Mutations in Sodium Channel β1- and β2-Subunits Associated With Atrial Fibrillation

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Background—We and others have reported mutations in the cardiac predominant sodium channel gene SCN5A in patients with atrial fibrillation (AF). We also have reported that SCN1B is associated with Brugada syndrome and isolated cardiac conduction disease. We tested the hypothesis that mutations in the 4 sodium channel β-subunit genes SCN1B–SCN4B contribute to AF susceptibility.

Methods and Results—Screening for mutations in the 4 β-subunit genes was performed in 480 patients with AF (118 patients with lone AF and 362 patients with AF and cardiovascular disease) and 548 control subjects (188 ethnically defined anonymized subjects and 360 subjects without AF). The effects of mutant β-subunits on SCN5A mediated currents were studied using electrophysiological studies. We identified 2 nonsynonymous variants in SCN1B (resulting in R85H, D153N) and 2 in SCN2B (R28Q, R28W) in patients with AF. These occur at residues highly conserved across mammals and were absent in control subjects. In 3 of 4 mutation carriers, the ECGs showed saddleback-type ST-segment elevation in the right precordial leads. Transcripts encoding both SCN1B and SCN2B were detected in human atrium and ventricle. In heterologous expression studies using Chinese hamster ovary cells, the mutant β1- or β2-subunits reduced SCN5A-mediated current and altered channel gating compared with coexpression of wild-type subunits.

Conclusions—Loss of function mutations in sodium channel β-subunits were identified in patients with AF and were associated with a distinctive ECG phenotype. These findings further support the hypothesis that decreased sodium current enhances AF susceptibility. (Circ Arrhythmia Electrophysiol. 2009;2:268-275.)

Key Words: arrhythmia ■ sodium channel ■ electrophysiology ■ genetics ■ mutations ■ atrial fibrillation

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mia diseases including the long-QT syndrome, the Brugada syndrome, progressive cardiac conduction disease, and sick sinus syndrome. Moreover, mutations in SCN1B and SCN4B have also been implicated in the Brugada syndrome and/or conduction disease, and long-QT syndrome, respectively. In addition, SCN5A mutations and polymorphisms have been associated with AF, and we recently reported SCN5A mutations in 5.9% of patients with AF. Taken together, these data suggest β-subunits as candidates for AF pathogenesis. Therefore, we have tested this hypothesis by screening sodium channel β-subunit genes for variants in patients with AF and control subjects.

Methods

Study Subjects

The study protocol was approved by the Institutional Review Board of Vanderbilt University, and all subjects gave informed consent. This study included 2 sets of patients with AF: (1) 375 patients including 118 patients with lone AF and 257 patients with AF and cardiovascular disease from the Vanderbilt AF Registry (356 whites [94%], 19 blacks [5%], 3 Hispanics [0.8%], 1 Asian [0.3%]), and (2) 105 patients from the Vanderbilt Cardiac Surgery Registry (101 whites [96%], 3 blacks [3%], 1 Hispanic [1%]) who had not had AF before or during surgery and in whom AF was documented in the postoperative period.

Control Populations

There were 3 sets of controls in this study:

1. There were 188 ethnically identified but otherwise anonymized subjects (white, black, Hispanic, Asian; n=47 for each group) from the Coriell Cell Repositories (Camden, NJ).
2. For the lone AF control subjects, we used 94 subjects (51 whites [54%], 43 blacks [46%]) who on screening had no personal or family history of AF and no post cardiac surgery or conduction disease, and long-QT syndrome, respectively. In addition, SCN1B, SCN2B, and SCN4B have also been implicated in the Brugada syndrome.
3. For the group with AF in association with heart disease or other risk factors, we included 266 patients (211 whites [81%], 51 blacks [19%]) from the Cardiac Surgery Registry who had no personal or family history of AF and no post cardiac operative AF. The patients were matched on the basis of age, sex, and ethnicity to the lone AF cohort.

Resequencing and Follow-Up Genotyping

The coding regions and flanking intronic sequences of all 4 β-subunit genes, including exon 3A of SCN1B, SCN2B, SCN3B, and SCN4B, were resequenced in these 4 patients carrying β2 subunit mutations and polymorphisms.

Quantitative Real-Time Polymerase Chain Reaction

Poly A+ RNA pooled separately from atria and ventricles of healthy hearts from ≥15 whites (Clontech, Mountain View, Calif) was analyzed. cDNA was synthesized from 2 μg of the RNA and used as template. Genes of interest subcloned into the pEFGP-IREs vector (SCN1B, SCN2B, SCN5A; Clontech) or the pRC-CMV vector (β-actin; Invitrogen, Carlsbad, Calif) were used for absolute quantification. Real-time polymerase chain reaction (PCR) was performed with predesigned TaqMan assays (SCN1B, Hs00168897_m1; SCN2B, Hs00394952_m1; SCN5A, Hs00965681_m1; β-actin, Hs00999909_m1) using the 7900HT Real-Time Instrument (Applied Biosystems, Foster City, Calif).

Functional Analysis

Full-length human SCN1B cDNA (Gen Bank accession No. NM_001037) and SCN2B cDNA (NM_004588) subcloned into a bicistronic vector (pEFGP-IREs, Clontech) also carrying GFP were supplied by Dr Alfred George, Jr. Mutations were prepared using the QuickChange II XL site-directed mutagenesis kit (Stratagene, La Jolla, Calif) and were verified by resequencing. The SCN5A CDNA (NM_198056) was subcloned into the pBK-CMV vector (Stratagene). SCN1B or SCN2B constructs (1 μg) were cotransfected with the plasmid encoding SCN5A (1 μg) in Chinese hamster ovary (CHO) cells. When SCN5A DNA was transfected without β-subunits, the pEFGP-IREs vector was cotransfected to identify fluorescent cells for voltage-clamp.

Whole-cell voltage-clamp was performed at room temperature using an Axopatch 200B amplifier and pClamp9.2 software (Molecular Devices, Union City, Calif) as described previously. Transfected cells were clamped with −1.0-mol/L Li1 glass microelectrodes and were held at a resting potential of −120 mV. Data for voltage dependence were fitted with the Boltzmann equation: y = (1 + exp([V1/2]−V/k))/2, where V1/2 is the voltage required to achieve half-maximal conductance or channel availability and k is the slope factor. Pulse protocols are shown as insets in the Figures.

Statistical Analysis

Data are presented as mean±SEM. Student unpaired t test, 1-way ANOVA, or Fisher exact test was used to test for significant differences. A value of P<0.05 was considered statistically significant. The authors had full access to the data and take responsibility for its integrity. All authors have read and agreed to the manuscript as written.

Results

Resequencing the AF population identified 2 nonsynonymous variants in the reference SCN1B sequence and 2 in SCN2B in 3 white and 1 black subject. These variants were absent in the Coriell controls and in the AF population control subjects, including a total of 309 whites and 141 blacks. Resequencing SCN5A in these 4 patients carrying β-subunit mutations did not identify any coding region or splice junction variant. No AF-unique nonsynonymous variant was identified in SCN3B or SCN4B.

Clinical Features

Clinical features are described in Table 1 and as follows:

- Patient 1: A heterozygous missense mutation in exon 3 of SCN1B (c.254G→A) resulting in p.R85H was identified in a 68-year-old white woman with paroxysmal AF and moderate aortic stenosis (pressure gradient, 31 mm Hg; aortic valve area, 0.82 cm²) (Figure 1). There was no history of hypertension. AF was diagnosed when she was 58 years old. The 12-lead ECG showed saddleback-type ST-segment elevation in leads V1 to V3 (Figure 1). The
ST-segment elevation was evident both during AF and sinus rhythm with beat-to-beat and day-to-day variability. She did not have ischemic heart disease, congestive heart failure, electrolyte abnormality, or antiarrhythmic drug therapy to explain the ST-segment elevation. Amiodarone failed to maintain sinus rhythm and did not exacerbate ST-segment elevation. Echocardiography revealed left atrial enlargement. No family member had documented AF, although her grandmother and daughter had a history of stroke. Her father had a history of myocardial infarction. Mutations in \textit{SCN1B} have been previously reported in the generalized epilepsy with febrile seizures plus (GEFS+) syndrome, and R85H was initially found in a patient with GEFS+.\cite{19,20} However, there was no personal or family history of seizures in this or any of the other 3 patients having \textit{\beta}-subunit mutations described here.

- Patient 2: A heterozygous missense mutation in exon 4 of \textit{SCN1B} (c.457G→A) resulting in p.D153N was identified in a 57-year-old black woman with paroxysmal AF (Figure 1). AF was initially diagnosed when she was 35 years old. Her ECG was normal and did not show ST-segment elevation in the right precordial leads or any conduction abnormality. Echocardiography revealed left atrial enlargement. When she was 54 years old, she had episodes of paroxysmal AF with rapid ventricular responses, unresponsive to sotalol, propafenone, and amiodarone; there was no ST-segment elevation during therapy with antiarrhythmic drugs. She underwent atrioventricular nodal ablation followed by implantation of dual-chamber pacemaker. There was no family history of AF, although her mother had hypertension and a pacemaker.

- Patient 3: A heterozygous missense mutation in exon 2 of \textit{SCN2B} (c.82C→T) resulting in p.R28W was identified in a 61-year-old white man with paroxysmal AF and hypertension (Figure 2). AF was initially diagnosed when he was 55 years old. The ECG showed saddleback-type ST-segment elevation in the right precordial leads during sinus rhythm with a prolonged PR interval of 220 ms. The magnitude of ST-segment elevation showed day-to-day variability. Echocardiography was normal. Holter recording during sinus rhythm did not reveal atrial tachycardia. Sotalol failed to maintain sinus rhythm and did not exacerbate ST-segment elevation. There was no family history of AF.

- Patient 4: A heterozygous missense mutation in exon 2 of \textit{SCN2B} (c.83G→A) resulting in p.R28Q was identified in a 57-year-old white man with paroxysmal AF (Figure 2). AF was initially diagnosed when he was 57 years old. There was saddleback-type ST-segment elevation in the right precordial leads. Echocardiography revealed slight

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Sex</th>
<th>Age at Onset, y</th>
<th>Type of AF</th>
<th>PR Interval, ms</th>
<th>QRS Interval, ms</th>
<th>QTc, ms</th>
<th>ST-Segment Elevation*</th>
<th>LVDD, mm</th>
<th>LVEF, %</th>
<th>LA, mm</th>
<th>Nucleotide Substitution</th>
<th>Amino Acid Substitution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>58</td>
<td>Paroxysmal</td>
<td>140</td>
<td>83</td>
<td>385</td>
<td>Yes</td>
<td>50</td>
<td>55</td>
<td>47</td>
<td>SCN1B 254G→A</td>
<td>R85H→H</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>35</td>
<td>Paroxysmal</td>
<td>132</td>
<td>84</td>
<td>400</td>
<td>No</td>
<td>46</td>
<td>65</td>
<td>48</td>
<td>SCN1B 457G→A</td>
<td>D153N→N</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>55</td>
<td>Paroxysmal</td>
<td>200</td>
<td>80</td>
<td>354</td>
<td>Yes</td>
<td>50</td>
<td>55</td>
<td>40</td>
<td>SCN2B 82C→T</td>
<td>R28W→W</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>57</td>
<td>Paroxysmal</td>
<td>180</td>
<td>88</td>
<td>398</td>
<td>Yes</td>
<td>49</td>
<td>60</td>
<td>42</td>
<td>SCN2B 83G→A</td>
<td>R28Q→Q</td>
</tr>
</tbody>
</table>

LA indicates left atrium; LVDD, left ventricular diastolic diameter; LVEF, left ventricular ejection fraction; QTc, corrected QT interval by Bazett formula.

*ST-segment elevation in the right precordial leads.

![Figure 1. SCN1B mutations in patients with AF. A, 12-lead ECG in patient 1 shows ST-segment elevation in leads V1 to V3. B, Heterozygous single-nucleotide change in SCN1B (c.254G→A) results in p.R85H in patient 1. Left and right panels indicate sequences in a control subject and the patient, respectively. C, Heterozygous single-nucleotide change in SCN1B (c.457G→A) results in p.D153N in patient 2. Arrows in B and C indicate heterozygous mutations. D, Alignment of \textit{\beta}1 amino acid sequences in human, mouse, rat, and dog. Sites of the mutations are indicated by boxes. E, Locations of mutations in the predicted topology of the \textit{\beta}1-subunit (circles).](http://circep.ahajournals.org/content/270/11/2176/F1.large.jpg)
left atrial enlargement. He did not receive any antiarrhythmic drugs to restore AF. His father and mother had AF and coronary heart disease.

There was no history of ventricular tachyarrhythmias or syncope in any of the 4 patients. Electrophysiological study has not been performed in any of the patients. DNA was not available in any family members of the 4 probands.

**ST-Segment Elevation in Lone AF**

Right precordial ST-segment elevation during sinus rhythm was identified more frequently in patients with lone AF (8/118, 6.8%) than in control subjects (1/94, 1.1%, \( P < 0.05 \)). The 8 patients in the lone AF group included 1 with the SCN2B mutation described above and 1 with a H445D SCN5A mutation.\(^{13} \) None of the 8 patients except for the SCN2B mutation carrier with a long PR interval (described above) showed a conduction abnormality.

**Conservation of Mutated Amino Acids**

The sites of the mutations identified here, R85 and D153 in \( \beta_1 \), and R28 in \( \beta_2 \) (Figure 1 and 2) are completely conserved across human, dog, rat, and mouse sequences, suggesting that these amino acids are functionally important.

**Real-Time PCR in Human Heart**

As a first step to establishing the functional significance of SCN1B and SCN2B in the genesis of AF, we studied their expression in atrial tissue. Figure 3 shows that the transcripts were readily detected in both atrium and ventricle. The abundance of SCN1B and SCN5A transcripts was greater in ventricle than atrium (68% and 35% of ventricle, respectively), but SCN2B transcript levels were similar in the 2 chambers.

**Electrophysiology**

Each of the 4 mutant subunits generated a loss of function phenotype. Peak sodium current amplitude was increased by 75% at a test pulse of \(-30 \text{ mV}\) when wild-type \( \beta_1 \) was coexpressed with SCN5A (Table 2, Figure 4, \( P < 0.001 \)). This effect was markedly blunted with the D153N mutation (24% increase versus SCN5A alone, \( P < 0.05 \)) and absent with the R85H mutation, resulting in smaller sodium current amplitude for the mutants than wild-type \( \beta_1 \) (\( P < 0.001 \) for each). D153N did not affect the voltage dependence of sodium channel activation or inactivation compared to wild-type \( \beta_1 \). However, R85H resulted in a positive shift of both voltage dependence of activation (\(+10.6 \text{ mV}, P < 0.001\)) and of inactivation (\(+6.2 \text{ mV}, P < 0.001\)) compared with wild-type \( \beta_1 \). There was no difference in persistent sodium current among wild-type (1.0±0.1%), D153N (0.9±0.1%), and R85H \( \beta_1 \) (1.1±0.2%).

In contrast to \( \beta_1 \), wild-type \( \beta_2 \) did not increase peak sodium current amplitude compared with SCN5A alone (Table 3, Figure 5, \( P = \text{NS} \)). However, coexpression of R28W or of R28Q reduced peak current amplitude by 30% (\( P < 0.05 \)) and by 36% (\( P < 0.01 \)) at \(-30 \text{ mV}\), respectively, compared with wild-type \( \beta_2 \). In addition, R28W produced a positive shift in the voltage dependence of activation (+5.1 mV, \( P < 0.001 \)) compared with wild-type but did not affect the voltage dependence of inactivation (\( P = \text{NS} \)). R28Q produced
a positive shift in both of the voltage dependence of activation (+7.4 mV, \( P < 0.001 \)) and of inactivation (+2.8 mV, \( P < 0.01 \)) compared with wild-type \( \beta 2 \). There was no difference in persistent sodium current among wild-type \( \beta 1 \) (1.2 ± 0.3%), R28W (1.1 ± 0.3%), and R28Q \( \beta 2 \) (1.2 ± 0.2%).

### Discussion

In this report, we describe rare nonsynonymous variants in \( SCN1B \) and \( SCN2B \) in patients with AF. These variants affect highly conserved residues and were not present in large control populations. Thus, \( SCN1B \) and \( SCN2B \) are candidate genes for increasing AF susceptibility. The findings that \( SCN1B \) and \( SCN2B \) are expressed in atrium, and that mutant \( \beta 1 \) and \( \beta 2 \) produced loss-of-function effects on \( SCN5A \)-mediated currents further supports the association of the variants with AF.

The reported effects of coexpressing \( \beta 1 \) on \( SCN5A \) channels are controversial. Some groups have reported that \( \beta 1 \) increases \( SCN5A \) currents without affecting the voltage dependence of gating or kinetics, whereas others have reported \( \beta 1 \)-mediated changes in channel gating and/or kinetics. In some reports, \( \beta 1 \) has no effect on \( SCN5A \)-mediated current. In \( \beta 1 \) null mice, an increase in sodium current amplitude without a change in channel gating or kinetics has been reported. In our experiments, wild-type \( \beta 1 \) increased \( SCN5A \) currents and modulated channel gating, and the p.R85H and p.D153N mutants showed loss of \( \beta 1 \) function with significantly decreased current amplitudes.

The effects of \( \beta 2 \) on \( SCN5A \) currents have been less extensively studied. Whereas one group reported that \( \beta 2 \) has no effect on \( SCN5A \) currents using heterologous expression, another group reported a negative shift of the voltage dependence of activation. Sinus node dysfunction has been reported in \( \beta 2 \) null mice. In the present study, whereas \( \beta 2 \) had no effects on \( SCN5A \) currents except for a minor positive shift of the voltage dependence of inactivation, both the p.R28W and p.R28Q mutants strikingly decreased peak sodium current amplitude. The patients had no evidence of sinus node dysfunction.

### Table 2. Biophysical Parameters for \( \beta 1 \) Variants Associated With AF

<table>
<thead>
<tr>
<th></th>
<th>Peak Current Density at −30 mV (pA/pF)</th>
<th>Voltage Dependence of Activation</th>
<th>Voltage Dependence of Inactivation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( V_{1/2} ) (mV)</td>
<td>( k ) (mV)</td>
<td>( n )</td>
</tr>
<tr>
<td>SCN5A</td>
<td>−160 ± 20.4 18</td>
<td>−38.7 ± 0.5 18</td>
<td>−86.9 ± 1.5 18</td>
</tr>
<tr>
<td>SCN5A/WT ( \beta 1 )</td>
<td>−281 ± 21.7 37</td>
<td>−48.9 ± 0.8* 37</td>
<td>−93.6 ± 0.8* 37</td>
</tr>
<tr>
<td>SCN5A/R85H ( \beta 1 )</td>
<td>−158 ± 16.8† 35</td>
<td>−38.3 ± 0.7† 35</td>
<td>−87.4 ± 0.6† 34</td>
</tr>
<tr>
<td>SCN5A/D153N ( \beta 1 )</td>
<td>−200 ± 18.2† 27</td>
<td>−50.8 ± 0.9* 27</td>
<td>−95.0 ± 0.7* 26</td>
</tr>
</tbody>
</table>

* \( P < 0.05 \) versus SCN5A.  
† \( P < 0.05 \) versus SCN5A/WT \( \beta 1 \).

![Figure 4](https://circarrhythmiaelectrophysiology.org/)

Figure 4. Electrophysiological characteristics of \( \beta 1 \)-subunit variants in CHO cells expressing SCN5A and \( \beta 1 \)-subunits. A, Representative current traces. B, Current-voltage relationships of SCN5A alone (filled circles), SCN5A coexpressed with wild-type (WT) \( \beta 1 \) (open circles), SCN5A coexpressed with R85H \( \beta 1 \) (open triangles), and SCN5A coexpressed with D153N \( \beta 1 \) (open squares). Voltage dependence of activation (C) and inactivation (D) are shown.
All of 4 mutations identified in SCN1B and SCN2B were located in the extracellular domain, which has a critical role in modulation of cell surface expression and gating of sodium channel. In previous studies of skeletal muscle and neuronal sodium channel \( \beta \)-subunits, deletion of the intracellular domain of the \( \alpha \)-subunit had no effect on its modulation of \( \alpha \)-subunit function, whereas deletions within the extracellular domain block modulation. Our recent study, which describes loss-of-function mutations in SCN1B in the extracellular domain, supports functional importance of the extracellular domain. However, it is also possible that specific residues may not be as important as preservation of overall structural motifs because \( \beta \)-subunit modulates sodium channel via the membrane anchor plus additional intracellular or extracellular regions.

Variation in SCN5A is associated with AF. Loss-of-function mutations in SCN5A have been associated with AF as well as with dilated cardiomyopathy, sinus node dysfunction, and/or conduction disease. Screening for SCN5A variants in a large AF cohort, which was also used for this study, found SCN5A mutations in 5.9% of those with AF. A common polymorphism in SCN5A (H558R) has also been associated with AF susceptibility, although this was not reproduced in another study.

Sodium channels play a critical role not only in the initiation of the action potential but also in the maintenance of the action potential dome, and loss of sodium channel function can cause shortening of refractoriness and slowing of conduction. Shortening of refractory period by a reduction in inward current and/or an increase in outward current has been proposed as creating a substrate for reentry, and this concept has been supported by evidence that loss-of-function mutations in SCN5A or gain-of-function mutations in potassium channel genes that shorten action potential duration contribute to AF susceptibility. Slow conduction, which is also promoted by decreased sodium current, is another important substrate for reentry. Thus, mutations in SCN1B and SCN2B that reduce sodium current can generate an AF-prone substrate through multiple mechanisms even in the presence of other susceptibility modifiers.

The clinical features of the AF cases we identified here appear to share molecular and pathophysiologic characteristics with the Brugada syndrome, characterized by ST-segment elevation in the right precordial leads, episodes of ventricular fibrillation, and occasionally AF. Moreover, loss-of-function mutations in SCN5A have been reported in

Table 3. Biophysical Parameters for \( \beta \) Variants Associated With AF

<table>
<thead>
<tr>
<th>Peak Current Density at (-30) mV</th>
<th>Voltage Dependence of Activation</th>
<th>Voltage Dependence of Inactivation</th>
</tr>
</thead>
<tbody>
<tr>
<td>pA/pF</td>
<td>( V_{1/2} ) (mV) k (mV) n</td>
<td>( V_{1/2} ) (mV) k (mV) n</td>
</tr>
<tr>
<td>SCN5A</td>
<td>(-157.0\pm13.6) 25</td>
<td>(-39.6\pm0.5) 7.6 0.2 25</td>
</tr>
<tr>
<td>SCN5A/( \beta ) WT</td>
<td>(-161.6\pm13.3) 27</td>
<td>(-38.0\pm0.6) 6.2 0.2 27</td>
</tr>
<tr>
<td>SCN5A/R28W ( \beta )</td>
<td>(-112.6\pm12.2^*) 31</td>
<td>(-32.9\pm0.8^*) 8.3 0.4 31</td>
</tr>
<tr>
<td>SCN5A/R28Q ( \beta )</td>
<td>(-103.4\pm11.8^*) 29</td>
<td>(-30.6\pm0.6^*) 6.3 0.2 29</td>
</tr>
</tbody>
</table>

\(^*P<0.05\) versus SCN5A.
\(^*P<0.05\) vs SCN5A/WT \( \beta \).

Figure 5. Electrophysiological characteristics of \( \beta \)-subunit variants in CHO cells expressing SCN5A and \( \beta \)-subunits. A, Representative current traces. B, Current-voltage relationships of SCN5A alone (filled circles), SCN5A coexpressed with wild-type (WT) \( \beta \) (open circles), SCN5A coexpressed with R28W \( \beta \) (open triangles), and SCN5A coexpressed with R28Q \( \beta \) (open squares). Voltage dependence of activation (C) and inactivation (D) are shown.
the Brugada syndrome as well as in AF,4,5,10 and we have recently reported a loss-of-function mutation in SCN1B in the Brugada syndrome.12 Brugada-type ST-segment elevation, similar to this study, has been reported in patients with lone AF, and a genetic etiology is suggested by a high frequency of a family history of AF, although no molecular mechanism was identified in previous studies.17,18 Taken together, these data implicate loss of sodium channel function due to β-subunit mutations as a further mechanism underlying the Brugada-type ECG and AF susceptibility. We also identified ST-segment elevation in other subjects with AF (more commonly than in control subjects), but mutations in SCN5A, SCN1B, or SCN2B were only identified in a minority; thus other genetic mechanisms probably play a role.10

Sodium channel–blocking drugs are widely used to restore and maintain sinus rhythm in paroxysmal AF. They are also used to exaggerate or unmask ST-segment elevation in Brugada syndrome, where they can increase ventricular arrhythmia susceptibility. Therefore, these drugs may be proarrhythmic (or at least ineffectual) in cases of AF—such as those we describe here—in which decreased sodium current plays a role in pathogenesis of the arrhythmia. ST-segment elevation in the right precordial leads may be useful to identify such patients.

Mutations in SCN1B were originally identified in familial epilepsy, GEFS+.19 However, there was no history of epilepsy in our patients carrying mutations including R85H, previously reported as an epilepsy mutation.20 In addition, there was no history of seizure disorder in patients with SCN1B mutations in conduction disease and Brugada syndrome that we have recently described.12 Conversely, to our knowledge, defects in cardiac function have not been investigated in SCN1B mutation carriers presenting with epilepsy, and AF has not been described in the family with R85H and seizures.19,20 The mechanism underlying this difference between the brain and heart phenotypes is not known, but sex, age, and genetic modifiers (eg, common polymorphisms) are commonly invoked as modulators of such clinical phenotypes. One possibility is that the Sudden Unexpected Death in Epilepsy (SUDEP) syndrome is a cardiac arrhythmia manifestation of β-subunit or other mutations contributing to epilepsy.37

Limitations

Screening for β-subunit genes was performed in large cohorts including ethnically defined and population-matched controls, and mutations were identified only in patients with AF. However, it is difficult to have controls definitely free from AF. We believe a cohort of patients with heart disease undergoing cardiac surgery but without AF is a very robust control set. Linkage or segregation analysis was not conducted because DNA was not available in family members of affected patients. The variants are rare and thus genetic variants in β-subunit genes may not be responsible in a large number of patients with AF. Evidence supporting a critical role of β-subunits in AF includes expression of SCN1B and SCN2B in atrium and loss of sodium channel function in the heterologous expression studies. The functional analyses used a conventional heterologous expression system, where the environment is different from that in the native cardiomyocyte, and other proteins associated with the sodium channel complex (including other β-subunits) are absent. Nevertheless, the in vitro characteristics of the mutations were consistent with the phenotype in the patients, further supporting the disease causality of the mutations. The observed alterations in gating indicate that the mutant subunits are expressed and probably coassemble with SCN5A to form dysfunctional channels.

Conclusions

In summary, we have identified mutations in sodium channel β1- and β2-subunit genes in patients with AF and have shown that sodium currents were reduced and channel gating was altered when the mutant β1 or β2 was coexpressed with SCN5A, compared with coexpression with wild-type β-subunits. Three of 4 mutation carriers showed ST-segment elevation in the right precordial leads, further implicating loss of sodium current as a disease mechanism for AF. We speculate that sodium channel blockers may have proarrhythmic effects in cases of AF in which decreased sodium current plays a role in pathogenesis of the arrhythmia.

Acknowledgments

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Disclosures

None.

References


### CLINICAL PERSPECTIVE

There is a positive family history in many patients with atrial fibrillation (AF), especially lone AF. Recent genetic studies have identified both rare and common genetic variants that appear to predispose to the arrhythmia, and this includes variants in *SCN5A*, encoding the cardiac sodium channel pore-forming alpha-subunit. Sodium channels are multiprotein complexes, and so in this study, 4 function-modifying sodium channel beta-subunit genes (*SCN1B* to *SCN4B*) were screened for mutations in a large number of patients with lone AF and AF associated with cardiovascular disease. This screening effort identified 4 subjects with mutations resulting in changes in amino acids highly conserved across species in *SCN1B* and *SCN2B*. All 4 mutations showed decreased sodium current, a change similar to that seen with loss-of-function mutations in *SCN5A* and *SCN1B* in Brugada syndrome. AF is relatively common in the Brugada syndrome, and 3 of the 4 AF patients carrying a mutation in a beta-subunit gene showed Brugada syndrome-like ST-segment elevation, further reinforcing the idea that loss of sodium channel function increases AF susceptibility. Indeed, in some series, saddleback or other ST-segment deformities are reported in up to 10% of patients with lone AF, suggesting that these patients represent a distinct subgroup of AF due to reduced sodium current through mutations in *SCN5A*, *SCN1B*, *SCN2B*, or other sodium channel-associated protein genes. Exposure to sodium channel blockers could be used to identify this subgroup, although long-term therapy with these drugs would be undesirable because they can increase ventricular arrhythmia susceptibility in Brugada syndrome.
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