Confirmation of Cause and Manner of Death Via a Comprehensive Cardiac Autopsy Including Whole Exome Next-Generation Sequencing

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• Annually, the sudden death of thousands of young people remains inadequately explained despite medicolegal investigation. Postmortem genetic testing for channelopathies/cardiomyopathies may illuminate a potential cardiac mechanism and establish a more accurate cause and manner of death and provide an actionable genetic marker to test surviving family members who may be at risk for a fatal arrhythmia. Whole exome sequencing allows for simultaneous genetic interrogation of an individual's entire estimated library of approximately 30 000 genes. Following an inconclusive autopsy, whole exome sequencing and gene-specific surveillance of all known major cardiac channelopathy/cardiomyopathy genes (90 total) were performed on autopsy blood–derived genomic DNA from a previously healthy 16-year-old adolescent female found deceased in her bedroom. Whole exome sequencing analysis revealed a R249Q-MYH7 mutation associated previously with familial hypertrophic cardiomyopathy, sudden death, and impaired β–myosin heavy chain (MHC-β) actin-translocating and actin-activated ATPase (adenosine triphosphatase) activity. Whole exome sequencing may be an efficient and cost-effective approach to incorporate molecular studies into the conventional postmortem examination.

(*Arch Pathol Lab Med*. 2014;138:1083–1089; doi: 10.5858/arpa.2013-0479-SA)

In the United States, an estimated 300 000 to 400 000 individuals die suddenly each year, mostly involving the elderly and cardiac abnormalities identifiable on autopsy.[1] With an incidence of between 1.3 and 8.5 per 100 000 patient years, sudden death in infants, children, adolescents, and young adults is relatively uncommon.[2] Yet, annually, an estimated 1000 to 5000 young people between 1 and 35 years of age die suddenly.

While the cause and manner of many sudden deaths in the young are explained after a comprehensive medicolegal investigation that includes a conventional autopsy examination, up to 50% of these cases of sudden death in the young remain unexplained, with no definite cardiac etiology identified after gross and microscopic inspection of the heart.[3] Such deaths are often termed *autopsy-negative sudden unexplained death* (SUD).[4]

Potentially lethal and heritable cardiac channelopathies, such as long QT syndrome (LQTS), catecholaminergic polymorphic ventricular tachycardia (CPVT), and Brugada syndrome (BrS), are typically associated with grossly and histologically normal hearts. This often leaves medical examiners and coroners in a position to postulate only that a fatal cardiac arrhythmia was responsible for the sudden death in an otherwise healthy young individual, leaving the family with little or no insight into the ramifications for family members.[3,5] In the case of sudden death–associated cardiomyopathies, such as hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), and arrhythmogenic cardiomyopathy (ACM), the lack of uniform diagnostic criteria has led to uneasiness in reporting with confidence when these conditions are present, given the serious implications for the decedent's family members. This is made even more complicated by the fact that in their earlier

Accepted for publication October 7, 2013.
Published as an Early Online Release December 2, 2013.

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Dr Ackerman is a consultant for Transgenomic, Inc (Omaha, Nebraska), which provides one of the commercially available clinical genetic tests for long QT syndrome (LQTS) called FAMILION-LQTS. In addition, Dr Ackerman (significant) and Mr Tester (modest) receive royalty payments from Mayo Clinic (Rochester, Minnesota) for intellectual property/technology agreements made between Mayo Clinic and Transgenomic, Inc. The other authors have no relevant financial interest in the products or companies described in this article.

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.

The abstract was presented in poster format by Mr Tester at the American Society of Human Genetics Annual Meeting; November 8, 2012; San Francisco, California.

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Table 1. List of the 90 Cardiac Channelopathy/Cardiomyopathy-Associated Genes Surveyed[a,b]

No.	Gene	Protein Name	Cardiac Channelopathy and/or Cardiomyopathy Disease Association
1	*ABCC9*	ATP-binding cassette, subfamily C (CFTR/MRP), member 9	DCM
2	*ACTC1*	Actin, α, cardiac muscle 1	HCM, DCM
3	*ACTN2*	Actinin, α 2	HCM, DCM
4	*AKAP9*	A kinase (PRKA) anchor protein (yotiao) 9	LQTS
5	*ANK2*	Ankyrin 2	LQTS
6	*ANKRD1*	Ankyrin repeat domain 1 (cardiac muscle)	HCM, DCM
7	*BAG3*	Bcl2-associated athanogene 3	DCM
8	*CACNA1C*	Calcium channel, voltage-dependent, L type, α 1C subunit	BrS, LQTS
9	*CACNA2D1*	Calcium channel, voltage-dependent, α 2/δ subunit 1	BrS
10	*CACNB2*	Calcium channel, voltage-dependent, β 2 subunit	BrS
11	*CALM1*	Calmodulin 1	LQTS, CPVT
12	*CALM2*	Calmodulin 2	LQTS
13	*CALR3*	Calreticulin 3	HCM
14	*CASQ2*	Calsequestrin 2 (cardiac muscle)	CPVT
15	*CAV3*	Caveolin 3	LQTS
16	*CRYAB*	Crystallin, α B	DCM
17	*CSRP3*	Cysteine- and glycine-rich protein 3 (cardiac LIM protein)	HCM, DCM
18	*DES*	Desmin	DCM
19	*DMD*	Dystrophin, muscular dystrophy	DCM
20	*DSC2*	Desmocollin 2	ACM
21	*DSG2*	Desmoglein 2	ACM
22	*DSP*	Desmoplakin	ACM
23	*EMD*	Emerin (Emery-Dreifuss muscular dystrophy)	DCM
24	*EYA4*	Eyes absent homolog 4 (Drosophila)	DCM
25	*FCMD*	Fukuyama-type congenital muscular dystrophy (fukutin)1	DCM
26	*FXN*	Frataxin	HCM
27	*GATA4*	GATA-binding protein 4	HCM
28	*GLA*	Galactosidase, α	HCM
29	*GPD1L*	Glycerol-3-phosphate dehydrogenase 1–like	BrS
30	*HCN4*	Hyperpolarization-activated cyclic nucleotide–gated potassium channel 4	BrS
31	*ILK*	Integrin-linked kinase	DCM
32	*JAG1*	Jagged 1	HCM
33	*JPH2*	Junctophilin 2	HCM
34	*JUP*	Junction plakoglobin	ACM
35	*KCND3*	Potassium voltage-gated channel, Shal-related family, member 3	BrS
36	*KCNE1*	Potassium voltage-gated channel, Isk-related family, member 1	LQTS
37	*KCNE2*	Potassium voltage-gated channel, Isk-related family, member 2	LQTS
38	*KCNE3*	Potassium voltage-gated channel, Isk-related family, member 3	BrS
39	*KCNH2*	Potassium voltage-gated channel, subfamily H (eag-related), member 2	LQTS
40	*KCNJ2*	Potassium inwardly rectifying channel, subfamily J, member 2	LQTS
41	*KCNJ5*	Potassium inwardly rectifying channel, subfamily J, member 5	LQTS
42	*KCNJ8*	Potassium inwardly rectifying channel, subfamily J, member 8	BrS
43	*KCNQ1*	Potassium voltage-gated channel, KQT-like subfamily, member 1	LQTS
44	*LAMP2*	Lysosome-associated membrane glycoprotein 2	HCM
45	*LBD3*	LIM binding domain 3 (ZASP)	HCM, DCM
46	*LMNA*	Lamin A/C	DCM
47	*MYBPC3*	Myosin-binding protein C, cardiac	HCM, DCM
48	*MYH6*	Myosin, heavy chain 6, cardiac muscle, α	HCM, DCM
49	*MYH7*	Myosin, heavy chain 7, cardiac muscle, β	HCM, DCM
50	*MYL2*	Myosin, light chain 2, regulatory, cardiac, slow	HCM
51	*MYL3*	Myosin, light chain 3, alkali; ventricular, skeletal, slow	HCM
52	*MYOM1*	Myomesin 1, 185 kDa	HCM
53	*MYOZ2*	Myozenin 2	HCM
54	*MYPN*	Myopalladin	HCM, DCM
55	*NEBL*	Nebulette	DCM
56	*NEXN*	Nexilin (F actin–binding protein)	HCM, DCM
57	*NKX2.5*	NK2 transcription factor–related 5	HCM
58	*PDLIM3*	PDZ and LIM domain 3	DCM
59	*PKP2*	Plakophilin 2	ACM
60	*PLN*	Phospholamban	HCM, DCM
61	*PRKAG2*	Protein kinase, AMP-activated, 2 noncatalytic subunit	HCM
62	*PTPN11*	Protein tyrosine phosphatase, nonreceptor type 11	HCM
63	*PSEN1*	Presenilin 1	DCM
64	*PSEN2*	Presenilin 2	DCM
65	*RAF1*	v-raf-1 murine leukemia viral oncogene homolog 1	HCM
66	*RBM20*	RNA-binding motif protein 20	DCM
67	*RANGRF*	RAN guanine nucleotide release factor	BrS
68	*RYR2*	Ryanodine receptor 2 (cardiac)	CPVT, ACM
69	*SCN1B*	Sodium channel, voltage-gated, type I, β	BrS
70	*SCN3B*	Sodium channel, voltage-gated, type III, β	BrS
71	*SCN4B*	Sodium channel, voltage-gated, type IV, β	LQTS

Table 1. Continued

No.	Gene	Protein Name	Cardiac Channelopathy and/or Cardiomyopathy Disease Association
72	*SCN5A*	Sodium channel, voltage-gated, type V, α	LQTS, BrS, DCM
73	*SGCD*	Sarcoglycan, δ (dystrophin-associated glycoprotein)	DCM
74	*SNTA1*	Syntrophin, α 1	LQTS
75	*TAZ*	Tafazzin	DCM
76	*TBX1*	T-box 1	HCM
77	*TBX5*	T-box 5	HCM
78	*TCAP*	Titin-cap (telethonin)	HCM, DCM
79	*TGFB3*	Transforming growth factor, β 3	ACM
80	*TMEM43*	Transmembrane protein 43	ACM
81	*TMPO*	Thymopoietin	DCM
82	*TNNC1*	Troponin C type 1	HCM, DCM
83	*TNNI3*	Troponin I type 3 (cardiac)	HCM, DCM
84	*TNNT2*	Troponin T type 2 (cardiac)	HCM, DCM
85	*TPM1*	Tropomyosin 1 (α)	HCM, DCM
86	*TRDN*	Triadin	CPVT
87	*TTN*	Titin	HCM, DCM
88	*TTR*	Transthyretin	HCM, DCM
89	*TXNRD2*	Thioredoxin reductase 2	DCM
90	*VCL*	Vinculin	HCM, DCM

Abbreviations: ACM, arrhythmogenic cardiomyopathy; BrS, Brugada syndrome; CPVT, catecholaminergic polymorphic ventricular tachycardia; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; LQTS, long QT syndrome; QT, interval between the start of the Q wave and end of the T wave in the cardiac electrical cycle as seen on an electrocardiogram.

[a] Genes are listed in alphabetical order.

[b] Channelopathies: BrS, CPVT, and LQTS. Cardiomyopathies: ACM, DCM, and HCM.

forms, these cardiomyopathies may manifest only subtle features.[3]

Postmortem genetic testing for channelopathies/cardiomyopathies may illuminate a potential cardiac mechanism and thereby establish a more accurate cause and manner of death and provide an actionable genetic marker by which to test surviving family members who may be potentially at risk for their own fatal arrhythmia. In fact, recently proposed guidelines for autopsy investigations of SUD in the young have suggested that postmortem genetic testing, for both structural and nonstructural genetically determined heart disease, should become the new standard of care in the evaluation of young SUD cases.[6–8]

While molecular autopsies involving the 4 major cardiac channelopathy genes (*KCNQ1* [LQT1], *KCNH2* [LQT2], *SCN5A* [LQT3, BrS1], and *RYR2* [CPVT1]) have implicated LQTS, CPVT, and BrS as the underlying pathogenetic basis for an estimated 25% to 30% of SUD cases,[9–11] to date there are at least 28 channelopathy-susceptibility genes. In addition, at least 64 genes have been associated with the major cardiomyopathies, that is, HCM, DCM, and ACM. Given the current financial landscape of the typical medical examiner's/coroner's office and the current lack of participation among major insurance companies and third-party payers for postmortem genetic testing, the daunting task of a comprehensive cardiac channelopathy/cardiomyopathy workup appears to be out of reach.

However, next-generation whole exome sequencing (WES), which allows for simultaneous genetic interrogation of an individual's entire estimated library of 30 000 genes, using a small amount of DNA, can be completed at a research cost of $1000 to $2000 in a matter of weeks to months. Whole exome sequencing may represent an efficient and cost-effective approach for postmortem genetic testing, compared to the estimated 10-fold higher cost of performing standard "1 gene, 1 exon" at-a-time approach of Sanger sequencing. Presently, there are more than 90 known cardiac channelopathy- and cardiomyopathy-susceptibility genes that could be included in a "comprehensive" sudden death gene panel. Moreover, WES can generate data that may be interrogated in the future as research uncovers new genetic mutations responsible for sudden death. Herein, we illustrate how WES established the definitive pathogenic cause and likely manner of death for a young victim of sudden death.

MATERIALS AND METHODS

Medical Examiner's Case of Sudden Death in an Adolescent Female

An autopsy was performed on a previously healthy 16-year-old white young woman (weight, 78.3 kg; height, 165.0 cm; body mass index, 28.2 kg/m^2) who was found deceased in her bedroom. Significant findings from her autopsy included a moderately enlarged heart (352 g; expected range, 119–290 g) but with normal ventricular septum to left ventricular free wall ratio (1.0; normal, $<$1.3). Histologically, the myocardium exhibited marked myocyte hypertrophy, myocyte disarray, and mild interstitial fibrosis, most notably in the anterolateral left ventricle. There was no identifiable mitral valve contact lesion in the left ventricular outflow tract. Additional findings included a patent foramen ovale (6 mm, potential diameter), mild cerebral edema, and fatty streaks present in the thoracic aorta. Of particular note, the postmortem toxicologic screen results were all negative.

Because of the absence of overt septal hypertrophy, the medical examiner deemed the autopsy *inconclusive* for a specific cardiomyopathy and specifically did not render a necropsy diagnosis of HCM. Instead, the autopsy pathologist and local medical examiner submitted a specimen to Mayo Clinic's Windland Smith Rice Sudden Death Genomics Laboratory in Rochester, Minnesota, for postmortem genetic mutational analysis in hopes of finding a cardiomyopathy mutation that might establish probable/definitive pathogenic cause.

Whole Exome Next-Generation DNA Sequencing

Three micrograms of genomic DNA isolated from 10 mL of blood, by using the Gentra Puregene Blood Kit (Qiagen,

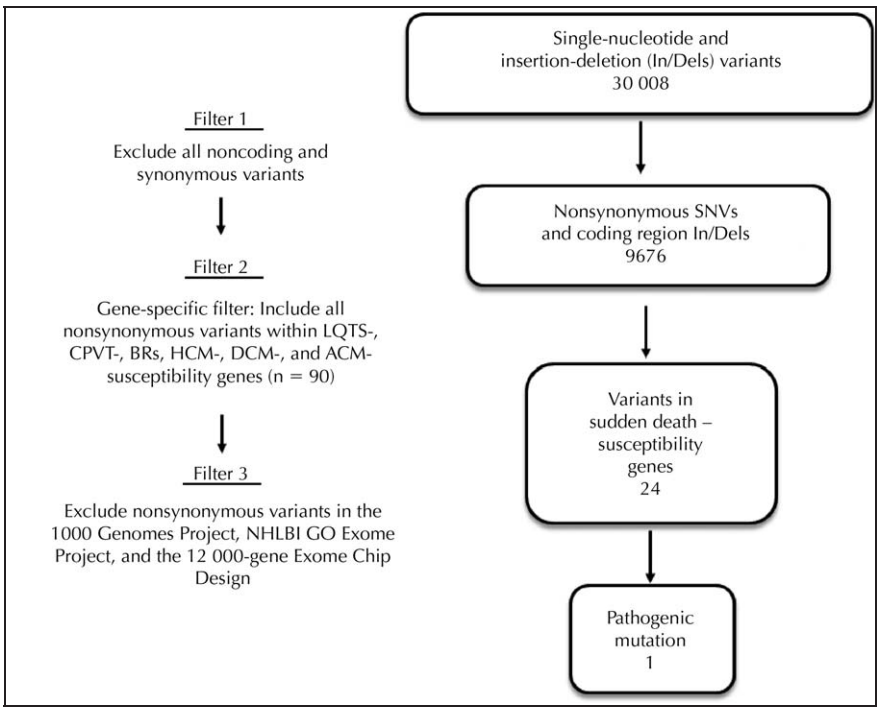

Figure 1. *Whole exome sequencing for sudden cardiac death variant filtration flow chart. Shown is the stepwise variant filtration process for evaluating the exome in cases of sudden unexpected death, resulting in the identification of a single putative pathogenic mutation. Abbreviations: ACM, arrhythmogenic cardiomyopathy; BrS, Brugada syndrome; CPVT, catecholaminergic polymorphic ventricular tachycardia; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; LQTS, long QT syndrome; NHLBI GO, National Heart, Lung, and Blood Institute Grand Opportunity; QT, interval between the start of the Q wave and end of the T wave in the cardiac electrical cycle as seen on an electrocardiogram; SNVs, single-nucleotide variants.*

Germantown, Maryland) and following the manufacturer's protocol, was submitted to Mayo Clinic's Medical Genome Facility (Rochester, Minnesota), supported by the Mayo Center for Individualized Medicine for WES. Following exome capture with the SureSelect XT Human All Exon V4 plus UTR Target Enrichment System (Agilent, Santa Clara, California), 71-Mb paired-end sequencing at 96% coverage with a read depth of 35× was carried out on the Illumina HiSeq 2000 platform (San Diego, California) using V3 reagents. Variant alignment to the latest available human genome (hg19), Mapping and Assembly with Quality (Maq) single-nucleotide variant (SNV) detection,[12] Burrows-Wheeler alignment insertion/deletion (INDEL) detection,[13] Maq and Genome Analysis Toolkit–based SNV/INDEL calling, SeattleSeq/Sorting Intolerant From Tolerant (SIFT) annotation, and allele frequencies for variants in the Single Nucleotide Polymorphism Database (dbSNP) and 1000 genomes were carried out by using the automated Targeted RE-sequencing Annotation Tool (TREAT) analytic pipeline developed at Mayo Clinic (Rochester, Minnesota).[14]

An annotated list of all SNVs/INDELs that met quality control standards was provided in an Excel (Microsoft, Redmond, Washington) spreadsheet with links for variant visualization, tissue expression, and biologic pathway/process provided. Following WES and variant annotation, variant filtration involving the exclusion of all noncoding regions and synonymous variants (ie, a DNA nucleotide alteration that does not alter the amino acid sequence of the protein) and gene-specific surveillance of all known LQTS, CPVT, BrS, HCM, DCM, and ACM genes (90 total; Table 1 and Figure 1) were performed to identify possible pathogenic mutation(s).

To be considered a possible pathogenic mutation that may have contributed to the sudden death, any variant, discovered within the cardiomyopathy/channelopathy-associated gene subset, must be absent in a large panel of ethnically matched controls (595 white, 319 African American, 134 Asian, and 118 Hispanic persons) and absent in 3 publicly available exome databases including the 1000 Genomes Project (n = 1094 subjects; 381 white, 246 African American, 286 Asian, and 181 Hispanic subjects),[15] the National Heart, Lung, and Blood Institute (NHLBI) Grand Opportunity Exome Sequencing Project (n = 5379 subjects; 3510 white and 1869 African American subjects),[16] and the Exome Chip Design (n = 12 000 subjects).[17]

Possible pathogenic mutations were confirmed in the SUD case's genomic DNA by using standard polymerase chain reaction and Sanger DNA sequencing methods. Polymerase chain reaction primers, conditions, and sequencing methods are available upon request.

RESULTS

The SUD victim's exome contained 30 008 variants. Of these, 98 genetic variants resided within the 90 channelopathy/cardiomyopathy-associated genes surveyed (Table 1). Twenty four variants within 15 genes were nonsynonymous variants (ie, a DNA alteration leading to a protein product with an altered amino acid sequence; Table 2). Most of these variants were common polymorphisms with a heterozygote frequency of 1% or greater in the public exome databases. However, 1 missense mutation (R249Q-MYH7; Table 2 and Figure 2) was absent in all publicly available exome databases including the 1000 Genomes Project (n = 1094), the NHLBI Exome Sequencing Project (n = 5379), and the 12 000 Exome Chip Design (n = 12 000).

Figure 1 depicts our WES-based sudden cardiac death stepwise variant filtration process for mutation identification. The R249Q-MYH7 mutation was confirmed in the decedent's genomic DNA by using standard Sanger DNA sequencing methods. Mutations in the *MYH7*-encoded β–myosin heavy chain (MHC-β) is one of the most common causes of genetically identifiable HCM and has been reported rarely in DCM also. Specifically, R249Q-MYH7 has been associated previously with familial HCM, sudden death, and impaired MHC-β actin-translocating and actin-activated ATPase (adenosine triphosphatase) activity.[18,19]

Once the pathogenic mutation was determined, the cause of death was revised to R249Q-MYH7–mediated cardiomyopathy. The surviving family members were then referred to a board-certified genetic counselor with specific expertise in

Table 2. Summary of All Nonsynonymous Variants Identified Within the 90-Gene Cardiac Channelopathy/Cardiomyopathy Panel[a]

Gene	Protein	Gene-Disease Association	dbSNP132	Codon Change	Amino Acid Substitution	Genotype	Present in Exome Databases	SIFT Prediction	PolyPhen Prediction
AKAP9	A kinase (PRKA) anchor protein (yotiao) 9	LQTS	rs6964587	ATG-ATT	M463I	TT	Yes	Tolerated	Benign
AKAP9	A kinase (PRKA) anchor protein (yotiao) 9	LQTS	rs6960867	AAT-AGT	N2792S	GG	Yes	Tolerated	Possibly damaging
CASQ2	Calsequestrin 2 (cardiac muscle)	CPVT	rs4074536	ACG-GCG	T66A	AG	Yes	Tolerated	Benign
DSG2	Desmoglein 2	ACM	rs2278792	AGA-AAA	R773K	GA	Yes	Tolerated	Benign
DSP	Desmoplakin	ACM	rs6929069	CGA-CAA	R1738Q	GA	Yes	Tolerated	Benign
EYA4	Eyes absent homolog 4 (Drosophila)	DCM	rs9493627	GGC-AGC	G277S	AA	Yes	Tolerated	Probably damaging
JUP	Junction plakoglobin	ACM	rs1126821	ATG-TTG	M697L	AT	Yes	Tolerated	Benign
MYBPC3	Myosin-binding protein C, cardiac	HCM, DCM	rs3729989	AGC-GGC	S236G	GG	Yes	Tolerated	Benign
MYH7	**Myosin, heavy chain 7, cardiac muscle, β**	**HCM, DCM**	**rs3218713**	**CGA-CAA**	**R249Q**	**GA**	**No**	**Damaging**	**Unknown**
MYOM1	Myomesin 1, 185 kDa	HCM, DCM	rs1962519	TCT-CCT	S181P	TC	Yes	. . .	Possibly damaging
PSEN1	Presenilin 1	DCM	rs17125721	GAA-GGA	E318G	AG	Yes	Damaging	Benign
RYR2	Ryanodine receptor 2 (cardiac)	CPVT	rs34967813	CAA-CGA	Q2942R	AG	Yes	Tolerated	Benign
TBX1	T-box 1	DCM	rs72646967	AAC-CAC	N397H	AC	Yes	Tolerated	Benign
TMEM43	Transmembrane protein 43	ACM	rs4685076	AAA-AAT	K168N	AT	Yes	Tolerated	Benign
TMEM43	Transmembrane protein 43	ACM	rs2340917	ATG-ACG	M179T	TC	Yes	Tolerated	Benign
TNNT2	Troponin T type 2 (cardiac)	HCM, DCM	rs3730238	AAG-AGG	K258R	AG	Yes	Tolerated	Benign
TRDN	Triadin	CPVT	rs2873479	ATT-AGT	I438S	TG	Yes	Tolerated	Benign
TRDN	Triadin	CPVT	rs9490809	ACT-AGT	T33S	CG	Yes	Tolerated	Unknown
TTN	Titin	HCM, DCM	rs6723526	AAA-GAA	K5255E	AG	Yes	. . .	Unknown
TTN	Titin	HCM, DCM	rs72648970	GAC-CAC	D6352H	GC	Yes	. . .	Unknown
TTN	Titin	HCM, DCM	rs72648907	CGT-CAT	R4917H	GA	Yes	Tolerated	Benign
TTN	Titin	HCM, DCM	rs35813871	ACA-ATA	T811I	CT	Yes	. . .	Unknown
TXNRD2	Thioredoxin reductase 2	DCM	rs1139793	ATA-ACA	I370T	TC	Yes	Tolerated	Benign
TXNRD2	Thioredoxin reductase 2	DCM	rs5748469	GCC-TCC	A66S	GT	Yes	Damaging	Benign

Abbreviations: ACM, arrhythmogenic cardiomyopathy; CPVT, catecholaminergic polymorphic ventricular tachycardia; dbSNP, Single-Nucleotide Polymorphism Database; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; LQTS, long QT syndrome; PolyPhen, polymorphism phenotyping; QT, interval between the start of the Q wave and end of the T wave in the cardiac electrical cycle as seen on an electrocardiogram; SIFT, sorting intolerant from tolerant.

[a] Bolded values indicate the putative pathogenic mutation identified in the 16-year-old sudden death victim.

cardiovascular diseases, for genetic counseling and for the orchestration and commencement of clinical cardiac evaluations and family-specific R249Q-MYH7 conformational mutation analysis to identify other family members at risk for sudden cardiac death. Considering the strength of the evidence for its assignment as a definite pathogenic mutation, predictive testing can be offered to the family. Accordingly, a mutation-negative relative with a normal echocardiogram finding can be dismissed from cardiology, whereas yearly cardiac evaluations would be considered for mutation-positive relatives. Further, given the positive family history of premature sudden death, a prophylactic implantable cardioverter defibrillator would be considered for a relative with so-called genotype-positive (ie, mutation positive for R249Q)/phenotype-positive (ie, diagnostic echocardiogram or cardiac magnetic resonance imaging findings) disease.

COMMENT

Potentially lethal cardiac channelopathies (LQTS, CPVT, and BrS) and cardiomyopathies (HCM, DCM, and ACM) are heritable genetic disorders that often exhibit variable expressivity ranging from a lifelong asymptomatic course, to mild cardiac episodes (ie, palpitations or syncope), to sudden cardiac death as the first manifestation, even in a single pedigree. In fact, the postmortem examination may represent the first opportunity to establish the presence and identity of potentially lethal genetic heart disease.[20] Tragically, unforeseen sudden deaths especially involving the young, leave a devastating void and exact an overwhelming psychological toll on living family members.

While a conventional autopsy may indicate an underlying pathologic process, a negative or inconclusive autopsy leaves a family without explanation and with anxiety that

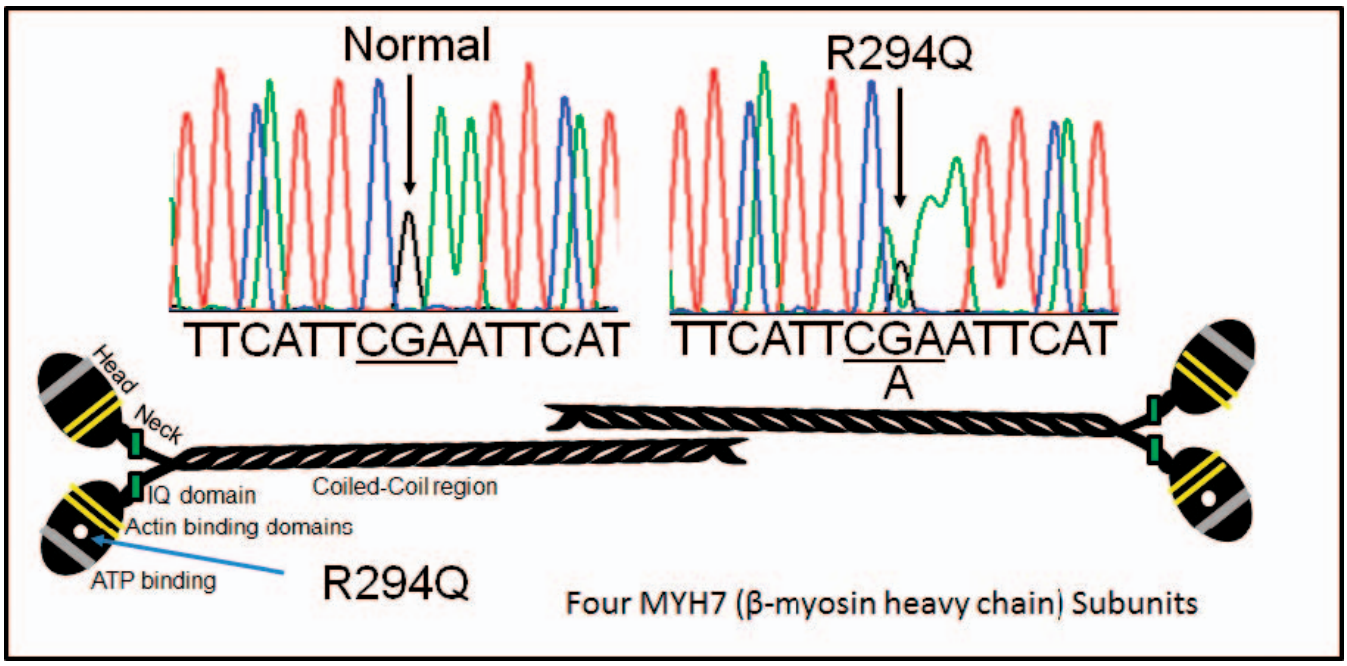

Figure 2. MYH7 *mutation confirmation by Sanger DNA sequencing. Shown are both a normal (right) and abnormal (left) DNA sequence chromatogram illustrating a heterozygote single-nucleotide substitution of a guanine (G, black peak) to an alanine (A, green peak) that results in an amino acid substitution of arginine (R, codon CGA) for a glutamine (Q, codon CAA) at amino acid position 294 of the β–myosin heavy chain protein. Also shown is schematic representation of MYH7 (β–myosin heavy chain) subunits with the mutation location depicted. Abbreviations: ATP, adenosine triphosphate; IQ, isoleucine glutamine.*

other family members could see a similar fate. Genetic testing in the postmortem setting can expose the underlying mechanism for the sudden death and identify or confirm the suspicion of an inherited sudden death–predisposing disorder.[4,9] Therefore, a more complete cardiac investigation would empower caregivers and families to reduce the risk of additional sudden deaths through preventative measures.[6]

Recently, the Association for European Cardiovascular Pathology has recommended strongly the use of postmortem genetic analysis for both structural and nonstructural genetically determined heart disease as a part of the requirements for an adequate postmortem evaluation of a sudden cardiac death.[7] In 2008, guidelines to ensure standardization of autopsy practices for sudden unexpected deaths in the young, and the preservation of appropriate material for all cases of sudden cardiac death to be used for genetic testing, was put forward by members of the Trans-Tasman Response AGAinst Sudden Death in the Young (TRAGADY).[6] In 2011, the Heart Rhythm Society and the European Heart Rhythm Association recommended genetic testing in autopsy-negative SUD cases, especially when clinically relevant presentations existed among the decedent or family members.[8] However, this level of care has been extremely difficult for medical examiners and coroners to provide owing to the cost and time-consuming nature of current genetic testing efforts.

However, WES is becoming increasingly available at a reasonable price and may be more efficient and cost-effective than previous approaches. In fact, while the cost of performing research-grade WES for this case, using Mayo Clinic's core facility, was only $1528, it would have cost an estimated $16 121 ($9.50/exon) to perform traditional DNA Sanger sequencing for all 1697 coding regions exons of 90 unique genes that have been implicated in sudden death–associated channelopathies/cardiomyopathies.

In this report, we provide proof-of-principle for WES-based postmortem genetic testing for a 16-year-old otherwise healthy young woman who died suddenly. Given the inconclusive gross and histologic findings on autopsy and the lack of a significant family history, a test to help confirm the presence of disease was highly desired. After WES and gene-specific surveillance, we identified an R249Q-MYH7 mutation that has been associated previously with familial HCM and sudden death.[18,19]

Using WES, we were able to complete our analysis of all 90 genes, verify R249Q-MYH7 in a CLIA (Clinical Laboratory Improvement Amendments of 1988)–approved laboratory, commence genetic counseling, cascade genetic testing in surviving family members, and initiate surveillance for those family members at risk for HCM, all within a few months. As a result, 6 additional family members were found to be at risk for HCM and were advised of recommendations for both clinical cardiac and genetic testing. Thus far, 3 family members have been informed that they are negative for the familial R249Q-MYH7 mutation and are therefore not at increased risk for either disease expression or future disease transmission, and have been dismissed from further cardiac follow-up.

To perform WES-based postmortem genetic testing, it is imperative that medical examiners and coroners adhere to current sample collection and retention guidelines. To ensure an adequate amount of DNA is available for WES, it is recommended that at least 5 to 10 mL of blood in EDTA (ie, purple-top tube) or 5 g of fresh tissue from the heart, liver, or spleen be collected at autopsy.[5,7] While WES may be performed on DNA isolated from blood spot cards, the amount of DNA isolated from this source is often

inadequate for a WES-based strategy. If blood spot cards are the only retainable source of DNA, owing to logistical concerns, then we would recommend collecting at least 4 to 8 "US quarter"–size spots of blood on a card specifically formulated for DNA extraction.

While the comprehensive nature of WES is beneficial, it also brings the daunting task of sifting through hundreds to thousands of nonsynonymous genetic variants for each individual exome, many of which might be predicted in silico to be deleterious. With WES, there is also great potential for the unintended identification of noncardiac disease–associated genetic variants not responsible for the sudden death, but that may be of substantial concern for surviving family members. Hypothetically, WES analysis of an SUD case could reveal the presence of a cancer-susceptibility *BRCA1* or *BRCA2* mutation that may be present in surviving family members who are currently unaware of their increased risk for cancer.

However, using bioinformatics and data filtering, one can focus variant analysis on targeted genes and avoid unintended incidental findings while conserving resources only for identifying the cause of sudden cardiac death. This is particularly important in cases of a targeted sudden death WES investigation, as surviving family members would not likely have the opportunity or full ability to make a well-informed decision as to the vast scope of information that may become available through WES (carrier status, risk for late-onset conditions). Focused testing limits the ethical dilemma and responsibility as to what is reported to the family and allows subsequent genetic counseling to concentrate on the family's understanding and adaptation to the cause of death, and on the implications to other family members, and to support the coping and grieving process.

It is therefore critical that the continued evaluation of a SUD case, using WES-based molecular testing, involve not only the medical examiner—and ideally, when available, also a cardiac pathologist responsible for performing a careful gross and histologic examination and for procurement of appropriate samples for genetic testing—but also a multidisciplinary health care team. This team should include a cardiologist with expertise in the clinical management of cardiac channelopathies and cardiomyopathies, who is conversant in the interpretation of cardiac genetic test results as a probabilistic predictor of pathogenicity, and a board-certified genetic counselor specifically trained in cardiovascular diseases and in the complex nature of WES.[5,21,22]

This report details the first utilization of WES to confirm the underlying pathogenic substrate most likely responsible for the sudden unexpected death of an otherwise healthy adolescent. Moreover, it allowed for the rapid identification and triaging of her at-risk living family members and dismissal of her mutation-negative relatives. Whole exome sequencing followed by gene-specific surveillance may be a highly efficient and cost-effective approach for a truly comprehensive cardiovascular autopsy.

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