The Novel Activity of Carbamazepine as an Activation Modulator Extends from Na\(\gamma\)1.7 Mutations to the Na\(\gamma\)1.8-S242T Mutant Channel from a Patient with Painful Diabetic Neuropathy

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ABSTRACT

Neuropathic pain in patients carrying sodium channel gain-of-function mutations is generally refractory to pharmacotherapy. However, we have shown that pretreatment of cells with clinically achievable concentration of carbamazepine (CBZ; 30 \(\mu\)M) de-polarizes the voltage dependence of activation in some Na\(\gamma\)1.7 mutations such as S241T, a novel CBZ mode of action of this drug. CBZ reduces the excitability of dorsal root ganglion (DRG) neurons expressing Na\(\gamma\)1.7-S241T mutant channels, and individuals carrying the S241T mutation respond to treatment with CBZ. Whether the novel activation-modulating activity of CBZ is specific to Na\(\gamma\)1.7, and whether this pharmacogenomic approach can be extended to other sodium channel subtypes, are not known. We report here the novel Na\(\gamma\)1.8-S242T mutation, which corresponds to the Na\(\gamma\)1.7-S241T mutation, in a patient with neuropathic pain and diabetic peripheral neuropathy. Voltage-clamp recordings demonstrated hyperpolarized and accelerated activation of Na\(\gamma\)1.8-S242T. Current-clamp recordings showed that Na\(\gamma\)1.8-S242T channels render DRG neurons hyperexcitable. Structural modeling shows that despite a substantial difference in the primary amino acid sequence of Na\(\gamma\)1.7 and Na\(\gamma\)1.8, the S242 (Na\(\gamma\)1.8) and S241 (Na\(\gamma\)1.7) residues have similar position and orientation in the domain I S4-S5 linker of the channel. Pretreatment with a clinically achievable concentration of CBZ corrected the voltage dependence of activation of Na\(\gamma\)1.8-S242T channels and reduced DRG neuron excitability as predicted from our pharmacogenomic model. These findings extend the novel activation modulation mode of action of CBZ to a second sodium channel subtype, Na\(\gamma\)1.8.

Introduction

Sodium channels underlie the initiation and propagation of action potentials, and gain-of-function mutations of sodium channels have been linked to a spectrum of human pain disorders (Dib-Hajj et al., 2010, 2015, 2017; Bennett and Woods, 2014). The Na\(\gamma\)1.8-tetrodotoxin-resistant (TTX-R) sodium channel is preferentially expressed in dorsal root ganglion (DRG) neurons (Akopian et al., 1996), has been shown to contribute most of the sodium current underlying the rising phase of action potentials (Renganathan et al., 2001; Blair and Bean, 2002), and supports high-frequency firing in response to depolarization (Renganathan et al., 2001). Na\(\gamma\)1.8 has been linked to pain in a rat model of diabetes (Mert and Gunes, 2012). Electrophysiological recordings have shown increased TTX-R sodium currents from small-diameter DRG neurons from diabetic rats, accompanied by shifts in the voltage dependence of activation and steady-state fast-inactivation of the channel (Hong et al., 2004). The exposure of DRG neurons to methylglyoxal, a glucose metabolite that accumulates in diabetes, has been shown to depolarize voltage

ABBREVIATIONS: CBZ, carbamazepine; CI, confidence interval; D, domain; DMSO, dimethylsulfoxide; DPN, diabetic peripheral neuropathy; DRG, dorsal root ganglion; G, conductance; I, inward current; IEM, inherited erythromelalgia; IENFD, intraepidermal nerve fiber density; MAF, minimal allele frequency; MOE, Molecular Operating Environment; Na\(\gamma\), voltage-gated sodium channel; QST, quantitative sensory testing; S242T, Na\(\gamma\)1.8-S242T; TCSS, Toronto Clinical Scoring System; TTX-R, tetrodotoxin-resistant; \(V_{1/2}\), half-maximal potential; \(V_{1/2,act}\), potential at which activation is half maximal; WT, Na\(\gamma\)1.8-WT.
dependence of the fast inactivation of Na\textsubscript{V}1.8 and produce a reduction in rheobase, which is consistent with a contribution of Na\textsubscript{V}1.8 to the hyperexcitability of these neurons in diabetic models (Bierhaus et al., 2012). Recently, gain-of-function Na\textsubscript{V}1.8 mutations have been identified in patients with painful small fiber neuropathy, including subjects with diabetic peripheral neuropathy (DPN) (Faber et al., 2012b; Huang et al., 2013; Han et al., 2014).

Although carbamazepine (CBZ), a Food and Drug Administration–approved antiepileptic Na\textsubscript{V} blocker, is not recommended as a first-line treatment of neuropathic pain (Finnerup et al., 2015), it is still used clinically in selected patients with some success. Importantly, CBZ has demonstrated effectiveness in the treatment of the pain disorder trigeminal neuralgia (Maarbjergerg et al., 2017). The classic action of CBZ is to inhibit Na\textsubscript{V}s in a voltage- and use-dependent manner (Tanelian and Brose, 1991; Kuo et al., 1997; Kuo, 1998; Yang et al., 2010). Although CBZ action on Na\textsubscript{V} is indisputable, especially at clinically achievable concentrations, it has been shown to also act on other ion channels and receptors (Ambrosio et al., 2002) that might contribute to its analgesic effect. Recently, we have demonstrated a novel action of CBZ as an activation modulator of mutant Na\textsubscript{V}1.7 channels, in which clinically achievable concentrations of CBZ (10–30 \mu M) depolarize the voltage dependence of activation; plasma levels in patients with epilepsy treated with CBZ range from 0.92 to 16 mg/l, which corresponds to 3.89–67.5 \mu M (Breton et al., 2005). CBZ (10–30 \mu M) depolarizes the activation of mutant Na\textsubscript{V}1.7 channels including Na\textsubscript{V}1.7-S241T (Yang et al., 2012), Na\textsubscript{V}1.7-V400M (Fischer et al., 2009), and Na\textsubscript{V}1.7-I234T (Yang et al., 2018), but not wild-type Na\textsubscript{V}1.7, and reduces the excitability of DRG neurons that express these mutant Na\textsubscript{V}1.7 channels (Yang et al., 2012, 2018; Geha et al., 2016). Open-label use of CBZ was reported to be beneficial in treating inherited erythromelalgia (IEM) patients carrying the Na\textsubscript{V}1.7-V400M mutation (Fischer et al., 2009) and Na\textsubscript{V}1.7-I234T mutation (Meijer et al., 2014). In a placebo-controlled, double-blind clinical study, we showed that CBZ (plasma concentration of 3.6–6.0 mg/l, which corresponds to 15.22–25.37 \mu M) was effective in treating patients with IEM carrying the Na\textsubscript{V}1.7-S241T mutation (Geha et al., 2016). However, except for the pharmacogenomic targeting of Na\textsubscript{V}1.7 carrying specific CBZ-responsive mutations (Fertleman et al., 2006; Fischer et al., 2009; Meijer et al., 2014; Geha et al., 2016), to date, CBZ has not been used to target other Na\textsubscript{V} isoforms based on their primary sequence.

The identification of gain-of-function mutations in Na\textsubscript{V}1.8 in individuals with painful peripheral neuropathy suggests that Na\textsubscript{V}1.8 channels contribute to neuropathic pain in peripheral neuropathy (Faber et al., 2012b; Huang et al., 2013; Han et al., 2014); thus, inhibitors of this channel may have therapeutic benefits. Whether the novel mode of action of CBZ extends to other Na\textsubscript{V}s is not known. We describe here, in an individual with painful diabetic neuropathya novel mutation in Na\textsubscript{V}1.8, S242T. Using patch-clamp recordings, we investigated the effects of the mutation at the channel level by voltage clamp, and the effects of expressing mutant channels on the excitability of DRG neurons by current clamp. We extrapolated from studies of CBZ interaction with the homologous Na\textsubscript{V}1.7-S241T mutation to ask whether the novel mechanism of action for this drug as activation modulator extends to Na\textsubscript{V}1.8-S242T (referred to as S242T), and to determine whether CBZ at clinically achievable concentrations reduces the hyperexcitability of DRG neurons expressing the mutant channel. Our studies ultimately aim to determine whether the novel mode of action of CBZ is isofom specific.

### Materials and Methods

#### Patient and Clinical Examinations

A 67-year-old male patient with diabetes was recruited as part of the Pain in Neuropathy Study (Themistocleous et al., 2016), an observational cross-sectional multicenter study approved by the National Research Ethics Service of the UK (No. 10/H0706/35). All participants signed informed consent forms, and the consent of Pain in Neuropathy Study participants (2010–2015) was made on the basis that they would not be told the result of the sequencing of Na genes. A detailed description of the study protocol was given previously (Themistocleous et al., 2016).

Clinical assessment included clinical history, structured neurologic examination, skin biopsy to determine the intraepidermal nerve fiber density (IFND), and quantitative sensory testing (QST).

Pain intensity was reported on an 11-point numerical rating scale, with 0 being no pain and 10 the worst pain imaginable, and pain location was indicated on a body map. The Douleur Neuropathique 4 Questionnaire (Bouhassira et al., 2005) was used as a screening tool for neuropathic pain. A comprehensive structured upper and lower limb neurologic examination was performed to detect clinical signs of a peripheral neuropathy (Kleyweg et al., 1991; Compston, 2010). The examination included the assessment of light touch, pinprick sensation, proprioception, vibration perception, deep-tendon reflexes, muscle bulk, and motor power. The clinical findings were quantified with the Toronto Clinical Scoring System (TCSS) (Bril and Perkins, 2002). TCSS is a screening tool for DPN and correlates with diabetic neuropathy severity.

The determination of IFND followed the European Federation of Neuromyopathological Societies/Peripheral Nerve Society Guideline on the utilization of skin biopsy (Lauria et al., 2010). QST is a measure of sensory perception in response to a given stimulus and can be used to demonstrate abnormalities in sensory function. QST was performed over the dorsum of both feet, in line with the published protocol of the German Research Network of Neuropathic Pain (Rolke et al., 2006). Raw QST data were transformed into z-scores to normalize for age, sex, and the body location of testing (Rolke et al., 2006; Maier et al., 2010). A z-score of zero is equal to the mean of the population. A score of greater or less than 2 S.D.s from the mean indicates gain of function or loss of function, respectively. Additional drug, laboratory, and clinical investigation data were retrieved from the clinical medical records.

#### Sequencing of Sodium Channels

Sequencing of the coding regions of SCN9A (encodes Na\textsubscript{V}1.7), SCN10A (encodes Na\textsubscript{V}1.8), and SCN11A (encodes Na\textsubscript{V}1.9) was undertaken by next-generation sequencing using the HaloPlex Target Enrichment System (Agilent Technologies, Santa Clara, CA) and the MiSeq Sequencing Platform (Illumina, Inc., San Diego, CA) after DNA extraction from blood. Variants were called using an in-house bioinformatics pipeline (further details available on request). Any variant that was both: 1) present at >1% allele frequency in the Exome Variant Database (http://evs.gs.washington.edu/EVS) or Exome Aggregation Consortium (http://exac.broadinstitute.org), and 2) not previously reported in the literature in association with painful neuropathy was considered unlikely to be pathogenic and was not investigated further. Rare variants that were present at <1% allele frequency in population databases were considered for further analysis, irrespective of whether they were previously reported in the literature in association with painful neuropathy or not. Variants of potential interest were confirmed by Sanger sequencing by capillary electrophoresis using the 3730 DNA Analyzer (Applied Biosystems, Foster City, CA). The in silico prediction of the functional effect of the variant was performed using multiple algorithms: Align GVGD (http://agvgd.hci.utah.edu/).
SIFT (http://sift.jcvi.org), and PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/).

Structural Modeling. Homology models for both the Na\textsubscript{v}1.8 and Na\textsubscript{v}1.7 were developed based on the recently published electric eel sodium channel structure (Yan et al., 2017) by using the homology modeling tool in Molecular Operating Environment (MOE) software (MOE, 2013; Chemical Computing Group ULC, Montreal, QC, Canada).

Functional Assessments of Na\textsubscript{v}1.8 S242T Mutant. The \textit{pcDNA}5-GFP-2A-Na\textsubscript{v}1.8 plasmid encoding enhanced green fluorescent protein and a "StopGo" 33-amino acid 2A linker upstream of the human Na\textsubscript{v}1.8 ATG codon was previously described (Faber et al., 2012b; Huang et al., 2013; Han et al., 2014). The nucleotide substitution c.724T\textrightarrow\text{A}, which leads to the amino acid substitution Ser242Thr was introduced into the construct using QuikChange Lightning site-directed mutagenesis (Agilent Technologies).

Primary Mouse DRG Neuron Isolation and Transfection. Animal studies were approved by US Veterans Affairs West Haven Medical Center Animal Care and Use Committee. DRG neurons were isolated, as previously reported (Faber et al., 2012b; Huang et al., 2013; Han et al., 2014), from homozygous Na\textsubscript{v}1.8-cre mice (4–8 weeks of age, both male and female) that lack functional endogenous Na\textsubscript{v}1.8 channels. In compliance with recent National Institutes of Health guidelines and our own practice, we have used both male and female mice as the source for DRG neurons. Briefly, DRGs were harvested, incubated at 37\textdegree C for 20 minutes in complete saline solution (in mM: 137 NaCl, 5.5 KCl, 25 NaHCO\textsubscript{3}, 10 \text{CaCl}_2, and 10 HEPES, adjusted to pH 7.2 with NaOH), containing 0.5 U/ml Liberase TM (Sigma-Aldrich, St. Louis, MO) and 0.6 mM EDTA, followed by a 15-minute incubation at 37\textdegree C in complete saline solution containing 0.5 U/ml Liberase TL (Sigma-Aldrich), 0.6 mM EDTA, and 30 U/ml papain (Worthington Biochemical, Lakewood NJ). Tissue was then centrifuged and triturated in 0.5 ml of DRG media: Dulbecco's modified Eagle's medium/F12 (1:1) with 100 U/ml penicillin, 0.1 mg/ml streptomycin (Life Technologies, Grand Island, NY), and 10% fetal bovine serum (Hyclone, Logan, UT), containing 1.5 mg/ml bovine serum albumin (low endotoxin; Sigma-Aldrich) and 1.5 mg/ml trypsin inhibitor (Sigma-Aldrich). After trituration, Na\textsubscript{v}1.8-WT (referred to as WT) or S242T mutant channel constructs were transfected into DRG neurons in suspension using a Nucleofector HS Electroporator (Lonza, Walkersville, MD) and Maxima SCN Nucleofector reagents (VСП1-1003, Lonza). After electroporation, 100 \mu l of calcium-free Dulbecco's modified Eagle's medium (Lonza) was added, and cells were incubated at 37\textdegree C for 5 minutes in a 95% air/5% CO\textsubscript{2} (v/v) incubator to allow neurons to recover. The cell mixture was then diluted with DRG media containing 1.5 mg/ml bovine serum albumin (low endotoxin; Sigma- Aldrich) and 1.5 mg/ml trypsin inhibitor (Sigma-Aldrich), seeded onto poly-L-lysine/laminin-coated coverslips (Corning, Discovery Labware, Bedford, MA) and incubated at 37\textdegree C to allow DRG neurons to attach to the coverslips. After 40 minutes of incubation, DRG media were added to each well to a final volume of 1.0 ml. For current-clamp recording, medium was supplemented with 50 ng/ml mouse nerve growth factor (Alomone Laboratories, Jerusalem, Israel) and 50 ng/ml recombinant human glial cell line–derived neurotrophic factor (PeproTech, Rocky Hill, NJ), and the DRG neurons were maintained at 37\textdegree C in a 95% air/5% (v/v) CO\textsubscript{2} incubator for 40–48 hours before recording.

Electrophysiology and Pharmacology. Voltage-clamp and current-clamp recordings were obtained with an EPC-10 Amplifier (HEKA Electronics, Holliston, MA) from small transfected mouse DRG neurons (diameter, \textless 25 \mu m) with robust green fluorescence at room temperature (\textcircled{22}\textdegree C), 40–48 hours after transfection, as described previously (Faber et al., 2012b; Huang et al., 2013; Han et al., 2014, 2015). As in our previous studies (Faber et al., 2012a), to minimize the variability of DRG neuron cultures prepared at different times, we carried out head-to-head comparisons of cells transfected with WT or mutant channels and cells treated with CBZ or vehicle, from cultures prepared contemporaneously by the same researcher. Voltage-Clamp Recording from Transfected Na\textsubscript{v}1.8-Null Mouse DRG Neurons. Fire-polished electrodes (1–2 M\Omega) were fabricated from 1.6 mm-inner-diameter borosilicate glass micropetites (World Precision Instruments, Sarasota, FL). The pipette potential was adjusted to zero before seal formation, and liquid junction potential was not corrected. Capacitive transients were cancelled, and voltage errors were minimized with 80–90\% series resistance compensation; cells were excluded from analysis if the predicted voltage error exceeded 4 mV. Leakage current was digitally subtracted online using hyperpolarizing control pulses, applied before the test pulse (P/6 subtraction). Currents were acquired with PatchMaster software (HEKA Electronics), 5 minutes after establishing whole-cell configuration, sampled at a rate of 50 kHz and filtered at 2.9 kHz. The pipette solution contained the following (in mM): 140 CsF, 10 NaCl, 1 EGTA, and 10 HEPES, at pH 7.3 with CsOH (adjusted to 315 mOsmol/l with dextrose). The extracellular bath solution contained the following (in mM): 70 NaCl, 70 choline chloride, 3 KCl, 1 MgCl\textsubscript{2}, 1 CaCl\textsubscript{2}, 5 CaCl\textsubscript{2}, 20 TEA chloride, pH 7.32 with NaOH (327 mOsmol/l). TTX (0.5 \mu M), CdCl\textsubscript{2} (0.1 mM), and 4-aminopyridine (1 mM) were added in the bath solution to block endogenous TTX-sensitive voltage-gated sodium currents, calcium currents, and potassium currents, respectively. Endogenous Na\textsubscript{v}1.9 currents were suppressed by holding the cells at −70 mV, a step that inactivates Na\textsubscript{v}1.9 channels and minimizes their contribution to the TTX-R current (Cummins et al., 1999).

For current-voltage relationships, cells were held at −70 mV and stepped to a range of potentials (−70 to +40 mV in 5-mV increments) for 100 milliseconds with a 5-second interval between each sweep. Peak inward currents (I) were plotted as a function of depolarization potential to generate I–V curves. Activation curves were obtained by converting I to conductance (G) at each voltage (V) using the equation \( G = I/(V - V_m) \), where \( V_m \) is the reversal potential that was determined for each cell individually. The activation curve of each cell was then fit with Boltzmann functions in the form of \( G_{\text{max}}/(1 + \exp[(V - V_{1/2,\text{act}})/k]) \), where \( G_{\text{max}} \) is the maximal sodium current value, \( V_{1/2,\text{act}} \) is the potential at which activation is half maximal, \( V \) is the test potential, and \( k \) is the slope factor. The fit parameters such as \( V_{1/2,\text{act}} \) are grouped and then evaluated for significance between groups using two-tailed t tests.

Steady-state fast inactivation was achieved with a series of 500-millisecond prepulses (−90 to −10 mV in 5-mV increments) and the remaining noninactivated channels were activated by a 40-millisecond step depolarization to 0 mV. The protocol for slow-inactivation consisted of a 30-millisecond step to potentials varying from −120 to −20 mV, followed by a 30-millisecond step to −70 mV to remove fast inactivation and a 20-millisecond step to 0 mV to elicit a test response. Peak \( V \) values obtained from steady-state fast-inactivation and slow-inactivation protocols were normalized by the maximum current amplitude for each cell and were fit with a Boltzmann equation of the form \( I/I_{\text{max}} = A + (1 - A)/(1 + \exp([-V_{1/2,\text{inh}} - V]/k)) \), where \( V \) represents the inactivating prepulse potential, and \( V_{1/2,\text{inh}} \) represents the midpoint of the inactivation. The fit parameters such as inactivation \( V_{1/2,\text{inh}} \) are grouped and then evaluated for significance between groups using two-tailed t tests.

Deactivation was estimated from current decay, using a 3-millisecond short-depolarizing pulse to 0 mV followed by a 100-millisecond repolarizing pulse to potentials ranging from −110 to −20 mV in 5-mV increments, and the holding potential for deactivation was −70 mV. Deactivation kinetics were calculated by fitting the decaying currents with a single exponential function. Differences between groups are evaluated point by point using unpaired two-tailed t tests with Bonferroni correction for multiple comparisons. For current clamp data, the results are grouped and are presented as a percentage of the maximal transient peak current. Ramp currents were elicited with slow ramp depolarization over a 600-millisecond period at 0.2 mV/ms. The amplitude of ramp current was presented as a percentage of the maximal peak current. Differences between groups are evaluated point by point
using unpaired two-tailed $t$ tests with Bonferroni correction for multiple comparisons.

**Current-Clamp Recording from Transfected Na$_v$1.8-Null Mouse DRG Neurons.** To reduce observer bias, current-clamp recordings were performed in a blind manner where the electrophysiologist was not aware whether the neurons were transfected with wild-type or mutant channels. Electrodes had a resistance of 1–3 MΩ when filled with the pipette solution, which contained the following (in mM): 140 NaCl, 3 KCl, 2 MgCl$_2$, 2 CaCl$_2$, and 10 HEPES, pH 7.3 with NaOH (adjusted to 320 mM with dextrose). The extracellular solution contained the following (in mM): 140 NaCl, 3 KCl, 2 MgCl$_2$, 2 CaCl$_2$, and 10 HEPES, pH 7.3 with NaOH (adjusted to 320 mM with dextrose). Whole-cell configuration was obtained in voltage-clamp mode before proceeding to the current-clamp recording mode. The current threshold was determined by the first action potential elicited by a series of 200-millisecond depolarizing current injections that increased in 5-pA increments with the sweep interval of 10 seconds. Cells with stable resting membrane potentials more negative than $-40$ mV and overshooting action potentials (>80 mV resting membrane potential to peak) were used for additional data collection. Action potential frequency was determined by quantifying the number of action potentials elicited in response to depolarizing current injections (500 milliseconds).

**CBZ Treatment.** CBZ was purchased from Sigma-Aldrich, and a stock is prepared by dissolving in dimethylsulfoxide (DMSO). For pharmacology experiments, CBZ aliquots were added to produce a working solution of a clinically achievable concentration of CBZ (30 $\mu$M) (Breton et al., 2005) in 0.1% DMSO (final concentration). Cells were treated with CBZ (30 $\mu$M) or 0.1% DMSO in incomplete DRG media (without the 10% fetal bovine serum) for 30 minutes before recording, and the CBZ or DMSO concentration was maintained in the bath solution during the recording as described previously (Fischer et al., 2009; Yang et al., 2012, 2018; Geha et al., 2016). Shorter treatment periods of 2 and 5 minutes were not enough to produce a depolarizing shift in activation of Na$_v$1.7-S241T channels (data not shown), whereas a 30-minute treatment period was effective and was thus implemented in the current studies, which is consistent with prior observations on the effect of CBZ on mutant Na$_v$1.7 channels (Fischer et al., 2009; Geha et al., 2016).

**Statistics.** Voltage-clamp and current-clamp data were analyzed using FitMaster (HEKA Electronics) and OriginPro 8.5 (OriginLab Corporation, Northampton, MA). As described above, we carried out head-to-head comparisons of cells transfected with WT or mutant channels, and of cells treated with CBZ or vehicle, from cultures prepared contemporaneously by the same researcher to minimize culture-to-culture variability. Except where noted, statistical significance was determined using an independent two-tailed $t$ test with unequal variance, with Bonferroni correction for multiple comparisons when appropriate. The percentages of spontaneously firing cells were compared using the $z$ test. The Mann-Whitney test was used to compare current threshold. The number of evoked action potentials between two different groups was evaluated using two-way ANOVA. All electrophysiology data are presented as the mean ± S.E.M., and error bars in the figures represent the S.E.M. A $P$ value of less than 0.05 was considered statistically significant.

**Results**

**Case History**

The 67-year-old male patient had received a diagnosis of type 2 diabetes mellitus at the age of 42 years, and his recent glycated hemoglobin A1c level was 8.2%. He developed pain, paresthesia, and numbness in both of his feet 12 years prior to assessment for this study. At the time of assessment, he reported pain and paresthesia in his feet and hands. He rated pain intensity over the 24 hours before clinical assessment as 3 of 10, with a Douleur Neuropathique 4 Questionnaire score of 7 (>4 indicative of neuropathic pain). He was not taking any analgesic medication. He did not complain of autonomic symptoms. His deceased father had also suffered from painful diabetic neuropathy, but no other family members were available for segregation analysis.

Neurologic examination identified a severe length-dependent sensory neuropathy that extended to his knees and wrists bilaterally with no weakness. Deep tendon reflexes were absent at the knees and ankles bilaterally. The TCSS score was 17, which is indicative of a severe neuropathy. QST revealed bilateral hyposensitivity in relation to both thermal and mechanical detection thresholds. The IENFD assessed at 10 cm above the lateral malleolus was markedly reduced (0 fibers/mm). The clinical presentation was therefore of a severe painful sensory neuropathy in a patient with a long-standing history of type 2 diabetes mellitus. Because of the family history of painful sensory neuropathy, we screened for gene variants in those genes encoding Na$_v$s that have previously been linked to painful sensory neuropathy: SCN9A, SCN10A, and SCN11A.

**Molecular Genetics and Structural Modeling**

Generally, our interest is the discovery of rare variants (<1%) as we are looking for variants that potentially have a high impact on channel function, recognizing that not every rare variant will have a large effect, and that there is a small number of relatively common variants that confer gain-of-function attributes on Na$_v$s in the context of painful neuropathy (Waxman et al., 2014).

Sequencing of SCN9A and SCN11A genes, encoding for the Na$_v$1.7 and Na$_v$1.9s, respectively, did not identify any pathogenic variants; there were no rare variants (<1%) in these channels or variants that had previously been linked to painful neuropathy. However, a potentially pathogenic variant (c.724T$\rightarrow$A, rs140288103) was found in SCN10A, the gene that encodes Na$_v$1.8. The c.724T$\rightarrow$A missense variant changes the amino acid 242 of Na$_v$1.8 channel from a serine to a threonine (S242T). The variant was present at a low frequency (MAF 0.0002) in both the Exome Variant Database and Exome Aggregation Consortium databases. Prediction programs Align GVGD, SIFT, and PolyPhen-2 classified the substitution probably damaging, respectively. S242 is located in the DI/S4-S5 linker and the orientation of the side chain matches exactly the Na$_v$1.7-S241T residue (Fig. 1B), which
obtained from DRG neurons from NaV1.8-null mice transfected with WT or S242T mutant channels. Figure 2A shows that of WT channels (WT: 325 ± 6 pA/pF, n = 31; P = 0.0006; t test) (Fig. 2C). The kinetics of activation were investigated by measuring the time-to-peak of the transient current. Considering the observed shift in V_{1/2} of activation induced by the S242T mutation, we expected faster activation depending on the step voltage. Activation of S242T channels from −40 to −10 mV was statistically significantly (two-tailed t test with Bonferroni correction, *P < 0.05) faster than that of WT channels (Fig. 2D). We also measured the kinetics of deactivation. As shown in Fig. 2E, the rates of current decay of S242T mutant channels were statistically significantly (two-tailed t test with Bonferroni correction, *P < 0.05) slower than those of WT channels at deactivation potentials from −30 to −10 mV.

The V_{1/2} of fast inactivation for S242T channels (−38.24 ± 1.08 mV, n = 25) was hyperpolarized by 3.8 mV compared with that for WT channels (−34.47 ± 0.86 mV, n = 24, P = 0.0088, t test) (Fig. 2F). As Fig. 2G shows, steady-state slow inactivation of S242T channels was enhanced, compared with WT channels. The V_{1/2} of S242T channels (−56.11 ± 1.21 mV, n = 13) was hyperpolarized by 8.9 mV compared with that of WT channels (V_{1/2} = −47.20 ± 1.56 mV, n = 14; P = 0.0001, two-tailed t test).

We measured the response of WT and S242T channels to a slow ramp stimulus. The ramp current for S242T channels (22.60% ± 1.45% of peak current, n = 26) was not different from that of WT channels (22.54% ± 0.92% of peak current, n = 25; P = 0.974, two-tailed t test); however, the average voltage where the peak of ramp current occurs was shifted by −6.4 mV for S242T channels (−22.77 ± 1.44 mV, n = 26) compared with that for WT channels (−16.34 ± 0.80 mV, n = 25; P = 0.0004, two-tailed t test) (Fig. 2H). Figure 2I compares the normalized amplitudes of persistent currents in DRG neurons expressing WT and S242T channels. The persistent currents of S242T channels were statistically significantly (two-tailed t test with Bonferroni correction, *P < 0.05) larger than the persistent currents of WT channels over the voltage range from −50 to −25 mV, whereas the S242T mutant channels produced smaller persistent currents than WT channels at more depolarized voltages between −10 and −20 mV.

**Current-Clamp Analysis.** We assessed the effect of the S242T mutant channels at the cellular level using current-clamp recordings on DRG neurons from NaV1.8-null mice transfected with either WT or S242T channels. The resting membrane potential of DRG neurons expressing S242T channels (−55.6 ± 1.4 mV, n = 29) was comparable to that of DRG neurons expressing WT channels (−54.9 ± 1.1 mV, n = 31; P = 0.728, t test). The input resistance of DRG neurons expressing S242T channels (362 ± 32 MΩ, n = 29) was also comparable to that of DRG neurons expressing WT channels (319 ± 38 MΩ, n = 31; P = 0.391, t test).

Current threshold, the minimal stimulus required to produce a single all-or-none action potential, was reduced in DRG neurons expressing S242T mutant channels. Figure 3A shows traces from a representative DRG neuron expressing WT channels. In response to ≤105 pA subthreshold current injections, the neuron only generated small, graded membrane potential depolarization. The first all-or-none action potential required a stimulus of 110 pA (current threshold for this neuron). Figure 3B shows recordings from a
representative DRG neuron that expressed S242T mutant channels. For this neuron, the current injection required to produce the first all-or-none action was 70 pA. Figure 3C presents a comparison of current threshold for a population of these two groups of neurons. The average threshold for DRG neurons expressing S242T mutant channels ($64 \pm 6$ pA, $n = 29$; 95% CI: $34–93$ pA) was smaller compared with that of neurons expressing WT channels ($124 \pm 21$ pA, $n = 31$; 95% CI: $81–166$ pA; $P = 0.0079$, Mann-Whitney test). There was no difference for action potential amplitude (WT: $110.4 \pm 1.6$ mV, $n = 31$; S242T: $111.4 \pm 1.9$ mV, $n = 29$; $P = 0.700$, t test) or the half-width of the action potential (WT: $8.28 \pm 0.73$ milliseconds, $n = 31$; S242T: $8.01 \pm 0.58$ milliseconds, $n = 29$; $P = 0.770$, t test) between the two groups of DRG neurons.

The effect of WT and S242T channels on the repetitive firing properties of DRG neurons was assessed by applying a series of 500-millisecond current injections to the two groups of DRG neurons. Representative action potentials triggered by increasing current injections of 150, 300, and 450 pA in neurons transfected with either WT or S242T channels are illustrated in Fig. 3, D–I. Cells expressing S242T (Fig. 3, G–I) fired with a higher frequency than cells expressing WT channels (Fig. 3, D–F) in response to similar stimuli. The average numbers of action potentials in cells expressing WT or S242T elicited by graded current injections in 50-pA steps are plotted in Fig. 3J. Compared with WT neurons expressing S242T channels produced higher firing frequencies at every stimulus level across the broad range studied, which reached statistical significance (two-way ANOVA, genotype $\times$ stimulus strength, $P < 0.001$). In addition, we observed that among both groups of DRG neurons, a subpopulation of neurons fired spontaneously. The proportion of spontaneously firing neurons for the group of DRG neurons expressing S242T channels (23 of 52 cells, 44.2%) was not different from that of
Fig. 3. Current-clamp analysis of effects of S242T mutant channels on DRG neuron excitability. (A and B) Representative action potential (AP) traces recorded from DRG neuron expressing WT (A) or S242T mutant (B) channels. Action potentials were elicited by 200-millisecond step depolarizing current injections from resting membrane potential $(V_m)$. (C) Comparison of current threshold for DRG neurons expressing WT ($n = 31$) and S242T ($n = 29$) mutant channels. Expression of S242T channels reduces the current threshold significantly. Two-tailed $t$ test, $^{*}P < 0.05$. Responses of a representative DRG neuron expressing WT channels (D–F) or S242T mutant channels (G–I) to 500-millisecond depolarization current steps that are 150 pA (left), 300 pA (middle), and 450 pA (right). (J) Comparison of responses (number of impulses evoked by a 500-millisecond stimulus) for the populations of DRG neurons expressing WT ($n = 29$) or S242T ($n = 24$) channels across a range of step current injections from 50 to 500 pA. Two-way ANOVA, $^{***}P < 0.001$. 
DRG neurons expressing WT channels (15 of 46 cells, 32.6%; \( P = 0.235 \), two-portion \( z \) test).

We would note that our recordings were made in DRG neurons transfected with WT or mutant \( \text{Na}_V1.8 \) channels, and thus express these channels at higher than normal levels, which is why, as in our prior studies (Faber et al., 2012a,b; Huang et al., 2013; Han et al., 2014, 2015), we performed a head-to-head comparison of the effects of the expression of these channels in these neurons. We consider the difference in the neuronal behavior as the indicator of increased excitability induced by mutant channels.

**Responsiveness of S242T Mutant Channels to CBZ**

**CBZ Rescues the Hyperpolarizing Shift of Activation of S242T Mutant Channels.** We have previously shown that the pretreatment of cells with a clinically achievable concentration of CBZ (30 \( \mu \)M) (Breton et al., 2005) for 30 minutes depolarizes the voltage dependence of the activation of \( \text{Na}_V1.7 \) channels that carry the S241T substitution (Yang et al., 2012) at the analogous position to the S242T in \( \text{Na}_V1.8 \) (Fig. 1A). Since the homology structure model shows that the spatial location of the S242T residue corresponds with that of the \( \text{Na}_V1.7 \)-S241T residue (Fig. 1B), we hypothesized that S242T might also be CBZ responsive. To test this hypothesis, DRG neurons expressing S242T mutant channels were pretreated with DMSO or 30 \( \mu \)M CBZ for 30 minutes before the recordings. Figure 4, A and B, shows representative traces recorded from DRG neurons expressing S242T mutant channels after treatment with DMSO or CBZ, respectively. As shown above, the expression of S242T mutant channels increased the excitability of DRG neurons. Because pretreatment with CBZ depolarized the activation of S242T mutant channels, shifting their \( V_{1/2} \) value to a voltage close to that of WT channels (Fig. 4C), we hypothesized that pretreatment with CBZ should attenuate the

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\text{CBZ Does Not Shift Activation of WT Channels.} \\
\text{To determine whether CBZ treatment shifts the activation of} \\
\text{Na}_V1.8-\text{WT channels, we incubated DRG neurons expressing} \\
\text{WT channels with 30 \( \mu \)M CBZ or DMSO. Figure 5, A and B,} \\
\text{shows representative traces recorded from DRG neurons} \\
\text{expressing WT channels after being treated with DMSO or} \\
\text{CBZ, respectively. CBZ treatment did not shift the voltage} \\
\text{dependence of activation of WT channels (} V_{1/2,act} \text{) compared with} \\
\text{the treatment with DMSO (Fig. 5D) (} V_{1/2,act} \text{DMSO,} \\
\text{−13.49 ± 2.13 mV,} \text{n = 10; CBZ,} \\
\text{−11.44 ± 1.64 mV,} \text{n = 14;} \\
\text{P = 0.855, two-tailed} \text{t test). The treatment with 30 \( \mu \)M} \\
\text{CBZ did not shift the steady-state fast inactivation of} \\
\text{WT channels (Fig. 5D) (} V_{1/2,fast} \text{DMSO,} \\
\text{−2.86 mV,} \text{n = 10; CBZ,} \\
\text{−2.36 mV,} \text{n = 14;} \\
\text{P = 0.793, two-tailed} \text{t test).} \\
\text{CBZ Reduces Excitability of DRG Neurons Expressing} \\
\text{S242T Mutant Channels. As shown above, the expres-} \\
\text{sion of S242T mutant channels increased the excitability} \\
\text{of DRG neurons. Because pretreatment with CBZ depolarized} \\
\text{the activation of S242T mutant channels, shifting their} \\
\text{\( V_{1/2,act} \) value to a voltage close to that of WT channels (Fig. 4C), we} \\
\text{hypothesized that pretreatment with CBZ should attenuate the} \\
\text{to −7.39 mV,} \text{P = 0.023,} \text{t test). The treatment of DRG neurons} \\
\text{with 30 \( \mu \)M CBZ did not shift steady-state fast inactivation of} \\
\text{S242T mutant channels (Fig. 4D). The} V_{1/2} \text{of steady-state fast} \\
inactivation for CBZ treatment (−38.20 ± 2.93 mV,} \text{n = 11) was} \\
\text{not different from that for DMSO treatment (−37.54 ±} \\
\text{2.86 mV,} \text{n = 7;} \text{P = 0.875,} \text{t test). Treatment with 30 \( \mu \)M} \\
\text{CBZ also did not cause a statistically significant shift in the} \\
\text{steady-state slow inactivation of S242T mutant channels. The} \\
\text{\( V_{1/2} \) of steady-state slow inactivation after DMSO treat-} \\
m\text{ment and CBZ treatment were −50.84 ± 1.59 mV,} \text{n = 13 and} \\
\text{−56.14 ± 3.11 mV,} \text{n = 11;} \text{P = 0.150,} \text{t test), respectively.} \\
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increased excitability of DRG neurons expressing S242T mutant channels. Current-clamp recording was implemented to evaluate the effect of CBZ pretreatment on the excitability of DRG neurons expressing S242T mutant channels.

Pretreatment of transfected DRG neurons with 30 μM CBZ for 30 minutes did not alter the resting membrane potential of DRG neurons expressing S242T channels compared with the DMSO control (DMSO: −52 ± 1.1 mV, n = 28; CBZ: −54.5 ± 0.8 mV, n = 29; P = 0.078, two-tailed t test). The input resistance of DRG neurons expressing S242T channels was not different between cells treated with CBZ (315 ± 55 MΩ, n = 29) or DMSO (366 ± 62 MΩ, n = 28; P = 0.545, two-tailed t test). Figure 6A shows traces of a representative neuron expressing S242T mutant channels treated with DMSO. The current threshold for this neuron was 55 pA. In contrast, Fig. 6B shows traces of a representative neuron expressing S242T mutant channels treated with CBZ, and the current threshold for this neuron was 90 pA. On average, CBZ treatment doubled the current threshold for DRG neurons expressing S242T mutant channel (DMSO: 55 ± 8 pA, n = 28; 95% CI, 39–71 pA; CBZ: 114 ± 21 pA, n = 29; P = 0.012; 95% CI, 71–157 pA; P = 0.037, Mann-Whitney test; Fig. 6C). However, there was no difference in action potential amplitude between the CBZ- and DMSO-treated neurons (DMSO: 104.5 ± 1.8 mV, n = 28; CBZ: 106.4 ± 1.5 mV, n = 29; P = 0.417, t test). The half-width of action potential was also not different between the two groups of DRG neurons (DMSO: 83.3 ± 0.83 mV, n = 28; CBZ: 9.78 ± 1.25 mV, n = 29; P = 0.338, two-tailed t test).

We further investigated the effect of CBZ treatment on the firing frequency of DRG neurons expressing S242T mutant channels. As shown in Fig. 6, D–F, a neuron treated with DMSO produced multiple action potentials in response to 500-millisecond depolarizing current steps of 150, 300, and 450 pA. In contrast, a neuron treated with CBZ (Fig. 6, G–I) generated fewer action potentials in response to the same current injections. Compared with the treatment with DMSO, the treatment with CBZ caused a marked reduction in the firing frequencies for DRG neurons expressing S242T mutant channels, which reached statistical significance (two-way ANOVA, treatment × stimulus strength, P < 0.001) (Fig. 6J). Although we observed a reduction in the proportion of spontaneously firing neurons treated with CBZ (DMSO: 85.1%; 23 of 35 cells; CBZ: 35.1%; 16 of 45 cells), this difference did not reach statistical significance (P = 0.315, two-portions z-test). These data show that CBZ treatment attenuated the hyperexcitability of DRG neurons expressing S242T mutant channels.

**CBZ Does Not Cause Reduction in the Excitability of DRG Neurons Expressing WT Channels.** We evaluated whether treatment with 30 μM CBZ for 30 minutes attenuates the excitability of DRG neurons expressing WT channels. Figure 7, A and B, shows traces of two representative neurons expressing WT channels after treatment with DMSO or 30 μM CBZ, respectively. Current threshold for these two neurons were 155 pA (DMSO) (Fig. 7A) and 135 pA (CBZ) (Fig. 7B). On average, the current threshold for DRG neurons expressing WT channels after treatment with CBZ (137 ± 23 pA, n = 27) was not different from that of neurons treated with DMSO (132 ± 24 pA, n = 25; P = 0.912, Mann-Whitney test) (Fig. 7C). We also found that treatment with CBZ had no effects on resting membrane potential (DMSO: −54.8 ± 1.4 mV, n = 25; CBZ: −55.1 ± 1.3 mV, n = 27; P = 0.875, t test), input resistance (DMSO: 313 ± 43 MΩ, n = 25; CBZ: 264 ± 41 MΩ, n = 27), amplitude (DMSO: 105.7 ± 1.5 mV, n = 25; CBZ: 107.8 ± 1.2 mV, n = 27; P = 0.277, t test), and half-width (DMSO: 9.51 ± 1.07 milliseconds, n = 25; CBZ: 9.55 ± 1.11 milliseconds, n = 27; P = 0.977, t test) of action potentials.

We compared the firing frequency of DRG neurons expressing WT channels after treatment with CBZ or DMSO. Figure 7, D–I, shows responses of two representative DRG neurons expressing WT channels after treatment with DMSO (Fig. 7, D–F) or CBZ (Fig. 7, G–I). Both neurons produced the same or nearly the same number of spikes in response to 150-, 300-, or 450-pA current injection. As summarized in Fig. 7J, across the range of current stimulis we studied, treatment with CBZ resulted in a small but statistically significant difference in the firing frequency of DRG neurons expressing WT channels compared with treatment with DMSO (two-way ANOVA, treatment × stimulus strength, P < 0.05). In addition, compared with DMSO treatment, CBZ treatment did not change the proportion of spontaneously firing neurons (DMSO: 13 of 38 cells, 34.2%; CBZ: 11 of 38 cells, 28.9%; P = 0.619, two-portions z-test).

**Discussion**

We report here the novel S242T mutation in a patient with neuropathic pain and DPN. Voltage-clamp recordings in DRG neurons show that S242T confers a complex phenotype at the channel level, manifesting both gain-of-function attributes, including a hyperpolarizing shift of activation, and loss-of-function attributes, including a hyperpolarizing shift in slow inactivation. Current-clamp recordings demonstrated that S242T mutant channels render DRG neurons hyperexcitable, indicating a dominant role for the gain-of-function attributes, consistent with neuropathic pain in this patient. We previously showed (Yang et al., 2012) that the homologous S241T mutation in Na\(v\)1.7 endows this channel with a unique mode of responsiveness to CBZ: depolarization of voltage dependence of activation. We demonstrate here that CBZ depolarizes the voltage dependence of activation of the S242T mutant channel and reduces the excitability of DRG neurons expressing these channels. These data suggest that the novel activity of CBZ as an activation modulator extends from Na\(v\)1.7 to Na\(v\)1.8.

The presence of this mutation could not be assessed in the deceased father, preventing analysis of genetic segregation. Nevertheless, our data suggest that S242T might be "pathogenic" or “probably pathogenic” based on criteria that have been established for the analysis of variants in Na\(v\)s (Waxman et al., 2014). The variant was absent in the cohort of patients with diabetes who did not have pain and is exceedingly rare in the genomic databases (MAF 0.0002). In silico analysis identified S242T as damaging and deleterious. The S242T mutation occurs at a position in the DI/S4-5 linker where there is a homologous gain-of-function mutation in Na\(v\)1.7 that causes IEM (Michiels et al., 2005; Lampert et al., 2006; Geha et al., 2016). As shown in this study, functional assessment of S242T shows that it confers gain-of-function attributes on Na\(v\)1.8 and renders DRG neurons hyperexcitable. One possible explanation for the late onset of symptoms is that an altered metabolic milieu secondary to diabetes, for example elevated methylglyoxal, which has been shown previously to depolarize Na\(v\)1.8 inactivation (Bierhaus et al., 2012), further
Fig. 6. Current-clamp analysis of the effects of CBZ treatment on DRG neurons expressing S242T mutant channels. Representative action potential (AP) traces recorded from DRG neuron expressing S242T mutant channels after treatment with DMSO (A) or 30 μM CBZ (B). (C) Comparison of current threshold for DRG neurons expressing S242T mutant channels after treatment with CBZ (n = 29) or DMSO (n = 28) control. CBZ treatment increased current threshold significantly. Two-tailed t test, *P < 0.05. Responses of a representative DRG neuron expressing S242T mutant channels after the treatment with DMSO (D–F) or CBZ (G–I) to 500-millisecond depolarization current steps of 150 pA (left), 300 pA (middle), and 450 pA (right). (J) Comparison of responses (number of impulses evoked by a 500-millisecond stimulus) in the population of DRG neurons expressing S242T mutant channels with treatment with CBZ (n = 26) or DMSO (n = 23) control across a range of step current injections from 50 to 500 pA. Two-way ANOVA, ***P < 0.001. V_m, membrane potential.
Fig. 7. Current-clamp analysis of the effects of CBZ treatment on DRG neurons expressing WT channels. Representative action potential (AP) traces recorded from DRG neurons expressing WT channels after treatment with DMSO (A) or 30 μM CBZ (B). (C) Comparison of current threshold for DRG neurons expressing WT channels after treatment of CBZ (n = 27) or DMSO (n = 25) control. CBZ treatment did not have a significant effect on current threshold. Responses of the representative DRG neurons expressing WT channels after treatment with DMSO (D–F) or CBZ (G–I) to 500-millisecond depolarization current steps of 150 pA (left), 300 pA (middle), and 450 pA (right). (J) Comparison of responses (number of impulses evoked by a 500-millisecond stimulus) in the population of DRG neurons expressing WT channels with treatment with CBZ (n = 25) or DMSO (n = 20) control across a range of step current injections from 50 to 500 pA. Two-way ANOVA, *P < 0.05. V_m, membrane potential.
enhancing the gain-of-function attributes of the mutant channel, may have been required for the full manifestation of clinical symptoms. However, this is a small study with genetic and clinical data available for only one patient, and, given the fact that DPN is potentially a gene-environment interaction, large cohorts will be needed for replication and to establish pathogenicity.

The S242T substitution within the DI/S4-S5 linker is homologous to the NaV1.7-S241T mutation, which has been identified in subjects with IEM (Michiels et al., 2005; Geha et al., 2016). Homology modeling based on the cryogenic electron microscopy structure of the electric eel sodium channel (Yan et al., 2017) shows a conserved position and orientation of the S/T substitution in NaV1.7 and NaV1.8. The S4-5 linker transduces the movement of the voltage sensor into a conformational change in the pore module during opening of the activation gate (Payandeh et al., 2011), which suggests that the activation properties of the NaV1.8 mutant channel would be altered by this substitution. Indeed, voltage-clamp recordings showed that, similar to the effect of the S241T substitution on NaV1.7, S242T channel activation is hyperpolarized and accelerated, and deactivation is slowed, all of which are gain-of-function features. However, unlike the NaV1.7-S241T channels, S242T channels displayed enhanced fast and slow inactivation, both of which are loss-of-function features. Enhanced fast inactivation has not been previously reported for mutations of the S4-5 linkers in NaV1.7-S241T or NaV1.7-I234T; however, the NaV1.7-I234T mutation in the S4-5 linker manifests massive enhancement of slow inactivation by about ~20 mV (Ahn et al., 2010). Local structural alterations due to intramolecular interactions of the S/T substitution or altered interactions of the threonine mutant residue with phospholipids in the plasma membrane might produce channel isoform-specific effects on gating.

Because the S242T mutation confers both gain-of-function (hyperpolarized and accelerated activation and slower deactivation) and loss-of-function (enhanced fast and slow inactivation) biophysical attributes on NaV1.8, it was not possible to predict a priori whether it would increase neuronal excitability. Current-clamp analysis showed that the S242T channels rendered DRG neurons hyperexcitable, which was manifested as reduced current threshold and increased evoked firing. It is well established that DRG neurons sit at the resting potentials in the −50 to −60 mV range (Fang et al., 2006; Faber et al., 2012a,b; Hendrich et al., 2012), and a hyperpolarizing shift in activation of NaV1.8 and an increase in persistent current close to the resting potential of these neurons would be expected to increase excitability (Huang et al., 2018). Hyperpolarized and accelerated activation and increased persistent currents at relatively hyperpolarized potentials of S242T compared with WT channels would be expected to increase the excitability of DRG neurons. The increased excitability of DRG neurons that we observed (Fig. 4) is consistent with the pain reported by the patient carrying the S242T mutation.

Building upon the observation that CBZ depolarizes the voltage dependence of the activation of NaV1.7-S241T channels (Yang et al., 2012) and attenuates the excitability of DRG neurons expressing these channels (Geha et al., 2016), we reasoned that the S242T mutation in NaV1.8, which corresponds to S241T in NaV1.7, would be CBZ responsive. Our data show that the clinically achievable concentration of CBZ (30 μM) also depolarizes the voltage dependence of the activation of S242T channels (Fig. 4C) and attenuates the firing of DRG neurons expressing the mutant channel (Fig. 6). By contrast, similar treatment with CBZ of DRG neurons expressing WT NaV1.8 channels did not depolarize the voltage dependence of activation of the channel (Fig. 5) and only slightly reduced the firing of DRG neurons (Fig. 7). Although a CBZ concentration of 30 μM could act on other sodium channels, our data suggest that the attenuation of firing of neurons expressing the mutant channel is more likely due to the effect of CBZ as an activation modifier. This conclusion is supported by the observation that CBZ pretreatment of DRG neurons that expressed the NaV1.7-F1449V mutant channels, whose activation is not modified by CBZ, did not attenuate the firing of these neurons (Yang et al., 2012).

The normalizing effect of CBZ on the activation of NaV1.7 mutant channels I234T, S241T, and V400M (Fischer et al., 2009; Yang et al., 2012, 2018; Geha et al., 2016) is novel, and has not been previously reported for drugs that bind at the NaV local anesthetic binding site. This effect is also distinct from classic state-dependent blocking of NaV channels in that it requires pretreatment with CBZ (Fischer et al., 2009; Yang et al., 2012, 2018; Geha et al., 2016). Whether this novel mode of action of CBZ on NaV1.7 and NaV1.8 mutations is due to the formation of a new binding site is not known. The fact that DI/S4-5 S/T substitution in both channels responded to CBZ in the same manner, however, suggests a common mechanism, despite differences in the primary sequence of the two channels even within the highly conserved linker sequence (Fig. 1A). Elucidation of the molecular determinants of productive drug-target interactions in channels other than NaV1.7 might be useful in establishing molecular and structural requirements for this novel mode of action of CBZ.

A recent systematic review (Finnerup et al., 2015) found that the evidence for efficacy of CBZ in neuropathic pain treatment was inconclusive and noted dose-limiting side effects. CBZ is not therefore used as a first-line treatment of neuropathic pain (first-line treatments include gabapentinoids, tricyclic antidepressants, and serotonin-noradrenaline reuptake inhibitors); however, CBZ is still used clinically in selected patients with some success, especially because of its demonstrable effectiveness in patients with trigeminal neuralgia (Maarbjerg et al., 2017). It is increasingly clear that there are multiple pathophysiological drivers of neuropathic pain, and we need to find better ways of stratifying patients to aid treatment selection (Themistocleous et al., 2018). A recent study (Demant et al., 2014) reported that patients with a specific pattern of sensory dysfunction termed the “irritable nociceptor” (defined by mechanical hypersensitivity and preserved small fiber function on QST) had a better treatment response to oxcarbazepine compared with those without this feature. A further approach to patient stratification is to use molecular genetics. Recently, patients with S241T and I234T were shown to respond to CBZ treatment, which was predicted using an approach that employed atomic-level structure modeling, mutant cycle analysis, and in vitro pharmacological testing (Yang et al., 2012, 2018; Geha et al., 2016). Thus far, it has been unclear whether this precision medicine approach can be extended to mutations in other sodium channels. Although we could not test the efficacy of CBZ in this subject because of the limitations imposed by the approved protocol, our current-clamp recordings demonstrate that 30 μM CBZ
attenuates S242T-induced hyperexcitability of DRG neurons, suggesting that CBZ treatment might be beneficial for individuals carrying this mutation.

In summary, we have described a novel gain-of-function Na\textsubscript{V}1.8 mutation in a patient with diabetes and neuropathic pain. S242T mutant channels manifest dominant gain-of-function attributes and render DRG neurons hyperexcitable. Exposure to a clinically achievable concentration of CBZ corrects the mutation-induced hyperpolarized shift of voltage dependence of activation and attenuates the hyperexcitability of DRG neurons expressing S242T channels. Our data suggest that the novel mode of action of CBZ can be extended from Na\textsubscript{V}1.7 to Na\textsubscript{V}1.8, and more generally suggest that the precise medicine approach capitalizing on this finding might produce beneficial outcomes to individuals carrying CBZ-responsive Na\textsubscript{V}1.8 mutations.

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