compared to classical sodium channel blockers. Using standard recording protocols, AEDs have been found to enhance both fast and slow inactivation, while lacosamide has been reported to selectively enhance voltage-dependence of slow inactivation. According to this model, lacosamide has been proposed to either bind to a different site, or at significantly slower binding rates than other anticonvulsants (Errington et al., 2008; Sheets et al., 2008). However, using a different recording protocol and a concentration of 100 μM, lacosamide has been
reported to effectively enhance steady state fast inactivation when exposed for long depolarizations, possibly due to binding to the fast inactivated state but at a very slow rate (Jo and Bean, 2017). In the present study, we observed that, at clinically achievable concentration of 30 µM, lacosamide does not affect fast inactivation in Na\(_{v}\)1.7 wild-type channels when standard recording protocols were used (Fig. 5A). Lacosamide failure to alter fast inactivation in wild-type channels in our study is likely due to the different lacosamide concentration and recording conditions, compared to those used by Jo and Bean (2017).

Lacosamide binding activity is known to share several properties with well-established sodium channel inhibitors. For instance, lacosamide has been found to compete with carbamazepine and lidocaine for channel inhibition in vitro, suggesting that they likely bind at the same site, but with different pharmacodynamics (Jo and Bean, 2017). Classical sodium channel blockers have been shown to share the same binding site located in the pore-forming region of the channel, within S6 of DI, DIII and DIV (Ragsdale \textit{et al}., 1994; Yarov-Yarovoy \textit{et al}., 2001, 2002; Catterall and Swanson, 2015). Genetic alterations at these sites have been shown to fully abolish both voltage- and use-dependent block (Fozzard \textit{et al}., 2011), highlighting the importance of maintaining the integrity of these regions for potent inhibition. Topological mapping shows that none of the five variants described in this study map to the pore domain itself, but instead they map to the VSD (Fig. 2). Therefore, the variants might not directly interfere with the drug binding site in the ion conduction pathway.

While lacosamide did not affect fast inactivation in wild-type channels (Fig. 5A) or any of the three non-responder variants (Fig. 5B, E and F), it caused a significant and substantial hyperpolarizing shift in the two variants identified in responsive patients (W719C and I739V; Fig. 5C and D). These differences in response to lacosamide may result from a novel emerging mechanism of action, conferred by these specific variants. This type of phenomenon has previously been reported in inherited erythromelalgia, where multiple examples of Na\(_{v}\)1.7 variants underlying inherited erythromelalgia pathogenicity have been shown to be treatment-responsive to carbamazepine in a disorder in which most patients are treatment-resistant. Three inherited erythromelalgia-causing variants mapping to DI (I234T, S241T and V400M) have been shown to respond to carbamazepine via a depolarizing (corrective) shift in activation (Fischer \textit{et al}., 2009; Meijer \textit{et al}., 2014; Geha \textit{et al}., 2016). Importantly, none of the mutations described above are located near the carbamazepine/local anaesthetic binding site. These findings, together with the present results, indicate that mutations outside the pore domain can alter the response of the mutant channel to drugs traditionally...
considered to be pore-binding, and suggest that functional testing of these variants in vitro may predict treatment responsiveness.

While fast inactivation seems to be the parameter that discriminates the responders from the non-responders, effects of lacosamide on slow inactivation and use-dependence might also be required for complete and sustained pain relief. In agreement with the known effects of lacosamide on slow inactivation, the wild-type channel as well as all but one mutant channel (W1538R) underwent a significant hyperpolarizing shift in the voltage-dependence of slow inactivation (Fig. 4). Interestingly, the W1538R variant was also found to be the strongest non-responder, with one carrier reporting better pain improvement from placebo than from treatment itself (Fig. 1). This variant specifically has been mapped to the VSD of DIV, and is part of the binding site of the new class of aryl sulfonamide Na\textsubscript{1.7} blockers, such as PF-05089771, which targets both the fast and slow inactivated states of Na\textsubscript{1.7} with high selectivity (McCormack et al., 2013; Focken et al., 2016; Theile et al., 2016). This observation raises the possibility of a potential common mechanism of action between the two drugs and suggests that a disruption at this site might alter lacosamide function.

Whole-exome sequencing (WES) of the two patients carrying the W1538R variant identified additional Na\textsubscript{1.7} mutations in both of these subjects. Both individuals carried the M932L/V991L variant, which has been associated with DRG neuron hyperexcitability (Faber et al., 2012), and one of these subjects also carried the I739V mutation (which also has been shown to produce DRG neuron hyperexcitability) (Faber et al., 2012; Han et al., 2012). WES analysis as carried out in this study does not allow the identification of the distribution of these mutations between the two Na\textsubscript{1.7} alleles, which requires analysis of DRG-specific RNA sequences from these patients. Such analysis is beyond the scope of this study because it requires access to sensory tissues from these patients or the development of induced pluripotent stem cells that can then be differentiated into nociceptors in vitro. Nonetheless, as other subjects in this cohort carrying either the I739V alone or the M932L/V991L compound variants responded to lacosamide (Supplementary Table 1), and fast inactivation of I739V mutant channels was hyperpolarized by exposure to lacosamide (Fig. 5), the W1538R mutation potentially acts as a dominant contributor to the poor efficacy of lacosamide in these subjects carrying the compound genotypes. Future clinical studies on patients carrying only the W1538R mutation will be needed to provide a more definitive conclusion that carriers of this mutation will not respond to treatment with lacosamide.

Interestingly, one of the patients carrying solely the I739V variant was unresponsive to lacosamide, while the four other patients were previously reported to respond well to lacosamide (Fig. 1) (de Greef et al., 2019). Furthermore, in the current study we demonstrated lacosamide to significantly shift all three gating parameters of I739V channels (Table 1). Thus, the cause for the lack of response of one carrier with the I739V mutation is not channel intrinsic, but is likely to be affected by additional genetic, epigenetic or environmental factors. Notably, the non-responder carrier had a relatively low level of pain severity, which may have limited the detection of lacosamide efficacy.

Many factors can impact on an individual’s response to a drug. While focusing on the interaction between drug and target molecule, our data suggest that in vitro pharmacological and biophysical analysis of Na\textsubscript{1.7} mutations may predict the likelihood of specific carriers to be responsive to treatment, which could be important in the future as an approach to personalized treatment based on the patient’s genetic background. Building on the first clinical trial of lacosamide for treatment of patients with SFN and carriers of Na\textsubscript{1.7} variants (LENSS) (de Greef et al., 2019), the present results suggest that, at least for strong positive responders and non-responders, pharmacogenomic analysis in vitro may correlate with clinical responsiveness. The relatively small number of variants that were studied here, two responders and three non-responders, necessitates caution in generalizing these data to suggest that SFN patients who carry Na\textsubscript{1.7} variants that enhance fast inactivation upon exposure to lacosamide will necessarily be responsive to treatment. If supported by studies in larger numbers of patients tested with lacosamide and if extended to other potential medications, the pharmacogenomic approach described in this paper might contribute to the development of selective, individualized pain treatment strategies.

Acknowledgements

We thank Fadia Dib-Hajj and Daniel Sosniak for excellent technical assistance.

Funding

This work was supported by Center Grant B9253-C from the U.S. Department of Veterans Affairs Rehabilitation Research and Development Service. This project also received funding from the Molecule-to-Man Pain Network, a European Commission Multi-Center Collaborative Projects through the European Union’s Horizon 2020 research and innovation program under grant agreement No. 721841. The clinical study was supported by a grant from the Prinses Beatrix Spierfonds. The Center for Neuroscience and Regeneration Research is a Collaboration of the Paralyzed Veterans of America with Yale University.

Competing interests

The authors report no competing interests.
Supplementary material is available at Brain online.

References

Bennett DL, Clark AJ, Huang J, Waxman SG, Dib-Hajj SD. The role of voltage-gated sodium channels in pain signaling. Physiol Rev 2019; 99: 1079–151.

Brouwer BA, Merkies IS, Gerrits MM, Waxman SG, Hoeijmakers JG, Faber CG. Painful neuropathies: the emerging role of sodium channelopathies. J Peripher Nerv Syst 2014; 19: 53–65.

Catterall WA, Swanson TM. Structural basis for pharmacology of voltage-gated sodium and calcium channels. Mol Pharmacol 2015; 88: 141–50.

Cawello W. Clinical pharmacokinetic and pharmacodynamic profile of lacosamide. Clin Pharmacokinet 2015; 54: 901–14.

Cregg R, Laguda B, Werdehausen R, Cox JJ, Linley JE, Ramirez JD, et al. Novel mutations mapping to the fourth sodium channel domain of Nav1.7 result in variable clinical manifestations of primary erythromelalgia. Neuromod Med 2013; 13: 265–78.

de Greef BTA, Hoeijmakers JG, Geerts M, Oakes M, Church TJE, Waxman SG, et al. Lacosamide in patients with Nav1.7 mutations-related small fibre neuropathy: a randomized controlled trial. Brain 2019; 142: 263–75.

de Greef BTA, Hoeijmakers JG, Gorissen-Brouwers CML, Geerts M, Faber CG, Merkies I. Associated conditions in small fiber neuropathy—a large cohort study and review of the literature. Eur J Neurol 2018; 25: 348–55.

de Greef BT, Merkies IS, Geerts M, Faber CG, Hoeijmakers JG. Efficacy, safety, and tolerability of lacosamide in patients with gain-of-function Nav1.7 mutation-related small fiber neuropathy: study protocol of a randomized controlled trial—the LENSST study. Trials 2016; 17: 306.

Dib-Hajj SD, Waxman SG. Sodium channels in human pain disorders: genetics and pharmacogenomics. Annu Rev Neurosci 2019; 42: 87–106.

Dib-Hajj SD, Yang Y, Black JA, Waxman SG. The Na(V)1.7 sodium channel: from molecule to man. Nat Rev Neurosci 2013; 14: 49–62.

Eijkenboom I, Sopacua M, Hoeijmakers JGJ, de Greef BTA, Lindsey P, Merkies IS, et al. Yield of peripheral sodium channels gene screening in pure small fibre neuropathy. J Neurol Neurosurg Psychiatry 2019; 90: 342–52.

Errington A, Coyne L, Stohr T, Selve N, Lees G. Seeking a mechanism of action for the novel anticonvulsant lacosamide. Neuropsychopharmacology 2006; 50: 1016–29.

Errington A, Stohr T, Heers C, Lees G. The investigational anticonvulsant lacosamide selectively enhances slow inactivation of voltage-gated sodium channels. Mol Pharmacol 2008; 73: 157–69.

Estacion M, Han C, Choi JS, Hoeijmakers JG, Lauria G, Drenth JP, et al. Intra- and interfamily phenotypic diversity in pain syndromes associated with a gain-of-function variant of NaV1.7. Mol Pain 2011; 7: 92.

Faber CG, Hoeijmakers JG, Ahn HS, Cheng X, Han C, Choi JS, et al. Gain of function Nav1.7 mutations in idiopathic small fiber neuropathy. Ann Neurol 2012; 71: 26–39.

Finnerup NB, Attal N, Haroutounian S, McNicol E, Baron R, Dwarkin RH, et al. Pharmacotherapy for neuropathic pain in adults: a systematic review and meta-analysis. Lancet Neurol 2015; 14: 162–73.

Fischer TZ, Gilmore ES, Estacion M, Eastman E, Taylor S, Melanson M, et al. A novel Nav1.7 mutation producing carbamazepine-responsive erythromelalgia. Ann Neurol 2009; 65: 733–41.

Focken T, Liu S, Chahal N, Dauphinais M, Grimwood ME, Chowdhury S, et al. Discovery of aryl sulfonamides as isoform-selective inhibitors of NaV1.7 with efficacy in rodent pain models. ACS Med Chem Lett 2016; 7: 277–82.

Fozzard HA, Sheets MF, Hanck DA. The sodium channel as a target for local anesthetic drugs. Front Pharmacol 2011; 2: 68.

Fu W, Wang SJ, Zhou GD, Liu W, Cao Y, Zhang WJ. Residual undifferentiated cells during differentiation of induced pluripotent stem cells in vitro and in vivo. Stem Cells Dev 2012; 21: 521–9.

Geha P, Yang Y, Estacion M, Schulman BR, Tokuno H, Apkarian AV, et al. Pharmacotherapy for pain in a family with inherited erythromelalgia guided by genomic analysis and functional profiling. JAMA Neurol 2016; 73: 659–67.

Han C, Hoeijmakers JG, Ahn HS, Zhao P, Shah P, Lauria G, et al. Nav1.7-related small fiber neuropathy: impaired slow-inactivation and DRG neuron hyperexcitability. Neurology 2012; 78: 1635–43.

Han C, Hoeijmakers JG, Liu S, Gerrits MM, Te Morsche RH, Lauria G, et al. Functional profiles of SCN9A variants in dorsal root ganglion neurons and superior cervical ganglion neurons correlate with autonomic symptoms in small fibre neuropathy. Brain 2012; 135: 2613–28.

Han C, Themistocleous AC, Estacion M, Dib-Hajj FB, Blesnaic I, Macala L, et al. The novel activity of carbamazepine as an activation modulator extends from NaV1.7 mutations to the Nav1.3-5242T mutant channel from a patient with painful diabetic neuropathy. Mol Pharmacol 2018; 94: 1256–69.

Jo S, Bean BP. Lacosamide inhibition of Nav1.7 voltage-gated sodium channels: slow binding to Fast-inactivated states. Mol Pharmacol 2017; 91: 277–86.

Kapetis D, Sassone J, Yang Y, Calbardi B, Xenakis MN, Westra RL, et al. Network topology of NaV1.7 mutations in sodium channel-related painful disorders. BMC Syst Biol 2017; 11: 28.

Kim DW, Kim HK, Baek EK. Switching from traditional sodium channel blockers to lacosamide in patients with epilepsy. Seizure 2019; 65: 172–5.

Kuo CC, Lu L. Characterization of lamotrigine inhibition of Na+ channels in rat hippocampal neurons. Br J Pharmacol 1997; 121: 1231–8.

Mantegazza M, Curia G, Biagini G, Ragsdale DS, Avoli M. Voltage-gated sodium channels as therapeutic targets in epilepsy and other neurological disorders. Lancet Neurol 2010; 9: 413–24.

McCormack K, Santos S, Chapman ML, Krafft DS, Marron BE, CW, et al. Voltage sensor interaction site for selective small molecule inhibitors of voltage-gated sodium channels. Proc Natl Acad Sci U S A 2013; 110: E2724–32.

Meijer IA, Vanasse M, Nizard S, Robitaille Y, Rossignol E. An atypical case of SCN9A mutation presenting with global autonomic symptoms in small fibre neuropathy. Brain 2012; 116: 1559–68.

Mourat A, Yang X, Li W, Gilbraith KB, Luo S, Cai S, et al. CRISPR/Cas9 editing of NFI gene identifies CRMP2 as a therapeutic target in neurofibromatosis type 1-related pain that is reversed by (S)-Lacosamide. Pain 2015; 158: 2301–19.

Namer B, Schmidt D, Eberhardt E, Maroni D, Dorfmeister E, Kleggetveit IP, et al. Pain relief in a neuropathy patient by lacosamide guided by genomic analysis and functional profiling. JAMA Neurol 2016; 73: 659–67.

Nishikawa N, Nomoto M. Management of neuropathic pain. J Gen Fam Med 2017; 18: 56–60.

Niespodziany I, Leclere N, Vandenplas C, Foerch P, Wolff C. Comparative study of lacosamide and classical sodium channel blocking antiepileptic drugs on sodium channel slow inactivation. J Neurosci Res 2013; 91: 436–43.

Nishikawa N, Nomoto M. Management of neuropathic pain. J Gen Fam Med 2017; 18: 56–60.

Persson AK, Liu S, Faber CG, Merkies IS, Black JA, Waxman SG. Neuropathy-associated Nav1.7 variant E228M impairs integrity of dorsal root ganglion neuron axons. Ann Neurol 2013; 73: 140–5.

Ragsdale DS, McPhee JC, Scheuer T, Catterall WA. Molecular determinants of state-dependent block of Na+ channels by local anesthetics. Science 1994; 265: 1724–8.

Downloaded from https://academic.oup.com/brain/advance-article-abstract/doi/10.1093/brain/awaa016/5721421 by guest on 05 February 2020
Rauck RL, Shaibani A, Biton V, Simpson J, Koch B. Lacosamide in painful diabetic peripheral neuropathy: a phase 2 double-blind placebo-controlled study. Clin J Pain 2007; 23: 150–8.

Shaibani A, Biton V, Rauck R, Koch B, Simpson J. Long-term oral lacosamide in painful diabetic neuropathy: a two-year open-label extension trial. Eur J Pain 2009a; 13: 458–63.

Shaibani A, Fares S, Selam JL, Arslanian A, Simpson J, Sen D, et al. Lacosamide in painful diabetic neuropathy: an 18-week double-blind placebo-controlled trial. J Pain 2009b; 10: 818–28.

Sheets PL, Heers C, Stoehr T, Cummins TR. Differential block of sensory neuronal voltage-gated sodium channels by lacosamide [(2R)-2-(acetylamino)-N-benzyl-3-methoxypropanamide], lidocaine, and carbamazepine. J Pharmacol Exp Ther 2008; 326: 89–99.

Sopacua M, Hoeijmakers JGJ, Merkies ISJ, Lauria G, Waxman SG, Faber CG. Small-fiber neuropathy: expanding the clinical pain universe. J Peripher Nerv Syst 2019; 24: 19–33.

Theile JW, Fuller MD, Chapman ML. The selective Nav1.7 inhibitor. Mol Pharmacol 2016; 90: 540–8.

Wallis Y, Payne S, McAnulty C, Bodmer D, Sistermans E, Robertson K, et al. Practical guidelines for the evaluation of pathogenicity and the reporting of sequence variants in clinical molecular genetics. Assoc Clin Genom Sci (ACGS) 2013. Available from https://www.acgs.uk.com/media/10791/evaluation_and_reporting_of_sequence_variants_bpgs_june_2013_-_finalpdf.pdf (15 September 2019, date last accessed).

Waxman SG, Merkies ISJ, Gerrits MM, Dib-Hajj SD, Lauria G, Cox JJ, et al. Sodium channel genes in pain-related disorders: phenotype-genotype associations and recommendations for clinical use. Lancet Neurol 2014; 13: 1152–60.

Wilson SM, Khanna R. Specific binding of lacosamide to collapsin response mediator protein 2 (CRMP2) and direct impairment of its canonical function: implications for the therapeutic potential of lacosamide. Mol Neurobiol 2015; 51: 599–609.

Wymer JP, Simpson J, Sen D, Bongardt S, Lacosamide S. Efficacy and safety of lacosamide in diabetic neuropathic pain: an 18-week double-blind placebo-controlled trial of fixed-dose regimens. Clin J Pain 2009; 25: 376–85.

Yang Y, Adi T, Effraim PR, Chen L, Dib-Hajj SD, Waxman SG. Reverse pharmacogenomics: carbamazepine normalizes activation and attenuates thermal hyperexcitability of sensory neurons due to Nav 1.7 mutation I234T. Br J Pharmacol 2018; 175: 2261–71.

Yang Y, Dib-Hajj SD, Zhang J, Zhang Y, Tyrrell L, Estacion M, et al. Structural modelling and mutant cycle analysis predict pharmacoresponsiveness of a Na(V)1.7 mutant channel. Nat Commun 2012; 3: 1186.

Yang Y, Huang J, Mis MA, Estacion M, Macala L, Shah P, et al. Nav1.7-A1632G mutation from a family with inherited erythromelalgia: enhanced firing of dorsal root ganglia neurons evoked by thermal stimuli. J Neurosci 2016; 36: 7511–22.

Yarov-Yarovoy V, Brown J, Sharp EM, Clare JJ, Scheuer T, Catterall WA. Molecular determinants of voltage-dependent gating and binding of pore-blocking drugs in transmembrane segment IIS6 of the Na(+) channel alpha subunit. J Biol Chem 2001; 276: 20–7.

Yarov-Yarovoy V, McPhee JC, Idsvoog D, Pate C, Scheuer T, Catterall WA. Role of amino acid residues in transmembrane segments IS6 and IIIS6 of the Na+ channel alpha subunit in voltage-dependent gating and drug block. J Biol Chem 2002; 277: 35393–401.