

# Na<sub>v</sub> channel variants in patients with painful and nonpainful peripheral neuropathy

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Samir Wadhawan, PhD  
Saumya Pant, PhD  
Ryan Golhar, PhD  
Stefan Kirov, PhD  
John Thompson, PhD  
Leslie Jacobsen, MD  
Irfan Qureshi, MD  
Senda Ajroud-Driss, MD  
Roy Freeman, MD  
David M. Simpson, MD  
A. Gordon Smith,  
MD, FAAN  
Ahmet Hoke, PhD, MD  
Linda J. Bristow, PhD  
On behalf of the  
Foundation for  
Peripheral Neuropathy  
and the Peripheral  
Neuropathy Research  
Registry (PNRR)  
Consortium

Correspondence to Dr. Bristow:  
Linda.Bristow@Sunovion.com

## ABSTRACT

**Objective:** To examine the incidence of nonsynonymous missense variants in *SCN9A* (Na<sub>v</sub>1.7), *SCN10A* (Na<sub>v</sub>1.8), and *SCN11A* (Na<sub>v</sub>1.9) in patients with painful and nonpainful peripheral neuropathy.

**Methods:** Next-generation sequencing was performed on 457 patient DNA samples provided by the Peripheral Neuropathy Research Registry (PNRR). The patient diagnosis was as follows: 278 idiopathic peripheral neuropathy (67% painful and 33% nonpainful) and 179 diabetic distal polyneuropathy (77% painful and 23% nonpainful).

**Results:** We identified 36 (*SCN9A*), 31 (*SCN10A*), and 15 (*SCN11A*) nonsynonymous missense variants, with 47.7% of patients carrying a low-frequency (minor allele frequency <5%) missense variant in at least 1 gene. The incidence of previously reported gain-of-function missense variants was low (≤3%), and these were detected in patients with and without pain. There were no significant differences in missense variant allele frequencies of any gene, or *SCN9A* haplotype frequencies, between PNRR patients with painful or nonpainful peripheral neuropathy. PNRR patient *SCN9A* and *SCN11A* missense variant allele frequencies were not significantly different from the Exome Variant Server, European American (EVS-EA) reference population. For *SCN10A*, there was a significant increase in the alternate allele frequency of the common variant p.V1073A and low-frequency variant p.S509P in PNRR patients compared with EVS-EA and the 1000 Genomes European reference populations.

**Conclusions:** These results suggest that identification of a genetically defined subpopulation for testing of Na<sub>v</sub>1.7 inhibitors in patients with peripheral neuropathy is unlikely and that additional factors, beyond expression of previously reported disease “mutations,” are more important for the development of painful neuropathy than previously discussed. *Neurol Genet* 2017;3:e207; doi:10.1212/NXG.000000000000207

## GLOSSARY

**bp** = base pair; **CL** = confidence limit; **EUR** = European; **EVS-EA** = Exome Variant Server, European American; **FDR** = false discovery rate; **GOF** = gain of function; **HWE** = Hardy-Weinberg; **IEM** = inherited erythromelalgia; **MAF** = minor allele frequency; **NCV** = nerve conduction velocity; **NGS** = next-generation sequencing; **NIST** = National Institute of Standards and Technology; **OR** = odds ratio; **PNRR** = Peripheral Neuropathy Research Registry; **SFN** = small fiber neuropathy; **SNP** = single nucleotide polymorphism.

Human genetics provides compelling evidence that the voltage-gated sodium channel, Na<sub>v</sub>1.7, plays a critical role in pain. Gain-of-function (GOF) mutations in *SCN9A*, the gene encoding Na<sub>v</sub>1.7, cause inherited erythromelalgia (IEM) and paroxysmal extreme pain disorder, rare familial diseases associated with excruciating pain.<sup>1-3</sup> By contrast, loss-of-function *SCN9A*

Supplemental data  
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From the Department of Translational Biomarkers and Computational Genomics (S.W., S.P., R.G., S.K., J.T.), Bristol-Myers Squibb, Hopewell Site, Pennington, NJ; Department of Genetically Defined Diseases Discovery Biology (L.J.B.), Bristol-Myers Squibb, Wallingford, CT; Department of Innovative Clinical Development (I.Q., L.J.), Bristol-Myers Squibb, Lawrenceville, Princeton, NJ; Department of Neurology (S.A.-D.), Northwestern Medical Faculty Foundation, Chicago, IL; Department of Neurology (R.F.), Beth Israel Medical Center, Harvard School of Medicine, Boston, MA; Department of Neurology (D.M.S.), Icahn School of Medicine at Mount Sinai Medical Center, New York, NY; Department of Neurology (A.G.S.), University of Utah School of Medicine, Salt Lake City, UT; Department of Neurology (A.H.), Johns Hopkins University, Baltimore, MD. S.P. is currently affiliated with Biocon Bristol-Myers Squibb Research Center, Bangalore, India.

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mutations cause congenital insensitivity to pain, a rare autosomal recessive disease characterized by loss of pain sensation.<sup>1,2</sup> These findings have triggered renewed efforts to develop novel, selective Na<sub>v</sub>1.7 inhibitors for the treatment of pain<sup>4</sup> and expanded Na<sub>v</sub> channel sequencing to patients with more prevalent chronic pain conditions. In this regard, rare missense variants have been reported in *SCN9A*,<sup>5,6</sup> *SCN10A*,<sup>6,7</sup> and *SCN11A*,<sup>8</sup> genes encoding Na<sub>v</sub>1.7, Na<sub>v</sub>1.8, and Na<sub>v</sub>1.9, respectively, in patients with painful small fiber neuropathy (SFN). It is thought that enhanced Na<sub>v</sub> channel activity may directly contribute to the pain experienced by these patients because several of the missense variants identified exert GOF effects in cell-based electrophysiology assays.<sup>9</sup>

The identification of patients carrying pathologic Na<sub>v</sub> missense variants provides an opportunity to select subjects whose pain may be more effectively treated with novel Na<sub>v</sub> agents. In this study, we collaborated with the Foundation for Peripheral Neuropathy to obtain DNA samples from patients with idiopathic or diabetic peripheral neuropathy enrolled in the Peripheral Neuropathy Research Registry (PNRR). The objective was to identify missense variants in *SCN9A*, *SCN10A*, and *SCN11A* and to examine their frequency in patients whose peripheral neuropathy was painful or nonpainful.

**METHODS Patients.** The study was performed at Bristol-Myers Squibb using whole blood DNA samples obtained from patients enrolled in the PNRR from 2011 to February 2015. Clinical information and patient DNA samples were provided by the Neurology Departments at John Hopkins University, Northwestern Medical Faculty Foundation, Beth Israel Medical Center-Harvard Medical School, and Icahn School of Medicine at Mount Sinai Medical Center. Consenting patients were evaluated using a comprehensive patient examination form and patient health questionnaire and received peripheral nerve workup including nerve conduction studies and, in some cases, analysis of skin biopsies. A copy of the patient health questionnaire is provided in the supplemental information and included questions about sensory, motor, and autonomic symptoms and medication. Patients were instructed to answer pain-related questions from the perspective of their neuropathy only, and those with additional complex medical issues or neurologic diseases were excluded from the registry. A DNA sample for next-generation sequencing (NGS) was obtained from 457 patients; 278 patients diagnosed with idiopathic peripheral neuropathy (186 painful and 92 nonpainful) and 179 patients

diagnosed with diabetic distal polyneuropathy (138 painful and 41 nonpainful). The PNRR patient sample comprised 61% of males and 83% of Caucasian ethnicity, and patients with painful peripheral neuropathy were significantly younger (by on average 6–7 years) than those without pain (table e-1 at [Neurology.org/ng](http://Neurology.org/ng)).

**Standard protocol approvals, registrations, and patient consents.** At each consortium site, institutional review board approval was obtained based on a unified protocol developed as a consensus by the consortium members. Written informed consent was obtained from all patients presenting to the neurology clinics that agreed to enroll in the PNRR.

**Na<sub>v</sub>-targeted sequencing and variant calling.** Agilent SureSelect hybrid capture probes were designed based on GRCh 37.3 to span entire 50 kb flanking, exonic, and intronic regions of *SCN9A*, *SCN10A*, and *SCN11A* totaling to 682,262 base pairs (bps). National Institute of Standards and Technology (NIST) standard NA12878 and NA18507 HapMap cell line DNAs were used as assay and informatics controls. PNRR patient DNA samples were randomized for processing and across NGS runs and plates using criteria of age, race, pain, and several clinical criteria including numbness, weakness, walk balance, diabetes, smoking, and alcohol consumption. Libraries were prepared using a standard Agilent SureSelect protocol and were sequenced on an Illumina HiSeq 2000 system with 100 bp-paired end reads to attain a minimal 200X coverage. The average coverage obtained was 400X per base in the targeted regions, with 10%–20% of bases missing coverage across all samples. The sequencing reads were mapped to the human genome build hg19 (GRCh 37),<sup>10</sup> and the variant calling was performed using Genome Analysis Toolkit (GATK) best practices (<https://software.broadinstitute.org/gatk/best-practices>).<sup>11–13</sup> The variants were annotated using snpEff.<sup>14</sup> Performance of the variant calling pipeline was assessed using NIST NA12878, looking at sensitivity (TP/TP + FN) and specificity (FP/FP + TN), where TP are variants called and present in NIST, FP are variants called but not present in NIST, FN are variants not called but present in NIST, and TN are variants not called and not present in NIST. A 93% sensitivity was observed, and positive predictive value and false discovery rate (FDR) were 51% and 49%, respectively. Variant calls were filtered to include variants with FILTER = “PASS”[“VQSR-TrancheSNP99.90to100.00”][“VQSRTrancheSNP99.00to99.90.”

**Variant classification and allele frequency comparisons.** PNRR variants were designated as common (minor allele frequency [MAF] > 5%) or low frequency (MAF < 5%) based on the alternate allele frequency reported in the NHLBI Exome Sequencing Project Exome Variant Server, European American (EVS-EA) population.<sup>15</sup> To compare PNRR variant allele frequencies in patients with or without pain, or to reference populations (EVS-EA and 1000 Genomes Project<sup>16</sup> global or European (EUR) populations), the Fisher exact test was performed using allele counts, and the Benjamini and Hochberg<sup>17</sup> FDR was used to correct for multiple testing. All analyses were performed in R, and results were reported as the Fisher P and Q values.

**SCN9A haplotype analysis.** PNRR single nucleotide polymorphisms (SNPs) with MAF > 10% were used for haplotype analysis. Genotypes were phased using BEAGLE V4.1.<sup>18</sup> Haplotype block definition and frequency comparison were performed in Haploview.<sup>19</sup> TAGGER within the Haploview package was used to find tag SNPs for each haplotype blocks to refine haplotype block definitions. A  $\chi^2$  test was used to compare haplotype frequencies.

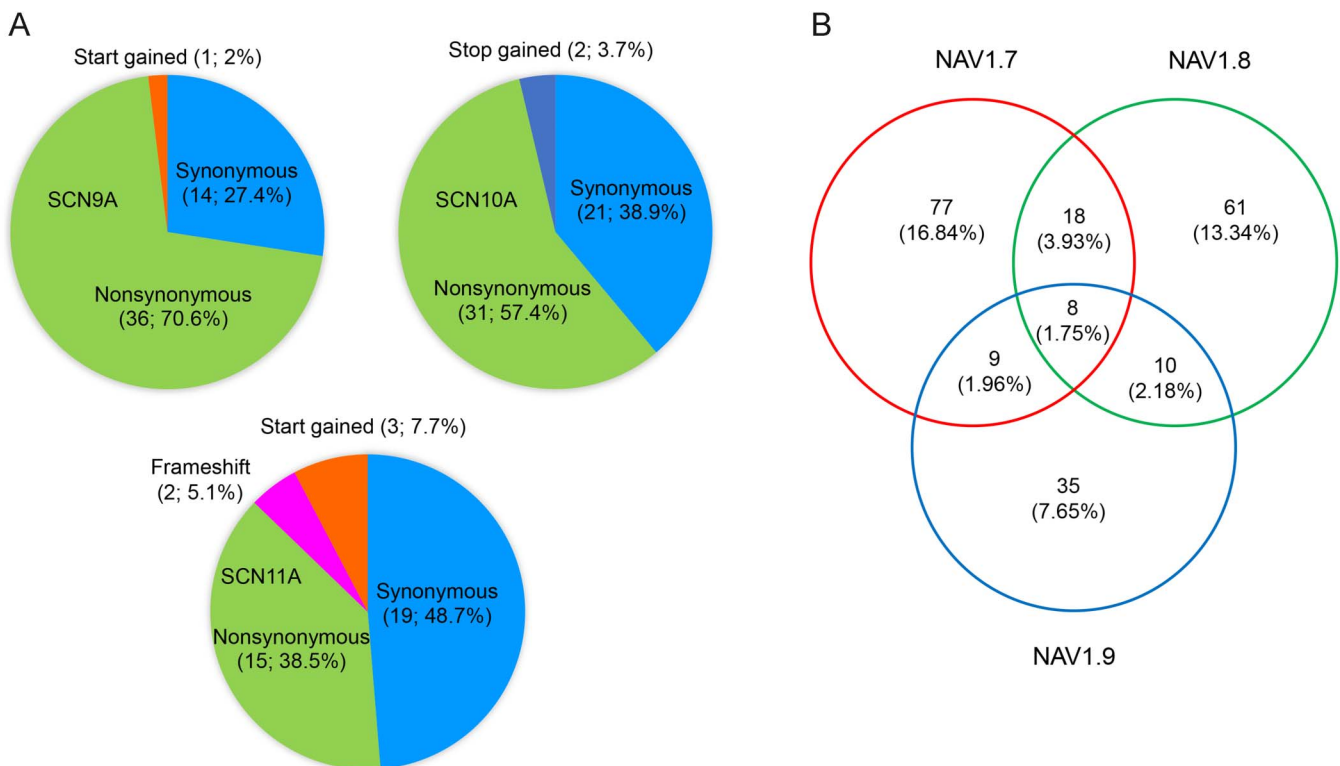
**Data and statistical analysis.** Height, weight, and year of birth were analyzed by a 2-tailed *t* test to compare patients with diabetic or idiopathic peripheral neuropathy with pain vs without pain (GraphPad Prism v7). Statistical analysis methods for nonsynonymous variant and haplotype comparisons are described above.

**RESULTS Incidence of genetic variants in the PNRR cohort.** NGS of *SCN9A*, *SCN10A*, and *SCN11A* identified >1000 variants in each gene, with most (79%–87%) occurring in intronic regions (table e-2). Most of the protein-coding variants we identified were nonsynonymous, missense variants that changed the amino acid sequence (figure 1A). Details of the gene location and alternate allele frequency for each nonsynonymous missense variant we identified are reported in the supplementary section (figure e-1 and tables e-3 and e-4). Variants were designated as low frequency (MAF <5%) or common (MAF >5%) based on the alternate allele frequency reported in the EVS-EA database. A low-frequency missense variant in at least 1 gene was present in 47.7% (218/457) of patients with 9.8% carrying low-frequency variants in multiple genes (figure 1B). For individual genes, the number of patients carrying at least 1 low-frequency variant was 24.5%, 21%, and

13.6% for *SCN9A*, *SCN10A*, and *SCN11A*, respectively. Patients were heterozygous carriers of low-frequency missense variants except for 5 subjects who were homozygous carriers of the *SCN10A* variants p.P1045T (n = 1), p.S509P (n = 2), or p.R14L (n = 1) or the *SCN11A* variant p.T1609I (n = 1).

**Comparison with reference populations.** For each gene, the analysis included all common and low-frequency nonsynonymous missense variants reported in PNRR patients, and the reference population being examined. The reference populations and the number of missense variants included in each analysis were as follows: EVS-EA: *SCN9A* (26), *SCN10A* (21), and *SCN11A* (10); 1000 Genomes (1K) global and EUR: *SCN9A* (23), *SCN10A* (21), and *SCN11A* (9). For *SCN10A*, an increase in the alternate allele frequency was observed for p.V1073A (odds ratio [OR] = 1.2519; 95% confidence limits [CLs] 1.0851–1.4443; *p* = 0.0021) and p.S509P (OR = 1.9225; 95% CL 1.3994–2.6411; *p* = 0.0001) compared with EVS-EA (table 1, table e-5). Similar results were also seen in comparison to the 1K-EUR population (p.V1073A: OR = 1.5783; 95% CL 1.3066–1.9064; *p* < 0.0001; p.S509P: OR = 2.0098; 95% CL 1.2432–3.2488; *p* = 0.0044) (table 1, table e-5).

**Figure 1 Protein-coding variants identified in *SCN9A*, *SCN10A*, and *SCN11A***



(A) Variants were classified based on changes in amino acid sequence. For each gene, results show the number (%) of variants identified in each class. (B) Number (%) of patients in the Peripheral Neuropathy Research Registry (PNRR) cohort (n = 457) that carry a low-frequency missense variant in *Nav1.7*, *Nav1.8*, and/or *Nav1.9*. Variants identified in PNRR patients were designated as low frequency (minor allele frequency <5%) based on the alternate allele frequency reported in the Exome Variant Server, European American reference population.

**Table 1** Missense variants with significant Q values compared with reference populations

| Gene   | Variant  | 1K global Q value | 1K-EUR Q value    | EVS-EA Q value  |
|--------|----------|-------------------|-------------------|-----------------|
| SCN9A  | p.D1908G | <b>1.30E-13</b> ↓ | <b>0.0283</b> ↑   | 0.4525          |
| SCN9A  | p.N1245S | <b>0.0194</b> ↑   | 1                 | 0.6291          |
| SCN9A  | p.V991L  | <b>1.33E-05</b> ↓ | <b>0.042</b> ↑    | 0.4525          |
| SCN9A  | p.M932L  | <b>1.33E-05</b> ↓ | <b>0.042</b> ↑    | 0.4525          |
| SCN9A  | p.I739V  | <b>0.0364</b> ↑   | 0.9584            | 0.7226          |
| SCN9A  | p.P610T  | <b>1.33E-05</b> ↑ | 0.9584            | 0.4951          |
| SCN9A  | p.S490N  | <b>1.28E-05</b> ↓ | 0.1457            | 0.1548          |
| SCN10A | p.V1073A | <b>1.32E-09</b> ↓ | <b>4.51E-05</b> ↑ | <b>0.0323</b> ↑ |
| SCN10A | p.P1045T | <b>2.62E-05</b> ↑ | 0.9116            | 0.8329          |
| SCN10A | p.I962V  | <b>0.0145</b> ↑   | 1                 | 0.9170          |
| SCN10A | p.S509P  | <b>4.26E-14</b> ↓ | <b>0.0464</b> ↑   | <b>0.0059</b> ↑ |
| SCN10A | p.I206M  | <b>0.0004</b> ↓   | 0.9116            | 1               |
| SCN10A | p.R14L   | <b>0.0187</b> ↑   | 1                 | 0.6513          |
| SCN11A | p.T1609I | <b>3.65E-07</b> ↓ | 0.1167            | 0.0823          |
| SCN11A | p.V909I  | <b>0.0001</b> ↑   | 0.5096            | 0.3559          |
| SCN11A | p.G481E  | <b>0.0239</b> ↑   | 0.8316            | 1               |

Abbreviations: 1K = 1000 Genomes; EUR = European; EVS-EA = Exome Variant Server, European American.

Significant Q values are shown in bold text. The arrows indicate if the alternate allele frequency is increased (↑) or decreased (↓) in the Peripheral Neuropathy Research Registry cohort compared with the reference population.

Several additional variants identified in *SCN9A*, *SCN10A*, and *SCN11A* had alternate allele frequencies that were significantly different from the 1K global population, but few remained significant compared with 1K-EUR, and none remained significant compared with the EVS-EA population (table 1, tables e-5, e-6, and e-7). The alternate allele frequencies in reference populations for previously reported GOF variants in *SCN9A*, *SCN10A*, and *SCN11A* are shown in tables e-3 and e-4 and were not significantly different in PNRR patients compared with the EVS-EA population.

**Relationship to pain status in PNRR patients.** We next focused on examining the incidence of nonsynonymous missense variants in PNRR patients with painful or nonpainful peripheral neuropathy. Evaluation of clinical information identified a small number of subjects (17/457; 3.7%) with discrepancies between patient self-reported and physician-reported pain which were excluded from further analysis. The resulting distribution of patients was as follows: 270 idiopathic (183 painful and 87 nonpainful) and 170 diabetic (134 painful and 36 nonpainful). For all missense variants identified in each gene, analysis of allele frequency showed no significant difference between patients with or without pain (tables e-8, e-9, and e-10). We also saw no significant difference

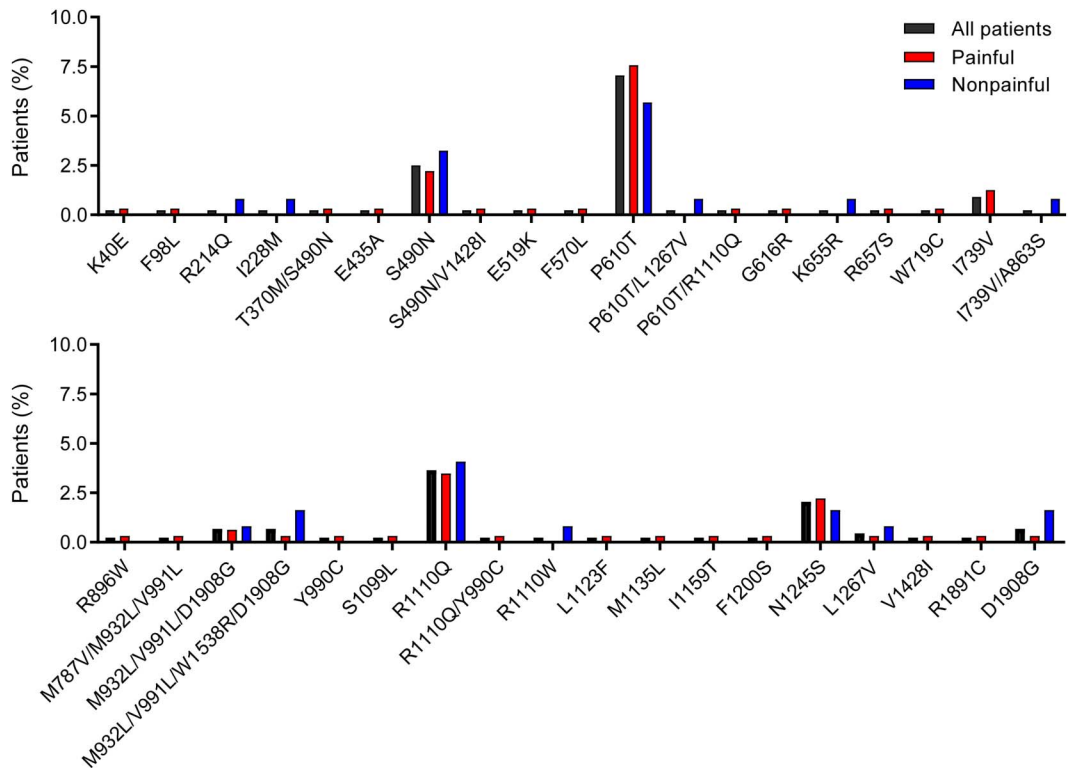
in the number of missense variants carried in each gene or all 3 genes combined in patients with or without pain (figure e-2). Consistent with results for the complete PNRR cohort, the number of patients carrying at least 1 low-frequency missense variant in *SCN9A*, *SCN10A*, or *SCN11A* was 25%, 21%, and 13%, respectively, with most of the individual variants or variant combinations present in only 1 patient (tables e-11, e-12, and e-13). For low-frequency variants present in >2% of patients, the incidence was similar in painful and nonpainful conditions (figure 2, figures e-3 and e-4) and in patients with diabetic and idiopathic peripheral neuropathy (figures e-4, e-5, and e-6). Finally, we selected a subset of patients with idiopathic peripheral neuropathy with probable, painful SFN using 2 approaches: (1) patients with idiopathic peripheral neuropathy with pain, autonomic symptoms but without weakness based on the patient history questionnaire (71 subjects) and (2) patients with idiopathic peripheral neuropathy with pain, normal nerve conduction velocity (NCV) but with abnormal skin biopsies with length-dependent patterns (19 subjects). The incidence of low-frequency missense variants in these groups was similar to the complete PNRR cohort (*SCN9A* 20%–26%, *SCN10A* 20%–21%, and *SCN11A* 14%–21%; table e-14).

**Incidence of previously reported low-frequency missense variants.** For *SCN9A*, 30/457 patients (6.6%) carried a low-frequency missense variant previously reported in patients with painful SFN,<sup>5,6</sup> painful diabetic peripheral neuropathy,<sup>20</sup> or (primary) IEM<sup>21,22</sup> of which 14 (3.1%) carried GOF variant(s) based on cell-based electrophysiology results (p.I228M,<sup>23</sup> p.G616R,<sup>21</sup> p.I739V,<sup>24,25</sup> and p.M932L/p.V991L<sup>5</sup>/p.W1538R<sup>22</sup>; table e-15). For *SCN10A* and *SCN11A*, previously reported variants<sup>6–8</sup> were detected in 4 or 2 patients, respectively, with only 2 (0.4%) or 1 (0.2%) patient(s) carrying a known GOF variant (p.G1662S<sup>26</sup> (*SCN10A*) or p.L1158P<sup>8</sup> (*SCN11A*); table e-15). Although the incidence was low, collectively, these variants were detected in both patients with idiopathic and diabetic peripheral neuropathy and in both painful and nonpainful conditions (table e-15).

**SCN9A haplotypes in PNRR patients.** To determine whether specific haplotypes are enriched in PNRR patients with painful vs nonpainful peripheral neuropathy, we performed haplotype analysis and compared haplotype frequencies between both groups. Specifically, 196 common *SCN9A* SNPs (MAF >10%) sequenced within the PNRR patient population were selected and phased using BEAGLE V4.1.<sup>18</sup> One hundred eighty-five SNPs passed the Hardy-Weinberg (HWE) filter and were used to define 14 haplotype blocks in PNRR patients, with the



**Figure 2** Frequency of individual Nav1.7 missense variants in Peripheral Neuropathy Research Registry patients



Results show the % of all patients, % of patients with painful peripheral neuropathy, or % of patients with nonpainful peripheral neuropathy that carry each individual *SCN9A* low-frequency missense variant or variant combination identified.

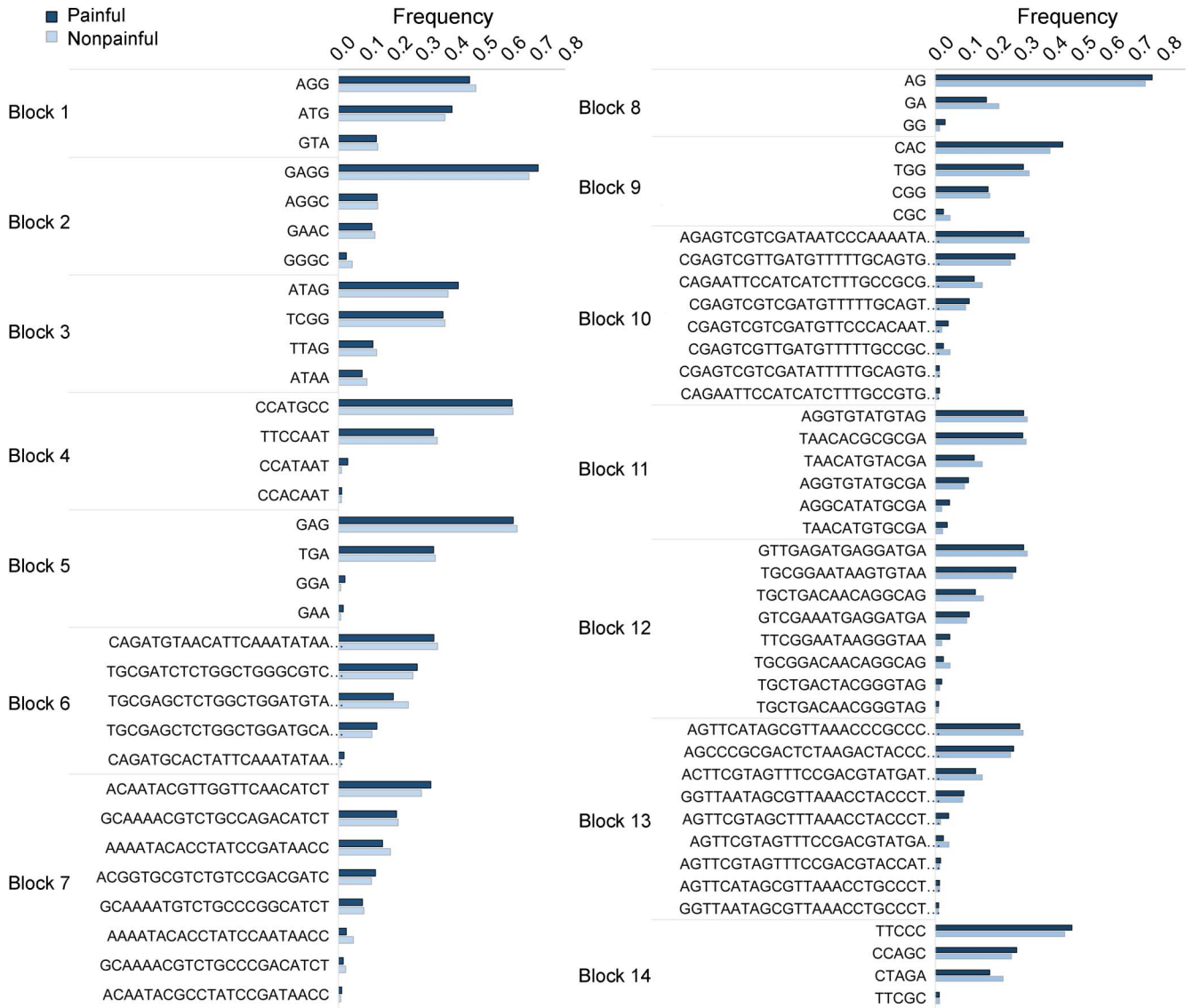
majority consisting of 2 most frequently observed haplotypes (see Methods). No significant differences in haplotype frequencies were seen between patients with painful or nonpainful peripheral neuropathy (figure 3). Because haplotype block definitions are altered by the number of SNPs and may affect frequency estimates, we also used TAGGER to find tag SNPs and redefine haplotype blocks. TAGGER identified 30 tag SNPs that were used to define 7 haplotype blocks. Again, we observed no significant differences in haplotypes between patients with painful or nonpainful peripheral neuropathy (figure e-7). Finally, we examined whether specific haplotypes were enriched in the PNRR cohort compared with the 1000 Genomes, Utah residents with Northern and Western European ancestry (1K-CEU) population. This reference population was selected because of the similarity in genetic background to the PNRR patient sample and because it provides a greater number of intronic and exonic SNPs for analysis than the EVS-EA population for which only exome sequencing data are available. Phased genotypes for 180 of the 186 PNRR SNPs were obtained from the 1K-CEU population data set of which 69 passed HWE filtering and were used to define 8 haplotype blocks. In comparison to the 1K-CEU reference population, there were 2 minor haplotypes

that had increased frequencies in the PNRR patient population ( $p < 0.01$ ; figure 4); however, they were present in less than 5% of the individuals, suggesting that these haplotypes may be functionally inconsequential.

**DISCUSSION** In a cohort of 457 patients diagnosed with peripheral neuropathy, low-frequency missense variants in *SCN9A*, *SCN10A*, and/or *SCN11A* were common, with almost 50% of subjects carrying a variant in at least 1 gene. While the likelihood of carrying a low-frequency variant was high, most (~75%) of the individual variant/variant combinations we identified were present in only 1 patient. Furthermore, for each gene, missense variant allele frequencies were not significantly different in patients whose peripheral neuropathy was painful or nonpainful. Finally, *SCN9A* haplotype analysis showed no significant differences in haplotype frequencies based on pain status, indicating no enrichment of specific haplotypes in patients with painful peripheral neuropathy.

Human genetics provides compelling evidence that Nav1.7 plays a critical role in pain. Furthermore, identification of Nav1.9 mutations in rare genetic pain disorders<sup>27–29</sup> and GOF missense variants in Nav1.7, Nav1.8, and Nav1.9 in patients with painful

**Figure 3** *Nav1.7* haplotypes in Peripheral Neuropathy Research Registry patients



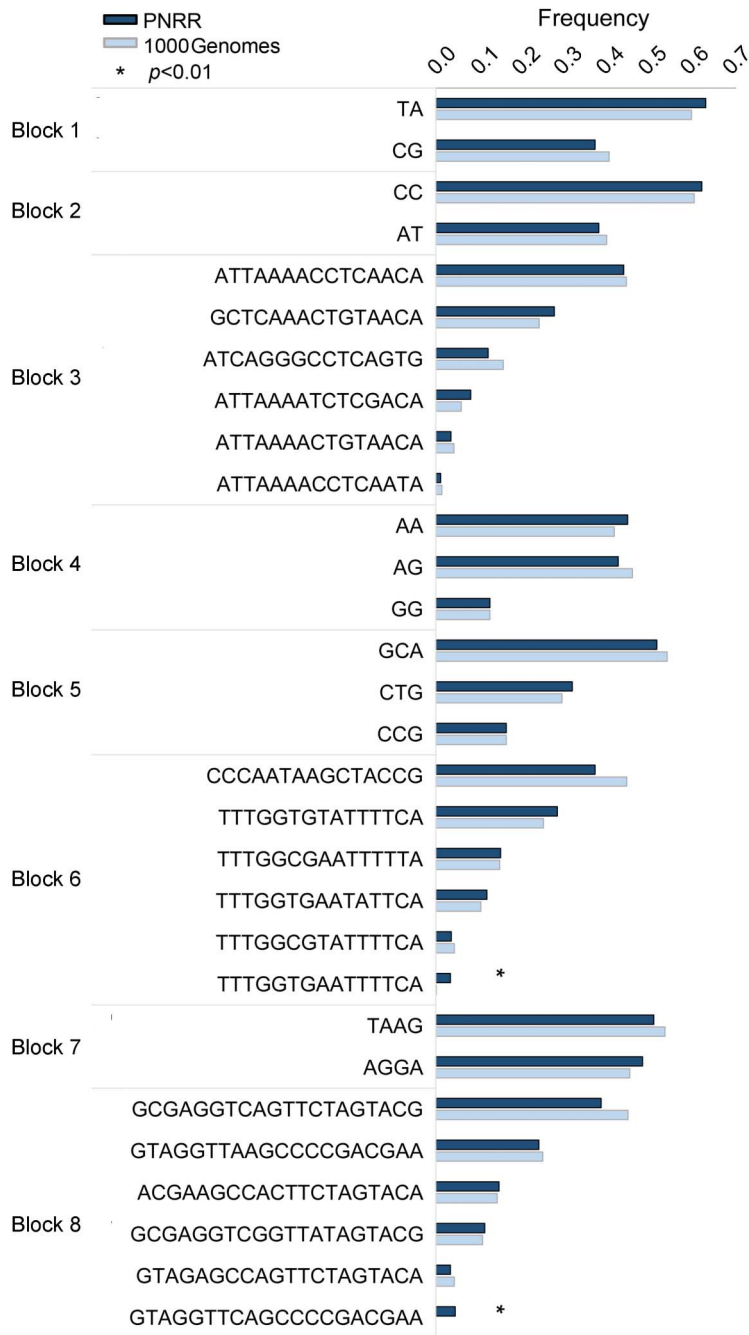
Results show the frequency of individual haplotypes, within 14 different haplotype blocks, in Peripheral Neuropathy Research Registry patients with painful or nonpainful peripheral neuropathy.

SFN<sup>5-8</sup> suggest that each *Nav* channel subtype has the potential to contribute toward the pain experienced by patients harboring mutations. To date, the only study reporting sequencing results for all 3 genes in the same patient cohort showed that missense variants in *SCN9A*, *SCN10A*, or *SCN11A* were detected in 8.7%, 3.8%, and 2.8% of patients with painful, predominantly pure SFN, respectively.<sup>8</sup> In comparison, the incidence of low-frequency missense variants in PNRR patients was ~ 3–5-fold higher, and 10% carried variants in more than 1 gene. It is possible that differences in sequencing platforms, sequencing depth, and approaches to variant calling/identification may explain these results. Of interest, we detected *SCN9A* low-frequency variants in 26% of PNRR patients with probable painful idiopathic SFN based on symptoms, NCV, and abnormal skin

biopsies, a result similar to the incidence reported in patients meeting strict clinical criteria for idiopathic pure SFN (29%).<sup>5</sup> However, the incidence in our subset of PNRR patients was not different from the complete idiopathic group (24%), patients with diabetes (26%), or patients with painful (25%) or nonpainful (24%) peripheral neuropathy regardless of etiology, suggesting that *SCN9A* missense variants do not occur more frequently in patients with idiopathic SFN.

With respect to the individual missense variants identified in PNRR patients, many were known SNPs, but several novel variants were also detected, most notably in *SCN11A* (27% novel variants). Of particular interest was the identification of patients carrying known GOF variants previously reported in patients with SFN or patients with IEM.

**Figure 4** Nav1.7 haplotype comparison with a reference population



Results show the frequency of individual haplotypes, within 8 different haplotype blocks, in Peripheral Neuropathy Research Registry (PNRR) patients or the 1000 Genomes CEU population. Results were analyzed by the chi-square test, \* $p < 0.01$ .

Collectively, the incidence of carrying a known GOF missense variant in the PNRR cohort was very low, and they were present in some patients with a non-painful phenotype. These results contrast with those reported in a small group of patients with strictly defined idiopathic SFN where all 8 *SCN9A* missense variants identified conferred GOF effects in cell-based electrophysiology assays.<sup>5</sup> Thus, while the likelihood of carrying a low-frequency *SCN9A* missense variant was similar (see above), previous results suggest an

enrichment of GOF variants in patients with idiopathic SFN, which was not apparent in PNRR peripheral neuropathy patients. It should be acknowledged that very few PNRR patients met criteria for probable idiopathic, pure SFN and that the biological impact of most of the missense variants we identified is unknown. Further investigation of patients with strictly defined pure idiopathic SFN is therefore important, given the potential attractiveness of this clinical population for testing of novel Nav1.7 agents.

To compare missense variant allele frequencies in PNRR patients with reference populations, we selected the EVS-EA population for our primary analysis.<sup>15</sup> The EVS database contains allele frequency information for exome sequencing variants identified in 4,300 Americans of European descent, a population closest in ancestry to the PNRR cohort.<sup>15</sup> While no differences were noted for *SCN9A* or *SCN11A*, a significant increase in the alternate allele frequency of the *SCN10A* variants p.V1073A and p.S509P was seen in PNRR patients. While the impact of p.S509P is unknown, the p.V1073A common variant has a GOF effect, and expression of the A1073 protein produces larger peak currents, slower fast inactivation, and larger persistent currents compared with V1073 in cell-based electrophysiology assays.<sup>30,31</sup> Of interest, genetic association studies also report that rs6795970 (p.V1073A) is strongly associated with prolongation of the PR interval and QRS complex of the electrocardiogram,<sup>32</sup> atrial fibrillation,<sup>31</sup> and Brugada syndrome.<sup>30</sup> It should be noted that the EVS-EA population represents a mixture of phenotypes including controls, specific cardiac and lung diseases, and specific traits (low-density lipoprotein and blood pressure). However, p.V1073A and p.S509P alternate allele frequencies were also higher in PNRR patients compared with the 1000 Genomes EUR population comprising 503 self-reported healthy individuals. These results suggest an association of p.V1073A and p.S509P with the peripheral neuropathy phenotype, although there was no association with pain status within the PNRR patient group itself.

Previous studies have also reported an association of the *SCN9A* missense variants p.D1908G and p.V991L/p.M932L with neuropathic pain in patients with painful diabetic peripheral neuropathy.<sup>20</sup> We also detected these variants in PNRR patients; however, we did not see any difference in allele frequencies compared with the EVS-EA reference population. It should be noted that patients with diabetic painful distal polyneuropathy comprised only 30% of the PNRR population, while the previous study focused on patients with diabetes enrolled in clinical trials with strict inclusion criteria including pain intensity scores. In addition, the reference

population selected was important; we consider EVS-EA to be the most appropriate comparator; however, a significant difference in the alternate allele frequency was observed when compared with the 1K-EUR population. Finally, with respect to the 1K global population, we consider the marked heterogeneity in MAF across the different ethnic groups comprising this population to be the likely explanation for both the increased number of variants and discrepancies in the direction of the frequency change observed when compared with results from the EVS-EA analysis.

Results from this study showed marked allelic heterogeneity in *SCN9A*, *SCN10A*, and *SCN11A* in patients with idiopathic or diabetic distal polyneuropathy. While Nav1.7 inhibitors have the potential for the treatment of peripheral neuropathic pain, identification of a specific, genetically defined subpopulation for drug testing in patients with peripheral neuropathy appears unrealistic. Additional factors, beyond expression of an Nav channel missense variant, appear to be important because there was no relationship between the presence of missense variants and pain state in PNRR patients. This approach may have utility for the exploration of other disease-related genes and the identification of druggable molecular targets in patients with neuropathic pain.

#### AUTHOR CONTRIBUTIONS

Dr. Wadhawan: drafting of the manuscript and analysis and interpretation of data. Dr. Pant: drafting of the manuscript, study design, and analysis and interpretation of data. Dr. Golhar: analysis and interpretation of data. Dr. Kirov: drafting of the manuscript and analysis and interpretation of data. Dr. Thompson: drafting of the manuscript, study design, and analysis and interpretation of data. Dr. Jacobsen: study concept and design. Dr. Qureshi: analysis and interpretation of data. Dr. Ajroud-Driss, Dr. Freeman, Dr. Simpson, Dr. Smith, and Dr. Hoke: drafting of the manuscript, study concept, and interpretation of data. Dr. Bristow: drafting of the manuscript, study concept and design, and analysis and interpretation of data.

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#### DISCLOSURE

S. Wadhawan, S. Pant, R. Golhar, and S. Kirov have been employees of Bristol-Myers Squibb. J. Thompson has been an employee of, has received research support from, and owns stock/stock options in Bristol-Myers Squibb. L. Jacobsen and I. Qureshi have been employees of and own stock/stock options in Bristol-Myers Squibb. S. Ajroud-Driss has served on the scientific advisory boards of MT Pharma and Alnylam; receives publishing royalties from UpToDate; has served on the speaker's bureau of MT Pharma; and has received research support from Alnylam Viomed and the Foundation for Peripheral Neuropathy. R. Freeman has served on the scientific advisory boards of Abide, Acetylon, Astellas, Biogen, Daiichi Sankyo, Dong, Grace, Grunenthal, Insys, Lundbeck, Nestle, Novartis, Pfizer, Vertex, and Spinifex; has served on the editorial boards of *Autonomic Neuroscience*, *Clinical Autonomic Research*, and the *Clinical Journal of Pain*; receives publishing royalties from UpToDate; has received research support from Impeto, Nestle, Pfizer, the NIH, and Multiple System Atrophy Consortium; and owns stock/stock options in Spinifex. D.M. Simpson has served on the scientific advisory boards of Allergan, Merz, Ipsen, and DSMB; Axsome; has received speaker honoraria from Allergan, Merz, and Ipsen; has served on the editorial board of *AIDS Patient Care*; has received publishing royalties from Oxford University Press; has been an employee of the Icahn School of Medicine at Mount Sinai; has been a consultant for, has served on the speakers' bureaus of, and has received research support from Allergan, Merz, Ipsen; has received research support the NINDS and the Foundation for Peripheral Neuropathy; and has been involved in legal proceedings regarding Proctor and Gamble. A.G. Smith has served on the scientific advisory board of the Celgene Data Monitoring Committee; has served on the editorial board of *NeuroLearn*; has been a consultant of Regeneis, Allergan, and Viomed; and has received research support from Impeto Medical SAS and the NIH. A. Hoke has served on the editorial board of *Experimental Neurology* and *Annals of Clinical Translational Neurology*; has been an employee of Johns Hopkins University; has been a consultant of Neurocrine Inc.; has received research support from the NIH, the Foundation for Peripheral Neuropathy, and the Dr. Miriam and Sheldon G. Adelson Medical Research Foundation; and was involved in the following legal proceedings: National Vaccine Injury Program—gave expert testimony on GBS and Arnold & Porter Kaye Scholer LLP—gave expert testimony on peripheral neuropathy. L.J. Bristow has been an employee of, has received research support from, and owns stock/stock options in Bristol-Myers Squibb. Go to [Neurology.org/ng](http://Neurology.org/ng) for full disclosure forms.

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## **Nav channel variants in patients with painful and nonpainful peripheral neuropathy**

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