SCN10A/Nav1.8 modulation of peak and late sodium currents in patients with early onset atrial fibrillation

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Aims
To test the hypothesis that vulnerability to atrial fibrillation (AF) is associated with rare coding sequence variation in the SCN10A gene, which encodes the voltage-gated sodium channel isoform NaV1.8 found primarily in peripheral nerves and to identify potentially disease-related mechanisms in high-priority rare variants using in-vitro electrophysiology.

Methods and results
We re-sequenced SCN10A in 274 patients with early onset AF from the Vanderbilt AF Registry to identify rare coding variants. Engineered variants were transiently expressed in ND7/23 cells and whole-cell voltage clamp experiments were conducted to elucidate their functional properties. Resequencing SCN10A identified 18 heterozygous rare coding variants (minor allele frequency ≤ 1%) in 18 (6.6%) AF probands. Four probands were carriers of two rare variants each and 14 were carriers of one coding variant. Based on evidence of co-segregation, initial assessment of functional importance, and presence in ≥ 1 AF proband, three variants (417delK, A1886V, and the compound variant Y158D-R814H) were selected for functional studies. The 417delK variant displayed near absent current while A1886V and Y158D-R814H exhibited enhanced peak and late (I_{Na-L}) sodium currents; both Y158D and R818H individually contributed to this phenotype.

Conclusion
Rare SCN10A variants encoding NaV1.8 were identified in 6.6% of patients with early onset AF. In-vitro electrophysiological studies demonstrated profoundly altered function in 3/3 high-priority variants. Collectively, these data strongly support the hypothesis that rare SCN10A variants may contribute to AF susceptibility.

Keywords
Atrial fibrillation • SCN10A • Nav1.8 • Late sodium current • Cardiac electrophysiology

1. Introduction
Atrial fibrillation (AF) is associated with substantial morbidity and increased mortality.¹ Although multiple acquired risk factors for AF have been identified, in ~30% of patients, AF occurs in the absence of underlying heart disease and has previously been defined as ‘lone’ AF.² Genome wide association studies (GWAS) have identified common AF susceptibility loci, and positional cloning and candidate gene approaches have implicated rare variants encoding cardiac ion channels and signalling molecules as genetic risk factors for AF.³

While PR interval prolongation has been associated with increased risk for developing AF, it was only recently that GWAS showed that single nucleotide polymorphisms (SNPs) in SCN10A modulated both PR and QRS duration.⁴⁻⁷ SCN10A encodes NaV1.8, a voltage-gated sodium channel expressed in sensory neurons within dorsal root ganglia (DRG),⁸,⁹ and has recently been identified in human cardiomyocytes and intracardiac neurons.¹⁰⁻¹² Functional studies have suggested a role for NaV1.8 channels as a component of the late sodium current (I_{Na-L}) in cardiomyocytes.

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Here, we tested the hypothesis that rare SCN10A coding variants are over-represented in patients with early onset AF and are associated with increased susceptibility to the arrhythmia by resequencing the entire SCN10A gene and functionally characterizing high-priority variants with in-vitro electrophysiology.

2. Methods

2.1 Study cohort

We identified European American (EA) and African American (AA) probands who presented with early onset AF according to the Vanderbilt AF registry (2002–2011).13 The first control population comprised EA and AA individuals identified from the Vanderbilt Cardiac Surgery Registry who underwent cardiac surgery, had no personal or family history of AF, and did not develop AF after cardiac surgery (Table 1) (see Supplementary material online).14 This study was performed according to the principles outlined in the Declaration of Helsinki. The Vanderbilt University Institutional Review Board (IRB) approved the protocols used in this study and participants were enrolled after informed written consent was obtained. The second control population comprises a total of 983 population controls of European ancestry as previously described.15

2.2 SCN10A resequencing

The coding sequence and flanking introns of SCN10A were amplified by polymerase chain reaction using primers designed to obtain fragments of appropriate size for mutational analysis (see Supplementary material online, Methods). All rare SCN10A variants identified were orthogonally validated using Sequenom sequencing.

2.3 Rare variants

Amino acid coding (AAC) variants (missense, non-synonymous, or indels) were identified by SCN10A resequencing. Post-cardiac surgery controls (n = 717) from the same population were then genotyped for the presence of the rare variants identified in early onset AF probands. Variants with a minor allele frequency (MAF) ≤1% in the non-AF control population and publicly available databases (dbSNP134, the 1000 Genomes Project, and the NHLBI ESP of 6500 individuals) were defined as rare. For all variants, we assessed conservation and predicted functional effects using SIFT, GERP, Grantham scores, and PolyPhen2 using the SeattleSeq Genomic Variation Server (http://snp.gs.washington.edu/SeattleSeqAnnotation134/).

3. Electrophysiological studies

Site-directed mutagenesis was used to generate three high-priority mutants (see Supplementary material online, Methods). The variant constructs studied were confirmed by re-sequencing. Voltage-clamp experiments were performed in ND7/23 cells transiently transfected with plasmids containing SCN10A wild-type (WT) or a rare variant identified in the lone AF cohort. The cells were co-transfected with another plasmid containing the construct for the green fluorescent protein (GFP). The voltage-clamp experiments were conducted at room temperature on transfected cells that expressed the GFP (‘green cells’) after 48 h incubation at 37°C. For the 417delK variant, a subset of transfected cells underwent a modified incubation protocol that consisted of an initial 24 h incubation at 37°C followed by a 24 h incubation at 28°C to determine whether the smaller current measured was due to lower membrane expression of the channel. Recordings were performed in the presence of tetrodotoxin 200 nM, nisoldipine 1 μM, and NiCl2 200 μM (see Supplementary material online, Methods).

Table 1 Clinical characteristics of European American patients with early onset AF and cardiac surgery and population controls

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>EA ancestry</th>
<th>Cardiac surgery controls (n = 654)</th>
<th>Population controls (n = 983)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male (%)</td>
<td>193 (71.8)</td>
<td>440 (67.3)</td>
<td>492 (50%)</td>
</tr>
<tr>
<td>Age at enrolment, years</td>
<td>50.5 ± 13.0</td>
<td>56.4 ± 13.8</td>
<td>50.8 ± 6.7</td>
</tr>
<tr>
<td>Age of diagnosis, years</td>
<td>43.3 ± 12.3</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Age of onset, years</td>
<td>40.9 ± 13.7</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Positive family history (%)</td>
<td>99 (36.8)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>29.1 ± 5.8</td>
<td>28.9 ± 5.7</td>
<td>23.5 ± 2.1</td>
</tr>
<tr>
<td>LV ejection fraction (%)</td>
<td>57.5 ± 7.5</td>
<td>47.1 ± 11.3</td>
<td>–</td>
</tr>
<tr>
<td>PR interval, ms</td>
<td>168.0 ± 28.7</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>QT (ms)</td>
<td>403.7 ± 41.9</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>QTc (ms)</td>
<td>412.0 ± 33.3</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>QRS duration (ms)</td>
<td>86.0 ± 30.7</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Heart rate (b.p.m.)</td>
<td>69 ± 21</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Type of AF (%)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Presented are mean ± SD. AA, African American; AF, atrial fibrillation; b.p.m., beats per minute; EA, European American; LV, left ventricular.

3.1 Data and statistical analysis

Electrophysiological data were analysed and plotted using a combination of Clampfit 9.2 and Origin 6.1. Results are expressed as mean ± SD. If only two groups were being compared, we used the Student’s t-test. For comparisons among means of more than two groups, analysis of variance was used with post hoc pairwise comparisons by Duncan test. If significant differences among means were detected, either two-sided P < 0.05 or P < 0.01 were considered to be statistically significant.

4. Results

4.1 Study cohort

We identified 269 EA patients with early-onset AF with an average age of AF onset of 40.9 ± 13.7 years. Paroxysmal AF was the most common subtype identified (75.1%), and family history of AF was present in 36.8%. Clinical characteristics of the EA AF probands, cardiac surgery, and population controls are shown in Table 1 (see Supplementary material online, Table S1 for data on the AA lone AF probands and controls).

4.2 Prevalence of rare SCN10A variants in lone AF patients

Resequencing 274 AF probands (269 EA and 5 AA) identified 18 rare heterozygous SCN10A variants (6.6%); the MAF of these rare variants
was 0.8% in 717 screened cardiac surgery controls (654 EA and 63 AA), 2.5% in additional Caucasian population controls and publicly available databases; compound variants (i.e. two rare SCN10A mutations) were identified among 4 out of 18 early onset AF probands. Of the variants tested among non-AF controls, 6 EAs and 0 AA had a rare SCN10A variant, respectively (see Supplementary material online, Table S2). Seventeen of the coding variants were identified among EA AF probands and one variant (A1886V) was identified in an AA AF proband (AF527). Thus, the proportion of EA AF probands with a rare coding SCN10A variant was 6.3% (17/269). The predicted conservation, in-silico functional predictions and the reported MAFs for the 18 rare variants identified are listed in Supplementary material online, Table S2 and shown in Figure 1.

4.3 Genotype–phenotype relationships in familial AF

Four of the 18 probands with a rare SCN10A variant had additional family members that were used for co-segregation analyses (Figure 2). The A1886V variant co-segregated with AF in an AA family (AF527), while in the remaining three AF families [AF737 (variants: Y158D, R814H), AF972 (variants: Y158D, R814H), and AF1044 (variant: 417delK)] co-segregation with AF could not be assessed due to limited numbers of first-degree relatives (Figure 2C and D show two families with both Y158D and R814H variants transmitted from parent to child, suggesting they are on the same allele). Table 2 displays the clinical characteristics of the 18 early onset AF probands with rare SCN10A variants.

4.4 Electrophysiological studies of SCN10A variants expressed in ND7/23 cells

We selected three rare heterozygous variants for functional characterization based on: (i) evidence for genotype–phenotype co-segregation (A1886V); (ii) likely to be functionally important (e.g. frame-shift mutations) (417delK), or (iii) if ≥ 1 early onset AF proband was affected by the same rare SCN10A variant (Y158D and R814H). After transient transfection and expression of the variants in ND7/23 cells, voltage-clamp

![Figure 1](image1.png) Representation of Nav1.8 channel protein and corresponding localization of the 18 rare variants identified in patients with early onset AF.

![Figure 2](image2.png) Pedigrees of the four AF families in which rare SCN10A variants were identified.
Table 2: AF probands with rare SCN10A variants in familial AF

<table>
<thead>
<tr>
<th>Family</th>
<th>Variant</th>
<th>Rare SCN10A variant co-segregating with AF</th>
<th>Gender</th>
<th>Diagnosis age, years</th>
<th>Rhythm</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>AF14</td>
<td>G1662S</td>
<td>–</td>
<td>Male</td>
<td>46</td>
<td>Persistent AF</td>
<td>None</td>
</tr>
<tr>
<td>AF200</td>
<td>F1115L</td>
<td>–</td>
<td>Male</td>
<td>58</td>
<td>Persistent AF</td>
<td>CCB,</td>
</tr>
<tr>
<td>AF213</td>
<td>R1268Q</td>
<td>–</td>
<td>Male</td>
<td>16</td>
<td>Paroxysmal AF</td>
<td>None</td>
</tr>
<tr>
<td>AF309</td>
<td>E285K</td>
<td>–</td>
<td>Female</td>
<td>48</td>
<td>Paroxysmal AF</td>
<td>CCB,</td>
</tr>
<tr>
<td>AF496</td>
<td>C1523Y</td>
<td>–</td>
<td>Male</td>
<td>47</td>
<td>Persistent AF</td>
<td>BB, DCCV</td>
</tr>
<tr>
<td>AF527ab</td>
<td>A1886V</td>
<td>Yes</td>
<td>Male</td>
<td>17</td>
<td>PAF, SVR</td>
<td>None</td>
</tr>
<tr>
<td>AF640</td>
<td>V1548F</td>
<td>–</td>
<td>Male</td>
<td>28</td>
<td>Paroxysmal AF, SVR</td>
<td>Flecainide</td>
</tr>
<tr>
<td>AF683</td>
<td>H506N, R1380Q</td>
<td>–</td>
<td>Male</td>
<td>60</td>
<td>Paroxysmal AF</td>
<td></td>
</tr>
<tr>
<td>AF737ab</td>
<td>Y158D, R184H</td>
<td>No</td>
<td>Female</td>
<td>48</td>
<td>Paroxysmal AF</td>
<td>None</td>
</tr>
<tr>
<td>AF857</td>
<td>R1268Q</td>
<td>–</td>
<td>Male</td>
<td>25</td>
<td>Persistent AF</td>
<td>DCCV</td>
</tr>
<tr>
<td>AF858</td>
<td>F719S, R1896G</td>
<td>–</td>
<td>Male</td>
<td>52</td>
<td>Persistent AF</td>
<td>BB, Propafenone</td>
</tr>
<tr>
<td>AF972ab</td>
<td>Y158D, R184H</td>
<td>No</td>
<td>Male</td>
<td>34</td>
<td>Persistent AF</td>
<td>RFA</td>
</tr>
<tr>
<td>AF1005</td>
<td>V1135R</td>
<td>–</td>
<td>Female</td>
<td>29</td>
<td>Permanent AF, SVR</td>
<td>Clopidogrel</td>
</tr>
<tr>
<td>AF1044b</td>
<td>417delK</td>
<td>No</td>
<td>Male</td>
<td>31</td>
<td>Persistent AF</td>
<td>PPM</td>
</tr>
<tr>
<td>AF1062</td>
<td>V1287I</td>
<td>–</td>
<td>Male</td>
<td>20</td>
<td>Persistent AF</td>
<td>BB, Flecainide, DCCV</td>
</tr>
<tr>
<td>AF1095</td>
<td>R14L</td>
<td>–</td>
<td>Male</td>
<td>51</td>
<td>Paroxysmal AF</td>
<td>None</td>
</tr>
<tr>
<td>AF1120</td>
<td>G1810W</td>
<td>–</td>
<td>Male</td>
<td>52</td>
<td>Persistent AF</td>
<td>DCCV</td>
</tr>
<tr>
<td>AF1277</td>
<td>V1287I</td>
<td>–</td>
<td>Male</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Of African-American ancestry.
b Families eligible for rare variant AF co-segregation analysis.

AF, atrial fibrillation; BB, beta blocker; CCB, calcium channel blocker; DCCV, direct current cardioversion; RFA, radiofrequency ablation; PPM, permanent pacemaker; SVR, slow ventricular rate in AF.

Experiments were conducted to characterize their biophysical properties when compared with the WT variant. In all cases, adequate expression of the channels in transfected ND7/23 cells was confirmed by PCR (see Supplementary material online, Figure S2A). We also excluded SCNSA expression in ND7/23 cells by PCR (see Supplementary material online, Figure S2B). Figure 3 shows representative whole-cell current traces recorded for WT channel (Figure 3A) and the rare variants studied (Figure 3B–F). All observed currents displayed slow kinetics, in agreement with previous results for this channel.11,16,17 With the exception of 417delK, all other variants generated sodium currents that were significantly larger than those recorded for the WT channel (Figure 4). A1886V (103.3 ± 52.4 pA/pF, n = 10) and Y158D (−81.9 ± 19.1 pA/pF, n = 7) variants were associated with peak currents that were ~2-fold greater than WT (−42.8 ± 14.7 pA/pF, n = 9, P = 0.009 and P = 0.002, respectively), while R814H variant generated a peak current that was ~4 times larger than WT (−187.1 ± 93.1 pA/pF, n = 17, P < 10−5). In addition, rare variants exhibited persistent (late) current (I_short) significantly larger than WT channel (Table 3 and Figure 6). Because Y158D and R814H variants occurred on the same allele and were identified in two probands, we included both variants in the same construct, transfected ND7/23 cells with the plasmid containing both SCN10A variants and examined their combined effects (compound variant) on the sodium current. The simultaneous presence of both variants on the same allele yielded a significant increase in peak current compared with WT, similar to their effect when expressed individually; thus, both rare variants contribute to the electrical property of the channel. Interestingly, the magnitude of the peak current was close to the sum of the averaged value of the peak currents of each variant expressed individually (Table 3).

The 417delK variant exhibited significantly smaller peak current when compared with WT (−7.5 ± 3.9 pA/pF (n = 7) vs. −42.8 ± 14.7 pA/pF (n = 9), P = 0.002). To determine whether the smaller current was due to lower membrane expression, we incubated 417delK-expressing cells at 28°C overnight.18,19 The peak current measured after this intervention was significantly larger than the peak current measured after 37°C incubation (−21 ± 8.2 pA/pF, n = 7, P = 0.001) (see Supplementary material online, Figure S3) but still significantly smaller than the WT current measured at 28°C. Incubation at lower temperature did not affect WT peak current (Table 3 and see Supplementary material online, Table S3). These findings are consistent with an impaired cell surface expression for 417delK variant at 37°C (physiological temperature) and its partial rescue at 28°C.

WT-Na1.8 and all variants studied activated at more positive voltages than the cardiac isoform Na1.5 (Figure 4). The voltage at which the maximum activation of WT-Na1.8 and rare variants was reached occurred between +5 mV and +15 mV (Table 3). This is strikingly different from what is observed for Na1.5, where maximum activation occurs at −20 mV. This is also in agreement with our previous reported results.11 Y158D, R814H (alone and combined), and 417delK achieved maximum activation at a more positive voltage than WT-Na1.8.

The voltage-dependence of activation and inactivation for WT and rare variants are shown in Figure 5. The area of overlap (dotted boxes) under the activation and inactivation voltage relationship enables a ‘window current’. A1886V, Y158D, R814H, and the compound variant (Y158D-R814H) all exhibited increased window currents and impaired inactivation, which would also account for enhanced late current when compared with WT (Figure 6). The voltage dependence of activation was obtained from the data shown in Figures 3 and 4. The V1/2 of activation for...
the WT-NaV1.8 channel was $-18.1 \pm 3.0$ mV ($n = 9$). Similar voltage-dependence of activation was observed for the A1886V variant and the compound Y158D-R814H variant. In contrast, individual expression of each one of the variants present in the compound variant elicited significantly more depolarized voltage-dependence of activation, $-13.1 \pm 5$ mV ($P = 0.03$) and $-13.9 \pm 4.5$ mV ($P = 0.003$) for Y158D and R814H, respectively. There were no significant differences in the inactivation $V_{1/2}$ among rare variants expressed individually when compared against WT. However, the compound variant (Y158D-R814H) caused a significant hyperpolarizing shift in the voltage-dependence of inactivation (Table 3).

**Figure 3** Representative traces obtained for the wild-type (WT) channel and rare variants Y158D, R814H, Y158D-R814H, A1886V, and 417delK expressed in ND7/23 cells ($n = 7–10$ cells). Inset in A: voltage-clamp protocol used.

**Figure 4** Current–voltage relationships obtained for human WT-Nav1.8 and rare variants expressed in ND7/23 cells ($n = 7–10$ cells) (A–E). Inset in A: voltage-clamp protocol used. Averaged data for the WT channel are presented in each panel for comparison purposes with rare SCN10A variants.
Table 3 Biophysical properties of WT, Nav1.8 and A1886V, Y158D, R814H, and 417delK Nav1.8 variants

<table>
<thead>
<tr>
<th>Parameter</th>
<th>WT</th>
<th>A1886V</th>
<th>Y158D</th>
<th>R814H</th>
<th>Y158D + R814H</th>
<th>R814H + 417delK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak I$_{Na-L}$ (pA/pF)</td>
<td>103.3 ± 18.8 (P = 0.0002)</td>
<td>103.3 ± 18.8 (P = 0.0002)</td>
<td>103.3 ± 18.8 (P = 0.0002)</td>
<td>103.3 ± 18.8 (P = 0.0002)</td>
<td>103.3 ± 18.8 (P = 0.0002)</td>
<td>103.3 ± 18.8 (P = 0.0002)</td>
</tr>
<tr>
<td>V at peak I$_{Na-L}$ (mV)</td>
<td>52.4 ± 81.9 (P = 0.81 x 10$^{-4}$)</td>
<td>52.4 ± 81.9 (P = 0.81 x 10$^{-4}$)</td>
<td>52.4 ± 81.9 (P = 0.81 x 10$^{-4}$)</td>
<td>52.4 ± 81.9 (P = 0.81 x 10$^{-4}$)</td>
<td>52.4 ± 81.9 (P = 0.81 x 10$^{-4}$)</td>
<td>52.4 ± 81.9 (P = 0.81 x 10$^{-4}$)</td>
</tr>
<tr>
<td>Peak I$_{Na-L}$ (% of peak WT)</td>
<td>0.002</td>
<td>0.002</td>
<td>0.002</td>
<td>0.002</td>
<td>0.002</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Figure 6A depicts the normalized currents recorded at +10 mV for WT and the rare variants studied. Figure 6B shows the average I$_{Na-L}$ represented as a percentage of the peak current for WT and each of the variants. For most rare variants, I$_{Na-L}$ was significantly larger when compared with WT. On average, I$_{Na-L}$ for the WT channel was 7.8 ± 3.9%. This value was ~2-fold higher for A1886V (14.6 ± 6%) and R814H (15.4 ± 3.7%) variants, 4.5-fold higher for Y158D (37.7 ± 7.7%), and 3-fold increase for the compound variant Y158D-R814H (24.5 ± 5.6%). When Y158D and R814H were expressed on the same protein, I$_{Na-L}$ was close to the average of both variants expressed separately (Table 3). I$_{Na-L}$ for 417delK variant is not shown because though the I$_{Na-L}$ expressed as a percentage of the peak current was close to 9%, the absolute values for the peak current and I$_{Na-L}$ were significantly smaller that the values for the WT channel (see Supplementary material online, Table S3).

5. Discussion

We identified 18 rare SCN10A coding variants in cohort of patients with early onset AF with a frequency of 6.6%, but only one variant (A1886V) co-segregated with AF. Possible explanations for the lack of co-segregation with AF in the other kindreds include variable penetrance of familial AF and the coexistence of modifier gene alleles altering AF susceptibility. The low frequency of a rare variant in an appropriately matched local and population controls in conjunction with functional studies demonstrating changes in the biophysical properties of the channel variants provide strong evidence that rare SCN10A variants may contribute to increased AF susceptibility.

Previous studies have shown that some SCN10A variants are associated with changes in P-wave duration, PR interval, and QRS duration and a common gain-of-function variant has been associated with heart block and ventricular arrhythmias. SCN10A is widely expressed in DRG and is the dominant voltage-gated sodium channel driving action potential (AP) upstroke and conduction in unmyelinated peripheral nerves. More recently, SCN10A expression has also been identified in human cardiomyocytes and intracardiac neurons. The cellular localization of Nav1.8 channel within the heart appears highly variable across cardiac chambers with enhanced expression in the conduction system.11 In 2011, Facer et al. confirmed the presence of Nav1.8 in human atrial cardiomyocytes. We then showed that the Nav1.8 specific blocker A-803467 selectively blocked the I$_{Na-L}$ in mouse and rabbit ventricular cardiomyocytes, shortens the AP duration (APD) at lower heart rates and in the presence of the sodium channel opener ATX-II enhanced I$_{Na-L}$ generated arrhythmogenic long-lasting early after depolarizations (EADs). We also demonstrated that Scn10a transcripts were readily detected in both atria and right ventricle but were markedly reduced in left ventricle of mouse hearts. Simultaneously, Verkerk et al. reported the presence of Nav1.8 in intracardiac neurons and myocardium in mouse heart tissue sections, further supporting the presence and participation of Nav1.8 sodium channel in cardiac electrophysiology. More recently, Qi et al. demonstrated that blockade of Nav1.8 channels by A-803467 suppressed vagally mediated effect on cardiac conduction and AF inducibility supporting a neural effect. Collectively, these studies not only support the presence of Nav1.8 sodium channel in intracardiac neurons but also in cardiomyocytes, and suggest a role for this channel in cardiac electrophysiology and modulating susceptibility to arrhythmias.

Non-coding intronic SCN10A genetic variants have consistently been associated with cardiac conduction disease, PR interval duration, and Brugada syndrome. A recent study by van den Boogaard et al. demonstrated that the SCN10A intronic variants were associated with AF, indicating a potential role for genetic variation in SCN10A in AF development.
examined the Scn10a-Scn5a locus in murine hearts using high-resolution 4C-seq analyses. They identified a common non-coding SNP within the Scn10a enhancer that interacts with the Scn5a promoter that is critical for cardiac conduction. Deletion of the Scn10a enhancer showed that it is essential for Scn5a expression in cardiac tissue. This study provides a potential mechanism by which common non-coding SCN10A genetic

Figure 5 Voltage dependence of activation and inactivation for WT-Nav1.8 and rare variants expressed in ND7/23 cells (n = 7–10 cells) (A–D). Inset in A: voltage step protocol used. Data are presented as peak currents normalized by peak $I_{Na}$ obtained at test potential $V_{test} = -10$ mV. Averaged data for the WT channel are presented in each panel for comparison with the variants. Sodium window current (defined as the current range between complete inactivation and beginning of activation) is identified with a dotted box in each panel.

Figure 6 (A) Normalized current traces by the peak current for WT-Nav1.8 and the three high-priority rare variants studied (currents measured at $+10$ mV) (n = 7–10 cells). (B) Average $h_{Na,L}$ (expressed as a percentage of peak $h_{Na}$) for WT, A1886V, Y158D, R814H, and Y158D-R814H rare variants measured 100 ms after applying the triggering pulse. Averaged data for WT channel are presented in each panel for comparison with the rare variants.
variation may regulate susceptibility to arrhythmias. However, it is unknown if rare coding SCN10A variants (such as the ones identified in this study) also modulate SCN5A expression even if they do not compromise the integrity of the SCN10A enhancer. We previously resequenced SCN5A in a large cohort of patients with early onset AF (n = 118) and AF associated with heart disease (n = 257) and identified 8 novel rare variants in 10 AF probands (2.7%).29 These variants were highly conserved and in six familial AF kindreds cosegregated with AF. In addition, our group has identified rare variants in SCN1B and SCN2B that associate with AF and functionally characterized these as loss of function mutations.30 All these studies underline the potential role of the cardiac sodium current for the development of AF.

One major challenge in contemporary genomics is determining which rare variants are pathogenic. Several criteria including the absence of the variant in an appropriate matched control population, racial matching, conservation, type of variant, cosegregation, and functional characterization of the variant have been proposed to distinguish pathogenic variants in Mendelian diseases from background genetic noise.31,32 While large public databases and in-silico prediction can provide some guidance, functionally characterizing the variants in vitro can provide critical information attesting to their contribution to disease causation. Here we selected three rare SCN10A variants based on co-segregation with AF (A1886V), a high predicted probability of being pathogenic (417delK), and when >1 AF proband carried the same rare variant (Y158D and R814H) and functionally characterized them. While the A1886V and the Y158D-R814H compound variants exhibited significantly increased peak and hNa-L, the 417delK variant showed a marked decrease in the peak current (~80%) when compared with the WT. To explore a potential trafficking defect, a subset of 417delK-expressing cells underwent a secondary overnight incubation at 28°C. Voltage-clamp studies demonstrated that the peak sodium current was significantly larger than that obtained after incubation at 37°C, although the loss of current was not fully restored by this intervention. These data strongly suggest that the decreased sodium current associated with the 417delK variant at 37°C is associated with impaired cell surface expression, which is partially rescued by a lower temperature (28°C). While additional experiments such as surface-membrane vs. intracellular protein expression studies may further define the mechanism of the trafficking defect associated with the 417delK loss-of-function SCN10A variant, the poor specificity of currently available Nav1.8 antibodies makes these experiments challenging. Our group and others have tested several commercially available Nav1.8 antibodies but have not convincingly demonstrated expression of Nav1.8 (data not shown).

In our study, none of the individuals carrying rare SCN10A gain-of-function variants (Y158D, R814H, Y158D-R814H, and A1886V) exhibited QT prolongation.33 The data provided here suggest that the SCN10A rare variants examined may mediate increased AF susceptibility by modulating not only the Nav1.8 current but also neuronal input into myocardium. The major goal of this study was to show that rare SCN10A variants are associated with AF and can modulate the biophysical properties of Nav1.8. Enhanced hNa-L in particular can disrupt the repolarization of cardiac cells and prolong atrial APD, which in turn can increase the risk for EADs.11 The functional data provided here for the SCN10A rare variants provide potential underlying cellular mechanisms by which their expression in the heart may contribute to increased AF susceptibility.

5.1 Limitations of the study
Our AF families are small. The lack of clinical data and DNA from all family members limited our ability to perform segregation analyses. Furthermore, variable SCN10A penetrance may impact segregation analyses. It is possible that rare variants may not segregate with AF in the absence of a ‘second hit’ (a modifier SNP such as a common AF risk allele or a clinical risk factor).3,21 We functionally characterized the three variants most likely to be pathogenic based on cosegregation with AF, a high predicted probability of being causative and when >1 early onset AF proband carried the same rare variant. Although these three high-priority variants profoundly altered electrophysiological function of the hNa-L and support our hypothesis that some rare SCN10A variants may be causative for AF, these results should not necessarily be extrapolated to the remaining rare variants identified in our patients with early onset AF. It is likely that some of the rare SCN10A variants identified may be benign rare polymorphisms and not linked with AF.

While we stratified our study cohort into EA and AA, the latter group was relatively small and extrapolation to larger AA cohorts should be considered with caution. In addition, the paroxysmal nature and variable symptoms in AF can make the assignment of the clinical phenotype a major challenge given the high prevalence of AF in the general population, the influence of environmental and epigenetic factors, and the late age of onset for the arrhythmia. We functionally characterized the three variants most likely to be pathogenic based on cosegregation with AF, a high predicted probability of being causative, and when >1 early onset AF proband carried the same rare variant. While it is challenging to predict if the remaining rare variants modulate the peak and hNa-L, we are planning on functionally characterizing these additional rare SCN10A variants but consider this to be beyond the scope of the present study.

Additional limitations relate to the in-vitro studies. The ionic current measured for each rare SCN10A variant reported here reflects the effect of a particular variant on the pore-forming α-subunit of the channel expressed in a heterologous expression system. The α-subunit is normally regulated by β-subunits, which combine with the α-subunit in a particular stoichiometry in cardiac myocytes. Although ND7/23 cells express endogenous β-subunits, the stoichiometry with which they interact with the transfected SCN10A α-subunit remains unknown. Another limitation relates to the effect of rare SCN10A variants on the excitability and the APD of the cardiac myocyte. It has previously been demonstrated that ventricular myocytes isolated from SCN10A–/– mice lack hNa-L and the APs are significantly shorter than those measured in WT myocytes,11 which supports the contribution of Nav1.8 sodium current to the cardiomyocyte electrophysiology. While here we demonstrated enhanced peak and hNa-L for A1886V and the compound Y158D and R814H rare SCN10A variants and loss of peak current for 417delK, we can only speculate about their direct effects on atrial APD. Gain-of-function variants can prolong the APD and increase the risk for EADs;3 loss-of-function variants can decrease the APD and increase the risk for delayed-after-depolarizations.

In summary, we identified rare SCN10A variants in 6.6% of patients with early onset AF. Electrophysiological characterization of three high-priority SCN10A variants demonstrated a marked reduction in peak INa-L for the 417delK variant and significantly enhanced peak and hNa-L for the A1886V, Y158D, R814H, and the Y158D-R814H compound variants. Collectively our findings support the association between SCN10A/Nav1.8 and AF and suggest potential disease-related mechanisms for increased susceptibility to the arrhythmia.

Supplementary material
Supplementary material is available at Cardiovascular Research online.
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