Whole-Exome Molecular Autopsy After Exertional Sudden Cardiac Death
Not a Panacea but a Step in the Right Direction

Virginie Beauséjour Ladouceur, MD; Dominic J. Abrams, MD, MRCP

The sudden death of a seemingly healthy child is a loss from which many families never recover. Despite a Medical Examiner’s autopsy, a significant proportion of such deaths remain unexplained or ascribed to natural causes, a conclusion that only exacerbates the deep sense of confusion, disbelief, and continued distress faced by the family.1 Identification of the underlying cause behind sudden cardiac death in the young not only provides an explanation for the family, but given the high likelihood of a genetic cause facilitates testing in first-degree family members and the implementation of protective measures in those considered at risk.

When faced with a negative conventional autopsy, a molecular autopsy therefore provides a welcome second line of investigation, a strategy deemed worthy of consideration in the 2011 joint expert consensus statement by the Heart Rhythm Society and European Heart Rhythm Association.2 A comprehensive molecular analysis on exertional sudden death cases, as performed by Anderson et al3 from the Mayo Clinic and reported in this edition of Circulation Cardiovascular Genetics, adds further understanding to potential causal mechanisms that underlie sudden death in children. The association between cardiac arrhythmia and exercise is firmly established for both long-QT syndrome type 1 (LQTS1) and catecholaminergic cardiac arrhythmia and exercise is firmly established for both LQTS1 and CPVT,10 which if proven by confirmed parental testing adds further weight to the case for pathogenicity. Additionally, all identified variants reported in this study are located within 4 functionally important domains of RyR2, 3 of which are unexplained or ascribed to natural causes, a conclusion that only exacerbates the deep sense of confusion, disbelief, and continued distress faced by the family.1 Identification of the underlying cause behind sudden cardiac death in the young not only provides an explanation for the family, but given the high likelihood of a genetic cause facilitates testing in first-degree family members and the implementation of protective measures in those considered at risk.

When faced with a negative conventional autopsy, a molecular autopsy therefore provides a welcome second line of investigation, a strategy deemed worthy of consideration in the 2011 joint expert consensus statement by the Heart Rhythm Society and European Heart Rhythm Association.2 A comprehensive molecular analysis on exertional sudden death cases, as performed by Anderson et al3 from the Mayo Clinic and reported in this edition of Circulation Cardiovascular Genetics, adds further understanding to potential causal mechanisms that underlie sudden death in children. The association between cardiac arrhythmia and exercise is firmly established for both long-QT syndrome type 1 (LQTS1) and catecholaminergic cardiac arrhythmia and exercise is firmly established for both LQTS1 and CPVT,10 which if proven by confirmed parental testing adds further weight to the case for pathogenicity. Additionally, all identified variants reported in this study are located within 4 functionally important domains of RyR2, 3 of which are
located within the larger intracytoplasmic section responsible for sensing cytoplasmic events that are transmitted to the fourth, transmembrane domain, which controls cellular calcium homeostasis by release from the sarcoplasmic reticulum. Similar variant clustering has been found in patients with a robust CPVT phenotype, adding further support that these are disease-causing mutations.

The authors are to be congratulated on their work, yet several important questions remain, largely inherent to the process of investigating sudden cardiac death. In an ideal world, the optimal strategy should be a comprehensive, multifaceted process incorporating deep phenotyping of the proband and wider family, coupled with detailed genetic analysis to identify associated causal variant(s). First, this process should begin with a detailed autopsy and phenotypic characterization of the proband, by definition the one definitively affected member of the family. The pathological investigation of suspected inherited cardiac disease, like its clinical counterpart, is best performed by a specialist cardiac pathologist familiar with the potentially subtle nuances associated with these conditions at an early stage in the disease process. Yet in less than half of young sudden death cases are autopsies performed, and the adequacy of those performed by medical examiners suggest that a significant proportion of these examinations fall below what is considered best practice. Mechanisms should therefore exist whereby in cases of sudden unexpected death in the young, a secondary expert cardiac review is a mandatory part of the process because a nonspecialist autopsy may miss critical findings that are key to the underlying diagnosis.

Second is a detailed clinical review of the wider family, starting with first-degree relatives and cascaded further across the pedigree as appropriate, cognizant of the varied penetrance and phenotypes common to many inherited cardiac disorders. This is best performed within the confines of a specialized cardiac genetics program with all the clinical, genetic, and psychological infrastructure that entails, and previous studies have shown a clear correlation between the number of family members studied and identification of a clinical diagnosis. The Dutch experience of 372 familial assessments after sudden cardiac death between 1996 and 2011 yielded a clinical diagnosis in 93 families (25%) supported by a genetic diagnosis in 52 (56%), with an average 5.5 people per family found to carry the identified variant. This demonstrates that a significant proportion of at-risk individuals can be identified through a process of combined clinical and genetic cascade testing. Despite the rapid advances in the understanding of molecular genetics and associated technology, the family itself remains a powerful tool in the validation of any identified variant, and ideally clinical and genetic testing should be used as widely as possible to determine segregation between the 2 across multiple family members. However, this approach within a sufficiently sized pedigree to demonstrate genotype–phenotype segregation of sufficient power to attribute definitive causality (ie, logarithm of odds score >3) is rarely achievable. In the initial molecular autopsy analysis of 173 sudden death cases, only 25% of families opted to undergo cascade genetic testing (notably all carried the variant identified in the proband although clinical evaluation was not reported), whereas the families of all 3 cases identified by whole-exome molecular autopsy in this study declined further investigation. Somewhat surprisingly, the uptake of clinical review in a specialized cardiogenetics clinic after familial sudden cardiac death remains low (<10%) despite community interventions to improve the situation.

Finally, in the absence of comprehensive pathological and familial clinical evaluation, classification of variants identified at molecular autopsy as pathogenic (and hence an explanation for the event) necessitates the use of criteria as set out in the ACMG guidelines and used by the authors in this study. However, the increasing recognition of variants identified within inherited cardiovascular disease–associated genes in control populations (including more radical splice, insertion/deletion, and truncating variants) only reinforces the need for caution, and the authors wisely urge vigilance in the overinterpretation of variants where there is no strong evidence for pathogenicity. Variants in disease-concordant genes appear attractive causal candidates, although the identification of definitively affected individuals within pedigrees who do not carry the presumed disease-causing familial variant supports the need for clinical as well as genetic evaluation of potentially at-risk family members. A study of several kindreds with Brugada syndrome who carried a presumed disease-causing SCN5A variant identified in the proband and at least 4 other members found individuals with the Brugada phenotype who did not carry the familial variant in 5 of 13 families. Similar findings have been reported with RyR2 variants associated with exertional cardiac arrest and desmosomal variants in arrhythmogenic right ventricular cardiomyopathy. Functional analysis may also add supportive evidence of pathogenicity; however, in vitro evidence of channel dysfunction associated with specific variants may not necessarily directly translate into a clinical phenotype in the complex biological environment of the human cardiovascular system. The T1304M variant in SCN5A has been implicated in LQTS type 3 and sudden death in infancy, an age at which LQTS exhibits a severe phenotype. Functional analysis demonstrates both a significant persistent sodium current (1.6% of peak current) and depolarizing shift in voltage-dependent inactivation, yet the same variant has an allelic frequency of 0.03% in the ExAC European control population compared with a LQTS population prevalence of 0.05%. Such findings point to a polygenic and/or environmental complexity beyond a simple monogenic effect in at least a proportion of cases—easy to infer yet difficult to definitively identify.

Overall, the findings of this study are a welcome addition and will be of considerable help to those faced with investigating families after the exertional sudden death of a child or young adult, allowing for a targeted clinical and genetic approach. Whole-exome sequencing is now readily available and affordable, yet the key lies not in the execution of genetic testing in the form of a molecular autopsy, but in its judicious interpretation ideally in concert with clinical findings across the wider pedigree, to ensure the family receives appropriate and considered counseling in the face of the extreme medical and psychological stress of sudden cardiac death.
Sources of Funding
The Inherited Cardiac Arrhythmia Program at Boston Children’s Hospital is generously supported by the Mannion and Roberts families.

Disclosures
None.

References

Keywords: Editorials; autopsy; genotype; phenotype; sudden death; syncope
Whole-Exome Molecular Autopsy After Exertional Sudden Cardiac Death: Not a Panacea but a Step in the Right Direction

Virginie Beauséjour Ladouceur and Dominic J. Abrams

doi: 10.1161/CIRCGENETICS.116.001484

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circgenetics.ahajournals.org/content/9/3/210

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation: Cardiovascular Genetics can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation: Cardiovascular Genetics is online at:
http://circgenetics.ahajournals.org//subscriptions/