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The Definition of Sarcomeric and Non-Sarcomeric Gene Mutations in Hypertrophic Cardiomyopathy Patients: A Multicenter Diagnostic Study Across Türkiye

ORIGINAL INVESTIGATION

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ABSTRACT

Background: Hypertrophic cardiomyopathy is a common genetic heart disease and up to 40%-60% of patients have mutations in cardiac sarcomere protein genes. This genetic diagnosis study aimed to detect pathogenic or likely pathogenic sarcomeric and non-sarcomeric gene mutations and to confirm a final molecular diagnosis in patients diagnosed with hypertrophic cardiomyopathy.

Methods: A total of 392 patients with hypertrophic cardiomyopathy were included in this nationwide multicenter study conducted at 23 centers across Türkiye. All samples were analyzed with a 17-gene hypertrophic cardiomyopathy panel using next-generation sequencing technology. The gene panel includes *ACTC1, DES, FLNC, GLA, LAMP2, MYBPC3, MYH7, MYL2, MYL3, PLN, PRKAG2, PTPN11, TNNC1, TNNI3, TNNT2, TPM1,* and *TTR* genes.

Results: The next-generation sequencing panel identified positive genetic variants (variants of unknown significance, likely pathogenic or pathogenic) in 12 genes for 121 of 392 samples, including sarcomeric gene mutations in 30.4% (119/392) of samples tested, galactosidase alpha variants in 0.5% (2/392) of samples and *TTR* variant in 0.025% (1/392). The likely pathogenic or pathogenic variants identified in 69 (57.0%) of 121 positive samples yielded a confirmed molecular diagnosis. The diagnostic yield was 17.1% (15.8% for hypertrophic cardiomyopathy variants) for hypertrophic cardiomyopathy and hypertrophic cardiomyopathy phenocopies and 0.5% for Fabry disease.

Conclusions: Our study showed that the distribution of genetic mutations, the prevalence of Fabry disease, and *TTR* amyloidosis in the Turkish population diagnosed with hypertrophic cardiomyopathy were similar to the other populations, but the percentage of sarcomeric gene mutations was slightly lower.

Keywords: Hypertrophic cardiomyopathy, genetic mutation, Fabry disease, next-generation sequencing

INTRODUCTION

Hypertrophic cardiomyopathy (HCM) is the most common hereditary cardiomyopathy with a marked clinical heterogeneity that may range from an asymptomatic course to the development of arrhythmias, heart failure, and sudden cardiac death (SCD), particularly in young adults and young athletes.[1-3] The prevalence of HCM has been reported to range from 1 : 200 to 1 : 500 in Western countries.[4] The HCM is defined by a wall thickness ≥15 mm in one or more left ventricular myocardial segments as measured by any imaging modality, that is not explained entirely by loading conditions.[5,6]

The HCM is considered a predominantly monogenic disease, while due to extreme heterogeneity, the disease-causing genes remain unknown in nearly 25%-40% of cases.[1,7] In up to 60% of adults with HCM, mutations in cardiac sarcomere protein genes are responsible for the disease, while in 5%-10% of cases, mutations in non-sarcomere genes lead to HCM associated with neuromuscular disease, metabolic disorders, or genetic syndromes.[7] Left ventricular hypertrophy (LVH) may also be

the result of inherited syndromes (HCM mimics) such as Fabry disease (FD) and Noonan syndrome, and diagnosis of HCM mimics is challenging since the extracardiac manifestations that would raise the clinical suspicion of a systemic syndrome in these cases may be subtle or even absent.[8]

Fabry disease is a rare lysosomal storage disorder caused by mutations in the *GLA* gene resulting in deficiency of the enzymatic activity of α-galactosidase A and the lysosomal accumulation of globotriaosylceramide and other related glycosphingolipids which ultimately leads to multiorgan damage.[9-11] It is the most common metabolic disorder in adults with HCM and its prevalence is around 0.5%-1% in patients older than 35-40 years.[12] Phenotypes of FD vary from the classic pediatric-onset phenotype with multiorgan involvement to later-onset phenotypes with manifestations that may be confined to the heart.[10,11,13] Cardiac involvement is present in over 60% of adult cases of FD.[14-16]

In general, patients with a sarcomeric protein mutation present earlier and have a higher prevalence of family history of HCM and SCD than those without a mutation.[8] They also tend to have more severe hypertrophy, microvascular dysfunction, and myocardial fibrosis.[17] Several studies have shown that some sarcomeric protein mutations are associated with a poorer prognosis than others, but these observations are based on small numbers of affected patients and are inconsistent between studies, while they are also limited by the rarity of individual mutations.[18,19] This situation may improve as better data are collected on individual mutations in national and international databases.

Genetic testing has become increasingly used in the clinical management of HCM to allow pre-symptomatic diagnosis and implementation of primary prevention strategies in high-risk individuals.[7,15,16,20] In recent years, some commercial next-generation sequencing (NGS)-based panels have also been expanded to include several known HCM mimics.[20] The NGS technology, enhancing the identification of gene mutations causing disorders for which targeted therapy is available, is considered a good platform to identify novel HCM-associated mutations and thus to improve the clinical care of affected patients.[21]

This NGS-based genetic diagnosis study aimed to identify sarcomeric and non-sarcomeric gene mutations (including those for treatable forms such as FD and *TTR*-related amyloidosis) and to confirm the final molecular diagnosis in patients diagnosed with HCM.

METHODS

Study Population

A total of 392 patients with HCM were included in this multicenter NGS diagnostic study conducted between January 2022 and June 2022 at 23 centers across Türkiye. Patients with left ventricular (LV) wall thickness ≥ 15 mm in one or more LV myocardial segments, as measured by any imaging technique (echocardiography, cardiac magnetic resonance imaging, or computed tomography), in the absence of abnormal loading conditions were enrolled in the study. Patients with LVH due to severe hypertension or aortic stenosis or those with confirmed etiology of HCM were excluded from this study.

Written informed consent was obtained from each subject following a detailed explanation of the objectives and protocol of the study which was conducted in accordance with the ethical principles stated in the "Declaration of Helsinki" and approved by İstanbul University-Cerrahpaşa Noninterventional Clinical Research Ethics Committee (Date of Approval: 05/01/2022, Protocol no: 306589-2022/04).

HIGHLIGHTS

- Hypertrophic cardiomyopathy (HCM) is mainly inherited as an autosomal dominant trait, caused by variants in sarcomeric gene mutations.
- Cardiologists have an important role in screening and distinguishing HCM patients.
- Secondary etiologies such as Fabry disease and cardiac amyloidosis should be kept in mind in the differential diagnosis of patients diagnosed with HCM.

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Procedures and Sequencing Analysis

A peripheral dried blood spot (DBS) from patients was collected on a filter paper and the samples without any information on patient identification were processed at a genetic disease diagnosis center.

The NGS panel was chosen based on worldwide prevalence, national and regional epidemiology, and local technical capacity. Fast-HCM NGS Kit (Multigen, İzmir, Türkiye) was used for target enrichment. The classification of pathogenicity of variants was based on the American College of Medical Genetics and Genomics (ACMG), being classified as pathogenic (P), likely pathogenic (LP) (probability greater than 90% of being pathogenic), uncertain significance, likely benign (more than 90% likely to be benign), and benign. A series of criteria defined by the ACMG was used to establish a scoring system based on the variant information (e.g., protein effect, position in the transcript, information about literature, functional assays, prediction software, and database). The presence or absence of certain traits was weighted differently, helping in determining the classification of variants. The HCM panel was developed for the detection of single nucleotide variants (SNVs) and small insertion/deletion (indels; up to 15-25 base pairs) located in the DNA coding sequences, flanking regions (25 bp adjacent to each exon), and known splice regions of the targeted genes, using DBS samples. The details of the 17-gene HCM panel are summarized in Table 1.

Table 1. The 17-Gene Hypertrophic Cardiomyopathy Panel Associations

Main Characteristics	Locus	Exons Sequenced	OMIM
ACTC1	15q14	7	102540
DES	2q35	9	125660
FLNC	7q32.1	48	102565
GLA	Xq22.1	7	300644
LAMP2	Xq24	10	309060
MYBPC3	11p11.2	35	600958
MYH7	14q11.2	40	160760
MYL2	12q24.11	7	160781
MYL3	3p21.31	7	160790
PLN	6q22.31	2	172405
PRKAG2	7q36.1	22	602743
PTPN11	12q24.13	16	176876
TNNC1	3p21.1	6	191040
TNNI3	19q13.42	8	191044
TNNT2	1q32.1	17	191045
TPM1	15q22.2	14	191010
TTR	18q12.1	4	176300

OMIM, Online Mendelian Inheritance in Man.

Library Preparation and Next-Generation Sequencing

Fast-HCM NGS Kit (Multigen) was used for target enrichment according to the manufacturer's protocol. PCR-based amplicon enrichment was performed. Nextera XT (Illumina, CA, USA) kit was used for library preparation for sequencing. Amplicon-based target enrichment kit amplifies all coding exons and exon–intron boundaries at least 25 bp in introns. Due to the nature of amplicon-based target enrichment, every amplicon is enriched successfully in every test. The limitation of this test is it cannot detect deep intronic mutations and copy number variants. The prepared library was sequenced on Illumina MiniSeq system using Mid-Output cartridge (Illumina). Gene2Info bioinformatic software analysis pipeline was utilized to align the sequencing data against the human genome reference sequence version GRCh38 (hg38) and obtain relevant information. Variants in the panel genes with a frequency of less than 0.5% in public databases (e.g., GnomAD) were evaluated. The variants which had a read depth below 100× were excluded. For variant description, the International FD Genotype-Phenotype Database (dbFGP; http://dbfgp.org/dbFgp/fabry/Mutation.html) and Human Gene Mutation Database (HGMD) were used. Varsome and Franklin Genoox websites were used to classify variants according to the ACMG 2015 criteria.

Statistical Analysis

Statistical Package for the Social Sciences software (version 23, SPSS Inc, Chicago, Ill, USA) was used for all statistical calculations. All data were expressed as mean ± SD for continuous variables and as percentage for categorical variables. Kolmogorov–Smirnov test was used to identify the distribution of variables normally. Independent samples *t*-test was used to compare continuous variables distributed normally and Mann–Whitney U-test was used to compare continuous variables distributed non-normally. The chi-square test was used to compare categorical data. For all tests, *P* value of <0.05 was considered statistically significant.

RESULTS

Demographic and Clinical Characteristics

Overall, mean (SD) patient age was 50.2 (15.9) years, and 266 (67.9%) of 392 patients were males. The mean age of 121 patients with positive variants was 49.0 (15.8) years and 78 (64.5%) were males. The mean age of 87 patients with HCM variants was 49.3 (SD 15.0) years, and 55 (63.2%) were males. The mean age of 2 patients (1 male, 1 female) with galactosidase alpha (GLA) variants was 68.0 (12.7) years (Table 2).

Demographic and clinical data in genotype-positive and genotype-negative patients are summarized in Table 3. Overall genotype positive (n=121) and genotype negative (n=271) patients had similar demographics and clinical characteristics as well as similar findings on echocardiography and blood analysis.

Prevalence of Total Positive Variants and Those with Molecular Diagnosis

The positive genetic variants [variants of unknown significance (VUS), LP, or P] were identified in 121 (30.9%) of 392 samples, including 119 (30.4%) samples positive for HCM variants or HCM phenocopies (including 1 sample positive for TTR-related amyloidosis) and 2 samples with GLA variants (Figure 1).

Table 2. Demographic Characteristics of Overall Patients (n = 392) and Those Identified with Positive Variants (n = 121)

			Patients with Positive Genetic Variants (n = 121)				
		Overall (n = 392)	Total (n = 121)	HCM Variants[a] (n = 87)	HCM Phenocopies[b] (n = 27)	Both (n = 5)	GLA Variants (n = 2)
Age (year)	Mean (SD)	50.2 (15.9)	49.0 (15.8)	49.3 (15.0)	46.8 (17.5)	47.4 (20.3)	68.0 (12.7)
	Median (min-max)	52 (1-84)	51 (15-82)	50 (17-82)	49 (15-76)	59 (24-67)	68.0 (59-77)
Gender, n(%)							
Female		126 (32.1)	43 (35.5)	32 (36.8)	7 (25.9)	3 (60.0)	1 (50.0)
Male		266 (67.9)	78 (64.5)	55 (63.2)	20 (74.1)	2 (40.0)	1 (50.0)

[a]HCM variants: MYBPC3, MYH7, TNNI3, TNNT2, TPM1, and MYL3.
[b]HCM phenocopy variants: DES, FLNC, LAMP2, PRKAG2, TNNC1, and TTR. FD, Fabry disease; HCM, hypertrophic cardiomyopathy.

Final molecular diagnosis was established in 69 (57.0%) of 121 samples with positive variants, corresponding to 17.6% of the total 392 samples analyzed. The diagnostic yield was 17.1% (15.8% for HCM variants) for HCM and HCM phenocopies and was 0.5% for FD (Figure 1).

The list of positive variants overall and those (LP/P) with a molecular diagnosis is provided in Table 4 and Figure 2, while the classification and description of variants identified within the total population are provided in Table 5.

Table 3. Demographic and Clinical Data in Genotype-Positive and Genotype-Negative Patients

	Genotype Positive (n = 121)	Genotype Negative (n = 271)	*P*
Variables			
Age (year), mean ± SD (*)	54.0 ± 11.0	58.0 ± 9.0	.891
Gender (male), n (%) (*)	77 (64.0)	168 (62.0)	.724
BMI (kg/m^2), mean ± SD (*)	28.0 ± 7.0	27.0 ± 5.0	.931
LVEF (%),mean ± SD (*)	58.0 ± 12.0	57.0 ± 9.0	.801
IVS (mm), mean ± SD (*)	18.0 ± 3.0	18.0 ± 3.0	.991
LVOTO (n, %) (*)	32 (26.0)	65 (24.0)	.635
Type 2 diabetes (n, %) (*)	5 (4.0)	14 (5.0)	.543
Family history of sudden cardiac death, n (%) (*)	4 (3.0)	6 (2.0)	.362
Atrial fibrillation (n, %) (*)	7 (6.0)	11 (4.0)	.224
GFR (mL/min), mean ± SD (*)	77.0 ± 12.0	74 ± 16	.320
Hemoglobin (g/dL), mean ± SD (*)	13.0 ± 3.0	12.0 ± 4.0	.218
Hematocrit (%), mean ± SD (*)	40.0 ± 5.0	38.0 ± 6.0	.200
WBC (10^3/μL), mean ± SD (*)	7.0 ± 2.0	8.0 ± 3.0	.140
PLT (10^3/μL), mean ± SD (**)	245.0 ± 67.0	285.0 ± 33.0	.751
LDL-C (mg/dL) mean ± SD (**)	114.0 ± 39.0	123.0 ± 17.0	.094
Heart rate (bpm), mean ± SD (**)	73.0 ± 11.0	69.0 ± 20.0	.688

*Independent samples *t*-test was used to compare continuous variables distributed normally and the chi-square test was used to compare categorical data.
**Mann–Whitney *U* test was used to compare continuous variables distributed non-normally. BMI, body mass index; GFR, glomerular filtration rate; IVS, interventricular septum; LDL-C, low-density lipoprotein-cholesterol; LVEF, left ventricular ejection fraction; LVOTO, left ventricular outflow tract obstruction; PLT, platelet; WBC, white blood cells.

Specifically, among 121 samples, 133 variants (94 were different and 43 were novel variants) were identified in 12 genes including *MYBPC3* gene (46 variants, 32 different variants in 45 cases), *MYH7* gene (38 variants, 24 different variants in 36 cases), *FLNC* gene (23 variants, 18 different variants in 22 cases), *MYL3* gene (7 variants, 5 different variants in 7 cases), *TNNI3* gene (3 variants, all were different in 3 cases), *DES* gene (4 variants, 2 different variants in 4 cases), *LAMP2* gene (3 variants, all were different in 3 cases), *GLA* gene (2 variants, all were different in 2 cases), *TPM1* gene (3 variants, 2 different variants in 3 cases), *TTR* gene (1 variant in 1 case), *TNNT2* gene (1 variant in 1 case), and *PRKAG2* gene (2 variants, 1 different variant in 2 cases). More than 1 mutation was noted in 12 samples, while homozygote variants were identified in 9 samples including *MYH7* (n = 6), *FLNC* (n = 1), *DES* (n = 1), and *MYL3* (n = 1) genes (Table 4).

Of 133 mutations, 98 (73.7%) were HCM gene variants, 33 (24.8%) were HCM phenocopies (including TTR variant in 1 [0.8%] sample) and 2 (1.5%) were GLA variants. Overall, 76 of 121 cases with positive variants had mutations either in the *MYBPC3* (42 [34.7%]) gene or in the *MYH7* (34 [28.1%]) gene (Table 4 and Figure 2).

The LP or P variants were found in 69 (57.0%) of 121 samples with variants (17.6% of all 392 cases), leading to a confirmed diagnosis. These 69 samples with LP or P variants involved *MYBPC3* (n = 30), *MYH7* (n = 28), *DES* (n = 4), *GLA* (n = 2), *TPM1* (n = 2), *LAMP2* (n = 2), *TNNT2* (n = 1), and *TNNI3* (n = 1) genes (Tables 4, 5, and Figure 2).

The diagnostic yield of FD was 0.5% with 1 patient having the missense c.679C>T mutation, which is associated with the classic phenotype, and 1 patient having the missense c.161T>A mutation associated with a later-onset phenotype (Table 5). One patient with FD had significant LVH (maximal wall thickness 21 mm) along with abnormal renal functions including proteinuria and reduced glomerular filtration rate (GFR), while the other patient had concentric LVH (maximal wall thickness 17 mm) and cryptogenic stroke.

In subgroup analysis, the percentage of patients having a family history of HCM was relatively low (n = 94, 24%) and the diagnostic yield of HCM was similar between patients with and without family history for HCM (19/94, 20% vs. 50/298, 17% $P = .286$). Interestingly, patients with a family history of HCM were younger than those without a family history of HCM (46 ± 12 vs. 54 ± 15 $P < .05$).

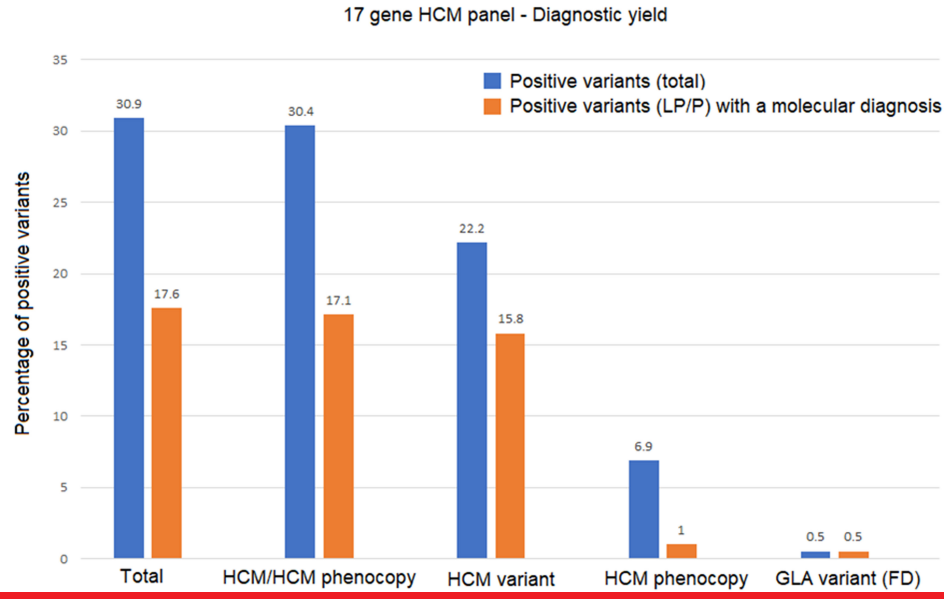

Figure 1. Percentage of positive variants overall and those with a molecular diagnosis.

DISCUSSION

In this diagnostic NGS study using a 17-gene HCM panel in patients with HCM, the LP or P variants were identified in 57.0% of 121 positive samples (17.6% of all 392 cases) which yielded a confirmed molecular diagnosis. The diagnostic yield was 17.1% (15.8% for HCM variants) for HCM and HCM phenocopies and was 0.5% for FD.

The success rate of NGS/HCM-related gene panels in HCM patients is considered to vary markedly among populations, with the diagnostic yields reported to range from 0.5% to 6.7% for FD and to range from 37.6% and 93.3% for HCM.[14,22-25] The diagnostic yield of FD (0.5%) in the current study is similar to some other NGS studies investigated across different geographic regions (0.5% to 0.9%),[23,25,26] while the yield of genetically confirmed HCM or HCM phenocopies (30.4%) is lower than the sarcomeric gene mutations reported in high-risk testing projects (40%-60%).[5,26]

Previous studies from Türkiye regarding sarcomeric gene mutations in HCM patients are generally limited to a specific gene mutation, while the number of patients is also

Table 4. List of Genes with Positive Variants Overall and Those with a Molecular Diagnosis

	Positive Variants Overall (n = 133)	Cases with Positive Variants (n = 121)*	Positive Variants with a Molecular Diagnosis (n = 69)
Genes with Variants	**n (%)**	**n (%)**	**n (%)**
HCM variants	98 (73.7)	89 (73.5)	
MYBPC3	46 (34.6)	42 (34.7)	30 (43.5)
MYH7	38 (28.6)	34 (28.1)	28 (40.6)
MYL3	7 (5.3)	7 (5.8)	-
TNNT2	1 (0.8)	1 (0.8)	1 (1.5)
TPM1	3 (2.3)	3 (2.5)	2 (2.9)
TNNI3	3 (2.3)	2 (1.7)	1 (1.5)
HCM phenocopies	33 (24.8)	30 (24.8)	
FLNC	23 (17.3)	21 (17.4)	-
DES	4 (3.0)	4 (3.3)	4 (5.8)
LAMP2	3 (2.3)	2 (1.7)	2 (2.9)
PRKAG2	2 (1.5)	2 (1.7)	-
TTR	1 (0.8)	1 (0.8)	-
GLA variants	2 (1.5)	2 (1.7)	
GLA	2 (1.5)	2 (1.7)	2 (2.9)

This NGS-based HCM panel test was designed to detect variants in exonic regions and exon-intron boundaries. Amplicon-based target enrichment technique was used. Due to the target enrichment method that was used in the panel and direct visual analysis of all genes in the panel, all variants (single nucleotide variants/frameshift variants) in the exonic regions and exon–intron boundaries were detected reliably. Deep intronic variants and gross deletions/duplications on exon/gene level were not detected with this test.
*Excluding the cases with multiple variants.HCM, hypertrophic cardiomyopathy; NGS, next-generation sequencing.

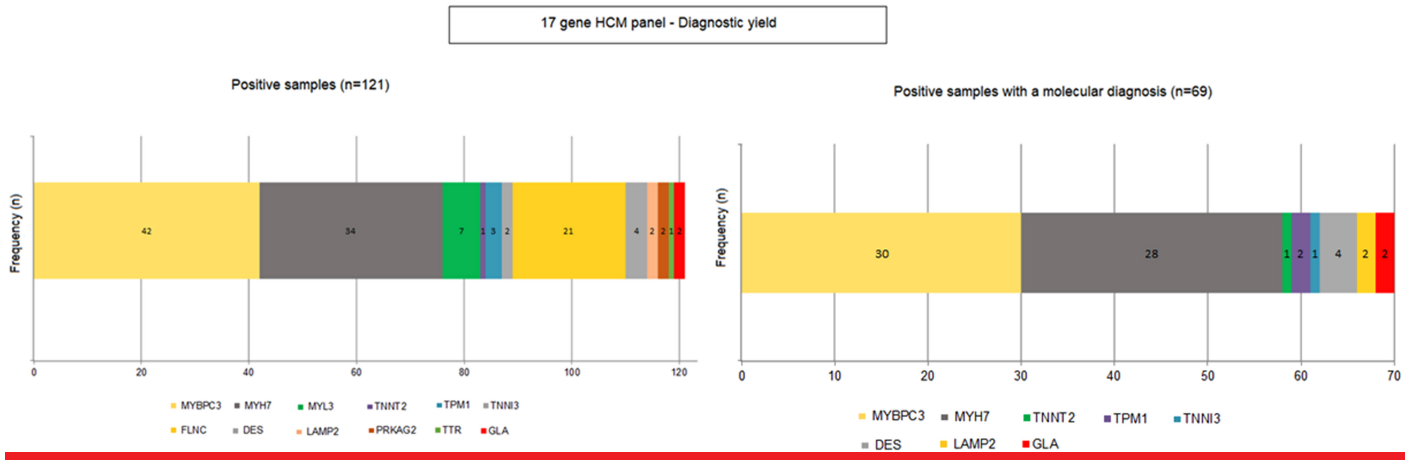

Figure 2. The list of genes with positive variants overall and those with a molecular diagnosis.

relatively small. In a study by Bilgic[27] in 21 patients with HCM who were evaluated via NGS, P or LP variants were detected in 6 patients in the genes *MYH7* (p.R663C, p.A423V), *MYBPC3* (p.P955fs*95, p.K301fs*31), *TNNT2* (p.R154Q), and *TNNI3* (p.R204C).[27] In the LVH-TR (LVH in Turkish Population) study, Kis et al[28] reported that the rate of unrelated HCM was 18.8% and the prevalence of FD was 1.58%. Also, in a screening study from Türkiye by Barman et al[29] in 80 adult patients with HCM without left ventricular outflow tract, hemizygous mutations associated with FD were detected in 2 (2.5%) male patients. Barman et al[30] also reported that the ratio of FD in patients with idiopathic LVH was 1.05%. The differences between the populations screened and the screening methods used to diagnose FD may explain the different FD rates reported by screening studies performed in various populations.

Non-genetic causes such as metabolic, infiltrative, neuromuscular, or endocrine disorders may play an important role in the etiology of HCM. Several genetic disorders such as Noonan syndrome and TTR-related amyloidosis may present with LVH, but the diagnosis of these clinical entities may be easier since extracardiac signs and symptoms are more dominant most of the time. In our study, *TTR* gene mutation was diagnosed only in 1 patient, while none of the patients were diagnosed with Noonan syndrome. In a study by Hoss et al[20] the use of extended panels including HCM mimics (*GLA, TTR, PRKAG2, LAMP2, PTPN11, RAF1,* and *DES*) in 1731 unrelated HCM patients revealed a P or LP variant in one of these genes in 1.45% of cases, which included a yield of 1% for FD, 0.3% for familial amyloidosis, 0.15% for *PRKAG2*-related CMP, and 1 patient with Noonan syndrome. Other studies on the analysis of 3 genes separately have identified P/LP variants in 0.4%-1% of *GLA*, 0.6% of *TTR*, and 0.4% of *PRKAG2* genes.[31-33]

Currently, the main clinical role of genetic testing in HCM is considered to facilitate familial screening to allow the identification of individuals carrying a risk for the disease.[34] It is also preferred to diagnose genocopies, such as lysosomal, infiltrative, and glycogen storage disease which have different treatment strategies, rate of disease progression, and prognosis.[35] The role of genetic testing in predicting prognosis is limited, but emerging data suggest that knowledge of the genetic basis of disease will provide an important role in disease stratification and offer potential targets for disease-modifying therapy in the future.[36]

Hence, this national multicenter diagnostic NGS study provides a valuable opportunity to acquire a deeper understanding of HCM and HCM phenocopies, specifically for the treatable forms such as TTR-related amyloidosis and FD, by investigating 392 patients from 23 centers across Türkiye, and demonstrates the importance of genetic testing for multiple diseases with overlapping phenotypes.

Notably, the 69 positive samples with LP or P variants in our study involved *MYBPC3* (n=31) and *MYH7* (n=28) genes in the majority of cases, while GLA (n=2), *DES* (n=3), and *LAMP2* (n=1) were the genes involved only in 6 cases. Likewise, data from FinHCM Genetic Study on the targeted sequencing of 59 genes revealed P/LP mutations in 38% of HCM cases, and the 4 most frequent sarcomeric mutations (*MYBPC3, MYH7, TPM1,* and *MYL2*) accounted for 28% of the HCM cases.[22] However, P/LP variants in non-sarcomeric genes were rare and found only in *GLA* and *PRKAG2* genes in 3 patients.[22] In a study on the genetic testing by NGS in 104 unrelated probands for 23 HCM-related genes, the mutation detection rate was reported to be 43.4%, and the mutations in *MYBPC3* accounted for 38.6%, followed by *TPM1* (20.5%), *MYH7* (18.2%), *TNNT2* (9.1%), *TNNI3* (4.5%), and *MYL2* (2.3%), while a mutation in *GLA* associated with FD was found in 1 patient.[24]

Although the use of large-scale sequencing methods has been associated with an increase in the spectrum of the putative HCM-related genes into non-sarcomeric genes, the mutations in non-sarcomeric genes are considered to be rare causes of HCM and are mainly classified as VUS.[22,24,37,38] In our study, while the percentage of LP and P variants was 30.8%, the diagnostic yield was 17.1% for HCM and HCM phenocopies, which seems to reflect that many mutations in sarcomeric or non-sarcomeric genes are mainly considered as VUS and studies targeting these mutations may increase the diagnostic ability.

Table 5. Classification and Description of Variants Identified Within the Total Population (n = 133)

Gene	DNA Variant: nucleotide	Zygosity	Pathogenicity According to ACMG 2015	Novelty
HCM Variants (n = 98)				
MYBPC3	c.2504_2505delGCinsTT	het	LP	Previously defined
MYBPC3	c.2504_2505delGCinsTT	het	LP	Previously defined
MYBPC3	c.2827C>T	het	P	Previously defined
MYBPC3	c.1458-17C>G	het	VUS	Previously defined
MYBPC3	c.927-9G>A	het	P	Previously defined
MYBPC3	c.2827C>T	het	P	Previously defined
MYBPC3	c.1731G>A	het	P	Previously defined
MYBPC3	c.1685C>T	het	VUS	Previously defined
MYBPC3	c.1731G>A	het	P	Previously defined
MYBPC3	c.787G>A	het	P	Previously defined
MYBPC3	c.1457+5G>T	het	VUS	Novel
MYBPC3	c.3697C>T	het	P	Previously defined
MYBPC3	c.1731G>A	het	P	Previously defined
MYBPC3	c.2941C>T	het	P	Previously defined
MYBPC3	c.2992C>G	het	VUS	Previously defined
MYBPC3	c.1504C>T	het	P	Previously defined
MYBPC3	c.3789_3812del	het	LP	Novel
MYBPC3	c.1293delC	het	P	Novel
MYBPC3	c.2962G>A	het	P	Previously defined
MYBPC3	c.1457+5G>T	het	VUS	Previously defined
MYBPC3	c.3569G>A	het	VUS	Previously defined
MYBPC3	c.2827C>T	het	P	Previously defined
MYBPC3	c.2309-1_2309dup	het	LP	Novel
MYBPC3	c.3535G>A	het	VUS	Previously defined
MYBPC3	c.1670G>A	het	VUS	Novel
MYBPC3	c.2827C>T	het	P	Previously defined
MYBPC3	c.2994+1G>A	het	P	Novel
MYBPC3	c.2864_2865del	het	P	Previously defined
MYBPC3	c.305_306insGAGG	het	LP	Novel
MYBPC3	c.3620delG	het	P	Previously defined
MYBPC3	c.2562A>G	het	VUS	Novel
MYBPC3	c.2535_2536insAGAC	het	LP	Novel
MYBPC3	c.2864_2865del	het	P	Previously defined
MYBPC3	c.1543_1545del	het	P	Previously defined
MYBPC3	c.2864_2865del	het	P	Previously defined
MYBPC3	c.2308G>A	het	LP	Previously defined
MYBPC3	c.3737T>C	het	VUS	Previously defined
MYBPC3	c.3787C>T	het	VUS	Novel
MYBPC3	c.2504_2505delGCinsTT	het	LP	Previously defined
MYBPC3	c.2864_2865del	het	P	Previously defined
MYBPC3	c.839_840del	het	LP	Novel
MYBPC3	c.1409G>A	het	VUS	Previously defined
MYBPC3	c.2962G>A	het	VUS	Previously defined
MYBPC3	c.1351+3G>T	het	VUS	Novel
MYBPC3	c.1351+3G>T	het	VUS	Novel
MYBPC3	c.1351+3G>T	het	VUS	Novel
MYH7	c.2609G>A	het	P	Previously defined
MYH7	c.3637G>A	het	VUS	Previously defined

(*Continued*)

Table 5. Classification and Description of Variants Identified Within the Total Population (n = 133) (*Continued*)

Gene	DNA Variant: nucleotide	Zygosity	Pathogenicity According to ACMG 2015	Novelty
MYH7	c.4817G>A	het	P	Previously defined
MYH7	c.1816G>A	het	P	Previously defined
MYH7	c.2548G>A	het	LP	Previously defined
MYH7	c.2783A>T	het	P	Previously defined
MYH7	c.1268C>T	het	LP	Previously defined
MYH7	c.1268C>T	het	LP	Previously defined
MYH7	c.632C>T	hom	VUS	Previously defined
MYH7	c.2631G>A	het	P	Previously defined
MYH7	c.632T>C	het	P	Previously defined
MYH7	c.4363G>A	het	VUS	Previously defined
MYH7	c.1750G>A	het	P	Previously defined
MYH7	c.3301G>A	het	VUS	Previously defined
MYH7	c.1988G>A	het	P	Previously defined
MYH7	c.2783A>T	hom	P	Previously defined
MYH7	c.4377G>T	het	VUS	Previously defined
MYH7	c.976G>C	het	P	Previously defined
MYH7	c.2631G>A	het	LP	Novel
MYH7	c.4985G>A	het	P	Previously defined
MYH7	c.1988G>A	het	P	Previously defined
MYH7	c.4985G>A	het	VUS	Previously defined
MYH7	c.1541A>G	het	VUS	Novel
MYH7	c.632T>C	het	P	Previously defined
MYH7	c.5786C>A	het	VUS	Novel
MYH7	c.2593A>G	het	LP	Previously defined
MYH7	c.2593A>G	het	LP	Previously defined
MYH7	c.1511A>G	het	LP	Novel
MYH7	c.632C>T	het	P	Previously defined
MYH7	c.2146G>A	het	P	Previously defined
MYH7	c.5710G>A	het	VUS	Novel
MYH7	c.1988G>A	het	P	Previously defined
MYH7	c.2783A>T	hom	P	Previously defined
MYH7	c.2783A>T	hom	P	Previously defined
MYH7	c.2783A>T	hom	P	Previously defined
MYH7	c.1988G>A	het	P	Previously defined
MYH7	c.428G>A	het	P	Previously defined
MYH7	c.2783A>T	hom	P	Previously defined
MYL3	c.452C>T	het	VUS	Previously defined
MYL3	c.170C>A	het	VUS	Previously defined
MYL3	c.170C>A	hom	VUS	Novel
MYL3	c.451G>A	het	VUS	Novel
MYL3	c.170C>A	het	VUS	Novel
MYL3	c.466G>A	het	VUS	Previously defined
MYL3	c.532G>A	het	VUS	Previously defined
TNNI3	c.202C>A	het	VUS	Novel
TNNI3	c.422G>C	het	LP	Novel
TNNI3	c.547-1G>A	het	VUS	Previously defined
TNNT2	c.341C>T	het	LP	Novel
TPM1	c.842T>C	het	P	Previously defined
TPM1	c.842T>C	het	P	Previously defined
TPM1	c.77A>C	het	VUS	Novel

(*Continued*)

Table 5. Classification and Description of Variants Identified Within the Total Population (n = 133) (*Continued*)

Gene	DNA Variant: nucleotide	Zygosity	Pathogenicity According to ACMG 2015	Novelty
HCM phenocopies (n = 33)				
DES	c.1353C>G	het	P	Previously defined
DES	c.1372-3dup	hom	LP	Novel
DES	c.1372-3dup	het	LP	Novel
DES	c.1372-3dup	het	LP	Novel
FLNC	c.904A>G	het	VUS	Novel
FLNC	c.5763_5764delTGinsCA	het	VUS	Novel
FLNC	c.6977G>A	het	VUS	Novel
FLNC	c.1102G>A	het	VUS	Novel
FLNC	c.3502G>C	het	VUS	Novel
FLNC	c.6484G>C	het	VUS	Novel
FLNC	c.4421G>A	het	VUS	Novel
FLNC	c.4763C>G	het	VUS	Novel
FLNC	c.5763_5764delTGinsCA	het	VUS	Novel
FLNC	c.3458G>T	het	VUS	Previously defined
FLNC	c.8174C>T	het	VUS	Novel
FLNC	c.2092G>A	het	VUS	Novel
FLNC	c.7952G>T	het	VUS	Novel
FLNC	c.15C>A	het	VUS	Novel
FLNC	c.5657A>G	het	VUS	Novel
FLNC	c.2092G>A	het	VUS	Novel
FLNC	c.5763_5764delTGinsCA	hom	VUS	Novel
FLNC	c.977T>C	het	VUS	Novel
FLNC	c.3449G>A	het	VUS	Novel
FLNC	c.3649A>T	het	VUS	Previously defined
FLNC	c.4061G>A	het	VUS	Novel
FLNC	c.4061G>A	het	VUS	Novel
FLNC	c.4061G>A	het	VUS	Novel
LAMP2	c.1093+1G>A	het	P	Previously defined
LAMP2	c.877C>T	het	P	Previously defined
LAMP2	c.449T>A	het	VUS	Novel
PRKAG2	c.634C>T	het	VUS	Novel
PRKAG2	c.634C>T	het	VUS	Novel
TTR	c.209G>A	het	VUS	Previously defined
GLA Variants (n = 2)				
GLA	c.161T>A	hem	LP	Novel
GLA	c.679C>T	het	P	Previously defined

dbFGP, International Fabry Disease Genotype-Phenotype Database; DLE, Diagnósticos Laboratoriais Specialized; DNA, deoxyribonucleic acid; *GLA*, 𝛼-galactosidase A gene; LP, likely pathogenic; P, pathogenic; VUS, variants of unknown significance.

Nonetheless, given the diagnostic effectiveness of expanded NGS panels in terms of HCM mimics necessitating different patient management, routine screening with expanded NGS panels involving non-sarcomeric genes rather than early panels targeting only fully validated sarcomeric HCM genes is suggested to allow the prompt recognition of probands with HCM-mimicking diseases.[39]

However, it should be noted that the NGS-based technique increases the detection rate of not only disease-causing variants but also VUSs which requires a rigorous process of interpretation to avoid misclassification.[40-43] Only variants in a limited set of genes are highly actionable and interpretable in the clinic, suggesting that larger panels offer limited additional sensitivity.[39,40]

Study Limitations

The pathogenicity of the genetic variants was determined according to the present classifications in the dbFGP and HGMD which consolidates data from peer-reviewed publications, known databases, and available patient clinical and biochemical findings. However, the classification was not available for all rare variants, and some of the variants classified as VUS may actually have caused Fabry symptoms in

patients having such a variant. Challenges to more precisely classifying VUSs include the lack of sufficient population-based statistical evidence, scarcity of reported functional evidence, and variability in clinical evaluations performed by clinicians and researchers. Moreover, the common finding of VUS in screenings of FD mandates the continuous re-evaluation of the prevalence results as growing knowledge of genetics comes to establish the pathogenic or benign nature of these variants.[14,44] Another limitation of the study is the lack of analyses on specific subgroups, which could provide methods to increase the yield of testing and therefore prevent wasting valuable resources.

In this panel, only SNVs were detected in exonic regions and exon–intron boundaries. Patients with normal genetic results might have copy number variants at exon/gene level which might be detected with MLPA or a similar technique and deep intronic mutations. Because of that normal genetic results must be evaluated carefully for further genetic evaluation. Finally, the family history of HCM in patients included in the study was only verbally confirmed which may be the reason for finding similar results between those with a positive family history of HCM and those without.

CONCLUSION

In conclusion, we showed that VUS/LP/pathologic HCM variants were less common in Turkish patients, while the prevalence of genetic variants of FD was similar to other populations. Supporting the importance of genetic testing for multiple diseases with overlapping phenotypes, our findings emphasize the effectiveness of the targeted NGS gene panel in improving the diagnosis of HCM mimics and the value of including treatable forms such as FD and TTR-related amyloidosis in the HCM differential diagnosis in terms of timely initiation of therapy or family screening in those with a confirmed diagnosis.

Ethics Committee Approval: This study was approved by the İstanbul University-Cerrahpaşa Noninterventional Clinical Research Ethics Committee (Date of Approval: 05/01/2022, Protocol no: 306589-2022/04).

Informed Consent: Written informed consent was obtained from all participants.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – V.O., H.O.; Design – V.O., H.O.; Supervision – V.O., H.O.; Resources – All authors; Materials – All authors; Data Collection and/or Processing – All authors; Analysis and/or Interpretation – All authors; Literature Search – All authors; Writing – V.O., H.O.; Critical Review – V.O., H.O.

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