JOURNAL OF THE AMERICAN COLLEGE OF CARDIOLOGY VOL. 72, NO. 20, 2018

Formin Homology 2 Domain Containing 3 *(FHOD3)* Is a Genetic Basis for Hypertrophic Cardiomyopathy

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ABSTRACT

BACKGROUND The genetic cause of hypertrophic cardiomyopathy remains unexplained in a substantial proportion of cases. Formin homology 2 domain containing 3 *(FHOD3)* may have a role in the pathogenesis of cardiac hypertrophy but has not been implicated in hypertrophic cardiomyopathy.

OBJECTIVES This study sought to investigate the relation between *FHOD3* mutations and the development of hypertrophic cardiomyopathy.

METHODS *FHOD3* was sequenced by massive parallel sequencing in 3,189 hypertrophic cardiomyopathy unrelated probands and 2,777 patients with no evidence of cardiomyopathy (disease control subjects). The authors evaluated protein-altering candidate variants in *FHOD3* for cosegregation, clinical characteristics, and outcomes.

RESULTS The authors identified 94 candidate variants in 132 probands. The variants' frequencies were significantly higher in patients with hypertrophic cardiomyopathy (74 of 3,189 [2.32%]) than in disease control subjects (18 of 2,777 [0.65%]; $p < 0.001$) or in the gnomAD database (1,049 of 138,606 [0.76%]; $p < 0.001$). *FHOD3* mutations cosegregated with hypertrophic cardiomyopathy in 17 families, with a combined logarithm of the odds score of 7.92, indicative of very strong segregation. One-half of the disease-causing variants were clustered in a small conserved coiled-coil domain (amino acids 622 to 655); odds ratio for hypertrophic cardiomyopathy was 21.8 versus disease control subjects (95% confidence interval: 1.3 to 37.9; $p < 0.001$) and 14.1 against gnomAD (95% confidence interval: 6.9 to 28.7; $p < 0.001$). Hypertrophic cardiomyopathy patients carrying (likely) pathogenic mutations in *FHOD3* ($n = 70$) were diagnosed after age 30 years (mean 46.1 ± 18.7 years), and two-thirds (66%) were males. Of the patients, 82% had asymmetric septal hypertrophy (mean 18.8 ± 5 mm); left ventricular ejection fraction <50% was present in 14% and hypertrabeculation in 16%. Events were rare before age 30 years, with an annual cardiovascular death incidence of 1% during follow-up.

CONCLUSIONS *FHOD3* is a novel disease gene in hypertrophic cardiomyopathy, accounting for approximately 1% to 2% of cases. The phenotype and the rate of cardiovascular events are similar to those reported in unselected cohorts. The *FHOD3* gene should be routinely included in hypertrophic cardiomyopathy genetic testing panels. (J Am Coll Cardiol 2018;72:2457–67) © 2018 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

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ISSN 0735-1097 https://doi.org/10.1016/j.jacc.2018.10.001

ABBREVIATIONS AND ACRONYMS

DCM = dilated cardiomyopathy

FHOD3 = formin homology 2 domain containing 3 gene

GWAS = genome-wide association studies

HCM = hypertrophic cardiomyopathy

LOD = logarithm of the odds

MAF = minor allele frequency

SCD = sudden cardiac death

Hypertrophic cardiomyopathy (HCM) is the most common inherited cardiomyopathy and is characterized by clinical variability and genetic heterogeneity (1). The introduction of massive parallel sequencing has improved the understanding of the disease, but >40% of genetic studies reveal no pathogenic mutation, suggesting that new disease-associated genes remain to be discovered. One potential candidate is formin homology 2 domain containing 3 (*FHOD3*) gene.

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Formins are a family of proteins containing a series of conserved domains and functional motifs that regulate actin dynamics (2). FHOD3 protein is expressed exclusively in the heart and plays an important role in sarcomere organization, myofibrillogenesis, and maintenance of the contractile apparatus in cardiomyocytes (3,4). Functional and genome-wide association studies (GWAS) have suggested a potential role of *FHOD3* in the pathogenesis of cardiac hypertrophy (5), but no pathogenic variants clearly associated with HCM have been reported.

At the beginning of 2014, we included *FHOD3* in genetic panels used to screen patients with inherited cardiomyopathies. After detecting an *FHOD3* variant that segregated with HCM in a large Spanish family, we performed a systematic evaluation of *FHOD3* mutations in a larger cohort of patients and in a control population.

METHODS

From February 2014 to August 2017, *FHOD3* was sequenced using next-generation sequencing in 7,881 consecutive unrelated probands with a diagnosis of different inherited cardiac conditions referred to our center for molecular genetic diagnosis. The phenotypes were established by each center prior to the genetic studies. Patients' samples were referred mainly from centers from Spain, followed by centers from the United Kingdom, Denmark, United States, Germany, and Argentina.

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Manuscript received February 25, 2018; revised manuscript received August 6, 2018, accepted August 14, 2018.

Of the probands, 3,189 had a diagnosis of HCM. An additional 2,777 index cases with no evidence of structural cardiac disease (mainly channelopathies and aortic diseases) were used as control subjects. The predominant ethnicity was European (>90% of the probands), and there were no differences between HCM probands and control subjects. The variants' frequencies in the general population were extracted from the gnomAD database version r2.0, August 2017 (6).

In the initial screening phase, candidate variants in *FHOD3* were identified and their frequencies compared in HCM versus control subgroups. We applied an MAF (minor allele frequency) threshold of 5×10^{-5} to consider a variant a candidate, following the same arguments used by Walsh et al. (7) to reassess gene pathogenicity in cardiomyopathies; we also excluded variants with an MAF $\geq 1\times 10^{-4}$ in any subpopulation of gnomAD to avoid variants detected in cases could be enriched in 1 specific population. Only unrelated index cases were included in the screening phase of the study. Patients with cardiomyopathies other than HCM and sudden cardiac death (SCD) victims were excluded from the analysis.

In the second phase, HCM probands carrying candidate variants in *FHOD3* were invited to participate in segregation studies. Clinical and genetic familial cascade screening was performed following written informed consent in those who agreed to participate. Carriers of variants in other sarcomeric genes that could be related to the phenotype (pathogenic, likely pathogenic, or of uncertain significance in a priority gene) were excluded. The clinical characteristics and outcomes in carriers of pathogenic or likely pathogenic variants in *FHOD3* with HCM (including probands and relatives) were assessed. The study protocol was approved by the Research Ethics Committee of A Coruña-Ferrol (registry code 2015/576).

GENETIC STUDIES, VARIANT FILTERING, AND VARIANT CLASSIFICATION. Coding exons and intronic boundaries of 213 genes related to inherited cardiovascular diseases and SCD (Online Table 1) were captured using a custom probe library (SureSelect Target Enrichment Kit for Illumina paired-end multiplexed sequencing method, Agilent Technologies, Santa Clara, California) and sequenced using the HiSeq 1500 platform (Illumina, San Diego, California) following lllumina protocols. The read depth (number of times that a base was sequenced by independent reads) of every nucleotide of genes related to the referring phenotype (including *FHOD3*) was >30× (mean 250× to 400×). Exons that did not fulfill this standard were complementary sequenced using the Sanger method. Only likely protein-altering variants (missense, in-frame insertions/deletions, frameshift, nonsense, and consensus splice site mutations) in the most relevant, longest transcript of the *FHOD3* gene (NM_001281740.1; 1,644 amino acids) were analyzed. Bioinformatics analysis was performed by means of a custom pipeline including software for variant calling, genotyping, and annotation.

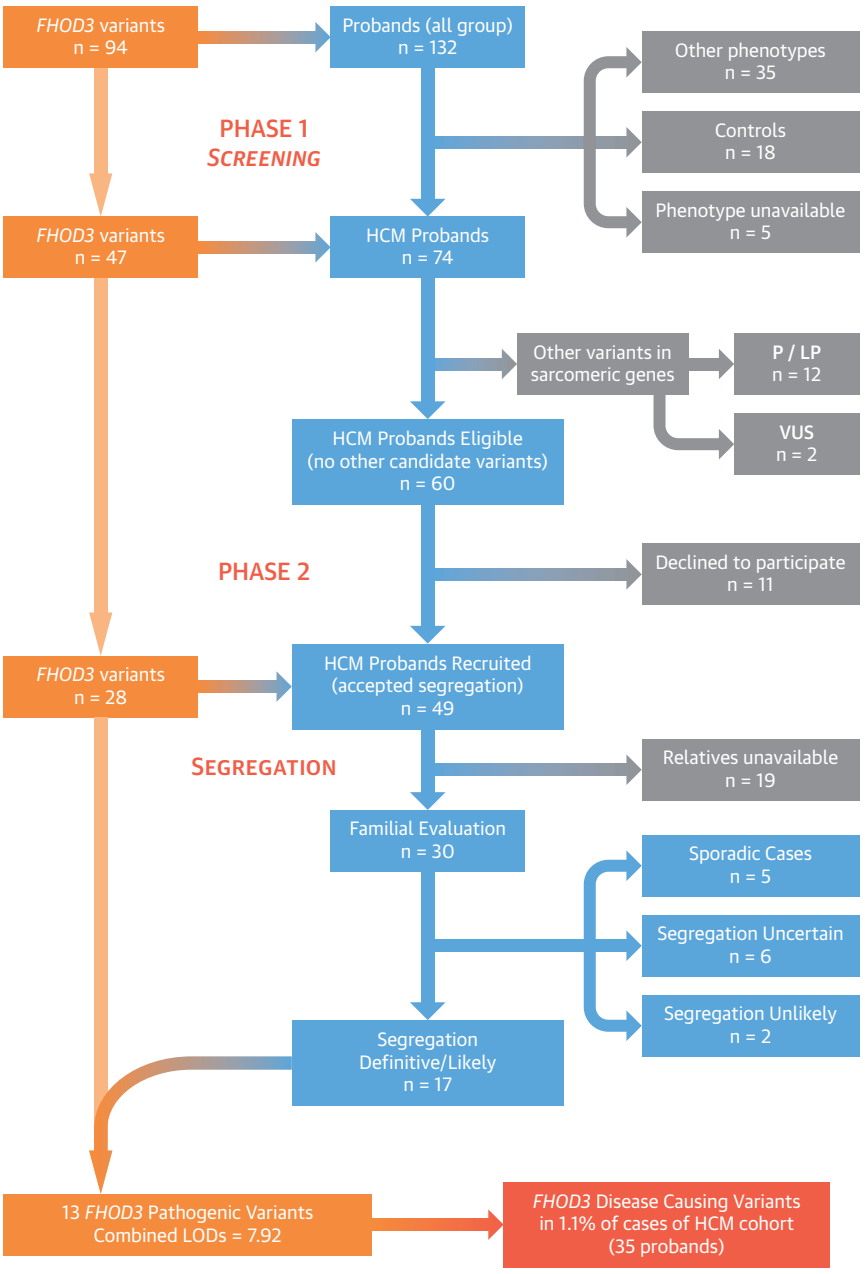
To establish the pathogenicity of identified variants, we developed a customized classification scheme based on the recommendations of the American College of Medical Genetics and Genomics (Online Table 2) (8); the final classification of each variant was agreed by consensus between 2 cardiologists with experience in interpretation of genetic variants.

STATISTICAL ANALYSIS. Continuous variables were expressed as mean ± SD, and comparison between groups was performed using the Student's *t*-test or the Mann-Whitney *U* test according to values distribution. Noncontinuous variables were expressed as an integer number (percent of total) and compared using the chi-square test or Fisher exact test, as appropriate. A 2-sided p value <0.05 was considered to indicate statistical significance. Analysis was performed using R version 3.4.3 (R Foundation for Statistical Computing, Vienna, Austria).

LOGARITHMS OF ODDS SCORE CALCULATION. We calculated 2-point logarithm of the odds (LOD) score for informative families by using the PARAMLINK package for R software (9). The model was set with $\theta = 0$, phenocopy rate = 0.005, and 2 different penetrance values: 0.80 and 0.95. An indeterminate status was assigned to family members with a confounding cardiac diagnosis, as well as to men younger than age 45 years and women younger than age 50 years who did not meet clinical criteria for HCM and could develop the disease afterwards.

SURVIVAL ANALYSIS. The cumulative probability of cardiovascular death in carriers of disease-causing mutations in *FHOD3* after a diagnosis of HCM (follow-up) was estimated using the Kaplan-Meier method. Cardiovascular death was defined as the presence of sudden cardiac death, appropriate defibrillator shock, heart failure death, or heart transplantation. The beginning of the follow-up was established as the first clinical visit when the patient had a diagnosis of the disease (unaffected carriers and SCD cases in which HCM was diagnosed postmortem were excluded from this analysis).

FIGURE 1 Flow Chart of the Screening and Segregation Phases of the Study

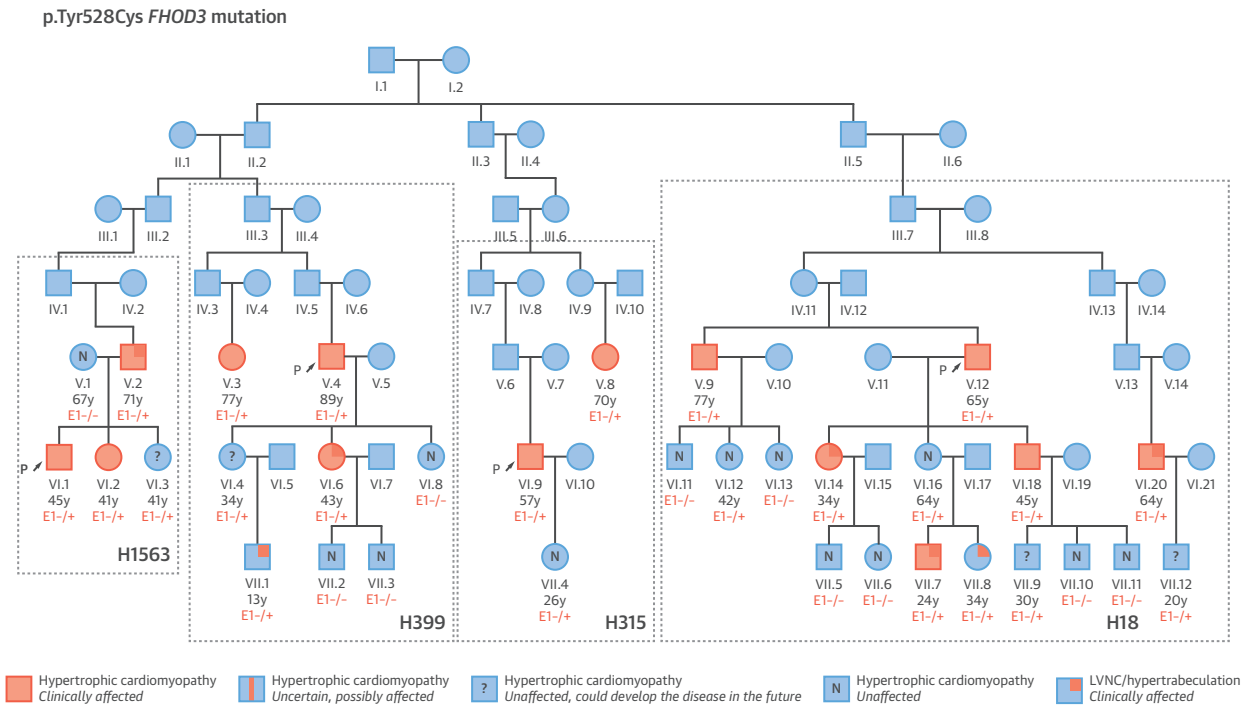


FHOD3 = formin homology 2 domain containing 3 gene; HCM = hypertrophic cardiomyopathy; P/LP = pathogenic/likely pathogenic; SCD = sudden cardiac death; VUS = variant of uncertain significance.

RESULTS

STUDY POPULATION. A total of 94 candidate variants in *FHOD3* distributed in 132 probands were identified, representing 1.67% of the 7,881 consecutive unrelated probands who were sequenced. Of these variants, 90 were nontruncating (88 missense, 2 in-frame deletion/insertion) and 4 were truncating (3 nonsense, 1 frameshift). HCM was the diagnosis in 74 of the 132 probands (56.1%) who carried a candidate variant in *FHOD3*. In 5 probands the phenotype was unavailable, and 18 individuals were control subjects without evidence of structural cardiac disease. A total of 35 patients were excluded from the

FIGURE 2 Pedigree of a Multigenerational Spanish Family



The p.Tyr528Cys variant in *FHOD3* was identified independently in 4 index cases belonging to 4 nuclear subfamilies **(arrows)** from the same region of Murcia, Spain. A subsequent genealogic analysis determined that they were descendants of a common ancestor born 7 generations ago.

analysis: 27 patients had a diagnosis of a cardiomyopathy other than HCM (18 dilated cardiomyopathy [DCM], 5 arrhythmogenic cardiomyopathy, 3 left ventricular noncompaction, and 1 restrictive cardiomyopathy), while 8 patients were SCD victims. A detailed description of the variants, population frequencies, bioinformatics predictors, initial estimation of pathogenicity, and the phenotype in carriers are shown in Online Table 3 and Online Figure 1.

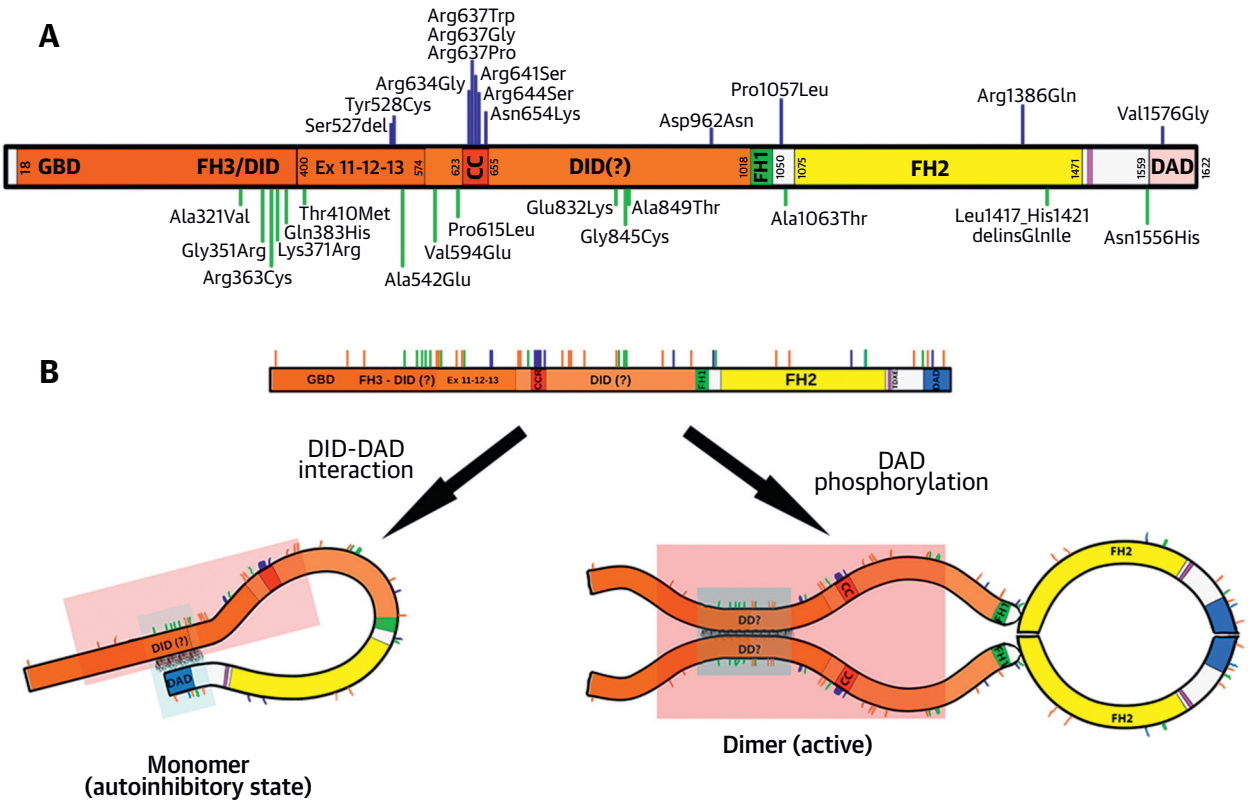
The prevalence of candidate variants in *FHOD3* was higher in the HCM cohort (2.32%; 47 variants in 74 of 3,189 probands) than in disease control subjects (0.65%; 17 variants in 18 of 2,777) or in the gnomAD database (0.76%; 587 variants in 1,049 of 138,606 individuals), with an odds ratio (OR) of 3.64 (95% confidence interval [CI]: 2.17 to 6.11; $p < 0.001$) and 3.02 (95% confidence interval: 2.45 to 3.95; $p < 0.001$), respectively.

SEGREGATION STUDY. Of the 74 probands with a diagnosis of HCM, 14 were excluded because they were carriers of additional variants in a sarcomeric gene that could be related to the phenotype: 12 of 74 (16.2%) a pathogenic or likely pathogenic variant (9 in *MYBPC3* and 3 in *MYH7*) and 2 of 74 (2.7%) a variant of unknown clinical significance (1 in *MYBPC3* and 1 in *MYL2*) (Online Table 4).

Finally, 49 HCM probands carrying 27 candidate variants in *FHOD3* recruited from 27 different centers accepted to participate in the segregation phase of the study, as shown in the study flow chart (**Figure 1**). Clinical assessment was possible in the relatives of 30 probands (Online Figure 2, Online Table 5). The presentation was familial (at least 1 affected family member) in 25 families (83%), and 5 cases (17%) were sporadic (there was no family history, and none of the evaluated relatives were affected).

After clinical evaluation and genotyping of 129 members from 25 families, a final combined LOD score of 7.92 was obtained, equivalent to a p value $<3 \times 10^{-7}$, indicative of very strong cosegregation (Online Table 6) (9). A total of 13 variants were considered pathogenic or very likely pathogenic; in all of them, there was evidence of cosegregation with HCM in at least 1 family (Online Table 7). Disease-causing variants were distributed in 35 HCM probands without additional variants that could explain the disease in sarcomeric genes,

FIGURE 3 Molecular Organization of FHOD3 Protein and Relevant Domains for Actin Binding and Protein-Protein Interactions



(A) The distribution across the FHOD3 protein of the mutations considered pathogenic/likely pathogenic **(blue, top)** and of uncertain significance **(green, bottom)** in HCM patients who participated in the segregation study. **(B)** The same distribution can be observed in the schematic representation of the protein, with the addition of the variants detected in HCM probands who did not participate in the Segregation Phase of the study **(orange)**. The intramolecular interaction between the DID and DAD domains, which prevents formin from nucleating actin filaments, is relieved by the phosphorylation of serine/threonine DAD residues by the ROCK protein **(B)**. The regulation of dimerization to an active state may be more complex, including a dimerization domain (DD). Although in some formin proteins, such as FHOD1 and mDia1, DID and DD domains are clearly concentrated in specific regions, in FHOD3 it could be more complex and involve a wider zone **(pink)**. CCR = coiled-coil region; DAD = diaphanous auto-regulatory domain; DD = dimerization domain; DID = diaphanous inhibitory domain; FH = formin homology domain; GBD = GTPase-binding domain.

representing 1.1% (35 of 3,189) of the entire HCM cohort.

In 6 families, segregation analysis was uncertain, and was unlikely in 2 (the probands were carriers of the variants p.Lys371Arg and p.Glu832Lys) (Online Figure 2, panel 1B). It was not possible to determine the pathogenicity of 14 variants that were finally classified as of unknown clinical significance.

The most commonly identified variants affected consecutive amino acids. The variant p.Tyr528Cys was identified in 6 probands, 4 of whom came from the same region of Spain and were proved to be descendants of a common ancestor born 7 generations ago (**Figure 2**). In this multigenerational family, the variant cosegregated with the disease with the highest LOD score (3.82) for an individual family. The other 2 probands came from centers from the United Kingdom and Denmark. The variant p.Ser527del, affecting the previous residue, was detected in 12 HCM probands from different geographical areas in Spain, Denmark, and the United Kingdom as well. None of these probands had additional variants in sarcomeric genes that could explain the disease, and cosegregation with the phenotype was confirmed in the 5 families with relatives available for evaluation. Among the remaining variants, 7 were clustered in a conserved small coiled-coil domain (amino acids 622 to 655) (**Figure 3A**, Online Figure 3). The OR of the presence of a candidate variant in *FHOD3* in this coiled-coil domain in HCM was 21.8 against control subjects (95% confidence interval [CI]: 1.3 to 37.9; $p < 0.001$) and 14.1 against gnomAD individuals

(95% CI: 6.9 to 28.7; p < 0.001). The complete list of the pathogenic or likely pathogenic variants and the distribution along the FHOD3 protein is summarized in Figure 3A. The variants identified in control subjects or in phenotypes other than HCM were randomly distributed throughout the gene, but none of them was identified in the coiled-coil domain or affecting residues 527 to 528 (Online Figure 1).

Only 2 truncating variants in *FHOD3* were identified in HCM cases: p.Lys1433Serfs*10 and p.Arg1597*. Both were present at very low frequencies in control subjects (MAF% 0.002 and 0.0004, respectively). The p.Lys1433Serfs*10 variant was identified in a patient who carried an additional variant, p.Arg17Gln, in *MYBPC3;* although it was excluded from the analysis, segregation was evaluated in the family, but the results were inconclusive (Online Figure 2). The nonsense variant p.Arg1597* was identified in 2 probands: 1 was a sporadic case with a nonsevere phenotype diagnosed after age 75 years and the second was a patient with restrictive cardiomyopathy (the variant did not segregate with the disease in this family). Both variants were considered of uncertain significance.

CLINICAL CHARACTERISTICS OF HCM PATIENTS CARRYING DISEASE-CAUSING VARIANTS IN *FHOD3*. Demographic, clinical, echocardiographic, and electrocardiographic characteristics of HCM carriers of disease-causing variants in *FHOD3* are shown in Table 1. Two-thirds (66.7%) of the probands were males; this predominance was also observed in affected relatives (56.8% vs. 43.2%). There was no difference in the age at diagnosis between index cases and relatives. Most of the patients were diagnosed after age 30 years (median 58 years in women and 48 years in men). Incomplete penetrance was observed: at age 70 years, 15% of men and 32% of women were clinically unaffected (Figure 4A).

Approximately 40% of the patients were symptomatic, with dyspnea as the most common symptom. The predominant HCM subtype was asymmetric septal hypertrophy (82%). The degree of hypertrophy was mild to moderate (mean 18.8 ± 5 mm), and the presence of massive hypertrophy (≥30 mm) was exceptional. Left ventricular outflow tract obstruction and systolic anterior movement of the mitral valve were present in 21% and 12.5% of the patients, respectively. Left ventricular systolic dysfunction (ejection fraction <50%) was described in 14% of the patients. Hypertrabeculation of the left ventricle was present in 16% (8 of 49) of the patients, one-half of

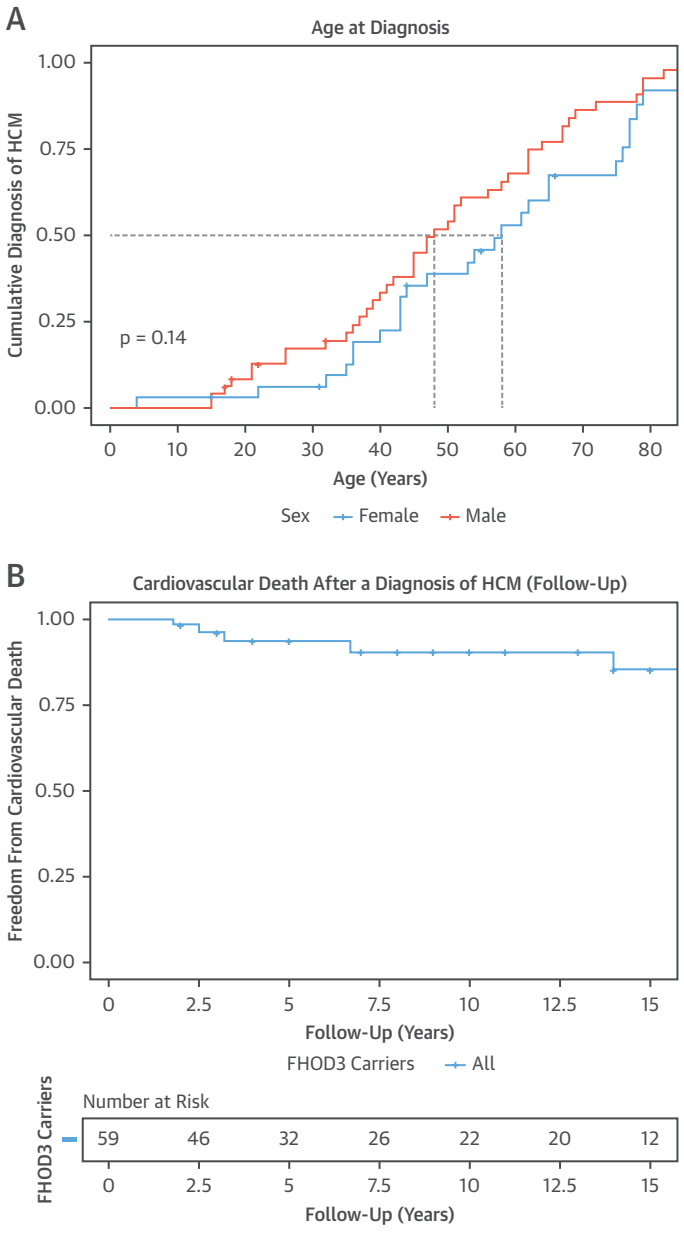
TABLE 1 Clinical Characteristics of HCM Patients Carrying Pathogenic or Likely Pathogenic Variants in *FHOD3* Stratified by Sex

	Overall (N = 70)	Male (n = 43)	Female (n =27)	p Value
Cardiac structural affection				
HCM subtype				
Septal asymmetric	35/41 (85.4)	22/25 (88)	13/16 (81.3)	NS
Concentric	4/41 (9.8)	2/25 (8.0)	2/16 (12.5)	NS
Apical	2/41 (6.3)	1/25 (4.0)	1/25 (4.0)	NS
LVMWT, mm	18.8 ± 5.4	18.9 ± 5.4	18.6 ± 5.6	NS
LVMWT >30 mm	3/64 (4.7)	1/39 (2.6)	2/25 (8.0)	NS
LVMWT >25 mm	7/64 (10.9)	4/39 (10.3)	3/25 (12.0)	NS
LV dilatation	2/55 (3.6)	0/31 (0.0)	2/24 (8.3)	NS
LVEDD, mm	45.7 ± 5.9	46.8 ± 5.2	43.8 ± 6.7	NS
LV dysfunction (EF <50%)	6/46 (13.0)	1/25 (4.0)	5/21 (23.8)	0.079
EF, %	61.5 ± 11.9	64.8 ± 8.5	57.5 ± 14.2	0.046
Hypertrabeculation	8/49 (16.4)	5/27 (18.5)	3/22 (13.6)	NS
Positive LVNC criteria	4/49 (8.2)	1/27 (3.7)	3/22 (13.6)	NS
Negative LVNC criteria	4/49 (8.2)	4/27 (14.8)	0/22 (0.0)	NS
LA dilatation	19/39 (48.7)	11/22 (50.0)	8/17 (47.1)	NS
LA, mm	41.7 ± 8	44.4 ± 8.2	38.2 ± 7.9	0.026
LVOTO	9/42 (21.4)	4/24 (16.7)	5/18 (27.8)	NS
SAM (MV)	5/40 (12.5)	4/22 (18.2)	1/18 (5.6)	NS
CMR LGE	11/17 (64.7)	6/10 (60.0)	5/7 (71.4)	NS
ECG and arrhythmias				
ECG rhythm				
Sinus rhythm	48/55 (87.3)	26/30 (86.7)	22/25 (88.0)	NS
Atrial fibrillation	6/55 (10.9)	4/30 (13.3)	2/25 (8.0)	NS
Pacemaker	1/55 (1.8)	0/30 (0.0)	1/25 (4.0)	NS
ECG LVH	39/49 (79.6)	19/27 (70.4)	20/22 (90.9)	0.076
Atrial fibrillation history				
Permanent	8/58 (13.8)	5/33 (15.2)	3/25 (12.0)	NS
Paroxysmal	3/58 (5.2)	0/33 (0.0)	3/25 (12.0)	NS
NSVT	11/41 (26.8)	4/23 (17.4)	7/18 (38.9)	NS
Frequent PVC	6/40 (15.0)	3/23 (13.0)	3/17 (17.6)	NS
ICD implanted	10/58 (17.2)	5/33 (15.2)	5/25 (20.0)	NS
History and symptoms				
Age at diagnosis	46.1 ± 18.7	43.6 ± 18.2	50.9 ± 19.2	0.047
Hypertension	8/43 (18.6)	3/26 (11.5)	5/17 (29.4)	NS
Dyspnea				
NYHA functional class II	19/59 (32.2)	9/34 (26.4)	10/25 (40.0)	NS
NYHA functional class III–IV	6/59 (10.1)	1/34 (2.9)	5/25 (20.0)	0.069
Syncope	5/56 (8.9)	4/31 (12.9)	1/25 (4.0)	NS
Events				
Cardiovascular death (overall)	13/68 (19.1)	10/41 (24.4)	3/27 (11.1)	NS
Sudden cardiac death	11/68 (16.2)	9/41 (22.0)	2/27 (7.4)	NS
Heart failure death/heart transplantation	2/68 (2.9)	1/41 (2.4)	1/27 (3.7)	NS
Stroke	5/64 (7.8)	2/38 (5.3)	3/26 (11.5)	NS

Values are n/N (%) or mean ± SD.

CMR LGE = late-gadolinium enhancement in magnetic resonance images; ECG = electrocardiogram; EF = ejection fraction; ICD = implantable cardioverter-defibrillator; LA = left atrium; LV = left ventricle; LVEDD = left ventricular end-diastolic diameter; LVH = left ventricular hypertrophy; LVMWT = maximal left ventricular wall thickness; LVNC = left ventricular noncompaction; LVOTO = left ventricular outflow tract obstruction; NSVT = nonsustained ventricular tachycardia; NYHA = New York Heart Association; PVC = premature ventricular contractions; SAM (MV) = systolic anterior movement of the mitral valve.

FIGURE 4 Cumulative Distribution of Age at Diagnosis and Survival Free of Cardiovascular Death During Follow-Up After the Diagnosis of HCM



(A) The cumulative distribution of age at diagnosis (penetrance) stratified by sex. **(B)** The freedom from cardiovascular death of the patients after a diagnosis of HCM was made (only affected carriers with clinical follow-up are included in this analysis). *FHOD3* = formin homology 2 domain containing 3 gene; HCM = hypertrophic cardiomyopathy.

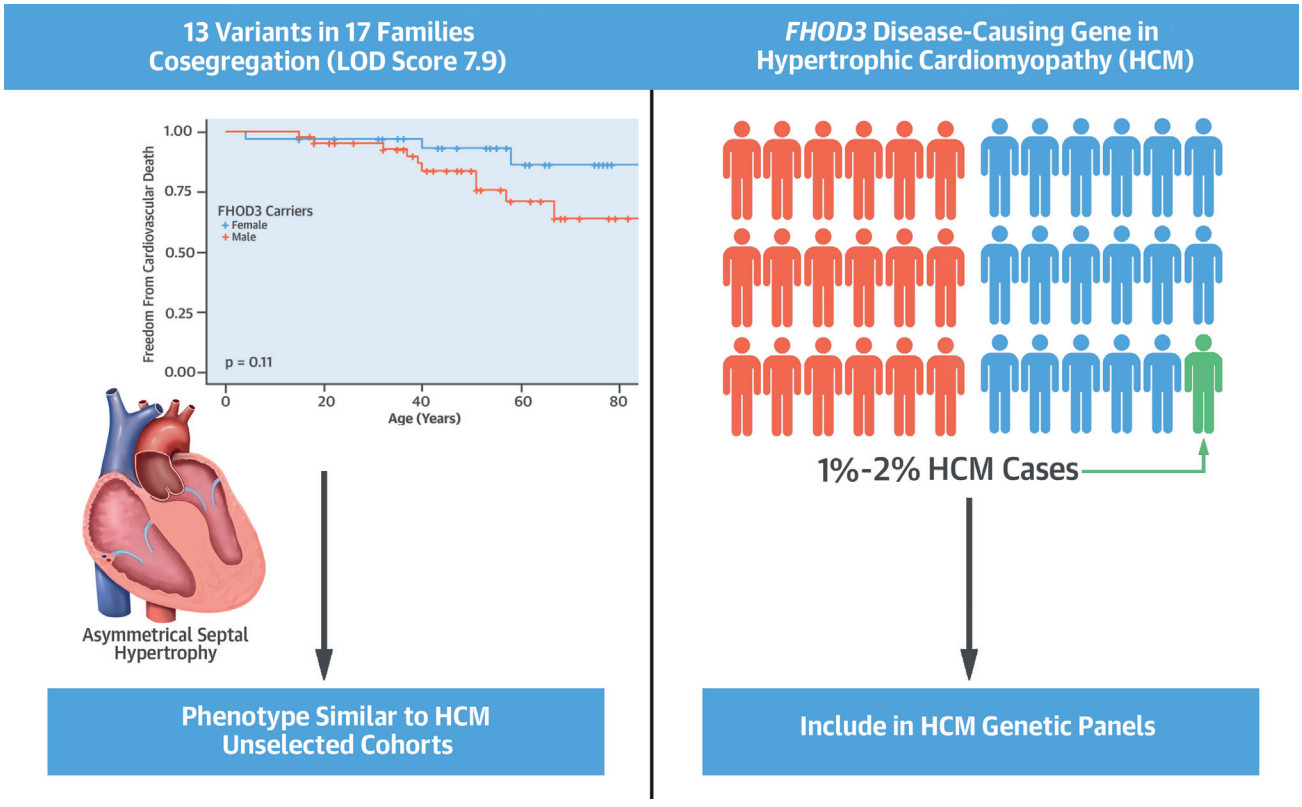
whom (4 of 49; 8%) fulfilled the criteria for left ventricular noncompaction.

EVENTS. A total of 13 of the 81 carriers/affected relatives (16%) experienced a cardiovascular death. The incidence was higher in men (10 of 48; 20.1%) than in women (3 of 33; 7.3%), but did not reach statistical significance (p = 0.16). Events were rare before the age of 30 years. The annual cardiovascular death incidence after the diagnosis of HCM was approximately 1% per year (**Figure 4B**) with a median of follow-up of 5 years in the group (range 1.8 to 18 years). SCD was the most frequent cause (11 of 13; 85%), being the first manifestation of the disease in 4 probands; the diagnosis was made after successful resuscitation from cardiac arrest in 2 and on autopsy in 2 other cases, 1 of whom was a compound heterozygous carrier of p.Arg1386Gln and p.Pro615Leu in *FHOD3* (Online Figure 2). The other 7 were relatives in whom genetic testing was unavailable. Two patients (2 of 13; 15%) experienced heart failure death: a 58-year-old woman who had a concomitant diagnosis of rheumatic severe mitral stenosis and a 37-year-old man with a restrictive filling pattern.

DISCUSSION

In this study, we demonstrated a clear relationship between *FHOD3* mutations and the development of HCM (**Central Illustration**). Almost 7,800 probands were screened for the presence of very rare candidate variants in *FHOD3*, and an excess of these variants in the HCM cohort with respect to control subjects was observed (OR: 3). This ratio is lower than those obtained by other groups using the same approach for nontruncating variants in *MYBPC3* (OR: 5.7) or *MYH7* (OR: 12) (7), reflecting that not all of the very rare candidate variants in *FHOD3* are necessarily associated with disease. As in other sarcomeric genes, it is difficult to predict if a novel nontruncating variant is pathogenic or not; in these cases, segregation analysis in the family is mandatory. In our study, cosegregation of several variants in *FHOD3* was confirmed, yielding a combined LOD score of 7.95, which is indicative of very strong cosegregation (highly significant linkage) (10). Disease-causing variants in *FHOD3* accounted for at least 1% of HCM cases. Taking into account that in this study we used a very strict strategy to consider a variant disease-causing (only variants with evidence of segregation), that segregation was evaluable in only one-half of the candidate variants, and that cases with additional potentially pathogenic variants in other sarcomeric genes were excluded, it is likely that *FHOD3* mutations account for approximately 2% of HCM cases and 4% of positive genetic studies. This prevalence would be similar to or greater than the reported prevalence of mutations in established sarcomeric protein genes, such as *TPM1, MYL2, MYL3, ACTC1*, and *TNNC1* (11,12).

CENTRAL ILLUSTRATION ***FHOD3* is a Novel Disease-Causing Gene in HCM**



Ochoa, J.P. et al. J Am Coll Cardiol. 2018;72(20):2457–67.

Diagram illustrating the results and main messages of the study. Around 50% of the genetic tests performed in hypertrophic cardiomyopathy (HCM) are negative; *FHOD3* plays a role in sarcomere organization, but was considered only a candidate gene. After evaluating >7,800 probands, we identified disease-causing variants in *FHOD3* cosegregating with HCM in several families and accounting for approximately 1% to 2% of HCM cases. *FHOD3* should be routinely included in HCM genetic testing panels. *FHOD3* = formin homology 2 domain containing 3 gene; LOD = logarithm of the odds.

The phenotype associated with *FHOD3* mutations in our study was relatively mild, with a low rate of adverse events in young individuals and an overall rate of cardiovascular endpoints similar to that described in unselected HCM patient cohorts (12,13). Two-thirds of the index cases were men, and the diagnosis in female carriers was made 10 years later than in males. Late onset of the disease and incomplete penetrance were observed in both sexes.

The association between *FHOD3* and HCM has been postulated in a GWAS study (5). The authors identified 2 frequent polymorphisms in *FHOD3* (1 deep intronic variant and the missense variant p.Val1326Ile, both with MAFs ≥30%) that were more frequent in HCM cases than in control subjects, but failed to demonstrate a difference in phenotype expression between homozygous and heterozygous carriers of the p.Val1326Ile variant. These findings can be considered an association (a signal in the GWAS study) between the gene and the phenotype, but no description of any variant in *FHOD3* causing HCM has been published to date. The explanation for this might be that most diagnostic laboratories do not include *FHOD3* in their sequencing panels; thus, very rare protein-altering candidate variants are not being identified. Alternatively, it can be difficult to detect an association in a GWAS when mild phenotypes with incomplete penetrance are present, and this type of study focuses on detecting frequent polymorphisms but is not designed to detect very rare variants.

A possible association of *FHOD3* mutations with DCM has also been suggested; there is a report of a small Japanese family (only 2 members were genotyped) in whom an in vivo functional study showed that the identified variant impaired actin filament assembly (14), and previous functional studies in animal models have demonstrated that FHOD3 plays

a role in heart development (15). Similarly, an exome-wide association study identified *FHOD3* as 1 of 8 loci independently associated with sporadic DCM (16). Our study was not designed to determine the possible causal relationship between *FHOD3* and DCM. Of the 130 probands carrying candidate variants in *FHOD3*, 18 had this phenotype (they were excluded in the screening phase of the study). These variants were not clustered in any particular region of the gene, and most of them were classified as of uncertain significance at initial evaluation. Further studies are needed to establish the clinical relevance of *FHOD3* mutations and their relationship to DCM.

The relationship between FHOD3 and ventricular hypertrophy has been recently explored in a study of angiotensin II–induced cardiac hypertrophy (17). Under basal conditions, the FHOD3 protein exists in an auto-inhibited state due to the interaction between its diaphanous autoregulatory domain and the diaphanous inhibitory domain (**Figure 3B**). The phosphorylation of specific phospho-acceptor residues located in the autoregulatory domain (Ser1590, Ser1596, and Thr1600) by the RhoA/ROCK pathway seems to inhibit the interaction with the inhibitory domain, allowing the FHOD3 protein to form active dimers that enable actin nucleation and assembly of myofibrils in cardiomyocytes, thereby causing cellular hypertrophy (18). Several *FHOD3* candidate variants in this study were located in the autoregulatory and inhibitory domains or their surrounding residues (**Figure 3A**, Online Figure 1); 1 hypothesis is that these mutations alter the normal interaction, leading to a predominance of FHOD3 protein in an activated state, a mechanism that has been described for mutations in the paralogue *FHOD1* (19,20).

FHOD3 protein dimerization to an activated state may be more complex, including a dimerization domain and a helical region (coiled-coil domain) that forms a cross-bridge between the opposing chains and provides the conformational flexibility required for the stair-stepping actin polymerization mechanism (21). One-half of the disease-causing variants found in our study were located in the coiled-coiled domain that seems to be specific for a HCM phenotype (no disease controls or patients with cardiomyopathies other than HCM where identified in it). The exact location and functional characterization of the inhibitory and dimerization domains in FHOD3 are still not well understood; 2 clearly pathogenic variants that cosegregated with HCM in several families (p.Ser527del and p.Tyr528Cys) were found near these domains. They also affect an exon that is only present in the longest transcript of *FHOD3*, which is currently considered the most important isoform in the adult ventricular myocardium, because it contains an additional exon that is required for targeting the FHOD3 protein to the myofibrils in cardiomyocytes (22). Our findings support the relevance of this isoform in the pathogenesis of HCM, although functional studies are needed to determine the exact mechanisms underlying the development of HCM in *FHOD3* mutation carriers.

STUDY LIMITATIONS. One of the limitations of our study is that segregation was limited in some families because of the small number of relatives available for screening, and clinical assessment was incomplete in some carriers. Another possible limitation is that patients were screened only for genes previously associated with inherited cardiac conditions; therefore, the presence of mutations in other genes contributing to the phenotype cannot be ruled out.

CONCLUSIONS

In this study, we have demonstrated that *FHOD3* is a novel disease-causing gene in HCM. Pathogenic mutations would account for approximately 1% to 2% of HCM cases, a prevalence that is similar or greater to that described in other secondary sarcomeric protein genes. The associated phenotype and the rate of cardiovascular events are similar to that described for unselected cohorts of patients with HCM; thus, a clinical follow-up is recommended in affected carriers. The *FHOD3* gene should be routinely included in genetic testing panels for HCM.

ACKNOWLEDGMENTS The authors thank Pilar Molina (Hospital Universitario y Politécnico La Fe, Valencia, Spain), Petros Syrris (UCL Institute of Cardiovascular Science, London, United Kingdom), Carmen Benito-Lopez y Sara Franco-Freire (Hospital Regional Universitario "Carlos Haya"), Juan de D. García-Diaz (Hospital Universitario Príncipe de Asturias, Alcalá de Henares, Madrid, Spain), Cristina Gomez-Ramirez, (Hospital Universitario Cruces, Barakaldo, Vizcaya, Spain), Iria C. Duro (Hospital Clínico Universitario de Valladolid, Valladolid, Spain), and Javier Limeres-Freire (Hospital Val d'Hebron, Barcelona, Spain), as well as Alicia Pallas Lozano and Radek Suchac for their help with English editing.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: *FHOD3* accounts for approximately 1% to 2% of cases of HCM, and its prevalence is similar to or greater than that of mutations in established sarcomeric protein genes.

TRANSLATIONAL OUTLOOK: Collaborative studies of a larger number of patients are necessary to identify an association with other phenotypes and expose mechanisms underlying the development of HCM in carriers of *FHOD3* disease-causing variants.

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KEY WORDS cardiomyopathies, *FHOD3*, formins, genetics, hypertrophic cardiomyopathy, sudden death

APPENDIX For a list of the recruiting hospitals of the GENESCOPIC research group and research centers (genetics, molecular biology, and data-analysis) of the GENESCOPIC research group as well as supplemental tables and figures, please see the online version of this paper.