

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

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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.

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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 polymorphic ventricular tachycardia (CPVT), typically leading to exertional syncope or seizures,^{4,5} although sudden death as the sentinel event is well recognized. The clinical diagnosis is made electrocardiographically in both LQTS1 and CPVT, and cardiac pathological and histological examination will by definition be normal, making these attractive cases for molecular autopsy. Given that the molecular architecture for LQTS1 and CPVT is better understood than for many other cardiovascular genetic disorders and the phenotype can be elicited in surviving relatives, identified variants in 2 major disease-associated genes, *KCNQ1* or *RyR2*, can be used as part of comprehensive cascade screening within the wider family.

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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 desmosomal⁶ and lamin-mediated disease,⁷ 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 al³ 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*.⁸ 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 recommendation for variant classification,⁹ 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.¹⁰ The absence of a family history in many cases is also concordant with the high rate of de novo variants associated with 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.¹¹ 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,¹² and the adequacy of those performed by medical examiners suggest that a significant proportion of these examinations fall below what is considered best practice.¹³ 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 program¹⁴ 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.¹⁵ 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 on 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.¹⁶ 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,⁸ 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.¹²

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)^{17,18} 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.¹⁹ Similar findings have been reported with *RyR2* variants associated with exertional cardiac arrest⁷ and desmosomal variants in arrhythmogenic right ventricular cardiomyopathy.²⁰ 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,²¹ yet the same variant has an allelic frequency of 0.03% in the ExAC European control population¹⁸ 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.

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