Investigation on susceptible variants to arrhythmia in forensic autopsy cases of SCDs caused by cardiomyopathy: assessment with the candidate SNPs

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Abstract

Background: Fatal arrhythmia is a common cause of sudden cardiac death (SCD) in patients with cardiomyopathies. Nowadays, associations of many genetic variants with cardiomyopathy have gradually been highlighted, however, whether the arrhythmia-related single nucleotide polymorphisms (SNPs) are associated with SCDs caused by cardiomyopathies is elusive. To identify the genetic susceptible factors in cardiomyopathy, a retrospective study was conducted from the autopsy cases. **Methods:** The present study was composed of 223 forensic autopsy cases including 51 SCDs caused by cardiomyopathies and 172 controls. The cardiomyopathy cases were made up of arrhythmogenic right ventricular cardiomyopathy (ARVC, $n = 27$) and Non-ARVC [$n = 24$, including hypertrophic cardiomyopathy, dilated cardiomyopathy, restrictive cardiomyopathy, and unclassified cardiomyopathy]. Four arrhythmia-related candidate SNPs: rs4687718, rs7366407, rs4292933, and rs2824292 were genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. Association between genotypes and the risk of SCD was assessed by stratification of the cardiomyopathy (ARVC cases vs. Non-ARVC cases). **Results:** The A-allele and dominant model (AA + AG vs. GG) of rs4687718 on transketolase gene (TKT) were significantly associated with cardiomyopathies (ORA allele = 2.481, 95% CIA allele: 1.262 - 4.876, PA allele = 0.007; ORAA+AG genotype = 2.580, 95% CIAA+AG genotype: 1.206 - 5.519, PAA+AG genotype value = 0.012). After stratification by cardiomyopathy subtypes, the allelic and genotypic frequencies of rs4687718 in Non-ARVC group were significantly different compared to controls, and between ARVC group and controls the difference was only duplicated in genotypic frequency. In the light of analyses of the different genetic models, however, there were no significant differences between Non-ARVC group and controls, but the AA + AG vs. GG model was associated with ARVC group. No significant differences were detected for other three SNPs. **Conclusion:** Our results revealed an association between TKT rs4687718 and SCDs caused by cardiomyopathy in Chinese autopsy cases. These findings highlighted the important role of molecular testing and may play in propensity toward in clinical prediction and forensic identification of cardiomyopathy SCDs. It also provided the basis for the classification of different types of cardiomyopathy at the genetic level.

Background

Sudden cardiac death (SCD) is a sudden, unexpected death caused by loss of cardiac function [1]. In China, the incidence of SCD is 41.8/100,000, while the total number is estimated as 544,000 per year [2]. Two-third of the SCD cases have been reported to have structural abnormalities of the heart, and cardiomyopathy was the one of the major components [3, 4]. Cardiomyopathy is defined as a myocardial disorder in which the heart muscle is structurally and functionally abnormal, in the absence of coronary artery disease, hypertension, valvular disease and congenital heart disease sufficient to cause the observed myocardial abnormality [5]. It can be classified as hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), arrhythmogenic right ventricular cardiomyopathy (ARVC), restrictive cardiomyopathy (RCM), and nonclassifiable cardiomyopathy [6].

Heart failure (HF) is the direct cause of death for cardiomyopathy in clinical, while in the practice of forensic medicine, fatal arrhythmia is the first to be responsible for the SCD [7, 8]. Because of the different classifications, the mechanisms of SCD in cardiomyopathies are not the same, but three theories including abnormal energy metabolism of cardiomyocytes, dysfunction of ion channels and viral infection are widely accepted [9-11]. As the highlighting of genetic contribution to cardiomyopathy, thousands to millions of candidate single nucleotide polymorphisms (SNPs) in genome-wide association studies (GWAS) have been reported to be associated with cardiomyopathy SCDs, in genes encoding several categories of proteins, including those of sarcomeres, desmosomes, cytoskeleton, and nuclear envelope [12, 13]. These results not only contribute to the clinical diagnosis and treatment of cardiomyopathy, and also expect to make some breakthrough in the current status of forensic cardiomyopathy identification, which mainly based on the different pathological features under the premise of excluding other causes of death [14]. Although many facts have suggested that there must have some consequent relationship between cardiomyopathy and arrhythmias, arrhythmia-related variants have never been explored in patients with cardiomyopathy. Therefore, we chose candidate SNPs of four genes based on the mechanism of sudden death of cardiomyopathy, and conducted a systematic genetic association investigation in case-control study of SCD with cardiomyopathy.

Methods

Subjects and sample collection

The study protocol was approved by the ethics committee of China Medical University (no. [2017]062). A total of 223 forensic autopsy cases from Department of Forensic Pathology, School of Forensic Medicine of China Medical University were enrolled, including 51 SCD cases caused by cardiomyopathies and 172 controls. The cardiomyopathy cases were made up of ARVC ($n = 27$) and Non-ARVC [$n = 24$, including HCM ($n = 13$), DCM ($n = 5$), RCM ($n = 2$), and unclassified cardiomyopathy ($n = 4$)] in this study. The causes of death were determined on the basis of a comprehensive medico-legal investigation, including autopsy examination and histological, toxicological and biochemical analyses.

Worldwide, pathological features of heart are the most important and primary basis for forensic identification and classification of cardiomyopathies, and related literatures can also provide complementary supports. The key points of different types of cardiomyopathies used commonly in China are as follows: ARVC [15], according to Protonotarios and Basso's diagnostic criteria, severely dilated right ventricle involving advanced myocardial atrophy with fibrofatty replacement in the right ventricular free wall, and not accompanied by lesion of conducting system and coronary arteries; HCM, a) increased thickness of inter ventricular septum and left ventricular wall, the ratio of interventricular septum to left ventricular wall is more than 1.3. b) increased weight of heart, often exceeds 500 g. c) incrassated endocardium and white plaque forms; DCM, increased heart volume, weight, and four chambers of the heart, ventricular cavity to expand more apparent; RCM, thickened left ventricular posterior wall and interventricular septum, decreased ventricular cavity, and increased subendocardial myocardial interstitial fibrous tissue; unclassified cardiomyopathy, arrhythmias and cardiac conduction disorders were the main manifestations. For the control group, 172 victims with non-related cardiac causes of death, including traffic accidents and high falls, were selected. All of the control victims were without any known cardiovascular disease, according to the anatomical and pathological findings, postmortem biochemical detection results (including NT-pro brain natriuretic peptide, troponin T, troponin I, and creatine kinase-MB), and clinical records (if provided).

The basic conditions of gender, age, and heart weight of the cardiomyopathy and control groups were well documented. The results were blind evaluated by three pathologists. Samples of 35 cases with cardiomyopathy were obtained from formalin-fixed paraffin-embedded (FFPE) tissue, and those of the remaining 16 cases with cardiomyopathy and all controls were obtained from body fluids, including freeze-thaw blood, urine and pericardial fluid.

Genomic DNA isolation and genotyping

The genomic DNA was extracted by the sodium dodecyl sulfate-protease K-phenol-chloroform method. The freeze-thaw blood and FFPE samples were pretreated before DNA extraction. In brief, for freeze-thaw blood, centrifuged (10000 rpm, 10 min) to collect the underlying sediments. While as for FFPE samples, 20 μ m thick sections of FFPE tissue blocks were collected into sterile microtubes. Removed the paraffin by adding 1 mL xylene in 56°C for 30 min followed by vortex and centrifugation (10000 rpm, 10 min), the supernatant was removed with a sterile transfer pipette. This step was repeated. After removing the xylene, the samples were washed twice with pure ethanol, and air dried.

The primers and restriction endonucleases used for polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) were based on the published sequences in GenBank. Considering that there were some FFPE tissue samples have been stored for several years, combined with the results of the preliminary experiments, the length of all amplified products were controlled within 140 bp. The primers used for SNPs detection, restriction endonucleases used for genotyping and amplified products are shown in Table 1.

PCR-RFLP technique was used to identify the SNPs. PCR was conducted in a final volume of 20 μ L PCR buffer containing 2 μ L genomic DNA (approximately 20 - 50 ng/ μ L), 10 μ L 2 $\times$ PowerTaq PCR StarMix with Loading Dye (GenStar, Beijing, China), 0.5 μ L forward and reverse primers and 7 μ L ddH₂O. PCR was carried out on a PCR Thermal Cycler Dice (Takara, Japan) with initial denaturation at 94°C for 1 min, followed by 35 cycles of denaturing for 15 s at 94°C, annealing for 30 s (55°C for rs4687718 and rs4292933; 60°C for rs7366407 and rs2824292), extension at 72°C for 20 s; and final extension at 72°C for 1 min. For restriction enzyme digestion, 1 μ L PCR product and 0.25 μ L restriction endonuclease (Takara, Dalian, China) were mixed in 10 μ L corresponding buffer. PCR products were incubated at 37°C for more than 1 h and the digestion products were separated by 10% polyacrylamide gel electrophoresis. Fragments were visualized by the Molecular Imager Gel DocXR+System (Bio-Rad, Hercules, CA, USA) after ethidium bromide staining.

Statistical analysis

The Hardy-Weinberg equilibrium (HWE) test for frequency distribution was calculated using PowerMarker version 3.0. Results were analysed using the SPSS version 19.0 software. The differences among groups were assessed using one-way analysis of variance (ANOVA) and Chi-square/Fisher's exact tests. A two-tailed P -value ≤ 0.05 was considered significant. Odds ratios (ORs), with their corresponding 95% confidence intervals (CIs), were used to evaluate the strength of the association between the polymorphisms and cardiomyopathy risk.

Results

Case profiles

The demographics and heart weights of each group were shown in Table 2. Gender compositions and age distributions between the cardiomyopathies and controls did not show statistically significant differences ($P_{\text{gender}} = 0.539$; $P_{\text{age}} = 0.304$). The weights of hearts in cardiomyopathy group were heavier than the controls ($P < 0.001$). It is worth to be explained that in the control group, a small number of decedents had a more than 500 g heart, however, they were not diagnosed as cardiomyopathy after pathological anatomy and biochemical examination, and therefore those were not eliminated in this study.

Genotyping the four SNPs

The mismatched PCR amplifications were first performed, and sequences containing the *Hind* III, *Nco* I and *Afa* I recognition sites were confirmed by DNA sequencing. Subsequently, genotypes in the four loci were examined by PCR-RFLP method in all samples. The results showed that the mismatched PCR-RFLP assays were successful and genotypes in the four SNPs were determined in all investigated samples.

Genetic association of four SNPs with cardiomyopathy

The distributions of genotypic and allelic frequencies of four SNPs in cardiomyopathy cases and controls are summarized in Table 3. The actual HWE P values of rs4687718, rs7366407, rs4292933, and rs2824292 in control group was 0.172, 0.649, 0.724, and 0.661, respectively. The genotypic and allelic frequencies of rs4687718 were significantly different between cardiomyopathy cases and controls ($P_{\text{genotype}} = 0.038$; $OR_{A \text{ allele}} = 2.481$, 95% $CI_{A \text{ allele}}$: 1.262 - 4.876, $P_{A \text{ allele}} = 0.007$). Association analyses of rs7366407, rs4292933, and rs2824292 showed that there were no significant differences between cardiomyopathy cases and controls. The difference in genotypes of rs4687718 locus between cardiomyopathy cases and controls was significant in the dominant model of AA + AG vs. GG ($OR = 2.580$, 95% CI : 1.206 - 5.519, $P = 0.012$), but not in the other three models (P values: 0.186, 0.634, and 0.225, respectively) (Table 4).

Genetic association of four SNPs by stratification of the cardiomyopathy

We analyzed the ARVC case as a specific type of cardiomyopathy, and the other four types of cardiomyopathies (including HCM, DCM, RCM and unclassified cardiomyopathy) were collectively referred to Non-ARVC group. There were no significant difference in genotypic and allelic frequencies of four SNPs between ARVC cases and Non-ARVC cases. In order to figure out the source of differences between the cardiomyopathy group and the controls, we also compared ARVC and Non-ARVC cases to the controls, respectively. After stratification, the genotypic and allelic frequencies of rs4687718 were significant different between the Non-ARVC cases and controls ($P_{\text{genotype}} = 0.047$; $OR_{A \text{ allele}} = 2.667$, 95% $CI_{A \text{ allele}}$: 1.123 - 6.334, $P_{A \text{ allele}} = 0.044$). The genotypic frequency of

rs4687718 was significant different between ARVC cases and controls ($P_{\text{genotype}} = 0.039$), but not in allelic frequency. When analyzed by different genetic models, the compared data between the two groups were quite impressive. The results showed that the dominant model (AA + AG vs. GG) was significant associated with ARVC cases (OR = 2.871, 95% CI: 1.222 - 7.345, $P = 0.047$), while no differences were confirmed in all four models in Non-ARVC group. Association analyses of rs7366407, rs4292933, and rs2824292 showed that there were no significant differences in genotypes and alleles between ARVC cases and controls, as well as Non-ARVCs and controls.

Discussion

Based on the relationship between arrhythmias and SCD, several arrhythmia-related mutations in multiple genes have been identified in a variety of cardiovascular diseases, including coronary heart disease, cardiac channelopathies, and sudden unexplained nocturnal death syndrome [11, 16-18]. In the current study, we selected four SNPs, which have been proved associated with arrhythmias under GWAS's profile, to evaluate their role as predisposing factors for cardiomyopathy SCDs in forensic autopsy cases. An association between the rs4687718 on transketolase gene (*TKT*) and cardiomyopathy SCDs was found. However, our results didn't show significant differences in the genotypic and allelic frequencies of rs7366407, rs4292933, and rs2824292 between the cardiomyopathy cases and controls.

Transketolase is a reversible, thiamine-dependent enzyme in the pentose phosphate pathway, and associated with energy metabolism and the regulation of cardiac contraction [19, 20]. Mutations in *TKT* could cause a syndrome which performance for congenital heart defects, short stature, and developmental delay [21]. Researchers have also shown that rs4687718 located on the intron 5 of *TKT* was associated with QRS interval of ECG, and could increase the risk of SCD in European-ancestry populations [22]. In our study, the results for the rs4687718 showed statistically significant difference between cardiomyopathy cases and controls, revealing the potential relationship between arrhythmias and cardiomyopathies. The results for the genetic model demonstrated that the A-allele of rs4687718 and dominant models (AA + AG vs. GG) played a strong risk role in cardiomyopathies in northern Chinese population. Interestingly, genetic studies of rs4687718 on the *TKT* with cardiac function are usually conducted in conjunction with calcium voltage-gated channel subunit alpha 1D gene (*CACNA1D*) [22]. *CACNA1D* encode the $\alpha 1$ subunits of cardiac L-type calcium channels (LTCCs), and many mutations found in *CACNA1D* were associated with ventricular arrhythmias [23]. It was observed that markers located at a physical distance of less than 10 Mb on the same chromosome were easily LD. The linkage location implies that rs4687718 on *TKT* is a mirror of some unknown associated SNPs on *CACNA1D*, which may form functional haplotype and merits further evaluation.

Numerous investigations have shown that ions in the myocardium, especially calcium (Ca^{2+}) ions, are closely related to the occurrence of arrhythmias [17, 24]. Given the expanding body of literature linking alterations in Ca^{2+} -handling with myocardial remodeling, investigators have begun to explore whether mutations in genes encoding Ca^{2+} -handling proteins might be associated with cardiomyopathies [25]. Calsequestrin 2 gene (*CASQ2*) encodes the intra-sarcoplasmic reticulum Ca^{2+} binding protein cardiac calsequestrin, and nitric oxide synthase 1 adaptor protein gene (*NOS1AP*) is also involved in LTCCs by encoding a cytosolic protein that binds to the neuronal nitric oxide synthase (nNOS) [26]. It has been demonstrated by independent groups and in different populations that allelic variants in *CASQ2* were associated with ventricular tachycardia, HF and SCD [27-29]. Mutations of the *NOS1AP* are strongly associated with abnormalities of the QT interval of the ECG, and some SNPs on the gene are also associated with the risk of SCD [26, 30-33]. Researchers have found the rs7366407 near *CASQ2* and rs4292933 in *NOS1AP* were associated with SCD in European, however it has not been verified in Chinese cardiomyopathy patients [26, 34, 35]. Though these two SNPs did not result in a statistically significant association with cardiomyopathy in the present study, it was also the first to investigate the polymorphism's influence on cardiomyopathy patients from the Chinese mainland.

A recent GWAS for SCD in the arrhythmia genetics in the Netherlands population has led to the attention of a SNP rs2824292 might be in arrhythmia susceptibility [36]. The rs2824292 locates on the vicinity of the *CXADR* gene in chromosome 21q21, and it is in complete LD with rs2824293 [37]. *CXADR* encodes the coxsackie and adenovirus receptor (CAR), which plays a role in the electrical conduction of the heart [38, 39]. Although the present result didn't show significant difference between cardiomyopathy cases and controls, considering the arrhythmia susceptibility in chromosome 21q21, this *CXADR* still deserves further study [36].

To better explore the association between arrhythmias and cardiomyopathy SCD, we set ARVC as an independent subgroup. ARVC is a familial cardiac disease characterized by ventricular arrhythmias and SCD, and it is the most common cause of SCD in young people [40]. As the sudden death is often the first symptom of this disease, it is urgent to explore the cause and mechanism of the disease in clinic. On the other hand, patients who die of ARVC usually only show nonspecific myocardial atrophy with fibrofatty replacement in the right ventricular in autopsy. Thus, SNPs in the candidate genes is likely to be an auxiliary indicator for both clinical diagnosis and forensic identification. Until now, associations of many genes with ARVC have been demonstrated, in which the structural proteins of desmosome occupied a significantly higher proportion [41]. However, to our knowledge, there were few reports on the association of arrhythmia-related mutations with ARVC, particularly in these four genes of autopsy cases. Our results showed that the A-allele of rs4687718 was significantly associated with Non-ARVC cases but not with ARVC cases. Besides, compared to controls, although rs4687718 on *TKT* showed different genotype frequencies in both ARVC and Non-ARVC cases, but the dominant model (AA + AG vs. GG) was significant associated with ARVC, while not with Non-ARVC. The results from the genetic models by stratification suggested that the dominant model plays a major role in the differences of genotypes in ARVC cases. It provided some evidences for the genetic testing as a potential method to both clinical prevention and forensic identification of SCD caused by ARVC. Furthermore, the differences in allelic and genotypic frequencies between ARVC and controls as well as Non-ARVC and controls also suggested that investigation on more susceptible genes in more samples may be helpful for identify different types of cardiomyopathy at the genetic level.

In summary, some genetic variants associated with arrhythmias may be also associated with cardiomyopathy SCDs. These findings highlight the important role of molecular testing and may play in propensity toward in clinical prevention and forensic identification of cardiomyopathy. To clinical, associations between particular SNPs and the SCDs caused by cardiomyopathy provide us unparalleled opportunities to find key susceptible and pathogenic genes, which can also improve the prediction of risk of adverse events in patients. On the other hand, the discovery of high-risk SNPs provides a new direction for forensic

identification of SCDs caused by cardiomyopathy, especially by ARVC. Therefore, if these findings are replicated in other populations, variants in these genes may provide more evidences for forensic identification and clinical prediction of SCDs with cardiomyopathies.

The size of the cardiomyopathy cases in the present study was a considerable limitation. As we all know, the inclusion of more samples makes more meaningful for genetic analysis. In consideration of this point, we prudently enrolled all cases of SCD with cardiomyopathy from our previous autopsies and screened at the same time control samples from the cases without cardiomyopathy as many as possible, to increase the cogency of the data. It is valuable that further studies of cardiomyopathy will be conducted in larger samples.

Conclusions

Overall, this is the first to investigate the association between cardiomyopathy SCDs and arrhythmia-related mutations in Chinese autopsy cases. These finding revealed that rs4687718 on *TKT* may be associated with cardiomyopathy. The detection of genes might be a new indicator for the forensic identification of cardiomyopathy. Further study in other cardiomyopathy-associated SNPs and in other populations may obtain more important insights into the identification to cardiomyopathy in forensic expertise.

Abbreviations

AIC: Arrhythmia-induced cardiomyopathy; ANOVA: One-way analysis of variance; ARVC: Arrhythmogenic right ventricular cardiomyopathy; *CACNA1D*: Calcium voltage-gated channel subunit alpha 1D gene; CAR: Coxsackie and adenovirus receptor; *CASQ2*: Calsequestrin 2 gene; CIs: Confidence intervals; DCM: Dilated cardiomyopathy; FFPE: Formalin-fixed paraffin-embedded; GWAS: Genome-wide association studies; HCM: Hypertrophic cardiomyopathy; HF: Heart failure; HWE: Hardy-Weinberg equilibrium; LD: linkage disequilibrium; LTCCs: L-type calcium channels; nNOS: Neuronal nitric oxide synthase; *NOS1AP*: Nitric oxide synthase 1 adaptor protein gene; OR: Odds ratio; PCR-RFLP: Polymerase chain reaction-restriction fragment length polymorphism; RCM: Restrictive cardiomyopathy; SCD: Sudden cardiac death; *TKT*: Transketolase gene.

Declarations

Ethics approval and consent to participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Consent for publication

Not applicable

Availability of data and materials

The dataset analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no conflict of interest.

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Authors' contributions

MHT&HP set up the study design and interpreted the data. MHT, MMZ, ZXH, YQJ &HP performed the statistical analyses, interpreted the data and drafted the manuscript. BLZ, HP&YQJ revised the manuscript critically and provided continuous guidance throughout the study. MHT, YQJ,SYZ, XNZ &XRY collecting the data and do the experiments. All authors read and approved the final manuscript.

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Tables

Table 1 Characteristics of four SNPs in the investigated genes and information used for PCR-RFLP genotyping

SNP	Gene	Nucleotide changes	Primer direction	Sequence (5'→3')	Fragment size (no. bp)	Restriction endonuclease
rs4687718	<i>TKT</i>	G>A	Forward Reverse	CGGCCTGCCAGCAGCAGAGCTAGGATAAG GTGCATTTTGTTCATCCATTCACaAG*	74 (28, 48)	<i>Hind</i> III
rs7366407	<i>CASQ2</i>	T>A	Forward Reverse	TTTTCTTGCTCCCATTCTGTTATTG AAAGGCTCTGGCTGTTGAATGGTAT	130 (58, 72)	<i>Mbo</i> I
rs4292933	<i>NOS1AP</i>	A>G	Forward Reverse	GAGCCTCA GTCCTGTAGC TGCCG GGCTGTGTTCCCTTTCTGAAGCcCA*	93 (26, 67)	<i>Nco</i> I
rs2824292	<i>CXADR</i>	G>A	Forward Reverse	CCTCCTACCTGCTGAAGCTGGAGACC CTGTGCACCTGTTCTTGCCTGgAT*	94 (24, 70)	<i>Afa</i> I

bp, base pairs; SNP, Single nucleotide polymorphism.

* Small letters show the mismatched bases used to artificially introduce endonuclease sites.

Table 2 General profiles in the case and control groups

Cause of death	N	% Male	Age (year)		Heart weight (g)	
			Mean (SD)	Range	Mean (SD)	Range
Cardiomyopathy	51	72.55%	46.00 (17.08)	20-87	471.17 (168.04)	229-900
ARVC	27	59.26%	44.26 (14.00)	23-71	369.70 (96.44)	229-567
Non-ARVC	24	87.50%	42.62 (20.29)	19-80	594.00 (154.50)	340-900
Control	172	68.02%	46.97 (18.27)	20-87	351.31 (82.89)	126-590
Total	223	69.06%	48.16 (15.51)	19-87	378.08 (118.43)	126-900

ARVC, arrhythmogenic right ventricular cardiomyopathy; Non-ARVC, including hypertrophic cardiomyopathy, dilated cardiomyopathy, restrictive cardiomyopathy and unclassified cardiomyopathy

Table 3 Distribution of genotypic and allelic frequencies of four SNPs in cardiomyopathy cases and controls

SNP (Gene)	Variants	Cardiomyopathy (%)	ARVC (%)	Non- ARVC (%)	Control (%)	Cardiomyopathy vs. Control			ARVC vs. Control			Non-ARVC vs. Control		
						P_1 value	OR_1 (95% CI)	$Power_1$	P_2 value	OR_2 (95%CI)	$Power_2$	P_3 value	OR_3 (95%CI)	$Power_3$
rs4687718 (TKT)	A	16 (0.157)	8 (0.148)	8 (0.167)	24 (0.07)	0.007*	2.481 (1.262 - 4.876)	0.734	0.089	2.319 (0.983 - 5.468)	0.195	0.044*	2.667 (1.123 - 6.334)	0.634
	G	86 (0.843)	46 (0.852)	40 (0.833)	320 (0.930)									
	AA	2 (0.040)	0 (0)	2 (0.083)	2 (0.012)	0.038*	NA	NA	0.039*	NA	NA	0.047*	NA	NA
	AG	12 (0.235)	8 (0.296)	4 (0.167)	20 (0.116)									
	GG	37 (0.725)	19 (0.704)	18 (0.75)	150 (0.872)									
rs7366407 (CASQ2)	A	39 (0.38)	19 (0.352)	20 (0.417)	122 (0.355)	0.609	1.126 (0.714 - 1.778)	0.082	0.968	0.988 (0.542 - 1.807)	0.050	0.402	1.300 (0.703 - 2.404)	0.139
	T	63 (0.62)	35 (0.648)	28 (0.583)	222 (0.645)									
	AA	7 (0.137)	3 (0.111)	4 (0.167)	23 (0.134)	0.793	NA	NA	0.910	NA	NA	0.688	NA	NA
	AT	25 (0.490)	13 (0.482)	12 (0.5)	76 (0.442)									
	TT	19 (0.373)	11 (0.407)	8 (0.333)	73 (0.424)									
rs4292933 (NOS1AP)	A	61 (0.598)	31 (0.574)	30 (0.625)	221 (0.642)	0.414	0.828 (0.526 - 1.303)	0.134	0.332	0.750 (0.419 - 1.343)	0.168	0.814	0.928 (0.497 - 1.732)	0.058
	G	41 (0.402)	23 (0.426)	18 (0.375)	123 (0.358)									
	AA	14 (0.275)	7 (0.259)	7 (0.292)	70 (0.407)	0.087	NA	NA	0.282	NA	NA	0.169	NA	NA
	AG	33 (0.647)	17 (0.630)	16 (0.667)	81 (0.471)									
	GG	4 (0.078)	3 (0.111)	1 (0.042)	21 (0.122)									
rs2824292 (CXADR)	A	45 (0.441)	21 (0.389)	24 (0.5)	147 (0.427)	0.804	1.058 (0.678 - 1.652)	0.057	0.595	0.853 (0.474 - 1.534)	0.080	0.342	1.340 (0.732 - 2.454)	0.160
	G	57 (0.559)	33 (0.611)	24 (0.5)	197 (0.573)									
	AA	6 (0.118)	2 (0.074)	4 (0.167)	30 (0.174)	0.204	NA	NA	0.335	NA	NA	0.261	NA	NA
	AG	33 (0.647)	17 (0.630)	16 (0.667)	87 (0.506)									
	GG	12 (0.235)	8 (0.296)	4 (0.167)	55 (0.320)									

* significant differences.

P value, genotypic frequencies between cardiomyopathy cases and controls; OR, odds ratio; CI, confidence interval.

Table 4 Analysis of genotype distributions for rs4687718 between cardiomyopathy and control groups under four genetic models

Genetic model	Cardiomyopathy vs. Control			ARVC vs. Control			Non-ARVC vs. Control			ARVC vs. Non-ARVC		
	P_1 value	OR_1 (95% CI)	$Power_1$	P_2 value	OR_2 (95%CI)	$Power_2$	P_3 value	OR_3 (95%CI)	$Power_3$	P_4 value	OR_4 (95%CI)	$Power_4$
Homozygous co- dominant (AA vs. GG)	0.186	4.054 (0.553 - 29.738)	0.374	0.615	1.013 (0.995 - 1.032)	0.051	0.067	8.333 (1.105 - 62.821)	0.428	0.487	1.111 (0.960 - 1.286)	0.051
Heterozygous co- dominant (AA vs. AG)	0.634	1.667 (0.207 - 13.428)	0.087	0.377	1.100 (0.964 - 1.255)	0.055	0.191	5.000 (0.535 - 46.719)	0.361	0.165	1.500 (0.852 - 2.641)	0.062
Recessive (AA vs. AG+GG)	0.225	3.469 (0.476 - 25.268)	0.476	0.573	1.012 (0.995 - 1.128)	0.059	0.074	7.727 (1.036 - 57.651)	0.774	0.216	1.091 (0.967 - 1.231)	0.051
Dominant (AA+AG vs. GG)	0.012*	2.580 (1.206 - 5.519)	0.914	0.047*	2.871 (1.122 - 7.345)	0.853	0.197	2.273 (0.814 - 6.344)	0.757	0.142	2.667 (0.707 - 10.0520)	0.641

*significant
differences.

P value, genotypic frequencies between cardiomyopathy cases and controls; OR, odds ratio; CI, confidence interval.