Association of FHOD3 Frameshift Variant with Hypertrophic Cardiomyopathy

Roxanne Oh

Molecular Cardiology Research Institute, Tufts Medical Center

Dr. Gordon Huggins

Introduction

Hypertrophic cardiomyopathy (HCM) is a condition characterized by the abnormal thickening of the heart muscle, often most affecting the left ventricle septum, not explained by excess physiological demand. HCM is found in 1 out of 200 to 1 out of 500 people and can be a familial disorder triggered by a sarcomere gene mutation. While rare variants in sarcomere genes myosin heavy chain (MYH7) and myosin binding protein-C (MYBPC) are most commonly responsible for "sarcomeric HCM", this disease is genetically heterogeneous with more than ten genes implicated in its cause. People with HCM who are not found to have a sarcomere gene mutation are diagnosed with "nonsarcomeric HCM", the etiology of which is an area of continued controversy. Individuals with sarcomeric HCM tend to be diagnosed at a younger age due to earlier presentation and greater severity of symptoms. Among individuals with HCM and even within families, HCM often exhibits incomplete penetrance and variable expressivity(Wooten, 2012).

Formin 2 Homology Domain 3 (FHOD3) is a protein that is physically tethered to the sarcomere and has two isoforms created by differential exon usage. In contrast to the FHOD3 Short isoform, the FHOD3 Long isoform contains additional exons. The unique FHOD3 Long amino acid sequences include a casein kinase 2 (CK2) phosphorylation site (Iskratsch, 2010) and a domain necessary for interaction with MYBPC3. The FHOD3 Long isoform regulates sarcomere organization in cardiomyocytes and early myofibrillogenesis (Kan-O, 2012). Both rare and common FHOD3 variants are associated with HCM (Choi, 2025; and Wooten). Rare FHOD3 variants associated with HCM are commonly found in exons encoding the FHOD3 "coiled-coil" domain (Ochoa, 2018). Such variants are of interest as they play a crucial role in forming the secondary structure of FHOD3. Currently, the role of exons specific to FHOD3 Long are undefined.

The purpose of this study was to characterize the clinical phenotype and association of rare FHOD3 variants found in the Tufts Medical Center HCM Database. Our study tested two hypotheses. First we hypothesized that FHOD3 variants, particularly those located in its coiled-coil domain, would be more prevalent in the Tufts HCM Cohort compared with the reported frequencies in gnomAD. Second, because the FHOD3 Long isoform is physically attached to the sarcomere, we hypothesized that people with HCM who carry a FHOD3 variant would have a form of HCM more similar to people with sarcomeric HCM.

Methods

Tufts Medical Center's database of 926 patients was used for this study. Data collected on these patients included age at diagnosis, family history of HCM, presence of health conditions and echocardiogram data.

Statistical Analyses

Patients were divided into groups based on sarcomeric and non-sarcomeric HCM. Several clinical phenotypes were compared including the age of clinical diagnosis of HCM and the age at which a clinically significant event occurred following diagnosis with HCM. A chi-squared analysis was used to determine significance between categorical variables while an independent sample t-test was used to determine significance between continuous variables. All statistical analyses were performed in Jasp. $p<0.05$ was considered significant. NS=not significant.

Next, the sample frequency for each rare FHOD3 mutation was calculated. Since the cohort was predominantly white, the sample frequency was compared to the general population frequency of the European population in the gnomAD database. A chi-squared analysis was done to determine significance. A Bonferroni calculation was performed to determine the threshold of significance. Since 27 mutations were examined, a significant p-value was classified as $p<5.0x10^{-3}$.

Subjects with nonsarcomere HCM (and denoted "Sarc (-) FHOD3 (-)") that did not carry a rare sarcomere gene variant or a FHOD3 variant were used as a control or reference group. The same variables used in Table 1 were compared between our control group, nonsarcomeric patients carrying a rare FHOD3 variant outside of the coiled-coil region, and nonsarcomeric patients carrying a rare FHOD3 variant located in the coiled-coil region. Patients that had both sarcomeric HCM and a rare FHOD3 variant were classified under the sarcomeric category. Significance was tested using chi-squared analysis for categorical variables and an ANOVA for continuous variables.

Characteristics of the heart, such as maximum thickness, ejection fraction, and LVEDD were measured using echocardiogram. These variables were compared between the control group, sarcomeric patients, and nonsarcomeric patients with a FHOD3 variant. An ANOVA was used to determine significance. The same analysis was performed to examine the difference between nonsarcomeric patients with versus without a FHOD3 variant, specifically at position 637 or 638. Analysis was performed using an independent sample t-test.

Similarly, phenotypic features, demographics, and echocardiogram data was compared between the control group and patients with the frameshift variant rs 144071785. Significance was determined using an independent sample t-test.

PCR of Mutations

We also created two different plasmids: one with the FHOD3 Arg637Gln mutation and another with the FHOD3 638 R to W mutation. A forward and reverse primer were generated for each respective mutation. The mutation was introduced into the forward primer for both mutations. Then, a PCR protocol was followed using the New England BioLabs mutagenesis kit to transform this plasmid into *E. coli* cells. Colonies were picked and miniprepped using the QIAprep Spin Miniprep Kit. Samples were left in an incubator shaker overnight to grow. Then, viability of each sample was determined using a NotI digest. Viable samples were submitted to Plasmidsaurus for sequencing.

Lastly, three patients with frameshift mutation rs144071785 and three patients without the mutation were identified in the cohort. Forward and reverse primers were generated to amplify the region of interest. PCR was performed using the Invitrogen PCR Purification Kit. Plasmid was transformed into *E. coli* cells. Colonies were picked, miniprepped, and grown in an incubator shaker overnight. Viable samples were submitted to Plasmidsaurus for sequencing.

Mutation	Forward Primer	Reverse Primer

R to Q	GAGAGAGAGGcagCGGCAGGAGA	TCTGCTGCCAGTGACTCTTG
R to W	AGAGAGGCGGtggCAGGAGAGAG	CTCTCTGCTGCCAGTGACTCTTG
Frameshift	ACCACCTTTTCTCCTGCGAG	TTTGGCTGACTGTGCTTCCT

Lowercase letters indicate the site of mutation.

Results

Tufts HCM Cohort

We compared the clinical phenotypes of subjects with sarcomeric and nonsarcomeric HCM. Subjects with sarcomeric HCM group had a younger age at diagnosis (p<0.001) and lower composite score (p=0.003) than the nonsarcomeric group (Table 1). In addition, subjects with nonsarcomeric HCM had a higher frequency of hypertension and hyperlipidemia compared with sarcomeric HCM. There was a significantly greater ratio of men to women in the nonsarcomeric group and a significantly higher incidence of familial history of HCM in the sarcomeric group.

Category	Sarc (-)	Sarc (+)	p-value
total	763	163	-
men	492 (64.5%)	88 (54.0%)	0.012
Family History	186 (24.7%)	54 (33.1%)	0.027
HTN	303 (39.9%)	43 (26.4%)	0.001
HLD	330 (43.5%)	42 (31.3%)	0.008
CKD	20 (2.6%)	0 (0%)	NS
DM	84 (11.1%)	9 (6.8%)	NS
Age at Diagnosis	47.6 (15.0)	40.7 (17.1)	<.001

Composite	52.2 (13.4)	48.0 (17.3)	0.003

Table 1. *Comparison of Demographic and Clinical Variables of Individuals with Sarcomeric HCM versus Individuals with Nonsarcomeric HCM*

Prevalence of FHOD3 Variants

Next, we analyzed each subject's whole exome sequence to identify FHOD3 variants and to assign all affected amino acid position. Using a Bonferroni corrected threshold of significance ($p<5.0x10^{-3}$), variant frequency in the Tufts HCM Cohort were compared with gnomAD; variants found in only a single person were considered to be non-significant. The most prevalent mutations were at positions 637 and 638 (Table 2). Frequency of frameshift rs 1444071785 was extremely significant. In agreement with previous observations (Ochoa), several FHOD3 variants were found to be significantly more prevalent in the Tufts HCM cohort compared with gnomAD. Two novel variants were found: a S/G mutation at position 448 and an N/S mutation at position 1556.

Location	Number of Individuals Observed	Amino Acid/BP Mutation	Variant Prevalence (%)	General Population Frequency (%)	p-value
27	2	R/P	0.22%	0.05%	NS
80	1	R/W	0.11%	0.02%	NS
225	5	A/T	0.54%	0.09%	9.99E-06
310	1	D/N	0.11%	0.00%	1.44E-07
341	1	R/Q	0.11%	0.00%	2.33E-08
356	1	G/S	0.11%	0.01%	1.93E-04
375	1	S/L	0.11%	0.03%	NS
448	1	S/G	0.11%	0.00%	N/A
464	1	L/F	0.11%	0.12%	NS
603	1	S/P	0.11%	0.03%	NS
605	2	P/R	0.22%	0.03%	6.68E-04
622	1	S/F	0.11%	0.00%	1.22E-278
637	39	R/Q	4.21%	0.39%	2.05E-77
638	10	R/W	1.08%	0.10%	3.01E-21
648	3	I/T	0.32%	0.01%	3.13E-19
653	2	R/I	0.22%	0.00%	4.40E-09
654	1	N/D	0.11%	0.00%	6.42E-94
658	1	R/G	0.11%	0.03%	NS

708	1	S/L	0.11%	0.00%	1.98E-10
783	1	A/T	0.11%	0.01%	5.60E-04
1105	1	R/C	0.11%	0.00%	3.94E-15
1221	1	S/N	0.11%	0.12%	NS
1243	1	A/T	0.11%	0.01%	NS
1459	2	R/Q	0.22%	0.01%	3.76E-08
1550	5	G/A	0.54%	0.74%	NS
1556	1	N/S	0.11%	0.01%	1.77E-03
1605	1	T/N	0.11%	0.00%	N/A
rs144071785	68	CAG-->C	7.34%	0.03%	0.00E+00

Table 2. *Frequency of FHOD3 mutations within Tufts HCM Cohort versus Population Frequency of FHOD3 mutations*

An additional finding was the frequency of rs 144071785, a frameshift characterized by two nucleotide deletions, being significantly higher in the Tufts HCM Cohort compared with control (Table 2).

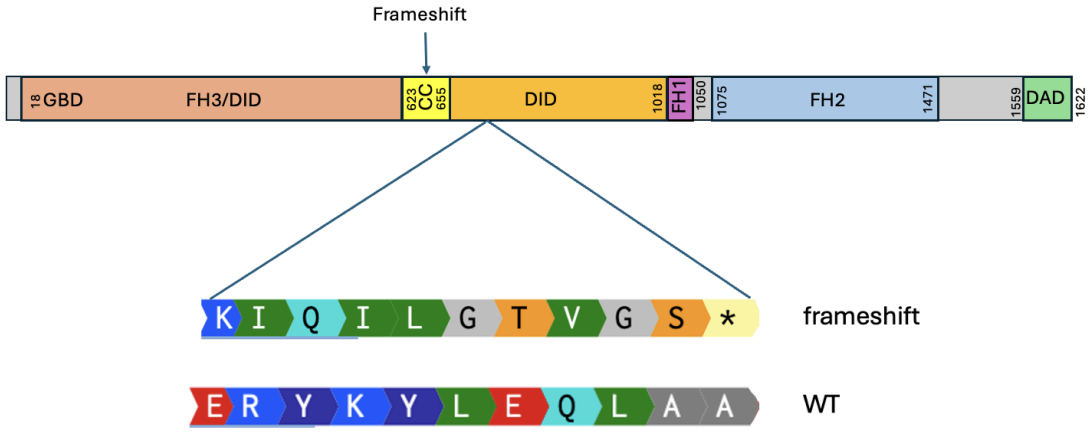

Figure 1. *Location of frameshift mutation is within the coiled-coil (CC) region of FHOD3 unique to the Long isoform around amino acids 634-636. Letters represent amino acid sequences for frameshift variant versus wildtype (WT). * denotes stop codon. Two base-pair deletions cause formation of early stop codon at amino acid 683.*

Clinical Phenotypes of FHOD3-Associated HCM

Clinical parameters of the heart were also investigated and compared across groups. Of significance, sarcomeric HCM patients had a greater maximum wall thickness than nonsarcomeric HCM patients without a FHOD3 mutation. Within the nonsarcomeric group, patients carrying a FHOD3 variant had a slightly larger LVEDD than those who did not.

Next, we compared demographic and clinical information between nonsarcomeric HCM patients with a coiled coiled (CC) FHOD3 variant, nonsarcomeric patients with a non-coiled coiled (non-CC) FHOD3 variant and FHOD3(-) Sarc(-) patients. No difference was found in any of the variables across the three groups (Table 3). This confirmation led us to investigate phenotypes, echocardiogram data and clinical parameters of individuals belonging to this group. There was no significant difference in most variables. However, there was a borderline significant difference in LVEDD size between the control group (Sarc (-) and FHOD3 (-)) and individuals carrying a 637 and 638 mutation.

Category	Sarc (-) FHOD3 (-) N=667	Sarc (+) N=163	FHOD3 (+) N=72	637+638 N=42	p-value
Maximum Thickness	18.4 (4.1)	20.1 (5.7)	18.8 (4.0)	19.3 (4.5)	p_{tukey} (Sarc+ vs Sarc-FHOD3-) <.001
Ejection Fraction	64.2 (6.2)	63.4 (5.7)	64.4 (4.9)	64.0 (4.6)	NS
LA size (mm)	41.8 (7.0)	40.9 (6.7)	41.7 (6.7)	41.7 (6.7)	NS
LVEDD (mm)	42.2 (6.4)	42.2 (7.8)	44.1 (5.9)	44.1 (5.7)	p_{tukey}(FHOD3+ vs Sarc-FHOD3-)=0.054 *p* (637+638 vs Sarc-FHOD3-)=0.059
LVESD (mm)	26.6 (5.5)	26.1 (6.7)	27.8 (5.4)	28.2 (5.1)	NS

Table 3. *Comparison of Heart Characteristics of Nonsarcomeric Individuals without a FHOD3 variant, Sarcomeric Individuals, Nonsarcomeric Individuals with a FHOD3 Variant, and Nonsarcomeric Individuals with a FHOD3 variant at position 637 or 638.*

When demographic, clinical, and heart characteristics of individuals with the frameshift mutation were compared with those of our control group, there was no significant difference in any variables.

Category	Sarc (-) FHOD3 (-)	Frame Shift (+)	p-value
total	689	48	-
men	408 (63.6%)	14 (29.8%)	NS
Family History	154 (24.4%)	14 (29.8%)	NS
HTN	264	14	NS

	(41.4%)	(29.8%)	
HLD	275 (43.2%)	21 (44.7%)	NS
CKD	18 (2.8%)	0 (0%)	NS
DM	877 (12.1%)	2 (4.2%)	NS
Age at Diagnosis	47.8 (15.1)	47.9 (14.0)	NS
Composite	52.2 (13.6)	50.2 (12.6)	NS

Table 4. *Comparison of Demographic and Clinical Variables of Nonsarcomeric Individuals without a FHOD3 variant versus Nonsarcomeric Individuals with a Frameshift Variant*

Category	Sarc (-) FHOD3 (-)	Frame Shift	p-value
Maximum Thickness	18.4 (4.1)	18.7 (3.3)	NS
Ejection Fraction	64.1 (6.3)	65.3 (3.8)	NS
LA Size (mm)	41.8 (7.0)	42.9 (7.9)	NS
LVEDD (mm)	42.2 (6.4)	42.9 (6.3)	NS
LVESD (mm)	26.6 (5.6)	27.1 (4.9)	NS

Table 5. *Comparison of Heart Characteristics of Nonsarcomeric Individuals without a FHOD3 variant versus Nonsarcomeric Individuals with a Frameshift Variant*

Discussion

In this study, we investigated the role of FHOD3 in HCM presentation and compared it to sarcomeric HCM. First, our analyses support that sarcomeric HCM tends to have a more severe phenotype compared to nonsarcomeric HCM, more frequently associated with familial disease, and diagnosis at a younger age. Most notably, patients with HCM demonstrated a greater maximum wall thickness and an overall lower composite score. By comparison, hypertension and hyperlipidemia were higher in the sarcomeric HCM group than the nonsarcomeric HCM group. Our results also affirmed the literature, which has established that sarcomeric HCM tends to be diagnosed at a younger age (Choi, 2025).

Furthermore, our study corroborates Ochoa's findings that there is an association between FHOD3 variants and HCM. More specifically, the most frequent rare FHOD3 variants are found at Arg637Gln and

Arg638Trp, both of which reside in the coiled-coil region. As variants that are localized in the coiled-coil region, it is possible that the change from a negatively charged amino acid to an uncharged amino acid impacts the secondary structure of FHOD3. As a result, improper formation of the sarcomere occurs and presents as a more severe form of HCM. Hernandez et al. 2025 cites these two mutations as intermediate-effect variants (IEVs) that modulate expression of nonsarcomeric HCM. In fact, Arg637Gln was found to be the most frequent variant in patients with nonsarcomeric IEVs, appearing in 49.3% of people with FHOD3-HCM. IEVs were associated with younger age at diagnosis and greater left ventricular wall thickness.

While we did not find these coiled-coil variants to be associated with younger age at diagnosis, our studies corroborate Hernandez's findings that FHOD3 HCM carriers have a significantly greater left ventricular maximum wall thickness. While the exact biological mechanism behind this is not known, FHOD3 is an actin filament regulatory protein whose function is necessary for normal contractile function in cardiomyocytes (Wooten, 2013). Thus, a mutation could cause loss of function, resulting in fewer or shorter sarcomeres that alter the structure of the heart. Consequently, LVEDD increases in order to compensate for weaker heart contractions and reduced stroke volume. Alternatively, it is possible that the mutation causes gain of function, resulting in more actin filaments or longer filaments that result in sarcomeric disarray. Despite FHOD3 being a protein whose Long isoform is localized to the sarcomere, our study demonstrates that nonsarcomeric HCM patients and patients with a rare FHOD3 variant have more similar HCM presentation regarding medical conditions and heart phenotypes. Moreover, the data show that the location of the FHOD3 variant, whether it is within or outside of the coiled-coil region, does not seem to impact clinical presentation. Our observations agree with similar studies that found the phenotype associated with FHOD3 to be relatively mild (Ochoa, 2018).

Interestingly, we identified a frameshift variant (rs144071785) with a significant prevalence in our cohort. In fact, 7.34% of the patients in our cohort carried this mutation despite being extremely rare in the general population. This mutation also occurs in the coiled-coil region of the FHOD3 Long isoform, around amino acids 634 to 636, and results from two base-pair deletions within 6 AG repeats located in exon 18. It is possible that this frameshift produces an early stop codon at amino acid 683, resulting in a protein that is too small. In addition, this frame shift could cause "nonsense mediated decay," in which quality control destroys this protein and complete loss of function occurs. In either case, the variant will decrease the amount of FHOD3 Long relative to FHOD3 short. Consequently, FHOD3 may be less able to create myofibrils due to the lower relative abundance of FHOD3 Long. Homozygosity for this gene could be lethal as mouse knockout studies have demonstrated that FHOD3-deficiency produces an embryonic lethal phenotype due to lack of sarcomere formation (Kan-O, 2012). This is supported by the absence of any rs 144071785 homozygotes in the gnomAD database. Our findings suggest the coiled-coil region as a hotspot for mutations in the FHOD3 gene that should be further investigated.

However, when we examined the clinical variables, there was no significant difference between carriers of the rs144071785 mutation and the control group, defined by having nonsarcomeric HCM and FHOD3 variants. Based on our analyses, there is no difference in clinical presentation between HCM individuals carrying this mutation and nonsarcomeric HCM. Biochemical functional studies are required to learn more about the mechanism by which FHOD3 variants may cause HCM. Current work in the Huggins

laboratory includes the production of expression plasmids with the 636 R to Q mutation and Arg637Trp mutations and characterization of the frame-shift variant described here.

Limitations

As a medical center of academic excellence, the cohort was a unique, pre-selected population of those with the most severe cases of HCM who sought out the best quality of care. Thus, it is possible that our data is not an accurate representation of the typical severity of HCM in the general population.

Conclusion

In this study, we confirmed that FHOD3 variants are more prevalent in the HCM population but do not resemble clinical characteristics of sarcomeric HCM. We identified the frame shift rs144071785 as a variant that is strongly associated with HCM. We recommend that our study be replicated in other well-established cohorts. Additionally, our study supports the genetic testing of FHOD3 variants on individuals with a family history of HCM. Future studies will examine the impact of the frameshift mutation on cardiac function.

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