Molecular autopsy in the sudden cardiac death of a young woman: A first Canadian report

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Standard autopsy of young victims with sudden cardiac death commonly does not identify a specific pathological diagnosis. In such cases, sudden cardiac death may be secondary to a genetic condition predisposing the patient to ventricular arrhythmias. Failure to identify a genetic etiology for an unexpected sudden death may leave surviving family members at risk for a similar tragedy. The case of a 21-year-old woman who died suddenly while at rest is presented. Molecular genetic analysis of tissue retrieved from the regional coroner's office identified a novel missense mutation in the *KCNH2* gene, a gene known to cause the long QT syndrome.

Key Words: Arrhythmia; Genetics; Long QT syndrome; Sudden cardiac death

Sudden cardiac death (SCD) in a young person is a tragic event. Postmortem examination is unremarkable in as many as 30% of cases (1), leaving surviving family members with uncertainty about the cause of death. Over the past decade, genetic research in families with inherited cardiac rhythm disturbances has established a molecular basis for ventricular arrhythmias that may occur in otherwise healthy, young individuals. Genes responsible for the long QT syndrome (LQTS), catecholamine-induced polymorphic ventricular tachycardia, Brugada syndrome and arrhythmogenic right ventricular cardiomyopathy have been identified. Postmortem molecular diagnosis, sometimes termed 'molecular autopsy', is now available to complement the standard autopsy in cases of unexplained sudden death. The information gained from molecular autopsy may provide the insight necessary to initiate prophylactic and potentially life-saving therapy in surviving family members. We present the case of a young woman who died suddenly and whose cause of death remained uncertain until a molecular autopsy was performed.

CASE PRESENTATION

A 21-year-old woman was found dead in her bed. She had a previous history of recurrent syncope, and in the hours preceding her death, she was studying for examinations. Her only medication was an unspecified "cold medication" for an upper respiratory tract infection. Previous cardiac investigations, including Holter monitoring and two-dimensional echocardiography, were reported to be

Une autopsie moléculaire après la mort cardiaque subite d'une jeune femme : Un premier rapport canadien

L'autopsie classique des jeunes victimes d'une mort cardiaque subite ne permet généralement pas de poser un diagnostic pathologique précis. Dans ces cas, la mort cardiaque subite peut être secondaire à un trouble génétique prédisposant le patient à des arythmies ventriculaires. Lorsqu'on omet de repérer une étiologie génétique à une mort cardiaque inattendue, la famille de la personne disparue risque de subir le même sort. On présente le cas d'une femme de 21 ans qui est morte subitement, au repos. L'analyse génétique moléculaire de tissus prélevés au bureau du coronar régional a permis de dépister une nouvelle mutation faux-sens du gène *KCNH2*, connu pour être responsable du syndrome du QT long.

normal. A 12-lead electrocardiogram (ECG) was never performed, and no specific etiology for her prior syncopal events was determined. There was no family history of SCD. A standard autopsy found a myxomatous mitral valve and a "septum at origin of the left main coronary artery". The remainder of the anatomical and toxicology examination was unremarkable, and the cause of death was listed as SCD, likely an arrhythmia secondary to the two structural anomalies.

In the year following this tragic SCD, the surviving sister, at 19 years of age, experienced a syncopal event prompting immediate cardiology consultation. Cardiac investigations included a 12-lead ECG, exercise stress test, and 24 h Holter monitor. These study results were within normal limits. A two-dimensional echocardiogram suggested the presence of a "dilated left main coronary artery". A subsequent coronary angiogram was normal. Despite these normal studies, the surviving sister had recurrent visits to her local emergency room for 'anxiety' and a second syncopal event, which prompted consultation with an arrhythmia specialist. Clinical history suggested a vagal origin for the syncopal events. However, in view of the SCD of her sister, further investigations were performed. A repeat 12-lead ECG remained within normal limits. A drug provocation study with intravenous procainamide and adrenaline was performed aimed to unmask the Brugada syndrome and LQTS, respectively. No evidence of a Brugada ECG pattern was evident. However, a borderline result for abnormal QT prolongation was observed.

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Figure 1) A 12-lead electrocardiogram of the deceased patient's mother, previously diagnosed with 'epilepsy', demonstrating a prolonged QT interval (corrected QT 483 ms). Note the notched T wave in lead V4 (arrow), consistent with the long QT syndrome type 2 (2)

Her family history was remarkable, with her mother having had a diagnosis of 'epilepsy', made 30 years previously on the basis of recurrent syncopal events with observed seizure-like activity. The patient had been maintained on phenytoin and had never undergone a 12-lead ECG. On evaluation in the arrhythmia clinic, her ECGs at the age of 53 years consistently demonstrated a prolonged corrected QT interval of approximately 480 ms (Figure 1). A diagnosis of LQTS was suspected, and she was initiated on a beta-blocker and weaned off phenytoin. Following genetic counselling of the mother and surviving sister, genetic testing was performed on the deceased for mutations associated with LQTS.

Autopsy samples were retrieved from the local coroner's office following a written request by the patient's mother. DNA was extracted from paraffin-embedded blocks of liver tissue. Genetic testing for LQTS entailed screening the two most common ion channel genes known to cause this condition, LQTS type 1 (LQT-1) and LQT-2. In the deceased patient, a novel missense mutation, alanine-85-proline, was identified in a 'mutation hotspot' region of the LQT-2 gene, a region known to alter the ion channel activity of this protein (Figure 2). DNA samples from the mother and sister were also tested. Molecular testing of the LQT-1 and LQT-2 genes was carried out on the mother and sister. The mutation was confirmed in the mother, who had ECG evidence of LQTS. DNA testing proved that the surviving sister did not carry this disease-causing mutation, providing significant reassurance.

DISCUSSION

Postmortem molecular genetic testing has not been previously reported for the use of autopsy diagnosis in Canada. In cases of SCD, determining a specific diagnosis enables the initiation of appropriate treatment and genetic counselling of surviving family members in the hope of preventing a similar tragedy. In the present case, the sudden death of a previously healthy, young woman was attributed to the autopsy findings of a myxomatous mitral valve and/or a coronary artery anomaly as possible causes of a sudden arrhythmia. A small proportion of patients with myxoid mitral valves have ventricular ectopy, and the risk for sudden death appears to be greatest in patients with more severe abnormalities, such as significant mitral regurgitation, a dilated left ventricle or ruptured chordae (3). The patient described had a two-dimensional echocardiogram before her death, which showed no evidence of mitral valve disease. The autopsy finding of a septum at the ostium of the left main coronary artery was not reported to be stenotic, and there was no evidence of thrombus. Furthermore, the patient had

Figure 2) Topology of KCNH2 ion channel. Mutations responsible for the long QT syndrome have been identified throughout all domains of the KCNH2 channel. A mutation 'hotspot' region is found in the N-terminus between amino acid residues 80 to 90, where nine unique mutations have now been identified in affected patients. Modified with permission from Michael Pasternack, University of Helsinki (Helsinki, Finland)

no history of exercise-induced chest pain or syncope, and her sudden death occurred within the confines of her university dormitory room. Without more evidence of symptoms that could be correlated with the mitral valve or coronary artery findings, it was difficult to attribute these anomalies to the cause of her sudden death.

Two years elapsed before genetic testing of autopsy tissue was performed, prompted by syncopal events in a surviving sibling and a suspicious history of 'epilepsy' in her mother. The molecular diagnosis of LQTS provided this family with important information about individual risks and medical management. Clinical testing alone was not sufficient in ruling out LQTS in the surviving sister. Inherited LQTS has a clinical penetrance of only 25%, and clinical evaluation by 12-lead ECG has only a 38% sensitivity in correctly identifying gene carriers in affected families (4). Although provocative intravenous drug testing with adrenaline may unmask LQT-1 caused by the *KCNQ1* gene, this test is of no value in the other multiple genetic causes that comprise more than 50% of LQTS cases. Not surprisingly, the result of intravenous adrenaline testing in the surviving sibling was equivocal, because the identified genetic defect was found in the LQT-2 gene, *KCNH2*. The limitations of clinical evaluation compare with a genetic test detection rate of up to 70% in affected individuals by current genetic testing methods (5). If DNA testing had been available sooner, the cardiac testing of family members might have been more directed and less costly. In addition, there are psychological benefits to the family and community to having a diagnosis, because the unexpected and unexplained death of a healthy young person often has profound impacts, including misplaced blame and guilt.

In Canada, blood and tissue samples are routinely stored in all coroners' cases, allowing for further testing. Blood samples are stored

for five years and then disposed of, whereas tissue samples, usually fixed in paraffin, are stored indefinitely. Samples from liver, spleen and lung provide good yields of DNA from a small quantity of tissue. Pathologists and coroners cannot be expected to have the sole responsibility for deciding which SCD cases may benefit from molecular testing. In addition, the selection of the most appropriate molecular test requires knowledge of the context of the sudden death, previous cardiac symptoms and the molecular testing methodology. While genetic testing for LQTS currently is only available through a small number of laboratories, as experience and evidence of the benefits of testing grows, its availability should increase. A policy may be developed for a multidisciplinary effort involving forensics, cardiology, genetics and public health to evaluate medical and family histories in cases of sudden cardiac death to provide the family with recommendations for medical evaluations.

CONCLUSIONS

In SCD cases, the clinical or family history can suggest an inherited arrhythmic condition and molecular testing of autopsy samples

can provide a specific diagnosis. By identifying a mutation causing LQTS, family members can be screened and given appropriate medical advice. Policies are needed to provide cost-effective molecular genetic testing in the sudden, unexplained deaths in young people.

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