Mutations in sodium channel β-subunit SCN3B are associated with early-onset lone atrial fibrillation

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Aims

Atrial fibrillation (AF) is the most frequent arrhythmia. Screening of SCN5A—the gene encoding the α-subunit of the cardiac sodium channel—has indicated that disturbances of the sodium current may play a central role in the mechanism of lone AF. We tested the hypothesis that lone AF in young patients is associated with genetic mutations in SCN3B and SCN4B, the genes encoding the two β-subunits of the cardiac sodium channel.

Methods and results

In 192 unrelated lone AF patients, the entire coding sequence and splice junctions of SCN3B and SCN4B were bidirectionally sequenced. Three non-synonymous mutations were found in SCN3B (R6K, L10P, and M161T). Two mutations were novel (R6K and M161T). None of the mutations were present in the control group (n = 432 alleles), nor have any been previously reported in conjunction with AF. All SCN3B mutations affected residues that are evolutionarily conserved across species. Electrophysiological studies on the SCN3B mutation were carried out and all three SCN3B mutations caused a functionally reduced sodium channel current. One synonymous variant was found in SCN4B.

Conclusion

In 192 young lone AF patients, we found three patients with suspected disease-causing non-synonymous mutations in SCN3B, indicating that mutations in this gene contribute to the mechanism of lone AF. The three mutations in SCN3B were investigated electrophysiologically and all led to loss of function in the sodium current, supporting the hypothesis that decreased sodium current enhances AF susceptibility.

Keywords

Lone AF • Genetics • Na⁺,1.5 • Na⁺-β-subunits • Sodium current

1. Introduction

Atrial fibrillation (AF) is the most common cardiac arrhythmia and is the cause of substantial mortality and morbidity.¹ In the general population, the prevalence of AF is estimated to be 1%, but is expected to increase within the next decades.² In recent years, the pathophysiology and the underlying mechanisms of AF have been the subject of intense research.³

Traditionally, various systemic and cardiac disorders have been regarded as causes of AF. These disorders influence the electrical and structural remodelling of the atria and are thought to be central in the AF pathogenesis.³ In 10–20% of the cases, AF is not associated with underlying cardiovascular and systemic disorders and is hence diagnosed as lone AF.⁴

A genetic component in common forms of AF has recently been recognized. In the Framingham Heart Study, the development of AF in the offspring was independently associated with parental AF, suggesting 30% of all patients with AF to be familial.⁵ A Danish twin study suggests a degree of hereditability as high as 62%.⁶ The identification of the genetic components underlying AF has lately been addressed intensively. The importance of single-nucleotide polymorphisms (SNPs) has been shown in a genome-wide association study, where a strong association between two SNPs on chromosome 4q25 and AF was found, strongest in those diagnosed at a
SCN3B mutations in lone AF

younger age. Furthermore, rare mutants in at least seven genes (SCN1A, KCNQ2, KCN10, KCN2, KCNE1, and KCNE2) encoding different ion channel subunits have been associated with AF. In addition to AF, mutations in SCN5A have also been linked to increased susceptibility to other arrhythmias such as long QT syndrome type 3, progressive cardiac conduction defect, sick sinus node syndrome, Brugada’s syndrome (BrS), and overlapping phenotypes. SCN5A encodes for Na\(\text{\textsubscript{a}}\),\(1,5\), the α-subunit of the sodium current (\(h\)). \(h\) is responsible for the early fast depolarization upstroke of the cardiac action potential. Mutations in SCN5A have recently been shown to play a central role in lone AF. In a cohort of 117 lone AF patients, four different SCN5A mutations were found, supporting the potential role of sodium current disturbances in the development of lone AF.

In mammalian hearts, the pore-forming α-subunits act in complex with modifying β-subunits and multiple other proteins to form the functional entity that carries the sodium current. In humans, four sodium channel β-subunits Na\(\text{\textsubscript{a}}\),β1–β4, encoded by SCN1–4B, have been identified. SCN1–4B all comprise large extracellular immunoglobulin-like domains, a single transmembrane spanning segment, and intracellular C-terminal domains. β-Subunits have been implicated in sodium channel expression at the cell surface, modulation of channel gating, and voltage dependency of the sodium current. Furthermore, they also seem to play a role in cell modulation of channel gating, and voltage dependency of the functional entity that carries the sodium current. In humans, four different SCN3–4B subunits have been associated with AF.

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2. Methods

2.1 Study Subjects

Patients were recruited from cardiology departments in eight hospitals in the Copenhagen region of Denmark. Patient records from all in- and outpatient activity in the past 10 years with the diagnose code (ICD-10) I48.9 (AF and atrial flutter) were collected and read. Only lone AF patients with the onset of disease before the age of 40 years were included. Patients were excluded from the study population if atrial flutter was the predominant or only arrhythmia documented. Patients with structural heart disease, ischaemic heart diseases, hypertension, diabetes, metabolic diseases, or other diseases related to AF were excluded. Patients with abnormal echocardiography were excluded. All patients with electrocardiogram (ECG) verified lone AF and onset of disease before the age of 40 years were given written information and offered to participate.

Blood samples, digitally recorded ECG, and clinical data were collected on all participating subjects. The study conforms to the principles outlined in the Declaration of Helsinki and was approved by the Scientific Ethics Committee of Copenhagen and Frederiksberg (KF 0131322) and all included patients gave written informed consent.

2.2 Control population

To distinguish between common genetic polymorphisms and disease-causing rare mutations, a group of 216 ECG documented healthy blood donors without cardiac symptoms was used.

2.3 Mutation screening

Genomic DNA was isolated from blood samples using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany).

The entire coding sequences and splice junctions of SCN3–4B (GenBank acc. nos NM\_018400.3 and NM\_174934.2, respectively) were bidirectionally sequenced with intronic primers [primers and polymerase chain reaction (PCR) conditions are available on request] using Big Dye chemistry (DNA analyzer 3730, Applied Biosystems, Foster City, CA, USA). All identified non-synonymous mutants were validated by resequencing in an independent PCR. We also screened 216 healthy controls, matched on the basis of ethnicity to the lone AF patients. The group of healthy controls was screened employing high-resolution melting curve analysis, and mutation carriers were included in doublet as positive controls (Light Scanner, Idaho Technology, Salt Lake City, UT, USA). For probes carrying non-synonymous mutations, bidirectional sequencing of SCNQ1 (NM\_000218), KCNQ2 (NM\_000335), KCN1E1/2/3/5 (NM\_000219/NM\_172201/NM\_005472.4/NM\_012282.2), and KCN2 (NM\_000891) was performed.

A variant was defined as mutation if present in less than 1% of the persons investigated.

Mutations were furthermore considered suspected disease-causing if (i) it resulted in a missense, shifted the reading frame, or affected a canonical splice site; (ii) it affected a conserved amino acid; (iii) the mutation co-segregated with the disease in affected family members; (iv) it was not identified among ethnically matched controls; (v) it was not described earlier as a polymorphism in public available databases; and (vi) the affected patient did not have mutations in any of the genes earlier associated with AF. In the absence of more than two affected family members for segregation analysis, disease association was presumed if all other criteria were met.

Suspected disease-causing mutations underwent electrophysiological evaluation as described below.

2.4 RNA preparation and reverse transcriptase reaction

We studied the expression of SCN1–4B in human myocardial tissue. Seven right atrial appendages were obtained from patients (mean age 64) undergoing coronary by-pass surgery and four left ventricular samples originating from healthy explanted hearts (mean age 42), where appropriate recipients were not found [ethical approval number 4991-010010-1018EKU (339/Pi1010)]. RNA extraction and cDNA synthesis was performed as described previously.

2.5 Quantitative real-time PCR

Quantitative real-time PCR was performed as described previously. The primers and probes targeting SCN2B(exon 3–4), SCN3B(exon 5–6), and SCN4B(exon 4–5) were designed and synthesized by Applied Biosystems, following submission of intron spanning sequences using Primer Express 3.0 software. For SCN1B, the custom-designed assay Hs00168897_m1 was used. Cyclophilin D (Assay Rn01458749_g1) was used for normalization.

2.6 Cloning of sodium channel subunits

The plasmid hNa\(\text{\textsubscript{a}},1.5\) in pcDNA3 was a kind gift from H. Abriel (U Lau- sanne and U Bern, Switzerland). The cDNA refers to isoform 2 (NM\_000335) and is also known as hNa\(\text{\textsubscript{a}},1\); i.e. it lacks Q1077 and carries the rare variant T559A. SCN3B cDNA encoding human Na\(\text{\textsubscript{a}},β3\) (NM\_018400) was amplified from EST clone DKFZp761F182Q (imaGenes, Berlin, Germany) and cloned into pcDNA3.1 engineered with a Kozak consensus site to optimize initiation of translation.

The point mutations R6K (aga \(\rightarrow\) aac), L10P (ctg \(\rightarrow\) ccg), and M161T (agt \(\rightarrow\) acg) in SCN3B were introduced using mutated oligonucleotide extension (Pfu Turbo Polymerase, Stratagene, La Jolla, CA, USA) from the plasmid template harbouring the cDNA of interest, digested with DpnI (Fermentas, St Leon, Germany) and transformed into and amplified in Escherichia coli XL1 Blue cells. All plasmids were verified by complete
DNA sequencing of the cDNA insert (Macrogen Inc., Seoul, Republic of Korea).

2.7 In vitro electrophysiology

For electrophysiological patch-clamp studies, CHO-Pro5 cells were transiently co-transfected with 0.3 µg pcDNA3-hSCN5A, 0.3 µg pcDNA3-hSCN1B, 0.3 µg pcDNA3-hSCN3B (WT or mutant), and 0.2 µg of pcDNA3-eGFP as a reporter gene, using Lipofectamine and Plus reagent (Invitrogen, USA) according to the manufacturer’s instructions. Patch-clamp experiments were performed at room temperature (20–22°C) 2–3 days after transfection. The internal pipette solution was (in mM): CsCl 60, CsAspartate 70, CaCl 2, MgCl 2, HEPES 10, EGTA 11, and Na 2ATP 5, pH 7.2, with CsOH; external solution (in mM): NaCl 130, CsCl 5, CaCl 2, MgCl 2, HEPES 10, and glucose 5, pH 7.4, with CsOH. Measurements were made with Pulse software (HEKA Elektronik, Lambrecht, Germany) and using an EPC-9 amplifier (HEKA Elektronik). Borosilicate glass pipettes (Module Ohm, Herlev, Denmark) were pulled on a DPZ-Universal puller (Zeitz Instruments, Munich, Germany) and had tip resistances of 1.5–2.5 MΩ when filled with intracellular solution. The series resistances recorded in the whole-cell configuration were compensated (80%) to minimize voltage errors. Data analyses were done as described previously. Briefly, peak current densities were measured during an activation protocol and Ihak densities (pA/pF) were obtained by dividing the peak Ihak by the cell capacitance. For the activation and steady-state inactivation curves, data from individual cells were fitted with a Boltzmann equation, \( y(x) = 1/(1 + \exp(x - \beta)/\gamma) \), in which \( \gamma \) is the normalized current or conductance, \( \beta \), the membrane potential, \( v_{1/2} \), the voltage at which half of the channels are activated or inactivated, and \( K \) the slope factor. Time-dependent recovery from inactivation curves and the onset of fast inactivation curves were fitted individually with a mono-exponential relationship. \( r \) values and half recovery time were obtained by fitting the decaying phase of individual current traces with a mono-exponential function and plotted vs. \( v_{m} \).

2.8 Statistical analysis

Data are presented as mean ± SEM. Student’s unpaired t-test, one-way ANOVA repeated measures with Dunnett’s post-test, or Fisher’s exact tests were 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.

3. Results

3.1 Study cohort

From November 2007 to April 2009, 823 patients with AF were identified. All patient record forms indicating AF/flutter and with onset of disease before 40 years of age were collected and read. All patients that fulfilled the inclusion criteria of lone AF were offered to participate (329 patients) and a total of 192 unrelated patients (82% males, 99% Caucasians) were included. The average onset of disease was 30.4 years and 31% of the patients had affected first-degree family members with a history of AF (Table 1).

3.2 Mutation screening

Direct DNA sequencing of SCN3–4B from the 192 index patients revealed three non-synonymous mutations in SCN3B (R6K, L10P, and M161T) (Figure 1 and Table 2). None of the mutations were present in the control group (n = 432 alleles), and none of them had previously been reported associated with AF. One not previously reported synonymous mutation was identified in SCN4B (c.639T > C). All genetically affected probands were men and heterozygous carriers. All amino acids in SCN3B for which missense mutations were identified are highly conserved across species, suggesting that these amino acids are functionally important (Figure 1).

All patients carrying the suspected disease-causing non-synonymous mutations were subsequently screened for mutations in genes that have already been associated with AF, namely KCNQ1, KCNH2, SCN5A, KCNE 1/2/3/5, KCNJ2, and SCN1B. We identified one variant SCN5A c.4786 T > A (F1596I) mutation in Patient 1. This mutation was not present in the control group (n = 216) nor had it been reported previously. We observed no changes in activation/inactivation parameters or in peak current density (data shown in Supplementary material online, Figure S1) and the pathogenicity of this mutation is therefore questionable.

3.3 Clinical data

Patient 1 had an onset of AF at the age of 39 years and has now persistent AF. His mother and aunt, both deceased, were affected by permanent AF late in life. The patient is highly symptomatic and has so far been DC converted 12 times and has undergone radiofrequency ablation for AF three times with some benefit. The sinus rhythm (SR) ECG was normal (ECG data: HR 86 b.p.m.; P-wave duration 92 ms; PR 154 ms; QRS 96 ms; QTc 428 ms) and a flecainide test did not induce a Brugada-like ECG pattern. Patient 2 had an onset of AF at the age of 35 years and has now persistent AF. He has no family history of AF but a mother with ECG findings of atrial premature complexes. She was free of inducible AF three times with some benefit. The sinus rhythm (SR) ECG was normal (ECG data: HR 86 b.p.m.; P-wave duration 131 ms; PR 160 ms; QRS 100 ms; QTc 412 ms). This patient did not agree on a flecainide test (Table 3).

Patient 3 had an onset of AF at the age of 36 years and has now paroxysmal AF. He has no family history of AF and the SR ECG was normal (ECG data: HR 64 b.p.m.; P-wave duration 110 ms; PR 152 ms; QRS 98 ms; QTc 408 ms). A Holter monitoring revealed a high number of ventricular premature complexes. This patient was not available for a flecainide test.
3.4 Real-time PCR in human heart and in vitro electrophysiology

Quantitative PCR analyses showed that SCN1–4B were all expressed in the atria and ventricle (Figure 2). SCN3B and SCN4B were expressed at the same level in the atria and ventricle. In the atria, SCN1B was found to be higher expressed than both SCN2B (P, 0.01) and SCN4B (P, 0.05). There was no difference in the expression level of SCN3B compared with SCN1B, SCN2B, or SCN4B in the atria (Figure 2). Data are expressed as mean ± SEM.

To further analyse the role of the three SCN3B mutants, we chose to characterize the SCN3B mutations electrophysiologically.

Co-expression of β3/R6K with β1/WT and Na+1.5 did not affect the voltage dependence of activation or peak current density when compared with control (Figure 3C and D, and Table 4) (P = NS), yet resulted in a negative shift of the voltage dependence of inactivation (−25.0 mV, P, 0.05) compared with WT control (Figure 3E and Table 4).

When β1/WT and Na+1.5 were co-expressed with β3/L10P, a 45% (P < 0.05) decrease in peak current density compared with control was observed (Figure 3C and Table 4). Co-expression of β3/L10P with β1/WT and Na+1.5 also resulted in a negative shift of voltage dependence of inactivation (−3.8 mV, P < 0.05) compared with cells expressing β1/WT, β3/WT, and Na+1.5 (Figure 3E and

Table 2 SCN3–4B mutants in young patients with lone AF

<table>
<thead>
<tr>
<th>Patient</th>
<th>Gene</th>
<th>cDNA</th>
<th>Amino acid</th>
<th>Reported/disease</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SCN3B</td>
<td>c.17G &gt; A</td>
<td>R6K</td>
<td>Novel</td>
<td>Loss of function</td>
</tr>
<tr>
<td>2</td>
<td>SCN3B</td>
<td>c.29T &gt; C</td>
<td>L10P</td>
<td>Known/BrS35</td>
<td>Loss of function</td>
</tr>
<tr>
<td>3</td>
<td>SCN3B</td>
<td>c.482T &gt; C</td>
<td>M161T</td>
<td>Novel</td>
<td>Loss of function</td>
</tr>
</tbody>
</table>

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We tested the hypothesis that mutations in SCN3B are involved in the pathophysiology of lone AF. Three mutations in SCN3B encoding the sodium channel β3-subunits were identified in three probands (R6K, L10P, and M161T), whereas no suspected disease-causing variants were found in SCN4B.

Mutations in SCN3B have not been linked to AF previously. To investigate the importance of the mutations, we addressed the expression of the sodium channel subunits β1–β4 in the human atrium. We are to our knowledge the first to show that SCN3B and SCN4B are expressed in the human atrium. Heterologous expression in CHO-Pro5 cells and patch-clamp analyses revealed that the mutations affected biophysical parameters of the conducted sodium current: M161T led to a decrease in peak current density, R6K affected steady-state inactivation, and L10P had effect on both. noteworthy, the M161T mutation caused the largest decrease in the peak current density which is in accordance with the high degree of evolutionary conservation of this particular amino acid. All of the mutations led to a loss of function of the sodium channel. Taking into account the atrial resting membrane potential of around −80 mV, even a small negative shift in steady-state inactivation will lead to a considerable decrease in the number of recruitable channels and thereby resulting in a significant loss of sodium current (Figure 3). These findings support the hypothesis that decreased sodium current or increased outward potassium currents may shorten the refractory period, creating a substrate for re-entry and thereby contributing to AF susceptibility. In line with this, recent mutation screening studies in AF patients showed that both loss-of-function mutations in SCN5A and gain-of-function mutations in genes coding for potassium channels led to shortened action potential duration, thereby substantiating the connection between shortening of the refractory period and development of AF.

Further support for a role of SCN3B in atrial electrophysiology comes from the knockout mouse model lacking the SCN3B gene (SCN3B−/−). Atrial burst pacing protocols induced atrial tachycardia and fibrillation in all SCN3B−/− but hardly any WT hearts. These findings demonstrate that a deficiency in SCN3B results in significant atrial electrophysiological abnormalities. Furthermore, SCN3B−/− mice had significantly shorter ventricular effective refractory periods. Programmed ventricular stimulation also could induce ventricular tachycardia in these mice, demonstrating the importance of SCN3B for the electrical stability of the heart. A recent study identified an SCN3B mutation in a survivor of idiopathic ventricular fibrillation, and the mutation was shown to cause a significant reduction in peak current density, thus confirming the importance of SCN3B for the electric stability of the heart. Mutations in SCN3B that reduce sodium current may cause an AF-prone substrate through two mechanisms: slowing of conduction and shortening of refractoriness, both of which will cause shortening of wavelength and thereby contribute to AF susceptibility. All three SCN3B mutations identified in our study led to a loss of function in the sodium channel which could result in an AF-prone substrate.
Figure 3  Na₃β3 mutants R6K, L10P, and M161T evoke electrophysiological changes. Whole-cell patch clamp analyses of transiently transfected CHO-Pro5 cells. Cells were transfected with Na₃,1.5Naβ1Naβ3 in a 1:1.5:1.5 molar ratio. (A) Representative current traces following a voltage step protocol with 5 mV increments from −70 to +15 mV, preceded by a −100 mV step. Na₁,5 and Na₃β1 co-expressed with wild-type (WT) Na₃β3 WT (filled circle), Na₁,5 and Na₃β1 co-expressed with R6K (filled square), and Na₁,5 and Na₃β1 co-expressed with L10P (filled triangle), and Na₁,5 and Na₃β1 co-expressed with M161T (filled inverted triangle). (B) Current–voltage (I–V) relationship, plotted as whole-cell current (corresponding to the activation currents analysed in Table 4). (C) Peak current density normalized to cell size. The absolute numbers are listed in Table 4. Steady-state activation (D) and inactivation (E) properties. The normalized values have been calculated by dividing the current level at the respective voltage by the largest current (I/I₉₅). Protocols applied in order to perform the analyses are inserted. (F) Onset of fast inactivation. The decaying phase of the current traces recorded following clamping to different potentials [as in (A)] was fitted to a one-exponential equation. (G) Time-dependent recovery from steady-state inactivation following four different pre-potentials, protocol shown in insert. Na₁,5-expressing cells were significantly different (P < 0.05) from the other groups at all potentials (Student’s unpaired t-test). *P < 0.05, **P < 0.01. (B, D–G) Analysed by one-way ANOVA repeated measures with Dunnett’s post-test. In (B), L10P and M161T are significantly different from WT (P < 0.01), and in (E), R6K and L10P are significantly different from WT (P < 0.05) (Table 4).
The measurements indicated that the mutations in SCN3B may indeed represent disease-causing mutations. The functional data and applying the definition criteria for disease-causing mutations (see Section 2) support the notion that all SCN3B mutations presented in this study might be considered as potentially disease-causing. Noteworthy, none of the mutations were detected in the control group (432 alleles) or reported in a cohort of AF patients consisting of 309 Caucasians and 141 African-Americans, further supporting both the novelty and the significance of the mutations.

Of the three index patients presented here, one had a family history of AF indicating that this family could be genetically predisposed. AF has only in a few families been documented to be inherited in a monogenic manner, implying that in most cases, the pattern of inheritance is more complex. The pathogenic process leading to AF is also influenced by other genetic variations, fibrosis, and ageing processes in general. Hence, the complex pattern of inheritance can be explained by the mutation being only a part of the pathogenic process leading to AF.

Noteworthy, Pappone et al. have recently reported that in 6% of a lone AF population (free of mutations in SCNA5A), a BrS ECG pattern could be induced by flecainide testing, thereby indicating that other proteins may be compromised in lone AF patients. Intriguingly, in a recent study by Hu et al., Na<sub>B3</sub>L10P was found in a patient with a flecainide induced BrS ECG pattern. Hence, we sought to investigate the proposed link between AF and BrS in our patient carrying this mutation, yet he declined the offered flecainide test. Thus, we cannot exclude that a BrS ECG pattern could also be induced in this patient, but the patient had not experienced any clinical events pointing to the syndrome.

The study by Hu et al. indicated that L10P gave rise to a trafficking defect in tsA201 cells. Noteworthy, co-expression of the mutant protein resulted in a loss of function of the sodium channel (82.6% decrease in peak current density and 9.6 mV negative shift in steady-state inactivation), which is in accordance with our findings, although to a smaller extent (45% decrease in peak current density and 3.8 mV negative shift in steady-state inactivation). We did not, however, observe a slowing in the steady-state recovery from inactivation. These differences may be explained by the different experimental conditions used in the studies, foremost the different expression systems (CHO-Pro5 vs. tsA201 cells), and the internal pipette solutions.

Both Na<sub>B3</sub>L10P and R6K reside in the extracellular domain (ECD) of the protein and are located within the signal peptide sequence. Intriguingly, Watanabe et al. investigated SCN1–4B in 118 patients with lone AF (onset of disease before 60 years) and in 362 patients with AF and cardiovascular disease and found four mutations, two in SCN1B (R85H and D153N) and two in SCN2B (R28Q and R28W). All those mutations reside in the ECD and confer a loss of function of the sodium channel. Together, their and our studies confirm the importance of this part of the protein for a normally functioning sodium current.

Watanabe et al. did not find non-synonymous mutations in SCN3–4B associated with AF. In their study, they included both lone and non-lone AF patients with an age limit of 60 years at the onset of disease, whereas we investigated a cohort of lone AF patients with onset of disease before the age of 40 years. It could be speculated that our younger lone AF patients are more genetically susceptible to AF than the mixed group of AF patients that Watanabe et al. investigated. Both studies found no non-synonymous mutations in SCN4B.

In conclusion, the present study is to our knowledge the first to associate mutations in SCN3B to lone AF. Taken together with the relative high percentage of positive family history in our cohort, our results indicate that lone AF in young patients has a genetic component.

### 4.1 Limitations

We limited our analysis to the coding regions of SCN3–4B in this study, and the possibility of mutations occurring in regions of the gene other than coding regions cannot be excluded. The functional analyses used a conventional heterologous expression system; hence, the environments differ from that in the native cardiomyocyte. When including patients in the study, it is possible that patients with a family history of AF were more willing to participate than patients without family history, and in most cases, presence of AF in family members relied on anamnestic information.

Genetic testing of family members was limited as some were deceased (those of Patient 1) or unavailable (of Patients 2 and 3). Co-segregation studies were therefore not possible.

### 4.2 Conclusion

In this study of 192 early-onset lone AF patients, we found three suspected disease-causing mutations in SCN3B, all leading to loss of function of the sodium channel. This study supports the hypothesis that disturbances in the sodium current may play a role in the development of lone AF. It is to our knowledge the first study to associate mutations in SCN3B with lone AF. SCN4B is not associated with lone AF in our study.

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### Table 4 Biophysical parameters for three SCN3B mutants associated with AF

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Peak current at −20 mV (pA/pF)</th>
<th>n</th>
<th>Steady-state activation V&lt;sub&gt;i&lt;/sub&gt; (mV)</th>
<th>Slope k value</th>
<th>n</th>
<th>Steady-state inactivation V&lt;sub&gt;i&lt;/sub&gt; (mV)</th>
<th>Slope k value</th>
<th>n</th>
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<tbody>
<tr>
<td>Na&lt;sub&gt;1.5&lt;/sub&gt; + β1 + β3</td>
<td>311 ± 57</td>
<td>20</td>
<td>−28.4 ± 1.4</td>
<td>6.6 ± 0.4</td>
<td>7</td>
<td>−79.2 ± 1.1</td>
<td>48 ± 0.2</td>
<td>10</td>
</tr>
<tr>
<td>Na&lt;sub&gt;1.5&lt;/sub&gt; + β1 + β3-R6K</td>
<td>273 ± 82</td>
<td>18</td>
<td>−31.2 ± 1.8</td>
<td>6.8 ± 0.6</td>
<td>6</td>
<td>−84.2 ± 1.5</td>
<td>48 ± 0.1</td>
<td>11</td>
</tr>
<tr>
<td>Na&lt;sub&gt;1.5&lt;/sub&gt; + β1 + β3-L10P</td>
<td>171 ± 42*</td>
<td>21</td>
<td>−28.5 ± 2.2</td>
<td>7.2 ± 0.9</td>
<td>6</td>
<td>−83.0 ± 0.9</td>
<td>48 ± 0.1</td>
<td>13</td>
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<tr>
<td>Na&lt;sub&gt;1.5&lt;/sub&gt; + β1 + β3-M161T</td>
<td>135 ± 39*</td>
<td>21</td>
<td>−26.1 ± 3.1</td>
<td>7.4 ± 1.0</td>
<td>4</td>
<td>−81.7 ± 0.5</td>
<td>44 ± 0.1</td>
<td>7</td>
</tr>
</tbody>
</table>

*Significantly different from Na<sub>1.5</sub> + β1 + β3 (*P < 0.05).  
#Significantly different from Na<sub>1.5</sub> + β1 + β3 (*P < 0.001).
Acknowledgments
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Supplementary material
Supplementary material is available at Cardiovascular Research online.

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References