Whole-Exome Molecular Autopsy After Exertion-Related Sudden Unexplained Death in the Young

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Background—Targeted postmortem genetic testing of the 4 major channelopathy-susceptibility genes (KCNQ1, KCNH2, SCN5A, and RYR2) have yielded putative pathogenic mutations in ≤30% of autopsy-negative sudden unexplained death in the young (SUDY) cases with highest yields derived from the subset of exertion-related SUDY. Here, we evaluate the role of whole-exome sequencing in exertion-related SUDY cases.

Methods and Results—From 1998 to 2010, 32 cases of exertion-related SUDY were referred by Medical Examiners for a cardiac channel molecular autopsy. A mutational analysis of the major long-QT syndrome–susceptibility genes (KCNQ1, KCNH2, and SCN5A) and catecholaminergic polymorphic ventricular tachycardia–susceptibility gene (RYR2) identified a putative pathogenic mutation in 11 cases. Whole-exome sequencing was performed on the remaining 21 targeted gene–negative SUDY cases. After whole-exome sequencing, a gene-specific surveillance of all genes (N=100) implicated in sudden death was performed to identify putative pathogenic mutation(s). Three of these 21 decedents had a clinically actionable, pathogenic mutation (CALM2-F90L, CALM2-N98S, and PKP2-N634fs). Of the 18 remaining cases, 7 hosted at least 1 variant of unknown significance with a minor allele frequency <1:20,000. The overall yield of pathogenic mutations was higher among decedents aged 1 to 10 years (10/11, 91%) than those aged 11 to 19 years (4/21, 19%, P=0.0001).

Conclusions—Molecular screening in this clinical scenario is appropriate with a pathogenic mutation detection rate of 44% using direct DNA sequencing followed by whole-exome sequencing. Only 5 of the 100 interrogated sudden death genes hosted actionable pathogenic mutations for more than one third of these exertion-related, autopsy-negative SUDY cases. (Circ Cardiovasc Genet. 2016;9:259-265. DOI: 10.1161/CIRCGENETICS.115.001370.)

Key Words: autopsy ■ exome ■ genetic testing ■ mutation ■ phenotype

Sudden death in children and young adults, affecting ≈1000 to 5000 individuals annually in the United States, is an uncommon but profoundly tragic event for families and physicians. These deaths are often secondary to an underlying cardiac abnormality with exertion preceding sudden death. Maron et al reported an analysis of the US National Registry of Sudden Death in Athletes composed of 1866 athletes and noted that death occurred in 82% of these individuals during or immediately after training/competition. Mellor et al went on to demonstrate that male sex and age <18 years were independently associated with exercise/stress-related deaths. Exertion as an inciting event raises concerns for associated cardiomyopathy, such as hypertrophic cardiomyopathy or arrhythmogenic right ventricular cardiomyopathy, or underlying channelopathy, including long-QT syndrome (LQTS) and catecholaminergic polymorphic ventricular tachycardia (CPVT).

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A comprehensive medicolegal investigation with conventional autopsy may lead to identification of a morphological abnormality. However, a significant number of sudden deaths will remain unexplained with no identifiable structural abnormality, as in patients with a channelopathy or subtle phenotypic features of a cardiomyopathy. These cases have been labeled autopsy-negative sudden unexplained death (SUD). When clinical suspicion for a diagnosis of an arrhythmia syndrome is present, primarily LQTS or CPVT, guidelines, by the Heart Rhythm Society and European Heart Rhythm Association, recommend a molecular autopsy investigation of the SUD case (class II indication).2

The cardiac channelopathy–centric molecular autopsy currently uses targeted postmortem genetic testing of the 4 major channelopathy-susceptibility genes (KCNQ1, KCNH2, SCN5A, and RYR2). This process has yielded putative pathogenic mutations in ≤30% of SUD cases in the young (SUDY).5–11 Tester et al noted a slightly higher mutation detection yield among SUDY patients with an exertional trigger (35%) in comparison to a nonspecific trigger (27%) or death during sleep (19%), but the difference did not reach statistical significance. The downside of direct DNA sequencing is the limitation of data based on a one gene at a time...
approach. This methodology is time intensive and involves a significant financial cost. As >100 known cardiac channelopathy–and cardiomyopathy-susceptibility genes have now been described, a comprehensive molecular evaluation seems out-of-reach for this standard practice. Therefore, the potential role of next-generation whole-exome sequencing (WES) to conduct a molecular autopsy has gained interest.

WES enables genetic interrogation of the coding region for an individual’s estimated library of 20,000+ genes at a relatively reasonable research cost, $1000 to $2000 per sample. This modality can provide a more comprehensive sudden death gene panel while allowing for future population-based epidemiological testing, potentially uncovering new disease-causing mutations or disease-predisposing single-nucleotide polymorphisms. However, the comprehensive nature of WES will lead invariably to identification of DNA variants which may or may not be involved in the clinical phenotype, termed variants of unknown/uncertain significance (VUS). This has led the American College of Medical Genetics (ACMG) to release a policy statement regarding the need for higher standards in reporting because of the risk of false-positive results burdening the consumer and healthcare system. Herein, we set out to evaluate the role of WES in exertion-related SUDY cases where a previous 4 channelopathy gene molecular autopsy remained negative.

### Materials and Methods

**Medical Examiner/Coroner Referred Cases for Autopsy-Negative SUD**

From September 1, 1998, through October 31, 2010, 32 unrelated autopsy-negative SUDY medical examiners/coroner cases who died during exertion between the ages of 1 and 19 years were referred to the Windland Smith Rice Sudden Death Genomics Laboratory at the Mayo Clinic in Rochester, Minnesota, for postmortem genetic testing (ie, molecular autopsy) after Mayo Clinic IRB approval and informed consent from the next of kin (Table 1).

All 32 cases had undergone previously a comprehensive mutational analysis via polymerase chain reaction, denaturing high-performance liquid chromatography, and DNA sequencing of the major LQTS- (*KCNQ1*, *KCNH2*, and *SCN5A*) and CPVT- (*RYR2*) susceptibility genes. A putative pathogenic mutation within these LQTS or CPVT genes was present in 11/32 (34%) cases. The remaining 21 cases remained genetically elusive.

### Table 1. Demographic Summary of the Exertion-Related SUDY Cohort

|                         | All Cases | Pathogenic Mutation-Positive Cases | Pathogenic Mutation-Negative Cases | P value |
|-------------------------|-----------|-----------------------------------|-----------------------------------|---------|
| Number of Individuals   | 32        | 14                                | 18                                |         |
| Sex                     |           |                                   |                                   |         |
| Male                    | 20        | 8                                 | 12                                | 0.72    |
| Female                  | 12        | 6                                 | 6                                 |         |
| Age at SUD              |           |                                   |                                   |         |
| Overall, mean±SD, y (range) | 11.5±5.1 (2–19) | 8.4±5.5 (2–17) | 13.8±3.2 (6–19) | 0.002   |
| Females, mean±SD, y (range) | 9.9±3.8 (5–17) | 8.3±3.7 (5–15) | 11.4±3.5 (6–17) | 0.17    |
| Males, mean±SD, y (range) | 12.4±5.6 (2–19) | 8.5±6.9 (2–17) | 15.0±2.5 (11–19) | 0.01    |
| Reported ethnicity      |           |                                   |                                   |         |
| White                   | 29        | 13                                | 16                                | 0.82    |
| Black                   | 2         | 1                                 | 1                                 |         |
| Hispanic                | 1         | 0                                 | 1                                 |         |
| Asian                   | 0         | 0                                 | 0                                 |         |
| Unknown                 | 0         | 0                                 | 0                                 |         |
| Personal history before SUD |         |                                   |                                   |         |
| Positive, n (%)         | 10 (31)   | 6 (43)                            | 4 (22)                            | 0.27    |
| Negative, n (%)         | 22 (69)   | 8 (57)                            | 14 (78)                           |         |
| Family history of cardiac events |         |                                   |                                   |         |
| Positive, n (%)         | 5 (16)    | 1 (7)                             | 4 (22)                            | 0.35    |
| Negative, n (%)         | 27 (84)   | 13 (93)                           | 14 (78)                           |         |
| Family history of SCD, n (%) | 3 (9)    | 1 (7)                             | 2 (11)                            | 1.00    |
| Personal or family history |         |                                   |                                   |         |
| Seizures, n (%)         | 2 (6)     | 2 (14)                            | 0                                 | 0.18    |
| Syncope, n (%)          | 5 (16)    | 3 (21)                            | 2 (11)                            | 0.63    |

Values in bold represent P values considered statistically significant (defined as P<0.05). SCD indicates sudden cardiac death; SUD, sudden unexplained death; and SUDY, sudden unexplained death in the young.
Whole-Exome Next-Generation DNA Sequencing

Three micrograms of genomic DNA isolated from an autopsy specimen (whole blood, blood spot card, or frozen tissue) from these 21 remaining SUDY cases was submitted to Mayo Clinic’s Medical Genome Facility (Rochester, MN) for WES. Exome capture was completed with SureSelect XT Human All Exon V4 plus UTR Target Enrichment System (Agilent, Santa Clara, CA). Exome sequencing was performed using the Illumina HiSeq 2000 platform (San Diego, CA) and V3 reagents. Parameters included 71-Mb paired-end sequencing at 96% coverage with a read depth of 35x.

The sequence alignment and analysis was completed using the SNP & Variation Suite v8.3.4 (Golden Helix, Inc, Bozeman, MT. www.goldenhelix.com). All variants were first filtered for a call quality score ≥20 and read depth ≥10. Variants occurring in noncoding regions outside of the canonical splice sites or representing synonymous variants were excluded from further analysis. Only insertion/deletions and single nucleotide variants causing a missense, splice-site error, or nonsense substitution underwent gene-specific surveillance for all known channelopathy- and cardiomyopathy-susceptibility genes (N=100; Table I in the Data Supplement).

Only ultrarare variants with an allele frequency <0.00005 (1:20 000 alleles) in the Exome Aggregation Consortium browser (n=60 706 unrelated individuals) and with an ACMG consensus variant classification of pathogenic were considered to be causative and actionable clinically.14 All variants designated as pathogenic were validated using Sanger DNA sequencing.

Statistical Analysis

Descriptive statistics (means, percentages, etc) were used to summarize the data. Cohorts were compared using the Fischer exact test on SAS statistical software version 9.3 (SAS Institute, Cary, NC). P values <0.05 were considered significant.

Results

Cohort Description

The details regarding these 32 cases of exertion-related SUDY are summarized in Table 1. Briefly, there were 20 men and 12 women, the mean age at death was 11±5 years and ranged from 2 to 19 years, and 91% of the decedents were white. Of the 32 cases, the sudden death was the sentinel event in 22 (69%), whereas 10 (31%) experienced symptoms before their sudden death (5 with syncope, 2 with arrhythmias, 2 with seizures, and 1 with palpitations). A family history of cardiac events was present in 5 of 32 cases (16%) with 3 cases notable for a family history of sudden cardiac death before 50 years of age.

Whole-Exome Molecular Autopsy

On average, each SUDY case had 9227±2098 nonsynonymous variants. Of these variants, 69±11 localized to the 100 surveyed genes. In total, 12 ultrarare (allele frequency <0.00005 in Exome Aggregation Consortium) nonsynonymous variants were identified in 10 of 21 cases (47.6%) (2 cases had 2 variants, Table 2). However, only 3 variants (CALM2-F90L, CALM2-N98S, and PKP2-N634fs) were classified as pathogenic according to the strict variant interpretation guideline recommendations outlined by the ACMG and therefore were considered clinically actionable.14 The remaining 9 ultrarare variants are still considered VUS. Comparison of victims with (n=7) and without (n=11) ultrarare VUS demonstrates no difference in clinical characteristics including age (14.3±4.6 and 13.5±2.3 years; P=0.63), sex (57% and 73% men; P=0.63), race (86% and 91% white; P=1.0), sudden death as the sentinel event (86% and 73%; P=1.0), and family history of cardiac events (42% and 10%; P=0.25).

Two of the decedents hosted mutations within the CALM2 gene. CALM2 is a member of the calmodulin gene family, which comprised 3 separate genes (CALM1, CALM2, and CALM3) that are unique at the nucleotide level and dispersed throughout the genome but all encode precisely the same calcium-binding protein. Mutations in all 3 calmodulin genes have been implicated previously in LQTS and CPVT.15-19 The CALM2-N98S mutation was identified in a 2-year-old boy who died while dancing. This CALM2-N98S was seen previously as a sporadic mutation in a 12-year-old Japanese boy with syncope and QT prolongation.18 The CALM2-F90L mutation was identified in a 5-year-old boy who died while playing. Although CALM2-F90L is novel, a CALM1-F90L mutation has been described previously.19 The third decedent is a 16-year-old boy who died suddenly while playing football. WES identified a frameshift deletion

| Age, y | Sex | Gene | Nucleotide Change | Amino Acid Change | ACMG Designation | ExAC Minor Allele Frequency | Personal History | Family History |
|-------|-----|------|-------------------|------------------|-----------------|-----------------------------|-----------------|---------------|
| 16    | M   | ACTN2| c.2356G>A         | p.G786S          | VUS             | …                           | Negative        | Negative       |
| 6     | F   | ANKR1| c.722G>A          | p.C241Y          | VUS             | 0.00001649                 | Negative        | Negative       |
| 5     | M   | CALM2| c.268T>C          | p.F90L           | Pathogenic      | …                           | Negative        | Negative       |
| 2     | M   | CALM2| c.293A>G          | p.N98S           | Pathogenic      | …                           | Negative        | Negative       |
| 18    | M   | CASO2| c.289A>G          | p.K97E           | VUS             | 0.00000841                 | Negative        | Negative       |
| 12    | F   | GATA1| c.157G>T          | p.G53W           | VUS             | 0.00002304                 | …               | SCD           |
| 19    | M   | MYBPC3| c.2374T>C         | p.W792R          | VUS             | 0.00004972                 | Negative        | Negative       |
| 12    | M   | MYBPC3| c.2500G>T         | p.R834W          | VUS             | 0.00001658                 | Syncope         | Syncope        |
| 17    | F   | MYL2 | c.430C>A          | p.P144T          | VUS             | 0.00000825                 | Negative        | SCD           |
| 16    | M   | PKP2 | c.1901delA        | p.N634fs         | Pathogenic      | …                           | Negative        | Negative       |

ACMG indicates American College of Medical Genetics; ExAC, Exome Aggregation Consortium; F, female; M, male; SCD, sudden cardiac death; SUDY, sudden unexplained death in the young; and VUS, variant of unknown significance.
mutation, p.N634fs, within the PKP2 gene. PKP2 encodes plakophilin-2, an essential armadillo-repeat protein in cardiac desmosomes, with mutations linked to arrhythmogenic right ventricular cardiomyopathy. Although this decedent’s specific mutation is novel, pathogenicity is linked to previous data demonstrating insertion–deletion mutations, nonsense mutations, and missense mutations within PKP2, resulting in arrhythmogenic right ventricular cardiomyopathy.20

The surviving family members of all decedents with newly identified pathogenic mutations were contacted. The implications of the decedent’s postmortem genetic test results were discussed, and the families were advised to undergo further cardiological evaluation that would include cascade genetic testing of the putative pathogenic mutation in a Clinical Laboratory Improvement Amendment certified facility. Notably, all 3 families have declined to pursue these recommendations thus far.

Spectrum and Prevalence of Rare, Putative Pathogenic Mutations

Including our previous findings, 14 of 32 (44%) of these autopsy-negative SUDY cases with an exertion-associated sudden death have a putative pathogenic and clinically actionable mutation (9 cases with an RYR2 mutation, 2 KCNQ1, 2 CALM2, and 1 PKP2; Table 3). One decedent hosted 2 RYR2 mutations (RyR2-H240R and RyR2-T4158P).

An interesting age effect was noted when comparing mutation-positive versus mutation-negative cases. Mutation-positive cases (n=14; 8.4±5.5 years; range: 2–17 years) were significantly younger than mutation-negative cases (n=18; 13.8±3.2 years; range: 6–19 years; P=0.002; Table 1). When these cases were subdivided further, there was a notable difference in age between mutation-positive male cases (n=8; 8.5±6.9 years) and mutation-negative male cases (n=12; 15.0±2.5 years; P=0.01; Table 1). This same relationship was not evident among mutation-positive female cases (n=6; 8.3±3.7 years) compared with mutation-negative female cases (n=6; 11.4±3.5; P=0.17; Table 1).

Figure 1. Age effect on the molecular autopsy detection yield of pathogenic mutations: illustrated is a bar graph demonstrating the yield of pathogenic mutation detection for decedents aged 1 to 10 years in comparison to those aged 11 to 19 years. The number in parenthesis (n=x) represents the total number of cases. Each bar represents the percent yield for each. F indicates female; and M, male.
19%; \(P=0.0001\); Figure 1). There was no difference statistically between the mutation-positive and mutation-negative cases with regard to sex, ethnicity, personal history of symptoms before SUD, or family history of cardiac events including sudden cardiac death.

**Discussion**

This is the largest single-center study demonstrating the use of a whole-exome molecular autopsy (WEMA) to identify pathogenic mutations in a population of exertionally related autopsy-negative SUDY victims. The mutation detection rate in this cohort improved from 34% with polymerase chain reaction, denaturing high-performance liquid chromatography, and direct DNA-sequencing analysis of only the 4 major channelopathy genes (\(KCNQ1\), \(KCNH2\), \(SCN5A\), and \(RYR2\)) to 44% after expansion to a WEMA. The identification of these genetic alterations assists in providing closure and clarity for the family, while ensuring completion of proper evaluation of at-risk family members including predictive, mutation-specific genetic testing (cascade screening).

The population of exertion-related autopsy-negative SUDY cases was targeted in this study because of our previous observations of an elevated probability for an underlying identifiable pathogenic mutation associated with a channelopathy.\(^5\) The rate of detection for a pathogenic mutation in exertion-related SUDY cases was greater for deceased children aged ≤10 years in comparison to those aged 11 to 19 years, especially among men. The same trend exists for women, but statistical significance was not ascertained, which was potentially limited because of sample size.

These results must be considered in the context of potential referral bias, with the study population potentially overestimating the true pathogenic mutation detection rate for this cohort and sample bias, with the overrepresentation of \(RYR2\) variants and the tendency for CPVT to present early in life. Even so, this information is of critical importance to the families of the decedents and pathologists, as the discussion to pursue a molecular autopsy is often limited because of financial constraints. Prioritization of cases may be necessary, and this study would suggest that victims with exertion-related SUDY before 10 years of age may have the highest pretest probability for a positive molecular autopsy.

We previously reported the first proof-of-principle case report on the role of a WEMA. A previously healthy 16-year-old girl with SUDY had a pathogenic \(MYH7\) mutation previously reported with hypertrophic cardiomyopathy and sudden death. Identification of this alteration allowed for appropriate genetic interrogation of her surviving at-risk family members.\(^12\) This study confirms the use of WES for a comprehensive molecular autopsy with the identification of 3 pathogenic mutations in patients previously thought to be genotype-negative exertion-related SUDY cases. These 3 mutations were associated with 2 genes, \(CALM2\) and \(PKP2\), previously implicated in SUD cases. The pathogenicity of c.293A>G in \(CALM2\) causing p.N98S and c.1901delA in \(PKP2\) causing p.N634fs has been established by previous investigations.\(^18,20\) The designation of pathogenicity is based on the presence of PS1/PS2 and PVS1/PS3 criteria per the ACMG guidelines for each variant, respectively.\(^14\)

The \(CALM2\) mutation, c.268T>C, causing p.F90L represents an interesting case. This nucleotide substitution in \(CALM2\) has not been reported. The calmodulin genes contain 8 highly conserved phenylalanine residues, including F90, encoding a helix-loop-helix calcium binding motif integral to signaling with target peptides.\(^19,21,22\) Mutations within this signaling domain have displayed aberrant interaction with ryanodine receptor type 2 calmodulin-binding domain peptide at low calcium concentrations.\(^23\) Decreased calmodulin–ryanodine receptor type 2 interaction would result in a dominant-negative effect, promoting an open ryanodine receptor type 2 configuration, as is seen in CPVT1-causing pathogenic mutations.\(^17\) A CALM1-F90L mutation has been described previously as a familial disease–causing mutation in a Moroccan family with episodes of ventricular fibrillation within the first 2 decades of life and mild QT prolongation. This mutation was present in the mother and 4 affected siblings, although being absent in the unaffected father, unaffected sibling, and 500 Moroccan controls.\(^24\) The designation of pathogenicity is therefore based on the presence of PS1/PS4 criteria per the ACMG guidelines.\(^14\)

To date, 5 of the 100 interrogated channelopathic and cardiomyopathic genes have hosted “actionable” pathogenic mutations for more than one third of these exertion-related, autopsy-negative SUDY cases. The addition of the calmodulin and the plakophilin genes should be considered for molecular autopsies currently using direct DNA-sequencing. The victim hosting the pathogenic \(PKP2\) variant in this study further supports that a standard forensic autopsy may fail to recognize subtle features of a cardiomyopathy or that sudden death may occur with subclinical disease. This supports the evaluation of cardiomyopathic genes in addition to channelopathy genes even in the setting of seemingly autopsy-negative SUD. With each addition of a targeted gene to the direct DNA-sequencing panel, the cost-effective nature of a WEMA becomes more apparent.

Although the idea of a WEMA is enticing, it is not without limitation. The technology is dependent on exome capture, sequencing quality, and read mapping with the potential for sequence artifact producing false-positive variants. Any putative mutation must therefore be validated via standard Sanger sequencing protocols. Furthermore, the comprehensive nature of WES will give rise to thousands of nonsynonymous genetic variants for each exome, many of which may be rare, predicted to be deleterious via various in silico tools, and even reside within biologically plausible genes. As evidenced in our cohort, half of the exertion-related SUDY cases have a VUS in one of the channelopathic or cardiomyopathic genes that remains stuck in genetic purgatory currently.\(^13\) Strict criteria must be met before reporting pathogenicity to prevent undue burden on the decedent’s family and the healthcare system.

The multitude of sequence variants identified through high-throughput next-generation sequencing led the ACMG, the Association for Molecular Pathology, and the College of American Pathologists to publish standards required for the interpretation of sequence variants.\(^14\) Criteria were developed for the classification of pathogenic and benign variants. The system is dynamic allowing for recategorization with the continued acquisition of knowledge. For example, confirmation of a de novo mutation in a patient with the
disease and no family history, functional studies supportive of a damaging effect on the gene, or prevalence studies documenting the presence of a mutation in the affected compared with controls may lead to reclassification of an otherwise ambiguous VUS to the designation of likely pathogenic or pathogenic. Uncertain significance is applied to variants that do not meet criteria for one of the classifications or when contradictory criteria between benign and pathogenic are present.

In our cohort, the currently ambiguous variants may one day be downgraded as benign or upgraded as a pathogenic, clinically actionable variant. Identification of a VUS result should not trigger familial testing of the decedent’s surviving first-degree relatives who have a normal cardiac evaluation without careful counseling. Specifically, the family must not conclude that a request for variant-specific testing of relatives implies that the root cause has been detected. Instead, the relatives must understand that an ambiguous variant has been found and that the VUS could be reclassified potentially, for example, if the VUS was determined to be a sporadic, de novo variant. Such a determination presumes correct maternity/paternity. Whether VUS testing of first-degree relatives is performed clinically or with informed consent on a research basis needs to be individualized. The key element that must be communicated in either setting is that the variant is being investigated further and that a conclusion of pathogenicity would be premature and incorrect. More work must be done. Until defined, these results produce a compendium of data allowing for ongoing evaluation in the search for new disease-associated mutations and multigenic models of disease. However, if appropriate restraint is not exercised, a VUS could be proclaimed erroneously and prematurely as the root cause. This could have disastrous consequences for the surviving family members. Arguably, the only thing worse than being unable to provide a family with a cogent explanation for their loved one’s death is to proclaim that the root cause has been found and be wrong.

Conclusion
Molecular screening in this clinical scenario, especially for the younger victim, is appropriate with a pathogenic mutation detection rate of 44%. Although WES may be a more cost-effective platform for obtaining genetic data than targeted gene-specific DNA sequencing, only 5 of the 100 so-called sudden death-associated genes have hosted actionable pathogenic mutations for more than one third of these exention-related, autopsy-negative SUDY cases. WES results must be scrutinized with great caution because most variants will be stuck in genetic purgatory as a VUS. Future population-based, molecular epidemiology studies of SUDY will be needed to confirm whether the yield of actionable pathogenic mutations is truly greater in 1 to 10-year-old children with an exention-related SUDY than in those aged 11 to 19 years.

Sources of Funding
This work was supported by the Mayo Clinic Windland Smith Rice Comprehensive Sudden Cardiac Death Program, the Sheikh Zayed Saif Mohammed Al Nahyan Fund in Pediatric Cardiology Research, the Dr Scholl Fund, and the Hannah M. Wernke Memorial Fund. This project was also supported in part by funding from Mayo Clinic’s Center for Individualized Medicine.

Disclosures
M.J. Ackerman is a consultant for Boston Scientific, Gilead Sciences, Medtronic, and St Jude Medical. M.J. Ackerman, D.J. Tester, and Mayo Clinic receive royalties from Transgenomic for their FAMILION-LQTS and FAMILION-CPVT genetic tests. However, none of these entities provided financial support for this study. The other authors report no conflicts.

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**CLINICAL PERSPECTIVE**

The use of whole-exome sequencing for a comprehensive molecular autopsy after direct DNA testing of the 4 major channelopathy-associated genes (*KCNQ1*, *KCNH2*, *SCN5A*, and *RYR2*) improved the yield of identifying a pathogenic mutation in the young patient with exertion-related sudden unexplained death. Prioritization of cases for a comprehensive molecular autopsy may be necessary, and when stratified by age, the overall yield of pathogenic mutations was higher among decedents aged 1 to 10 years, than those aged 11 to 19 years. In this setting of exertional sudden death in the young, the sudden death–causing pathogenic mutation (ie, smoking gun) was found in >40% of the cases enabling powerful closure for the family and clarity with respect to cascade screening of the decedent’s relatives. In contrast to closure and clarity, the whole-exome molecular autopsy can cause great confusion also. Ultrarare variants of unknown significance in a plethora of genes were identified in one third of cases after a whole-exome molecular autopsy. Although such variants may one day be upgraded to pathogenic or downgraded to benign, we must refrain from assuming that a variant of unknown significance is actionable clinically and from proclaiming prematurely and erroneously that it (the variant) is the root cause.