An Autopsy Case of Sudden Unexpected Death of a Young Adult in a Hot Bath: Molecular Analysis Using Next-Generation DNA Sequencing

Clinical Medicine Insights: Case Reports
Volume 10: 1–6

DOI: 10.1177/1179547617702884

Yukiko Hata, Koshi Kinoshita and Naoki Nishida

Department of Legal Medicine, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Toyama, Japan.

ABSTRACT: We report a case of sudden unexpected death of a young woman who was found in a bathtub of hot water. The autopsy concluded that all possible causes of sudden loss of consciousness, except cardiac origin, could be excluded. However, the heart did not show any obvious pathological changes. We used next-generation DNA sequencing (NGS) to examine 73 genes and detected 3 rare, potentially pathogenic variants with minor allele frequencies ⩽1.0%. The pathogenicity of these variants was evaluated using 8 in silico predictive algorithms, and *SCN5A*_p.Gly289Ser, *CACNB2*_p.Ser502Leu, and *MYH11*_p.Lys1573Glu were detected as possible pathogenic variants. Inherited heart disease is a likely cause of sudden unexpected deaths of young people in hot baths, even before the clinical manifestation of the disease. In the future, molecular analysis by NGS may help to predict young to early middle-aged people who could be at risk of sudden arrhythmogenic fatality in hot baths.

KEYWORDS: Arrhythmia, genetics, hot bath, next-generation sequencing, sudden unexpected death

RECEIVED: December 6, 2016. **ACCEPTED:** February 11, 2017.

PEER REVIEW: Four peer reviewers contributed to the peer review report. Reviewers' reports totaled 709 words, excluding any confidential comments to the academic editor.

TYPE: Case Report

FUNDING: The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported in part by a KAKENHI grant from JSPS, Japan, to YH (JP15k08867) and by Presidential Discretionary Funds, University of Toyama 2014, to NN.

DECLARATION OF CONFLICTING INTERESTS: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

CORRESPONDING AUTHOR: Naoki Nishida, Department of Legal Medicine, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, 2630 Sugitani, Toyama-shi, Toyama 930-0194, Japan. Email: nishida@med.u-toyama.ac.jp

Introduction

Mortality rates due to accidental drowning are higher in Japan than in Western countries,[1] largely resulting from a higher incidence of sudden unexpected death (SUD) of Japanese people in hot baths.[2–4] Although the precise mortality rates are unknown, approximately 10% of SUDs confirmed by the Tokyo Medical Examiner's Office occur at home, involving individuals who took deep hot baths.[2]

A few studies have examined the autopsies of victims of SUD associated with taking hot baths. Satoh et al[4] summarized the findings of 268 autopsy cases of SUD in hot baths. Most of the subjects were above 70 years of age. Pathological and serological examination of the 173 subjects who did not show decomposition revealed a high incidence of structural cardiac disorders, such as coronary artery disease and cardiomegaly. Only 7 of the subjects were below 50 years of age, but all 7 had a history of epilepsy.[4] Here, we present a rare autopsy case of SUD of a young subject found dead in a hot bath. We attempted genetic screening using next-generation DNA sequencing (NGS), which allows large numbers of samples to be sequenced simultaneously. It can be used for the comprehensive analysis of panels of 20 to 80 genes associated with inherited arrhythmia or cardiomyopathy[5] to detect arrhythmogenic potential in the victims whose hearts have no significant structural disorders.

Case Report

A 28-year-old female beauty therapist was found dead in a bathtub with her face submerged. Resuscitation was not successful. There was no clinical history of significant organ or functional disease, such as epilepsy, that could have caused SUD or syncope in any of the cases. There was no family history of heart disease, and no electrocardiography had been performed within the past 10 years.

During medicolegal autopsy, no traumatic injury was found, but signs of drowning, specifically froth in the upper airway and pulmonary edema, were evident. Low levels of ethanol (1.1 mg/mL) were detected in the blood, but the full toxicological examination was negative. We concluded that all possible causes of sudden loss of consciousness, other than those of cardiac origin, were excluded by the full autopsy examination as well as the investigation of the scene of death.

The heart weighed 200 g and was examined as described in a previous report,[6] but it did not show any significant pathological changes. Under microscopic examination, ischemic necrosis of myocytes, substantial coronary artery atherosclerosis with luminal narrowing greater than 50%, and myocardial disarray were not evident. Diffuse but very mild interstitial fibrosis of the left ventricle was found (Figure 1).

Molecular Testing

The ethical committee of Toyama University approved this study, which was performed in accordance with the ethical standards established in the 1964 Declaration of Helsinki.

The genetic analysis using NGS was conducted as described in a previous report.[7] Genomic DNA samples of the case were extracted directly from whole blood using the QIAamp DNA

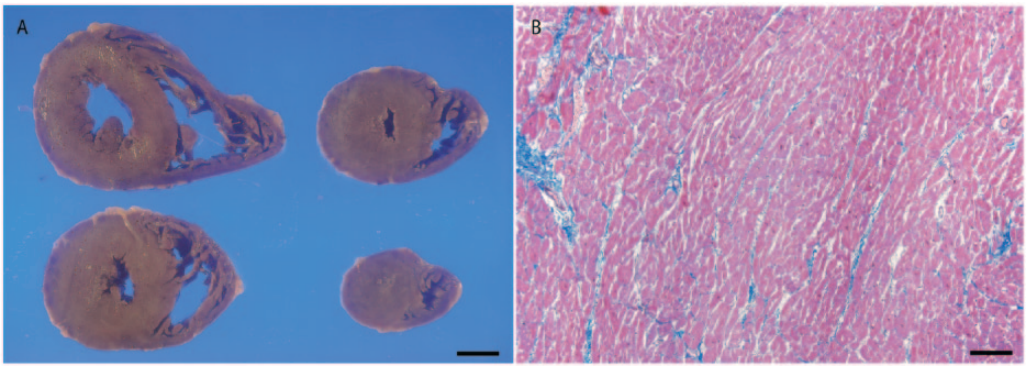

Figure 1. Gross and microscopic appearance of the deceased victim's heart (after fixation with formalin): (A) Horizontal section of both ventricles (scale bar = 1 cm) and (B) mild interstitial fibrosis of the left ventricle, visualized using Elastica-Masson staining (scale bar = 100 μm).

Table 1. List of the 73 analyzed genes associated with inherited cardiac diseases.

ABCC9, ACTC1, ACTN2, AKAP9, ANK2, BAG3, BMPR1A, CACNA1C, CACNB2, CALR3, CAPN3, CAV3, COL4A1, DES, DMD, DSC2, DSG2, DSP, ELN, EMD, GAA, GATA4, GLA, GPD1L, HCN4, JUP, KCNE1, KCNE2, KCNE3, KCNH2, KCNJ2, KCNQ1, KRAS, LAMP2, LDB3, LMNA, MYBPC3, MYH11, MYH6, MYH7, MYL2, MYL3, MYLK, MYOZ2, NKX2-5, NRAS, PKP2, PLN, PRKAG2, PTPN11, RAF1, RPS7, RYR2, SCN1B, SCN3B, SCN4B, SCN5A, SGCD, SLC25A4, SMAD3, SNTA1, SOS1, STARD3, TAZ, TBX5, TGFBR1, TGFBR2, TMEM43, TNNC1, TNNI3, TNNT2, TPM1, VCL

Mini Kit (Qiagen Sciences Inc., Germantown, MD, USA). We designed a custom AmpliSeq panel using Ion AmpliSeq Designer software (http://www.ampliseq.com) to target all exons of 73 cardiac disorder–related genes associated with cardiomyopathy and channelopathy (Table 1). This custom panel, which consisted of 2 separate polymerase chain reaction (PCR) primer pools and produced a total of 1870 amplicons, was used to generate the target amplicon libraries. Genomic DNA samples were PCR-amplified using the designed custom panel and the Ion AmpliSeq Library Kit v2.0 (Life Technologies, Carlsbad, CA, USA). Prepared libraries were pooled in equimolar concentrations for multiplexing. Emulsion PCR and Ion Sphere Particle enrichment were conducted with the Ion PGM Template OT2 200 Kit (Life Technologies). Ion Sphere Particles were loaded on an Ion 314 Chip Kit v2 and sequenced using an Ion PGM Sequencing 200 Kit (Life Technologies).

The Torrent Suite and Ion Reporter Software 5.0 (Life Technologies) were used to perform primary to tertiary analyses, including optimized signal processing, base calling, sequence alignment with the hg19 human genome reference (http://genome.ucsc.edu/), and variant analysis. For all variants detected, we consulted the East Asian (EAS) population database of 4327 individuals from the Exome Aggregation Consortium (http://exac.broadinstitute.org) to filter out those variants for which the minor allele frequency (MAF) was ⩾1.0% or undetermined in the EAS population.

For each genetic variation identified, we applied the Single Nucleotide Polymorphism Database (dbSNP) as a population database and the Human Gene Mutation database (HGMD) and ClinVar as reported disease-causing mutation databases. We also included 8 types of in silico predictive algorithms to evaluate the pathogenicity of identified variants. The URL for each database, in silico algorithms, and conditions used to evaluate pathogenicity are listed in Table 2.

Results of Molecular Testing

From the NGS analysis, *SCN5A*_p.Gly289Ser, *CACNB2*_p.Ser502Leu, and *MYH11*_p.Lys1573Glu were detected as rare variants in EAS, and the MAFs were 0%, 0.95%, and 0.035%, respectively. The sequences of *SCN5A*_p.Gly289Ser and *CACNB2*_p.Ser502Leu found in this case study are depicted in Figure 2.

*SCN5A*_p.Gly289Ser was previously reported as possibly pathogenic in an earlier study[8] and was evaluated as "conflicting interpretations of pathogenicity" in ClinVar. The other 2 variants were evaluated as having "uncertain significance" in ClinVar and are not noted in HGMD. After using our in silico predictive algorithm analyses, *SCN5A*_p.Gly289Ser was evaluated as possibly pathogenic twice, *CACNB2*_p.Ser502Leu was evaluated as possibly pathogenic 5 times, and *MYH11*_p.Lys1573Glu was evaluated as possibly pathogenic 6 times (Table 3).

Discussion

Previous reports indicate a number of heart conditions that may cause SUD in young adults, including structural heart disease such as coronary anomaly, hypertrophic cardiomyopathy, and arrhythmogenic right ventricular cardiomyopathy.[9,10] No evidence of these conditions were found in this particular case. Our study supports the findings of others with evidence that concealed cardiomyopathy and channelopathy may also result in SUD of young adults.[7,11,12]

Arrhythmogenic events and related SUD usually require both an abnormal myocardial substrate and an inciting trigger,

Table 2. Databases used for variant interpretation.

NAME	WEB SITE	CONDITION OF PATHOGENICITY
Population database		
Single Nucleotide Polymorphism Database (dbSNP)	http://www.ncbi.nlm.nih.gov/SNP	
Reported disease-causing mutation database		
Human Gene Mutation database (HGMD)	http://www.hgmd.cf.ac.uk	
ClinVar	http://www.ncbi.nlm.nih.gov/clinvar	
In silico prediction algorithm		
Functional Analysis Through Hidden Markov Models (FATHMM)	http://fathmm.biocompute.org.uk	Damaging
Mutation Assessor	http://mutationassessor.org	Medium, High
SIFT Sequence (SIFT)	http://sift.jcvi.org	Damaging
Align GVGD	http://agvgd.iarc.fr/index.php	⩾C15
MutationTaster	http://www.mutationtaster.org	Disease causing
PolyPhen-2	http://genetics.bwh.harvard.edu/pph2	Probably damaging, Possibly damaging
Protein Variation Effect Analyzer (PROVEAN)	http://provean.jcvi.org/index.php	Deleterious
Combined Annotation-Dependent Depletion (CADD)	http://cadd.gs.washington.edu	Score >10

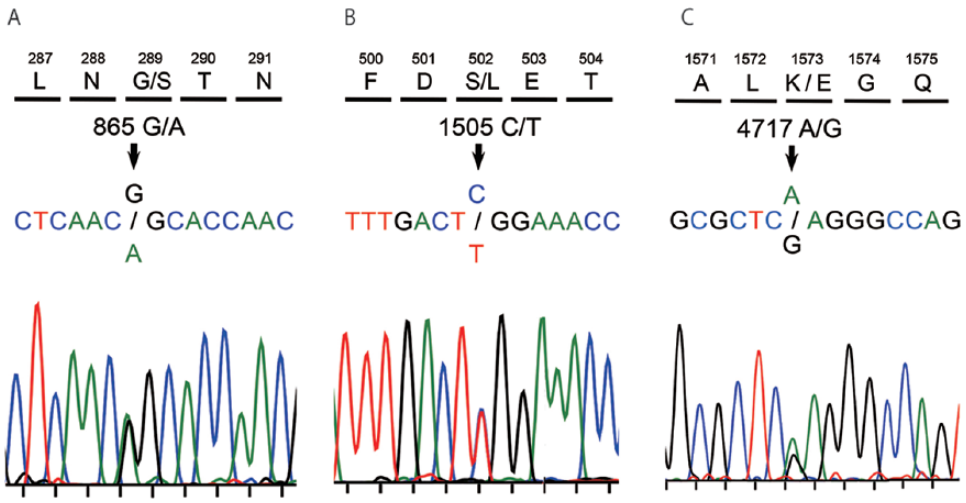

Figure 2. Sequences for possible channelopathy-related pathogenic variants from the deceased: (A) *SCN5A*, (B) *CACNB2*, and (C) *MYH11*.

such as exercise, being asleep, or emotional stress as seen in many cases.[13] Nagasawa et al[14] found that in the elderly, blood pressure and heart rate begin to rise immediately on immersion in a hot bath. These changes were associated with a temporary decrease in sympathetic activity without the compensatory parasympathetic suppression, resulting in hypotension and bradycardia. Chiba et al[3] showed that increased peripheral blood pressure and cardiac output occurred after bathing in both young and elderly subjects. In addition, asymptomatic ventricular tachycardia occurred in elderly individuals while sitting in hot water, and this arrhythmia developed within 5 minutes of immersion.[3] Many Japanese people love to take bath in water that reaches shoulder depth and soak in a sitting position, and a higher water temperature than in Western countries is generally used (approximately 40-42°C).[4] These studies, along with the case described here, show that immersion in deep hot water can trigger an arrhythmogenic event which could lead to drowning in the bath water in individuals with an inherited or acquired heart disease.

We recently reported the significance of postmortem genetic analysis using NGS for both SUD syndrome[7] and SUD with epilepsy cases[15] in young people to explore the cause of such death if pathological change of the heart is not evident. The present case is notable in that detection of rare gene variants

Table 3. Detected variants and results of in silico analysis.

	SCN5A P.GLY289SER	*CACNB2* P.SER502LEU	*MYH11* P. LYS1573GLU
Transcript	NM_198056.2	NM_000724.3	NM_002474.2
MAF(%)	0.0	0.95	0.035
dbSNP	rs199473084	rs137886839	rs151101824
Disease-causing mutation database			
ClinVar	Uncertain significance	Conflicting interpretations of pathogenicity	Uncertain significance
HGMD	Disease-causing mutation (long QT syndrome) CM097628	None	None
In silico prediction algorithm			
FATHMM	**Damaging**	**Damaging**	Tolerated
Mutation Assessor	Neutral	Low	**Medium**
SIFT	Tolerated	**Damaging**	**Damaging**
Align GVGD	C0 (the lowest risk grade)	C0 (the lowest risk grade)	C0 (the lowest risk grade)
MutationTaster	Polymorphism	**Disease causing**	**Disease causing**
Polyphen-2	Benign	**Possibly damaging**	**Probably damaging**
PROVEAN	Neutral	Neutral	**Deleterious**
CADD	**11.76**	**25.4**	**26.4**

Abbreviations: CADD, combined annotation dependent depletion; FATHMM, Functional Analysis Through Hidden Markov Models; MAF, minor allele frequency; PROVEAN, Protein Variation Effect Analyzer; SIFT, SIFT Sequence.
Bold type shows the pathogenic condition in each in silico algorithm.

suggested that the deceased might have had undiagnosed arrhythmogenic potential which could cause sudden loss of consciousness during bathing.

However, we should note the limitations involved in interpretation of the detected variants found by NGS analysis.[16,17] We frequently depend on population databases and in silico analyses to evaluate the pathogenicity of detected variants because functional or genetic analysis of family members, the criterion standard method for evaluating the pathogenicity of genetic variants, can be difficult in some cases. However, the evaluations obtained from different in silico analyses do not always correspond, as shown in our case. Guidelines for interpreting sequence variants recommend that several in silico analyses should be used to evaluate the pathogenicity of arrhythmia-related gene variants because most algorithms used for missense variant prediction are only 65% to 80% accurate when examining known disease variants.[18] In addition, Le Scouarnec et al[19] and Kapplinger et al[20] indicated that identification of a variant does not confirm the presence of the disease because many of the variants found in Brugada syndrome patients were also identified in the control population. Genetic analysis using NGS may provide significantly useful information about the mechanism of SUD in some conditions, but careful and comprehensive evaluation of the detected variants is needed when the evaluation for pathogenicity differs across a range of predictive procedures.

*SCN5A*_p.Gln289Ser is a very rare variant, not only in EAS but across the world. Most of the in silico tools evaluated this variant as "negative." However, 1 patient with long QT syndrome has been reported to have this variant.[8] Therefore, we have evaluated this variant as possibly pathogenic in our case. In addition, although the relevance of drinking alcohol to SUD occurring in hot baths is not fully understood, some researchers consider that ethanol intake may increase the chance of developing atrial fibrillation, a prolonged QT interval, and SUD[21] as the combination of pathogenic genetic variants and alcohol intake might increase the risk of sudden arrhythmogenic events occurring in hot baths.

The *CACNB2*_p.Ser502Leu variant is rare but has a relatively high incidence in EAS, yet 5 of 8 in silico tools evaluated this variant as pathogenic. The L-type calcium channel is composed of 4 subunits, and *CACNB2* codes one of 3 ancillary subunits. *CACNB2* is the dominant isoform known to play an essential role in the voltage dependence of the L-type calcium channel. Accelerated inactivation of the calcium current was found in 1 person who had a mutation in *CACNB2*, and the variant is also associated with Brugada syndrome, short QT, long QT 8 (Timothy syndrome), J wave syndrome, and sudden

death.[22] There are very few autopsy reports of SUD with *CACNB2* variants detected. Our results show that the variants of *CACNB2* may also have the potential to cause arrhythmogenic SUD in hot baths.

We should note that we cannot evaluate the pathogenic significance of the combined effect of the variants seen in present case. Currently available in silico tools can only indicate the pathogenicity of single genes; thus, the pathogenic significance of interactions between different gene variants cannot be fully evaluated. This victim's heart might have concealed an arrhythmogenic potential from the combination of 2 channelopathy-related pathogenic variants, even if structural abnormality was not evident.

The role of the variant of *MYH11* (which encodes a smooth muscle myosin heavy chain) is not well established. This gene belongs to the myosin heavy chain family and is a major contractile protein in smooth muscle cells.[19] Whereas mutations in *MYH11* have been identified in families with inherited patent ductus arteriosus and thoracic aortic aneurysms and dissections,[23,24] cases of arrhythmia or sudden death associated with this *MYH11* variant have not been reported. Examination of further cases involving this variant will be useful to determine how significant this variant is.

Given the high cost of genetic analysis, it is not feasible to conduct this analysis routinely for every case. In many circumstances, careful toxicological screening and histological examination of the heart and other organs should provide enough evidence to specify cause of death or might at least contribute to narrowing the list of target genes to explore. In particular, detection of minimal cardiac pathology, including necrosis, inflammation, and fatty infiltration into the ventricle, during an examination might be indicative of cardiomyopathy-related genetic variants. Such findings may prompt further genetic analysis, even if the observed pathological changes do not fulfill the commonly used diagnostic criteria of structural heart diseases.[7,15]

Conclusions

We report here a rare autopsy case of a young female adult who died suddenly and unexpectedly in her bathtub. Genetic analysis using NGS showed 2 previously unpredicted channelopathy-related variants with possible arrhythmogenic potential. A combination of the possible pathogenic channelopathy-related gene variants might have contributed to this unusual death in the bathtub, and the event may also have been triggered by bathing under the influence of alcohol. Although the evaluation of these detected variants is still complicated by our inability to completely assess pathogenicity, future molecular analysis by NGS may help to predict which young people could be at risk of SUD in hot baths.

Acknowledgements

We thank Ms Syuko Matsumori, Ms Tamae Sasakura, Mr Noboru Onozuka, and Mr Osamu Yamamoto for their technical assistance.

Author Contributions

YH and NN conceived and designed the experiments. YH and KK analyzed the data. YH wrote the first draft of the manuscript. KK and NN contributed to writing the manuscript. YH, KK, and NN agreed on the manuscript results and conclusions. NN made critical revisions and approved the final version. All the authors reviewed and approved the final manuscript.

Disclosures and Ethics

The authors have read and confirmed their agreement with the ICMJE authorship and conflict of interest criteria. The authors have also confirmed that this article is unique and not under consideration or published in any other publication, and that we have permission from rights holders to reproduce any copyrighted material. Any disclosures are made in this section. The external blind peer reviewers report no conflicts of interest.

REFERENCES

1. Health and Welfare Statistics Association Japan. World Health Statistics Annual. *J Health Welfare Stat*. 2008;55(suppl 9):58.
2. Abe H, Kohno R, Oginosawa Y. Characteristics of syncope in Japan and the Pacific rim. *Prog Cardiovasc Dis*. 2013;55:364–369.
3. Chiba T, Yamauchi M, Nishida N, Kaneko T, Yoshizaki K, Yoshioka N. Risk factors of sudden death in the Japanese hot bath in the senior population. *Forensic Sci Int*. 2005;149:151–158.
4. Satoh F, Osawa M, Hasegawa I, Seto Y, Tsuboi A. "Dead in hot bathtub" phenomenon: accidental drowning or natural disease? *Am J Forensic Med Pathol*. 2013;34:164–168.
5. Lubitz SA, Ellinor PY. Next-generation sequencing for the diagnosis of cardiac arrhythmia syndrome. *Heart Rhythm*. 2015;12:1062–1070.
6. Hata Y, Mori H, Tanaka A, et al. Identification and characterization of a novel genetic mutation with prolonged QT syndrome in an unexplained postoperative death. *Int J Legal Med*. 2014;128:105–115.
7. Hata Y, Kinoshita K, Mizumaki K, et al. Postmortem genetic analysis of sudden unexplained death syndrome under 50 years of age: a next-generation sequencing study. *Heart Rhythm*. 2016;13:1544–1551.
8. Kapplinger JD, Tester DJ, Salisbury BA, et al. Spectrum and prevalence of mutations from the first 2,500 consecutive unrelated patients referred for the FAMILION long QT syndrome genetic test. *Heart Rhythm*. 2009;6:1297–1303.
9. Vaartjes I, Hendrix A, Hertogh EM, et al. Sudden death in persons younger than 40 years of age: incidence and causes. *Eur J Cardiovasc Prev Rehabil*. 2009;16:592–596.
10. Basso C, Calabrese F, Corrado D, Thiene G. Postmortem diagnosis in sudden cardiac death victims: macroscopic, microscopic and molecular findings. *Cardiovasc Res*. 2001;50:290–330.
11. Tester DJ, Ackerman MJ. The role of molecular autopsy in unexplained sudden cardiac death. *Curr Opin Cardiol*. 2006;21:166–172.
12. Marsman RF, Tan HL, Bezzina CR. Genetics of sudden cardiac death caused by ventricular arrhythmias. *Nat Rev Cardiol*. 2014;11:96–111.
13. Hata Y, Kinoshita K, Kudo K, Ikeda N, Nishida N. Anomalous origin of the right coronary artery from the left coronary sinus with an intramural course: comparison between sudden-death and non-sudden-death cases. *Cardiovasc Pathol*. 2015;24:154–159.
14. Nagasawa Y, Komori S, Sato M, et al. Effects of hot bath immersion on autonomic activity and hemodynamics: comparison of the elderly patient and the healthy young. *Jpn Circ J*. 2001;65:587–592.
15. Hata Y, Yoshida K, Kinoshita K, Nishida N. Epilepsy-related sudden unexpected death: targeted molecular analysis of inherited heart disease genes using next-generation DNA sequencing [published online ahead of print May 2, 2016]. *Brain Pathol*. doi:10.1111/bpa.12390.
16. Bagnall RD, Das KJ, Duflou J, Semsarian C. Exome analysis-based molecular autopsy in cases of sudden unexplained death in the young. *Heart Rhythm*. 2014;11:655–662.
17. Amendola LM, Dorschner MO, Robertson PD, et al. Actionable exomic incidental findings in 6503 participants: challenges of variant classification. *Genome Res*. 2015;25:305–315.
18. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the association for molecular pathology. *Genet Med*. 2015;17:405–423.

19. Le Scouarnec S, Karakachoff M, Gourraud JB, et al. Testing the burden of rare variation in arrhythmia-susceptibility genes provides new insights into molecular diagnosis for Brugada syndrome. *Hum Mol Genet*. 2015;24:2757–2763.
20. Kapplinger JD, Giudicessi JR, Ye D, et al. Enhanced classification of Brugada syndrome-associated and long-QT syndrome-associated genetic variants in the SCN5A-encoded Na(v)1.5 cardiac sodium channel. *Circ Cardiovasc Genet*. 2015;8:582–595.
21. Chiuve SE, Rimm EB, Mukamal KJ, et al. Light-to-moderate alcohol consumption and risk of sudden cardiac death in women. *Heart Rhythm*. 2010;7:1374–1380.
22. Cordeiro JM, Marieb M, Pfeiffer R, Calloe K, Burashnikov E, Antzelevitch C. Accelerated inactivation of the L-type calcium current due to a mutation in CACNB2b underlies Brugada syndrome. *J Mol Cell Cardiol*. 2009;46:695–703.
23. Zhu L, Vranckx R, Khau Van Kien P, et al. Mutations in myosin heavy chain 11 cause a syndrome associating thoracic aortic aneurysm/aortic dissection and patent ductus arteriosus. *Nat Genet*. 2006;38:343–349.
24. Harakalova M, van der Smagt J, de Kovel CG, et al. Incomplete segregation of MYH11 variants with thoracic aortic aneurysms and dissections and patent ductus arteriosus. *Eur J Hum Genet*. 2013;21:487–493.