High Prevalence of Long QT Syndrome–Associated SCN5A Variants in Patients With Early-Onset Lone Atrial Fibrillation

Morten S. Olesen, MSc, PhD*; Lei Yuan, MD*; Bo Liang, MD, PhD; Anders G. Holst, MD; Nikolaj Nielsen, MSc; Jonas B. Nielsen, MD; Paula L. Hedley, MSc; Michael Christiansen, MD; Søren-Peter Olesen, MD, DMSc; Stig Haunsø, MD, DMSc; Nicole Schmitt, MSc, PhD; Thomas Jespersen, MSc, PhD, DMSc; Jesper H. Svendsen, MD, DMSc, FESC

Background—Atrial fibrillation (AF) is the most common cardiac arrhythmia. The cardiac sodium channel, NaV1.5, plays a pivotal role in setting the conduction velocity and the initial depolarization of the cardiac myocytes. We hypothesized that early-onset lone AF was associated with genetic variation in SCN5A.

Methods and Results—The coding sequence of SCN5A was sequenced in 192 patients with early-onset lone AF. Eight nonsynonymous mutations (T220I, R340Q, T1304M, F1596I, R1626H, D1819N, R1897W, and V1951M) and 2 rare variants (S216L in 2 patients and F2004L) were identified. Of 11 genopositive probands, 6 (3.2% of the total population) had a variant previously associated with long QT syndrome type 3 (LQTS3). The prevalence of LQTS3-associated variants in the patients with lone AF was much higher than expected, compared with the prevalence in recent exome data (minor allele frequency, 1.6% versus 0.3%; P=0.003), mainly representing the general population. The functional effects of the mutations were analyzed by whole cell patch clamp in HEK293 cells; for 5 of the mutations previously associated with LQTS3, patch-clamp experiments showed an increased sustained sodium current, suggesting a mechanistic overlap between LQTS3 and early-onset lone AF. In 9 of 10 identified mutations and rare variants, we observed compromised biophysical properties affecting the transient peak current.

Conclusions—In a cohort of patients with early-onset lone AF, we identified a high prevalence of SCN5A mutations previously associated with LQTS3. Functional investigations of the mutations revealed both compromised transient peak current and increased sustained current. (Circ Cardiovasc Genet. 2012;5:450-459.)

Key Words: atrial fibrillation ■ genes ■ long-QT syndrome ■ QT interval electrocardiography ■ SCN5A

Atrial fibrillation (AF) is the most prevalent sustained cardiac arrhythmia, affecting almost 7 million patients in the European Union and United States combined.1–4 In most cases, AF arises secondary to hypertension, ischemic, and/or structural heart disease relatively late in life.1,5 However, 10% to 20% of patients experiencing AF are younger than 60 years and free of traditional predisposing conditions. These patients are said to have lone AF. The mechanisms underlying lone AF are not fully understood, but interplay between multiple substrates and triggers may constitute the etiology of AF.6 As such, early-onset lone AF may be a primary electric disease caused by disturbances in ion channel function.7

Clinical Perspective on p 459

Familial predisposition for AF has recently been recognized. Fox et al8 showed that the development of AF in offspring is associated with parental AF. The importance of common genetic variants in the development of AF has been revealed in recent genome-wide association studies.9 Rare mutations in genes encoding potassium channels (KCNQ1, KCNH2, KCN5, KCNJ2,5, and KCNE1,2,3,5), sodium channels (SCN5A and SCN1-3B), a peptide hormone (ANP), a gap junction protein (GJA5), and a nuclear membrane protein (LMNA) have been linked to AF.10–14

Mutations in SCN5A, the gene encoding the α-subunit underlying the dominant cardiac sodium current, is composed...
of a central pore-forming α-subunit, Na_{1.5},^{15,16} and 2 β-subunits of the Na_{αβ} type. In addition to its implication in Brugada syndrome (BrS), long QT syndrome type 3 (LQTS3), and conduction defects, Na_{1.5} has recently a role in AF.^{17} However, functional characterizations of SCN5A mutations associated with AF have been sparse. Two studies reported mutations that increased the transient peak current but showed no effect on the sustained current.\textsuperscript{18,19} Another study described a mutation with decreased transient peak current.\textsuperscript{20} In a third study of a family affected by an LQTS3 mutation, resulting in increased sustained sodium current, some members also had AF, suggesting that an increased sustained current could also play a role in AF.\textsuperscript{21} A recent study of a human LQTS3 mutation expressed in a knock-in mouse model also suggested that the sustained sodium current could play a role in lone AF.\textsuperscript{22}

We hypothesized that patients with early-onset lone AF would carry a high prevalence of SCN5A mutations, because such individuals are particularly likely to have a primary genetic defect as a substrate for AF. Also, we aimed to characterize possible mutations electrophysiologically by a patch-clamp approach to elucidate the functional impact of these mutations because such experiments have not previously been performed systematically.

**Methods**

**Study Subjects**

Patients were recruited from cardiology departments in 8 hospitals in the Copenhagen region of Denmark. Patient records from all inpatient and outpatient activity in the past 10 years with the diagnose code International Statistical Classification of Diseases, 10th Revision (ICD-10) I48.9 (AF and atrial flutter) were included. Only patients with a history of lone AF before the age of 40 years (ie, absence of clinical or echocardiograph findings of other cardiovascular diseases, hypertension, or metabolic or pulmonary diseases) were included (Table 1). All patients were interviewed about family history of arrhythmia. Patients that carried mutations had a second interview specifically about family history of arrhythmia, sudden death, dilated cardiomyopathy, and other SCN5A associated disease. All mutation carriers were offered a flecainide provocation test (2 mg/kg flecainide intravenously) to exclude BrS.

To distinguish between common genetic polymorphisms, rare variants, and mutations, a group of ECG-documented healthy controls without cardiac symptoms were collected.

The study conforms to the principles outlined in the Declaration of Helsinki and was approved by the Scientific Ethics Committee (reference no. KF 0131322). All included patients gave written informed consent.

**Mutation Screening**

Genomic DNA was isolated from blood samples using the QiAamp DNA Blood Mini Kit (QIAGEN; Hilden, Germany). The entire coding sequence and splice junctions of SCN5A (NM_198056.2) (primers and polymerase chain reaction conditions are available on request) were amplified and analyzed using a high-resolution melting curve analysis, as previously described\textsuperscript{23} (Light Scanner, Idaho Technology; Salt Lake City, UT). Fragments with melting curves differing from the curves of wild-type DNA were purified and directly sequenced using Big Dye chemistry (DNA analyzer 3730, Applied Biosystems; Carlsbad, CA).

All identified nonsynonymous variants were validated by resequencing in an independent polymerase chain reaction. DNA from 22 of the patients was sequenced directly using Big Dye chemistry. The group of healthy controls was screened using high-resolution melting curve analysis, with probands included as positive controls. In probands with nonsynonymous variants, bidirectional sequencing of SCN1–3B (NM_001037.4, NM_004588, and NM_018400.3), KCNQ1 (NM_000218.2), KCNH2 (NM_000238), KCNQ3 (NM_002249.5), KCNQ5 (NM_002234.2), KCNE1/2/3 (NM_001127668, NM_172201, NM_005472.4, and NM_012282.2), KCNJ2,5 (NM_006891.2 and NM_000890.3), KCNJ3 (NM_002249.4),\textsuperscript{24} ANP (NM_006172.3), and LMNA (NM_0005572) was performed.

**Bioinformatics**

We performed species alignment (Figure 1) and Polyphen-2\textsuperscript{25} prediction analyses of variants.\textsuperscript{26} The Single Nucleotide Polymorphism Database (http://www.ncbi.nlm.nih.gov/projects/SNP) was searched for identified mutations and variants.

The National Heart, Lung, and Blood Institute GO Exome Sequencing Project (ESP) holds information on exome data from next-generation sequencing of all protein-coding regions in 3510 persons of European American ancestry.\textsuperscript{27} We compared the proportion of mutations or rare variants in SCN5A with minor allele frequency (MAF) <0.1% in ESP with those variants in the lone AF population also with an MAF <0.1% in ESP, assuming genetic homogeneity. We also compared the 2 populations regarding the proportion of previously published LQTS-associated variants, as reported in a recent comprehensive review by Hedley et al\textsuperscript{27} and in data from the Human Gene Mutation Database.\textsuperscript{28}

**In Vitro Electrophysiological Data**

Mutations were introduced into Na_{1.5} cDNA cloned in pcDNA3.1 (Invitrogen; Nærum, Denmark), using standard mutated oligonucleotide extension polymerase chain reaction. All constructs were verified by DNA sequencing. For patch-clamp studies, HEK293 cells were transiently cotransfected with 0.3 µg pcDNA3-HNa_{1.5} (wild-type or mutants) and 0.2 µg of pcDNA3-eGFP as a reporter gene, using Lipofectamine and Plus reagent (Invitrogen), according to the manufacturer’s instructions. Patch-clamp experiments were performed at room temperature (20°C–22°C) 2 to 3 days after transfection. Patch-clamp recordings were conducted using an internal solution containing the following (mmol/L): CsCl 60; CsAspartate 70; EGTA 11; MgCl$_2$ 1; CaCl$_2$ 1; HEPES 10; and Na$_2$-ATP 5, pH 7.2, with CsOH; external solution NaCl 130; CaCl$_2$ 2; MgCl$_2$ 1.2; CsCl 5; HEPES 10; and glucose 5, pH 7.4, with CsOH. Data analyses were performed as previously described.\textsuperscript{29}

**Table 1. Clinical Characteristics of the Lone AF Population (n=192)**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age of onset, median (IQR), y</td>
<td>31.5 (26–36)</td>
</tr>
<tr>
<td>Male sex, %</td>
<td>82</td>
</tr>
<tr>
<td>Height, cm</td>
<td>183±9</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>89±17</td>
</tr>
<tr>
<td>BMI, kg/m$^2$</td>
<td>26.7±4.6</td>
</tr>
<tr>
<td>Blood pressure, mm Hg</td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>131±13</td>
</tr>
<tr>
<td>Diastolic</td>
<td>78±9</td>
</tr>
<tr>
<td>AF type</td>
<td></td>
</tr>
<tr>
<td>Paroxysmal, %</td>
<td>55.9</td>
</tr>
<tr>
<td>Persistent, %</td>
<td>35.9</td>
</tr>
<tr>
<td>Permanent, %</td>
<td>8.2</td>
</tr>
<tr>
<td>Family history of AF</td>
<td></td>
</tr>
<tr>
<td>First-degree relatives with AF, %</td>
<td>31</td>
</tr>
</tbody>
</table>

Data are reported as mean±SD, unless otherwise noted. AF indicates atrial fibrillation; IQR, interquartile range; BMI, body mass index.

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Olesen et al Na$_1.5$ Mutations in Lone AF 451
A potential increase in sustained current was investigated by applying 30 µmol/L tetrodotoxin for the mutations R1626H and D1819N.

Data Analysis of Electrophysiological Experiments

Peak current densities were measured during an activation protocol, and sodium current $I_{Na}$ densities ($pA/pF$) were obtained by dividing peak $I_{Na}$ by cell capacitance. For activation and steady-state inactivation curves, data from individual cells were fitted with a Boltzmann equation,

$$y(V_m) = \frac{1}{1 + \exp\left(\frac{V_m - V_{1/2}}{K}\right)}$$

where $y$ is the normalized current or conductance; $V_m$ the membrane potential; $V_{1/2}$ the voltage at which half of the channels are activated or inactivated; and $K$, the slope factor. The decay characteristics of the fast transient current was fitted best with time constants using the following equation:

$$I = A_0 + A_1 \exp\left(-\frac{t}{T}\right)$$

where $t$ is the time from the beginning of the test pulse and $T$ is the time constant of current decay. Recovery curves from inactivation were obtained by giving a 50-ms, –20-mV depolarizing pulse, followed by clamping to 4 different prepotentials. Recovery was fitted with monoexponential function:

$$\frac{I_{test}}{I_{pre}} = Y_0 + A \exp\left(-\frac{T}{T}\right)$$

where $Y_0$ is the offset, $A$ is amplitude, and $T$ is the time constant. Data are presented as mean±SEM unless otherwise noted. A Student unpaired $t$ test, 1-way ANOVA, or Fisher exact tests were used to test for significant differences. Normal distribution of the data set was tested by Shapiro-Wilks normality test using GraphPadPrism 5.0 software. $P<0.05$ was considered statistically significant. The authors had full access to the data and take responsibility for its integrity.

Results

Study Cohort

The study population consisted of 192 patients with onset of AF ranging from 16 to 39 years without any concomitant disease. All included individuals were of Danish/white ethnicity. Clinical data are shown in Table 1.30

Mutation Screening

Screening of SCN5A in the 192 patients with lone AF revealed 10 nonsynonymous mutations (S216L, T220I, R340Q, T1304M, F1596I, R1626H, D1819N, R1897W, V1951M, and F2004L; Table 2), 5 that had not been functionally characterized before (see later). S216L was found in 2 patients with lone AF. S216L and F2004L were previously described in healthy controls (both with an MAF of 0.09%) and were, therefore, considered as rare variants.34 In addition, T220I, T1304M, and R1897W were identified with a low frequency in the ESP; however, the disease status of these individuals was unknown.

None of the mutations were present in our control population (n=216) and have not previously been reported in another large group of healthy controls (n=1100).34 All patients were heterozygous for the mutations.

Figure 1. A, DNA sequencing traces (chromatograms) for variants identified in SCN5A. B, Evolutionary conservation between species. The location of mutated amino acid is marked in red. C, The position of the mutations indicated in schematic of protein topology.
All patients carrying nonsynonymous mutations were subsequently screened for mutations in the whole coding region of the genes already known to be associated with AF: SCN1–3B, KCNQ1, KCNH2, KCNA5, KCNJ2-3, KCNA5, and KCNJ2-3; KCNE1,2,3,5, ANP, and LMNA, but no additional mutations, were found.

### Bioinformatics

All mutations were highly conserved across species, except for V1951M and F2004L, which are not conserved, and R340Q, which was conserved only in eutherian mammals (Figure 1). A PolyPhen2 prediction indicated that 7 of the 10 mutations and rare variants in SCN5A were predicted to be probably or at least possibly damaging (Table 2). We identified a significantly higher frequency of rare SCN5A variants (MAF < 0.1%) in the patients with lone AF when compared with the frequencies reported in ESP (MAF, 2.9% versus 1.1%; P=0.013). This was also the case regarding SCN5A variants previously associated with LQTS3 (MAF, 1.6% versus 0.3%; P=0.003).

### Family History

All patients carrying a mutation in SCN5A were interviewed specifically about family history of arrhythmias and SCN5A-related diseases, and 5 of the probands had a family history of arrhythmia. The proband carrying the mutation F1596I had a mother and a sister who were both affected by AF, but because they were both deceased, genetic screening was not possible. The proband carrying D1819N also had a family history of AF, but without cosegregation of the mutation. The proband carrying R1897W had a mother diagnosed as having postoperative AF at an old age, but she did not carry the mutation. The patient carrying the mutation V1951M had a father diagnosed as having nonsustained ventricular tachycardia (VT), although he was not a mutation carrier either. The proband

<table>
<thead>
<tr>
<th>Amino Acid Change</th>
<th>Nucleotide Change</th>
<th>Frequency in 102 Patients</th>
<th>Conserv.*</th>
<th>Exon</th>
<th>Location</th>
<th>Polyphen −2 Score</th>
<th>Reported Studies</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>S216L</td>
<td>c.647C&gt;T</td>
<td>2</td>
<td>HC</td>
<td>6</td>
<td>Transmembrane</td>
<td>Benign</td>
<td>LQTS27, SIDS31</td>
<td>Positive voltage shift in steady-state inactivation; increase in fast inactivation; negative voltage shift in recovery from inactivation; 8-fold increased sustained sodium current21</td>
</tr>
<tr>
<td>T220I</td>
<td>c.659C&gt;T</td>
<td>1</td>
<td>HC</td>
<td>6</td>
<td>Transmembrane, helical, voltage sensor</td>
<td>Probably damaging: 0.97</td>
<td>AF22, SSS33</td>
<td>Decreased peak current; negative voltage shift in steady-state inactivation; slower time-dependent recovery from inactivation23</td>
</tr>
<tr>
<td>R340Q</td>
<td>c.1018C&gt;G</td>
<td>1</td>
<td>CM</td>
<td>8</td>
<td>Extracellular</td>
<td>Possibly damaging: 0.93</td>
<td>LQTS27, SIDS31</td>
<td>Negative voltage shift in steady-state activation; negative voltage shift in steady-state inactivation; increased fast inactivation</td>
</tr>
<tr>
<td>T1304M</td>
<td>c.3911C&gt;T</td>
<td>1</td>
<td>HC</td>
<td>22</td>
<td>Transmembrane, voltage sensor</td>
<td>Probably damaging: 1.00</td>
<td>LQTS27, SIDS31</td>
<td>Positive voltage shift in steady-state activation; positive voltage shift in steady-state inactivation; increased fast inactivation; faster time-dependent recovery from inactivation; 7-fold increased sustained sodium current21</td>
</tr>
<tr>
<td>F1596I</td>
<td>c.4786T&gt;A</td>
<td>1</td>
<td>HC</td>
<td>26</td>
<td>Transmembrane, helical</td>
<td>Probably damaging: 0.96</td>
<td>AF7</td>
<td>Normal peak current parameter, sustained current not investigated7</td>
</tr>
<tr>
<td>R1626H</td>
<td>c.4877G&gt;A</td>
<td>1</td>
<td>HC</td>
<td>28</td>
<td>Transmembrane, helical, voltage sensor</td>
<td>Probably damaging: 1.00</td>
<td>Novel</td>
<td>Positive voltage shift in steady-state activation; negative voltage shift in steady-state inactivation; decreased fast inactivation; 2- to 3-fold increased sustained sodium current</td>
</tr>
<tr>
<td>D1819N</td>
<td>c.5455G&gt;A</td>
<td>1</td>
<td>HC</td>
<td>28</td>
<td>Intracellular</td>
<td>Probably damaging: 0.99</td>
<td>LQTS27</td>
<td>Negative voltage shift in steady-state inactivation; decreased fast inactivation; 6- to 10-fold increased sustained sodium current</td>
</tr>
<tr>
<td>R1897W</td>
<td>c.5689C&gt;T</td>
<td>1</td>
<td>HC</td>
<td>28</td>
<td>Intracellular</td>
<td>Probably damaging: 1.00</td>
<td>Novel</td>
<td>Negative voltage shift in steady-state inactivation</td>
</tr>
<tr>
<td>V1951M</td>
<td>c.5851G&gt;A</td>
<td>1</td>
<td>NC</td>
<td>28</td>
<td>Intracellular</td>
<td>Benign</td>
<td>AF27</td>
<td>Decreased fast inactivation; positive voltage shift in recovery from inactivation</td>
</tr>
<tr>
<td>L2004F</td>
<td>c.5851G&gt;A</td>
<td>1</td>
<td>NC</td>
<td>28</td>
<td>Intracellular</td>
<td>Benign</td>
<td>SIDS31</td>
<td>Positive voltage shift in steady-state inactivation; decreases fast inactivation; faster recovery from inactivation; 4-fold increased sustained sodium current21</td>
</tr>
</tbody>
</table>

AF indicates atrial fibrillation; LQTS, long QT syndrome; SIDS, sudden infant death syndrome.

*Conserv. is the degree of conservation for the mutated site among multiple species: CM, conserved among large mammals; HC, highly conserved; NC, not conserved.
carrying F2004L had a father with AF, but he was unavailable for genetic testing. For the other patients, there was no family history of AF and, therefore, no genetic testing of relatives was performed.

In Vitro Electrophysiological Data
SCN5A mutations not previously characterized electrophysiologically (R340Q, R1626H, D1819N, R1897W, and V1951M) were investigated for a potential functional impact. We expressed wild-type or mutant channels in mammalian HEK293 cells and addressed electrophysiological parameters by whole cell patch-clamp experiments (Figure 2 and Table 3). No significant difference in peak current density was observed in any of the mutants compared with control. However, differences were observed in steady-state activation and in several different inactivation parameters summarized in Table 3. In brief, R340Q showed a negative voltage shift of both steady-state activation and inactivation, together with a reduced time constant for onset (decay) of fast inactivation (Figure 2D, E, and B, respectively). R1626H gave a positive voltage shift of steady-state activation and a negative voltage shift of steady-state inactivation, together with a decreased onset of fast inactivation (Figure 2D, E, and B, respectively). D1819N revealed a minor change in the onset of fast inactivation parameters with an increase of the decaying time constant at depolarizing potentials (Figure 2B). R1897W showed a drastic negative voltage shift of the steady-state inactivation potential (Figure 2E), and V1951M gave a decrease of the time-dependent inactivation at different potentials and a decrease of onset of inactivation time constant (Figure 2B and F, respectively).

The R1626H mutant had a moderately increased sustained current component, whereas D1819N produced pronounced sustained sodium currents (Figure 3B–D).

Electrocardiographic Data
Flecainide provocation tests did not induce Brugada ECG patterns in any of the tested probands (Table 4). Of the 10 identified mutations and rare variants, 5 (S216L, R340Q, T1304M, D1819N, and V1951M) have previously been associated with LQT3 syndrome. Of the 2 patients carrying S216L, 1 had a borderline prolonged QTc interval of 469 ms, whereas the other proband, who was also a carrier of H558R, a variant previously able to rescue other mutations functionally, had a 43-ms increase in QTc interval. The V1951M proband had a QTc of 425 ms during the flecainide test. The patient also had a relatively large 43-ms increase in QTc interval. During the flecainide test, the QTc interval increased to 495 ms. The patient carrying R1626H had a borderline prolonged QTc interval of 467 ms. This is in contrast to a study by Darbar et al., who reported several SCN5A mutations that cosegregated with familial AF. We envision several explanations for this discrepancy. First, the 2 cohorts differed in sex, age, and size of the families. Second, because of the relative age of the probands’ parents, they may not have developed AF; however, they were predisposed for this. Third, a cohort selection bias may exist, in that patients with familial aggregation potentially more often could have been referred to the cohort described by Darbar et al. The proband carrying R1897W had a mother diagnosed as having postoperative AF at an old age and she did not carry the mutation; AF after surgery is common. The

Discussion
To our knowledge, this study is the first to comprehensively attempt to associate early-onset lone AF with mutations in SCN5A. In a cohort of 192 patients with onset of lone AF before the age of 40 years, we identified 8 mutations (T220I, R340Q, T1304M, F1896I, R1626H, D1819N, R1897W, and V1951M) in SCN5A. The high degree of conservation across species indicates that these residues are important for channel function. We also identified 2 rare SCN5A variants (S216L in 2 probands and F2004I in 1 proband). Of 11 SCN5A-positive probands, 6 (3.2% of the total population) carried a mutation or rare variant previously associated with LQT3 syndrome.

Genetic screening of the patients with lone AF revealed a much higher prevalence of mutations or rare variants in SCN5A, as expected from the prevalences in ESP (MAF, 2.9% versus 1.1%; P=0.013), representing the general population. Also, mutations or rare variants previously associated with LQT3 were present with a higher prevalence in the patients with lone AF patients compared with ESP (MAF, 1.6% versus 0.3%; P=0.003). Despite some limitations in comparing MAF in the 2 populations (different screening techniques, no possibility of matching on age and sex, and different geographic regions), this quantitative approach strongly supports the hypothesis that the present SCN5A mutations or rare variants identified in the patients with lone AF might be involved in the pathogenesis of AF.

All mutation carriers had a QTc interval within the normal range (<470 ms); however, 2 probands had a borderline prolonged QTc interval of 467 and 469 ms, respectively. Recently, individuals carrying an LQTS-associated mutation with a QTc interval within the normal range (<440 ms) also had an increased risk for life-threatening cardiac events. Hence, we speculate that the patients with lone AF in our cohort carrying mutations previously associated with LQT3 may have an increased risk of life-threatening arrhythmias. If patients with lone AF in general carry a high prevalence of LQTS3-associated variants, then as a group, they might have an increased risk of life-threatening arrhythmias. This novel finding could have potentially clinical implications for future risk stratification in patients with lone AF. However, further investigations are warranted to address a potential benefit of genetic screening of patients with lone AF in a clinical setting. Interestingly, in the present group of SCN5A genotype-positive patients, those patients carrying an LQTS3-associated variant also presented with the longest QTc intervals. The 2 patients with the shortest QTc intervals were the only 2 SCN5A-positive probands who carried the variant R558H (Table 4), which has rescued several other SCN5A variants (Table 2).

Our results from the present cohort of patients with early-onset lone AF indicate that SCN5A mutations only in rare cases produce highly penetrant monogenic forms of AF (Table 2). This is in contrast to a study by Darbar et al. who reported several SCN5A mutations that cosegregated with familial AF. We envision several explanations for this discrepancy. First, the 2 cohorts differed in sex, age, and size of the families. Second, because of the relative age of the probands’ parents, they may not have developed AF; however, they were predisposed for this. Third, a cohort selection bias may exist, in that patients with familial aggregation potentially more often could have been referred to the cohort described by Darbar et al. The proband carrying R1897W had a mother diagnosed as having postoperative AF at an old age and she did not carry the mutation; AF after surgery is common. The
Figure 2. Electrophysiological characterization of SCN5A mutants. A, Representative current traces obtained with a current/voltage protocol (inset in D) for wild-type (WT) and the 5 NaV1.5 mutations. B, Onset of fast inactivation. Single exponential fit to the decaying phase of the current traces (as shown in A). C, Current/voltage relationship of WT and NaV1.5 mutants. D, Steady-state activation curves. Activation properties were determined from I/V relationships by normalizing peak \( I_{\text{Na}} \) to driving force and maximal \( I_{\text{Na}}^* \), and plotting normalized conductance vs mV. E, Steady-state inactivation curves. Boltzmann curves were fitted to both steady-state activation and inactivation data. F, Time- and voltage-dependent recovery from inactivation. The time-dependent recovery from inactivation at different voltage potentials (inset) was fitted with a monoexponential relationship, and the \( \tau \) values were plotted. A and C-E, Averaged values and the numbers of cells measured are presented in Table 3. B and F, \( n=10 \) for each group. *\( P<0.05 \), **\( P<0.01 \), and ***\( P<0.001 \). ●WT, ◇R340Q, ▼R1626H, □D1819N, ○V1951M. Error bars represent the mean±SEM. In some figures, the SEM bars are smaller than the data symbols.

A patient carrying the mutation V1951M had a father diagnosed as having nonsustained VT, although he was not a mutation carrier either. A recent article provided important input to the discussion about reduced penetrance. In LQTS type 1 patients, variants in the 3’UTR-region of the KCNQ1 gene modify disease severity in an allele-specific manner, and this mechanism may also be important in other LQTS genes, such as SCN5A. The lack of familial cosegregation, in combination with the fact that both rare SCN5A variants and previously LQTS3-associated variants are highly
 Compromised Peak Sodium Current

To investigate whether the mutations reported herein are disease-causing, whole cell patch-clamp electrophysiological investigations were performed. We analyzed 5 SCN5A mutations that have not been functionally investigated previously (namely, R340Q, R1626H, D1819N, R1897W, and V1951M). None of the mutations had a significant effect on peak current density. Steady-state activation was altered for 2 mutations. Channels harboring the R340Q mutation showed a negative potential shift, which means that these channels open at more negative membrane potentials, increasing the availability of the channels (Figure 2 and Table 2). R1626H channels showed a positive voltage shift of activation, which is expected to reduce channel availability. Because Na\(_\text{v}1.5\) channels are activated at potentials close to the resting membrane potential of cardiomyocytes, small changes in steady-state inactivation are expected to give large impact on sodium channel availability. Indeed, for the R340Q, R1626H, and R1897W mutations, we observed a >5-mV negative shift in steady-state inactivation (Figure 2 and Table 3). We also investigated the onset (or decay) of inactivation, which is a measure of the width of the sodium peak. If the onset of inactivation time constant is decreased, the channel will close faster, resulting in a decreased depolarizing power of the mutated channel. Decreased time constants were found for R340Q and V1951M. A third inactivation property is the time-dependent recovery from inactivation. The V1951M mutation had a shorter recovery time at normal potentials. A faster recovery could be speculated to be proarrhythmic because the sodium channel complexes are released from inactivation earlier and can thereby contribute to wavelength shortening.\(^{17}\) In conclusion, all 5 mutations displayed altered phenotypes compared with wild-type Na\(_\text{v}1.5\). Most of the observed changes indicated a decreased transient sodium current.\(^4\) The 6 probands carrying delayed rectifier current and can, therefore, potentially unmask the hERG1 (K\(_\text{r}11.1\)) potassium channel, which is responsible for the fast delayed rectifier current and can, therefore, potentially unmask increased sustained sodium current.\(^{46}\) The 6 probands carrying an SCN5A mutation previously associated with LQTS3 may be predisposed for AF through increased sustained sodium current, a mechanism thought to at least partly underlie LQTS3. Indeed, for 3 (S216L, T1304M, and L2004F) of the 6 mutations, increased sustained sodium current has been reported (8-, 7-, and 4-fold, respectively).\(^{31}\) In addition, 3 of 7 patients carrying a mutation previously associated with LQTS3 had either a borderline prolonged QT\(_c\) interval at baseline or a higher increase in QT\(_c\) interval during flecainide testing.

### Increased Sustained Sodium Current

Flecainide has, apart from blocking I\(_{Na}\), blocked the K\(_r\)11.1 (hERG1) potassium channel, which is responsible for the fast delayed rectifier current and can, therefore, potentially unmask increased sustained sodium current.\(^{46}\) The 6 probands carrying an SCN5A mutation previously associated with LQTS3 may be predisposed for AF through increased sustained sodium current, a mechanism thought to at least partly underlie LQTS3. Indeed, for 3 (S216L, T1304M, and L2004F) of the 6 mutations, increased sustained sodium current has been reported (8-, 7-, and 4-fold, respectively).\(^{31}\) In addition, 3 of 7 patients carrying a mutation previously associated with LQTS3 had either a borderline prolonged QT\(_c\) interval at baseline or a higher increase in QT\(_c\) interval during flecainide testing.

### Table 3. Clinical Characteristics of Probands With SCN5A Variants

<table>
<thead>
<tr>
<th>No.</th>
<th>Patient Genotype</th>
<th>H558R Genotype</th>
<th>Sex</th>
<th>Phenotype</th>
<th>Onset, y</th>
<th>ECG at Inclusion</th>
<th>ECG During Flecainide Testing</th>
<th>Flecainide Testing</th>
<th>Family History of AF</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>S216L</td>
<td>HR</td>
<td>M</td>
<td>Persistent</td>
<td>36</td>
<td>Normal (QT(_c), 438 ms)</td>
<td>...</td>
<td>Not done</td>
<td>None</td>
</tr>
<tr>
<td>2</td>
<td>S216L</td>
<td>HH</td>
<td>M</td>
<td>Persistent</td>
<td>39</td>
<td>Normal (QT(_c), 469 ms)</td>
<td>...</td>
<td>Not done</td>
<td>None</td>
</tr>
<tr>
<td>3</td>
<td>T220I</td>
<td>HH</td>
<td>M</td>
<td>Paroxystic</td>
<td>35</td>
<td>Normal (QT(_c), 422 ms)</td>
<td>...</td>
<td>Not done</td>
<td>None</td>
</tr>
<tr>
<td>4</td>
<td>R340Q</td>
<td>HH</td>
<td>M</td>
<td>Paroxystic</td>
<td>26</td>
<td>Normal (QT(_c), 422 ms)</td>
<td>Not available</td>
<td>Negative</td>
<td>None</td>
</tr>
<tr>
<td>5</td>
<td>T1304M</td>
<td>HH</td>
<td>M</td>
<td>Paroxystic</td>
<td>37</td>
<td>r'-Wave in V1/V2 (QT(_c), 439 ms)</td>
<td>QRS increase 28 ms QT(_c), increase 30 ms</td>
<td>Negative</td>
<td>None</td>
</tr>
<tr>
<td>6</td>
<td>F1596I</td>
<td>HH</td>
<td>M</td>
<td>Persistent</td>
<td>39</td>
<td>Low voltage (QT(_c), 428)</td>
<td>QRS increase 15 ms QT(_c), increase 19 ms</td>
<td>Negative</td>
<td>Mother and mother’s sister with AF, both decreased</td>
</tr>
<tr>
<td>7</td>
<td>R1626H</td>
<td>HH</td>
<td>M</td>
<td>Paroxystic</td>
<td>37</td>
<td>Normal (QT(_c), 443 ms)</td>
<td>QRS increase 26 ms QT(_c), increase 52 ms</td>
<td>Negative</td>
<td>None</td>
</tr>
<tr>
<td>8</td>
<td>D1819N</td>
<td>HH</td>
<td>F</td>
<td>Paroxystic</td>
<td>25</td>
<td>Short PR of 106 ms (QT(_c), 467 ms)</td>
<td>QRS increase 4 ms QT(_c), increase 48 ms</td>
<td>Negative</td>
<td>Family history of AF, but no mutation cosegregation</td>
</tr>
<tr>
<td>9</td>
<td>R1897W</td>
<td>HH</td>
<td>M</td>
<td>Paroxystic</td>
<td>38</td>
<td>r'-Wave and J-point elevation in V1/V2 (QT(_c), 397)</td>
<td>GRS increase 20 ms QT(_c), increase 16 ms</td>
<td>Negative</td>
<td>Mother with postoperative AF, no mutation</td>
</tr>
<tr>
<td>10</td>
<td>V1951M</td>
<td>HR</td>
<td>M</td>
<td>Persistent</td>
<td>22</td>
<td>J-wave in II, III and avF (QT(_c), 425 ms)</td>
<td>QRS increase 18 ms QT(_c), increase 41 ms</td>
<td>Negative</td>
<td>Father diagnosed as having VT, no mutation</td>
</tr>
<tr>
<td>11</td>
<td>F2004L</td>
<td>HR</td>
<td>M</td>
<td>Chronic</td>
<td>38</td>
<td>AF</td>
<td>...</td>
<td>Not done</td>
<td>Father AF, not available for genetic testing</td>
</tr>
</tbody>
</table>

AF indicates atrial fibrillation; HH, H558H; HR, H558R.
than the expected value of 21±17 ms for healthy individuals (Table 4).41

In our cohort, the patient carrying the novel R1626H mutation had an unexpectedly large increase in QTc (52 ms) during flecainide testing and the patient carrying D1819N had a borderline prolonged QTc interval of 467 ms at baseline. Hence, we investigated the sustained sodium current for R1626H and D1819N. Patch-clamp experiments revealed a 2- to 3-fold increase in sustained current for the R1626H mutation, whereas the D1819N conducts a dramatically 6- to 10-fold increased sustained current. Hence, the in vitro investigations confirmed the effect of flecainide on the QTc interval observed in the 2 probands, indicating that the sustained component of the sodium current might play a role in the pathogenesis of AF. This is in line with a study using isolated atrial myocytes from patients with AF, which showed increased sustained sodium current.42 Furthermore, Lemoine et al22 have recently showed atrial action potential prolongation, atrial early after depolarizations, and triggered activity in a genetically modified animal model of human LQTS3. Treatment with ranolazine,

Figure 3. The sustained sodium current (\(I_{\text{NaL}}\)) of R1626H and D1819N is increased at different voltages. Representative recordings in the absence or presence of tetrodotoxin (TTX) for wild-type (WT) (n=6) (A), R1626H (n=6) (B), and D1819N (n=6) (C). Currents were activated by a 500-ms step to −20 mV from a holding potential of −100 mV. For comparison, the peak and late current are shown at different scales. The sustained currents were normalized to the peak current observed in each trace. D, Summarized data of \(I_{\text{NaL}}\) at different voltages. Currents were activated by a 500-ms step from −30 to 0 mV in 10-mV increments from a holding potential of −100 mV. Currents in the presence of TTX were subtracted from currents recorded in the absence of TTX to determine the TTX-sensitive current. \(I_{\text{NaL}}\) was measured as the mean current between 450 and 500 ms, and the ratio between the TTX-sensitive peak and the late current was calculated for WT, R1626H, and D1819N. At each condition, the difference in \(I_{\text{NaL}}\) between R1626H or D1819N and WT was significant. *\(P<0.05\), **\(P<0.01\), and ***\(P<0.001\).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Peak Current at −15 mV, pA/pF</th>
<th>No. of Experiments</th>
<th>Steady-State Activation (V_{1/2}), mV</th>
<th>Slope k Value</th>
<th>n</th>
<th>Steady-State Inactivation (V_{1/2}), mV</th>
<th>Slope k Value</th>
<th>n</th>
<th>No. of Experiments</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>−539±55</td>
<td>23</td>
<td>−27.9±1.3</td>
<td>6.6±0.3</td>
<td>22</td>
<td>−85.6±0.9</td>
<td>5.5±0.1</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>R340Q</td>
<td>−462±90</td>
<td>14</td>
<td>−34.1±1.5†</td>
<td>6.3±0.5</td>
<td>14</td>
<td>−91.1±1.3*</td>
<td>5.7±0.2</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>R1626H</td>
<td>−380±74</td>
<td>15</td>
<td>−23.5±1.8**</td>
<td>7.6±0.4**</td>
<td>14</td>
<td>−91.2±1.9*</td>
<td>5.6±0.2</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>D1819N</td>
<td>−385±59</td>
<td>15</td>
<td>−27.7±1.8</td>
<td>7.5±0.6</td>
<td>14</td>
<td>−85.4±0.5</td>
<td>5.9±0.2**</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>R1937W</td>
<td>−465±65</td>
<td>15</td>
<td>−29.5±1.6</td>
<td>7.2±0.5</td>
<td>15</td>
<td>−91.8±1.5*</td>
<td>5.5±0.2</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>V1951M</td>
<td>−616±108</td>
<td>10</td>
<td>−31.5±1.8</td>
<td>6.9±0.4</td>
<td>10</td>
<td>−85.6±1.0</td>
<td>5.6±0.2</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

*\(P<0.01\), **\(P<0.05\), significantly different from \(Na_{1.5}\)-WT.
a blocker of the sustained sodium currents, normalized the number of early after depolarizations in this model. Patients treated with ranolazine for angina pectoris had a lower incidence of supraventricular tachycardias.

Our study on human AF, together with the mice experiments by Lemoine et al., for the first time, to our knowledge, indicate a possible overlap between the mechanisms underlying LQT3 and lone AF with action potential prolongation as a substrate and early after depolarizations as triggers for arrhythmia. However, both decreased and increased transient peak sodium current have also been suggested as possible mechanisms for AF, and further studies are needed to reveal the electrophysiological mechanisms behind AF.

Limitations

We only analyzed the coding regions of SCN5A, and mutations occurring in gene regions other than coding regions cannot be excluded. We used a conventional heterologous expression system, which differs from native cardiomyocytes. Furthermore, for the electrophysiological parameters investigated, several changes of several parameters were observed. Although these data provide strong support for discussing whether a given mutation is preferentially a loss- or gain-of-function mutation, they cannot be regarded as conclusive.

Conclusions

We identified 8 mutations and 2 rare variants in SCN5A in 192 patients with early-onset lone AF. Many of these patients with lone AF carried a mutation or rare variant previously associated with LQT3, compared with the expected frequency in the general population (MAF, 1.6% versus 0.3%; P = 0.003). All identified variants have been investigated electrophysiological, and in 9 of them, compromised peak sodium current was found, whereas 5 variants showed increased sustained sodium current. Our results thereby indicate that both gain- and loss-of-function alterations in the electrophysiological properties of the cardiac sodium current may lead to the development of AF in young adults.

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This study was supported by The Danish Heart Foundation (grants 07–10-R60-A1815-B573–22398 and 11–04-R84-A3333–22660), the John and Birthe Meyer Foundation, the Lundbeck Foundation, the Carlsberg Foundation, the Research Grant from the Arvid Nilsson Foundation of the Heart Centre Rigshospitalet, the Arvid Nilsson Foundation, the John and Birthe Meyer Foundation, the Danish Heart Rhythm Study, and the European Society of Cardiology Committee for Practice Guidelines and the European Society of Cardiology Committee for Practice Guidelines (Writing Committee to Revise the 2001 Guidelines for the Management of Patients With Atrial Fibrillation). Rev Port Cardiol. 2002;26:383–446.


Intriguingly, 6 of these have previously been associated with LQTS, and LQTS variants exceeded the expected frequency with early-onset lone AF. In 192 subjects with early-onset lone AF, we identified 8 mutations and 2 rare variants in SCN5A. Atrial fibrillation (AF). To our knowledge, this study is the first to investigate the prevalence of SCN5A variants in patients with early-onset lone AF. The human cardiac sodium channel have been associated with inherited susceptibility to a plethora of diseases, such as long QT syndrome (LQTS), Brugada syndrome, sudden infant death syndrome, progressive cardiac conduction disorders, and atrial fibrillation. To our knowledge, this study is the first to investigate the prevalence of SCN5A variants in patients with genotype-confirmed long-QT syndrome and normal-range corrected QT intervals. Circulation. 2011;57:51–59.


The cardiac sodium channel is responsible for both the fast depolarization upstroke of the cardiac action potential (peak component) and the late phase of the action potential (sustained component). Mutations in the gene SCN5A encoding the human cardiac sodium channel have been associated with inherited susceptibility to a plethora of diseases, such as long QT syndrome (LQTS), Brugada syndrome, sudden infant death syndrome, progressive cardiac conduction disorders, and atrial fibrillation (AF). To our knowledge, this study is the first to investigate the prevalence of SCN5A variants in patients with early-onset lone AF. In 192 subjects with early-onset lone AF, we identified 8 mutations and 2 rare variants in SCN5A. Intriguingly, 6 of these have previously been associated with LQTS, and LQTS variants exceeded the expected frequency in the general population (minor allele frequency 1.6% versus 0.3%; P=0.003). All identified variants have been investigated electrophysiologically, and in 9 of them, a compromised peak sodium current was found, whereas 5 variants showed increased sustained sodium current. Our results thereby indicate that both gain- and loss-of-function alterations in the electrophysiological properties of the cardiac sodium current may lead to the development of AF, and that the same genetic variants that are involved in the LQTS syndrome are also involved in lone atrial fibrillation. To our knowledge, this study is the first to investigate the sustained sodium current in this context of AF and may indicate a role for the specific blocker of the sustained current sodium channel in clinical practice.

CLINICAL PERSPECTIVE
High Prevalence of Long QT Syndrome–Associated SCN5A Variants in Patients With Early-Onset Lone Atrial Fibrillation

Morten S. Olesen, Lei Yuan, Bo Liang, Anders G. Holst, Nikolaj Nielsen, Jonas B. Nielsen, Paula L. Hedley, Michael Christiansen, Søren-Peter Olesen, Stig Haunsø, Nicole Schmitt, Thomas Jespersen and Jesper H. Svendsen

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