Prevalence of Long-QT Syndrome Gene Variants in Sudden Infant Death Syndrome

Marianne Arnestad, MD*; Lia Crotti MD*; Torleiv O. Rognum, MD; Roberto Insolia, BSc; Matteo Pedrazzini, BSc; Chiara Ferrandi, BSc; Ashild Vege, MD; Dao W. Wang, MD; Troy E. Rhodes, MD, PhD; Alfred L. George, Jr, MD; Peter J. Schwartz, MD

Background—The hypothesis that some cases of sudden infant death syndrome (SIDS) could be caused by long-QT syndrome (LQTS) has been supported by molecular studies. However, there are inadequate data regarding the true prevalence of mutations in arrhythmia-susceptibility genes among SIDS cases. Given the importance and potential implications of these observations, we performed a study to more accurately quantify the contribution to SIDS of LQTS gene mutations and rare variants.

Methods and Results—Molecular screening of 7 genes (KCNQ1, KCNH2, SCN5A, KCNE1, KCNE2, KCNJ2, CAV3) associated with LQTS was performed with denaturing high-performance liquid chromatography and nucleotide sequencing of genomic DNA from 201 cases diagnosed as SIDS according to the Nordic Criteria, and from 182 infant and adult controls. All SIDS and control cases originated from the same regions in Norway. Genetic analysis was blinded to diagnosis. Mutations and rare variants were found in 26 of 201 cases (12.9%). On the basis of their functional effect, however, we considered 8 mutations and 7 rare variants found in 19 of 201 cases as likely contributors to sudden death (9.5%; 95% CI, 5.8 to 14.4%).

Conclusions—We demonstrated that 9.5% of cases diagnosed as SIDS carry functionally significant genetic variants in LQTS genes. The present study demonstrates that sudden arrhythmic death is an important contributor to SIDS. As these variants likely modify ventricular repolarization and QT interval duration, our results support the debated concept that an ECG would probably identify most infants at risk for sudden death due to LQTS either in infancy or later on in life.

(Circulation. 2007;115:361-367.)

Key Words: death, sudden • genetics • ion channels • long-QT syndrome

Sudden infant death syndrome (SIDS) remains the leading cause of mortality in the first year of life in the postnatal period. Despite many hypotheses, which mainly focus on respiratory or cardiac abnormalities, the underlying mechanisms remain elusive. Nonetheless, the view that SIDS is multifactorial and that several different mechanisms, which include metabolic and genetic disorders can lead to SIDS, is forcing the search for preventable causes.

Among preventable causes of SIDS are the life-threatening arrhythmias caused by long-QT syndrome (LQTS). LQTS is a genetic disorder caused by mutations in several genes that mostly encode cardiac ion channels and characterized by QT interval prolongation on the ECG and by propensity to lethal arrhythmias during sympathetic activation, rest, or sleep, according to the specific genotype. All features of LQTS, which include a negative postmortem examination, are compatible with the definitions of SIDS. As LQTS can be suspected or diagnosed by an ECG, and as very effective therapies are available to reduce overall mortality to 2%, the definition of the precise contribution of LQTS to SIDS would carry important practical implications.

The suggestion that the same mechanisms operant in LQTS could contribute to some of the SIDS victims was advanced in 1974 to 1976, but it took an 18-year prospective study with ECGs recorded in >33 000 infants to provide the first evidence that a prolonged QT interval on days 3 to 4 of life was associated with a significantly higher risk of SIDS. This

Received August 12, 2006; accepted November 10, 2006.

*Dr Arnestad and Dr Crotti contributed equally to this article.

The online-only Data Supplement, consisting of a table, is available with this article at http://circ.ahajournals.org/cgi/content/full/CIRCULATIONAHA.106.658021.DC1.

From the Institute of Forensic Medicine (M.A., T.O.R.), University of Oslo, Oslo, Norway; Molecular Cardiology Laboratory (L.C., R.I., M.P., C.F., P.J.S.), IRCCS Fondazione Policlinico S. Matteo, Pavia, Italy; Department of Cardiology (L.C., P.J.S.), University of Pavia and IRCCS Fondazione Policlinico S. Matteo, Pavia, Italy; Department of Laboratory Medicine (A.V.), Faculty of Medicine, Norwegian University of Science and Technology, Trondheim, Norway; and Departments of Medicine and Pharmacology (D.W.W., T.E.R., A.L.G.), Vanderbilt University, Nashville, Tenn.

Correspondence to Dr Peter J. Schwartz, Professor & Chairman, Department of Cardiology, IRCCS Fondazione Policlinico S. Matteo, Viale Golgi 19, 27100 Pavia, Italy. E-mail pjqt@compuserve.com

© 2007 American Heart Association, Inc.

Circulation is available at http://www.circulationaha.org

DO: 10.1161/CIRCULATIONAHA.106.658021

361
was followed by molecular demonstrations that de novo LQTS mutations created an arrhythmia-prone substrate responsible for cardiac arrest, as a result of documented ventricular fibrillation, in a typical case of near-miss for SIDS and of death of another infant caused by SIDS.14,15 Although these findings provided the necessary “proof of concept” that LQTS could indeed cause SIDS, their anecdotal nature limited immediate implications. A molecular screening of a cohort of 93 SIDS victims showed a prevalence of LQTS mutations of 5.2% among the 58 white infants (LQTS seems rare among blacks).16 Also, a recent report indicates that homozygosity for a SCN5A polymorphism common among blacks increases the risk of SIDS.17

To assess the prevalence of LQTS gene variants in sudden infant death, a molecular study in a large number of SIDS victims is required. We performed such a study in a cohort of 201 Norwegian SIDS victims, with adequate controls, and report here that genetic variants in LQTS genes are present in 9.5% of SIDS victims. This finding has direct clinical implications for the reduction of sudden death in infants and children.

Methods

Study Population

Between 1988 and 2004, 252 cases of sudden unexpected infant death from the southeastern region of Norway were investigated at the Institute of Forensic Medicine in Oslo. This involved evaluation of circumstances of death, review of medical and family history, full-body radiographic examination, toxicology, and a thorough autopsy with extensive histologic and microbiologic examinations, which include neuropathological examination.18 Each case was categorized according to the Nordic criteria19,20 as pure SIDS (no cause of death revealed), borderline SIDS (cases of sudden infant death in association with nonlethal ailments), or infectious and violent deaths. The latter served as controls. Infectious deaths in infants treated with QT-prolonging drugs (n=6) were excluded.

Blinded to diagnosis, investigators screened 246 cases: 140 pure SIDS, 61 borderline SIDS, 45 non-SIDS deaths, which included violent (n=17) and infectious deaths (n=28) (Table 1). As additional ethically matched controls, 137 adult unrelated cases of death from noncardiac causes from the same geographical regions as the SIDS cases were screened. We used adult controls because the finding of LQTS mutations in infants that died from accidental causes does not exclude the presence of asymptomatic LQTS. Thus, the total number of controls was 182. The present study was approved by the Norwegian regional ethics committee, which mandated complete anonymity of the samples and thus prevented contact with the families.

Molecular Screening

Genomic DNA was extracted from blood or frozen spleen samples with standard techniques, and the DNA stock solutions stored in a biobank registered and kept in accordance with Norwegian Biobank legislation. Anonymous DNA samples were transferred to Italy with permission of the Norwegian Biobank Authorities. Previously published intron primer pairs as well as in-house designed polymerase chain reaction primers (primer sequences available on request) were used to amplify all coding sequences of the 3 genes most commonly associated with LQTS (SCN5A, KCNH2, SCN5A) and 4 other genes less commonly associated with LQTS (KCNEL1, KCNE2, KCNJ2, CAV3). Denaturing high-performance liquid chromatography (Transgenomic Wave, Transgenomic, Inc, Omaha, Neb) analysis was used as a preliminary screening technique and samples with abnormal elution profiles were sequenced on an ABI Prism 310 Genetic Analyzer (Applied Biosystems, Foster City, Calif). TOPO

<table>
<thead>
<tr>
<th>TABLE 1. Sex, Age, and Cause of Death Among Cases and Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Original Diagnosis</strong></td>
</tr>
<tr>
<td>Total SIDS cases</td>
</tr>
<tr>
<td>Borderline SIDS</td>
</tr>
<tr>
<td>Slight infection</td>
</tr>
<tr>
<td>Nonlethal conditions*</td>
</tr>
<tr>
<td>Circumstances of death†</td>
</tr>
<tr>
<td>Infectious diseases</td>
</tr>
<tr>
<td>Pneumonia</td>
</tr>
<tr>
<td>Septicemia</td>
</tr>
<tr>
<td>Myocarditis</td>
</tr>
<tr>
<td>Other</td>
</tr>
<tr>
<td>Violent deaths</td>
</tr>
<tr>
<td>Suffocation</td>
</tr>
<tr>
<td>Injuries</td>
</tr>
<tr>
<td>Other</td>
</tr>
<tr>
<td>Adult deceased controls</td>
</tr>
<tr>
<td>Disease</td>
</tr>
<tr>
<td>Intoxication</td>
</tr>
<tr>
<td>Violent death</td>
</tr>
</tbody>
</table>

IQR indicates interquartile range.
*Minor brain malformations, etc, without clinical significance.
†Social conditions, etc.

TA Cloning (Invitrogen, Carlsbad, Calif) assay by sequencing of single-stranded DNA was performed to determine allele position of R954C and K897T in the KCNH2 gene because family members were unavailable.

All detected genetic variants were classified in 3 subsets: mutations, rare variants, and common genetic variants. Mutations are genetic variants identified neither in ethnically matched controls nor in previously reported control populations.21,22 Rare genetic variants are either those absent in Norwegian controls but found in <0.7% of other white control populations or at any frequency in nonwhite populations, or found in <0.7% of Norwegian controls and having evidence of a functional effect. Common genetic variants are those identified in >0.7% of Norwegian controls or of other white control populations.21,22 When a common variant was present in >8% of controls, we assayed for homozygosity of the minor allele. As the distribution of genetic variants did not differ between SIDS and borderline SIDS, the findings in the 2 groups are presented together.

Statistical Analysis

Comparison of groups identified on the basis of the genotype was performed in univariate analysis with the Fisher exact test. Binomial exact 95% confidence intervals were computed to assess the reliability of the estimated proportion of SIDS victims with mutations and rare genetic variants, given the sample size (n=201). Age at time of death was reported as median with interquartile ranges. Two-sided probability values <0.05 were considered statistically significant.

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

Results

The sex and age distribution of the SIDS victims (Table 1) followed traditional epidemiological data.1,2 Both maternal smoking and prone position were identified in the majority of
cases (59% and 62%, respectively) as previously described. SIDS cases with LQTS gene variants did not differ from the remaining SIDS population in terms of epidemiological variables or findings at autopsy (Table 2).

### Mutations and Rare Genetic Variants in Norwegian SIDS

Mutations and rare variants in LQTS genes (defined in Methods) were identified in 26 of 201 cases (12.9%). They were found most frequently in SCN5A (50%, 13 cases), followed by KCNQ1 (19%, 5 cases), KCNH2 (19%, 5 cases), CAV3 (11%, 3 cases), and KCNE2 (4%, 1 case). One subject carried variants in 2 genes (SCN5A, CAV3). No mutations were found in KCNE1 or KCNJ2. Among these 26 SIDS cases, there were 11 mutations (55%, 10 missense and 1 deletion) and 9 rare variants (45%; Table 3), mainly located in conserved regions of the encoded proteins (alignment data available on request).

### Sodium Channel Variants in SIDS

Four mutations and 5 rare variants were identified in 13 cases, predominantly in cytoplasmic domains (78%). Of the 4 mutations, 3 were novel (F1486L, R680H, delAL586-587) and 1 mutation (T1304M) was previously described in 2 LQTS families. F1486L, a novel mutation that affects a highly conserved phenylalanine residue, which is critical for fast inactivation.

---

**TABLE 2. SIDS With and Without Functionally Relevant Gene Variants Compared for Epidemiological Variables and Autopsy Findings**

<table>
<thead>
<tr>
<th></th>
<th>Mutated Cases</th>
<th>Nonmutated Cases</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at time of death</td>
<td>5.0 mo (median)</td>
<td>3.5 mo (median)</td>
<td>0.17</td>
</tr>
<tr>
<td>Cold season</td>
<td>9 of 19 (47)</td>
<td>107 of 182 (59)</td>
<td>0.34</td>
</tr>
<tr>
<td>Male infant</td>
<td>12 of 19 (63)</td>
<td>108 of 182 (59)</td>
<td>0.81</td>
</tr>
<tr>
<td>Prone sleeping</td>
<td>12 of 16 (75)</td>
<td>95 of 158 (60)</td>
<td>0.29</td>
</tr>
<tr>
<td>Bed sharing</td>
<td>2 of 17 (12)</td>
<td>47 of 179 (26)</td>
<td>0.25</td>
</tr>
<tr>
<td>Cold at time of death</td>
<td>4 of 16 (25)</td>
<td>62 of 155 (40)</td>
<td>0.29</td>
</tr>
<tr>
<td>Fever at time of death</td>
<td>3 of 17 (18)</td>
<td>22 of 143 (15)</td>
<td>0.73</td>
</tr>
<tr>
<td>Maternal smoking</td>
<td>5 of 12 (42)</td>
<td>64 of 105 (61)</td>
<td>0.23</td>
</tr>
<tr>
<td>Maternal age</td>
<td>25.5 y (median)</td>
<td>26 y (median)</td>
<td>0.62</td>
</tr>
<tr>
<td>Intrathoracic petechiae</td>
<td>14 of 19 (74)</td>
<td>135 of 182 (74)</td>
<td>0.71</td>
</tr>
</tbody>
</table>

Values are expressed as n of N (%), unless otherwise indicated.

**TABLE 3. Mutations and Rare Genetic Variants in LQTS Genes Identified in 26 SIDS Cases**

<table>
<thead>
<tr>
<th>Genetic Variants</th>
<th>Classification</th>
<th>Functional Effect</th>
<th>No. of SIDS Cases</th>
<th>No. of Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>KCNQ1</td>
<td>I274V Mutation</td>
<td>Gain-of-function</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>P448R Rare genetic variant</td>
<td>...</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>G460S Mutation</td>
<td>Loss-of-function</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>KCNH2</td>
<td>R273Q Mutation</td>
<td>Loss-of-function</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>V279M Mutation</td>
<td>...</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>R885C Mutation</td>
<td>...</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>R954C/K997T* Mutation</td>
<td>Loss-of-function</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>S1040G Mutation</td>
<td>...</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>SCN5A</td>
<td>S216L Rare genetic variant</td>
<td>Increased persistent sodium current</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>del AL 586-587 Mutation</td>
<td>Accelerated inactivation</td>
<td>1†</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>R680H Mutation</td>
<td>Increased persistent sodium current (in acidosis)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>R1193Q Rare genetic variant</td>
<td>Increased persistent sodium current</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>T1304M Mutation</td>
<td>Increased persistent sodium current</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>F1486L Mutation</td>
<td>Increased persistent sodium current</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>V1951L Rare genetic variant</td>
<td>Accelerated inactivation</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>F2004L Rare genetic variant</td>
<td>Increased persistent sodium current</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>P2006A Rare genetic variant</td>
<td>Increased persistent sodium current</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>CAV3</td>
<td>C72W Rare genetic variant</td>
<td>No functional data</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>T78M Rare genetic variant</td>
<td>Increased persistent sodium current</td>
<td>2‡</td>
<td>1</td>
</tr>
<tr>
<td>KCNE2</td>
<td>Q6E Rare genetic variant</td>
<td>Impaired I(_K) activation</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

Ellipses (…) indicate no evidence for functional effects.

*Both variants occur on the same KCNH2 allele.

†This case carries both SCN5A-delAL586-587 and CAV3-T78M.

‡One case carries both SCN5A-delAL586-587 and CAV3-T78M. One case has only CAV3-T78M.
was identified in an infant found dead in the first week of life. Retrospective analysis of an ECG performed on day 1 revealed 2:1 atrioventricular block with an extremely prolonged QTc (720 ms) that had not been recognized. The functional characterization of this mutation and of T1304M exhibited an increased persistent current, consistent with a LQTS phenotype. The 2 other novel mutations (delAL586-587 and R680H) exhibited increased persistent current only under conditions of internal acidosis (R680H) or when expressed in the context of the common splice variant, delQ1077 (delAL586-587).27

Among the 5 rare SCN5A variants identified, 3 (S216L, F2004L, F2006A) exhibited increased persistent sodium current typical of mutations associated with LQTS.27 The other 2 mutations, R1193Q and V1951L, have been described previously as disease-causing mutations. R1193Q is a rare variant (0.3%) in the white population22 that is reported in subjects with both acquired and congenital LQTS28,29 and in a child with frequent ventricular fibrillation episodes at 6 months of age whose dizygotic twin died unexpectedly during sleep at 4 months of age.30 When expressed in human embryonic kidney cells, R1193Q produces an abnormal level of persistent current.28 V1951L, described as a Brugada syndrome mutation,31 is a common variant (6.7%) in Hispanics.22 However, V1951L channels exhibit significantly increased persistent current in the delQ1077 background,27 which thus raises the possibility of a dysfunctional variant with ethnic-dependent phenotype expression.

### Potassium Channel Variants in SIDS

In 11 SIDS cases, 9 different mutations or rare variants were identified; 7 of 9 mutations (78%) were novel (Table 3). Four of the 9 mutations (6 cases) were not considered to be related to SIDS because of lack of evidence for channel dysfunction (Rhodes et al, unpublished observations). This includes KCNQ1-P448R because it is a common polymorphism in Asians32 without functional effects despite the fact that we did identify this allele in 3 SIDS cases and in none of the controls.

KCNH2-R273Q and the compound allele KCNH2-R954C/K897T (K897T is a polymorphism previously demonstrated to exaggerate the electrophysiological defect of a KCNH2 mutation33) exhibited significantly reduced activity when expressed in Chinese hamster ovary cells (Rhodes et al, unpublished observations). KCNQ1-G460S generated In, current with 40% smaller current density, which produced a partial loss-of-function phenotype; by contrast KCNQ1-I274V had a “gain-of-function” effect (Rhodes et al, unpublished observations). KCNE2-Q9E was detected in an infant found dead in the prone position and with a family history of sudden cardiac death. This variant, a cause of acquired LQTS,34 which was not found in Norwegian controls and in 2 other white control populations,31,33 increased the voltage dependence of channel activation in a coexpression functional study with human ether-a-go-go related gene.33

### CAV3 Variants in SIDS

Recently, CAV3 was indicated as a candidate gene for LQTS34 and SIDS.38 In our study, 2 SIDS cases and 1 Norwegian control subject were heterozygous for a missense CAV3 variant (T78M). One CAV3-T78M SIDS carrier was also heterozygous for the SCN5A-delAL586-587 mutation described above. This CAV3 allele was previously associated with isolated cases of either congenital LQTS34 or SIDS,35 and CAV3-T78M purportedly interacts with the cardiac sodium channel to promote increased persistent current. Another missense variant (C72W) was found in 1 SIDS case and in none of the Norwegian controls. In the absence of functional data, however, we have conservatively decided not to include this rare variant among those causally related to SIDS.

### Common Genetic Variants

Thirteen common variants were detected in this population (Table 4). SCN5A-A572D, previously described as a disease-causing mutation6 and not previously identified in control populations,22 was detected in 2.2% of our controls and in 3 SIDS cases (1.5%). We consider it a common variant in the Norwegian population and not associated with SIDS.

Four additional common variants (KCNH2-K897T, KCNH2-R1047L, SCN5A-H558R, KCNE1-G38S), with higher heterozygous frequencies among controls (8.2 to 44.5%), were identified without deviation from Hardy-Weinberg equilibrium. None of these alleles was correlated with SIDS except KCNH2-R1047L, whose minor allelic frequency was higher in controls compared with SIDS cases (4.7% versus 1.7%, P=0.02).

Two common variants, KCNH2-K897T and SCN5A-H558R, which may modify the functional phenotype of

---

**TABLE 4. Minor Allele Frequencies of Common Genetic Variants Identified in Cases and/or Controls**

<table>
<thead>
<tr>
<th>Common Genetic Variants</th>
<th>Minor Allele Frequency in SIDS Cases</th>
<th>Minor Allele Frequency in Controls</th>
<th>Cases vs Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>KCNH2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K897T</td>
<td>0.199</td>
<td>0.222</td>
<td>P=0.43</td>
</tr>
<tr>
<td>R1047L</td>
<td>0.017</td>
<td>0.047</td>
<td>P=0.02</td>
</tr>
<tr>
<td>SCN5A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H558R</td>
<td>0.239</td>
<td>0.214</td>
<td>P=0.44</td>
</tr>
<tr>
<td>A572D</td>
<td>0.007</td>
<td>0.011</td>
<td>P=0.71</td>
</tr>
<tr>
<td>KCNE1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G38S</td>
<td>0.308</td>
<td>0.321</td>
<td>P=0.75</td>
</tr>
<tr>
<td>D85N</td>
<td>0.007</td>
<td>0.016</td>
<td>P=0.32</td>
</tr>
<tr>
<td>CAV3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F97L</td>
<td>0.005</td>
<td>0.005</td>
<td>P=0.22</td>
</tr>
<tr>
<td>G56S</td>
<td>0.003</td>
<td>0.003</td>
<td>P=0.47</td>
</tr>
<tr>
<td>KCNJ2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R422L</td>
<td>0.003</td>
<td>0.003</td>
<td>P=0.47</td>
</tr>
<tr>
<td>KCNE2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T8A</td>
<td>0.002</td>
<td>0.011</td>
<td>P=0.20</td>
</tr>
</tbody>
</table>
mutations located elsewhere in the same gene, did not have different allele frequencies between SIDS cases and controls. However, when the analysis is limited to the SIDS cases with SCN5A mutations or rare variants that exhibited modest or latent functional effects (delAL586-587, R680H, V1951L, F2004L, P2006A), the R558 allele frequency was twice that of controls (44% versus 21%, \( P=0.059 \); online-only Data Supplement, Table).

Prevalence of Functional LQTS Gene Variants in SIDS

Our results indicate that 19 of the 201 SIDS victims (9.5%) were carriers of LQTS gene variants with a functional effect (Table 3). Seven cases were carriers of 1 mutation, 11 were carriers of 1 rare genetic variant, and 1 case carried 1 mutation and 1 rare variant. The prevalence of variants with functional effect in this SIDS series is a robust statistical observation as evidenced by the exact 95% confidence intervals, which range from 5.8 to 14.4%.

Discussion

The present study, based on the largest data set of DNA samples from SIDS victims reported to date, provides strong evidence that overt or latent functional LQTS gene variants are associated with 9.5% of SIDS cases. Furthermore, as disease-causing mutations are identified in only \( \approx 70\% \) of definite cases of LQTS, our data may underestimate the true prevalence. This finding should prompt an objective assessment of the implementation of feasible preventive strategies for reducing the burden of SIDS and of sudden death in children and adolescents.

The demonstration by our results that genetic arrhythmogenic disorders are implicated in nearly one tenth of rigorously defined SIDS reduces the number of sudden infant deaths which, because they occur in an apparently healthy infant and remain unexplained after thorough examination, would be hidden under the umbrella of SIDS.

Rationale for the Relationship Between LQTS and SIDS

Between 1974 and 1976, Schwartz proposed that some SIDS victims may have died because of a lethal cardiac arrhythmia caused by a mechanism similar to that which underlies the arrhythmias of LQTS. Ventricular fibrillation is the leading cause of death for men aged 30 to 65 years in the Western world, and it would be surprising if infants would be completely spared. Indeed, LQTS causes sudden death even in the first months of life, as exemplified by the very first report of the Romano-Ward variant of LQTS. Existing epidemiology and narratives indicate that SIDS victims often die almost instantaneously. Few disease mechanisms can kill as rapidly as a lethal arrhythmia, which makes arrhythmic death a most plausible and likely cause that contributes to SIDS. Autopsy is negative in arrhythmic deaths as it happens in SIDS. Almost two thirds of fatal cases of LQTS are without sentinel events, and syncope would escape notice in an infant already lying in its crib.

Other arrhythmogenic disorders of genetic origin can cause sudden death in infancy and also contribute to SIDS. Among them, the Brugada and the short-QT syndrome are identifiable by both ECG and molecular screening, whereas catecholaminergic polymorphic ventricular tachycardia is identifiable only by molecular screening.

Molecular Findings

The scope of the present study provides a reliable estimate of the prevalence of mutations and rare variants in the LQTS genes, as shown by the narrow 95% confidence intervals (5.8 to 14.4%), which indicate that at the lower end of the spectrum this prevalence would not be <6% and that in actual probability it exceeds 10%.

Other potential pitfalls include the possibility of confounding, ethnic-specific, genetic effects and questions that surround the functional consequences of the identified variants. We did restrict our population to Norwegian cases and controls that originated from the same geographic areas and did not include 3 novel KCNH2 mutations because they lacked functional effects when expressed in Chinese hamster ovary cells. For the same reason, we did not include KCNQ1-P448R or SCN5A-A572D. Conversely, we did include only those genetic variants that showed functional differences from the wild-type ion channel or that are known as LQTS-causing mutations. Thus, a total of 19 of 201 (9.5%) cases with mutations/rare variants with demonstrable functional effects were identified.

Most variants identified in SIDS cases were in SCN5A, which differs from findings in clinically determined LQTS families where SCN5A mutations are found in <10% of cases. The high prevalence of SCN5A mutations in SIDS fits with their established role in causing arrhythmias during sleep, when most SIDS deaths occur. The mechanism by which potassium channel variants can contribute to SIDS is related to the observation that several conditions increase sympathetic activity during sleep, such as REM sleep, mild respiratory infections that lead to hypoxemia and thereby to chemoreceptive reflexes, sudden noises and startle, and so on. The 15 variants identified in these 19 cases include those likely capable of causing a lethal arrhythmia by themselves and those which might have required the presence of facilitating factors according to the “fatal triangle” hypothesis, which implies a vulnerable stage of development, a predisposition, and a trigger event. Infants are exposed to several conditions that increase cardiac electrical instability: REM sleep with bursts of vagal and sympathetic activation, minor upper respiratory tract infections that in infants easily induce hypoxemia and trigger chemoreceptive reflexes, and the prone-sleeping position, which is associated with increased sympathetic activity. The coexistence of these events in infants with genetic variants that reduce the repolarization reserve could enhance the risk of lethal arrhythmias. Another possible “second hit” could be the presence of common variants that amplify the functional electrophysiological impairment of genetic variants located on the same gene. This could be the case for the 7 of 8 SIDS subjects that carried SCN5A-H558R in association with mutations/rare variants in SCN5A that exhibited a modest or latent functional effect.
Indeed, the R558 allele frequency was twice as large in this subgroup of SIDS cases compared with controls. Similarly, the combination of CAV3-T78M with SCN5A-deJAL586-587 in 1 SIDS victim may also illustrate this concept.

The common variant KCNH2-R1047L was less frequent in SIDS victims versus controls (P=0.02), but its distribution was opposite to that reported in another white population.16,21 Together with a functional characterization indistinguishable from wild-type,9 this suggests a weak role in SIDS.

SCN5A-V1951L and SCN5A-R1193Q are common genetic variants in Hispanic and Asian populations, respectively,22 but in white populations and in our series of SIDS victims they appear to be rare variants that have significant functional effects27,28 and have already been reported as disease-causing mutations.28,29,30 In contrast, SCN5A-A572D, previously described as a mutation that causes LQTS disease,6 appears to be a common polymorphism in the Norwegian population. This raises the intriguing hypothesis that factors associated with specific ethnicity may modulate the effect of a given genetic variant. This could explain why the same genetic variant, such as V1951L, may be pathological in one population and might not exhibit any pathological effect in another.

Study Limitations
The present study, like similar ones based on DNA investigation, suffers from the anonymity imposed by ethical committees, which results in the lack of familial segregation data. In addition, anonymity prevents the identification and prophylactic therapy of those family members of SIDS victims with LQTS mutations who also are mutation carriers exposed to the risk of sudden cardiac death.

Implications
By providing evidence that genetically mediated arrhythmias represent a significant and nondisposable cause of SIDS, these findings call for a cautious medicolegal differential diagnosis when dealing with multiple SIDS in the same family48 and suggest an approach for a feasible reduction of preventable sudden deaths in infants and children.

The fact that, especially in infancy, malignant LQTS is usually associated with marked QT prolongation should stimulate an objective assessment of the pros and cons of prophylactic therapy of those family members of SIDS victims with LQTS mutations who also are mutation carriers exposed to the risk of sudden cardiac death.

Acknowledgments
We are grateful to Catherine Klersy and Carla Spazzolini for statistical assistance, to Elisa Andreoli for assistance with the molecular screening, and to Pinuccia De Tomasi for expert editorial support.

Sources of Funding
This work was partially supported by the National Institutes of Health grant HL68880, by an Italian Ministry of Health grant (“Studio sulla prevalenza, il significato clinico e l’evoluzione delle anomalie ECG neonatali associate ad aritmie nell’infanzia”), and by grants from the Norwegian SIDS Society and the Norwegian Foundation for Health and Rehabilitation.

Disclosures
None.

References


CLINICAL PERSPECTIVE

The 30-year-old hypothesis that some cases of sudden infant death syndrome (SIDS) could be caused by long-QT syndrome (LQTS) has received growing support from molecular studies. The practical implications of this hypothesis depend, however, on the true prevalence of LQTS-caused SIDS, and the available data are still inadequate. To more accurately quantify the contribution of LQTS gene mutations and rare variants to SIDS, we screened 7 genes associated with LQTs by denaturing high-performance liquid chromatography and nucleotide sequencing of genomic DNA from 201 SIDS cases and from 182 infant and adult controls, all Norwegians. Based on their functional effect, we considered as likely contributors to sudden death 8 mutations and 7 rare variants found in 19 of 201 cases (9.5%; 95% CI, 5.8 to 14.4%). This establishes sudden arrhythmic death as an important contributor to SIDS and carries 2 important implications. The genetic origin of 1 in 10 SIDS cases implies that the traditional concept of recurrent SIDS being highly suspicious is no longer acceptable, as our findings call for a cautious medicolegal differential diagnosis when dealing with multiple SIDS in the same family. As these genetic variants likely prolong QT interval duration and as, especially in infancy, malignant LQTS is usually associated with marked QT prolongation (QTc >470 ms), our results support the concept that an ECG performed between the third and fourth weeks of life would probably identify most of the infants at risk for sudden death caused by LQTs, either in infancy or later on in life, which would thus allow the institution of preventive measures.
Prevalence of Long-QT Syndrome Gene Variants in Sudden Infant Death Syndrome
Marianne Arnestad, Lia Crotti, Torleiv O. Rognum, Roberto Insolia, Matteo Pedrazzini, Chiara Ferrandi, Ashild Vege, Dao W. Wang, Troy E. Rhodes, Alfred L. George, Jr and Peter J. Schwartz

_Circulation_. 2007;115:361-367; originally published online January 8, 2007;
doi: 10.1161/CIRCULATIONAHA.106.658021
_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2007 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/115/3/361

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org/subscriptions/