Long QT molecular autopsy in sudden infant death syndrome

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ABSTRACT

Objective To describe experience of long QT (LQT) molecular autopsy in sudden infant death syndrome (SIDS).

Design Descriptive audit from two distinct periods: (1) A prospective, population-based series between 2006 and 2008 (‘unselected’). (2) Before and after 2006–2008, with testing guided by a cardiac genetic service (‘selected’). LQT genes 1, 2, 3, 5, 6 and 7 were sequenced. Next of kin were offered cardiac evaluation.

Setting New Zealand.

Patients 102 SIDS cases.

Interventions Nil.

Main outcome measures Detection of genetic variants.

Results Maori 49 (47%), and Pacific island 24 (23%), infants were over-represented. Risk factors were common; bed sharing was reported in 49%. Rare genetic variants were commoner within the selected than unselected populations (5 of 31 infants (16%) vs 3 of 71 infants (4%) p < 0.05). In the selected population two infants had variants of definite or probable pathogenicity (KCNQ1, E146K; KCNH2, R1047L), two had novel variants of possible pathogenicity in SCN5A (I795F, F1522Y) and one had R1193Q in SCN5A, of doubtful pathogenicity. R1193Q was also the only variant in the three cases from the unselected population and occurred as a second variant with R1047L. Engaging families proved challenging. Only 3 of 8 (38%) variant-positive cases and 18 of 94 (19%) of variant-negative families participated in cardiac/genetic screening.

Conclusions LQT molecular autopsy has a very low diagnostic yield among unselected SIDS cases where risk factors are common. Diagnostic yield can be higher with case selection. Engagement of the family prior to genetic testing is essential to counsel for the possible uncertainty of the results and to permit family genotype-phenotype cosegregation studies.

INTRODUCTION

Sudden infant death syndrome (SIDS) accounts for 8% of postneonatal deaths and 0.5 deaths per 1000 live births in the USA1 and 13% and 0.7 per 1000, respectively, in New Zealand.2

Long QT syndrome (LQTS) is a group of inherited cardiac ion channelopathies, with 13 known genotypes, which predispose to sudden death due to ventricular tachycardia. Molecular genetic testing has now demonstrated variants in genes associated with LQTS in over 10% of SIDS cases.3–6 LQTS type 3 (linked to the SCN5A gene) predominates but types 1, 2, 6, 9 and 12 have also been described.7

Testing for LQTS genes for SIDS is not yet part of standard practice,7–9 and it is not known if genetic testing should for example be restricted to ‘pure’ (type 1A) SIDS10 11 or whether it may have a role in infants exposed to a potential arrhythmic trigger. For example, fatal arrhythmias can be caused by adrenergic stress in LQTS and fever in Brugada syndrome, a condition also linked to SCN5A.12 Co-sleeping (indicating type II SIDS) might cause stress and overheating. The role of racial origin is to be evaluated, and it is also not known if adding family cardiac screening will increase diagnostic yield, such as it does with sudden death investigations in the 1–40 age group.13–15

New Zealand (population 4.43 million) has an established national young sudden death registry and investigative programme, with national ethics committee approval.16 17 We wished to examine the value of the long QT molecular autopsy in our population, with the addition of family cardiac investigation, and to see how these results compared with those from sudden unexplained deaths in 1 to 40-year-olds.13

Original article

Table 1 Demographics, history, investigations, cardiac testing and final diagnosis in the 3 cases in which a genetic variant (all R1193Q in SCN5A) was identified during the prospective (‘unselected’) study period

<table>
<thead>
<tr>
<th>ID</th>
<th>Age</th>
<th>Ancestry</th>
<th>Circumstances of death</th>
<th>Sleeping arrangement</th>
<th>Sleep position</th>
<th>SIDS category</th>
<th>Previous medical history</th>
<th>Cardiac tests during life</th>
<th>Family history of syncope or SIDS</th>
<th>Social history</th>
<th>No. of family members investigated</th>
<th>Final cardiac diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>5 months</td>
<td>Asian</td>
<td>Found deceased in bed</td>
<td>Own cot</td>
<td>Supine</td>
<td>SIDS IB</td>
<td>Nil</td>
<td>Full term</td>
<td>Nil</td>
<td>No</td>
<td>No adverse social factors</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>1 months</td>
<td>Pacific Islander</td>
<td>Found deceased in bed</td>
<td>Not stated</td>
<td>Supine</td>
<td>SIDS IB</td>
<td>Nil</td>
<td>Nil</td>
<td>No</td>
<td>Nil</td>
<td>Unknown</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>&lt;1 months</td>
<td>Maori</td>
<td>Found deceased in bed</td>
<td>Co-sleeping</td>
<td>Not stated</td>
<td>SIDS II</td>
<td>Nil</td>
<td>Nil</td>
<td>No</td>
<td>Nil</td>
<td>adverse</td>
<td>0</td>
</tr>
</tbody>
</table>

SIDS, sudden infant death syndrome.

METHODS

Population

Data are presented from two distinct periods. The first period (the prospective study or ‘unselected’ period), 1 September 2006–31 August 2008, was a 2-year prospective population-based series where testing incurred no cost and referrals were not filtered.

The second period was from before and after the prospective study period when Cardiac Inherited Disease Group (New Zealand) (CIDG) investigated some SIDS cases on an ad hoc basis, with referrals from a variety of sources. The police report and full autopsy report was reviewed by the cardiac genetic team and accepted for testing if there was felt to be an increased likelihood of arrhythmic death.

Classification of SIDS

Cases were subdivided into three categories according to Krous et al11 SIDS IA, IB and II, on consensus review of autopsy and police reports by the first author and two senior forensic pathologists (PLM, SRS). IA comprises infants between 21 days and 9 months old with a normal clinical history, term delivery, normal growth and development, no similar previous deaths in the family, a negative scene examination, safe sleep environment and exclusion of an accidental or intentional death. Lethal pathological findings should be absent and there must be a comprehensive post-mortem examination including ancillary studies such as toxicology, microbiology, radiology, vitreous biochemistry and metabolic studies. The SIDS IB category differs from IA by the lack of one of these investigations. The SIDS category II includes deaths outside the SIDS I age range (21 days–9 months) and cases where accidental asphyxia could not be excluded.

Source of DNA

This work began in 2000. Before September 2006, blood or tissue for DNA extraction was taken at the discretion of the pathologist. Archived blood on a neonatal screening card was used as a source of DNA when no other sample was available, including cases where death occurred before CIDG existed.18

After September 2006, a suitable sample was obtained at the autopsy. Cardiac genetic investigation commenced once ancillary tests were completed and were negative.

Contact with next of kin

After September 2006, contact was made with the next of kin by a standard letter, followed by a phone call from either the coordinator or a designated clinician. If contact was successful and the family wished to engage in the process, the family was invited to a local cardiology clinic, full clinical and family history obtained and ECGs taken from parents and siblings. All results were reviewed by a central expert committee.

Genetic testing

Sequencing of the LQTS genes 1, 2, 3, 5 and 6 was performed in all and 7 in some, dependent on the laboratory. Three clinically accredited laboratories were involved at various times (LabPlus Auckland City Hospital, New Zealand; Rikshospitalet University Hospital, Norway; and Statens Serum Institut, Denmark). DNA was amplified using exon-specific primers and the subsequent amplicons were subjected to bidirectional capillary-based di-deoxy sequencing as previously described.19 Variants were categorised into one of three types regarding level of certainty of pathogenicity using combined evidence from previous reports, in vitro evidence and in silico evaluations into: 1 —definite or probable, 2 —possible or 3 —high level of uncertainty.

RESULTS

DNA was initially stored on 248 cases of sudden unexpected death in infancy. Year of death ranged from 1983 to 2012. The cause of death was SIDS in 146, 103 of which were referred for LQTS gene screening and family investigation. In one of these, DNA quality was too poor to test, leaving 102 cases.

Ethnicity

Maori 49 (47%), Pacific islander 24 (23%), 24 New Zealand European (23%), 5 Asian infants (5%) and 3 others. In the general population, New Zealand Europeans represent 68%, Maori 15% and Pacific islanders 7%, respectively.20

SIDS classification

SIDS IA category criteria were met in only 10% of cases, SIDS IB in 55% and SIDS II in 35%.11 Bed sharing was reported in 49% and major adverse social factors in 13%.

Age and activity at time of death

Age was from 4 days to 1 year (median 3 months); 80% of deaths occurred between 21 days and 9 months; 49% were male; 88% of infants died during sleep and six (6%) died while awake. In six cases activity at death was not stated.
Review of cases with variants in LQTS genes

Five rare missense variants in LQTS genes were found in 8 of the 102 infants (8%), 3 of 71 (4%) from the prospective study period 2006–2008 (unselected cases) and 5 of 31 (16%) from outside the prospective study period (selected cases) (p<0.05) (see tables 1 and 2).

Rare variants were identified in the SCN5A (LQT3) gene in seven infants and in the KCNQ1 (LQT1) and KCNH2 (LQT2) genes in one infant each. One infant had two variants. In silico analyses of each are presented in table 3.

Genetic variants identified outside from the prospective study period (71 unselected cases, 2006–2008)

No variants of likely pathogenicity were found. Three cases had R1193Q in SCN5A, of doubtful significance (discussed below). None of these families could be screened because they declined or repeatedly did not attend scheduled appointments.

Genetic variants identified from outside the prospective study period (31 selected cases)

<table>
<thead>
<tr>
<th>ID</th>
<th>Age</th>
<th>Ancestry</th>
<th>Circumstances of death</th>
<th>SDS category</th>
<th>Sleep position</th>
<th>Social history during life</th>
<th>Genetic variant</th>
<th>No variants of likely pathogenicity (3 cases)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 year</td>
<td>Asian</td>
<td>Found deceased in bed</td>
<td>SIDS II</td>
<td>Found prone</td>
<td>Nil</td>
<td>R1193Q in SCN5A</td>
<td>Unlikely LQTS</td>
</tr>
<tr>
<td>2</td>
<td>&lt;1 month</td>
<td>Pacific</td>
<td>Found deceased in bed</td>
<td>SIDS II</td>
<td>Found prone</td>
<td>Nil</td>
<td>I759F in KCNQ1</td>
<td>Uncertain Possible LQTS</td>
</tr>
<tr>
<td>5</td>
<td>3 months</td>
<td>Maori</td>
<td>Seizures then unresponsive</td>
<td>No</td>
<td>Not stated</td>
<td>Unknown</td>
<td>E146K in KCNQ1</td>
<td>Possible LQTS</td>
</tr>
<tr>
<td>7</td>
<td>7 months</td>
<td>NZ</td>
<td>Found deceased in bed</td>
<td>SBDS</td>
<td>Not stated</td>
<td>Unknown</td>
<td>H558R in SCN5A</td>
<td>No Normal ECG</td>
</tr>
<tr>
<td>8</td>
<td>&lt;1 month</td>
<td>European</td>
<td>Not stated</td>
<td>SBDS</td>
<td>Not stated</td>
<td>Nil</td>
<td>F1522Y in SCN5A</td>
<td>Equivocal QT prolongation</td>
</tr>
</tbody>
</table>

Novel variants of possible pathogenicity (2 cases)

Case 2, of Pacific island ancestry, was found deceased, in a co-sleeping environment. Genetic testing revealed the previously unreported I759F in the SCN5A gene. Efforts failed to contact the family. Case 8 was <1 month New Zealand European who suffered a cardiac arrest on day one of life while feeding, resulting in severe hypoxic-ischaemic encephalopathy and death. An ECG showed a prolonged QTc interval (0.49 msc) but it was taken early postarrest with encephalopathy which might explain the finding. The previously unreported F1522Y was found in the SCN5A gene.

Variant with a high degree of uncertainty regarding pathogenicity

Cases 1 and 5 carried the R1193Q variant in SCN5A. It has been reported in association with LQTS and Brugada syndrome, and is present in 0.3% of the Caucasian population.24 However, it is also a documented polymorphism that is common (8–10%) in the Asian/Han Chinese population.25 Case...
### Table 3  In silico analysis of long QT genetic variants found in eight victims of SIDS

<table>
<thead>
<tr>
<th>Gene</th>
<th>RefSeq accession numbers (transcript and protein) and UniProt number</th>
<th>Variant position (transcript, protein and exon)</th>
<th>Bioinformatic programmes</th>
<th>Mutation Assessor&lt;sup&gt;b&lt;/sup&gt; (Functional Impact)</th>
<th>I-MUTANT 3.0&lt;sup&gt;c&lt;/sup&gt;</th>
<th>PMut&lt;sup&gt;d&lt;/sup&gt;</th>
<th>MutPred&lt;sup&gt;e&lt;/sup&gt;</th>
<th>SNPs&amp;GO&lt;sup&gt;f&lt;/sup&gt;</th>
<th>PANTHER&lt;sup&gt;g&lt;/sup&gt;</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>KCNQ1</strong></td>
<td>NM_000218.2 NP_000209.2 P51787</td>
<td>c.436G&gt;A p.E146K E2</td>
<td>Benign (Scores: 0.250; 0.134) Neutral</td>
<td>Disease (RI 6.0) Neutral (RS 0)</td>
<td>Pdel: 0.859 Disease (RI 5.0) Pdel: 0.31</td>
<td>Pathogenic (LQTS1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>KCNH2</strong></td>
<td>NM_000238.2 NP_000229.1 Q12809</td>
<td>c.3140G&gt;T p.R1047L E13</td>
<td>Benign (Scores: 0.039; 0.020) Low</td>
<td>Disease (RI 2.0) Pathological (RS 8) Pdel: 0.373 Disease (RI 1.0) Pdel: 0.42</td>
<td>Pathogenic (LQTS2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>SCN5A</strong></td>
<td>NM_198056.2 NP_932173.1 Q14524</td>
<td>c.1673A&gt;G p.H558R E6 c.2275A&gt;T&lt;sup&gt;†&lt;/sup&gt; p.I759F E15 c.3578G&gt;A p.R1193Q E20 c.4565T&gt;A&lt;sup&gt;†&lt;/sup&gt; p.F1522Y E27</td>
<td>Benign (Scores: 0.000; 0.000) Neutral Benign (Scores: 0.003; 0.007) Medium Benign (Scores: 0.001; 0.006) Low&lt;sup&gt;†&lt;/sup&gt; Benign (Scores: 0.417; 0.116) Low</td>
<td>Neutral (RI 6.0) Neutral (RS 6) Disease (RI 5.0) Neutral (RS 3) Disease (RI 6.0) Pathological (RS 3) Disease (RI 1.0) Neutral (RS 8)</td>
<td>Pdel: 0.878 Disease (RI 7.0) Pdel: 0.54 Pathogenic (LQTS3) Pdel: 0.825 Neutral (RI 4.0) Pdel: 0.28 Uncertain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Pdel: 0.823 Disease (RI 4.0) –</td>
<td>Possibly pathogenic (LQTS3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> http://genetics.bwh.harvard.edu/pph2/
<sup>b</sup> http://mutationassessor.org/
<sup>c</sup> http://gpcr2.biocomp.unibo.it/cgi/predictors/I-Mutant3.0/I-Mutant3.0.cgi; RI refers to Reliability Index.
<sup>d</sup> http://mnm2.pcb.ub.es:8080/PMut/; RS refers to Reliability Score.
<sup>e</sup> http://mutpred.mudb.org/; Pdel refers to the probability that the variant is a deleterious mutation.
<sup>f</sup> http://snps-and-go.biocomp.unibo.it/snps-and-go/; RI refers to Reliability Index.
<sup>g</sup> http://www.pantherdb.org/tools/cmpScoreForm.jsp; Pdel refers to the probability that a given variant will cause a deleterious effect on protein function.

*Scores relate to predictions based on HumDiv and HumVar models.
†Not previously reported.
<sup>†</sup>Accelerates the inactivation of the sodium channel current and exhibits reduced sodium channel current at the end of phase I of the action potential (taken from report provided by Mutation Assessor).

LQTS, long QT syndrome; SIDS, sudden infant death syndrome.
1 had Asian ancestry but the others, including those from the prospective study period, were Pacific Island, Asian and Maori (two cases). ECG screening was only possible in the family of Case 1 and was normal.

Results of family screening

Despite extensive efforts, only three families of the eight variant-positive cases (38% described above) and 18 families of the 94 variant-negative SIDS cases (19%) could be screened, with only one family with QT prolongation. The reasons for failed screening were multiple but included 32 cases where the family did not wish to engage or did not reply to messages or attend appointments.

Familial LQTS with uninformative genetic testing

One family with uninformative genetic testing had family members with LQTS by clinical criteria (outside the prospective study period). A Maori infant died suddenly during feeding. A subsequent ‘near-miss’ SIDS episode occurred in another sibling who was noted to have a prolonged QT interval, as did a brother and parent. Affected individuals are treated with β-blockers.

DISCUSSION

Some authors recommend routine screening of LQTS genes in SIDS, whereas some advocate a more cautionary approach. Recent expert guidelines from an international consortium of heart rhythm groups state that an arrhythmia focused molecular autopsy can be useful in all victims of sudden unexplained death in infancy. Our experience, a diagnostic rate of effectively zero, demonstrates a reluctance to attend appointments or a mistrust of the system. It will be interesting to observe whether other countries with high prevalence of social deprivation (unlike Norway) have the same experience. This is the population at most risk of SIDS.

The risk of infant death increases with co-sleeping. Consensus recommendations now promote a broader definition of conditions favouring a safe sleep environment than just avoiding prone sleeping. This message still appears to require active dissemination, particularly to ethnic groups in which adult–infant bed sharing is the norm, such as Pacific peoples.

Our discovery of a kindred with LQTS based on prolongation of the QT interval, but lacking a demonstrable variant, reinforces the point that gene sequencing should not be a stand-alone test in those with a suggestive clinical history. Sequencing detects variants in only 70%–75% of individuals with definite LQTS.

Limitations of the study

This study applied a sequence-based analysis of LQTS genes for types 1, 2, 3, 5, 6 and 7. It did not include gene dosage testing, nor screening other LQTS genes (eg, Ankyrin B, CACNA1C, CAV3, SCN4B, AKAP9 and SNTA1) and other cardiac channelopathies linked to sudden infant deaths (eg, RYR2, NOS1AP and GDP1-L). Further, we acknowledge that some of the variants found (such as R1193Q) might exert influence alongside variants from other genes not tested for.

CIDG has a single part-time co-ordinator who covers the whole of New Zealand. A better-resourced service might have led to higher rates of engagement by families, particularly those with poor social circumstances and those with cultural mistrust of health services, among whom Maori and Pacific Islanders tend to be over-represented.

In a clinical service, we have reported that the quality of post-mortem investigation is variable. We cannot tell if New Zealand is better or worse than other countries in this respect, since no other similar reports are available. Improvements are needed in process, reporting and more consistency in following the recommended best practise guidelines.

Maori and Polynesians are over-represented in the SIDS cohort. This may limit the degree to which the findings here may be extrapolated to other countries.

CONCLUSIONS AND PRACTICE RECOMMENDATIONS

While we have adopted the molecular autopsy in sudden unexplained death in the 1–40-year age group, we now use it sparingly in infants. A prerequisite for testing now is that the family can be contacted and is willing to participate in full cardiac and genetic evaluation. The family must accept the potential implications and uncertainties of genetic testing.
Factors which make us more inclined to offer testing are (1) a family history of previous sudden deaths, syncope, seizures or proven cardiac arrhythmias. (2) Absence of known risk factors, particularly bed sharing with possible overlaying. (3) Sudden collapse while awake. (4) The autopsy and report excluding a recognizable cause of death is extensive and of high quality.  

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JMG wrote the manuscript and takes full responsibility for the manuscript. JMG, PML, JC, SRS, DBR, DBL, SRS, Conception and design. Revising the article. Final approval of the version to be published. JC

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