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. 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. A comprehensive molecular analysis on exertional sudden death cases, as performed by Anderson et al 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 polymorphic ventricular tachycardia (CPVT), typically leading to exertional syncope or seizures, although sudden death as polymorphic ventricular tachycardia (CPVT), typically leading to exertional syncope or seizures, although sudden death as

Article, see p 259

The opinions expressed in this article are not necessarily those of the editors or of the American Heart Association. From the Inherited Cardiac Arrhythmia Program, Division of Cardiac Electrophysiology, Boston Children’s Hospital, MA. Correspondence to Dominic J. Abrams, MD, MRCP. Boston Children’s Hospital, 300 Longwood Ave, Boston, MA 02115. E-mail dominic.abrams@chboston.org


Circ Cardiovasc Genet is available at http://circgenetics.ahajournals.org
DOI: 10.1161/CIRCGENETICS.116.001484

To date, the vast majority of variants identified at molecular autopsy have resided in the recognized disease-associated genes for LQTS, Brugada syndrome, and CPVT. Although cardiomyopathy-related death should be diagnosed at autopsy, life-threatening arrhythmia may precede the classical electrocardiographic, structural, and histological features in both desmosomal and lamin-mediated disease, suggesting that variants in these genes may also be identifiable after a negative autopsy, where subtle cardiomyopathic features may not be appreciated.

The advent of rapid and increasingly affordable whole-exome sequencing promises new hope in the molecular investigation of sudden death, with possible variant identification in new genes encoding proteins involved in established biological pathways, or even revealing new molecular mechanisms. In this study, Anderson et al sought to examine the yield of whole-exome molecular autopsy among 32 individuals aged 1 to 19 years with autopsy-negative, exertion-related sudden unexplained death during a 12-year study period. All cases initially underwent targeted comprehensive sequencing of the 3 major LQTS genes (KCNQ1, KCNH2, and SCN5A) and targeted sequencing of RYR2, as part of a larger molecular analysis in 173 sudden death cases. This identified 11 putative pathogenic variants (34%) in the exertional sudden death cases reported in this study, 9 in RYR2 and 2 in KCNQ1. The additional 21 cases that remained genetically elusive after this initial approach subsequently underwent whole-exome sequencing. In 3 cases, a further 3 variants were identified, 2 in CALM2 and 1 in PKP-2, therefore implicating only 5 of the 100 recognized cardiovascular disease genes. These 3 were ascribed pathogenic status based on the recent American College of Medical Genetics and Genomics (ACMG) and Association for Molecular Pathology consensus recommendations for variant classification, yielding a total positive finding in 14 of 32 cases (44%) with this tiered approach.

The results of this study provide important information into the molecular mechanisms that underlie exertional sudden cardiac death in the pediatric population. Specifically, that mutations in RYR2 in younger children are by far the most prevalent is concordant with the well-recognized natural history of CPVT and numerous previous studies that document the prevalence of sudden death in an untreated population. The absence of a family history in many cases is also concordant with the high rate of de novo variants associated with CPVT, 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 genetic cause. The Dutch experience of 372 familial cases has shown a clear correlation between these conditions and the presence of phenotype in at least 4 other members found indi-viduals 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

Key Words: 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/