ORIGINAL RESEARCH

Variant Spectrum of Formin Homology 2 Domain-Containing 3 Gene in Chinese Patients With Hypertrophic Cardiomyopathy

Guixin Wu (iD), MD*; Jieyun Ruan, MD*; Jie Liu (iD), MD*; Channa Zhang, BS; Lianming Kang, MD; Jizheng Wang (iD), PhD; Yubao Zou, MD (iD); Lei Song (iD), MD, PhD

BACKGROUND: The *FHOD3* (formin homology 2 domain-containing 3) gene has recently been identified as a causative gene of hypertrophic cardiomyopathy (HCM). However, the pathogenicity of *FHOD3* variants remains to be evaluated. This study analyzed the spectrum of *FHOD3* variants in a large HCM and control cohort, and explored its correlation with the disease.

METHODS AND RESULTS: The genetic analysis of *FHOD3* was performed using the whole exome sequencing data from 1000 patients with HCM and 761 controls without HCM. A total of 37 *FHOD3* candidate variants were identified, including 25 missense variants and 2 truncating variants. In detail, there were 27 candidate variants detected in 33 (3.3%) patients with HCM, which was significantly higher than in the 12 controls (3.3% versus 1.6%; odds ratio, 2.13; $P<0.05$). On the basis of familial segregation, we identified one truncating variant (c.1286+2delT) as a causal variant in 4 patients. Furthermore, the *FHOD3* candidate variant experienced significantly more risk of cardiovascular death and all-cause death (adjusted hazard ratio [HR], 3.71; 95%, 1.32–8.59; $P=0.016$; and adjusted HR, 3.02; 95% CI, 1.09–6.85; $P=0.035$, respectively).

CONCLUSIONS: Our study suggests that *FHOD3* is a causal gene for HCM, and that the presence of *FHOD3* candidate variants is an independent risk for cardiovascular death and all-cause death in HCM.

Key Words: cardiovascular events ■ formin homology 2 domain-containing 3 ■ genetic testing ■ hypertrophic cardiomyopathy

Hypertrophic cardiomyopathy (HCM) is one of the most common cardiovascular disorders, and is classically regarded as an autosomal dominant mendelian disease.[1] Characterized by its clinical variability and genetic heterogeneity, HCM is a worldwide disease, with a prevalence of at least 1 in 500.[2–4] Since *MYH7* was first identified as a gene associated with HCM, variants in 7 sarcomere genes (*MYBPC3*, *TNNT2*, *TNNI3*, *MYL2*, *MYL3*, *TPM1*, and *ACTC1*) have been reported to cause HCM.[5,6] However, causal variants located within these genes were not detected in around half of patients, suggesting that new disease-associated genes remained to be discovered.[7–9]

FHOD3 (formin homology 2 domain-containing 3) protein, a myocardial formin that localizes to thin actin filaments, is encoded by the *FHOD3* gene.[10] The formin homology-2 domain of FHOD3 protein terminates filament extension through blocking capping protein from binding the actin filament end.[11] The role of FHOD3 in regulating sarcomere organization, myofibrillogenesis, and contractility in cardiomyocytes suggests that *FHOD3* may be a potential candidate gene for HCM.[12,13] Previously, Wooten et al

Nonstandard Abbreviations and Acronyms

FHOD3	formin homology 2 domain-containing 3
HCM	hypertrophic cardiomyopathy
SCD	sudden cardiac death
VUS	variants of unknown significance

had reported *FHOD3* variants were associated with HCM in the Tufts HCM Cohort by genome-wide association study.[14] In another study, *FHOD3* was considered a novel genetic cause of HCM in a European cohort, accounting for ≈1% to 2% of patients with HCM.[15] More recently, an in-frame variant (NM_001281740, c.1578_1580del, p.Ser-527del) of *FHOD3* was identified as a causal variant in a Chinese family with HCM.[16] However, the pathogenicity of *FHOD3* variants has not been evaluated systemically. Herein, we analyzed the spectrum of *FHOD3* variants in a large cohort with HCM, and established a correlation of *FHOD3* variants with clinical manifestations.

METHODS

Because of privacy, the data, analytic methods, and study materials will not be made available to other researchers for purposes of reproducing the results or replicating the procedure.

Patient and Control Cohorts

From 2012 to 2018, 1039 patients with HCM and 823 controls without HCM were recruited through Fuwai Hospital, Chinese Academy of Medical Sciences, Beijing, China. HCM was defined by a maximal ventricular wall thickness ≥15 mm that was not solely explained by abnormal loading conditions. Patients with lesser degrees of maximal ventricular wall thickness (13–14 mm) were diagnosed as having HCM if they had a family history of disease.

Clinical evaluation was performed in all patients, including history of disease, systematic clinical examinations, and pedigree investigation. HCM was excluded in all of the controls by the lack of primary left ventricular hypertrophy in echocardiography.

The study was approved by the Ethics Committees of Fuwai Hospital, and complies with the principles of the Declaration of Helsinki. Written informed consent was obtained from all patients.

Genotyping

Targeted capture was performed using the Agilent Sure SelectXT Human All Exon V6 kit, followed by 2×150-bp paired-end sequencing on the Illumina NovaSeq platform using manufacturer's protocol. A total of 25 patients and 12 controls were removed with excess missing rates or excess heterozygosity, both defined by 1.5× interquartile range above the third quartile. Moreover, 14 patients and 50 controls were excluded with the mean identical-by-descent sharing >0.125 with any other individuals, which was estimated after linkage disequilibrium pruning using PLINK.[17] The sequencing achieved a mean coverage of 142× with >99.9% of targeted regions in the *FHOD3* gene. Variants detected in *FHOD3* were described according to the Human Genome Variation Society recommendations[18] and were annotated according to the longest transcript of *FHOD3* gene (NM_001281740.1). A modified classification scheme, based on the criteria of American College of Medical Genetics and Genomics, was constructed for *FHOD3* variant classification (Table S1).[19] The pathogenicity of detected variants was classified as pathogenic, likely pathogenic, variants of unknown significance (VUS), likely benign, or benign (Table S2). *FHOD3* variants of pathogenic, likely pathogenic, and VUS status were defined as candidate variants. All *FHOD3* candidate variants were verified by Sanger sequencing, and the primers for PCR amplification are listed in Table S3.

Variants in 8 sarcomere disease genes (*MYH7*, *MYBPC3*, *TNNT2*, *TNNI3*, *MYL2*, *MYL3*, *TPM1*, and *ACTC1*) were classified following the American College of Medical Genetics and Genomics guideline.[19] Patients were divided as *FHOD3* HCM and non-*FHOD3* HCM, including genotype-positive HCM and genotype-negative HCM based on whether patients carried any pathogenic, likely pathogenic, or VUS variants of sarcomere genes or not.

Pedigree Analysis

Patients with HCM with *FHOD3* candidate variants underwent pedigree analysis. Each family member underwent 12-lead electrocardiography and echocardiography to assess his/her cardiac condition. Candidate variants found in probands were tested among family members using Sanger capillary sequencing. Two-point logarithm of the odds scores were calculated using the PARAMLINK package for R software[20] with a parametric linkage model of autosomal dominance, θ=0, phenocopy rate=0.005, and 2 different penetrance values: 0.80 and 0.95.

Follow-Up and End Points

Follow-up was performed by a clinic visit or telephone interview for all patients with HCM until December 2018. The primary end point was cardiovascular death, defined as death caused by cardiogenic or vascular causes, including sudden cardiac death (SCD), heart failure (HF)–related death, and stroke-related death. The secondary end point was all-cause death. SCD was defined as witnessed sudden and unexpected death with or without documented ventricular fibrillation within 1 hour of new symptoms or nocturnal deaths with no antecedent history of worsening symptoms. HF-related death was defined as death proceeded by HF or heart transplantation in the end stage of HF.

Statistical Analysis

All statistical analysis was performed with SPSS version 24.0 software (SPSS, Chicago, IL) unless otherwise specified. Categorical variables are presented as number (percentage), and continuous variables are presented as mean±SD. The Pearson χ^2 test or the Fisher exact test was used for comparing categorical variables. The independent-sample *t* test was used for continuous variable comparisons, and the Mann-Whitney *U* test was used for abnormally distributed variables. Survival curves were constructed according to the Kaplan-Meier method, and comparisons were performed using the log-rank test. Cox regression with Firth penalized maximum likelihood models was used to calculate the hazard ratio (HR) and 95% CI to estimate the effect of candidate variants on end points using R software version 3.4.3 (R Core Team, Vienna, Austria) with the "coxphf" package (https://cran.r-project.org/web/packages/coxphf/coxphf.pdf). The characteristics with a *P*<0.05 in univariable analysis were chosen for the multivariable model, including left ventricular end-diastolic dimension, family history of SCD, maximal ventricular wall thickness, and left atrial diameter. *P* values are 2 sided and considered significant when <0.05.

RESULTS

FHOD3 Candidate Variants in the Study Population

There were a total of 1000 patients with HCM and 761 controls without HCM included in the final analysis of this study. The characteristics of the study population at enrollment are summarized in Table S4. The participants consisted of 1000 patients with HCM and 761 controls. The cohort with HCM was 64.5% men, with a median age of 47.9±14.6 years. There was no significant difference in sex and age between patients with HCM and controls.

A total of 37 *FHOD3* candidate variants were identified, including 25 missense variants and 2 truncating variants (Table 1).[21–23] In detail, 14 (37.8%) variants were reported in the Exome Aggregation Consortium database or the Genome Aggregation database, whereas 23 (62.2%) variants were first detected in patients with HCM or the total population. In our cohort, 27 candidate variants were detected in 33 (3.3%) patients with HCM (Table 1). Comparatively, 12 variants were found in 12 (1.6%) controls, which represented a significant difference (odds ratio [OR], 2.13; *P*<0.05). Furthermore, 6 variants were clustered in an exclusively cardiac isoform domain of the protein (amino acids 400–574), which maintained the 2 truncating variants (Figure S1). Notably, all these 6 variants were only detected in 9 patients with HCM.

Segregation Study

Pedigree analysis was performed in 6 of 33 patients with *FHOD3* candidate variants (Figure 1). Of these, 4 families included at least a second subject with HCM. Among these family members, all 6 affected patients were *FHOD3* candidate variant carriers (Figure 1). The novel truncating variant (c.1286+2delT) classified as likely pathogenic was detected in 4 patients, of whom none carried a probable pathogenic mutation in 8 sarcomere genes. In pedigree A, the variant was a de novo mutation because the cardiac structure was normally in both parents of the proband without this variant (Figure 1A). The cosegregation of HCM and the variant is shown in pedigree B (Figure 1B). The grandmother (I-1) and mother (II-3) of the proband are variant carriers, and present left ventricular hypertrophy. All 3 uncles without this variant have a normal phenotype. In addition, the variant is also detected in 2 daughters of the proband in pedigree C, who show cardiac hypertrophy (Figure 1C). No other patients were found in pedigree D, although the son of the proband, an 8-year-old boy, carried the variant but had a normal echocardiogram and 12-lead electrocardiography (Figure 1D). The combined logarithm of the odds score of the variant was

Table 1. Candidate Variants of *FHOD3* Detected in Patients With HCM and Controls

Transcript Effect (NM_001281740.1)	Protein (NP_001268669.1)	Type	Variant Classification*	SNP	FHOD3 Domain	CADD	SIFT	Polyphen	GnomAD MAF%	ExAC MAF%	In-House MAF%	Subject Identifier (Phenotype)	Sarcomere Gene Variants
c.274C>T	p.Arg92Trp	Missense	VUS	rs759696197	GBD/FH3	27.1	0.005	0.967	0.0032	0.0051	0.0284	H8267 (HCM)	MYH7, p.Arg453Cys
c.562C>T	p.Arg188Cys	Missense	VUS	rs143579901	GBD/FH3	24.2	0.001	0.015	0.0032	0	0.0284	H7105 (HCM)	None
c.646G>A	p.Val216Ile	Missense	VUS	rs551483382	GBD/FH3	26.6	0.004	0.758	0.0084	0.0084	0.0284	H7191 (HCM)	MYH7, p.Glu930Lys
c.776C>T	p.Thr259Met	Missense	VUS	rs770013602	GBD/FH3	26.2	0.008	0.414	0.0096	0.0165	0.0284	HT065 (HCM)	None
c.796A>G	p.Met266Val	Missense	VUS	Novel	GBD/FH3	24.1	0.187	0.21	0	0	0.0284	A073 (control)	None
c.1007G>A	p.Ser336Asn	Missense	VUS	Novel	GBD/FH3	13.15	NA	NA	0	0	0.0284	H8138 (HCM)	None
c.1063C>T	p.Arg355Trp	Missense	VUS	Novel	GBD/FH3	25.9	0.001	0.471	0	0	0.0284	S285 (HCM)	None
c.1097C>T	p.Ser366Leu	Missense	VUS	rs747730516	GBD/FH3	27.4	0.001	0.982	0.0032	0.0041	0.0568	H7597 (HCM)	None
												H8282 (HCM)	None
c.1157C>T	p.Pro386Leu	Missense	VUS	Novel	GBD/FH3	24.7	NA	NA	0	0	0.0284	Y398 (control)	None
c.1189C>T	p.Arg397Cys	Missense	VUS	rs760874847	GBD/FH3	20.7	0.091	0.001	0	0	0.0568	S101 (HCM)	MYBPC3,p.Gln469Ter
c.1286+2delT	NA	Spicing	Pathogenic	Novel	Ex				0	0	0.1136	H7177 (HCM)	MYH7, p.Arg1045His
												H8306 (HCM)	None
												H7571 (HCM)	None
												H7147 (HCM)	None
												H7104 (HCM)	None
c.1309C>T	p.Gln437Ter	Nonsense	LP	Novel	Ex	35	NA	NA	0	0	0.0284	H7115 (HCM)	None
c.1552G>A	p.Val518Met	Missense	VUS	Novel	Ex	25.7	NA	NA	0	0	0.0284	H1260 (HCM)	MYBPC3,p.Asp1091Gly
c.1580C>T	p.Ser527Phe	Missense	VUS	Novel	Ex	25.5	NA	NA	0	0	0.0284	H8136 (HCM)	MYH7, p.Leu832Phe
c.1640A>C	p.Glu547Ala	Missense	VUS	Novel	Ex	23.7	NA	NA	0	0	0.0284	H8194 (HCM)	MYBPC3, p.Glu611fs
c.1703G>T	p.Arg568Leu	Missense	VUS	Novel	Ex	20.9	NA	NA	0	0	0.0284	H7327 (HCM)	None
c.1733T>A	p.Phe578Tyr	Missense	VUS	Novel		19.2	NA	NA	0	0	0.0284	Y5332 (control)	None
c.1924G>A	p.Glu642Lys	Missense	VUS	Novel	CC	26.2	0.005	0.979	0	0	0.0284	S099 (HCM)	MYH7, p.Gly823Glu
c.2077C>T	p.Arg693Trp	Missense	VUS	rs533572045	DID	29.1	0.0	0.292	0.0064	0	0.0284	Y2722 (control)	None
c.2078G>A	p.Arg693Gln	Missense	VUS	rs148866621	DID	23.2	0.083	0.001	0.0096	0.0087	0.0284	H7520 (HCM)	MYBPC3,p. Glu542Lys

(Continues)

Table 1. Continued

Transcript Effect (NM_001281740.1)	Protein (NP_001268669.1)	Type	Variant Classification*	SNP	FHOD3 Domain	CADD	SIFT	Polyphen	GnomAD MAF%	ExAC MAF%	In-House MAF%	Subject Identifier (Phenotype)	Sarcomere Gene Variants
c.2090G>A	p.Arg697Gln	Missense	VUS	rs553344694	DID	23.2	0.006	0.811	0.0065	0.0095	0.0284	H8315 (HCM)	None
c.2429G>T	p.Gly810Val	Missense	VUS	Novel	DID	22.7	0.02	0.022	0	0	0.0284	H1341 (HCM)	None
c.2584G>A	p.Asp862Asn	Missense	VUS	rs544119818	DID	21.1	0.671	0.002	0.0032	0.0043	0.0568	H8819 (HCM)	None
												HTO20 (HCM)	MYH7, p.Arg663His
c.2746T>G	p.Ser916Ala	Missense	VUS	Novel	DID	20.8	0.091	0.711	0	0	0.0568	H1424 (HCM)	None
												T903 (control)	None
c.2824G>C	p.Glu942Gln	Missense	VUS	rs779000457	DID	27.1	0.016	0.986	0.0025	0.0035	0.0284	H8818 (HCM)	MYBPC3, p.Tyr842Ter
c.2837G>A	p.Ser946Asn	Missense	VUS	Novel	DID	15.46	0.247	0.058	0	0	0.0284	S122 (HCM)	MYH7, p.Ile263Thr
c.2954C>A	p.Ala985Asp	Missense	VUS	Novel	DID	25.9	0.003	0.996	0	0	0.0284	B014 (control)	None
c.3005A>T	p.Glu1002Val	Missense	VUS	Novel	DID	26.9	0.003	0.408	0	0	0.0284	H1253 (HCM)	None
c.3412T>C	p.Ser1138Pro	Missense	VUS	Novel	FH2	23.1	0.071	0.101	0	0	0.0284	HTO33 (HCM)	None
c.3478G>A	p.Ala1160Thr	Missense	VUS	rs746707013	FH2	24.6	0.004	0.777	0.0032	0	0.0284	Y3909 (control)	None
c.3587C>G	p.Thr1196Arg	Missense	VUS	Novel	FH2	25.2	0.01	0.999	0	0	0.0284	Y6233 (control)	None
c.3624G>C	p.Gln1208His	Missense	VUS	Novel	FH2	24.2	0.001	0.909	0	0	0.0284	H8258 (HCM)	None
c.3796A>G	p.Ile1266Val	Missense	VUS	Novel	FH2	25.2	0.276	0.028	0	0	0.0284	H8911 (HCM)	MYH7, p.Lys1485Arg
c.4270T>A	p.Tyr1424Asn	Missense	VUS	rs753641918	FH2	25.1	0.314	0.003	0.0012	0.0017	0.0568	H8004 (HCM)	None
												T303 (control)	None
c.4586C>T	p.Pro1529Leu	Missense	VUS	Novel		18.4	0.342	0.052	0	0	0.0284	Y1615 (control)	None
c.4702C>T	p.Arg1568Cys	Missense	VUS	rs770836110	DAD	28.1	0.187	0.917	0.0016	0.0008	0.0284	A2015 (control)	None
c.4787T>C	p.Leu1596Ser	Missense	VUS	Novel	DAD	24.1	0.0	0.994	0	0	0.0284	Y4609 (control)	None

CADD indicates combined annotation-dependent depletion score (phred; v1.3; August 2015)[21]; CC, coiled-coiled; DAD, diaphanous autoregulation domain; DID, diaphanous autoinhibitory domain; Ex, exclusively cardiac isoform; ExAC, Exome Aggregation Consortium (http://exac.broadinstitute.org); FH2, formin homology 2 domain; FHOD3, formin homology 2 domain-containing 3; GBD/FH3, GTPase-binding domain/formin homology 3 domain; GnomAD, Genome Aggregation (https://gnomad.broadinstitute.org); HCM, hypertrophic cardiomyopathy; LP, likely pathogenic; MAF, minor allele frequency; NA, not available; Polyphen, polymorphism phenotyping v2 (September 2014)[22]; SIFT, sorting intolerant from tolerant (computed from ENSEMBL 55; September 2014)[22]; SNP, single nucleotide polymorphism; and VUS, variants of uncertain significance.

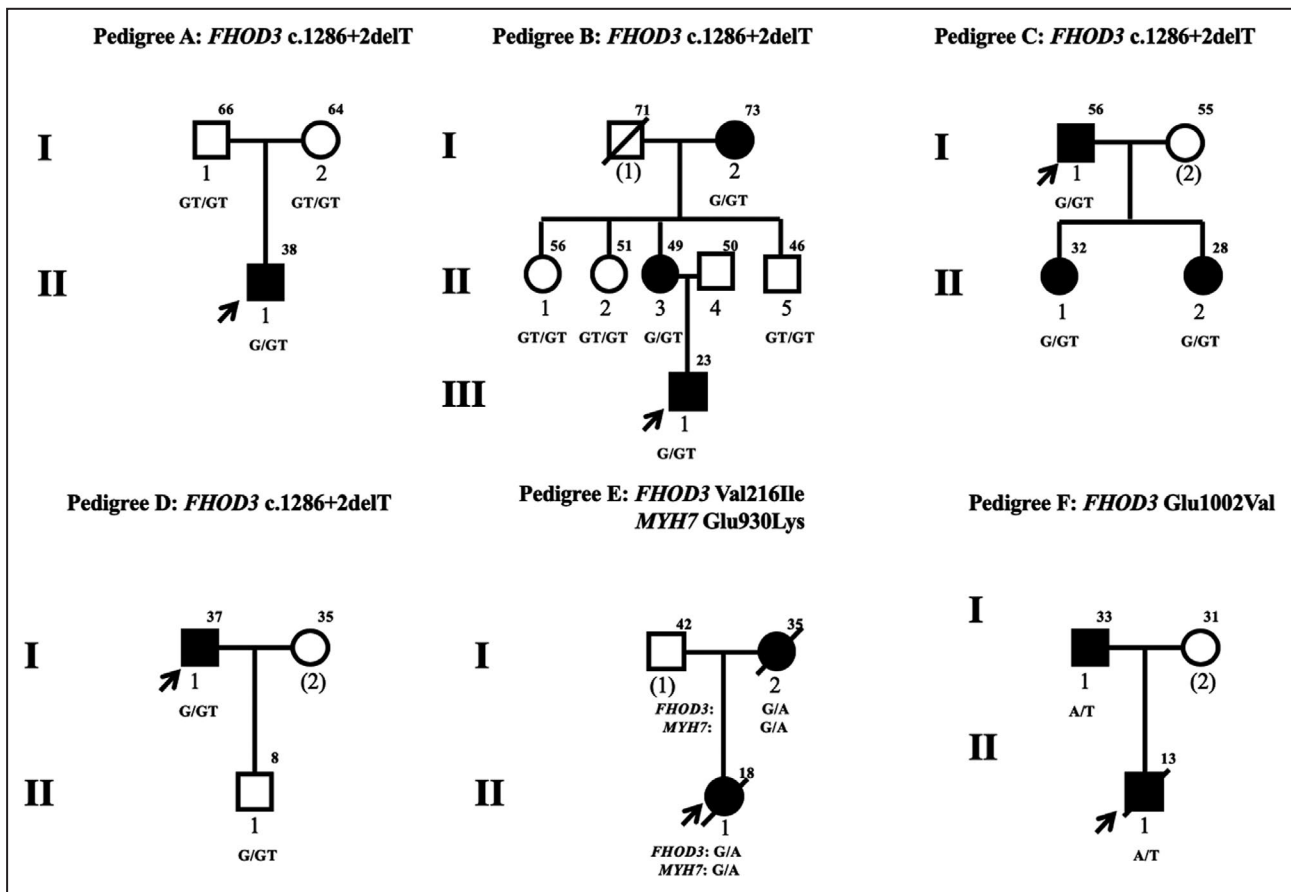*Determined according to criteria in Table S1.

Figure 1. Cosegregation of *FHOD3* (formin homology 2 domain-containing 3) candidate variants with hypertrophic cardiomyopathy.
Individuals affected by left ventricular hypertrophy are indicated by black symbols. Unfilled symbols represent individuals without ventricular hypertrophy. Arrows indicate the probands. Circles represent women; squares represent men. Symbols with a slash through them indicate deceased subjects. The current age or the age at death is indicated to the upper right of each symbol. Numbers in parentheses indicate individuals without DNA available.

1.44 in family linkage analysis of pedigrees B through D (Table S5). In general, the variant was defined as pathogenic variant and causative of disease onset in these patients.

The Val216Ile variant in *FHOD3* (*rs551483382*) was detected in 1 patient, but was also present in 10 (0.0084%) individuals in the Exome Aggregation Consortium database, and classified as VUS (Table 1). The proband also carried the Glu930Lys variant in *MYH7* (*rs397516171*), which was classified as pathogenic. Pedigree analysis showed that both *rs551483382* and *rs397516171* are present in the mother of the proband (I-2) diagnosed as having HCM (Figure 1E). The pathogenicity of *FHOD3* Val216Ile has not been determined.

The Glu1002Val variant, classified as VUS, was novel and not present in Exome Aggregation Consortium or Genome Aggregation database (Table S2). There was no probable sarcomere pathogenic mutation detected in the patient with the Glu1002Val variant. The father of the patient who

presented with left ventricular hypertrophy also carried *FHOD3* Glu1002Val variant, but whole exome sequencing found no sarcomere pathogenic mutation. We assumed that Glu1002Val variant might be the causal variant in this family, but the available evidence is insufficient to determine its pathogenicity.

Characteristics of Patients With *FHOD3* Candidate Variants

Similar clinical and echocardiographic characteristics were observed between patients with HCM with or without *FHOD3* candidate variants (Table 2). Patients with *FHOD3* variants presented a similar probability of outflow tract obstruction as patients without *FHOD3* variants. In addition, there was no difference of maximal ventricular wall thickness between patients with or without *FHOD3* candidate variants.

The mean age of patients with *FHOD3* candidate variants at diagnosis was 45.2±17.2 years, and 24

Table 2. Demographic and Clinical Characteristics of Patients With HCM With or Without *FHOD3* Candidate Variants

Variable	*FHOD3* Variant Carriers	Noncarriers	*P* Value*
Sample size	33	967	
Age at enrollment, y	45.2±17.2	48.0±14.5	0.282
Age at diagnosis, y	40.0±14.5	43.5±14.6	0.179
Men, n (%)	24 (72.7)	622 (64.3)	0.321
BMI, kg/m²	24.6±2.8	25.6±3.7	0.133
Family history of SCD, n (%)	4 (12.1)	112 (11.6)	0.847
Echocardiography			
MVT, mm	23.6±6.9	22.6±5.8	0.340
Left atrium, mm	43.0±6.8	41.7±7.2	0.300
LVEDD, mm	45.1±7.7	44.0±6.3	0.327
Ejection fraction, %	66.3±11.3	67.6±8.1	0.372
Outflow tract obstruction, n (%)	19 (57.6)	544 (56.3)	0.881

Continuous variables are presented as mean±SD; the categorical variable sex was presented as number (percentage). BMI indicates body mass index; *FHOD3*, formin homology 2 domain-containing 3; HCM, hypertrophic cardiomyopathy; LVEDD, left ventricular end-diastolic diameter; MVT, maximal left ventricular wall thickness; and SCD, sudden cardiac death.

*Continuous variables were compared by Student *t* test; the categorical variables were compared by χ² test.

(72.7%) patients were men. The clinical manifestations of male and female patients with *FHOD3* candidate variants were similar (Table S6).

A total of 64 subjects were lost to follow-up, including 1 patient with an *FHOD3* variant and 63 without. During the follow-up period of 2.6±1.6 years (2411 patient-years), 41 patients reached the primary end point, including 5 *FHOD3* candidate variant carriers and 36 noncarriers. In detail, all 5 (15.6%) *FHOD3* candidate variant carriers died of SCD, and 36 (4.0%) patients without *FHOD3* variants died of cardiovascular death, including 20 (2.2%) of SCD, 12 (1.3%) of HF-related death, and 5 (0.6%) of stroke-related death. Univariate analysis showed that the risk of cardiovascular deaths was significantly higher in patients with *FHOD3* candidate variants than those without (15.6% versus 4.0%; HR, 4.09; 95% CI, 1.48–9.22; *P*=0.009)

(Table 3). Moreover, the *FHOD3* candidate variant was associated with the risk of SCD (15.6% versus 2.2%; HR, 7.24; 95% CI, 2.54–17.36; *P*<0.001) (Table S7). Figure 2 displays Kaplan-Meier survival curves of freedom of cardiovascular death and SCD. Multivariate analysis showed the patients with *FHOD3* candidate variants experienced a significantly higher risk of cardiovascular death and SCD than those without (adjusted HR, 3.71; 95% CI, 1.32–8.59; *P*=0.016; and adjusted HR, 6.79; 95% CI, 2.26–17.35; *P*=0.001, respectively) (Table 3 and Table S7).

A total of 49 patients reached the secondary end point, including 5 (15.6%) patients with candidate variants and 44 (4.9%) without those variants. A total of 36 patients died of cardiovascular diseases, and another 8 patients without *FHOD3* variants died of cancer or accidents. Patients with *FHOD3* candidate variants had a higher risk of all-cause death (HR, 3.35; 95% CI, 1.22–7.46; *P*=0.022) (Table S8). Kaplan-Meier survival curves of freedom of all-cause death are displayed in Figure 2C. Multivariate analysis showed that the *FHOD3* candidate variant remained an independent predictor of all-cause death (adjusted HR, 3.02; 95% CI, 1.09–6.85; *P*=0.035) (Table S8).

Among 986 non-*FHOD3* variant carriers, a total of 482 patients were included in non-*FHOD3* genotype-positive group, carrying sarcomere gene mutations. Except for 29 patients who were lost to follow-up, there were 18 subjects reaching primary outcome, containing 12 patients who died of SCD, and 4 subjects reaching secondary outcome. Kaplan-Meier survival curves of primary and secondary outcomes were constructed for comparison between the prognosis of *FHOD3* variant carriers and non-*FHOD3* genotype-positive carriers. The results suggested *FHOD3* patients with HCM had a higher risk of reverse outcomes than non-*FHOD3* genotype-positive patients (Figure S2A through S2C).

DISCUSSION

FHOD3 is highly expressed in the heart, and plays an important role in maintaining normal cardiac

Table 3. Univariable and Multivariable Cox Regression Analysis of the Association Between *FHOD3* Candidate Variants and Cardiovascular Death in Patients With HCM

Variants	Crude HR (95% CI)	Crude *P* Value	Adjusted HR (95% CI)	Adjusted *P* Value
FHOD3 variants	4.086 (1.480–9.221)	0.009	3.707 (1.320–8.594)	0.016
LVEDD	1.066 (1.025–1.103)	0.002	1.071 (1.025–1.113)	0.003
Family history of SCD	2.258 (1.035–4.473)	0.041	2.276 (1.032–4.580)	0.042
MVT	1.034 (1.002–1.214)	0.042	1.046 (0.9991–1.101)	0.102
Left atrial diameter	1.047 (1.006–1.086)	0.025	1.020 (0.977–1.062)	0.361

FHOD3 indicates formin homology 2 domain-containing 3; HCM, hypertrophic cardiomyopathy; HR, hazard ratio; LVEDD, left ventricular end-diastolic diameter; MVT, maximal left ventricular wall thickness; and SCD, sudden cardiac death.
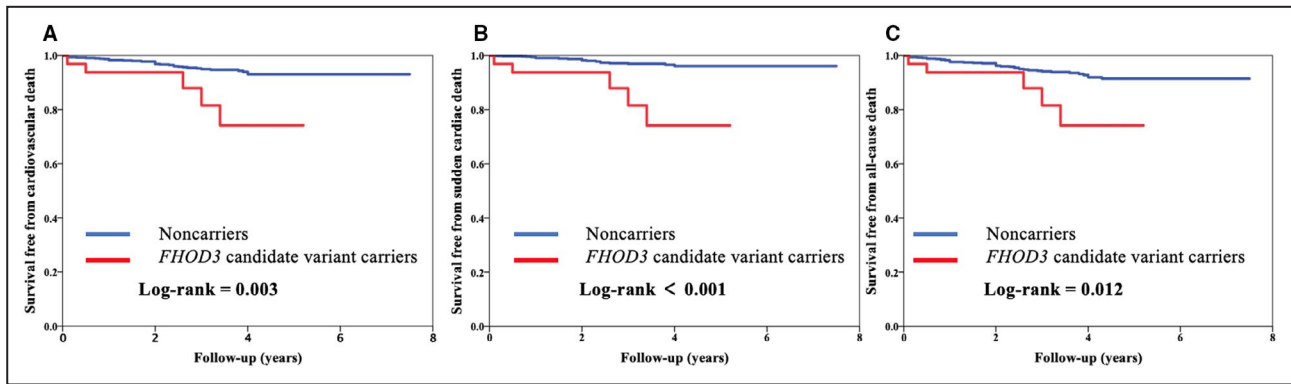
Figure 2. Cumulative Kaplan-Meier analysis showing that *FHOD3* (formin homology 2 domain-containing 3) candidate variants were associated with a higher risk of cardiovascular death (A), sudden cardiac death (B), and all-cause death (C). *P* values were calculated using the log-rank test.

function.[24,25] As a regulator of actin assembly in cardiac sarcomeres, the FHOD3 mutants Ile1127Ala and Lys1273Asp are defective in actin binding.[12,13] Matsuyama et al reported that aberrantly mislocalized FHOD3 was deleterious, and contributed to the pathogenesis of *MYBPC3* (cardiac myosin-binding protein C)-related cardiomyopathy by failing to directly interact with MYBPC3.[26] Recently, *FHOD3* mutations were shown to be associated with heart diseases; for example, the Tyr1249Asn variant was identified to cause a dilated cardiomyopathy family by interfering with actin filament assembly.[27] Moreover, as some *FHOD3* variants account for HCM cases, *FHOD3* is also regarded as causative of HCM.[15] In our study, the *FHOD3* gene was screened in a large Chinese cohort, and a total of 37 *FHOD3* candidate variants were detected in patients with HCM or controls. The OR of *FHOD3* candidate variants in patients with HCM was 2.13 with respect to controls, which is comparable with that reported by Ochoa et al.[15] Candidate variants of *FHOD3* gene detected in population databases or controls indicated that not all *FHOD3* candidate variants cause the HCM phenotype. Therefore, the pathogenicity of *FHOD3* variants remains to be validated by segregation and functional assays.[9] Under the pedigree analysis, the combined logarithm of the odds score of truncating variant c.1286+2delT reached 1.44 as evidence for suggestive linkage.[28] Moreover, this variant was the de novo mutation in pedigree A, as strong support for the pathogenicity of this variant, according to the American College of Medical Genetics and Genomics guideline.[19] Therefore, the variant was identified as a causal variant for HCM. However, other candidate variants were not evaluated because of the absence of second patients or small size of families. Considering the caveat of variable expressivity and probability of incomplete penetrance, the pathogenicity of these variants remains to be determined.

The c.1286+2delT variant is located within the exclusively cardiac isoform domain, which is expressed in the heart but spiced out in the kidney and brain.[29] In a previous study, the FHOD3 isoform without an exclusively cardiac isoform domain could not localize to the sarcomere, indicating that this domain is paramount in FHOD3 localization.[12] Notably, all 6 variants in the exclusively cardiac isoform domain were only found in patients with HCM in our study, further suggesting that this domain is crucial to HCM. The FHOD3 protein also contains 3 formin homology domains, a GTPase binding domain, a diaphanous autoinhibitory domain, a coiled-coil domain, and a diaphanous autoregulation domain.[30,31] The interaction of diaphanous autoinhibitory domain and diaphanous autoregulation domain was reported to be responsible for FHOD3 dimerization.[32] Half of the pathogenic variants identified by Ochoa et al were clustered in the coiled-coil domain, indicating that this is associated with HCM.[15] However, only Glu642Lys from the coiled-coil domain was detected in a patient with HCM in our study, whereas we did not determine the pathogenicity of variants in the diaphanous autoinhibitory domain or diaphanous autoregulation domain. Thus, the function of FHOD3 domains remains unclear and to be explored.

The genotype-phenotype correlation between mutations and prognosis in patients with HCM has varied in studies.[33–35] Phenotypes were found to be similar in patients with *MYH7* or *MYBPC3* mutations,[36] whereas patients with variants in genes encoding thin myofilament proteins presented with milder hypertrophy but a higher risk of systolic dysfunction.[37] Our study revealed the *FHOD3* candidate variants to be an independent predictor for cardiovascular death and all-cause death. The *FHOD3* variant carriers showed worse prognosis than non-*FHOD3* carriers or non-*FHOD3* genotype-positive carriers, indicating the *FHOD3* variants should be considered in the management and risk stratification

of patients with HCM. Ochoa et al described the sex difference that female carriers were diagnosed 10 years later than male carriers. Nevertheless, the sex difference did not reproduce in our study, which might be as a result of the difference of ethnicity or particular variants.

Our study has some limitations. First, the *FHOD3* sequence was limited within the exome region. Second, the pathogenicity of most of the candidate variants requires confirmation by further study; therefore, our finding underestimates the effect of the *FHOD3* gene in HCM.

In conclusion, our study screened the *FHOD3* gene in a large Chinese cohort, which consisted of 1000 patients with HCM and 761 controls without HCM, identifying a total of 37 *FHOD3* candidate variants. Among these variants, the truncating variant c.1286+2delT was identified as a causal variant in 4 patients. Thus, we verified the *FHOD3* gene was a causal gene for HCM. Finally, we found that *FHOD3* candidate variants increased the risk of cardiovascular death and all-cause death, suggesting that they should be included in the management and risk stratification of patients with HCM.

ARTICLE INFORMATION

Affiliations

From the State Key Laboratory of Cardiovascular Disease, Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China (G.W., J.R., J.L., C.Z., J.W., L.S.); Cardiomyopathy Ward, Fuwai Hospital, National Center for Cardiovascular Disease, Chinese Academy of Medical Science and Peking Union Medical College, Beijing, China (G.W., J.R., J.L., L.K., L.S.); National Clinical Research Center of Cardiovascular Diseases, Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China (L.S.); and Department of Cardiovascular Internal Medicine, Fuwai Hospital, National Center for Cardiovascular Disease, Chinese Academy of Medical Science and Peking Union Medical College, Beijing, China (Y.Z.).

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Disclosures

None.

Supplementary Material

Tables S1–S8
Figures S1–S2

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Supplemental Material

Table S1. Customized classification of pathogenic variants based on the recommendations of the ACMG guideline.

Classification	Major criteria	Supporting criteria
Pathogenic	1. Widely reported variant with conclusive evidence of genotype-phenotype association and with consensus about its pathogenicity. 2. Demonstrated co-segregation with a phenotype ($>$10 meiosis) 3. Co-segregation in at least 2 families ($\leqslant$10 meiosis), or present in at least 5 probands with the same phenotype and meeting at least 2 supporting criteria.	A. Protein-truncating variant in a gene where loss of function is a proven pathogenic mechanism B. Functional studies that supporting pathogenicity. C. De novo presentation in the setting of a novel disease in the family (maternity and paternity confirmed) D. Missense variant that generates the same amino-acid change as a previously reported pathogenic variant. E. Variant with very low frequency/absent in the control population (MAF$<$0.001%).
Likely pathogenic	1. Protein-trucating variant with very low frequency/absent in the control population (MAF$<$0.001%) that affects a gene where loss of function is not an established pathogenic mechanism or that does not meet criteria to be considered pathogenic. 2. Missense variant/in-frame insertion or deletion in a non-repetitive region of a gene which does not meet criteria to be considered pathogenic, but that meets at least 3 supporting criteria.	A. Variant with very low allelic frequency/absent in the control population (MAF$<$0.001%). B. De novo presentation in the setting of a novel disease in the family (maternity and paternity unconfirmed). C. Patient's phenotype or family history suggests that disease could be explained by mutations in the gene (gene with well-established phenotype-genotype association). D. Bioinformatics predictors agree that it would be deleterious. E. Located in a mutational hot-spot, functional domain, or relevant region of the codified protein. F. Reported in at least 2 unrelated individuals that presented the same phenotype.

Variants of uncertain significance	1.Variants with contradictory information about their pathogenicity 2.Variant that do not meet criteria for being included in another classification category	
Likely benign	1.Variant allele frequency in control populations is higher than the expected for diseases or has a MAF＞0.01%. 2.Absence of variant co-segregation with the phenotype in at least one family. 3.Meeting at least 2 supporting criteria.	A. Missense variant in a gene where only variants causing protein truncation have shown association with disease. B. Functional study showing that the variant does not affect the structure or function of the encoded protein. C. Bioinformatics predictors agree that the variant would not alter the function of the protein (including splicing variants outside the consensus region of the gene). D. In-frame insertion/deletions in a repetitive gene region without known function. E. Presence of the variant in homozygosis in control population.
Benign	1.MAF＞1% in any of the control population databases. 2.Previously reported in the literature with well-established evidence of consensus about its non-disease-causing classification, and with no contradictory data. 3.Absence of co-segregation with the disease in at least 2 reported families. 4.Meeting at least 2 supporting criteria.	A. Variant allele frequency in controls population is higher than expected for disease or has a MAF＞0.01% B. Absence of co-segregation of the variant with the phenotype in at least 1 family. C. Functional study showing that the variant does not affect the structure of function of the encoded protein. D. Presence of the variant in healthy unaffected subjects at an age at which the disease should be full penetrant (variant must be in homozygosis in recessively inherited disease, or in hemizygosis in X-linked diseases).

ACMG[19], American College of Medical Genetics and Genomics; MAF, minor allele frequency.

Table S2. The variants of *FHOD3* detected in HCM patients and controls.

Transcript effect (NM_ 001281740.1)	Protein (NP_001268669.1)	Type	Variant classification#	dsSNP	*FHOD3* domain	CADD	SIFT	Polyphen	GnomAD& MAF%	ExAC* MAF%	In-house MAF%	Phenotype (n)
c.274C>T	p.Arg92Trp	Missense	VUS	rs759696197	GBD/FH3	27.1	0.005	0.967	0.0032	0.0051	0.0284	HCM (1)
c.562C>T	p.Arg188Cys	Missense	VUS	rs143579901	GBD/FH3	24.2	0.001	0.015	0.0032	0	0.0284	HCM (1)
c.566A>G	p.Asn189Ser	Missense	LB	rs747688287	GBD/FH3	17.74	0.49	0.001	0.0008	0.0008	0.0852	HCM (1); control (2)
c.595A>G	p.Ile199Val	Missense	B	rs61735987	GBD/FH3	17.31	0.31	0.002	1.6533	3.2533	7.2414	HCM (136); control (112)
c.646G>A	p.Val216Ile	Missense	VUS	rs551483382	GBD/FH3	26.6	0.004	0.758	0.0084	0.0084	0.0284	HCM (1)
c.776C>T	p.Thr259Met	Missense	VUS	rs770013602	GBD/FH3	26.2	0.008	0.414	0.0096	0.0165	0.0284	HCM (1)
c.796A>G	p.Met266Val	Missense	VUS	Novel	GBD/FH3	24.1	0.187	0.21	0	0	0.0284	Control (1)
c.958G>T	p.Val320Leu	Missense	LB	rs571359036	GBD/FH3	22.1	0.247	0.047	0.0062	0.0092	0.1420	HCM (1); control (4)
c.1004C>G	p.Pro335Arg	Missense	B	rs117005081	GBD/FH3	23.0	0.058	0.159	1.2641	2.9346	0.7098	HCM (16); control (9)
c.1007G>A	p.Ser336Asn	Missense	VUS	Novel	GBD/FH3	13.15	NA	NA	0	0	0.0284	HCM (1)
c.1063C>T	p.Arg355Trp	Missense	VUS	Novel	GBD/FH3	25.9	0.001	0.471	0	0	0.0284	HCM (1)
c.1097C>T	p.Ser366Leu	Missense	VUS	rs747730516	GBD/FH3	27.4	0.001	0.982	0.0032	0.0041	0.0568	HCM (2)
c.1157C>T	p.Pro386Leu	Missense	VUS	Novel	GBD/FH3	24.7	NA	NA	0	0	0.0284	Control (1)
c.1189C>T	p.Arg397Cys	Missense	VUS	rs760874847	GBD/FH3	20.7	0.091	0.001	0	0	0.0568	HCM (2)
c.1286+2delT	NA	Spicing	P	Novel	Ex				0	0	0.1136	HCM (4)
c.1297G>A	p.Ala433Thr	Missense	B	rs62083981	Ex	0.945	NA	NA	2.3784	3.4027	0.1136	HCM (3); control (1)
c.1309C>T	p.Gln437Ter	Nonsense	LP	Novel	Ex	35			0	0	0.0284	HCM (1)
c.1364C>T	p.Ser455Leu	Missense	B	rs2848901	Ex	16.74	NA	NA	27.0205	38.1965	43.7923	HCM (486); control (343)
c.1411G>A	p.Gly471Arg	Missense	B	rs72895597	Ex	1.256	NA	NA	12.8884	10.1348	0.9938	HCM (11); control (24)
c.1552G>A	p.Val518Met	Missense	VUS	Novel	Ex	25.7	NA	NA	0	0	0.0284	HCM (1)

c.1580C>T	p.Ser527Phe	Missense	VUS	Novel	Ex	25.5	NA	NA	0	0	0.0284	HCM (1)
c.1640A>C	p.Glu547Ala	Missense	VUS	Novel	Ex	23.7	NA	NA	0	0	0.0284	HCM (1)
c.1703G>T	p.Arg568Leu	Missense	VUS	Novel	Ex	20.9	NA	NA	0	0	0.0284	HCM (1)
c.1733T>A	p.Phe578Tyr	Missense	VUS	Novel		19.2	NA	NA	0	0	0.0284	Control (1)
c.1754C>A	p.Ser585Tyr	Missense	LB	rs200702049		21.5	0.005	0.348	0.0046	0.0091	0.2271	HCM (5); control (3)
c.1844C>T	p.Pro615Leu	Missense	LB	rs199579476		2.072	1.0	0.0	0.0024	0.0016	0.0284	Control (1)
c.1912C>T	p.Arg638Trp	Missense	LB	rs141148037	CC	26.4	0.0	0.995	0.0478	0.0561	0.1136	HCM (4)
c.1924G>A	p.Glu642Lys	Missense	VUS	Novel	CC	26.2	0.005	0.979	0	0	0.0284	HCM (1)
c.2077C>T	p.Arg693Trp	Missense	VUS	rs533572045	DID	29.1	0.0	0.292	0.0064	0	0.0284	Control (1)
c.2078G>A	p.Arg693Gln	Missense	VUS	rs148866621	DID	23.2	0.083	0.001	0.0096	0.0087	0.0284	HCM (1)
c.2090G>A	p.Arg697Gln	Missense	VUS	rs553341694	DID	23.2	0.006	0.811	0.0065	0.0095	0.0568	HCM (2)
c.2129C>G	p.Ala710Gly	Missense	B	rs61735993	DID	22.2	0.057	0.197	13.873	13.5774	7.2961	HCM (118); control (102)
c.2260G>A	p.Glu754Lys	Missense	LB	rs139884505	DID	15.4	0.462	0.002	0.1721	0.1285	0.0284	HCM (1)
c.2321A>G	p.Gln774Arg	Missense	B	rs61735994	DID	6.648	0.423	0.0	2.2748	2.8685	0.0852	Control (3)
c.2429G>T	p.Gly810Val	Missense	VUS	Novel	DID	22.7	0.02	0.022	0	0	0.0284	HCM (1)
c.2464G>A	p.Val822Phe	Missense	LB	Novel	DID	12.27	NA	NA	0	0	0.0852	Control (3)
c.2584G>A	p.Asp862Asn	Missense	VUS	rs544119818	DID	21.1	0.671	0.002	0.0032	0.0043	0.0568	HCM (2)
c.2746T>G	p.Ser916Ala	Missense	VUS	Novel	DID	20.8	0.091	0.711	0	0	0.0568	HCM (1); control (1)
c.2824G>C	p.Glu942Gln	Missense	VUS	rs779000457	DID	27.1	0.016	0.986	0.0025	0.0035	0.0284	HCM (1)
c.2837G>A	p.Ser946Asn	Missense	VUS	Novel	DID	15.46	0.247	0.058	0	0	0.0284	HCM (1)
c.2915C>T	p.Pro972Leu	Missense	LB	rs551904999	DID	11.00	0.332	0.002	0.0064	0	0.0568	Control (2)
c.2954C>A	p.Ala985Asp	Missense	VUS	Novel	DID	25.9	0.003	0.996	0	0	0.0284	Control (1)
c.3005A>T	p.Glu1002Val	Missense	VUS	Novel	DID	26.9	0.003	0.408	0	0	0.0284	HCM (1)

						CADD	SIFT	POLYPHEN	gnomAD	ExAC	MAF	
c.3187G>A	p.Ala1063Thr	Missense	LB	rs560946106	FH2	13	0.515	0.003	0.0064	0.0041	0.0568	HCM (2)
c.3412T>C	p.Ser1138Pro	Missense	VUS	Novel	FH2	23.1	0.071	0.101	0	0	0.0284	HCM (1)
c.3478G>A	p.Ala1160Thr	Missense	VUS	rs746707013	FH2	24.6	0.004	0.777	0.0032	0	0.0284	Control (1)
c.3587C>G	p.Thr1196Arg	Missense	VUS	Novel	FH2	25.2	0.01	0.999	0	0	0.0284	Control (1)
c.3601G>A	p.Asp1201Asn	Missense	LB	rs554487359	FH2	24	0.023	0.493	0.0127	0.0034	0.0284	HCM (1)
c.3624G>C	p.Gln1208His	Missense	VUS	Novel	FH2	24.2	0.001	0.909	0	0	0.0284	HCM (1)
c.3796A>G	p.Ile1266Val	Missense	VUS	Novel	FH2	25.2	0.276	0.028	0	0	0.0284	HCM (1)
c.3976G>A	p.Val1326Ile	Missense	B	rs2303510	FH2	25.6	0.143	0.145	33.5504	34.1531	25.454	HCM (435); control (374)
c.4270T>A	p.Tyr1424Asn	Missense	VUS	rs753641918	FH2	25.1	0.314	0.003	0.0012	0.0017	0.0568	HCM (1); control (1)
c.4519G>A	p.Ala1507Thr	Missense	LB	rs574765321		25.9	0.052	0.946	0.0255	0.04	0.0852	HCM (1); control (2)
c.4586C>T	p.Pro1529Leu	Missense	VUS	Novel		18.4	0.342	0.052	0	0	0.0284	Control (1)
c.4667A>G	p.Asn1556Ser	Missense	LB	rs139930679		14.11	0.617	0.002	0.0223	0.0091	0.1136	HCM (2); control (2)
c.4702C>T	p.Arg1568Cys	Missense	VUS	rs770836110	DAD	28.1	0.187	0.917	0.0016	0.0008	0.0284	Control (1)
c.4708G>A	p.Val1570Ile	Missense	LB	rs201824593	DAD	27.2	0.005	0.991	0.0939	0.0064	0.0284	HCM (1)
c.4787T>C	p.Leu1596Ser	Missense	VUS	Novel	DAD	24.1	0.0	0.994	0	0	0.0284	Control (1)

#Determined according to criteria in Table S1. P, pathogenic; LP, likely pathogenic; VUS, variants of uncertain significance; LB, likely benign; B, benign.

&GnomAD: (the Genome Aggregation) https://gnomad.broadinstitute.org/ *ExAC: (Exome Aggregation Consortium) http://exac.broadinstitute.org

CADD, Combined Annotation Dependent Depletion score (phred); v1.3 (August, 2015)[21]; SIFT, SIFT (sorting intolerant from tolerant) algorithm; computed from ENSEMBL 55 (September, 2014)[22]; POLYPHEN, Polymorphism Phenotyping (v2; September 2014)[23].

HCM, hypertrophic cardiomyopathy; MAF, minor allele frequency; GBD/FH3, GTPase-binding domain/formin homology 3 domain; Ex, exclusively cardiac isoform; CC, coiled-coiled; FH2, formin homology 2 domain; DAD, diaphanous auto-inhibitory domain.

Table S3. Primer used for Sanger sequencing confirmation of *FHOD3* candidate variants.

	Forward primer	Reverse primer
FHOD3-E3	5' ATTTTCCCAACATGGTCAAGC 3'	5' CAGAAGAACCTCATCTACCCC 3'
FHOD3-E6	5' TTGGTGCCTTAATTGCATC 3'	5' CATTTATACTGTAACGGCTTG 3'
FHOD3-E7	5' CGATTCAGCACATACTCGTGTT 3'	5' CCTCTCCCAGGTAAGCTCAT 3'
FHOD3-E8	5' TGCCATCACTGGATACGTC 3'	5' TTCCAAATAAGCCCACAAGCA 3'
FHOD3-E10	5' GGGCAATCCTGAAATGCAGTCAC 3'	5' AAATCCACCGAGATGTTTGGC 3'
FH0D3-E11	5' CTCTTTTCCTGGCTTTGTCT 3'	5' AGTTCTCTAATGAAAACATGCTC 3'
FHOD3-E12	5' ACCTCCTTGCCCTCTATAAGTCT 3'	5' CTGTGTTCTCCTCCCCGAGT 3'
FHOD3-E13	5' CTGTGTTCTCCTCCCCGAGT 3'	5' GAGTTCTGATTTGCACACC 3'
FHOD3-E15P1	5' ATCTGAAACAAGAAGACCCGAG 3'	5' AGTTGTAAAGTCACATGCCTT 3'
FHOD3-E15P2	5' CCTGGAATACTTCTATAACTCCC 3'	5' GCCCAAGAATACATGAGTCCC 3'
MYH7-E17&18	5' CTCACACCCTACCTCCCCACAC 3'	5' GAGGTCCTGTTCCCAGGGCGGT 3'
FHOD3-E17	5' TGTGTGATGCTGCCATTTCCC 3'	5' AGTTGCTGTCTCAGTATTAGCCT 3'
FHOD3-E18	5' CCCTTCACAGCATTGCCTCGAT 3'	5' CCACACTCCTTGTCCCCAGACA 3'
FHOD3-E19P1	5' TGAGCCCAATGACAAGGTCCC 3'	5' AATCTCTTCAGCCCTACCCAAC 3'
FHOD3-E19P2	5' AGTCACTCCCATGTGTCAGGC 3'	5' ACTCTGTCTTCGGCTGCACC 3'
FHOD3-E21	5' CTTGCCTAGAATGTCCTATGTGT 3'	5' TCAATTCACCCTCCGTACCCT 3'
FHOD3-E22	5' GTCCAGAGCCCTTGTCACC 3'	5' CTACAGGATGAGGGATGGGG 3'
FHOD3-E28	5' AGCCCTCTGGATCTATCACTAGC 3'	5' CAACGTCAACAGCCAACCCT 3'

Table S4. Demographic and Clinical characteristics of the subjects in the discovery study.

Variable	HCM cases	Non-HCM controls	*P*-value*
Sample size	1000	761	
Age at enrolment, year	47.9 ± 14.6	47.7 ± 14.8	0.453
Male, n (%)	645 (64.5)	521 (68.5)	0.07
BMI, kg/m^2	25.5 ± 3.7	24.9 ± 3.4	0.001
Echocardiography			
MVT, mm	22.6 ± 5.8	9.8 ± 4.2	<0.001
Left atrial, mm	41.7 ± 7.2	26.1 ± 4.2	<0.001
LVEDD, mm	44.0 ± 6.3	42.2 ± 5.2	<0.001
Ejection fraction, %	67.5 ± 8.2	65.4 ± 7.7	0.25

Continuous variables were presented as mean ± standard deviation; the categorical variable sex was presented as number (n) and percentage (%).

*Continuous variables were compared by Student's *t*-test; the categorical variable sex was compared by chi-squared test.

HCM, hypertrophic cardiomyopathy; BMI, body mass index; MVT, maximum left ventricular wall thickness; LVEDD, left ventricular end-diastolic diameter.

Table S5. LOD score indicating linkage between the truncating variant c.1286+2delT of *FHOD3* and hypertrophy cardiomyopathy in Pedigrees.

Pedigree ID	Number of individuals genotyped	Number of carriers	LOD score 95%	LOD score 80%
A	3	1	NA	NA
B	7	3	1.141	0.966
C	4	3	0.301	0.301
D	3	2	0	0
Combined LOD score			1.441	1.267

LOD, Logarithm of the odds.

LOD score 95%: Logarithms of the odds score calculated for a disease penetrance of 95%.

LOD score 80%: Logarithms of the odds score calculated for a disease penetrance of 80%.

Table S6. Demographic and Clinical characteristics of HCM patients with *FHOD3* candidate variants.

Variable	Male	Female	*P*-value*
Sample size	24	9	
Age at enrolment, year	45.2 ± 18.1	45.3 ± 15.4	0.981
Age of diagnosis, year	40.5 ± 14.7	38.9 ± 14.9	0.787
BMI, kg/m^2	24.6 ± 3.0	24.4 ± 2.6	0.874
Family history of SCD, n (%)	3 (12.5)	1 (11.1)	0.913
Echocardiography			
MVT, mm	23.9 ± 7.4	22.7 ± 5.5	0.646
Left atrial, mm	43.6 ± 7.0	41.4 ± 6.7	0.430
LVEDD, mm	46.9 ± 7.4	43.4 ± 6.7	0.217
Ejection fraction, %	64.0 ± 12.2	72.3 ± 4.5	0.059
Outflow tract obstruction, n (%)	12 (50.0)	7 (77.8)	0.150

Continuous variables were presented as mean ± standard deviation; the categorical variable sex was presented as number (n) and percentage (%).

*Continuous variables were compared by Student's *t*-test; the categorical variables were compared by chi-squared test.

HCM, hypertrophic cardiomyopathy; BMI, body mass index; MVT, maximum left ventricular wall thickness; LVEDD, left ventricular end-diastolic diameter.

Table S7. Univariable and multivariable Cox regression analysis of the association between *FHOD3* candidate variants and SCD in patients with hypertrophy cardiomyopathy.

Variants	Crude HR (95% CI)	Crude *P*-value	Adjusted HR (95% CI)	Adjusted *P*-value
FHOD3 variants	7.245 (2.541-17.363)	<0.001	6.791 (2.268-17.353)	0.001
LVEDD	1.033 (0.971-1.087)	0.295	1.042 (0.975-1.107)	0.228
Family history of SCD	3.133 (1.256-7.050)	0.016	3.517 (1.382-8.167)	0.010
MVT	1.102 (1.035-1.168)	0.003	1.094 (1.028-1.159)	0.005
Left atrial diameter	1.037 (0.984-1.088)	0.167	1.011 (0.954-1.068)	0.688

SCD, sudden cardiac death; HR, hazard ratio; CI, confidence interval; MWT, maximal wall thickness;

LVEDD, left ventricular end-diastolic diameter.

Table S8. Univariable and multivariable Cox regression analysis of the association between *FHOD3* candidate variants and all-cause death in patients with hypertrophy cardiomyopathy.

Variants	Crude HR (95% CI)	Crude *P*-value	Adjusted HR (95% CI)	Adjusted *P*-value
FHOD3 variants	3.352 (1.224-7.459)	0.022	3.020 (1.090-6.852)	0.035
LVEDD	1.054 (1.014-1.090)	0.010	1.051 (1.007-1.092)	0.025
Family history of SCD	1.813 (0.842-3.519)	0.121	1.762 (0.811-3.458)	0.143
MVT	1.020 (0.971-1.070)	0.425	1.026 (0.974-1.078)	0.318
Left atrial diameter	1.047 (1.010-1.083)	0.014	1.029 (0.989-1.067)	0.155

SCD, sudden cardiac death; HR, hazard ratio; CI, confidence interval; MWT, maximal wall thickness;

LVEDD, left ventricular end-diastolic diameter

Figure S1. The schematic of FHOD3 protein and the distribution of candidate variants.
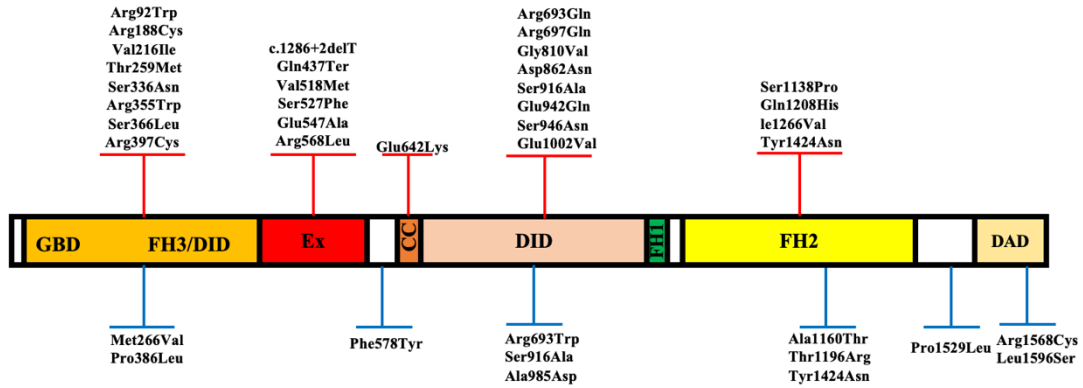
The schematic structure of FHOD3 protein is quoted from the paper by Ochoa et al[15]. The distribution of *FHOD3* candidate variants identified in patients with hypertrophy cardiomyopathy (up) and controls (down) were displayed. Ex, exclusively cardiac isoform; CC, coiled-coil region; DAD, diaphanous auto-regulatory domain; DID, diaphanous inhibitory domain; FH, formin homology domain; GBD, GTPase-binding domain.

Figure S2. Cumulative Kaplan–Meier analysis showing that *FHOD3* candidate variants were associated with a higher risk of outcomes than non-*FHOD3* genotype-positive patients (A-C).
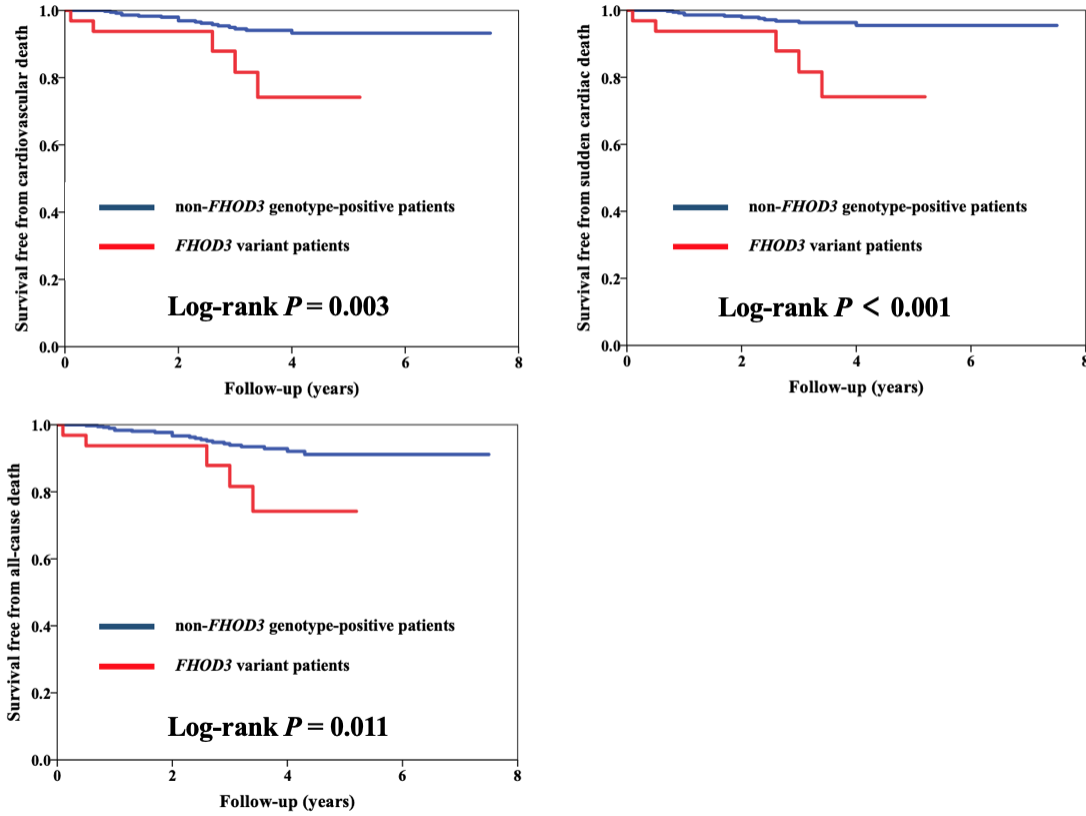
A, cardiovascular death; B; sudden cardiac death, C; all-cause death. *P* values were calculated using the log-rank test.