Common SCN10A variants modulate PR interval and heart rate response during atrial fibrillation

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Aims
SCN10A encodes the sodium channel Nav1.8 implicated by genome-wide association studies as a modulator of atrioventricular conduction (PR interval). In a cohort of patients with atrial fibrillation (AF), we examined whether there was an association between common variants in SCN10A and both the PR interval during normal sinus rhythm and the heart rate response during AF.

Methods and results
Patients prospectively enrolled in the Vanderbilt AF registry with electrocardiograms in normal sinus rhythm and/or AF within 1 year of enrollment were genotyped for two common SCN10A variants rs6795970 and rs12632942. Both variants were associated with the PR interval duration in a gene-dose effect on unadjusted analysis; after adjustment for the covariates age, gender, body mass index, hypertension, congestive heart failure, and medication usage, the association remained for rs6795970 only (P = 0.012, partial R² = 0.0139). On unadjusted analysis, heart rate response during AF was associated with rs6795970 (P = 0.035, partial R² = 0.015), but not with rs12632942 (P = 0.89), and neither association was significant after adjustment for covariates.

Conclusion
The common variant rs6795970 in SCN10A is associated with the PR interval duration among healthy patients and those with AF. In addition, this single nucleotide polymorphism trended towards an association with heart rate response during AF indicating the importance of this common SCN10A polymorphism as a marker of atrioventricular conduction.

Keywords
Atrial fibrillation • SCN10A • PR interval

Introduction
Atrial fibrillation (AF) is the most common sustained arrhythmia in clinical practice, with the lifetime risk of development of AF ranging from 20% to 25%.1 Common risk factors for the development of AF include age, gender, body mass index (BMI), hypertension (HTN), congestive heart failure (CHF), and cardiac valvular dysfunction.2 Additionally, PR interval prolongation on the electrocardiogram (ECG) has also been validated as a risk factor and serves as an endophenotype for the development of AF.3,4 The PR interval is highly heritable, and this finding has been confirmed by genome-wide association (GWA) studies identifying common loci associated with PR interval duration.5–10

Consistently identified among these GWA studies has been the 3p22.2 locus, a region containing two sodium channels, SCN5A encoding the Nav1.5 sodium channel and SCN10A encoding Nav1.8. A common coding variant, rs6795970, resulting in A1073V was identified by multiple studies7,8,10 as a key tag single nucleotide polymorphism (SNP) in this region, while the work of Pfeufer et al.9 implicated the intronic SNP rs6800541. The SNPs, located in SCN10A, are in strong linkage disequilibrium but not with SNPs in SCN5A, suggesting that this is an independent SCN10A signal. In addition, SNPs at the SCN10A locus have consistently been associated with QRS duration further supporting a role for the Nav1.8 channel in cardiac conduction.11 Interestingly, it appears that tag SNPs may play a part in both SCN10A and SCN5A expression as it has recently been demonstrated that this region serves as a binding site for transcription factors TBX3/TBX5.12

SCN10A expression has been established in the dorsal root ganglion (DRG), nociceptive nerve fibers, and retina.13–16 Recent
studies indicate that SCN10A is expressed in cardiomyocytes by immunostaining\textsuperscript{17} and quantitative reverse transcriptase- polymerase chain reaction (qRT-PCR),\textsuperscript{8,11,18} although Western blots have been inconclusive. In addition, reports of mouse models evaluating SCN10A function have reported varying results: ECG monitoring of SCN10A \textsuperscript{-/-} mice was reported to reveal shortened PR interval,\textsuperscript{8} yet when wild-type mice were treated with A-803467, a specific Nav1.8 blocker, both the PR interval and QRS duration were prolonged.\textsuperscript{11} In cardiomyocytes, studies with A-803467 and in SCN10A \textsuperscript{-/-} mice suggested that Nav1.8 enhances the late sodium current with the resultant prolongation of the action potential duration.\textsuperscript{18}

While SCN10A appears to be an important modulator of cardiac conduction in community based studies\textsuperscript{7-9} and in electronic medical record cohorts,\textsuperscript{10} it remains to be determined whether SCN10A variants modulate the PR interval in patients with AF due to underlying substrate modification.\textsuperscript{19,20} In addition when patients are in AF, ventricular rate is primarily reliant on atrioventricular conduction.\textsuperscript{21} While SCN10A expression in these tissues has not been studied, it seems reasonable to hypothesize that SCN10A variants will regulate heart rate during AF. Accordingly, among the Vanderbilt AF Registry patients, we tested whether rs6795970 (A1073V), a coding variant in high linkage disequilibrium with the original tagged SNP (rs6800541) modulates the PR interval and heart rate response during AF. In addition we tested a second coding variant in moderate linkage disequilibrium with the index SNP, rs12632942 (L1092P).

**Methods**

**Study populations**

**Vanderbilt atrial fibrillation registry**

Patients were enrolled prospectively and were required to have documented AF by ECG or telemetry recording; details have been described previously.\textsuperscript{22} Exclusion criteria included age < 18 years and AF secondary to cardiac surgery. Upon enrollment, a detailed medical history was obtained and a blood sample or cheek swab was collected for future genotyping. Race was self-reported. Current enrollment includes over 1500 patients with AF, of which 304 have lone AF. Written informed consent was obtained prior to enrollment under a protocol approved by the Vanderbilt University Review Board.

Baseline demographic information including age, gender, BMI, ethnicity, prevalent HTN, and prevalent CHF were recorded at the time of enrollment. This study was limited to individuals of European descent. The PR interval in normal sinus rhythm (NSR) and the heart rate in AF were measured manually from 12-lead ECGs. Both ECGs were required to have been recorded within one year of enrollment into the study, and at the time of ECG acquisition medications known to affect atrioventricular (AV) nodal conduction were recorded including: β blockers, non-dihydropyridine calcium channel blockers, digoxin, and class III antiarrhythmics. The ECGs were reviewed by two physicians blinded to patient’s genotype.

**Genotyping**

A total of 1223 probands underwent genotyping for rs6795970 (cDNA 3218) and rs12632942 (cDNA 3275) SNPs with Taqman (Applied Biosystem) or Sanger sequencing. For Sanger sequencing the coding and splice junctions of SCN10A were amplified by PCR using primers designed to obtain fragments of appropriate size. The PCR-amplified DNA fragments were then directly sequenced using ABI 3730xl DNA Analyzer (Vanderbilt DNA Sequencing Facility). Approximately 2.5% (n = 51) of individuals failed genotyping of both SNPs and were excluded from further analysis resulting in a study sample size of 1192 individuals. The minor allele frequency (MAF) in our cohort was 34% for rs6795970 and 25% for rs12632942. The genotype frequencies for all SNPs did not deviate significantly from Hardy-Weinberg equilibrium.

**Statistical analysis**

Patient demographics, medical history and medications data were summarized with median and interquartile range (IQR) for continuous variables and with percentages for categorical variables for all patients (N = 1192), for patients with a NSR ECG (N = 852) and for patients with an ECG documenting AF (AF, N = 502). Three hundred and twenty six had both an ECG with NSR and an ECG with AF. The outcomes were PR interval in NSR patients and heart rate in AF patients and were described with the median and IQR. Since the residuals for the PR interval and heart rate models were right skewed, log transformed all outcomes for regression modeling. For each outcome, models were fit with and without adjustment for age, gender, BMI, AF nodal blocking medication usage, prevalent HTN, and prevalent congestive heart failure. In addition to analyses of all patients in AF, analyses were conducted on a subgroup of 101 patients who were not observed to be on AV nodal blocking medications. To determine the impact of SNP variable on the outcome, the squared semi-partial correlation coefficient was calculated to assess the change in the R\textsuperscript{2} when each SNP variable was removed from the model.

We believe it is worth noting that while a standard linear regression analysis parameter describes the additive change (i.e. the difference) in the mean responses associated with a change in a risk factor, a parameter in a linear regression model with log-transformed response describes a different quantity. In particular, if the residuals for the model with the log-transformed response are approximately symmetric as they were in the present analyses, an exponentiated parameter estimate (exp(beta)) can be interpreted as the multiplicative-change or fold-change in the median response value associated with a change in the risk factor. Thus, if X is a risk factor and Y is a response that is log transformed for a regression analysis, then the exponentiated parameter associated with X is equal to the median response value for X = x + 1 divided by the median response value for X = x (i.e. it is a median ratio). For the present analyses, we report estimated fold-changes in the median response (i.e. median ratios) and 95% confidence intervals associated with changes in all risk factors as well as the semi-partial coefficient of determination. Analyses were performed using the R programming language version 2.15.0 (Vienna, Austria).

**Results**

**Demographics**

Among the 1192 subjects, the median age of diagnosis was 58 years (IQR: 50, 65 years) and the majority was male (N = 805, 68%)
Table 1 Baseline demographic data for entire cohort and subgroups with PR interval Measurements and/or heart rate response in AF

<table>
<thead>
<tr>
<th></th>
<th>All (N = 1192)</th>
<th>PR interval measured (N = 852)</th>
<th>AF HR recorded (N = 502)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age*</td>
<td>58 (50.65)</td>
<td>58 (50.65)</td>
<td>60 (51.66)</td>
</tr>
<tr>
<td>Gender (% male)</td>
<td>67.5</td>
<td>65.3</td>
<td>68.3</td>
</tr>
<tr>
<td>BMI (kg/cm²)*</td>
<td>30.1 (26.4,35.0)</td>
<td>30.2 (26.335.4)</td>
<td>30.7 (26.6.36.2)</td>
</tr>
<tr>
<td>HTN (%)</td>
<td>70.9</td>
<td>72.4</td>
<td>72</td>
</tr>
<tr>
<td>CHF (%)</td>
<td>19.7</td>
<td>14.9</td>
<td>22.2</td>
</tr>
<tr>
<td>% Taking AV nodal blocking agent</td>
<td>–</td>
<td>89.7</td>
<td>79.5</td>
</tr>
<tr>
<td>Beta blocker</td>
<td>–</td>
<td>51</td>
<td>46.4</td>
</tr>
<tr>
<td>Calcium channel blocker</td>
<td>–</td>
<td>32.5</td>
<td>23.4</td>
</tr>
<tr>
<td>Digoxin</td>
<td>–</td>
<td>13.7</td>
<td>18.8</td>
</tr>
<tr>
<td>Class 3 Antiarrhythmic</td>
<td>–</td>
<td>38.4</td>
<td>20.2</td>
</tr>
</tbody>
</table>

*Continuous values are represented by median and the interquartile range (IQR).

The median BMI for this group was 30.1 kg/cm² (IQR: 26.4, 35.0 kg/cm²) and 844 (71%) had a history of HTN and 234 (20%) had a history of CHF. For the subset of individuals with a measured PR interval (N = 852) and/or AF ECG (N = 502), the baseline covariates are similar to those of the entire cohort. Among the subset with a normal sinus rhythm ECG, 89.7% were taking medications with AV nodal blocking properties while 79.5% of those with an AF ECG were also on an AV nodal blocking medication.

PR interval analysis

In unadjusted analysis rs6795970 was associated with a prolongation of the PR interval (AA vs. GG: median ratio = 1.058, 95% CI 1.02–1.098; AG vs. GG: median ratio 1.018, 95% CI 0.993–1.0444 P = 0.010, partial R² = 0.0123) (Figure 1, Table 2). For rs12632942, the minor allele was also associated with the PR interval on unadjusted analysis (GG vs AA: median ratio = 0.956, 95% CI 0.917–0.996; GA vs. AA: median ratio 0.977, 95% CI 0.954–1 P = 0.033, Partial R² = 0.008); however, the direction of effect differed. After adjustment for age, gender, BMI, HTN, CHF, medication usage, the association with rs6795970 remained (AA vs. GG median ratio = 1.062, 95% CI 0.954–1 P = 0.033, Partial R² = 0.008) (Figure 1, Table 2).

Heart rate in atrial fibrillation analysis

For rs6795970, in unadjusted analysis, the minor allele was associated with slower heart rate response while in AF (AA vs. GG: median ratio = 0.91, 95% CI 0.846–0.98; AG vs. GG: median ratio = 0.961, 95% CI 0.914–1.01 P = 0.035, partial R² = 0.015) (Figure 2, Table 3). For rs12632942, the minor allele was not significantly associated with heart rate response on unadjusted analysis (P = 0.89). After adjustment for age, gender, BMI, HTN, CHF, medication usage, an association for both SNPs was not observed (rs6795970: P = 0.23; rs12632942: P = 0.56).

Among the subset of patients off all AV node blocking medications (N = 101), rs6795970 was found to approach significance with heart rate response in AF (P = 0.067) without adjustment of covariates; however, this association was not seen after adjusting for covariates as listed above (P = 0.22).

Discussion

Common variants in SCN10A have previously been associated with PR interval duration, and among patients with AF we found that...
this association remains for the common variant, rs6795970 (A1073V), although the underlying substrate has been structurally and electrically remodelled. For the rs12632942 (L1092P) SNP, the contribution to the PR interval and AF risk are less strong, likely reflecting the moderate linkage disequilibrium with the index SNP as described in prior studies.9,10 Additionally, our results showed a trend towards an association of A1073V and ventricular response during AF. Taken together these results indicate that common variation in SCN10A modulates AV conduction.

Comparison to the NHLBI Exome Sequencing Project revealed rs6795970 to be of slightly lower frequency than that reported for individuals of European ancestry.34% vs. 39%) and for rs12632942 the frequency was comparable (25% vs. 26%) (http://evs.gs.washington.edu/EVS/). The variation in MAF for rs12632942 may have resulted from inclusion of individuals with mixed ancestry as we relied on self-reporting of race to limit our study to those of European ancestry. Hence, this may question the extension of these results to a population outside of our local population.

As in prior studies, we found the PR interval to be modified by the tag SNP, A1073V, in both the unadjusted and adjusted analysis. For rs12632942, the unadjusted analysis did find an association; however, after adjustment for covariates, this association was no longer seen, indicating that this SNP is most likely not a modifier of the PR interval among patients with AF. For both SNPs, increasing age, male gender, BMI, and prevalent CHF were found to be important determinants in predicting PR interval. Age and gender have been previously associated with PR interval.23,24 Additionally, increasing BMI and CHF are commonly known to be associated with PR interval prolongation.25–27

Assessment of heart rate response in AF yielded an association between the tag SNP A1073V on the unadjusted analysis; however, after adjustment this result was not sustained. The covariates on analysis associated with reduced heart rate response in AF included use of an AV nodal blocking medication, male gender, and CHF. Contrary to the PR interval response, age was not found to be associated with AF heart rate response. The finding of female gender and elevated heart rate response in AF has been reported previously.28 Congestive heart failure with AF has been associated with remodelling leading to abnormalities in conduction and tissue refractoriness, and it is plausible that this could lead to reduced ventricular response while in arrhythmia.29 A secondary analysis of those individuals not on AV nodal blocking agents (n = 101), additionally trended towards an association with the A1073V SNP and heart rate response; however, when adjustment was made for covariates, the association was not significant.

Table 2 Unadjusted and adjusted tests of association for SCN10A-A1073V and SCN10A-L1092P and PR interval

<table>
<thead>
<tr>
<th>SNP</th>
<th>rs ID</th>
<th>N</th>
<th>Compare</th>
<th>Unadjusted Median ratio (95% CI)</th>
<th>P value</th>
<th>Partial R2</th>
<th>Adjusted* Median ratio (95% CI)</th>
<th>P value</th>
<th>Partial R2</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1073V</td>
<td>rs6795970</td>
<td>743</td>
<td>AA vs. GG</td>
<td>1.058 (1.02, 1.098)</td>
<td>0.010</td>
<td>0.0123</td>
<td>1.062 (1.021, 1.105)</td>
<td>0.012</td>
<td>0.0139</td>
</tr>
<tr>
<td>L1092P</td>
<td>rs12632942</td>
<td>836</td>
<td>GG vs. AA</td>
<td>0.956 (0.917, 0.996)</td>
<td>0.033</td>
<td>0.008</td>
<td>0.966 (0.921, 1.012)</td>
<td>0.15</td>
<td>0.005</td>
</tr>
</tbody>
</table>

CI, confidence interval.
*Adjusted for age, gender, BMI, medications, HTN, and CHF.
Table 3  Unadjusted and adjusted tests of association for SCN1A-A1073V and SCN1A-L1092P and heart rate response in atrial fibrillation for all individuals and subgroup of individuals off AV-node blocking medication

<table>
<thead>
<tr>
<th>SNP</th>
<th>Group Studied</th>
<th>N</th>
<th>Compare</th>
<th>Unadjusted Median ratio (95% CI)</th>
<th>P value</th>
<th>Partial R²</th>
<th>Adjusted# Median ratio (95% CI)</th>
<th>P value</th>
<th>Partial R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1073V</td>
<td>All</td>
<td>438</td>
<td>AA vs. GG</td>
<td>0.91 (0.845, 0.98)</td>
<td>0.035</td>
<td>0.015</td>
<td>0.936 (0.865, 1.012)</td>
<td>0.23</td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td>Subgroup off meds</td>
<td>87</td>
<td>AG vs. GG</td>
<td>0.961 (0.914, 1.01)</td>
<td></td>
<td></td>
<td>0.975 (0.925, 1.027)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>AA vs. GG</td>
<td>0.786 (0.626, 0.986)</td>
<td>0.067</td>
<td>0.0623</td>
<td>0.806 (0.618, 1.052)</td>
<td>0.22</td>
<td>0.0406</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>GG vs. GG</td>
<td>0.904 (0.799, 1.024)</td>
<td></td>
<td></td>
<td>0.925 (0.801, 1.069)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L1092P</td>
<td>All</td>
<td>496</td>
<td>GG vs. AA</td>
<td>1.02 (0.938, 1.108)</td>
<td>0.89</td>
<td>0.0004</td>
<td>1.027 (0.941, 1.121)</td>
<td>0.82</td>
<td>0.0009</td>
</tr>
<tr>
<td></td>
<td>Subgroup off meds</td>
<td>99</td>
<td>GA vs. AA</td>
<td>1.004 (0.957, 1.052)</td>
<td></td>
<td></td>
<td>0.999 (0.95, 1.05)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>GG vs. AA</td>
<td>0.907 (0.742, 1.11)</td>
<td>0.56</td>
<td>0.012</td>
<td>0.893 (0.715, 1.116)</td>
<td>0.56</td>
<td>0.014</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>GA vs. AA</td>
<td>0.957 (0.851, 1.076)</td>
<td></td>
<td></td>
<td>0.956 (0.834, 1.095)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Cl. confidence interval.
#Adjustment for age, gender, BMI, hypertension, congestive heart failure and medication (medication not included in model for the subgroup off medication).

Limitations

The vast majority of patients in this study were on medications modifying conduction through the AV node. Although we included use of AV nodal blocking medications in the linear regression models, we were unable to quantify the amount of AV nodal blocking medication that the patients were taking and in addition, responses to these medications are known to vary across individuals. It would have been preferable to evaluate the effect of the SNPs on a population of individuals not on medications. When we did perform this analysis, our sample size was significantly reduced. Finally, while we limited our study to those who self-reported their race as Caucasian, it is possible that we included patients with variable genetic ancestry, which would alter our results.

Conclusions

The common polymorphism rs6795970 encoding for the nonsynonymous variant SCN1A-A1073V is associated with the PR interval in both healthy individuals and those with AF and SCN1A remains an important modulator of cardiac conduction. This SNP may also be a modifier of heart rate response during AF; however, clinical factors such as gender and prevalent CHF have a stronger effect.

Funding

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Conflict of interest: none declared.

References

Acute respiratory distress syndrome following straightforward pulmonary vein isolation

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A 65-year-old man, healthy, no allergies, with paroxysmal atrial fibrillation underwent pulmonary vein isolation (PVI) using irrigated radiofrequency ablation (total 1500 mL saline during procedure) under general anaesthetic. The patient was in sinus rhythm with a structurally normal heart. He was therapeutically anticoagulated. Thrombus was excluded on trans-oesophageal echocardiogram. During ablation, the patient developed persistent junctional rhythm. Hours after the uncomplicated procedure he developed marked dyspnoea and pleuritic chest pain with bilateral lung base crackles. Echocardiography showed good left ventricular function. Heart Rhythm 2007;4:743–9.


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