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Postmortem Long QT Syndrome Genetic Testing for Sudden Unexplained Death in the Young

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Objectives

This study sought to determine the spectrum and prevalence of long QT syndrome (LQTS)-associated mutations in a large cohort of autopsy-negative sudden unexplained death (SUD).

Background

Potentially heritable arrhythmia syndromes may explain a significant proportion of SUD in the young. Here, comprehensive postmortem LQTS genetic testing was performed in a cohort of SUD cases.

Methods

From September 1998 to March 2004, 49 cases of SUD (30 male patients, average age at death 14.2 ± 10.9 years) were referred by medical examiners/coroners to Mayo Clinic’s Sudden Death Genomics Laboratory. Using polymerase chain reaction, denaturing high-performance liquid chromatography, and direct DNA sequencing, open reading frame/splice site mutational analysis was conducted for all 8 genes implicated in the pathogenesis of either LQTS (LQT1 to LQT6) or multisystem disorders involving either QT or QU prolongation.

Results

Ten LQTS-associated mutations (4 novel) were discovered in 10 SUD cases (20%, 8 female patients, average age at death 18.0 ± 11.8 years). The LQTS susceptibility mutations LQT1 (5), LQT2 (3), and LQT3 (2) were far more common among women (8 of 18, 44%) than men (2 of 30, 6.7%, p < 0.008). The activities at the time of SUD included sleep (5), exertion (2), auditory arousal (1), and undetermined (2). Sudden death was the sentinel event in two-thirds of the cases.

Conclusions

In this cardiac channel-focused molecular autopsy investigation of SUD, over one-third of decedents harbored a putative cardiac channel mutation: 7 previously reported to host mutations in the RyR2-encoded calcium release channel and now 10 with LQTS susceptibility mutations. Accordingly, postmortem cardiac channel genetic testing should be pursued in the evaluation of autopsy-negative SUD. (J Am Coll Cardiol 2007;49:240–6) © 2007 by the American College of Cardiology Foundation

In developed countries, sudden cardiac death is one of the most common causes of death. In the U.S., for example, an estimated 1,000 individuals die suddenly each day, with the vast majority of deaths occurring in the elderly and most often secondary to coronary artery disease. Sudden death in the young is relatively uncommon, with an incidence between 1 and 5 per 100,000 patient-years, and nearly one-half occur without warning, thus precipitating the need for a detailed medicolegal investigation, including autopsy (1). A postmortem analysis may detect a noncardiac basis of sudden unexplained death (SUD), such as asthma, epilepsy, or pulmonary embolism. However, structural cardiovascular abnormalities identifiable at autopsy including hypertrophic cardiomyopathy, arrhythmogenic right ventricular cardiomyopathy, congenital coronary artery anomalies, and myocarditis account for the majority of autopsy-positive sudden deaths (1). However, for at least 10% and perhaps as many as 30% of sudden deaths involving previously healthy children, adolescents, and young adults, no abnormalities are evident at autopsy (2). These autopsy-negative deaths are referred to as SUD syndrome after the first year of life (2–4).

Potentially lethal channelopathies, such as catecholaminergic polymorphic ventricular tachycardia (CPVT) and long QT syndrome (LQTS), leave no evidence to be found during a comprehensive medicolegal autopsy, leaving coroners, medical examiners, and forensic pathologists only to conjecture that a fatal arrhythmia might be responsible for
the SUD (5–7). However, a postmortem genetic analysis (i.e., molecular autopsy) may potentially substantiate the pathogenic basis for a SUD (8–12). Previously, we discovered pathogenic mutations involving the CPVT1-associated cardiac ryanodine receptor (RyR2) in 7 of 49 (14%) cases of SUD (13). Recent reports have implicated mutations in the LQTS-associated genes KCNQ1 and KCNH2 in small cohorts of SUD (8,10,12,14). Our previous population-based molecular autopsy of sudden infant death syndrome (SIDS) implicated LQTS-causing channel mutations in approximately 5% of infants (11,15). In addition, Crotti et al. (16) recently reported putative LQTS-associated mutations in 8.3% of SIDS patients derived from a cohort comprising approximately 200 infants. Although LQTS genetic testing is a clinically available diagnostic test, postmortem mutational analysis, or molecular autopsy, is performed only in specialized research laboratories at the present time.

Congenital LQTS comprises a distinct group of cardiac channelopathies characterized by delayed repolarization of the myocardium, QT prolongation, and increased risk for syncope, seizures, and sudden cardiac death in the setting of a structurally normal heart. Aborted cardiac arrest or sudden death is the sentinel event in 5% to 10% of LQTS cases (17). Approximately 75% of LQTS is caused by mutations in 5 cardiac channel-encoding genes: KCNQ1 (LQT1), KCNH2 (LQT2), SCN5A (LQT3), KCNE1 (LQT5), and KCNE2 (LQT6), whereas mutations in ANK2-encoded ankyrin B (LQT4) account for the extremely rare, nonchannel subtype of LQTS (18). Mutations in the KCNJ2-encoded Kir2.1 potassium channel are responsible for Andersen-Tawil syndrome (ATS1, formerly called LQT7), whereas a mutation in the CACNA1C-encoded L-type calcium channel mediates Timothy syndrome (TS1, formerly called LQT8) (19–21).

As such, LQTS may represent an arrhythmogenic disorder able to escape suspicion, detection, and apprehension by either a standard medicolegal autopsy or a careful evaluation of those surviving first-degree and second-degree relatives left behind (22,23). Here, in our cohort of 49 medical examiner/coroner-referred cases of SUD, we now focus on these LQTS susceptibility genes as candidate genes responsible for SUD.

**Methods**

**Medical examiner/coroner-referred cases of SUD.** From September 1998 to March 2004, 49 medical examiner/coroner cases of SUD from 42 medical examiner offices were referred to Mayo Clinic’s Sudden Death Genomics Laboratory for a cardiac channel molecular autopsy. By definition, to be accepted as a case of SUD, the decedent had to be >1 year old and the death had to be sudden, unexpected, and unexplained after the conclusion of a comprehensive medicolegal autopsy. Decedents with a premortem diagnosis of a cardiac channelopathy either in self or in a family member were excluded from this study.

This study was approved by the Mayo Foundation Institutional Review Board. Although informed consent is waived for investigations involving decedents, written informed consent from the decedent’s parents or appropriate next-of-kin was obtained.

**Postmortem genetic analysis of the LQTS susceptibility cardiac channel genes.** DNA was extracted from autopsy blood using standard phenol-chloroform procedures or from frozen tissue using the Qiagen DNeasy Tissue Kit (Qiagen, Inc., Valencia, California). Using previously published polymerase chain reaction primers, comprehensive postmortem LQTS genetic testing, using polymerase chain reaction, denaturing high-performance liquid chromatography (WAVE, Transgenic Inc., San Jose, California) and direct DNA sequencing, was conducted for the entire coding region (61 translated exons) of the LQTS susceptibility genes: KCNQ1, KCNH2, SCN5A, KCNE1, KCNE2, and KCNJ2, as previously described (8,11,24,25).

In addition, a targeted analysis of 10 ANK2 exons implicated in LQT4 and a mutation-specific analysis for TS1 (LQT8) involving CACNA1C were also conducted (23). Primers, polymerase chain reaction, and denaturing high-performance liquid chromatography conditions for mutation analysis are available on request. Previously, we performed a targeted mutational analysis of the CPVT-associated genes: RyR2-encoded ryanodine receptor/calcium release channel and CASQ2-encoded calsequestrin, in this cohort of SUD cases (13). In the current study, complete LQTS mutational analysis was performed for all 49 SUD referral cases in Mayo Clinic’s Sudden Death Genomics Laboratory regardless of their RyR2 mutation status.

**Results**

Table 1 summarizes the demographics and clinical phenotype of the 49 SUD cases that were referred by 42 medical examiners/coroner offices throughout North America to Mayo Clinic’s Sudden Death Genomics Laboratory for postmortem genetic testing during this 6-year study period. The mean age at death for this mostly white (92%) cohort was 14.2 ± 10.9 years (mean ± SD; range 1 to 43 years). A family history of sudden cardiac death (SCD) or syncope was explicitly documented by the medical examiner in 26 cases (13 positive and 13 negative) but was not specified in 23 cases. A personal history of syncope, seizure-like activity, or cardiac arrest before the SUD was
reported in 7 cases. Importantly, however, no decedent or relative had received a clinical diagnosis before death of a suspected cardiac channelopathy or heritable arrhythmia syndrome. When specified, most of the deaths occurred during sleep (17) or with exertion (12). Among the 19 female cases of SUD, 2 died suddenly during the postpartum period.

Postmortem genetic testing for LQTS susceptibility mutations showed 10 putative SUD-associated channel mutations/polymorphisms (4 novel) in 10 cases (20%) (Table 2, Fig. 1). Eight of the 10 mutations were true LQTS susceptibility mutations: LQT1 (5), LQT2 (2), and LQT3 (1), affecting highly conserved residues with nonconservative amino acid substitutions and absent in over 3,000

<table>
<thead>
<tr>
<th>Case</th>
<th>Gender</th>
<th>Age at SCD (yrs)</th>
<th>Ethnic Class</th>
<th>Gene</th>
<th>Exon</th>
<th>Nucleotide Change</th>
<th>Amino Acid Change</th>
<th>Event at SUD</th>
<th>Sentinel Event</th>
<th>Family History</th>
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<tr>
<td>1</td>
<td>M</td>
<td>17</td>
<td>W</td>
<td>KCNQ1</td>
<td>3</td>
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<td>SUD</td>
<td>POS</td>
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<td>43</td>
<td>W</td>
<td>KCNQ1</td>
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<td>NEG</td>
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<td>KCNQ1</td>
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<td>p.G584S†</td>
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<td>NEG</td>
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<td>KCNH2</td>
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<td>SUD</td>
<td>NEG</td>
</tr>
</tbody>
</table>

* Denotes a novel mutation. † Denotes a polymorphism.

B = black; Del = deletion; DUP = duplication; F = female; M = male; N/A = not available; NEG = negative; POS = positive; SUD = sudden unexplained death; W = white.
reference alleles (Fig. 1). In addition 2 mutations, R176W-KCNH2 and S1103Y-SCN5A, are functional polymorphisms that confer increased arrhythmia susceptibility to a vulnerable host (26–28). No pathogenic mutations in the genes associated with LQT4-6, ATS1, or TS1 were seen.

In conjunction with S1103Y-SCN5A, case 9 was also shown to harbor the uncommon, relatively black-specific KCNQ1 duplication of amino acids 52-54 (API, Table 2) (29). We speculate that the combination of this duplication and the S1103Y-SCN5A functional polymorphism may have conferred susceptibility for a fatal arrhythmia. In addition to cases 1 to 10, case 11 hosted an established CPVT1-associated mutation (S2246L-RyR2) as well as 2 nonsynonymous polymorphisms in LQTS-associated genes: G643S-KCNQ1 and D85N-KCNE1, which is seen in 1% to 2% of the population (Table 2). No RyR2 variants were seen in the 10 decedents with primary LQTS susceptibility mutations (cases 1 to 10).

Clinically, 8 of the 10 decedents were female and the average age at death was 18.0 ± 11.8 years. Overall, 8 of 18 female subjects (44%) compared with only 2 of 30 male subjects (6.7%) hosted LQTS-associated mutations (p < 0.008). The events at SUD included sleep (5 cases; LQT1 [2], LQT2 [1], LQT3 [2]) exertion (2 cases with LQT1), auditory trigger (1 case with LQT2), and undetermined (2 cases; LQT1 [1], LQT2 [1]) (Table 2). In contrast to the known link between exertional triggers and LQT1, 2 of the 5 LQT1-associated SUD cases had their sudden death during sleep. Consistent with the link between LQT2 and the postpartum period (8,11,24), one decedent experienced her SUD 4 months postpartum and was found at molecular autopsy to host the R176W-KCNH2 functional polymorphism.

Personal and family history data were available for 9 of the 10 cases of SUD hosting a LQTS susceptibility mutation/polymorphism. Sudden death was the sentinel event in 6 of the 9 cases (67%), whereas 3 cases (33%) had a personal history of either syncope or seizures (Table 2). Five of 9 (56%) had a family history positive for cardiac events. First-degree and second-degree relatives for 7 of the LQTS genotype–positive decedents have had confirmatory genetic testing. The identified mutations were familial in all 7 cases, and a total of 23 genotype-positive family members have been identified so far. Although incomplete penetrance and variable expressivity is the norm, each relative tested who contributed the positive family history of cardiac events...
hosted the LQTS-associated mutation, thus confirming proper co-segregation.

Figure 2 summarizes the overall yield of the postmortem genetic analysis, including the previous targeted analysis of the RyR2-encoded cardiac ryanodine receptor/calcium release channel that confers susceptibility for CPVT1. Overall, 17 of 49 decedents (35%) hosted a cardiac channel mutation, including 9 of 18 female subjects (50%) and 8 of 30 male subjects (27%). There was a significant cardiac channelopathy-gender effect, with 80% of the LQTS-associated mutations detected in females, whereas 6 of 7 (86%) CPVT1-associated mutations occurred in male decedents ($p < 0.001$). Consistent with the slightly later manifestation observed in LQTS compared with CPVT clinically, the decedents hosting LQTS-associated mutations tended to be older (18.0 ± 11.8 years) than those with CPVT1-associated mutations (13.6 ± 11.2 years), but this failed to achieve statistical significance secondary to the small sample size.

Discussion

Here, in this large molecular autopsy series of medical examiner/coroner-referred cases of autopsy-negative SUD, we provide molecular evidence implicating a cardiac channelopathy as the pathogenic basis for 35% of SUD cases, including 15% with CPVT1 and now 20% with LQTS susceptibility mutations. In addition, consistent with the known natural history of both CPVT and LQTS, there was a striking gender predilection for the elucidated channelopathies, with CPVT1-associated mutations found predominantly among male decedents, whereas LQTS-associated mutations predominantly involved female decedents (30,31).

Certainly, this study is not a population-based study of SUD. The reasons prompting the request for postmortem mutational analysis for these SUD cases from the 42 medical examiner offices represented herein and not others is unknown. The total number of autopsies performed or autopsies labeled as SUD by the 42 medical examiner offices over this study period is unknown. Nevertheless, the point estimate of 35% is remarkably congruent with both population-based epidemiology studies of SCD and clinical studies involving surviving family members of a sudden death victim (2,22,32,33).

In one of the most comprehensive epidemiologic studies of sudden death in the young to date, Puranik et al. (2) examined the autopsy reports from 427 young victims of unexpected death over a 10-year period in eastern Sydney, Australia. More than one-half were determined to be cardiac in origin, with autopsy-negative SCD and a presumed primary arrhythmia (29%) leading the way. Further, a 25-year review of autopsies in military recruits showed a nontraumatic sudden death rate of 13 per 100,000 recruit years with more than one-third (35%) of the sudden deaths representing autopsy-negative SUD (32). In retrospect, several cases were identified as having a family history of sudden premature death, suggesting a heritable predisposition to a malignant cardiac arrhythmia (32).

In 2003, Behr et al. (22) performed a cardiologic assessment of first-degree relatives for 32 cases of sudden arrhythmic death syndrome and showed that 22% of these families had evidence of inherited cardiac disease, with the majority having clinical features suggestive of LQTS. Similarly, in 2005, Tan et al. (33) found that 28% of families had an identifiable cardiac channelopathy after a clinical assessment of first-degree relatives of young SUD victims. Together, these reports indicate that identifiable and treatable cardiac channelopathies account for approximately one-third of autopsy-negative sudden deaths in the young.

In addition, our data suggest that many cases of autopsy-negative SUD may be preventable. Although sudden death was the sentinel event in the majority of mutation positive cases, many had a positive family history of cardiac events, yet no family members carried the diagnosis of LQTS. Further, 3 decedents had a personal history of syncope or seizure, but no premortem diagnosis was established. Overall, approximately half of the 17 decedents with a cardiac channel mutation detected by postmortem genetic testing showed potential warning signs, either personally or in the family. It is critical that such warning signs be heeded and thoroughly investigated. Given the efficacy of LQTS-related therapies, it is expected that a premortem diagnosis of LQTS might have thwarted the sudden death.

Although some of these sudden deaths may have been prevented with astute recognition of possible warning signs, the SUD was nevertheless the sentinel event for two-thirds of the LQTS-associated cases and for nearly all of the previously published CPVT1-associated cases (13). Inevitably, these data may renew consideration of a primary screening program to identify potentially at-risk subjects before such a tragic sentinel event. For CPVT, a
universal screening program is difficult to envision because the resting electrocardiogram (ECG) is always normal and exercise/catecholamine stress testing is required. On the other hand, individuals with LQTS most at risk for a lethal arrhythmia typically have manifest QT prolongation on a resting ECG. Although the practicalities, such as cost and feasibility of an ECG screening program in the young, remain debatable, it unavoidably follows that if at a relatively young age an ECG had been performed on the 10 decedents hosting LQTS-causing mutations, these individuals might have been diagnosed with LQTS, enabling the initiation of probable life-saving pharmacotherapy or device-related therapy.

Finally, a molecular autopsy can have a profound influence on surviving family members and perhaps should be considered in all cases of SUD (34). In addition, these data support the recommendation of a comprehensive cardiologic evaluation for surviving first-degree relatives (35). However, variable expressivity and incomplete penetrance are trademarks in arrhythmia syndromes such as LQTS and CPVT and should be expected in this evaluation (36).

Indeed, we have performed pedigree expansion of over half of genotype-positive decedents so far and have found that most of the mutations are familial. Only one spontaneous germline mutation has been identified in this cohort. Confirmatory genetic testing of family members has established genotype-positive status for at least 30 relatives, enabling the initiation of prophylactic therapy and other preventative measures, such as avoidance of medications with an unwanted effect on the QT interval. As shown here, the elucidation of a channelopathy susceptibility mutation in a postmortem sample may provide molecular confirmation regarding the cause and manner of death and a prospective life saving clue for the evaluation and management of those left behind.

Acknowledgments

The authors thank the medical examiners, forensic pathologists, coroners, and families who supported this research. Genetic testing in hopes of establishing the cause and manner of death for their patient or family member. Hopefully, these discoveries will translate into refined evaluation of those left behind and ultimately in steps to prevent sudden unexplained death in the young.

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