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State of Postmortem Genetic Testing Known as the Cardiac Channel Molecular Autopsy in the Forensic Evaluation of Unexplained Sudden Cardiac Death in the Young

Michael J. Ackerman, M.D., PH.D.

From the Long QT Syndrome Clinic and the Mayo Clinic Windland Smith Rice Sudden Death, Genomics Laboratory, Mayo Clinic, Rochester, Minnesota

Abstract

Background—Thousands of infants, children, adolescents, and young adults die sudden and unexpectedly each year in the United States. A significant proportion are autopsy negative and are classified as autopsy negative sudden unexplained death (SUD) after the first year of life and as sudden infant death syndrome (SIDS) if prior to their first birthday. Postmortem genetic testing known as the cardiac channel molecular autopsy is capable of identifying the subset of channelopathic SUD/SIDS.

Methods—Review of the literature and analysis of the state of such postmortem genetic testing in the evaluation of SUD/SIDS.

Results—Although still confined to anecdotal reports, relatively small case series of coroner/medical examiner-referred cases of SUD/SIDS, and one population-based cohort of SIDS, it is estimated that approximately 25–35% of autopsy-negative SUD and approximately 10% of SIDS may stem from mutations in either long QT syndrome (LQTS)- or catecholaminergic polymorphic ventricular tachycardia (CPVT)-susceptibility genes.

Discussion—Whether the cardiac channel molecular autopsy should become the standard of care in the postmortem evaluation of autopsy negative SUD or SIDS will require further scrutiny. Cost effectiveness analyses of a more intense postmortem focus on the decedent compared to the current battery of tests recommended for the deceased SUD victim's first degree relatives should be performed.

Conclusion—If deemed justified to upgrade such postmortem genetic testing from “investigational” to clinically indicated, uniform “standard operating procedures” to ensure that tissue is acquired and archived in a manner that is “DNA friendly” and insurance coverage that extends beyond one's final breath will be needed. (PACE 2009; 32:S86–S89)

Keywords

channelopathies; genetic testing; ion channels; Long QT Syndrome; sudden death

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Address for reprints: Michael J. Ackerman, M.D., Ph.D., Guggenheim 501, Mayo Clinic, Rochester, MN 55905. Fax: 507-284-0101, 507-284-3757; ackerman.michael@mayo.edu.

Conflicts of interest: Dr. Ackerman is a consultant for PGx-Health with respect to the FAMILION™ genetic tests for channelopathies and cardiomyopathies. Intellectual property derived from MJA's research program resulted in license agreements in 2004 between Mayo Clinic Health Solutions (formerly Mayo Medical Ventures) and PGxHealth (formerly Genaisance Pharmaceuticals).

Introduction

In the developed countries, sudden cardiac death (SCD) is one of the most common causes of death. SCD is defined by the American Heart Association as the sudden, abrupt loss of heart function in a person who may or may not have diagnosed heart disease whereby the time and mode of death are unexpected and the death occurs either instantly or shortly after symptoms appear. In the United States, for example, SCD claims an estimated 300,000–400,000 individuals each year with the vast majority involving the elderly. In comparison, sudden death in infants, children, adolescents, and young adults is relatively infrequent with an incidence between 1.3 and 8.5 per 100,000 patient years.¹ However, tragically, thousands of Americans under the age of 20 years die suddenly each year. Fortunately, in many cases, cause and manner of death can be established from a comprehensive medicolegal investigation, including autopsy. SCD in the elderly, for example, is often secondary to coronary artery disease. However, the epidemiology of sudden death in the young is less apparent.

Following a death scene and medicolegal investigation including autopsy, sudden death in infancy can be attributed to infection, cardiovascular anomalies, child abuse/negligence, accidents, homicide, or metabolic/genetic disorders. However, 70–80% of sudden unexpected deaths in infancy have no identifiable cause following a postmortem investigation and are labeled as sudden infant death syndrome (SIDS). The pathophysiological mechanisms responsible for SIDS remain poorly understood.

For nearly half of young victims from 1 to 35 years of age, there are no apparent warning signs and sudden death often occurs as the sentinel event, thus placing extreme importance on the medicolegal investigation and autopsy to determine cause and manner of death. A postmortem examination may reveal/suggest a noncardiac basis for the sudden death such as asthma, epilepsy, or pulmonary embolism. However, SCD is the prevailing cause of sudden death in the young, with structural cardiovascular abnormalities often evident at autopsy, including hypertrophic cardiomyopathy, arrhythmogenic right ventricular cardiomyopathy, congenital coronary artery anomalies, and myocarditis.^{2,3}

Not all SCD has an obvious attributable cause that can be determined at autopsy. It is estimated that at least 3% and perhaps as much as 30% of sudden deaths involving previously healthy children, adolescents, and young adults have no identifiable morphologic abnormalities found at autopsy, and the SCD is labeled as autopsy negative sudden unexplained death (SUD).^{2,4–7} The exact prevalence of SUD, particularly in children, is unclear. There is a paucity of large population-based explorations of SCD in the young that are needed to better elucidate the frequency and potential etiologies of such heartbreaking events.

Potentially lethal and heritable channelopathies such as congenital long QT syndrome (LQTS), catecholaminergic polymorphic ventricular tachycardia (CPVT), and Brugada syndrome (BrS) leave no trace to be found by a comprehensive medicolegal autopsy, leaving coroners, medical examiners, and forensic pathologists only to speculate that a fatal arrhythmia might lie at the heart of an SUD. However due to molecular advances, a cardiac channel molecular autopsy may potentially elucidate such a pathogenic mechanism and establish probable cause and manner for SUD in infants (SIDS), children, adolescents, and young adults.^{8–12}

State of the Cardiac Channel Molecular Autopsy for SUDs and SIDS

Following the sentinel discovery of cardiac channel mutations as the pathogenic basis for LQTS in 1995,^{13,14} research-based genetic testing ensued for the next decade until maturing

into a commercially available, clinical diagnostic test in 2004. Comprehensive open reading frame/splice site analysis of five LQTS-susceptibility genes provides the putative pathogenic mutation for approximately 75% of LQTS.¹⁵ BrS genetic testing of *SCN5A* is also available commercially and *SCN5A* analysis explains approximately 20% of BrS.¹⁶ CPVT genetic testing, consisting of either targeted or comprehensive analysis of the 105 translated exons of the *RYR2*-encoded cardiac ryanodine receptor or calcium release channel, has also completed the maturation from an “investigational” test to a clinical diagnostic test, and mutations in *RYR2* explain approximately 50–60% of CPVT.¹⁷

The first ever report of a postmortem molecular diagnosis of an arrhythmia disorder through the use of a molecular autopsy occurred in 1999 when we reported the diagnosis of inherited LQTS in a 19-year-old woman who died after a near-drowning.¹⁸ Subsequently, Tester et al. provided proof of principle that some cases of unexplained drownings harbor mutations in the cardiac ryanodine receptor associated with CPVT1 following a cardiac channel molecular autopsy in two medical examiner-referred cases.¹⁹ A familial mutation was identified in a 16-year-old female who drowned during swim team practice, and a sporadic de novo mutation was identified in a 9-year-old apparently healthy boy who failed to surface while diving into a lake with friends at summer camp.

In August 1999, a young decedent's mother brought her 13-year-old son to the Mayo Clinic for an evaluation and asked, “Does my 13-year-old son have what killed my 17-year-old son 5 months ago?”¹⁰ The 17-year-old had been found dead in bed. The results of autopsy and toxicology were negative. The results of a standard clinical assessment of the decedent's immediate family were negative for LQTS, with family members having normal electrocardiograms. However, a molecular autopsy provided the answer to the mother's query. Genetic testing of autopsy material identified a familial 5-bp deletion in *KCNQ1*, which provided a definitive answer to the mother's question. Since these preliminary case reports of molecular autopsies, investigators have sought to determine the spectrum and prevalence of pathogenic cardiac ion channel mutations in unique series of SUD cases.

In 2004, Chugh et al. identified 12 cases of SUD following a comprehensive postmortem analysis of a consecutive series of 270 adult (age ≥ 20 years) cases of SCD occurring over a 13-year period.¹² Postmortem genetic analysis of the LQTS-susceptibility genes revealed an identical *KCNH2* mutation in two of 12 (17%) cases of autopsy negative SUD. Similarly, Di Paolo et al. performed LQTS molecular autopsies on 10 cases of juvenile (ages 13–29 years) SUD and identified *KCNQ1* mutations in two individuals.²⁰

Recently, we completed the largest molecular autopsy series of SUD to date. A targeted analysis of 23 of the 105 translated exons of the CPVT1-associated, *RYR2*-encoded cardiac ryanodine receptor was conducted in a series of 49 medical examiner-referred cases of SUD, revealing *RYR2* mutations in 15%.²¹ Subsequently, 20% were positive for mutations following comprehensive mutational analysis of all 60 translated exons in the LQTS-associated genes: *KCNQ1*, *KCNH2*, *SCN5A*, *KCNE1*, and *KCNE2*.²² Thus, over one-third of SUD cases hosted a potentially pathogenic cardiac channel mutation.

This postmortem observation converges concordantly with rigorous clinical evaluations of family members of an autopsy-negative SUD victim. In 2003, Behr et al. performed a detailed cardiovascular evaluation of 109 first-degree relatives for 32 cases of SUD, and showed that 22% of these families had evidence of inherited cardiac disease, with the majority having clinical features suggestive of LQTS.⁵ Similarly, in 2005, Tan et al. found that 28% of families had an identifiable cardiac channelopathy including CPVT and LQTS following a clinical assessment of first-degree relatives of young SUD victims.²³ Together,

these reports suggest that identifiable and potentially treatable cardiac channelopathies account for up to one-third of autopsy-negative SUD in the young.

With respect to autopsy-negative SUD during the first year of life (otherwise known as SIDS), two independent research programs have established that approximately 10% of SIDS may stem from pathogenic mutations involving the three most common LQTS-susceptibility genes.^{11,24–27} In the United States, there are approximately 3,000 cases of SIDS each year. Thus, an estimated 300 SIDS cases might have a positive LQTS genetic test if such testing were performed on the decedent. In addition, we have recently demonstrated mutations in the *CAV3*-encoded caveolin 3 (LQT9), *RYR2*, and *GPD1L*-encoded glycerol phosphate dehydrogenase 1-like protein (BrS2) in SIDS.^{28–30} Taken together, we currently estimate that 10–15% of SIDS stems from a primary cardiac channelopathy.

Discussion

Molecular Autopsy for SUD/SIDS: The New Standard of Care

Cardiac channel postmortem genetic testing (also known as molecular autopsy) has not yet been transformed from a research test into a routine, standard part of the conventional autopsy when the coroner, medical examiner, or forensic pathologist is faced with an SUD. Considering that autopsy-negative SUD accounts for a significant number of sudden deaths in the young and that epidemiological, clinical, and now postmortem genetic analyses all attest that approximately one-third of SUD after the first year of life may stem from a lethal cardiac channelopathy, should the cardiac channel molecular autopsy be viewed as the standard of care for the postmortem evaluation of SUD? Unfortunately, it is profoundly difficult for the medical examiner/coroner/forensic pathologist to provide this level of care for several reasons. Most importantly, insurance companies generally do not accept any responsibility for providing coverage beyond the grave. Explicitly, most insurance companies accept no responsibility to pay for a molecular autopsy of a deceased person regardless of the implications to his or her living relatives.

Thus, postmortem genetic testing, which provides an answer 35% of the time that could save another family member's life, is available only to families who are willing to pay for cardiac channel genetic testing out-of-pocket. The only other alternative now is for the medical examiner/coroner/forensic pathologist to enroll the decedent's sample in Institutional Review Board-approved, research-based genetic testing that, although free, can be and usually is a painfully slow process.

Regardless of whether commercial or research, the role of the medical examiner/coroner/forensic pathologist is vital as current “standard-operating procedures” for the conduct of an autopsy do not ensure that a postmortem sample is acquired in a DNA-friendly fashion. With rare exceptions, both formalin-fixed and paraffin-embedded tissues constitute suboptimal sources for this critical test.³¹ In contrast, blood collected in EDTA (purple top tube) or frozen heart, liver, or spleen provides the greatest source of DNA, enabling the successful conduct of postmortem cardiac channel genetic testing.

Conclusions

To be sure, an accurate diagnosis, derived from either a clinical assessment of surviving relatives or a molecular autopsy, enables informed genetic counseling for families and guides the appropriate commencement of preemptive strategies targeted toward the prevention of another tragedy among those left behind.

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