Role of SCN5A Y1102 Polymorphism in Sudden Cardiac Death in Blacks

Allen Burke, MD; Wendy Creighton, MD; Erik Mont, MD; Ling Li, MD; Susan Hogan, MD; Robert Kutys, MS; David Fowler, MD; Renu Virmani, MD

Background—The Y1102 polymorphism of the cardiac sodium channel (SCN5A) gene has been found in 13% of black Americans. It has been linked to lethal arrhythmias in black families with ventricular tachycardia. The prevalence of the Y1102 polymorphism in a series of sudden death in blacks is unknown.

Methods and Results—We investigated the incidence of the Y1102 polymorphism in a series of 289 sudden deaths in blacks by sequencing an amplified segment of DNA that contained the polymorphic site extracted from prospectively sampled frozen splenic tissue. The deaths were classified as noncardiac controls (n=107), cardiac arrhythmias with clear anatomic substrate (n=117), cardiac arrhythmias with no anatomic substrate except mild to moderate cardiac hypertrophy (n=40), and unexplained cardiac arrhythmias (n=25). Cause of death was determined after complete forensic autopsy and postmortem cardiac examination. The overall frequency of the Y1102 polymorphism was 9.0%. The frequency was 5.6% in noncardiac deaths, 4.3% in cardiac deaths with obvious anatomic substrate, 20.0% in arrhythmias with moderate hypertrophy, and 28% in unexplained arrhythmias. Adjusted for age and sex, the relative risk of an unexplained arrhythmic death was 8.4 (95% CI 2.1 to 28.6, P=0.001) with the Y1102 allele compared with noncardiac deaths. The relative risk for cardiac arrhythmias with mild cardiac hypertrophy was 4.9 (95% CI 1.3 to 13.4, P=0.01).

Conclusions—The Y1102 allele is a risk factor in blacks for sudden cardiac death in the absence of obvious morphological findings or mild to moderate cardiomegaly. (Circulation. 2005;112:798-802.)

Key Words: ion channels ■ epidemiology ■ polymerase chain reaction ■ sudden death ■ tachyarrhythmias

The SCN5A gene encodes the α-subunit of the voltage gated cardiac sodium channel.1 Mutations in this gene have been associated with sudden death in the long-QT3 syndrome2 and in Brugada syndrome.3 More recently, a single nucleotide polymorphism, which results in a substitution of tyrosine (Y1102) for serine (S1102, wild type) has been identified in blacks. Although generally considered benign, the Y1102 allele is disproportionately represented in a group known to be at high risk for lethal ventricular arrhythmias.4

Sudden unexpected death due to cardiac causes is more frequent in blacks than in their white counterparts.5–7 Although the rates of sudden unexpected death due to severe coronary atherosclerosis are similar in whites and blacks, sudden deaths due to nonatherosclerotic heart disease, especially hypertensive left ventricular hypertrophy, are far more frequent in blacks.7

In a minority (approximately 10% to 20%) of complete autopsies in young adults dying suddenly of apparent cardiac arrhythmias, no anatomic substrate is identified. In a larger subset, only minor anatomic substrates, such as moderate left ventricular hypertrophy, are identified. The role of occult abnormalities in the cardiac ion channels in sudden unexpected deaths without obvious morphological cause is currently unknown.

The purpose of this study was to determine the frequency of the Y1102 allele in a series of sudden unexpected deaths in blacks. An increase in this allele in sudden unexpected deaths without clear anatomic substrate should provide evidence that the allele contributes to lethal arrhythmias in sporadic sudden deaths in blacks, possibly accounting for the increased risk for sudden death in this racial group.

Methods

During the 6 years from 1996 to 2001, we prospectively examined cases of unexpected sudden deaths in blacks aged >10 years seen in consultation from the Office of the Chief Medical Examiner in Maryland. The criteria for collection were cases of apparent sudden unexpected death (witnessed arrest or arrest within 1 hour of normal state of health, or deceased found dead within 12 hours of having been in normal state of health, in apparent normal activity, such as...
sleep, work, or exercise). Cases were included only after full forensic autopsy disclosed no definitive noncardiac cause of death. During the study period, there were 631 total autopsies in blacks aged >10 years who died suddenly and unexpectedly of cardiac disease. There were 254 black men with the cause of death determined as coronary atherosclerosis, 75 black women with atherosclerosis, 196 black men with hypertensive heart disease and cardiomyopathies, 45 black women with hypertensive heart disease and cardiomyopathies, 37 black male arrhythmic deaths, and 24 black female arrhythmic deaths. During the same period, 1051 inspections were performed in blacks younger than 60 years of age for whom the cause of death was attributed to heart disease (generally atherosclerotic heart disease) in the absence of autopsy. From the autopsied cases, we were able to collect DNA samples on 182 (29%) of the total 631 for inclusion in the present study. The reason that only 29% were collected was due to study limitations; procedures for case collection were in place from 2 to 4 days weekly during the study period. These days were typically but not always during weekdays. There was no other bias in case collection, because cases were selected uniformly during days assigned for collection.

During the same period and collected at the same time, 107 cases were collected in a similar manner to serve as controls (group 1). The final diagnosis in these cases were drug overdoses (n=31), end-stage alcoholism (n=17), infections (n=11), trauma (n=11), bleeding (n=9), pulmonary embolism (n=8), aortic dissection (n=7), diabetic ketoacidosis (n=5), end-stage renal disease (n=3), anaphylaxis (n=2), asthma (n=1), complex congenital heart disease (n=1), and carcinomatosis (n=1).

We have previously reported the findings of sudden coronary deaths in blacks of a subset of these cases autopsied in 1999 and earlier, and heart study including determination of heart weight was performed as described previously.7,8 The determination of alcoholism was based on history during the scene investigation, and the hypertension was performed based on scene investigation including medication usage and microscopic evaluation of renal vasculature as described previously.9

Causes of death in the noncontrol cardiac cases (sudden cardiac death cases) were classified into 4 groups after the results of all autopsy data were available and before determination of SCNS5A polymorphism. There were 117 cardiac deaths with cardiac morphological substrate (group 2), 40 cardiac deaths with no morphological substrate other than mild to moderate left ventricular hypertrophy (group 3), and 25 cardiac arrhythmic deaths without morphological substrate (group 4, unexplained arrhythmias). Mild to moderate cardiomegaly was defined as heart weight increased for body weight as determined by population-based tables in the absence of marked cardiomegaly. Marked cardiomegaly was defined as heart weight greater than 600 g for men and 500 g for women. In the last category, cardiac arrhythmia was determined on the basis of exclusion of other causes of death by full forensic autopsy.

**Determination of Y1102 Allele Presence**

DNA was isolated from splenic tissue collected prospectively. Genomic DNA was isolated from spleens by standard procedures. DNA samples were amplified by polymerase chain reaction (PCR) for exon 18 of SCNS5A using the following oligonucleotides: F1, 5′-AGGGTCTAAACCCCAAGGCGTCA-3′, and R1, 5′-CCCCACGCTCGCCTCAAGGGCACAAA-3′. PCR products were labeled fluorescently with the BigDye Terminator Cycle Sequencing Ready Reaction Kit (ABI Prism). The labeled samples were then analyzed on an ABI377 automated DNA sequencer (Applied Biosystems). DNA sequences from sudden death victims were compared with those in GenBank databases to determine the genotypes at 1102. The genomic determination was done in a blinded fashion, without knowledge of causes of death but only with numeric identifier.

**Determination of Control Marker of African Origin**

FY T-46C genotyping (for the determination of the FY-B null polymorphism for the Duffy antigen/receptor for chemokine) was performed as described previously.12,13 PCR reactions were conducted with human genomic DNA samples between primer P3, 3′-AGGGTCTAAACCCCAAGGCGTCA-3′, and primer P39, GGCATAGGGCGAGGGACT. PCR products were purified on Microcon (Millipore) and sequenced directly with an ABI 377 automated sequencer.

Differences in allele frequencies between sudden coronary death cases and controls were assessed by logistic regression (nominal logistic fit; JMP software, SAS Institute). Group 1 was used as the control group, and the allele frequency was compared with groups 3 and 4 in 2 separate analyses. There were 2 nominal variables: occurrence of sudden death (as opposed to control death; dependent variable), and the presence of Y1102 allele (as opposed to wild-type allele). For multivariable analysis, independent variables were in Y1102 allele, sex, age, hypertension, heart weight, and alcoholism, and the dependent variable was occurrence of sudden death. All variables were nominal with the exception of age and heart weight (continuous). Because women were underrepresented in groups 3 and 4, a sex/Y1102 allele interaction term was included as a parameter in the regression analysis. Probability values were 2-sided.

**Results**

The rate of sudden unexpected deaths in blacks during the study period in the state of Maryland was 12 per 100 000 in the fourth and 75 per 100 000 in the fifth decades (data available at www.census.gov/population/estimate). Given that the rate of autopsies performed at the Medical Examiner was 78% in the fourth decade and 54% in the fifth decade of life, 47% of sudden cardiac deaths were attributed to coronary artery disease; of the remainder, 48% of deaths were caused by cardiomyopathies or severe hypertensive heart disease, 20% by mild to moderate cardiomegaly, 13% by cardiac arrhythmias of uncertain cause, 11% by valve disease, 6% by coronary nonatherosclerotic heart disease, and 2% by complex congenital heart disease. The specific causes of death in the 117 cardiac cases (group 2) in the study were as follows: coronary thrombosis due to acute plaque rupture (31), severe coronary atherosclerosis without thrombosis with healed myocardial infarction (28), coronary thrombosis due to acute plaque erosion (16), severe coronary atherosclerosis with marked cardiomegaly (4), left ventricular hypertrophy with marked cardiomegaly (8), dilated cardiomyopathy with marked cardiomegaly (12), hypertrophic cardiomyopathy (5), anaplastic left coronary artery origin (4), severe aortic stenosis with marked left ventricular hypertrophy (2), arrhythmogenic right ventricular dysplasia (2), mitral valve prolapse (3), cardiac sarcoidosis (1), and extensive myocarditis with scarring (1).

The mean ages and gender distribution of the 4 study groups with complete heart dissection and genetic analysis are given in Table 1. The mean age of group 4 patients (unexplained arrhythmic deaths in the absence of morphological findings) was significantly less than group 2 (cardiac deaths; P=0.02).

### Table 1. Mean Age, Gender Distribution, and Percent of Y1102 Polymorphism in the 4 Groups

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Mean Age, y</th>
<th>M:F Ratio</th>
<th>% Heterozygote for Y1102 Allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (controls)</td>
<td>107</td>
<td>44.5±11.6</td>
<td>78:22</td>
<td>5.6</td>
</tr>
<tr>
<td>Group 2 (cardiac deaths)</td>
<td>117</td>
<td>46.3±11.5</td>
<td>91:26</td>
<td>4.3</td>
</tr>
<tr>
<td>Group 3 (mild cardiac abnormalities)</td>
<td>40</td>
<td>44.4±8.9</td>
<td>32:68</td>
<td>20.0</td>
</tr>
<tr>
<td>Group 4 (unexplained arrhythmias)</td>
<td>46</td>
<td>50.2±13.0</td>
<td>14:11</td>
<td>28.0</td>
</tr>
</tbody>
</table>

AGGCTTGTGCAGGCAGTG, and primer P39, GGCATAGGGCGAGGGACT. PCR products were purified on Microcon (Millipore) and sequenced directly with an ABI 377 automated sequencer.
The Y1102 polymorphism of the SCN5A gene has recently been identified at high frequency in black families at risk for sudden death, including individuals with syncope, aborted atrial septal defect repair.

No patient was homozygous for the Y1102 allele. The frequency of Y1102 heterozygosity is given in Table 1. By univariate analysis, the frequency of heterozygosity was significantly higher in group 4 than in group 1 ($P=0.0005$) or group 2 ($P=0.0007$). The frequency of heterozygosity was significantly higher in group 3 than in group 1 ($P=0.01$) or group 2 ($P=0.004$). When group 1 and group 4 were combined (arrhythmic deaths versus controls), the Y1102 allele was independently associated with arrhythmic death, adjusted for age, sex, presence of hypertension, body mass index, alcoholism, presence of alcoholism, and heart weight ($P=0.001$). The relative risk for cardiac arrhythmias with mild cardiomegaly was 4.9 (95% CI 1.3 to 13.4, $P=0.01$) with patients with the Y1102 allele compared with noncardiac deaths (group 3 versus group 1).

The frequency of the FY*B null allele (T46–C substitution) was similar in all 4 groups (150 in group 1, 153 in group 2, 156 in group 3, and 152 in group 4, per 100 000 individuals, $P>0.9$). Clinicopathological data pertaining to heterozygous group 3 and 4 individuals are presented in Tables 2 and 3, respectively.

**Discussion**

The present study demonstrates in an autopsy series that the Y1102 polymorphism of SCN5A was disproportionately represented in sudden deaths without morphological abnormalities compared with those who died of noncardiac causes or of sudden death with a clear morphological abnormality. This increased risk was seen only in a small subset of sudden death patients: those with no or mild cardiac structural defects. Whether this difference represents a truly increased risk of sudden deaths in patients with this polymorphism, as suggested by Splawski et al., remains to be determined. The present study does demonstrate that the underlying cardiac morphological abnormality in blacks with the Y1102 polymorphism dying of unexplained arrhythmias is mostly likely no abnormality or mild to moderate cardiomegaly.

Genetic defects in the cardiac potassium and sodium ion channels result in a heterogeneous group of diseases that manifest as long-QT interval, ventricular tachyarrhythmias (characteristically, torsade de pointes), and sudden death. Recently, the Brugada syndrome has been linked to abnormalities of the sodium channel, and mutations in the ryanodine receptor have been implicated in sudden death in families with familial polymorphous ventricular tachycardia. Ryanodine receptor mutations result in increased sensitivity of calcium-induced activation of the calcium-release (L-type calcium channel) complex, without long-QT intervals. The diverse genetic defects related to cardiac ion channels may lead to lethal ventricular arrhythmias in the absence of any morphological findings in the heart or conduction system at autopsy.

Abnormalities of the sodium channel gene have been implicated in sudden death in both long-QT3 syndrome and Brugada syndrome. In the former, there is an increase in the sodium current activation, and in the latter, a decrease. Both syndromes are characterized by sudden death at rest; in contrast, potassium channel and ryanodine receptor defects often result in ventricular tachyarrhythmias in response to exertion or adrenergic or auditory stimuli.

The Y1102 polymorphism of the SCN5A gene has recently been identified at high frequency in black families at risk for sudden death, including individuals with syncope, aborted atrial septal defect repair.

**TABLE 2. Findings in Sudden Deaths With Y1102 Polymorphism and Mild to Moderate Cardiomegaly**

<table>
<thead>
<tr>
<th>Age, y</th>
<th>Sex</th>
<th>Heart Weight, g</th>
<th>Cardiac Findings</th>
<th>History</th>
<th>Activity at Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>26</td>
<td>Male</td>
<td>520</td>
<td>Concentric left ventricular hypertrophy</td>
<td>Obesity, asthma (not seen at autopsy)</td>
<td>Sleeping</td>
</tr>
<tr>
<td>37</td>
<td>Female</td>
<td>490</td>
<td>Dilated cardiomyopathy (mild)</td>
<td>Obesity</td>
<td>Shopping</td>
</tr>
<tr>
<td>42</td>
<td>Male</td>
<td>590</td>
<td>Concentric left ventricular hypertrophy</td>
<td>Alcoholism</td>
<td>Riding on bus</td>
</tr>
<tr>
<td>43</td>
<td>Male</td>
<td>540</td>
<td>Concentric left ventricular hypertrophy; moderate atherosclerosis</td>
<td>Obesity</td>
<td>Driving</td>
</tr>
<tr>
<td>45</td>
<td>Male</td>
<td>480</td>
<td>Concentric left ventricular hypertrophy</td>
<td>No history</td>
<td>Collapsed on street</td>
</tr>
<tr>
<td>47</td>
<td>Male</td>
<td>470</td>
<td>Concentric left ventricular hypertrophy</td>
<td>Hypertension</td>
<td>Found in kitchen</td>
</tr>
<tr>
<td>51</td>
<td>Male</td>
<td>445</td>
<td>Concentric left ventricular hypertrophy; remote atrial septal defect repair</td>
<td>Atrial septal defect, remote drug use</td>
<td>Sleeping</td>
</tr>
<tr>
<td>70</td>
<td>Male</td>
<td>580</td>
<td>Concentric left ventricular hypertrophy</td>
<td>Syncope, AICD placement</td>
<td>Riding on bus</td>
</tr>
</tbody>
</table>

AICD indicates automatic implantable cardioverter defibrillator.
sudden death, medication- or bradycardia-associated prolongation of the QTc interval, and documented ventricular tachyarrhythmias. The single-nucleotide polymorphism Y1102 consists of a transversion of C to A in codon 1102 of the SCN5A gene, which results in a substitution of serine (S1102, wild type) to tyrosine (Y1102). Splawski et al. screened control populations of different races for Y1102 and found the single-nucleotide polymorphism in 13.2% of blacks (27/205) and 0% of whites (0/511) and Asians (0/578). The authors then compared the allele frequency between a study population of black individuals thought to be at high risk for arrhythmias with a control group of healthy blacks and found Y1102 to be disproportionately represented in the high-risk group (47.8% versus 13% of controls). They further examined the extended family of 1 proband from the study population and demonstrated linkage between Y1102 and an “arrhythmia phenotype.” Cell transfection studies demonstrated accelerated channel activation with the variant allele, which suggests a mechanism for abnormal cardiac action potential and arrhythmia.

The present study is the first to investigate the possible role of the SCN5A Y1102 polymorphism in sporadic arrhythmic sudden deaths in blacks. In deaths with obvious cardiac abnormality, there was no increase in the allele over the control group; however, in cases without anatomic abnormality, the allele was present in more than one fourth of the subjects, and in patients dying with mild to moderate left ventricular hypertrophy, it was present in one fifth of subjects, more than twice the frequency seen in control subjects. By logistic regression, these results indicated a several-fold increase in the relative risk of sudden death in blacks compared with those without the polymorphism. Because the rate of sudden unexpected death in blacks in the target age range (30 to 50 years) was approximately 50 per 100,000,7 and of these, ~20% were due to mild to moderate cardio-megaly or idiopathic arrhythmias, 10 of 100 000 deaths in blacks aged 30 to 50 years are caused by conditions that may be related to the Y1102 polymorphism. Given a population in the state of Maryland of 200 000 blacks aged 30 to 50 years, the number of such arrhythmic deaths would be 20 yearly,7 ~20% of which would harbor the Y1102 allele. Therefore, there may be nearly 4 black deaths yearly in Maryland attributable in part to the SCN5A polymorphism.

In the present study, we found the Y1102 allele in 9.0% of all blacks and in 5.7% of controls. The only other study addressing the frequency of the Y1102 allele in blacks, to the best of our knowledge, is that by Splawski et al.,4 who found the allele present in 27 of 205 patients, or 13.2%. The lower frequency in cases in the present study, which was restricted to the state of Maryland, remains unexplained because variations of the Y1102 allele in different black populations is unknown. The frequency of the FY*B null allele, a marker of African descent, was 150 per 100 000, quite similar to the 145 per 100 000 found in the study by Zimmerman et al.,18 which indicates that the present study population of blacks demonstrated an expected incidence of a well-described marker of African descent.

Study Limitations

The relative risk calculations in the present study were based on a frequency of 5.7% for the Y1102 allele, which is lower than that found in the prior study by Splawski et al.4 Therefore, they may be elevated spuriously, if for some reason, our control population was skewed against having this genetic variation. Because the data were collected prospectively but studied retrospectively, no attempt could be made to identify kindreds and determine whether there was an increase in sudden death in first-degree family members. Furthermore, the potential role of other genetic defects, especially those of other cardiac ion channels or other regions of SCN5A, was not investigated in the present study, nor did we investigate the possibility of population stratification causing a biased result. Finally, not every case of unexpected death was analyzed, and a collection bias in the study cases cannot be excluded completely.

Because this is an autopsy series, the control subjects could not be chosen randomly but by necessity were individuals of the same ethnic background who were autopsied at the same time as the test subjects (sudden cardiac death without overt causes or minimal morphological findings). Therefore, the concept of relative risk is that of dying of a presumed arrhythmia versus another noncardiac cause, and not that of a healthy control population. Because of the small number of Y1102 carriers, our estimates of odds ratios lack precision, as reflected by wide CIs. Because multiple hypothesis tests were conducted, there is a possibility of inflated type I error.

In summary, we describe an increased incidence of the SCN5A Y1102 polymorphism in a series of sudden unexpected deaths in blacks dying suddenly with no anatomic findings or mild to moderate cardiomegaly. The role of the allele in promoting sudden death in blacks with left ventricular hypertrophy, as well as its role, possibly in conjunction with other genetic defects, in sporadic sudden death in blacks dying without apparent anatomic cause needs to be studied further clinically.

References

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