ORIGINAL ARTICLE

Identification of pathogenic variants in genes related to channelopathy and cardiomyopathy in Korean sudden cardiac arrest survivors

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Pathogenic variants in genes related to channelopathy and cardiomyopathy are the most common cause of sudden unexplained cardiac death. However, few reports have investigated the frequency and/or spectrum of pathogenic variants in these genes in Korean sudden cardiac arrest survivors. This study aimed to investigate the causative genetic variants of cardiac-associated genes in Korean sudden cardiac arrest survivors. We performed exome sequencing followed by filtering and validation of variants in 100 genes related to channelopathy and cardiomyopathy in 19 Korean patients who survived sudden cardiac arrest. Five of the 19 patients (26.3%) had either a pathogenic variant or a likely pathogenic variant in *MYBPC3* ($n = 1$), *MYH7* ($n = 1$), *RYR2* ($n = 2$), or *TNNT2* ($n = 1$). All five variants were missense variants that have been reported previously in patients with channelopathies or cardiomyopathies. Furthermore, an additional 12 patients (63.2%) had more than one variant of uncertain significance. In conclusion, pathogenic or likely pathogenic variants in genes related to channelopathy and cardiomyopathy are not uncommon in Korean sudden cardiac arrest survivors and cardiomyopathy-related genes should be included in the molecular diagnosis of sudden cardiac arrest in Korea.
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INTRODUCTION

Sudden cardiac death (SCD) is a major cause of death affecting people of all ages, with an incidence of 1 per 1000 patient-years.[1] In the elderly, coronary artery disease is the main cause of SCD, whereas in younger individuals, various other causes are prevalent and a complete postmortem study often fails to determine the cause of death. These autopsy-negative sudden unexplained deaths (SUDs) are frequently explained by heritable cardiac diseases.[2] Heritable cardiac channelopathies such as long QT syndrome, catecholaminergic polymorphic ventricular tachycardia and Brugada syndrome (BrS) in a morphologically normal heart affect heart rhythm and cardiac electrical conduction and are associated with sudden cardiac arrest. Additionally, heritable cardiomyopathies, including hypertrophic cardiomyopathy, dilated cardiomyopathy and arrhythmogenic cardiomyopathy, are difficult to diagnose and are often considered indeterminable owing to the uncertain or minimal related changes; therefore, they may account for a significant proportion of SUD cases.[3]

The identification of dozens of genes related to heart disease coupled with whole-exome sequencing (WES), which enables accurate, thorough and cost-effective genetic analysis of pathogenic variants in the entire library of genes, appears to be useful for comprehensive molecular diagnosis. These advances also have an enormous impact on the surviving relatives of sudden cardiac arrest survivors or victims by enabling preventive treatment or presymptomatic follow-up.[4]

In a previous study of Korean sudden cardiac arrest survivors with structurally normal hearts, analysis of three cardiac ion channel genes, *SCN5A*, *KCNQ1* and *KCNH2*, showed that 3 of the 15 patients exhibited a pathogenic variant in *SCN5A*.[5] However, WES and gene panel studies have not yet been used to characterize genes that are related to channelopathy or cardiomyopathy in Korean patients with sudden cardiac arrest. Therefore, in this study, we tried to use WES to identify pathogenic or likely pathogenic variants in 100 genes related to channelopathy or cardiomyopathy in Korean sudden cardiac arrest survivors.

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MATERIALS AND METHODS

Study population

Patients who survived sudden cardiac arrest caused by idiopathic ventricular tachycardia or fibrillation were enrolled consecutively from January 2010 to March 2015 at a tertiary care hospital in Seoul, Korea. All sudden cardiac arrest survivor cases were clinically examined by electrocardiogram, two-dimensional echocardiography, coronary angiography or cardiac magnetic resonance imaging. In addition, medical history and family history were recorded. We excluded patients who had abnormal findings in coronary angiography, a positive spasm provocation test with ergonovine (if performed) or a history of coronary artery disease. Written informed consent was obtained from all study subjects. Our institutional review board approved this study (Samsung Medical Center 2014-08-111).

Whole-exome sequencing

Genomic DNA was extracted from peripheral blood leukocytes using the Wizard Genomic DNA Purification Kit following the manufacturer's instructions (Promega, Madison, WI, USA). SureSelect Human All Exon V5 (Agilent Technologies, Santa Clara, CA, USA) was used for library preparation; sequencing was performed using the Illumina NextSeq500 platform (Illumina Inc., San Diego, CA, USA), generating 2×150-bp paired-end reads. Alignment of sequence reads, indexing of the reference genome (hg19) and variant calling with a pipeline was based on Genome Analysis Tool Kit Best Practices. Alignment was performed using BWA-MEM (version 0.7.12); duplicated reads were marked with Picard (version 1.96, http://picard.sourceforge.net); local alignment, base quality recalibration and variant calling were performed with the Genome Analysis Tool Kit, version 3.2–2; and annotation was performed with Variant Effect Predictor, dbNSFP v3.02 (Supplementary Table S1).

Variant filtering steps and data analysis

The number of candidate variants was filtered and prioritized using a four-step strategy to generate a short candidate variant list for experimental validation (Figure 1). Initially, we removed variants with $<10\times$ coverage owing to less reliable variant calls. Next, variants were limited to those with low population frequency, assuming that rare variants are more likely to cause sudden cardiac arrest than common ones. The minor allele frequency (MAF) threshold was carefully chosen based on cardiac disease prevalence (Supplementary Table S2), and variants with an MAF ⩾ 1% in the 1000 Genomes Project database,[6] the National Heart, Lung and Blood Institute (NHLBI) Exome Variant Server,[7] the Exome Aggregation Consortium (ExAC) database[8] or our in-house database of 300 exomes were removed. The third step was to prioritize variants causing nonsynonymous amino-acid changes, start codon alterations, stop loss changes, in-frame insertions/deletions, frameshifts, nonsense variants or changes affecting the consensus splice site sequences. Finally, we performed gene-specific analysis with an *in silico* gene panel composed of 100 genes including 96 channelopathy and cardiomyopathy genes[4] and the recently reported Calmodulin genes (*CALM2* and *CALM3*)[9] as the causative genes for long QT syndrome, as well as genes specifically expressed in the heart muscle tissue that are related to cardiomyopathy or channelopathy (*NPPA* and *TNNIK3*). These genes were obtained by searching previous publications and the human protein atlas (http://www.proteinatlas.org/, Supplementary Table S3).[10]

Confirmation and validation of candidate variants

Candidate variants found in WES data were confirmed using standard PCR and Sanger sequencing methods (primer sequences available upon request). Sequence data were aligned to the reference sequence with the Sequencher software (Gene Codes Corporation, Ann Arbor, MI, USA).

Criteria for evidence-based classification of candidate variants

Candidate variants were classified according to the standards and guidelines by the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP).[11] These guidelines recommend for classifying variants into five categories: pathogenic variant (PV), likely pathogenic variant (LPV), variant of uncertain significance (VUS), likely benign
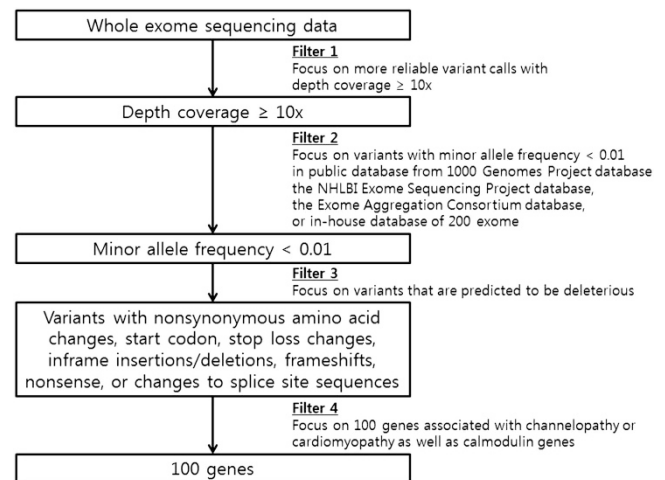
Figure 1 Workflow of whole-exome sequencing data analysis prioritizing candidate variants.

variant (BV), and BV based on the combination of many lines of weighted evidences, including population data, computational data, functional data, segregation data, *de novo* data, allelic data and others. To assess the frequency of a variant in a control or general population, we used the Korean Reference Genome Database,[8] which consists of publicly available race-matched control data from whole-genome sequencing of 622 Korean individuals, as well as other public databases such as the 1000 Genomes Project database,[6] the NHLBI Exome Variant Server and the ExAC database. A primary literature review was conducted using various sources cited in the Human Gene Mutation Database (HGMD) professional version, release 2016.1 (http://www.hgmd.org/), ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) and PubMed to determine the potential pathogenicity of all identified variants. A variety of *in silico* tools were used to assess the predicted impact of missense change, including FATHMM (http://fathmm.biocompute.org.uk), SIFT (http://sift.jcvi.org), Polyphen2 (http://genetics.bwh.harvard.edu/pph2), MutationTaster (http://www.mutation-taster.org), MutationAssessor (http://mutationassessor.org), PROVEAN (http://provean.jcvi.org/index.php) and CADD (http://cadd.gs.washington.edu), all of which use missense prediction algorithms, along with GERP (http://mendel.stanford.edu/sidowlab/downloads/gerp/index.html), PhastCons (http://compgen.bscb.cornell.edu/phast/) and PhyloP (http://compgen.bscb.cornell.edu/phast/), all of which use nucleotide-conservation prediction algorithms.

RESULTS

Clinical findings

Twenty Korean patients who survived sudden cardiac arrest were enrolled in the study. One patient was excluded because of positive findings in the spasm provocation test with ergonovine, indicating variant angina. Thus a total of 19 subjects were eligible for analysis. The demographic and clinical characteristics of our cohort are shown in Table 1. There were 16 males (84.2%) and 3 females; the average age at sudden cardiac arrest event was 34.4 ± 15.7 years (range, 8–66 years) with 32.5 ± 16.1 years (range, 8–66 years) for males and 44.7 ± 7.5 years (range, 34–50 years) for females. No patient had a contributing past medical history. One patient had chronic kidney disease (serum creatinine 1.8 mg dl^{-1}). Three had a history of hypertension. No patient had experienced a cardiac event prior to the sudden cardiac arrest. Three patients had a known family history of SCD, and two had a family history of cardiovascular disease such as acute myocardial infarction or congestive heart failure.

WES data analysis and classification of candidate variants

WES was performed in 19 sudden cardiac arrest survivors. The targeted coding sequences of the 100 genes included 89.8 Mb;

Table 1 Demographic and clinical characteristics of the 19 sudden cardiac arrest survivors

Case no.	Sex	Age at SCA, years	Smoking	Medical history	Diagnosis	Family history
SCAS-1	M	66	Ex-smoker	CKD (Cr 1.8)	Idiopathic VF	CHF—daughter
SCAS-2	M	48	Current	NS	Brugada phenocopy	None
SCAS-3	M	60	No	Hypertension	Idiopathic VF	SCA—father
SCAS-4	M	35	No	NS	Idiopathic VF	No
SCAS-5	M	18	No	NS	Long QT syndrome	SCA—father
SCAS-6	M	35	Current	NS	Idiopathic VF	None
SCAS-7	M	18	No	NS	CPVT	None
SCAS-8	M	39	No	NS	Idiopathic VF	None
SCAS-9	F	50	No	Hypertension	Idiopathic VF	None
SCAS-10	M	37	Ex-smoker	NS	Idiopathic VF	None
SCAS-11	F	50	No	NS	Idiopathic VF	None
SCAS-12	F	34	No	NS	Idiopathic VF	None
SCAS-13	M	21	Current	NS	Idiopathic VF	None
SCAS-14	M	12	No	NS	HCMP	None
SCAS-15	M	22	Current	NS	Brugada syndrome	SCA—maternal side
SCAS-16	M	8	No	NS	CPVT	None
SCAS-17	M	34	No	NS	Complete AV block	None
SCAS-18	M	33	No	NS	Complete AV block	None
SCAS-19	M	34	Ex-smoker	Hypertension	Idiopathic VF	AMI—father

Abbreviations: AMI, acute myocardiac infarction; AV, atrioventricular; CHF, congestive heart failure; CKD, chronic kidney disease; CPVT, catecholaminergic polymorphic ventricular tachycardia; F, female; HCMP, hypertrophic cardiomyopathy; M, male; NS, not significant; SCA, sudden cardiac arrest; SCAS, sudden cardiac arrest survivor; VF, ventricular fibrillation.

89.6% of the target bases were covered by ≥10 sequence reads. Detailed statistical data are given in Supplementary Table S4.

As shown in Table 2, a total of 2 675 336 single-nucleotide variants and insertion/deletions in the exome-targeted region were identified. The number of candidate variants was reduced using a four-step filtration and prioritization strategy to generate a list of candidate variants for further validation. Two patients had no candidate variants, whereas 53 candidate variants were identified in the remaining 17 patients (mean 3.1; range, 1–9). These 53 variants were classified according to the ACMG/AMP guidelines as follows: 2 PVs, 3 LPVs, 34 VUSs and 14 likely BVs. Finally, 2 PVs, 3 LPVs and 34 VUSs were confirmed via Sanger sequencing (Table 2).

The detailed information of candidate variants is listed in Table 3 according to ACMG/AMP guidelines. Five of the 19 patients (26.3%) had either a PV or LPV in *MYBPC3* (*n* = 1), *MYH7* (*n* = 1), *RYR2* (*n* = 2) and *TNNT2* (*n* = 1). All five variants were missense variants that were previously reported in patients with channelopathies or cardiomyopathies. The maximum allele frequencies from the ExAC and KRGDB databases for these variants were 0.0003 and 0.004, respectively.[12]

Furthermore, an additional 12 patients (63.2%) had more than one VUS in genes related to channelopathy and cardiomyopathy. A total of 34 VUSs were detected in 15 genes: *ANK2, CTNNA3, DSG2, DSP, MYBPC3, PKP2, PRDM16, RBM20, RYR2, SCN2B, SCN5A, TCAP, TNNI3, TNNI3K,* and *TTN.* Among these variants, one in *SCN5A* was categorized as disease-causing variant for atrial fibrillation in the HGMD while additional five were questionable variants (Table 3).

DISCUSSION

In this study, we identified cardiac disease-associated PV or LPV in 5 of the 19 sudden cardiac arrest survivors via WES focusing on 100 channelopathy- and cardiomyopathy-related genes. In one previous study of 57 families in London with a member who exhibited sudden arrhythmic death syndrome, inherited arrhythmia syndrome was detected in 53% of families via cardiogenetic screening for *KCNQ1, KCNE1, KCNH2, KCNE2, SCN5A, ANK215, KCNJ2, CAV3, CASQ2* and *RYR2.*[13] Moreover, in a gene-specific analysis of 117

channelopathy-, cardiomyopathy- or metabolic disorder susceptibility-related genes in 14 consecutively referred Caucasian autopsy-negative SUD in the young victims, 8 ultra-rare variants absent in 3 publically available exome databases were identified in 6 genes in 7 of the 14 cases (50%).[3] Furthermore, WES identified potentially lethal cardiac disease-associated variants in three of the five SUD victims in one study conducted at the Zurich Institute of Forensic Medicine in Switzerland.[2] In another investigation in the Netherlands, 17 of the 43 (40%) consecutive families with at least one SUD victim who died at ≤40 years of age had inherited cardiac disease.[14] Compared with these data, we observed a smaller proportion of inherited cardiac disease in Korean sudden cardiac arrest survivors and a lower diagnostic yield. We note that our study population consisted of survivors of sudden cardiac arrest, unlike the victims of SCD included in the abovementioned studies. The difference of study subjects might affect the results, although further studies are needed to explore this difference.

Of the five cardiac disease-associated PV or LPV identified in our cohort, two were detected in channelopathy genes and three were detected in cardiomyopathy genes. Several studies investigating cardiac channelopathy-associated genes causing lethal cardiac arrhythmia in SUD cases without any underlying structural pathology revealed that 26–53% of all cases were diagnosed with inherited cardiac channelopathies.[13,15,16] However, WES studies of cardiomyopathy- and channelopathy-related genes in autopsy-negative SUD cases revealed that 6 of the 14 studied cases harbored rare variants in cardiomyopathy-associated genes; interestingly, only 1 case exhibited a variant in a cardiac channelopathy gene, and 11 cardiomyopathy-related variants and 6 channelopathy-related variants were observed in other cases.[3,17] Autopsy or macroscopic evaluation may fail to identify areas with sufficient abnormal myocardium. Moreover, structurally faulty myocardial conditions genetically induced by cardiomyopathy genes may cause sudden death, even when insufficient to meet diagnostic criteria at a microscopic level.[13] In our cohort, two of the three patients (SCAS-1 and SCAS-2) with LPV in cardiomyopathy genes had no evidence of cardiomyopathy when evaluated on two-dimensional echocardiography and the remaining patient diagnosed with hypertrophic cardiomyopathy showed a mild

Table 2 Filtering and classification of variants in the 19 sudden cardiac arrest survivors

Variants (n)	SCAS-1	SCAS-2	SCAS-3	SCAS-4	SCAS-5	SCAS-6	SCAS-7	SCAS-8	SCAS-9	SCAS-10
Total variants from WES data	1 048 575	84 952	87 433	87 676	88 711	86 575	88 802	88 644	88 171	91 236
Step 1										
Remove variants with depth coverage <10	1 036 715	74 030	75 788	75 691	77 295	74 680	77 528	76 986	76 579	79 468
Step 2										
Select variants with MAF<0.01 in population databases	3067	2681	3040	2926	3325	3004	3318	3033	3132	3563
Step 3										
Select disease-causing variant types	360	368	417	339	370	382	370	375	357	337
Step 4										
Select variants in the 100 cardiac-associated genes	2	4	1	3	0	5	9	1	0	3
After application of the ACMG guidelines										
PV	0	0	0	0	0	0	0	0	0	0
LPV	1	1	0	0	0	0	1	0	0	0
VUS	0	0	1	3	0	4	3	1	0	3

Variants (n)	SCAS-11	SCAS-12	SCAS-13	SCAS-14	SCAS-15	SCAS-16	SCAS-17	SCAS-18	SCAS-19	Total
Total variants from WES data	89 508	89 464	88 765	95 751	94 460	93 846	96 013	93 521	93 233	2 675 336
Step 1										
Remove variants with depth coverage <10	77 985	77 135	76 616	81 031	80 610	79 903	81 988	79 653	79 258	2 438 939
Step 2										
Select variants with MAF<0.01 in the population databases	3375	3169	3252	3991	3879	3842	4148	3743	3673	64 161
Step 3										
Select disease-causing variant types	362	355	360	436	404	401	403	407	393	7196
Step 4										
Select variants for the 100 cardiac-associated genes	4	4	3	1	2	1	7	2	1	53
After application of the ACMG guidelines										
PV	0	0	0	1	0	1	0	0	0	2
LPV	0	0	0	0	0	0	0	0	0	3
VUS	4	3	3	0	1	0	5	2	1	34

Abbreviations: ACMG, American College of Medical Genetics and Genomics; LPV, likely pathogenic variant; MAF, minor allele frequency; PV, pathogenic variant; SCAS, sudden cardiac arrest survivor; VUS, variant of uncertain significance; WES, whole-exome sequencing.

hypertrophic manifestation of the cardiac septum. Therefore, minimal structural abnormalities that are deemed inconclusive or even missed completely may underlie a significant proportion of SUD cases. A study by Maron *et al.*[18] also showed that SCD may be the first manifestation of disease. Therefore, evaluation of cardiomyopathy genes should be considered in patients with a history of life-threatening sudden cardiac arrest and in the relatives of these patients, even when no evidence of cardiomyopathy is present. It is of note that SCAS-2 patient showed echocardiography patterns that were consistent with BrS at he time of sudden cardiac arrest resuscitation but was normalized at follow-up echocardiography test. This patient lacked symptoms that are characteristic to BrS such as palpitation, presyncope and syncope. No variants were identified in BrS-related genes either. Taken together, this patient might have Brugada phenocopy rather than BrS.[19]

Probands with PV or LPV in cardiomyopathy genes had no family history of cardiac disease except one daughter of SCAS-1 patient who was diagnosed as congestive heart failure. Although periodic review of family history is required, confirming cardiac disease in relatives can be complicated by many factors, including failure to undergo appropriate cardiac screening, reduced penetrance, early death from other causes before the onset of HCM and/or other social issues, and thus it may not be possible to confirm whether a proband with HCM is truly a simplex case or not (https://www.ncbi.nlm.nih.gov/books/NBK1768/). In addition, a PV or LPV in a proband with HCM may occur *de novo*. According to the study by Morita *et al.*,[20] the proportion of cases caused by *de novo* PV was unknown but could be estimated to be 30%, which were detected most commonly in *MYH7* and *MYBPC3*.

Table 3 Variants in the 19 sudden cardiac arrest survivors classified according to the 2015 American College of Medical Genetics and Genomics and the Association for Molecular Pathology guidelines

Case no.	Gene	Reference sequence	Nucleotide change	Protein change	HGMD phenotype	HGMD variant class	ACMG classification	MAF (ExAC)	MAF (KRGDB)
SCAS-1	**MYH7**	NM_000257.2	**c.4130C>T**	**p.Thr1377Met**	**HCM**	**DM**	**Likely pathogenic variant**	**0.00000**	**0.00000**
SCAS-2	**MYBPC3**	NM_000256.3	**c.1000G>A**	**p.Glu334Lys**	**HCM**	**DM**	**Likely pathogenic variant**	**0.00030**	**0.00400**
SCAS-3	TTN	NM_133378.4	c.60256G>C	p.Asp20086His	—	—	VUS	0.00004	0.00000
SCAS-4	DSG2	NM_001943.3	c.166G>A	p.Val56Met	ARVC	DM?	VUS	0.00187	0.00160
	PKP2	NM_004572.3	c.2150C>T	p.Pro717Leu	ARVC	DM?	VUS	0.00013	0.00720
	PKP2	NM_004572.3	c.1546_1584del	p.Lys516_Thr528del	—	—	VUS	0.00000	0.00000
SCAS-5				Not detected					
SCAS-6	ANK2	NM_001148.4	c.9689C>T	p.Thr3230Met	—	—	VUS	0.00001	0.00000
	CTNNA3	NM_013266.2	c.1853A>G	p.His618Arg	—	—	VUS	0.00002	0.00080
	SCN2B	NM_004588.4	c.250C>T	p.Arg84Cys	—	—	VUS	0.00005	0.00000
	TCAP	NM_003673.3	c.145G>A	p.Glu49Lys	DCM	DM?	VUS	0.00008	0.00160
SCAS-7	DSP	NM_004415.2	c.4943A>G	p.Gln1648Arg	—	—	VUS	0.00016	0.00000
	RYR2	NM_001035.2	**c.14311G>A**	**p.Val4771Ile**	**PVT**	**DM**	**Likely pathogenic variant**	**0.00000**	**0.00000**
	TTN	NM_133378.4	c.4874C>G	p.Ser1625Cys	—	—	VUS	0.00004	0.00080
	TTN	NM_133378.4	c.53585G>A	p.Cys17862Tyr	—	—	VUS	0.00006	0.00080
SCAS-8	CTNNA3	NM_013266.2	c.2260A>C	p.Asn754His	—	—	VUS	0.00000	0.00000
SCAS-9				Not detected					
SCAS-10	CTNNA3	NM_013266.2	c.1850T>C	p.Ile617Thr	—	—	VUS	0.00026	0.00560
	RBM20	NM_001134363.1	c.2089G>A	p.Gly697Arg	—	—	VUS	0.00066	0.00080
	TTN	NM_133378.4	c.84049T>G	p.Phe28017Val	—	—	VUS	0.00000	0.00000
SCAS-11	MYBPC3	NM_000256.3	c.1519G>A	p.Gly507Arg	HCM	DM?	VUS	0.00068	0.00400
	PRDM16	NM_022114.3	c.3461A>C	p.Glu1154Ala	—	—	VUS	0.00010	0.00560
	RYR2	NM_001035.2	c.12334G>A	p.Asp4112Asn	—	—	VUS	0.00000	0.00000
	TTN	NM_133378.4	c.43675G>C	p.Val14559Leu	—	—	VUS	0.00000	0.00080
SCAS-12	TNNI3	NM_000363.4	c.235C>T	p.Arg79Cys	—	—	VUS	0.00039	0.00400
	TTN	NM_133378.4	c.8069C>T	p.Thr2690Ile	—	—	VUS	0.00059	0.00480
	TTN	NM_133378.4	c.22100A>G	p.Asn7367Ser	—	—	VUS	0.00002	0.00000
SCAS-13	RYR2	NM_001035.2	c.4840C>T	p.Arg1614Cys	—	—	VUS	0.00005	0.00000
	TTN	NM_133378.4	c.27403G>A	p.Glu9135Lys	—	—	VUS	0.00000	0.00160
	TTN	NM_133378.4	c.89405C>A	p.Thr29802Asn	—	—	VUS	0.00005	0.00160
SCAS-14	**TNNT2**	**NM_001001430.2**	**c.274C>T**	**p.Arg92Trp**	**HCM**	**DM**	**Pathogenic variant**	**0.00001**	**0.00000**
SCAS-15	TTN	NM_133378.4	c.81041C>T	p.Ser27014Phe	—	—	VUS	0.00000	0.00000
SCAS-16	**RYR2**	NM_001035.2	**c.6737C>T**	**p.Ser2246Leu**	**PVT**	**DM**	**Pathogenic variant**	**0.00000**	**0.00000**
SCAS-17	PKP2	NM_004572.3	c.2150C>T	p.Pro717Leu	ARVC	DM?	VUS	0.00013	0.00720
	TTN	NM_133378.4	c.34052A>G	p.Asp11351Gly	—	—	VUS	0.00000	0.00000
	TTN	NM_133378.4	c.37246G>C	p.Gly12416Arg	—	—	VUS	0.00004	0.00000
	TTN	NM_133378.4	c.47690C>A	p.Ala15897Glu	—	—	VUS	0.00000	0.00000
	TNNI3K	NM_015978.2	c.2225_2227delCTT	p.Ser745del	—	—	VUS	0.00026	0.00400
SCAS-18	TTN	NM_133378.4	c.19712G>A	p.Arg6571Gln	—	—	VUS	0.00002	0.00080
	TTN	NM_133378.4	c.48691G>A	p.Ala16231Thr	—	—	VUS	0.00003	0.00080
SCAS-19	SCN5A	NM_198056.2	c.5851G>A	p.Val1951Met	AF	DM	VUS	0.00008	0.00800

Abbreviations: ACMG, American College of Medical Genetics and Genomics; AF, atrial fibrillation; ARVC, arrhythmogenic right ventricular cardiomyopathy; DCM, dilated cardiomyopathy; DM, disease-causing mutation; ExAC, Exome Aggregation Consortium; HCM, hypertrophic cardiomyopathy; HGMD, human gene mutation database; KRGDB, Korean Reference Genome Database; MAF, minor allele frequency; PVT, polymorphic ventricular tachycardia; VUS, variant of uncertain significance. Rows in bold are pathogenic or likely pathogenic variants.

It is also possible that the same PV that is shared by unrelated hypertrophic cardiomyopathy individuals might be due to independent occurrence of *de novo* variants.[21]

Perturbation of cardiac ion channels, which are critical in cardiac electrophysiology, can result in SCD owing to fatal ventricular arrhythmias.[22] The Heart Rhythm Society/European Heart Rhythm Association guidelines state that 'in the setting of autopsy-negative SUDs, comprehensive or targeted (*RYR2*, *KCNQ1*, *KCNH2*, and *SCN5A*) ion channel genetic testing may be considered in an attempt to establish probable cause and manner of death and to facilitate the identification of potentially at-risk relatives and is recommended if circumstantial evidence points toward a clinical diagnosis of long QT syndrome or catecholaminergic polymorphic ventricular tachycardia specifically'.[23] Compared with the role of cardiac channelopathy-associated genes in Caucasian SUD cases, *KCNQ1* and *KCNH2* seem to be very rare causative genes in Korean patients with sudden cardiac arrest.[13,16,17] Similarly, a previous study of Korean survivors of sudden cardiac arrest revealed no pathogenic variants in *KCNQ1* or *KCNH2*.

Interestingly, *MYBPC3*-Glu334Lys variant detected in SCAS-2 patient has an MAF of 0.004 in KRGDB. Although this MAF seems to be high compared with estimated disease prevalence, the penetrance of *MYBPC3* variants is known to be reduced.[24] According to the recently published studies, MAF of some variants that were previously reported to be pathogenic in the HGMD and/or ClinVar might be >1% either globally or in ethnicity-matched controls.[25,26] Although some of this excess can be attributed to BVs falsely assigned as pathogenic, other variants have genuine effects on disease susceptibility but confer reduced lifetime risks or penetrance. Therefore, it is no wonder that a variant with relatively high MAF than expected could be the cause of genetic disease owing to the reduced penetrance.

Most of the filtered variants from the WES data were categorized as VUSs according to the ACMG/AMP guidelines because they lacked sufficient evidences to demonstrate pathogenicity (such as *de novo* data, segregation data or functional data) as they were novel variants or had not been evaluated further. Although they exhibited very low MAF in the various population databases, their disease associations have not yet been conclusively determined.

Of note, this is the first study that used WES to investigate cardiac-specific genes associated with channelopathy or cardiomyopathy in Korean sudden cardiac arrest survivors. Using WES, we found that 5 of the 19 Korean sudden cardiac arrest survivors had PV or LPV in four genes, *MYBPC3*, *MYH7*, *RYR2* and *TNNT2*. Korean sudden cardiac arrest survivors appear to exhibit a smaller proportion of inherited cardiac disease and a lower diagnostic yield compared with subjects in other studies. However, this study has some limitations in that only survivors were investigated. According to 2015 National Emergency Department Information System for Cardiac Arrest data of Korea, 9.6% patients survived to discharge among resuscitation-attempted out-of-hospital cardiac arrests.[27] We also conclude that cardiomyopathy may be a significant cause of sudden cardiac arrest even in the absence of clinical findings, because channelopathy- and cardiomyopathy-associated genes were detected at similar proportions. However, further studies with larger Korean cohorts experiencing sudden cardiac arrest are needed to confirm the detection rate of these pathogenic variants and to definitively determine the causative cardiac-associated genes in Korean populations. Nevertheless, the results of this study would be useful for implementing cardiogenetic screening in the general Korean population.

CONFLICT OF INTEREST

The authors declare no conflict of Interest.

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