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

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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.

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Introduction

Painful peripheral neuropathy represents a major health burden and a globally unmet clinical need. Current first-line therapeutic strategies for the management of chronic pain associated with painful neuropathy, including tricyclic antidepressants, pregabalin, gabapentin or serotonin-noradrenaline reuptake inhibitors, are in many cases reported as unsatisfactory, in part because of adverse effects (Finnerup *et al.*, 2015; Nishikawa and Nomoto, 2017). Furthermore, the degree of pain improvement dramatically varies across the population (Sopacua *et al.*, 2019). More effective pharmacological management of neuropathic pain represents a high priority.
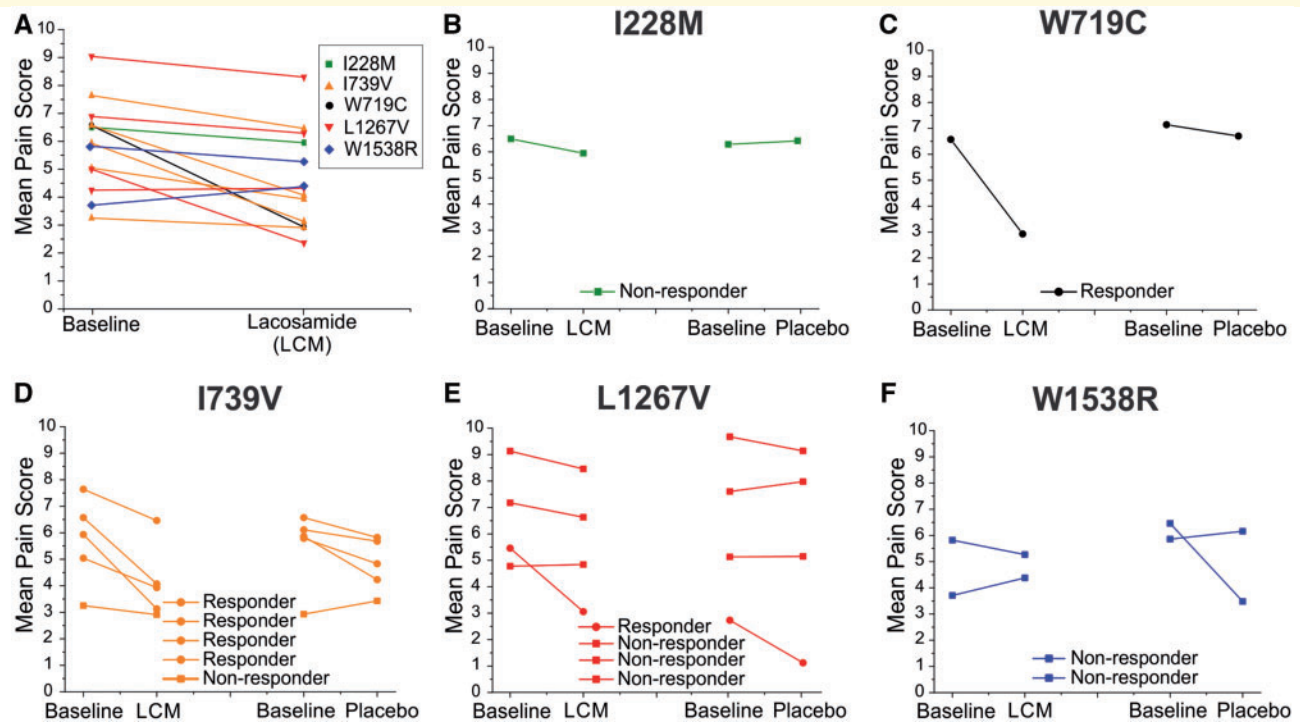
Genetic studies have provided compelling evidence regarding the contribution of altered voltage-gated sodium channel (VGSC) expression and function in neuropathic pain. The Na$_v$1.7 sodium channel has drawn particular interest because it is preferentially expressed in peripheral sensory and sympathetic neurons and has directly been linked to multiple human pain conditions: gain-of-function missense mutations in *SCN9A* (encoding the Na$_v$1.7 protein) have been reported in patients with inherited erythromelalgia, paroxysmal extreme pain disorder, and some forms of small fibre neuropathy (SFN). Conversely, loss-of-function mutations in Na$_v$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 Aδ-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$_v$1.7 (Errington *et al.*, 2006, 2008; Niespodziany *et al.*, 2013). However, a recent study has alternatively suggested that lacosamide binds to the fast-inactivated state of Na$_v$1.7, but at a slower rate (Jo and Bean, 2017). There is also evidence that lacosamide may alter the effect of CRMP2 (collapsin-response mediator protein 2) on Na$_v$1.7 channel trafficking (Wilson and Khanna, 2015; Moutal *et al.*, 2017). Furthermore, lacosamide has been found to have a higher affinity for tetrodotoxin-sensitive sodium channels over other ion channels and central receptors (Sheets *et al.*, 2008; Moutal *et al.*, 2017) that are targeted by traditional AEDs (Errington *et al.*, 2006), conferring increased selectivity. A retrospective study of patients with seizures who had switched from other AED drugs to lacosamide, because of deleterious effects from their original AED treatment, has shown successful conversion in many of these patients (Kim *et al.*, 2019), suggesting lacosamide to have improved tolerability. Lacosamide has also been tested in small clinical trials as a potential therapy for painful diabetic neuropathy where the majority of patients reported significant pain improvement with <10% reporting adverse effects (Rauck *et al.*, 2007; Shaibani *et al.*, 2009a, b; Wymer *et al.*, 2009). However, until recently, the clinical effects of lacosamide on patients with Na$_v$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$_v$1.7 variants. Fifteen different mutations were identified in this cohort, of which five were carried by several patients. Different subjects in this trial exhibited a spectrum of responses to treatment, with some patients reporting substantial pain relief while others did not show any improvement (Fig. 1). In a different study, lacosamide has been shown to produce pain relief in one patient with SFN (Namer *et al.*, 2019). However, the factors responsible for variable lacosamide responsiveness in the de Greef *et al.* (2019) study are not known. In the present study, we report the Na$_v$1.7 variants in these patients and assess the

Figure 1 Clinical response of patients carrying specific Na$_v$1.7 variants. (A) The changes in pain scores in response to either lacosamide (LCM) or placebo are plotted for all subjects carrying Na$_v$1.7 variants assessed in this study. Black and orange colours, adapted from de Greef *et al.* (2019), represent results from mostly responsive patients, whereas green, red and blue represent the results of mostly unresponsive patients. **(B–F)** The clinical responses for each variant. **(B)** The responses from a non-responder carrying variant I228M. **(C)** The responses from a patient with a large clinical response carrying variant W719C. **(D)** The responses from the patients carrying the I739V variant (five patients, four responders). **(E)** The responses from patients carrying the L1267V variant (four patients, three non-responders). **(F)** The responses from patients carrying the W1538R variant (two patients, both non-responders).

pharmacogenomic correlation between the Na$_v$1.7 genetic variants and the response to lacosamide in this series of patients. Using voltage-clamp recordings of HEK293 (human embryonic kidney) cells expressing variants from both responsive and non-responsive patients, we characterized the effect of lacosamide on the biophysical properties of the Na$_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 *SCN9A* gene (Na$_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$_v$1.7 wild-type isoform was rendered tetrodotoxin-resistant and fused to an eGFP-2A linker, as previously described (Yang *et al.*, 2016). The construct produces a green fluorescent protein with the Na$_v$1.7 channel as independent proteins from the same transcript, which enables the visual identification of transfected cells based on their fluorescence. Na$_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® XL site-directed mutagenesis

(Stratagene). HEK293 cells, grown under standard culture conditions (5% CO_2, 37°C) in Dulbecco's modified Eagle's medium supplemented with 10% foetal bovine serum, were transiently transfected by electroporation with a $Na_v1.7$ variant using Lipojet (SignaGen Laboratories). The transfected cells were resuspended and plated onto coverslips the next day and patch-clamp recordings were performed over the following 48 h.

Electrophysiology

Whole-cell voltage-clamp recordings were obtained at room temperature on isolated HEK293 cells showing green fluorescence. Recordings were alternatively performed on coverslips of HEK293 cells expressing wild-type or a $Na_v1.7$ variant, either treated with vehicle (extracellular solution) or lacosamide. Electrodes were pulled from 1.65-mm outer diameter borosilicate glass micropipettes (WPI) and had a resistance of 0.8–2.0 $M\Omega$ 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 dextrose). The extracellular solution contained (in mM): 140 NaCl, 3 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 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 capacitance 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 increments 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 conditioning 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 lacosamide 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 absolute 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, Northhampton, 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.
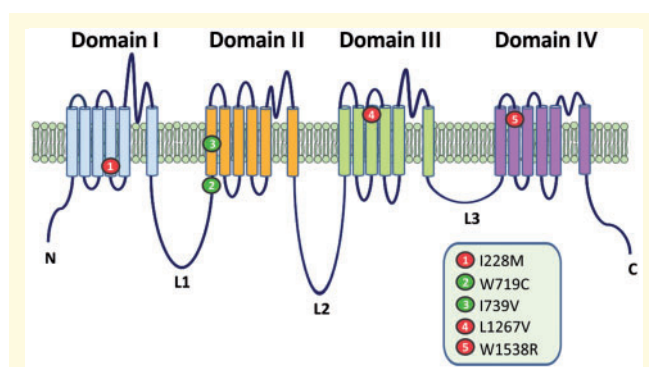
Results

Clinical characterization

Whole-exome sequencing of the patients' genomes identified 15 different *SCN9A* mutations, including five recurrent variants (Supplementary Table 1). Patients carrying different $Na_v1.7$ variants displayed a range of responses to lacosamide. We selected variants that were most representative of the range of clinical responsiveness for biophysical analysis. 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 response 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 previously (Fu *et al.*, 2012; Han *et al.*, 2012), was found in five different patients. While four I739V carriers displayed decreases (1.1 to 2.8 points) in their pain scores, one carrier did not exhibit any significant improvement while treated with lacosamide (Fig. 1D).

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$_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>C and c.2971G>T; 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$_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).

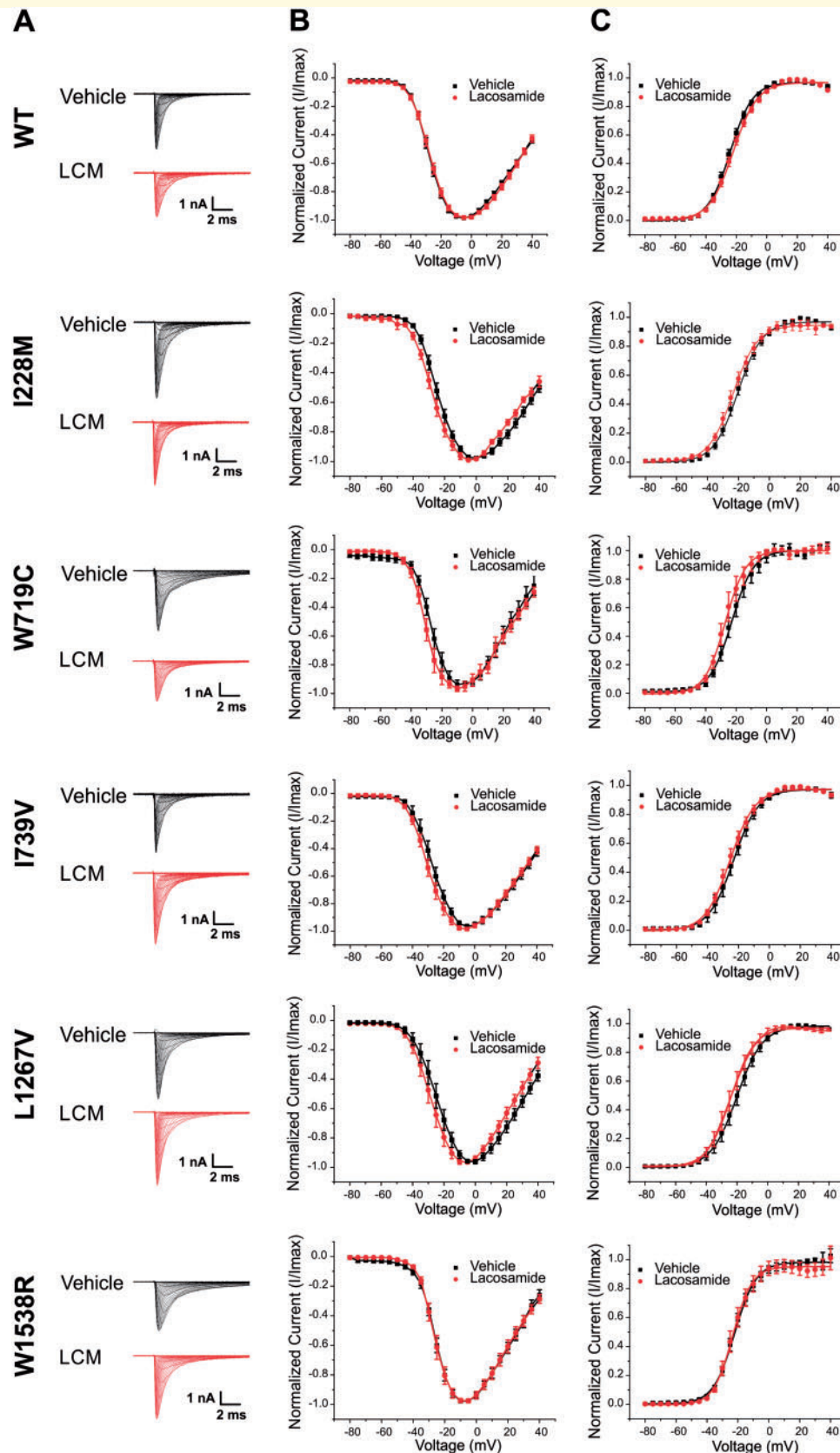## Lacosamide fails to alter the voltage-dependence of activation in responsive and non-responsive Na$_v$1.7 variants

Representative Na$_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$_v$1.7 variants
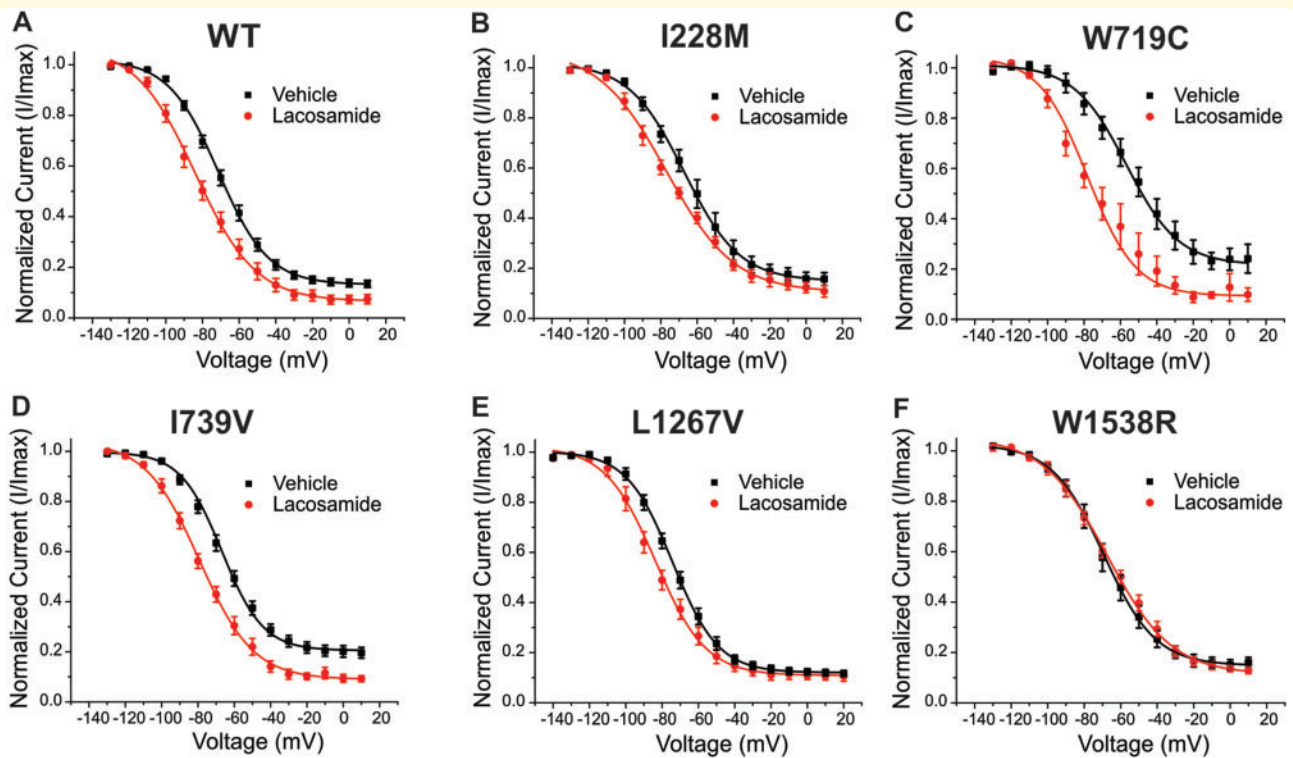
Lacosamide has previously been reported to have large effects on sodium channel slow inactivation (Errington *et al.*, 2006, 2008; Niespodziany *et al.*, 2013). To assess the potential impact of the variants on the lacosamide-mediated shift in slow inactivation, we compared the normalized slow inactivation curves from each of the variants examined in the presence of 30 µM lacosamide or vehicle control, as shown in Fig. 4. As expected, lacosamide shifted the voltage-dependence of slow inactivation of the wild-type channel to more hyperpolarized potentials (i.e. enhancing slow inactivation, thus reducing the number of Na$_v$1.7 channels that are available to open at physiological membrane potentials; Fig. 4A). The half-inactivation voltage ($V_{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$_v$1.7 wild-type

Figure 2 Topology of the human Na$_v$1.7 sodium channel, with the location of I228M, W719C, I739V, L1267V and W1538R. All but one mutation (W719C) are located in the VSDs of the channel. The two lacosamide-responsive mutations are shown in green. Both are located within the vicinity of Domain II: W719C is located at the end of Linker 1 and I739V in VSD II. Non-responsive mutations are indicated in red. All three variants (I228M, L1267V and W1538R) map to the VSD in Domains I, III and IV, respectively.

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.

Figure 4 Lacosamide effects on slow inactivation for each Na$_v$1.7 variant. The averaged voltage-dependence of slow inactivation curves in the presence (red) or absence (black) of lacosamide are shown for (**A**) wild-type (WT), (**B**) I228M, (**C**) W719C, (**D**) I739V, (**E**) L1267V and (**F**) W1538R. The average V$_{1/2}$s calculated from of the Boltzmann fits are listed in Table 1.
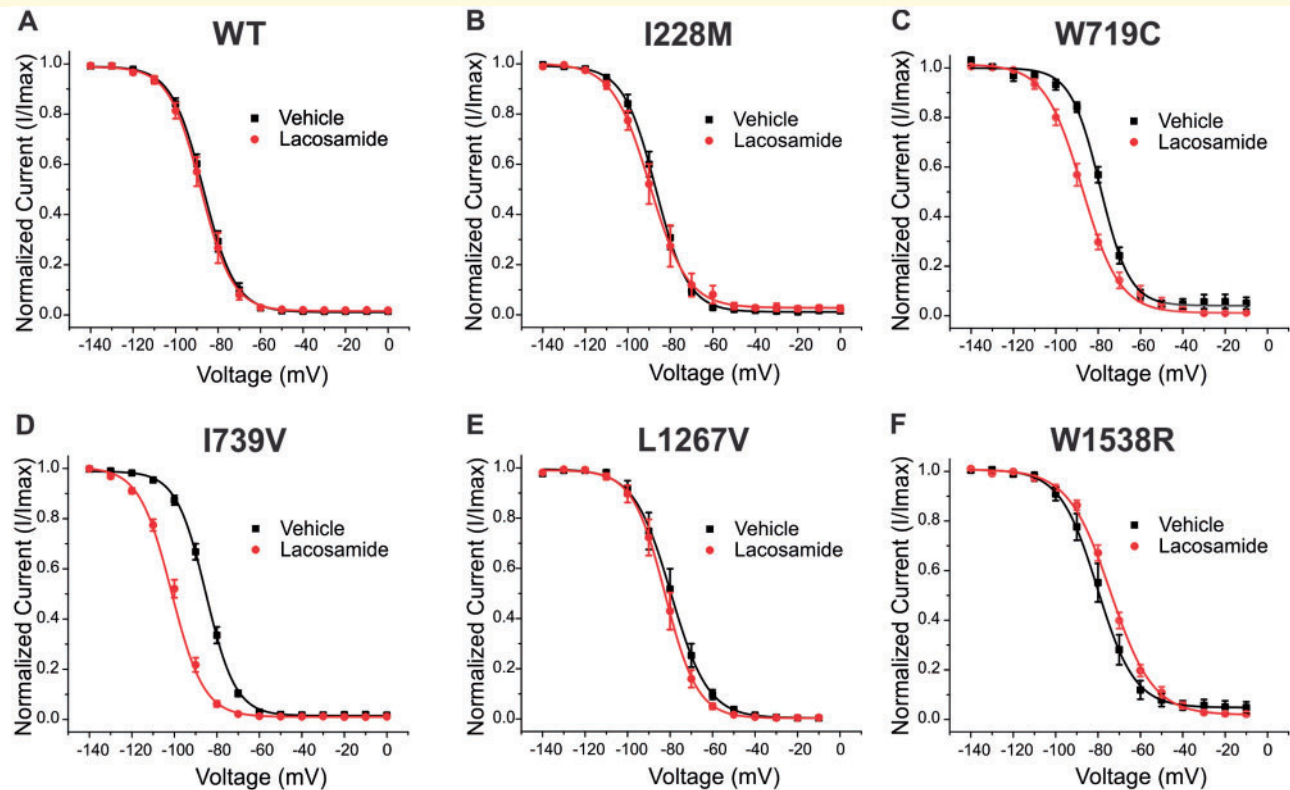
Table 1 Half inactivation voltage (V$_{1/2}$) and use-dependence of inhibition for the Na$_v$1.7 mutations to lacosamide

Variant	Slow inactivation (V$_{1/2}$)		Fast inactivation (V$_{1/2}$)		Use-dependence	
	Vehicle	Lacosamide	Vehicle	Lacosamide	Vehicle	Lacosamide
Wild-type	−70.6 ± 1.4 (n = 16)	−79.5 ± 2.1** (n = 9)	−86.8 ± 1.4 (n = 19)	−88.0 ± 1.9 (n = 8)	0.82 ± 0.02 (n = 20)	0.75 ± 0.02* (n = 9)
Responders						
W719C	−56.9 ± 4.4 (n = 8)	−77.1 ± 5.3* (n = 4)	−78.7 ± 0.8 (n = 11)	−88.4 ± 1.3** (n = 5)	0.96 ± 0.01 (n = 10)	0.87 ± 0.04** (n = 4)
I739V	−67.1 ± 1.6 (n = 12)	−79.3 ± 2.1** (n = 5)	−85.2 ± 1.0 (n = 11)	−99.8 ± 1.1** (n = 6)	0.82 ± 0.01 (n = 16)	0.73 ± 0.03** (n = 5)
Non-responders						
L1267V	−74.4 ± 2.1 (n = 9)	−83.7 ± 2.7* (n = 8)	−82.5 ± 2.4 (n = 7)	−87.1 ± 1.6 (n = 11)	0.89 ± 0.02 (n = 12)	0.87 ± 0.02 (n = 10)
W1538R	−69.4 ± 4.0 (n = 10)	−68.0 ± 2.8 (n = 8)	−79.5 ± 3.0 (n = 10)	−73.9 ± 1.2 (n = 8)	0.91 ± 0.02 (n = 11)	0.93 ± 0.01 (n = 8)
I228M	−66.0 ± 2.9 (n = 7)	−78.1 ± 2.5** (n = 7)	−87.1 ± 1.6 (n = 11)	−88.9 ± 2.8 (n = 6)	0.82 ± 0.03 (n = 6)	0.75 ± 0.01* (n = 7)

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.

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.

Figure 5 Lacosamide effects on steady state fast inactivation for each Na$_v$1.7 variant. The averaged fast inactivation curves in the presence or absence of lacosamide are shown for (**A**) wild-type (WT), (**B**) I228M, (**C**) W719C, (**D**) I739V, (**E**) L1267V and (**F**) W1538R. The average V$_{1/2}$s calculated from of the Boltzmann fits are listed in Table 1.

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 Na$_v$1.7 mutation is shown in Table 1.
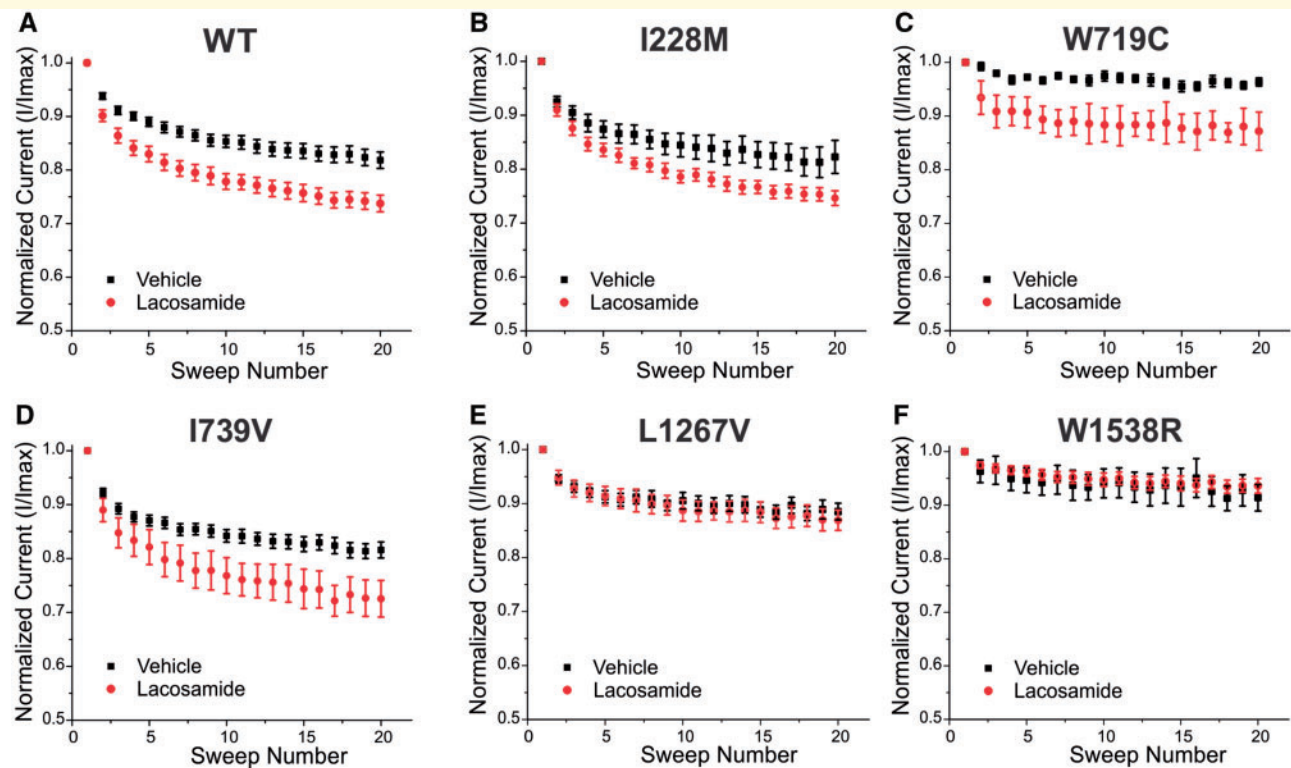
Discussion

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$_v$1.7 mutations. We have recently described pharmacogenomically-guided targeting of rare but drug-responsive Na$_v$1.7 mutations with carbamazepine in patients with inherited erythromelalgia (Yang *et al.*, 2012, 2018; Geha *et al.*, 2016; Han *et al.*, 2018). However, thus far there has not been a pharmacogenomic study of patients with more common pain disorders who carry Na$_v$1.7 channel mutations. In this study, we build on the findings of the LENSS trial (de Greef *et al.*, 2019), which investigated the efficacy of lacosamide for the treatment of patients with Na$_v$1.7-related SFN, and provide biophysical data that may explicate why a subset of Na$_v$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

Figure 6 Lacosamide effects on use-dependent block for each Na$_v$1.7 variant. The averaged use-dependent block to a train of 20 pulses at 20 Hz in the presence or absence of lacosamide are shown for (**A**) wild-type (WT), (**B**) I228M, (**C**) W719C, (**D**) I739V, (**E**) L1267V and (**F**) W1538R. The averages of the normalized responses are shown in Table 1.

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 *et al.*, 1994; Yarov-Yarovoy *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 *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 *et al.*, 2009; Meijer *et al.*, 2014; Geha *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 $Na_v1.7$ blockers, such as PF-05089771, which targets both the fast and slow inactivated states of $Na_v1.7$ 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 $Na_v1.7$ 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 $Na_v1.7$ 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 $Na_v1.7$ 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 $Na_v1.7$ 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 $Na_v1.7$ 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.

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Competing interests

The authors report no competing interests.

Supplementary material

Supplementary material is available at *Brain* online.

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