Postmortem Molecular Analysis of SCN5A Defects in Sudden Infant Death Syndrome

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Sudden infant death syndrome (SIDS) remains elusive in its causes and devastating in its consequences. Despite the impressive decline in the incidence of SIDS since the nationwide “Back-to-Sleep” campaigns and the recommendation to avoid the prone sleep position,1-4 SIDS remains a leading cause of death in the first year of life. In 1998, the rate of SIDS was 0.64 per 1000 live births in the United States.5 Thus, SIDS still claims more than 2500 infants each year and accounts for approximately 9% of the more than 28000 infants who die before reaching their first birthday.5

Despite numerous hypotheses for the causes of SIDS, including respiratory dysfunction, cardiorespiratory instability, cardiac dysrhythmias, and inborn errors of metabolism, the pathophysiological mechanisms responsible for SIDS remain poorly understood.6,7 Recently, the discovery of a deficit in a serotonergic pathway in the brainstem8,9 and the contribution of prone sleep position to the pathophysiological process have provided promising insights into possible mechanisms.10 In addition, investigators have postulated that ventricular arrhythmias and the congenital long QT syndrome (LQTS) may be responsible for some cases of SIDS.11-16 Clinically, LQTS affects approximately 1 in 5000 individuals; they can show on electrocardiography QT prolongation of greater than 460 milliseconds. Long QT syndrome presents with syncope, seizures, or sudden death if the LQTS substrate degenerates into a polymorphic ventricular tachyarrhythmia (torsade de pointes). Long QT syndrome is a primary cardiac channelopathy with 6 identified chromosomal loci and 5 cardiac ion channel genes implicated.17-18 Defects in the cardiac sodium channel gene (SCN5A) account for approximately 5% to 10% of LQTS and individuals with SCN5A mutations have an increased risk of cardiac events during sleep.19,20

In 1998, Schwartz and colleagues21 reported results from a 19-year electrocardiographic assessment of more than 34000 infants at day 3 or 4 of life. In this cohort, 24 infants subse-

Context  Fatal arrhythmias from occult long QT syndrome may be responsible for some cases of sudden infant death syndrome (SIDS). Because patients who have long QT syndrome with sodium channel gene (SCN5A) defects have an increased frequency of cardiac events during sleep, and a recent case is reported of a sporadic SCN5A mutation in an infant with near SIDS, SCN5A has emerged as the leading candidate ion channel gene for SIDS.

Objective To determine the prevalence and functional properties of SCN5A mutations in SIDS.

Design, Setting, and Subjects Postmortem molecular analysis of 93 cases of SIDS or undetermined infant death identified by the Medical Examiner’s Office of the Arkansas State Crime Laboratory between September 1997 and August 1999. Genomic DNA was extracted from frozen myocardium and subjected to mutational analyses. Missense mutations were incorporated into the human heart sodium channel α subunit by mutagenesis, transiently transfected into human embryonic kidney cells, and characterized electrophysiologically.

Main Outcome Measures Molecular and functional characterization of SCN5A defects.

Results Two of the 93 cases of SIDS possessed SCN5A mutations: a 6-week-old white male with an A997S missense mutation in exon 17 and a 1-month-old white male with an R1826H mutation in exon 28. These 2 distinct mutations occurred in highly conserved regions of the sodium channel and were absent in 400 control patients (800 alleles). Functionally, the A997S and R1826H mutant channels expressed a sodium current characterized by slower decay and a 2- to 3-fold increase in late sodium current.

Conclusion Approximately 2% of this prospective, population-based cohort of SIDS cases had an identifiable SCN5A channel defect, suggesting that mutations in cardiac ion channels may provide a lethal arrhythmogenic substrate in some infants at risk for SIDS.

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quently died of SIDS and 12 demonstrated a QTC of 440 milliseconds or more at the initial surveillance electrocardiogram. This observation generated intense dialogue in the pediatric community. More recently, Schwartz and colleagues provided molecular proof of principle in a case report of a 44-day-old infant who presented with an out of hospital cardiac arrest and was defibrillated successfully from ventricular fibrillation. Genetic testing demonstrated a sporadic, de novo SCN5A missense mutation (S941N).

We hypothesized previously that cardiac ion channel genes, particularly SCN5A, might be candidate gene(s) for SIDS. In this study, postmortem cardiac tissues from a large population-based cohort of SIDS were collected prospectively, frozen, and subjected to mutational analyses.

**METHODS**

**SIDS Cohort**

Arkansas has a population of approximately 2.5 million persons and had 36831 live births in 1998. During that year, there were a total of 339 infant deaths, with an infant mortality rate of 9.2 deaths per 1000 live births. The Medical Examiner’s Office of the Arkansas State Crime Laboratory conducted autopsies for more than 95% of the sudden, unexplained, and unexpected infant deaths.

Between September 1997 and August 1999, necropsy tissue was collected and frozen for 93 SIDS or possible SIDS cases. During this study period, there were 2 cases in which tissue was not properly archived, precluding a molecular autopsy. The death certificate was assigned the diagnosis of SIDS if autopsy, toxicology, and death-scene evaluation were all negative. The cause of death was ruled undetermined or possible SIDS if there was a negative autopsy but a scene investigation was either not conducted or revealed cosleeping and the possibility of suffocation or asphyxia could not be excluded. Infants whose cause of death was determined to be secondary to a specific disease or to asphyxia were excluded. The institutional review boards of the Mayo Foundation, Baylor College of Medicine, and the Arkansas State Crime Laboratory approved postmortem molecular analysis. Written informed consent was not required for this necropsy study. The molecular analyses were conducted with the investigators blinded to the identities of the SIDS victims.

**SCN5A Mutation Analyses**

Genomic DNA was extracted from frozen myocardium (sample weight approximately 25 mg) using the QIAamp DNA Mini Kit (Qiagen, Inc, Valencia, California). Protein-encoding exons of the cardiac sodium channel gene, SCN5A, were amplified from genomic DNA by polymerase chain reaction using the full-length genomic sequence and previously published intron–and exon-based primers. The mutations were detected by denaturing high performance liquid chromatography using a TransgenicOMEGA (Omaha, Nebraska) system, as previously described.

The precise sequences of the mutations were determined by manual, radiolabeled Thermo Sequenase sequencing (Amersham, Cleveland, Ohio) and independently confirmed by dye-terminator cycle-sequencing (ABI Prism 377).

Analysis of 400 control subjects including the 100 white human variation panel and the 100 black human variation panel from Coriell Cell Repositories (Camden, New Jersey) and the National Institute of General Medical Sciences verified putative disease-causing mutations. The other 200 controls were derived from our own control panel used in the LQTS studies and consisted primarily of white people who were either healthy or had been electrocardiographically screened with no evidence of QT prolongation.

**Mutant SCN5A Gene-Expression Studies**

Clones, mutagenesis, transfection, and voltage-clamp techniques were the same as those described in 1998 by Nagamoto et al. The wild type (WT) human heart sodium channel (hH1α) was provided by Hali A. Hartmann, PhD (Baylor College of Medicine, Houston, Texas). The A997S and R1826H mutations were introduced into the WT construct using site-directed polymerase chain reaction mutagenesis techniques (Stratagene, La Jolla, California). The polymerase chain reaction products containing the A997S or R1826H mutation were sequenced to verify the mutation and then subcloned into human heart sodium channel hH1α, a cDNA clone of human heart sodium channel. Transient transfection of WT-, A997S-, R1826H-hH1α subunits into human embryonic kidney 293 cells was achieved using a cationic lipidosome method. Cotransfection with green fluorescent protein in a 10:1 ratio allowed for selection of transfected cells. After 24 to 48 hours, the transfected cells were transferred directly to the experimental chamber. Macroscopic sodium current was recorded using the whole-cell patch-clamp technique at room temperature using an Axopatch 200 amplifier (Axon Instruments Inc, Union City, California) and previously verified conditions.

Current and kinetic data were fit to nonlinear regression model equations using pClamp v6.03 or Origin 6.0 as described previously. All determinations of statistical significance of mean data were performed by using a t test for comparisons of 2 means. A P value of <.05 was considered statistically significant.

**RESULTS**

**Postmortem Molecular Analyses of SIDS Cohort**

In this cohort of sudden unexplained infant deaths, 45 were diagnosed as SIDS and 48 were denoted undetermined or possible SIDS. The average age of the deceased infants was 3 months. The cohort included 51 males and 42 females with a racial distribution of 58 white, 34 black, and 1 Hispanic.

Two of the 93 SIDS or possible SIDS infants (2.15%) were found to possess mutations in SCN5A by denaturing high performance liquid chromatography and...
DNA sequencing. One missense mutation was detected in a 6-week-old white male. This infant was a well developed, well nourished, full-term infant. The cause of death was SIDS. A complete medicolegal autopsy including death scene evaluation was conducted. The infant was alive at the time of a 1 AM feeding, was placed subsequently in the supine position in bed between his parents away from pillows and was discovered not breathing 5 to 6 hours later. Figure 1A shows the abnormal chromatogram by denaturing high performance liquid chromatography for the polymerase chain reaction product from exon 17. This abnormal profile was the result of a single nucleotide substitution (nucleotide 2989 G>T) resulting in a missense mutation denoted A997S (alanine residue at amino acid position 997 changed to a serine residue, Figure 1B). The A997 amino acid is located in the cytoplasmic connector between the second and third domains of the sodium channel and is conserved highly across different species. This mutation was not detected in 800 alleles obtained from 400 unrelated normal controls.

The second mutation was discovered in a 42-day-old white male. This full-term previously healthy infant’s death was ruled undetermined because a death scene evaluation could not rule out the possibility of asphyxia secondary to overlaying. A single-nucleotide alteration (nucleotide 5477 G>A) in exon 28 resulting in the substitution of a histidine for the normal arginine at amino acid position 1826 (designated R1826H) was determined (Figure 2). The R1826H mutation resides in the cytoplasmic C-terminal region of the sodium channel near several reported LQTS-causing mutations.29 The R1826 amino acid is highly conserved, and the R1826H defect was not detected in 400 normal controls.

In addition, several single nucleotide polymorphisms and other polymorphic sequence variations were identified in this SIDS cohort. The specific single nucleotide polymorphisms detected and their frequencies are summarized in the TABLE.

Electrophysiological Phenotype of A997S- and R1826H-SCN5A Mutations

When transiently expressed into a mammalian cell line lacking intrinsic
currents (human embryonic kidney cells) and examined by whole cell voltage clamp, typical voltage-dependent sodium current was seen for both mutants containing either the A997S (Figure 3A) or the R1826H missense mutation (Figure 3C). Clearly, the rate of decay of the mutant currents was less rapid compared with WT currents. Another marked difference was an approximately 300% increase in the late sodium current for both A997S and R1826H compared with the WT current that was readily apparent on a more expanded time and amplitude scale (Figure 3B and D). With a depolarization to negative 20 mV, the late current measured at 240 milliseconds after the onset of depolarization was 0.7% (mean [SD] 0.4%) for WT (n = 5), 2.2% (0.6%) for A997S (n = 7), and 2.1% (0.4%) for R1826H (n = 4, P < .05). Summary data for the late current at different test potentials within the range of the action potential plateau show the substantial increase in late sodium current (Figure 4).

COMMENT

To our knowledge, this study represents the first population-based molecular study probing the cause of SIDS. Defects in the cardiac sodium channel gene, SCN5A, were discovered in 2% of infants from a 2-year population-based cohort of SIDS. Mechanistically, both the A997S and R1826H mutations confer a gain-of-function sodium channel phenotype characterized by persistent and increased inward sodium current similar to several other SCN5A defects reported in congenital LQTS (Figure 5).28,30 Cardiac channelopathies now join fatty acid oxidation disorders31 and medullary serotonergic network deficiency9 as definitive pathophysiological mechanisms for SIDS.

The anonymous nature of this initial necropsy study prevented a determination of whether the 2 infants with SCN5A mutations represent sentinel events for familial LQTS or sporadic, de novo mutations. Of the 5 known LQTS genes, more sporadic mutations have

Table. Single Nucleotide Polymorphisms

<table>
<thead>
<tr>
<th>Single Nucleotide Polymorphism</th>
<th>Amino Acid Polymorphism</th>
<th>Exon</th>
<th>Frequency, No. (%) (n = 93)</th>
</tr>
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<tbody>
<tr>
<td>717 C→T</td>
<td>I239I</td>
<td>7</td>
<td>1 (1.1)</td>
</tr>
<tr>
<td>2262 +33delG</td>
<td>Intron</td>
<td>14</td>
<td>3 (3.2)</td>
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<tr>
<td>2788→6 C→T</td>
<td>Intron</td>
<td>17</td>
<td>7 (7.5)</td>
</tr>
<tr>
<td>3183 G→A</td>
<td>E1061E</td>
<td>17</td>
<td>16 (17.2)</td>
</tr>
<tr>
<td>4299 + 28 C→T</td>
<td>Intron</td>
<td>24</td>
<td>1 (1.1)</td>
</tr>
<tr>
<td>4299 + 37-45delACCTGAGGC</td>
<td>Intron</td>
<td>24</td>
<td>2 (2.2)</td>
</tr>
<tr>
<td>4299 + 53 T→C</td>
<td>Intron</td>
<td>24</td>
<td>9 (9.7)</td>
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<tr>
<td>4509 C→T</td>
<td>S1503S</td>
<td>26</td>
<td>1 (1.1)</td>
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<tr>
<td>4848 C→T</td>
<td>F1616F</td>
<td>28</td>
<td>7 (7.5)</td>
</tr>
<tr>
<td>5091 C→T</td>
<td>F1697F</td>
<td>28</td>
<td>1 (1.1)</td>
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<tr>
<td>5457 T→C</td>
<td>D1819D</td>
<td>28</td>
<td>37 (39.8)</td>
</tr>
</tbody>
</table>

Figure 3. Electrophysiological Phenotype of the A997S-SCN5A and R1826H-SCN5A Sodium Channels

A and C, Sodium currents recorded in human embryonic kidney cells transiently expressing wild type (WT), A997S, or R1826H mutant sodium channels. Representative currents traces for WT and A997S (A) and WT and R1826H (C) were obtained for a test depolarization to −20 mV from a holding potential of −140 mV. Currents were scaled to have equal peaks. Decay rates over the first 200 milliseconds were obtained from the portion of the trace after the current reached 90% of peak by fitting to a sum of exponentials: INa (t) = 1−(Af exp −t/τf + As exp −t/τs) plus offset where t is time; τf and τs represent the time constant of the fast and slow components; and Af and As are amplitudes of fast and slow component, respectively. For WT hH1a, τf and τs were 7.2 and 7.8 milliseconds and Af and As were 67% and 33%, respectively. B and D, Similar recordings as in A and C, respectively, but recorded on different scales for time (x-axis) and magnitude of current (y-axis) to demonstrate the persistent and increased late current associated with the A997S and R1826H mutations. INa was normalized to the peak current to allow for comparison.
been identified in the SCN5A gene than in the other ion channel genes. In- deed, the case report of near-SIDS was a sporadic SCN5A gene defect.23 Spo-

Figure 4. Persistent Late Sodium Current Associated With the A997S-SCN5A and R1826H-SCN5A Sodium Channels

Summary data for the magnitude of the late INa at various test membrane potentials in the action potential plateau range. The current was measured 240 milliseconds after depolarization from a holding potential of -140 mV to the 3 membrane potentials shown. The bars represent the mean (SEM) for n=5, 7, and 4 experiments for wild type, A997S, and R1826H, respectively. Asterisk indicates a significant increase in late current amplitude in the mutant sodium channel compared with wild type (P<.05).

Figure 5. Schematic of SCN5A Cardiac Sodium Channel and Locations of LQTS-, Brugada/IVF-, and SIDS-Associated Mutations

A. The cardiac sodium channel alpha subunit is encoded by SCN5A, which resides on chromosome 3p21. SCN5A is composed of 4 domains designated DI to DIV with each domain containing 6 transmembrane spanning segments denoted S1 through S6 and a pore region between S5 and S6. The linear topology of SCN5A with its 2016 amino acids is drawn to scale. Exon-encoded mutations associated with a LQT3 phenotype are shown in pink dots whereas Brugada syndrome and idiopathic ventricular fibrillation (IVF) defects are shown in yellow (intronic variants are not displayed). The approximate locations of the 2 missense sudden infant death syndrome (SIDS) mutations in the cardiac ion channel genes may explain why electrocardiographic evaluations of the parents and siblings of SIDS victims have not demonstrated QT prolongation.23,32

The phenotype of LQTS results from defective cardiac ion channels resulting in a perturbation in the control of ventricular repolarization.17-18 Defects in the SCN5A gene comprise approximately 5% to 10% of LQTS. Interestingly, such individuals with SCN5A-based LQTS have more cardiac events including sudden death during non- arousing states like sleep19,20 and may not respond as favorably to the standard medical therapy for the treatment of LQTS, namely β-blockers.31 Besides LQTS, a defective SCN5A gene can produce a distinct arrhythmogenic disorder referred to as Brugada syndrome (Figure 5).34 Brugada syndrome has been implicated as another potential cause of sudden death in children.35 These observations of sporadic SCN5A gene defects and the association with sleep made this our leading candidate ion channel gene for SIDS. It remains to be determined whether defective potassium channel genes will be found in this population-based cohort of SIDS as well. Although not as striking as the association between sleep and SCN5A, we have genotyped several LQTS families with a history of sleep-triggered cardiac events to the more common LQTS potassium channel genes, KCNQ1 and HERG (KCNH2) (unpublished observations). Perhaps this initial 2% prevalence of cardiac ion channel defects in SIDS will be an underestimate. It will be interesting to see if future cohorts of SIDS will also reveal this same prevalence of ion channelopathies. Presently, we can surmise that approximately 50 of the 2500 annual infant deaths (ie, 2%) attributed to SIDS may be secondary to defects in the SCN5A gene. Whether or not these particular at-risk infants could have been identified premortem and their lives...
saying will continue to be debated. A routine newborn genetic test for SCN5A gene defects is unlikely in the near future. Routine newborn electrocardiographic screening may not meet the requirements of an effective screening tool with its poor positive predictive value. Nonetheless, the discovery of fundamental causes of the sudden infant death syndrome is an important step toward perhaps one day eliminating the occurrence of such deaths. Moreover, the use of molecular autopsies provides a glimpse of the impact the Human Genome Project will have on the forensic evaluation of sudden unexplained death.

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Reference:


