Cardiac Sodium Channel Gene Variants and Sudden Cardiac Death in Women

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Background—Several cardiac ion channel genes have been implicated in monogenic traits with a high risk of sudden cardiac death (SCD). Mutations or rare variants in these genes have been proposed as potential contributors to more common forms of SCD, but this hypothesis has not been assessed systematically.

Methods and Results—We directly sequenced the entire coding region and splice junctions of 5 cardiac ion channel genes, SCN5A, KCNQ1, KCNH2, KCNE1, and KCNE2, in 113 SCD cases from 2 large prospective cohorts of women (Nurses’ Health Study) and men (Health Professional Follow-Up Study). Controls from the same population were then screened for the presence of mutations or rare variants identified in cases, and sequence variants without prior functional data were expressed in Xenopus oocytes to assess their biophysical consequences. No mutations or rare variants were identified in any of the 53 subjects who were men. In contrast, in 6 of 60 women (10%), we identified 5 rare missense variants in SCN5A that either had been associated previously with long-QT syndrome (A572D and G615E), had been reported to alter sodium channel function (F2004L), or had not been reported previously in control populations (A572F and W1205C). Of the 4 variants without prior functional data, 3 variants were located in the I-II linker (A572D, A572F, and G615E), and all resulted in significantly shorter recovery times from inactivation. When compared with 733 control samples from the same population, the overall frequency of these rare variants in SCN5A was significantly higher in the SCD cases (6/60, 10.0%) than in controls (12/733, 1.6%; \( P = 0.001 \)).

Conclusion—Functionally significant mutations and rare variants in SCN5A may contribute to SCD risk among women. (Circulation. 2008;117:16-23.)

Key Words: death, sudden epidemiology genetics ion channels women
Although the mutations associated with these primary arrhythmic syndromes are thought to be rare, data exist to suggest that rare sequence variants in these same channels may be associated with more subtle phenotypes in a larger number of individuals. Carriers of such subclinical genetic variants may manifest ventricular arrhythmias only when the arrhythrogenic substrate is further destabilized beyond a particular threshold by an environmental exposure such as ischemia, QT-prolonging drugs, or electrolyte shifts. The contribution of such subclinical mutations or rare alleles to SCD in the general population has not been assessed systematically. To evaluate the hypothesis that mutations or rare variants in cardiac ion channel genes, particularly SCN5A, underlie cases of SCD within an adult population, we sought to determine both the prevalence and function of cardiac ion channel gene coding variants among unselected cases of SCD drawn from 2 large prospective cohorts of women and men.

Methods

Study Populations
The Nurses’ Health Study (NHS) and Health Professional Follow-Up Study (HPFS) are prospective cohort investigations among 121 700 female US registered nurses 30 to 55 years of age at baseline in 1976 (NHS) and 51 529 US male health professionals 40 to 75 years of age at baseline in 1986 (HPFS). Information about medical history, lifestyle choices, and incident disease is assessed biennially by self-administered questionnaires. The validity and reproducibility of the data collected have been reported in detail previously. Blood samples were similar to those who did not, although the men who provided samples were somewhat younger than those who did not.

Study Subjects

End Point Ascertainment
The main study end point comprised incident cases of SCD occurring after the blood sample collection and before February 1, 2004, in the HPFS and June 1, 2004, in the NHS. Deaths were either reported by next-of-kin or postal authorities or identified through a search of the National Death Index. Death certificates were obtained to confirm fatal CHD cases in a 2:1 fashion on the basis of age, smoking, and date of blood draw.

Genotyping
The entire coding sequence and splice junctions of SCN5A, KCNE1, KCNE2, KCNQ1, and KCNH2 were directly sequenced in 60 cases of SCD in women and 53 cases of SCD in men (Figure 1). DNA was extracted from the buffy coat fraction of centrifuged blood, and synthetic oligonucleotides were used to amplify each exon and its flanking splice site sequences directly from genomic DNA by polymerase chain reaction (PCR).

PCR was performed under the following conditions: 30 to 50 ng of genomic DNA was subjected to 35 cycles of amplification in a volume of 25 μL including 10 mmol/L Tris HCl (pH 8.3), 50 mmol/L KCl, 1.5 mmol/L MgCl2, 200 mmol/L dNTP, and 0.2 U Taq polymerase. For sequencing, PCR products were purified, and 25 to 50 ng of DNA was sequenced directly by use of the ABI PRISM dye terminator method (model 377, Applied Biosystems, Foster City, Calif). Electrophoreograms for each amplicon were assembled and analyzed with the use of the Phred/Phrap/Consed software package. Each exon and 10 base pairs upstream or downstream from each exon-intron boundary were analyzed for sequence variants and compared with reference traces. Allelic variants that resulted in amino acid changes in the encoded proteins and that were not identified in controls from the same cohort or in previously reported control populations were classified as mutations, whereas sequence variants found in ≤0.7% of the controls that had evidence for a functional effect were considered rare genetic variants. For those sequence variants not found in any of the control samples, an additional 240 control alleles from individuals of black or Asian-American descent were also screened for the mutation. Sequence variants were confirmed by reamplification, resequencing, and, where possible, by restriction digestion of the PCR products. Once confirmed, the exons containing coding sequence variants were also sequenced in the MI cases, and control samples from the same cohort and the allele frequencies were compared.

Electrophysiological Characterization of Sequence Variants
SCN5A sequence variants were introduced into a wild-type (WT) cDNA expression construct (psP64T-hH1) with the use of site-directed mutagenesis (QuickChange, Stratagene, La Jolla, Calif), and the coding region was resequenced to exclude the introduction of additional mutations. As previously described, the hH1 protein consists of 203 residues with a histidine at position 558, glutamine at position 1027, and a glutamine at position 1077 that is the result of an alternative splice acceptor site for exon 18. The hβ1 coding region was PCR cloned into pCR-TOPO II before subcloning into the pXOOM vector with the use of a HindIII/XbaI fragment. WT psP64T-hH1, mutant psP64T-hH1, and hβ1-pXOOM constructs were linearized with XbaI, and complementary RNA was synthesized from the S6 promoter for WT and mutant psP64T-hH1 or T7 promoter for the hβ1-pXOOM construct (mMessage Machine kit, Ambion, Inc, Austin, Tex). Approximately 5 ng of each complementary RNA was injected into stage IV Xenopus oocytes, and record-

Figure 1. Genomic structure of SCN5A, KCNQ1, KCNH2, KCNE1, and KCNE2. The region of each gene sequenced is indicated by the boxes and corresponds to individual exons and adjacent splice sites. All genes are indicated in a 5’ to 3’ direction.
nings were performed with the use of standard 2-electrode voltage clamp recording techniques 2 to 5 days after injection. Cells were continuously perfused with a bath solution consisting of modified ND-96 (96 mmol/L NaCl, 2.0 mmol/L KCl, 1.8 mmol/L CaCl2, 1.0 mmol/L MgCl2, 5 mmol/L HEPES, 2.5 mmol/L Na-pyruvate, pH 7.6). Signals were amplified with the use of a 2-electrode voltage clamp TEV-200A (Dagan, Inc, Minneapolis, Minn), digitized with a Digidata 1322A A/D converter (Molecular Devices, Inc, Sunnyvale, Calif), and analyzed with Clampex 9.2, Clampfit 9.2 (Molecular

Statistical Analysis

Means or proportions for baseline risk factors were calculated for cases and controls. The significance of associations was tested with the Student t test for continuous variables. We used the Fisher exact test to analyze the frequencies of coding-sequence variations between cases and controls. The significance of associations was tested with the Fisher exact test to analyze differences in the frequencies of coding-sequence variations between groups.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

The clinical characteristics of the SCD cases are outlined in Table 1. All of the SCD cases in women and 49 of those in men were white. The men who suffered SCD were older at the time of death and exercised more frequently than the women SCD victims in the NHS. As in the SCD cases, the majority of these women were white. The SCD cases and MI cases were similar with respect to baseline cardiac risk factors, except that by definition

Table 1. Clinical Characteristics of SCD and MI Cases and Controls

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Men, SCD Cases (n=53)</th>
<th>Women, SCD Cases (n=60)</th>
<th>Women, MI Cases (n=241)</th>
<th>Women, MI Controls (n=492)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean±SD, y*</td>
<td>66.5±8.1</td>
<td>60.8±6.1</td>
<td>60.4±6.5</td>
<td>60.3±6.5</td>
</tr>
<tr>
<td>White, n (%)</td>
<td>49 (96.1)</td>
<td>60 (100)</td>
<td>237 (98.3)</td>
<td>488 (99.2)</td>
</tr>
<tr>
<td>Current smoker, n (%)</td>
<td>8 (15.4)</td>
<td>17 (28.3)</td>
<td>75 (31.1)</td>
<td>155 (31.5)</td>
</tr>
<tr>
<td>Body mass index, mean±SD</td>
<td>26.2±3.9</td>
<td>26.9±5.2</td>
<td>26.8±5.9</td>
<td>25.4±4.1</td>
</tr>
<tr>
<td>Parental history of MI at &lt;60 y, n (%)</td>
<td>12 (22.6)</td>
<td>12 (20.0)</td>
<td>53 (22.0)</td>
<td>60 (12.2)</td>
</tr>
<tr>
<td>Postmenopausal, n (%)</td>
<td>...</td>
<td>51 (85.0)</td>
<td>207 (85.9)</td>
<td>411 (83.3)</td>
</tr>
<tr>
<td>Hormone replacement therapy use, n (%)</td>
<td>...</td>
<td>20 (33.3)</td>
<td>74 (30.7)</td>
<td>179 (36.0)</td>
</tr>
<tr>
<td>Aspirin use &gt;22 d/mo, n (%)</td>
<td>15 (29.4)</td>
<td>15 (25.0)</td>
<td>48 (19.9)</td>
<td>88 (17.9)</td>
</tr>
<tr>
<td>History of hypertension, n (%)§</td>
<td>31 (58.5)</td>
<td>34 (56.7)</td>
<td>138 (57.3)</td>
<td>144 (29.3)</td>
</tr>
<tr>
<td>History of diabetes mellitus, n (%)‡</td>
<td>9 (17.0)</td>
<td>9 (15.0)</td>
<td>48 (19.9)</td>
<td>33 (6.7)</td>
</tr>
<tr>
<td>Alcohol, median (IQR), g/d</td>
<td>3.3 (0–9.6)</td>
<td>0.9 (0–11.2)</td>
<td>0.9 (0–3.7)</td>
<td>1.8 (0–8.6)</td>
</tr>
<tr>
<td>Physical activity, median (IQR), MET-h/wk*</td>
<td>25.6 (8.6–50.5)</td>
<td>8.4 (2.5–25.7)</td>
<td>7.7 (2.9–20.2)</td>
<td>9.3 (3.7–20.4)</td>
</tr>
<tr>
<td>History of CVD, n (%)‡§</td>
<td>7 (13.2)</td>
<td>9 (15)</td>
<td>9 (3.7)</td>
<td>10 (2.0)</td>
</tr>
</tbody>
</table>

IQR indicates interquartile range; MET, metabolic equivalent.

*P<0.001 for comparison of men vs women SCD cases.
†P<0.003 for comparison of women SCD cases vs women MI cases.
‡P<0.05 for comparison of women SCD cases vs women MI controls.
§P<0.001 for comparison of women SCD cases vs women MI controls.

Table 1. Clinical Characteristics of SCD and MI Cases and Controls

*P<0.001 for comparison of men vs women SCD cases.
†P<0.003 for comparison of women SCD cases vs women MI cases.
‡P<0.05 for comparison of women SCD cases vs women MI controls.
§P<0.001 for comparison of women SCD cases vs women MI controls.

CVD defined as a reported history of coronary revascularization or confirmed angina, MI, or stroke before the blood draw.

One of the variants, F2004L, had previously been associated with sudden infant death syndrome in a Norwegian population. Two others had been associated with long-QT syndrome (A572D) and drug-induced torsades de pointes (G615E), and 2 were novel (A572F, W1205C). Two variants (F2004L, A572D) had also been reported in control populations. The W1205C transition is located at a highly conserved amino acid in the DI/II/SI transmembrane domain, and 3 others (A572D, A572F, G615E) were located in the DI-II linker. The F2004L substitution affects a residue in the extreme carboxyl terminus in a region of the protein with limited conservation at amino acid level. No other unique or rare coding sequence variants were identified in the remaining 5075 nucleotides from the other 4 ion channel genes among the 60 women.

The 6 women carrying the SCN5A variants died at ages ranging from 53 to 76 years. Three of the women with SCN5A variants had no known prior history of cardiac disease. The woman with the A572F variant had a history of cardiomyopathy, the woman with the A572D variant had a history of atrial fibrillation and congestive heart failure, and 1 of the women with the F2004L variants had a history of MI before the SCD. We did not routinely collect ECGs in these cohorts, and only the woman with the A572D variant had an ECG after resuscitation from a ventricular fibrillation arrest, which revealed an irregular wide-complex tachycardia of 2 morphologies.

To estimate the allele frequency of these variants in this population and to determine their specificity for SCD, we sequenced the exons containing these variants in a total of 241 MI cases and their 492 smoking- and age-matched controls from the NHS. As in the SCD cases, the majority of these women were white. The SCD cases and MI cases were similar with respect to baseline cardiac risk factors, except that by definition
the MI cases were free from cardiovascular disease at the time of the blood draw (Table 1). As expected, both the SCD and MI cases were more likely to have diabetes mellitus and hypertension and to be overweight compared with the control women without MI, associations previously reported in this cohort.

Among the 733 women (MI cases and controls), 3 of the variants were rare. The A572D, G615E, and F2004L variants were found in 2, 1, and 9 women, respectively, yielding estimated allele frequencies of 0.1%, 0.06%, and 0.6%. No differences were present in the frequency of these variants in MI cases versus controls. The 2 other sequence variants (A572F, W1205C) were considered mutations because they were not present in any of these control samples or in an additional 240 control alleles from individuals of black or Asian American descent. The carrier frequency of these 5 sequence variants in SCN5A was significantly higher in SCD cases (6/60, 10.0%) than in the combined MI cases and controls (12/733, 1.6%; \( P = 0.001 \)).

One of the rare variants, F2004L, has been demonstrated previously to have an effect on sodium channel function characterized by mildly increased persistent sodium current combined with depolarizing shifts in the voltage dependence of inactivation and faster recovery from inactivation. For the other 4 variants without clear functional data, electrophysiological characterization was performed in *Xenopus* oocytes. Expression of A572F, A572D, G615E, or W1205C all

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**Figure 2.** Topology, location, and conservation of SCN5A variants. A, Topology of SCN5A with the location of each variant. The sodium channel consists of a homotetrameric protein with 6 transmembrane spanning segments in each motif. B, Electrophoretograms of the WT and variant sequences. C, Conservation across species. The alignment was generated with the use of ClustalW and the following sequences: human (NP_000326.2), rat (AAK38884.1), dog (NP_001002994.1), mouse (CAB70096.1), and cow (CAC80974.1).
revealed robust currents, similar in magnitude to those observed with the WT SCN5A construct. Voltage-dependent activation was unchanged in each variant compared with the WT SCN5A construct (Table 2). A hyperpolarizing shift was present in the midpoint of voltage-dependent inactivation (V_{1/2}) in the A572F variant compared with the WT construct (−63.2±0.1 versus −60.8±0.2 mV; P=0.01) and a subtle depolarizing shift of V_{1/2} in the G615E variant (−59.1±0.1 versus −60.8±0.2; P=0.002) (Table 2 and Figure 3A). For each of the variants in the I-II linker, the fast time constant (τ_f) for recovery from inactivation was significantly faster than the WT variant (5.6±0.4, 5.3±0.5, 5.3±0.1 versus 7.3±0.6 ms for A572F, A572D, G615E, and WT, respectively; P<0.005 in each case). No significant difference existed in the time course of recovery in the W1205C variant compared with the WT variant (Table 2 and Figure 3B).

### Discussion

Although mutations or rare variants in cardiac ion channel genes were not a common cause of SCD in these 2 prospective cohorts, such variants were present in a significant minority (10%) of SCD cases among women. Despite the genetic heterogeneity observed in familial arrhythmic syndromes, all of the missense variants identified were in a single ion channel gene, the cardiac sodium channel SCN5A. Regional and transmural variation in sodium channel conductance is responsible for much of the normal heterogeneity of ventricular repolarization, and perturbations of this heterogeneity can result in life-threatening ventricular arrhythmias in diverse disease states. Mutations in SCN5A have been associated with multiple discrete phenotypes, many with a propensity for ventricular arrhythmias, most notably the long-QT syndrome (LQTS) and Brugada syndromes. Recently, mutations and rare variants in SCN5A were found to account for approximately half of the gene variants reported in the largest survey of sudden infant death syndrome victims, in which ion channel variants were found in 9.5% of sudden infant death syndrome cases. This study, unlike our own, also found variations in KCNQ1 and KCNH2, although to a lesser extent. Our data now suggest that mutations or rare variants with subtle effects on cardiac sodium channel function may also predispose to SCD among white women.

The F2004L variant found in 2 of our adult SCDs had previously been reported in 3 sudden deaths among infants in Norway. This substitution along with the 3 SCN5A

### Table 2. Voltage-Dependent Activation, Steady State Availability, and Time Course of Recovery for SCN5A Variants

<table>
<thead>
<tr>
<th>Variant</th>
<th>Voltage Dependence of Activation</th>
<th>Steady State Availability</th>
<th>Time Course of Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>V_{1/2}, mV</td>
<td>k</td>
<td>n</td>
</tr>
<tr>
<td>WT</td>
<td>−28.5±0.5</td>
<td>−6.6±0.4</td>
<td>9</td>
</tr>
<tr>
<td>A572F</td>
<td>−32.3±0.5</td>
<td>−6.0±0.6</td>
<td>15</td>
</tr>
<tr>
<td>A572D</td>
<td>−31.0±0.3</td>
<td>−5.5±0.3</td>
<td>8</td>
</tr>
<tr>
<td>G615E</td>
<td>−31.9±0.4</td>
<td>−5.9±0.4</td>
<td>9</td>
</tr>
<tr>
<td>W1205C</td>
<td>−31.1±0.4</td>
<td>−5.9±0.3</td>
<td>8</td>
</tr>
</tbody>
</table>

Values are mean±SE.

*p<0.01, †p=0.002, ‡p=0.005, §p=0.001.
variants located in the I-II linker all resulted in significantly shorter recovery times from fast inactivation. This same biophysical defect has also been observed in 6 other SCN5A variants associated with sudden infant death syndrome. More rapid recovery from fast inactivation was first reported in association with Brugada syndrome but subsequently has been reported in long-QT syndrome as well. In the latter disorder, as for many of the sudden infant death syndrome–associated mutations (including the F2004L variant), this defect typically occurs in association with other significant alterations in channel gating. Given the subtle nature of the associated biophysical findings compared with those generally observed in the long-QT and Brugada syndromes, we suspect that these variants may not act in isolation but rather require the presence of another genetic variant or environmental factor. The clustering of 3 of the variants in a single cytoplasmic domain of SCN5A also raises the possibility that a unifying mechanism may involve interacting partner proteins rather than just simple changes in sodium conductance.

The final variant (W1205C), located in a highly conserved transmembrane domain, did not have any detectable effect on ion channel function in a heterologous expression system. The absence of any electrophysiological phenotype suggests that this variant may not exert a physiological effect. Alternatively, effects of the variant on pathways other than ion conductance or, indeed, changes in conductance properties dependent on interacting proteins not represented in the Xenopus oocyte system potentially might result in a phenotype in vivo. It is also possible that the mutation may have exhibited an electrophysiological effect if expressed on the background of other common polymorphisms that have been shown to influence the functional effects of mutations in other studies. These fundamental uncertainties about the association of specific phenotypes with genomic variants serve to emphasize the need for efficient in vivo modeling or other rigorous functional tests that capture the complexity of channel partner–protein interactions to discriminate genetic noise from real signals.

Our finding that rare sequence variants in SCN5A were only present in SCD cases among women may be due to chance in this small sample. However, a similar paucity of SCN5A variants that result in subtle alterations in channel conductance.34 These data suggest that future studies of the genetic basis of arrhythmic risk will have to include not only analyses of common variation in ion channel genes but also coding and noncoding variants across the genome.47 A major challenge will be the development of functional assays to evaluate the significance of the resultant genetic data.

Several limitations of the present study warrant consideration. First, these large prospective cohort studies were not specifically designed to identify patients with arrhythmic disorders, and specific questions about these disorders, including systemic family histories and ECGs, were not obtained. As a result, we were not able to determine whether these SCN5A variants resulted in detectable ECG changes or were associated with a familial history of syncope or SCD. Second, the cohorts were recruited from health professionals, and this selectivity may limit the generalizability of the findings. These data also exemplify the difficulty in determining whether rare genetic variants found in patients with disease are pathogenic. In genetic screening for familial arrhythmic syndromes, the absence of a nonsynonymous variant in control populations is often considered adequate evidence to label the variant a disease-causing mutation; however, this will depend greatly on the number and type of controls screened. Two of the sequence variants initially reported to be absent in control samples were later detected, albeit at a lower frequency, in larger numbers of controls derived from the same population. In addition, the presence or absence of such rare variants in control populations does not always predict whether the variant will be associated with a functional alteration in channel properties.

In summary, mutations or rare variants in SCN5A may contribute to SCD risk in the general population, particularly in women. However, given the low overall prevalence of these variants (<2%), screening the general population for SCN5A mutations would not be warranted at this time. Further studies aimed at identifying genetic or functional markers of SCD risk and elucidating the mechanisms of such death in the general population are needed if we are to improve the prediction and prevention of such events.

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Disclosures
None.

References
CLINICAL PERSPECTIVE

Our present ability to identify individuals who are at risk for sudden cardiac death (SCD) in the general population is poor. Although SCD risk has a heritable component, our understanding of the genetic basis of SCD is most advanced in rare arrhythmic disorders such as the long-QT and Brugada syndromes, in which mutations in genes encoding cardiac ion channels result in increased susceptibility for SCD. The extent to which the heritable component of more common forms of SCD might be due to similar mutations or rare polymorphisms in these same genes is currently unknown. To address this question, we determined both the prevalence and function of mutations and rare coding sequence variants in 5 cardiac ion channel genes among 113 unselected cases of SCD drawn from 2 large prospective cohorts of women and men. No mutations or rare variants were identified in any of the 53 subjects who were men. In contrast, 2 mutations and 3 rare missense variants in a single ion channel gene, the cardiac sodium channel SCN5A, were found in 6 of 60 women (10%), and all but 1 resulted in significantly shorter recovery times from channel inactivation. The overall frequency of these rare variants in SCN5A was significantly higher in the SCD cases (6/60, 10.0%) compared with controls (12/733, 1.6%; P = 0.001) from the same population. These data suggest that functionally significant rare variants in SCN5A may contribute to SCD risk in the general population and particularly among women.
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