Letters

RESEARCH LETTER

Molecular Autopsy for Sudden Unexpected Death

Approximately 11 000 individuals younger than 45 years in the United States die suddenly and unexpectedly each year from conditions including sudden infant death, pulmonary embolism, ruptured aortic aneurysm, and sudden cardiac death (SCD). Sometimes the cause of death is not determined, even after a clinical autopsy, leaving living relatives with an inaccurate or ambiguous family health history. Moreover, the rate of clinical autopsy has declined from approximately 50% fifty years ago to less than 10% in 2008, contributing further to uncertain family health histories.[1] This uncertainty may be partially resolved with postmortem genetic testing ("molecular autopsy").[2] Initial studies, limited to cardiac channelopathy and epilepsy genes, have yielded molecular diagnoses in approximately 25% of cases.[3,4] A more comprehensive molecular autopsy program, expanded beyond SCD, has the potential to provide more accurate family health information to a wider spectrum of afflicted families. Here we report preliminary results from a systematic, prospective, family-based, molecular autopsy study.

Methods | Exome sequencing was performed on blood or tissue samples collected from deceased persons aged 45 years or younger, with sudden unexpected death, sequentially referred to Scripps Translational Science Institute by the medical examiner between October 2014 and November 2015. Deaths from an external cause or in persons with known comorbid conditions were excluded. Exome sequencing of saliva samples from parents, when available, was also performed. Full details of the genome sequencing, analysis, and interpretation methodology have been previously described.[5] Mutations were categorized as likely cause of death (mutation previously reported or expected in an SCD-related gene); plausible cause of death (mutation of unknown significance in an SCD gene); or speculative cause of death (mutation previously reported in other disorders). Reported allele frequencies are the highest population-specific frequencies observed in the Exome Aggregation Consortium Browser. The study was approved by the Scripps institutional review board. Written informed consent was obtained from all participants and from next of kin for deceased individuals.

Results | Twenty-five cases (80% male) were sequenced, with 9 including both parents of the deceased. Clinical autopsies discovered the likely cause of death in 5 cases. A likely cause of death was identified by molecular autopsy in 4 cases (16%), a plausible cause in 6 (24%), and a speculative cause in 7 (28%); no mutations were identified in 8 (32%) (**Table**). The likely genetic cause of death was corroborated with clinical autopsy findings in 2 of 5 cases. All other clinical autopsy findings (3 cases) could be linked to a plausible or speculative genetic cause. Seventy percent (7/10 cases) of likely and plausible pathogenic mutations were inherited from relatives who did not die suddenly, as determined either by direct observation of the variant in a family member[4] or inference based on previous observation in reference populations.[3]

Discussion | Molecular autopsy was able to uncover a likely or plausible cause of death in 40% of cases (10/25). Many of the findings were variants of unknown significance inherited from relatives not affected by sudden death and present at population frequencies incompatible with full disease penetrance. Although this study is limited by its small sample size and potentially by selection bias, the observation of likely and plausible pathogenic variants in unaffected relatives is consistent with recent large-scale studies that identified clinically relevant variants in living relatives of SCD cases.[4,6] Our study suggests similar findings may be observed in non-SCD sudden death. It should be noted that these speculative and plausible findings cannot definitively be linked to sudden death.

The ambiguity associated with some of these genetic findings should be balanced against the potential for clinical follow-up, active surveillance, or preventive interventions in living relatives. Although molecular autopsies may help identify genetic causes of sudden unexpected death, a comprehensive and systematic effort to collect and share genetic and phenotypic data is needed to more precisely define pathogenic variants and provide quantifiable risks to living relatives.

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Author Contributions: Dr Torkamani had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Concept and design: Torkamani, Muse, Spencer, Wagner, Topol.
Acquisition, analysis, or interpretation of data: All Authors.
Drafting of the manuscript: Torkamani, Muse, Wagner.
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Table. Primary Results of 25 Sequentially Referred Cases of Potentially Genetic Sudden Unexpected Death

	Variant as Cause of Death[a]			Supporting Information[b]				
Age, y	Likely	Plausible	Speculative	Evidence	Phenotype	Allele Frequency, %	Inheritance	Case Notes[c]
Female								
44								
2		JUP p.D136N			ARVD	0.02	Maternal	
34			ZFPM2 p.S657G	ClinVar	Congenital diaphragmatic defects	14	Unknown: likely inherited	Aneurysm observed in autopsy
25	PKP2 p.Q378X			ClinVar	ARVC	<0.001	Paternal	Pathology-confirmed ARVC
Male								
22			CYP3A4*1G	PharmGKB	Fentanyl toxicity	12	Unknown: likely inherited	Positive for fentanyl during toxicology screen
15								
1	HCN4 p.V759I			PMID:23623143	Brugada syndrome	0.5	De novo	Confirmed de novo via Sanger sequencing
17 and 24								Deceased brothers
3 mo		RYR2 p.L2534P			Ventricular tachycardia	<0.001	Paternal	
32	MYH6 p.A1004S			ClinVar; PMID:20864896; PMID:15998695	Hypertrophic cardiomyopathy	0.3	Unknown: likely inherited	Pathology confirmed cardiomyopathy
3 mo			SCN9A p.M932L / p.V991L	ClinVar; PMID:21698661; PMID:23280954	Epilepsy	23	Recessive	High allele frequency with functional validation
40								
44		TRPM4 p.A566V			Progressive familial heart block, Brugada syndrome	<0.001	Unknown: likely inherited	
		SCN10A p.A1235V				<0.001		
2 mo			SLC12A3 p.G741R	PMID:8528245	Gitelman syndrome	0.1	Unknown: likely inherited	
2 mo			SLC9A3R1 p.L110V	ClinVar	Hypophosphatemic nephrolithiasis	3.6	Unknown: likely inherited	High allele frequency with functional validation
3 mo								
10 mo			NDUFV1 c.1282-2A>G	Splice site acceptor	Mitochondrial complex I deficiency	<0.001	De novo	Confirmed de novo via Sanger sequencing
9 mo		SCN10A p.D248H			Brugada syndrome	<0.001	Unknown	
21								
33		THBD p.R101L			Thrombophilia	<0.001	Unknown: likely inherited	Family history of thrombosis
21								
9			IGHMBP2 p.Q51E	PMID:14681881	Juvenile-onset respiratory distress	0.9	Recessive	Signs of respiratory distress at death
			p.D974E			0.2		
28		RYR2 p.A979S			Ventricular tachycardia	0.2	Unknown	
29	TRPM4 p.W525X			Nonsense; PMID:25531103	Progressive familial heart block	0.2	Maternal	

Abbreviations: ARVC, arrhythmogenic right ventricular cardiomyopathy; ARVD, arrhythmogenic right ventricular dysplasia; SCD, sudden cardiac death.

[a] Likely pathogenic indicates previously reported or expected pathogenic variants in SCD genes. Plausible indicates variant of unknown significance in SCD genes. Speculative indicates previously reported or expected pathogenic in other disorders. Speculative findings are not considered positive but are included as plausible novel causes of sudden death, for example: (1) a 2-month old male with a known pathogenic mutation in a noncardiac ion channel *SLC12A3*—a gene that causes Gitelman syndrome, a kidney disorder that can increase the risk for ventricular arrhythmia, and (2) a male aged 10 months with an expected pathogenic mutation in *NDUFV1*, a gene that causes mitochondrial dysfunction and possible sudden death.

[b] Evidence column includes supporting evidence for previously reported pathogenic variants. ClinVar is available at http://www.ncbi.nlm.nih.gov/clinvar/. PMID indicates PubMed identifier available at http://www.ncbi.nlm.nih.gov/pubmed. PhamGKB indicates Pharmacogenomics Knowledgebase, available at https://www.pharmgkb.org/. Phenotype column reflects the phenotype conferred by the reported genetic variants. Allele frequency indicates the largest population-specific allele frequency observed in the exome aggregation consortium browser. Inheritance indicates the observed or inferred genetic variant inheritance pattern. Likely inherited refers to variants inferred to be inherited based on prior observation in reference populations.

[c] Findings during clinical autopsy. The majority of clinical autopsies were negative. No information in the case notes denotes an autopsy-negative case.

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COMMENT & RESPONSE

Escitalopram and Outcomes Among Patients With Depression and Heart Failure

To the Editor: In a placebo-controlled, randomized clinical trial, Dr Angermann and colleagues reported that escitalopram did not reduce all-cause death or hospitalization in depressed patients with heart failure.[1] It is not clear why the authors expected that it would, as it does not reduce mild to moderate levels of depression.

At baseline, patients had a mean Montgomery-Åsberg Depression Rating Scale (MADRS) score of 20 to 22 indicating mild to moderate severity of depression. After 12 weeks, MADRS scores in the placebo and the escitalopram group were 12.5 and 11.2, respectively.

In mild to moderate depression antidepressants are no better than placebo,[2] and a clinically significant difference can only be found in severe major depression, even in populations without severe somatic comorbidities.[3] In addition to the placebo effect, an estimated 20% of patients spontaneously remitted within weeks when enrolled in a wait list control group of a depression trial.[4] It is unlikely that the 2-week interval between depression screening and depression diagnosis in this study was sufficient to exclude patients with a high likelihood for spontaneous remission.

Thus, this trial does not, in our view, support "the concept of alternative pathophysiological mechanisms for mood disorders in somatic illnesses."[1] Rather, it adds to the mounting evidence that questions the risk-benefit ratio of prescribing antidepressants to patients with less than severe depression.

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In Reply Drs Ströhle and Rieckmann express skepticism about the Effects of Selective Serotonin Re-Uptake Inhibition on Morbidity, Mortality, and Mood in Depressed Heart Failure Patients (MOOD-HF) study hypothesis that escitalopram would reduce mortality and morbidity in depressed patients with heart failure. Depression is an established risk marker for incident cardiac disease and associated with adverse outcomes in cardiovascular patients.[1] In heart failure, it predicts death and rehospitalization with incidence rates rising in parallel with symptom severity.[2] Increasing depressive symptoms may worsen[3] and depression remission improve[4] cardiovascular outcomes. These observations provide an excellent rationale for investigating whether altering depression with an antidepressant would modify risk and improve prognosis in heart failure.

According to the *Diagnostic and Statistical Manual of Mental Disorders*, major depressive disorder (MDD) requires specific symptoms that persist for at least 14 days.[5] During baseline assessment in MOOD-HF, the diagnosis of MDD was established by psychiatrists utilizing a standardized interview; questionnaires were only used for longitudinal follow-up of the depressive symptoms. We are confident that the psychiatrists' diagnosis considered critical issues (eg, symptom duration).

Concerns expressed by Ströhle and Rieckmann that antidepressants are efficacious mainly for MDD episodes more severe on average than those in MOOD-HF led us to conduct further analyses regarding baseline depression severity and treatment response. The **Figure** compares patients treated with