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Postmortem genetic diagnosis of long QT syndrome in a case of sudden unexplained death of a young child: A case report and overview of regional guidelines for genetic testing

Routine procurement and freezing of tissue at autopsy is essential for diagnosis of genetic cardiac conditions and counseling of surviving relatives.

ABSTRACT: Many cases of sudden unexplained death in the young are due to heritable mutations that cause disturbances in cardiac conduction that cannot be diagnosed at the time of autopsy. Screening of immediate family members of the deceased can identify individuals affected with the same condition. The collection and freezing of unfixed tissue at autopsy is crucial for the genetic confirmation of heritable cardiac conduction disorders. The case of a young victim of sudden unexplained death demonstrates how electrocardiogram screening of relatives can identify a cardiac ion channel mutation in family members. Several North American guidelines address tissue retention in cases of sudden unexplained death of the young. Although similar guidelines have not been published for British Columbia, autopsies for most cases of unexplained death in infants and children under 16 years of age in BC are performed at BC Children's Hospital, where a standard autopsy approach is used and tissue is snap-frozen. To ensure every family can obtain the care they need, regardless of the jurisdiction, physicians performing autopsies in cases of sudden unexplained death in the young should use standard autopsy practices and retain tissue suitable for future genetic testing.

A death occurring instantaneously, or less than 24 hours after symptom onset, in an otherwise healthy individual, without a known cause, is known as a "sudden unexplained death" (SUD), a definition recognized in *ICD-10*.[1] Unexplained sudden cardiac death is a subset of SUD, in which the cardiac cause of the death is not detectable by basic autopsy techniques such as gross and microscopic examination. In individuals 1 year of age and older, such a death is referred to as "sudden unexplained death syndrome" (SUDS).[2] Where no cause of death is identified after a thorough autopsy, scene investigation, and toxicological evaluation,

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such a death is referred to as "sudden arrhythmic death syndrome" (SADS).[2] Sudden death in infants under 1 year of age is referred to as "sudden death in infancy" (SUDI).[2] When the autopsy, scene investigation, and toxicology results are negative, such a death is referred to as "sudden infant death syndrome" (SIDS)[2] (see box). The term "sudden unexpected death" is frequently found in the literature, but lacks specificity. The specific terms listed in the box have been endorsed by Heart Rhythm Society, the European Heart Rhythm Association, the Asia Pacific Heart Rhythm Society, the American College of Cardiology Foundation, the American Heart Association, the Pediatric and Congenital Electrophysiology Society, and the Association for European Pediatric and Congenital Cardiology.[2] Since both SADS and SIDS are, by definition, diagnoses of exclusion, these diagnoses cannot be made without performing a complete autopsy that includes a neuropathology examination.

In individuals younger than 35 years, including infants, the heritable cardiac arrhythmia disorders, including long QT syndrome, Brugada syndrome, short QT syndrome, and catecholaminergic polymorphic ventricular tachycardia (CPVT), are increasingly recognized as causes of sudden unexplained death. Independent studies from Asia, Europe, and North America have found causative mutations for these disorders in approximately 10% of deceased infants originally diagnosed with SIDS.[3-5] Similar findings were identified in individuals up to age 35, with one American study identifying cardiac ion channel gene mutations in 35% of referred-in cases.[6]

Since many cardiac conditions are inherited, systematic investigation of first-degree relatives can inform future clinical management and counseling. The value of screening relatives for heritable cardiac disorders after patients have died suddenly and unexpectedly has been well documented in several studies.[7-10] In these studies, the percentage of families affected by mutations ranged from 14%[11] to 53% (the data also included structural disorders such as hypertrophic cardiomyopathy).[8] Screening survivors of unexplained cardiac arrest and their relatives for cardiac arrhythmia syndromes is especially valuable because a significant proportion of them will be diagnosed with a cardiac disorder.[12]

Evidence indicates that inherited arrhythmias are responsible for a subset of sudden unexplained death cases, and that the fatal outcome of these channelopathies in asymptomatic mutation carriers can be prevented by lifestyle modification, medications, and discretionary use of an implantable cardiac defibrillator. Members of the death investigation team (coroner/medical examiner, pathologist, clinicians) should all maintain a high index of suspicion for inherited arrhythmias, especially because North American guidelines on the workup for SUDS and SUDI cases have not consistently addressed the need to retain unfixed tissue for future genetic testing.

In British Columbia, although autopsies are performed throughout the province, the retention of unfixed tissue in cases of autopsy-negative sudden death of young individuals is not a standardized practice except at BC Children's Hospital, and there are currently no established provincial guidelines that mandate this practice.

Terms used to describe sudden death

Sudden unexplained death (SUD)
Death occurring instantaneously, or less than 24 hours after symptom onset, in an otherwise healthy individual, without a known cause.

Sudden unexplained death syndrome (SUDS)
Death in an individual 1 year of age and older.

Sudden arrhythmic death syndrome (SADS)
Death in an individual 1 year of age and older where no cause is identified after a thorough autopsy, scene investigation, and toxicological evaluation; diagnosis of exclusion.

Sudden unexplained death in infancy (SUDI)
Death in an infant under 1 year of age.

Sudden infant death syndrome (SIDS)
Death in an infant under 1 year of age where no cause is identified after a thorough autopsy, scene investigation, and toxicology evaluation; diagnosis of exclusion.

Case data

A 10-year-old child collapsed at school. Bystander CPR was initiated promptly and the child was transported to hospital by ambulance. Resuscitation efforts were unsuccessful, and the child was pronounced dead. One month prior to the collapse, the child had experienced a syncopal episode in which the head had been struck; a head CT image showed no pathology. Other than this incident, the child had been completely well.

When an autopsy was performed, lymphocytic thyroiditis was the major finding, and no significant pathology

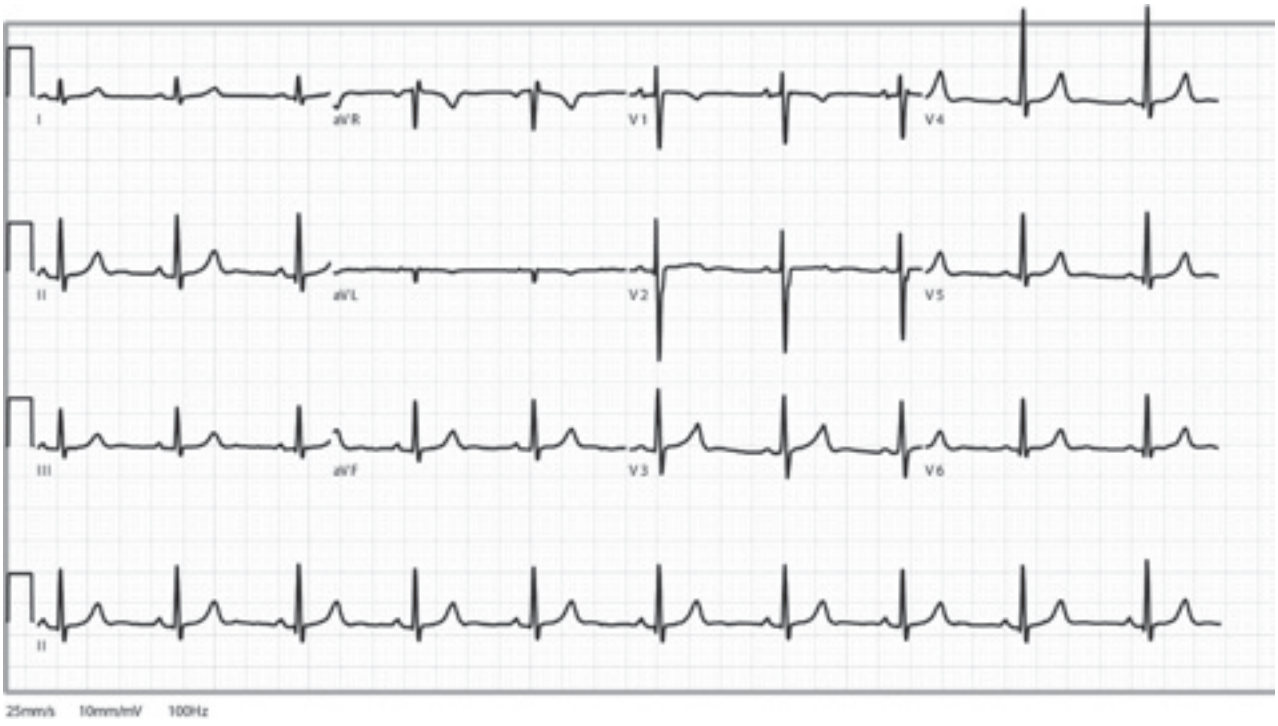

Figure. Twelve-lead electrocardiogram of the deceased's surviving sibling, recorded at initial cardiology assessment. Note the prolonged corrected QT interval of 530 msec.

in the heart or brain was identified. Blood tests revealed a low T4 level, elevated thyroid-stimulating hormone, and presence of thyroperoxidase antibody. Toxicological tests were negative. The lymphocytic thyroiditis and thyroid function abnormalities were consistent with Hashimoto thyroiditis, a cause of clinical hypothyroidism.

Since hypothyroidism can be associated with QT interval prolongation,[13-17] and is postulated to unmask latent primary long QT syndrome,[18] the final autopsy report recommended that the deceased's immediate family members be referred for consideration of possible primary long QT syndrome.

At cardiology evaluation, the deceased's sibling had a markedly prolonged corrected QT interval (QTc) of 530 msec (**Figure**). The ECGs of the parents were not diagnostic of long QT syndrome per se; the mother's QTc was 430 msec (normal), and the father's QTc was 450 msec (upper limit of normal for an adult male). However, given the surviving sibling's abnormal ECG results and the history of sudden death in the family, the father's ECG was considered suspicious for long QT syndrome. Together, findings for the father and surviving sibling were strongly suggestive of autosomal dominant long QT syndrome, although all surviving family members were asymptomatic. Therefore, the family was counseled that the deceased's cause of death was almost certainly long QT syndrome, exacerbated by hypothyroidism.

To confirm the hypothesis that the deceased had long QT syndrome, DNA was extracted from the tissue samples that had been procured at autopsy and frozen as part of the routine approach to sudden unexplained death in childhood. Sequencing of five genes that account for most of the variants of long QT syndrome was performed. A mutation was detected in the *KCNQ1* gene, previously reported in the literature as pathogenic.[19]

When the surviving family members were tested, the same mutation in *KCNQ1* was detected in the deceased's father and surviving sibling. Both individuals have since been treated with beta-blockers and are under clinical supervision, with no cardiac events or arrhythmias documented to date.

The snap-frozen retained tissue sample collected at autopsy allowed the medical team to determine the precise cause of death for this child through the identification of a pathogenic cardiac ion channel mutation. This allowed efficient screening of surviving relatives for the same mutation, leading to diagnosis, treatment, and close follow-up.

Discussion

Individuals with congenital long QT syndrome or other inherited cardiac ion channel mutations have grossly and microscopically normal cardiac tissue. Thus, autopsy frequently reveals no anatomical or toxicological cause of death.[20] When there is an autopsy-negative death and a low index of suspicion, this group of diseases can be missed as a cause of death, leading to lost opportunities to screen surviving relatives for disease.

In an attempt to increase the rate of diagnosis of inherited cardiac arrhythmia disorders in autopsies of young persons with no anatomical cause of death, a number of countries, jurisdictions, and medical organizations have developed guidelines, memoranda, protocols, and checklists for members of the death investigation team to help them carry out sudden death investigations systematically.[21-25]

A common feature of the various published guidelines is the requirement that the scene be thoroughly investigated by the coroner/medical examiner and the family history and medical history be appropriately documented. A Canadian survey-based study showed that there is heterogeneity in practices in sudden death investigation among the provincial/territorial agencies, particularly in regard to use of specific protocols for investigating sudden unexplained death, coding of cause/manner of death, and how conclusions/recommendations from death investigations are conveyed to families.[26] Although the term "sudden unexpected death" is less specific as those listed in the box on page 487, this term is frequently encountered in guidelines.

Collection of tissue or blood at autopsy for future DNA extraction is essential for investigating the possibility that the deceased had an inherited cardiac arrhythmia once other causes of death have been ruled out. Although the identification of a clinical syndrome in surviving relatives may obviate the need to confirm the genetic diagnosis in the deceased, testing the deceased's tissue remains the simplest and most direct way to establish a diagnosis. This may be done in the context of referring the deceased's family to specific clinical programs with subsequent testing, or may, in some jurisdictions, occur as part of the diagnostics in the autopsy itself. In some jurisdictions, privacy concerns may prevent relatives from being contacted. Limited health care services, a reality in many settings, may also result in an inability to screen relatives. If the deceased has a confirmed genetic diagnosis, this can allow the surviving relatives to be screened for the disorder and then counseled regarding management.

In spite of the potential benefits of genetic testing, tissue retention for genetic testing is not addressed consistently in the established guidelines or incorporated consistently into routine autopsy practice. Specifically, the type of tissue needed, and the method of tissue storage, is not well delineated. It is known that DNA extraction and molecular assays are best done on snap-frozen, unfixed tissue. Formalin fixation and paraffin-embedding of tissue, the traditional method of postmortem tissue storage, results in unacceptable levels of DNA degradation that can severely compromise the molecular assays.[27] This is especially true with assays that evaluate for mutations implicated in cardiac arrhythmia disorders.[27]

In cases of autopsy-negative sudden cardiac death, collection and storage of tissue, DNA, or both at autopsy for future molecular analysis is warranted.

Canadian consensus statement

A multidisciplinary panel for the Canadian Cardiovascular Society/Canadian Heart Rhythm Society[28] has reported on genetic testing of cardiac structural protein and ion channel diseases known to cause sudden cardiac death. The panel's 2011 consensus statement includes a section addressing testing of material retained in sudden cardiac death autopsies, and genetic testing of relatives of sudden cardiac death victims. Specifically, the panel states that based on current evidence, in cases of autopsy-negative sudden cardiac death, collection and storage of tissue, DNA, or both at autopsy for future molecular analysis is warranted. As well, testing of relatives should be performed if they display the clinical phenotype of a disease. The testing of tissue retained at autopsy is dependent on the outcome of clinical investigations of the relatives.

These recommendations antedated access to broad "sudden death" gene screening panels that are currently advocated in this circumstance.

Ontario guidelines

In 2008, the Office of the Chief Coroner of Ontario released a memorandum directed at pathologists and coroners of the province.[29] The appendix of that document contains guidelines for pathologists that apply to the autopsy investigation of deceased individuals under age 40. Pathologists are encouraged to pay special attention to the heart in cases of sudden unexpected/unexplained death, and consider saving the heart for expert cardiac examination. In cases where a cardiac event is suspected, the guidelines recommend that tissue be frozen and sent to the nearest molecular genetics laboratory.

US guidelines

In 2007, the National Association of Medical Examiners published a white paper[30] that specifies the minimum actions required in an investigation of sudden unexpected/unexplained infant death. These actions include a thorough scene investigation, completion of a medical history, identification of any criminal activity, X-ray imaging of the deceased, complete gross and microscopic examination at autopsy, additional tests, including vitreous fluid and DNA analysis, and evaluation of a postmortem blood spot card to rule out metabolic diseases. This document does not specifically address retention of frozen tissue, but given the requirement to perform DNA analysis, it is clear that frozen tissue or blood must be available for this purpose.

Under the auspices of the Centers for Disease Control and Prevention, and the National Center for Child Death Review, a formal registry was established in 2009 to improve knowledge of factors for sudden unexpected/unexplained infant death and improve investigative practices, and to ultimately develop better preventive and interventional strategies for reducing the occurrence of these deaths.[31] In a pilot project, individual states collected standardized information on sudden infant deaths for a national database, including both individual variables (for example, infant information, caregiver information, sleep environment) and systems variables (for example, completeness of the autopsy, ancillary studies, scene investigation). Information about whether genetic testing was performed was also collected. Following implementation of this formal program, the rate of complete data collection improved.

British Columbia practices

Although no provincial standards or guidelines have been published for BC, the majority of autopsies for unexpected death in persons under age 16 are performed at BC Children's Hospital, where the Department of Pathology and Laboratory Medicine has a long-established standard approach to complete autopsy with ancillary testing, which includes acylcarnitine profile analysis, microbiology studies, skeletal survey, and toxicology studies. In all cases, tissue is snap-frozen and retained for possible future genetic testing. Where medically appropriate (for example, if a structural cardiac disorder with a suspected genetic basis is diagnosed at autopsy, or if no anatomic cause of death is found at autopsy, increasing the likelihood for a heritable cardiac conduction disorder), the pathologist will recommend referral of surviving relatives to the British Columbia Inherited Arrhythmia Program for screening and assessment.

Summary

Up to 10% of SIDS cases and up to 35% of cases of sudden unexpected death in childhood and young adulthood are due to heritable cardiac arrhythmia conditions.[3-6] As the case presented here illustrates, the routine procurement and freezing of small amounts of tissue at autopsy is essential for diagnosis of genetic cardiac conditions, and greatly enhances clinical management of surviving relatives. Family screening is essential for the immediate relatives of the deceased to identify affected asymptomatic individuals, target them for interventions, and prevent future deaths. Standardization of autopsy practices, which vary widely across provincial, national, and international jurisdictions, is needed to give every family the care required, regardless of where a deceased child's autopsy is performed. At a minimum, small amounts of tissue (frozen, not formalin-fixed) should be retained for DNA testing as indicated during the autopsy of a child or young adult victim of sudden unexplained death. The members of the death investigation team (pathologists, clinicians, coroner/medical examiner), should work together with the family's primary care provider to ensure prompt referral of the family to inherited arrhythmia specialists.

Competing interests

None declared.

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