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Original Article
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Role of Genetic Testing in Diagnosis and Prognosis Prediction in Hypertrophic Cardiomyopathy in Korea

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ABSTRACT

Background: Hypertrophic cardiomyopathy (HCM) needs careful differentiation from other cardiomyopathies. Current guidelines recommend genetic testing, but genetic data on differential diagnoses and their relation with clinical outcomes in HCM are still lacking. This study aimed to investigate the prevalence of genetic variants and the proportion of other cardiomyopathies in patients with suspected HCM in Korea and compare the outcomes of HCM according to the presence of sarcomere gene mutation.
Methods: We enrolled 1,554 patients with suspected HCM having left ventricular hypertrophy on transthoracic echocardiography between April 2012 and February 2023. Patients who declined genetic testing or who had pure apical HCM without a familial history were excluded. Genetic testing was performed using a next-generation sequencing panel or whole-exome sequencing for cardiomyopathies. We performed cardiovascular magnetic resonance if the diagnosis was inconclusive. Genotype-positive HCM was defined as sarcomere gene mutations of pathogenic or likely pathogenic variants. Adverse clinical outcomes were defined as a composite of all-cause death, resuscitated cardiac arrest, heart failure-related admission, appropriate implantable cardioverter defibrillator shocks, and stroke.
Results: Of 492 patients (mean age 49.6 ± 14.7 years, 29.4% women) who underwent genetic testing, 214 (43.5%) had disease-causing gene mutations. After combining gene tests, multi-imaging modality, and clinical information, 447 (90.9%) had HCM, and 27 (5.5%) had Fabry disease. Among the HCM patients, 182 (40.7%) were genotype-positive, and 265 (59.3%) were genotype-negative. Kaplan–Meier curve analysis showed that genotype-positive HCM patients experienced more composite outcomes (log-rank, $P < 0.001$). In multivariable Cox analysis, non-sustained ventricular tachycardia (NSVT) (hazard ratio [HR], 1.91; 95% confidence interval [CI], 1.17–3.12; $P = 0.010$), left ventricular ejection fraction (LVEF) < 50% (HR, 5.50; 95% CI, 2.68–11.27; $P < 0.001$), LA reservoir strain (HR, 0.96; 95% CI, 0.93–0.99; $P = 0.037$), and positive sarcomere gene mutation (HR, 1.70; 95% CI, 1.04–2.78; $P = 0.034$) were significantly association with composite outcomes. Sarcomere gene mutation had incremental value for predicting adverse outcomes added on NSVT and LVEF < 50%.
Conclusion: Genetic testing is helpful in diagnosing HCM, and sarcomere gene mutations in HCM are significantly associated with clinical outcomes.

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Keywords: Cardiomyopathy, Hypertrophic; Genotype; Genetic Testing; Hypertrophy, Left Ventricular; Sarcomere Gene Mutations

INTRODUCTION

Hypertrophic cardiomyopathy (HCM) has various phenotypes. Therefore, patients with left ventricular hypertrophy (LVH) must be carefully differentiated from those with other inherited cardiomyopathies, infiltrative cardiomyopathy, or conditions with secondary hypertrophy attributable to pressure overload.[1] An accurate diagnosis is essential because we have entered an era in which specific treatments for HCM, Fabry disease, and Amyloidosis exist. However, this remains challenging even though various multimodal imaging techniques have been developed.

Genetic testing is helpful in the diagnosis of HCM mimics in patients with few or extracardiac manifestations.[2,3] Current guidelines recommend genetic testing in patients with an atypical clinical presentation of HCM or when another genetic condition is suspected to be the cause.[4,5] However, genetic testing has not been systematically established in clinical practice. In addition, disease-causing sarcomere variant identification in HCM remains unresolved, and there is heterogeneous data on the phenotypes of HCM according to the type of gene mutation.[6] Furthermore, genetic data on the differential diagnosis of suspected HCM and their relation with clinical outcomes are still lacking.

This study investigated the prevalence of genetic variants and the proportion of other cardiomyopathies in patients with suspected HCM in Korea. Additionally, we compared the clinical manifestations and outcomes between patients with genotype-positive and genotype-negative HCM.

METHODS

Study population

We retrospectively enrolled 1,554 patients with suspected HCM having a left ventricular (LV) wall thickness of 13 mm or more in those with a family history of HCM and 15 mm or more on transthoracic echocardiography (TTE) in those without a family history of HCM who were referred to the HCM and Fabry Clinic at Severance Hospital (Yonsei University College of Medicine, Seoul, Republic of Korea) from April 2012 to February 2023. We excluded patients who did not consent to genetic testing or had pure apical HCM without a family history. We performed genetic testing for HCM and other genetic diseases that cause cardiac hypertrophy. We performed cardiovascular magnetic resonance (CMR) in patients whose TTE was inconclusive or suspected of having an alternative diagnosis. The final clinical diagnosis was made based on the genetic results of patients with genetic mutations; otherwise, the diagnosis was made by combining TTE, CMR, and clinical data.

TTE and speckle-tracking echocardiography

We performed comprehensive TTE using commercially available equipment. We obtained standard two-dimensional measurements as recommended by the American Society of Echocardiography.[7] We evaluated the degree of myocardial hypertrophy, dynamic left ventricular outflow tract obstruction, mitral regurgitation, myocardial function, maximal

left ventricular wall thickness (LVWT), left ventricular ejection fraction (LVEF), and LV apical aneurysms. We used speckle-tracking echocardiography (STE) to evaluate the left atrial (LA) and LV myocardial functions. We measured the left ventricular global longitudinal strain (LVGLS) offline from two-dimensional images of the apical two-, three-, and four-chamber views using a vendor-independent software package (TomTec; Image Arena 4.6, Munich, Germany) by an independent investigator blinded to the clinical information.[8,9] We used apical four-chamber non-foreshortened views to measure LA strain.[10] We classified the patients into three groups according to the pattern and location of LVH: septal, apical mixed, and diffuse types.

Genetic testing

We performed genetic testing using the next-generation sequencing (NGS) panel or whole-exome sequencing (WES). The NGS panel using blood samples is a commercially available genetic testing for cardiomyopathy using a combination of oligonucleotide hybridization-based DNA sequencing and dideoxy-based DNA sequencing assessing eight HCM-associated myofilament encoding genes involving myosin-binding protein C (*MYBPC3*), b-myosin heavy chain (*MYH7*), essential and regulatory myosin light chains (*MYL2* and *MYL3*), cardiac troponin T (*TNNT2*), cardiac troponin I (*TNNI3*), a-tropomyosin (*TPM1*), and cardiac actin (*ACTC*) as well as three genes associated with metabolic cardiomyopathies: *GLA* for Fabry disease, *LAMP2* for Danon disease, *PRKAG2* for PRKAG2 cardiomyopathy *TTR* for amyloidosis, and *MT-TL1* for MELAS.[11] The NGS panel used in this study is displayed in **Supplementary Table 1**. The WES uses high-molecular-weight genomic DNA extracted from a patient's buccal swab sample. We performed this procedure according to the CAP/CLIA-validated standard operating protocol. All known exonic regions of human genes (~22,000) were captured by xGen Exome Research Panel v2 (Integrated DNA Technologies, Coralville, IA, USA) and were sequenced with Novaseq 6000 (Illumina, San Diego, CA, USA) as 150 bp paired-end reads. The base call (BCL) sequence files were converted and demultiplexed to FASTQ files using bcl2fastq v2.20.0.422 (Illumina). Sequence reads were aligned to the Genome Reference Consortium Human Build 37 (GRCh37) and Revised Cambridge Reference Sequence (rCRS) of the mitochondrial genome using BWA-mem 0.7.17 to generate BAM files. Aligned BAM files were sorted and extracted using the statistical metric by samtools (v.1.9).[12] Duplication was marked by Picard (v.2.20.8) (http://broadinstitute.github.io/picard/). Variant calling files for single nucleotide variants and small insertions/deletions (INDEL) were generated following the GATK best practices (GATK v.3.8).[13,14] Variants were then annotated with Ensembl Variant Effect Predictor (VEP) and classified according to the American College of Medical Genetics and Genomics (ACMG) guideline.[15] We defined a positive gene mutation as the presence of a gene mutation classified as pathogenic or likely pathogenic according to the American College of Medical Genetics and Genomics (AGMS) criteria. As previously described, the sequencing data was analyzed using 3billion's bioinformatics pipeline 'EVIDENCE'.[16]

CMR protocol and image analysis

We performed CMR imaging using a 3-T mitral regurgitation scanner (Magnetom Trio Tim, Siemens Healthcare, Erlangen, Germany) with a six-channel anterior body matrix coil and a spine matrix coil array. We acquired cine images in the short-axis plane orientation covering both ventricles using a retrospective electrocardiogram (ECG)-gated balanced steady-state free-precession (TrueFISP) sequence. We achieved cardiac localization using a steady-state free precession sequence under ECG gating. The CMR protocol included T1 mapping, T2 mapping, extracellular volume (ECV), and late gadolinium enhancement (LGE), and we made differential diagnoses according to these values.[17]

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Definition of genotype-positive and genotype-negative HCM and clinical outcomes

We defined HCM patients with sarcomere gene mutations of pathogenic or likely pathogenic variants as genotype-positive HCM and patients with no genetic mutations or with variants of uncertain significance as genotype-negative HCM. We defined the adverse clinical outcomes in patients with HCM as a composite of all-cause death, resuscitated cardiac arrest, heart failure (HF)-related admission, appropriate ICD shocks, and stroke. The follow-up endpoint was when the composite outcomes occurred, the day of heart transplantation, or the date of the last hospital visit.

Statistical analysis

Continuous variables are presented as mean ± SD or median (interquartile range), and categorical variables are given as a percentage of the group total. To compare continuous variables among groups according to screening, we used the Student's *t*-test. We performed χ^2 tests to compare nonparametric data. Univariable and multivariable Cox proportional hazard analysis was used to identify independent determinants for adverse clinical outcomes. Factors significantly associated with adverse clinical outcomes in univariable Cox analysis and known sudden cardiac death (SCD) risk factors were included in multivariable Cox analysis after confirming no multicollinearity problem. We estimated survival rates using the Kaplan-Meier survival method and analyzed differences using a log-rank test. All statistical tests were two-tailed, and 95% confidence interval (CI) was calculated. $P < 0.05$ was considered statistically significant. We performed all statistical analyses using R software (version 4.3.1; R Foundation for Statistical Computing, Vienna, Austria) with "survminer," "survival," and "lmtest" packages.

Ethics statement

The study was approved by the Institutional Review Board (IRB) of Yonsei University Health System (IRB number: 4-2012-0655) and conducted following the Declaration of Helsinki. Informed consent was waived because of the retrospective nature of the study.

RESULTS

Study population and baseline characteristics of suspected HCM patients

Four hundred ninety-two patients (mean age 49.6 ± 14.7 years, 29.4% women) underwent genetic testing. There were 292 (59.6%) patients with hypertension, 216 (44.1%) with diabetes mellitus, 81 (16.5%) with atrial fibrillation, and 48 (11.5%) with coronary artery disease. The most common LVH pattern was septal hypertrophy (73.7%), followed by apical mixed (17.6%) and diffuse hypertrophy (8.8%). The mean maximal LVWT was 20.0 ± 4.9 mm, and LVEF was 67.9 ± 9.5%. Laboratory tests showed that the mean serum NT-pro BNP level was 2,243.9 ± 6,985.2 pg/mL, Troponin-T level was 112.4 ± 736.3 pg/mL, and hemoglobin level was 14.6 ± 1.7 g/dL (**Table 1**).

Types of mutated genes in patients with suspected HCM

Disease-causing gene mutations were detected in 214 (43.5%) patients. Among them, 182 had HCM with sarcomere gene mutations, 27 had Fabry disease with *GLA* gene mutations, 3 had Amyloidosis with *TTR* gene mutations, and 2 had MELAS with *MT-TL1* gene mutations (**Fig. 1**). Of the 27 patients with a GLA gene mutation, 12 were males and 15 were females. All 12 male patients had significantly low alfa-galactosidase-A enzyme activity levels with a

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Table 1. Baseline characteristics of the study population

Variables	Total (N = 492)
Demographic parameters	
Age, yr	49.6 ± 14.7
Female	144 (29.4)
Hypertension	292 (59.6)
Diabetes mellitus	216 (44.1)
Dyslipidemia	189 (38.6)
Atrial fibrillation	81 (16.5)
CAD	48 (11.5)
Echocardiographic data	
Left ventricular hypertrophy distribution	
Septum	361 (73.7)
Apical predominant	86 (17.6)
Diffuse	43 (8.8)
Maximal LV wall thickness, mm	20.0 ± 4.9
IVSd, mm	15.5 ± 5.0
PWd, mm	11.0 ± 2.4
LVEF, %	67.9 ± 9.5
LVEDD, mm	47.2 ± 5.6
LVESD, mm	30.2 ± 5.9
E/e'	14.8 ± 6.4
LAVI, mL/m^2	40.4 ± 19.5
RVSP, mmHg	28.3 ± 10.0
CMR	
T1, ms	1,301.2 ± 92.4
T2, ms	42.6 ± 5.4
ECV, %	32.4 ± 7.3
LGE	344 (82.5)
Laboratory data	
NT-pro BNP, pg/mL	2,243.9 ± 6,985.2
Troponin-T, pg/mL	112.4 ± 736.3
Hemoglobin, g/dL	14.6 ± 1.7
Creatinine, mg/dL	1.0 ± 1.0

Values are presented as number (%) or mean ± standard deviation.
CAD = coronary artery disease, LV = left ventricle, IVSd = diastolic interventricular septum, PWd = diastolic posterior wall, LVEF = left ventricular ejection fraction, LVEDD = left ventricular end-diastolic dimension, LVESD = left ventricular end-systolic dimension, LAVI = left atrial volume index, RVSP = right ventricular systolic pressure, CMR = cardiovascular magnetic resonance, ECV = extracellular volume, LGE = late gadolinium enhancement.

mean of 1.6 ± 2.0 nmol/hr/mg protein (normal range: 35–100 nmol/hr/mg protein). Diagnoses of the 278 patients without disease-causing gene were made by combining multi-modality cardiac imaging results and clinical information. Finally, among the total of 492 patients, there were 447 (90.9%) HCM, 27 (5.5%) Fabry disease, 5 (1.0%) uremic cardiomyopathy, 4 (0.8%) cardiac amyloidosis, 4 (0.8%) hypertensive cardiomyopathy, 2 (0.4%) mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS) syndrome, 2 (0.4%) left ventricular non-compaction cardiomyopathy, and 1 (0.2%) cardiac sarcoidosis (**Table 2**).

The frequency of sarcomere gene mutation in HCM

Among the 447 patients with HCM, 182 (40.7%) were genotype-positive and 265 (59.3%) were genotype-negative. Of those with genotype-positive HCM, 99 (54.4%) had pathogenic variants, while 83 (45.6%) had likely pathogenic variants. The most common mutation was *MYBPC3* in 84 (46.2%) patients, followed by *MYH7* in 55 (30.2%), and *TNNI3* in 33 (12.1%). *MYL2* mutations were detected in 8 (4.4%), *MYL3* in 7 (3.8%), *TPM1* in 5 (2.7%), *TNNT2* in 2 (1.1%) patients, and *TNNC1* in one (0.5%) patient (**Fig. 2**). A double mutation was detected in two patients: one with pathogenic *MYH7* and *MYBPC3* variants and the other with pathogenic *MYL2* and likely pathogenic *MYBPC3* variants.

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Table 2. Differential diagnosis of HCM-suspected patients

Variables	Total (N = 492)
HCM	447 (90.9)
Genotype-positive	182 (40.7)
Genotype-negative	265 (59.3)
Fabry disease	27 (5.5)
Amyloidosis	4 (0.8)
TTR amyloidosis	3 (0.6)
AL amyloidosis	1 (0.2)
Uremic cardiomyopathy	5 (1.0)
Hypertensive cardiomyopathy	4 (0.8)
MELAS	2 (0.4)
LV non-compaction	2 (0.4)
Sarcoidosis	1 (0.2)

Values are presented as number (%).
HCM = hypertrophic cardiomyopathy, MELAS = mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes, LV = left ventricle.

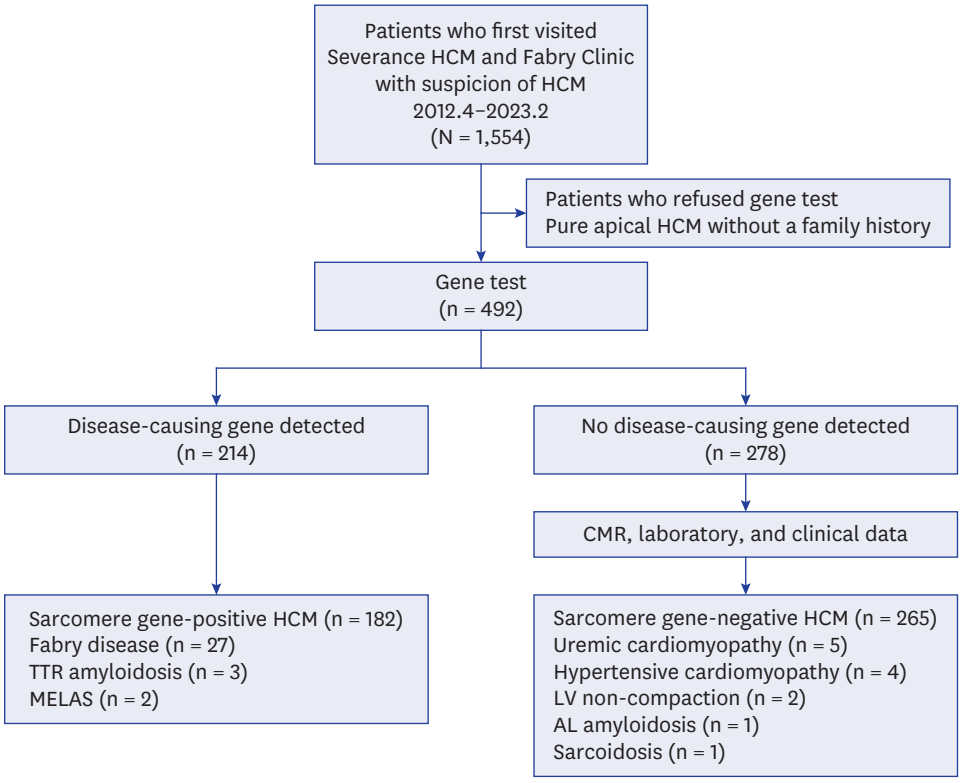

Fig. 1. Study flow chart and genetic test results for patients with suspected HCM.
HCM = hypertrophic cardiomyopathy, CMR = cardiovascular magnetic resonance, MELAS = mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes, LV = left ventricle.

Comparison of clinical and echocardiographic features between the sarcomere gene-positive and gene-negative HCM

Patients with genotype-positive HCM were significantly younger when LVH was first detected (45.7 ± 14.4 vs. 52.2 ± 14.8 years, $P < 0.001$) and more likely to have syncope (19.2% [n = 35] vs. 4.5% [n = 12], $P < 0.001$). In addition, they more frequently had a family history of HCM (53.3% [n = 97] vs. 12.5% [n = 33], $P < 0.001$) and a family history of SCD (33.3% [n = 60] vs. 12.1% [n =32], $P < 0.001$) (**Table 3**). Most of the patients with genotype-positive HCM had septal hypertrophy (95.6%), and only a few (3.9%) had apical predominant hypertrophy. In

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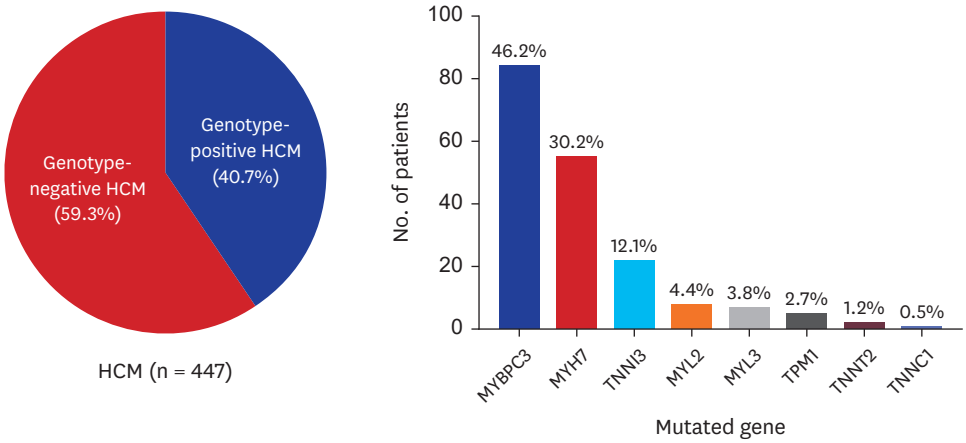

Fig. 2. Distribution of genotype-positive and genotype-negative HCM patients and prevalence of specific gene mutations.
HCM = hypertrophic cardiomyopathy.

Table 3. Comparison of baseline data between genotype-positive and genotype-negative HCM

Variables	Total HCM (N = 447)	Genotype-positive HCM (n = 182)	Genotype-negative HCM (n = 265)	*P* value
Demographic and clinical data				
Age at diagnosis, yr	49.6 ± 14.9	45.7 ± 14.4	52.2 ± 14.8	< 0.001
Female	123 (27.5)	58 (31.9)	65 (24.5)	0.110
HTN	270 (60.4)	105 (57.7)	165 (62.3)	0.383
Diabetes mellitus	210 (47.0)	89 (48.9)	121 (45.7)	0.563
Dyslipidemia	182 (40.7)	72 (39.6)	110 (41.5)	0.753
Atrial fibrillation	101 (22.6)	48 (26.4)	53 (20.0)	0.142
CAD	48 (12.5)	13 (7.8)	35 (16.1)	0.023
Syncope	47 (10.5)	35 (19.2)	12 (4.5)	< 0.001
Family history of HCM	130 (29.1)	97 (53.3)	33 (12.5)	< 0.001
Family history of SCD	92 (20.6)	60 (33.0)	32 (12.1)	< 0.001
Follow-up duration, mon	70.8 ± 56.3	71.2 ± 59.2	70.6 ± 54.3	0.913
Laboratory data				
NT-pro BNP, pg/mL	1,524.8 ± 2,813.0	1,324.4 ± 1,599.8	1,682.2 ± 3,478.4	0.262
Troponin-T, pg/mL	123.0 ± 785.4	109.9 ± 667.8	132.7 ± 864.5	0.821
Hemoglobin, g/dL	14.8 ± 1.6	14.7 ± 1.5	14.8 ± 1.7	0.811
Creatinine, mg/dL	1.0 ± 0.6	0.9 ± 0.2	1.0 ± 0.7	0.048

Values are presented as number (%) or mean ± standard deviation.
HCM = hypertrophic cardiomyopathy, HTN = hypertension, CAD = coronary artery disease, SCD = sudden cardiac death.

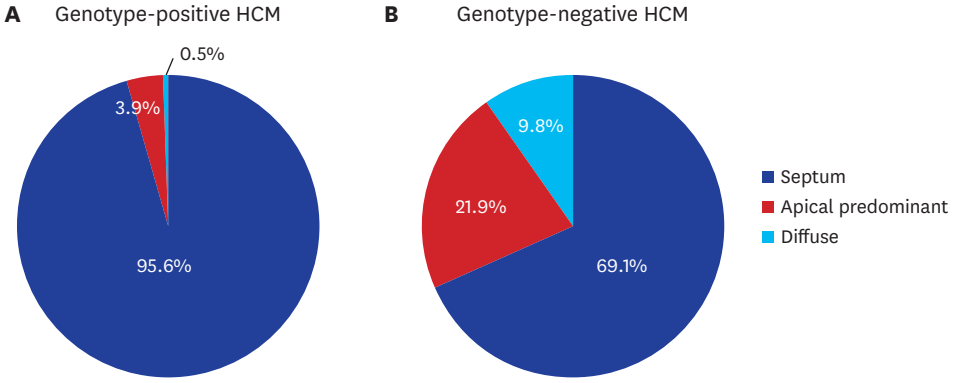

Fig. 3. Distribution of LVH patterns in genotype-positive and genotype-negative HCM patients. (**A**) Genotype-positive HCM. (**B**) Genotype-negative HCM.
HCM = hypertrophic cardiomyopathy.

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Table 4. Comparison of multimodal imaging data between genotype-positive and genotype-negative HCM

Variables	Total HCM (N = 447)	Genotype-positive HCM (n = 182)	Genotype-negative HCM (n = 265)	*P* value
Echocardiographic data				
Max. LVWT, mm	20.3 ± 4.8	22.1 ± 5.4	19.0 ± 4.0	< 0.001
LVEDD, mm	47.2 ± 5.7	46.1 ± 5.7	48.0 ± 5.6	0.001
LVESD, mm	30.2 ± 6.0	29.7 ± 6.3	30.4 ± 5.8	0.213
LVEF, %	67.6 ± 9.2	66.8 ± 9.6	68.1 ± 8.8	0.146
IVSd, mm	16.3 ± 5.3	17.9 ± 5.8	15.3 ± 4.6	< 0.001
PWd, mm	11.3 ± 2.3	10.9 ± 2.1	11.5 ± 2.4	0.004
E/e'	14.5 ± 6.3	14.3 ± 6.2	14.7 ± 6.3	0.555
LAVI	40.6 ± 19.8	43.0 ± 24.1	39.0 ± 16.2	0.034
RVSP, mmHg	28.2 ± 9.9	28.9 ± 10.1	27.7 ± 9.8	0.267
LVH distribution				< 0.001
Septum	357 (79.9)	174 (95.6)	183 (69.1)	
Apical predominant	84 (18.8)	7 (3.9)	77 (29.1)	
Diffuse	6 (1.3)	1 (0.5)	5 (1.9)	
RVH	83 (22.8)	49 (29.9)	34 (16.8)	0.005
LVOT obstruction	113 (25.3)	55 (30.2)	58 (21.9)	0.060
Apical aneurysm	45 (10.1)	19 (10.5)	26 (9.8)	0.939
Speckle-tracking echocardiography				
LVGLS, %	−13.3 ± 4.0	−13.9 ± 4.0	−12.9 ± 4.0	0.010
LA strain (reservoir), %	21.9 ± 11.0	22.2 ± 11.4	21.7 ± 10.7	0.647
LA strain (conduit), %	−13.0 ± 7.8	−13.6 ± 8.0	−12.7 ± 7.6	0.224
LA strain (contractile), %	−9.2 ± 5.6	−8.9 ± 5.6	−9.4 ± 5.5	0.359
CMR data				
T1, ms	1,320.2 ± 81.6	1,337.8 ± 97.4	1,305.0 ± 62.0	0.037
T2, ms	42.3 ± 4.8	42.5 ± 4.0	42.2 ± 5.6	0.858
ECV, %	32.3 ± 6.1	32.9 ± 5.6	31.9 ± 6.6	0.514
LGE	316 (85.9)	149 (93.1)	167 (80.3)	0.001

Values are presented as number (%) or mean ± standard deviation.
HCM = hypertrophic cardiomyopathy, LVWT = left ventricular wall thickness, LVEDD = left ventricular end-diastolic dimension, LVESD = left ventricular end-systolic dimension, LVEF = left ventricular ejection fraction, IVSd = diastolic interventricular septum, PWd = diastolic posterior wall, LAVI = left atrial volume index, RVSP = right ventricular systolic pressure, LVH = left ventricular hypertrophy, RVH = right ventricular hypertrophy, LVOT = left ventricular outflow tract, LVGLS = left ventricular global longitudinal strain, LA = left atrium, CMR = cardiovascular magnetic resonance, ECV = extracellular volume, LGE = late gadolinium enhancement.

contrast, patients with genotype-negative HCM had lesser septal hypertrophy (69.1%) and more apical predominant hypertrophy (21.9%) compared to those with genotype-positive HCM (**Fig. 3**). Patients with genotype-positive HCM had higher maximal LV walls (22.1 ± 5.4 vs. 19.0 ± 4.0, $P < 0.001$), and more RV involvement (29.9% [n = 49] vs. 16.8% [n = 34], $P = 0.005$). There were no significant differences in LVEF, E/e', RVSP, or LA strain between the two groups. Still, patients with genotype-positive HCM had better LVGLS (−13.9 ± 4.0% vs. −12.9 ± 4.0%, $P = 0.010$) than those with genotype-negative HCM. There were no significant differences in CMR parameters, including T1, T2, and ECV values between the two groups, except the presence of LGE was more frequent in genotype-positive HCM (93.1% [n = 149] vs. 80.3% [n = 167], $P = 0.001$) (**Table 4**).

Clinical outcomes in sarcomere gene-positive and negative HCM

During 5.9 ± 4.7 years, composite clinical outcomes occurred in 86 (19.2%) patients with HCM. These outcomes were significantly more frequent in patients with genotype-positive HCM compared to those with genotype-negative HCM (28.0%, [n = 51] vs. 13.2%, [n = 35], $P < 0.001$). The specific outcomes included HF-related admission (13.2%, [n = 24] vs. 5.3%, [n = 14], $P = 0.006$) and appropriate ICD shocks (8.2%, [n = 15] vs. 2.6%, [n = 7], $P = 0.014$). The rate of ICD implantation (32.4% [n = 59], vs 14.0% [n = 37], $P < 0.001$) was also higher in

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Table 5. Frequencies of clinical outcomes and interventions in HCM patients

Variables	Total HCM (N = 447)	Genotype-positive HCM (n = 182)	Genotype-negative HCM (n = 265)	*P* value
Clinical outcomes				
Composite outcomes	86 (19.2)	51 (28.0)	35 (13.2)	< 0.001
All-cause death	14 (3.1)	6 (3.3)	8 (3.0)	1.000
Resuscitated cardiac arrest	19 (4.3)	12 (6.6)	7 (2.6)	0.072
HF-related admission	38 (8.5)	24 (13.2)	14 (5.3)	0.006
Appropriate ICD shocks	22 (4.9)	15 (8.2)	7 (2.6)	0.014
Stroke	15 (3.4)	9 (4.9)	6 (2.3)	0.201
NSVT	88 (19.7)	48 (26.4)	40 (15.1)	0.005
Burn-out	66 (14.8)	26 (14.3)	27 (10.2)	0.243
Interventions				
ICD implantation	95 (21.4)	59 (32.4)	37 (14.0)	< 0.001
Septal myectomy	33 (7.4)	12 (6.6)	21 (7.9)	0.730
Heart transplantation	9 (2.0)	7 (3.8)	2 (0.8)	0.052

Values are presented as number (%).
HCM = hypertrophic cardiomyopathy, HF= heart failure, ICD = Implantable cardioverter defibrillator, NSVT = non-sustained ventricular tachycardia.

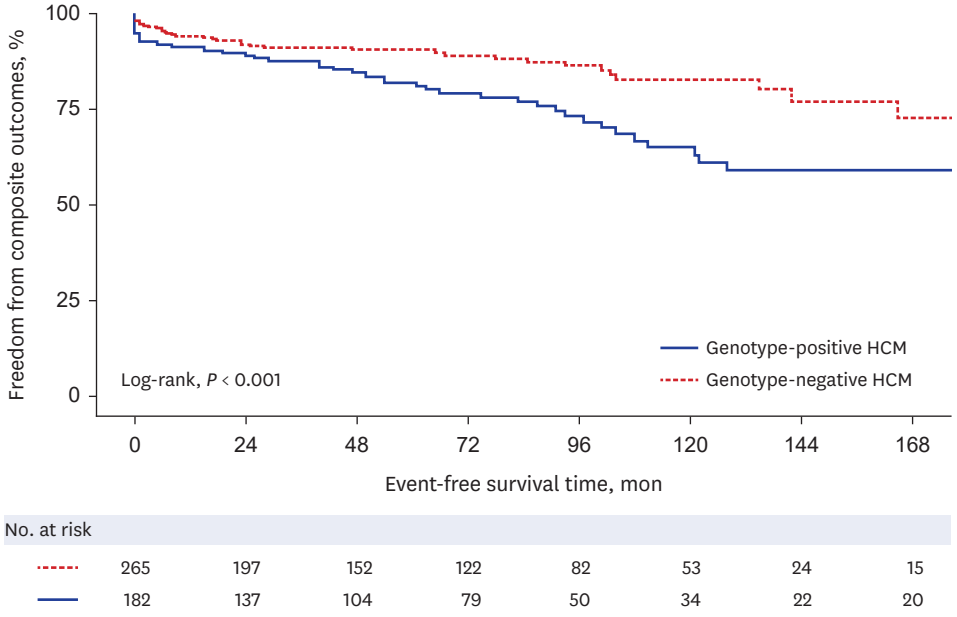

Fig. 4. Kaplan-Meier survival curve for composite outcomes in genotype-positive and genotype-negative HCM patients.
HCM = hypertrophic cardiomyopathy.

patients with genotype-positive HCM than patients with genotype-negative HCM (**Table 5**). Kaplan-Meier curve analysis revealed that patients with genotype-positive HCM experienced significantly more composite outcomes (log-rank test, $P < 0.001$) than patients with genotype-negative HCM (**Fig. 4**).

Factors associated with clinical outcomes in patients with HCM

After confirming that there was no multi-collinearity problem, age at diagnosis, atrial fibrillation, E/e', RVSP, LVGLS, LA reservoir strain, non-sustained ventricular tachycardia (NSVT), apical aneurysm, family history of SCD, LVEF < 50%, maximal LVWT ≥ 30 mm, and genotype-positive were included in the multivariable Cox analysis. In multivariable Cox analysis, NSVT (hazard ratio [HR], 1.91; 95% CI, 1.17–3.12; $P = 0.010$), EF lower than 50%

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Table 6. Factor associated with composite clinical outcomes in HCM patients

Variables	Univariate		Multivariate	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Demographic parameters				
Age at diagnosis	1.009 (0.994–1.025)	0.252	0.997 (0.977–1.016)	0.742
Female sex	1.233 (0.781–1.945)	0.368		
Hypertension	0.789 (0.512–1.214)	0.281		
Atrial fibrillation	2.614 (1.707–4.003)	< 0.001	1.251 (0.714–2.191)	0.434
DM	0.928 (0.608–1.418)	0.731		
Dyslipidemia	0.771 (0.500–1.189)	0.239		
CAD	1.493 (0.815–2.735)	0.195		
Echocardiographic parameters				
Max. LVWT	1.014 (0.975–1.054)	0.491		
LVEF	0.938 (0.920–0.956)	< 0.001		
E/e'	1.059 (1.031–1.087)	< 0.001	1.017 (0.986–1.048)	0.291
RVSP	1.035 (1.019–1.052)	0.001	1.008 (0.986–1.030)	0.473
LVGLS	0.926 (0.879–0.976)	0.004	1.026 (0.949–1.110)	0.517
LA reservoir strain	0.944 (0.922–0.966)	< 0.001	0.964 (0.931–0.994)	0.037
SCD risk factors				
NSVT	2.354 (1.514–3.660)	< 0.001	1.907 (1.165–3.123)	0.010
Apical aneurysm	1.440 (0.810–2.558)	0.214	1.315 (0.642–2.693)	0.455
Family history of SCD	1.084 (0.651–1.806)	0.755	0.791 (0.433–1.445)	0.446
LVEF < 50%	9.691 (5.502–17.068)	< 0.001	5.498 (2.682–11.268)	< 0.001
Max. LVWT ≥ 30 mm	1.669 (0.834–3.341)	0.148	1.997 (0.892–4.474)	0.093
HCM gene test				
Genotype-positive	2.096 (1.319–3.094)	0.001	1.699 (1.040–2.777)	0.034

HCM = hypertrophic cardiomyopathy, HR = hazard ratio, CI = confidence interval, DM = diabetes mellitus, CAD = coronary artery disease, LVWT = left ventricular wall thickness, LVEF = left ventricular ejection fraction, RVSP = right ventricular systolic pressure, LVGLS = left ventricular global longitudinal strain, LA = left atrium, SCD = sudden cardiac death, NSVT = non-sustained ventricular tachycardia.

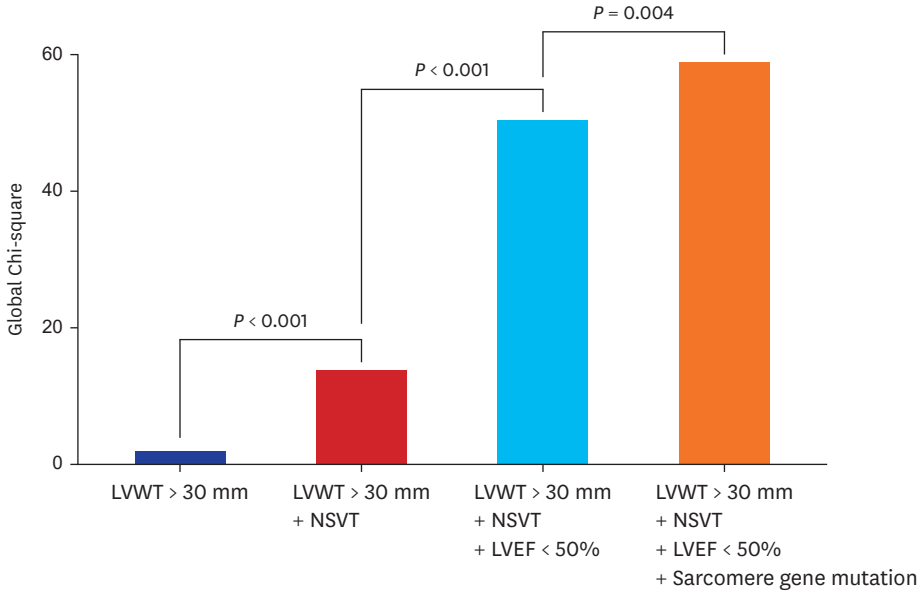

Fig. 5. Incremental value of genetic testing for predicting clinical outcomes in hypertrophic cardiomyopathy patients. LVWT = left ventricular wall thickness, NSVT = non-sustained ventricular tachycardia, LVEF = left ventricular ejection fraction.

(HR, 5.50; 95% CI, 2.68–11.27; $P < 0.001$), LA reservoir strain (HR, 0.96; 95% CI, 0.93–0.99; $P = 0.037$), and presence of sarcomere gene mutation (HR, 1.70; 95% CI, 1.04–2.78; $P = 0.034$) had significant association with the occurrence of composite outcomes (**Table 6**). Sarcomere

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Table 7. Comparison of patients with thick and thin filament gene mutation

Variables	Thick filament (n = 152)	Thin filament (n = 30)	*P* value
Age at diagnosis, yr	46.4 ± 14.2	42.5 ± 15.1	0.178
LVH distribution			0.893
Septum	145 (95.4)	29 (96.7)	
Apical predominant	6 (3.9)	1 (3.3)	
Diffuse	1 (0.7)	0 (0.0)	
Maximal LVWT, mm	22.3 ± 5.5	21.1 ± 5.2	0.297
Obstructive type	50 (32.9)	5 (16.7)	0.121
EF < 50% at diagnosis	7 (4.6)	4 (13.3)	0.157
Burn-out during follow-up	25 (16.4)	8 (26.7)	0.285
Composite outcomes	42 (27.6)	9 (30.0)	0.967
All-cause death	4 (2.6)	2 (6.7)	0.567
Resuscitated cardiac arrest	6 (3.9)	6 (20.0)	0.005
HF-related admission	21 (13.8)	3 (10.0)	0.788
Appropriate ICD shocks	12 (7.9)	3 (10.0)	0.984
Stroke	8 (5.3)	1 (3.3)	1.000

Values are presented as number (%) or mean ± standard deviation.
LVH = left ventricular hypertrophy, LVWT = left ventricular wall thickness, EF = ejection fraction, HF = heart failure, ICD = Implantable cardioverter defibrillator.

gene mutation results had incremental value for predicting the clinical outcomes over maximal LVWT ≥ 30 mm, NSVT, and LVEF < 50% when analyzed using the likelihood ratio test for Cox models (**Fig. 5**).

Phenotypes of patients according to thick and thin filament gene mutation

Among the 182 patients with genotype-positive HCM, 152 (83.5%) had thick-filament gene mutations, while 30 (16.5%) had thin-filament gene mutations (**Supplementary Table 2**). There were no significant differences in LVH pattern, maximal LVWT, prevalence of obstructive type or prevalence of reduced LVEF between the two groups. However, resuscitated cardiac arrest occurred significantly more often in patients with thin-filament mutations compared to those with thick-filament mutations (20.0% [n = 6], 3.9% [n = 6], $P = 0.005$) (**Table 7**).

DISCUSSION

The study's principal findings were as follows: 1) Genetic testing was helpful in the differential diagnosis of HCM from other cardiomyopathies. 2) Patients with genotype-positive HCM were younger at diagnosis, had higher maximal LVWT, more septal hypertrophic distribution, and were associated with poor clinical outcomes.

The prevalence of Fabry disease was much higher (5.5%) in our study group than was previously known (0.5–2%)[18] because we carefully screened patients at a higher risk of having other cardiomyopathies for genetic testing through our HCM and Fabry clinic, which makes it different from the general population. According to our previous study, the prevalence of Fabry disease reached 4.6–18.8% if the patients with HCM were carefully selected for the possibility of Fabry disease.[19]

In our Korean HCM study group, *MYBPC3* gene mutation was the most common at 46.2%, followed by *MYH7* gene mutation at 30.2%, similar to the known frequencies of genetic mutations worldwide. In current clinical practices, routine genetic testing to assess prognosis or risk stratification in HCM is not recommended yet. However, based on the results of this study that sarcomere gene mutations in HCM were associated with poor clinical outcomes

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and had incremental value in predicting clinical outcomes, genetic testing in HCM could be used as a routine process to predict prognosis.

In this study, there was a significantly higher prevalence of family history of HCMP and SCD with a higher proportion of young patients in genetic-positive HCM than genotype-negative HCM. This can be interpreted in two ways. Since it was not possible to confirm whether all of the study population was index patients, there is a possibility that family history was actively investigated after confirming the genotype-positive result. Another possibility is that, on the contrary, gene testing could be conducted more actively because there was a family history. Therefore, the results should be interpreted carefully, and further studies only with index patients are required.

The implications of non-sarcomere genes in HCM are still unknown, and information is continuously being updated. For example, the ALPK3 variants are receiving attention as a cause of HCM.[20,21] The pathogenic or likely pathogenic *ALPK3* gene mutation was detected in 8 (1.8%) patients in our HCM group. Their maximal LV wall thickness was 21.1 ± 4.7 mm, and 62.5% [n = 5] had a septal LVH pattern. They showed a good prognosis, with none experiencing clinical outcomes or heart transplantation.

LVGLS was better in genotype-positive HCM than in genotype-negative HCM, which can be explained by the fact that the genotype-positive HCM group consists of younger patients. LA reservoir strain was significantly associated with clinical outcomes in our study similar to previous research results, the lower the LA reservoir strain, the higher the risk of developing adverse cardiovascular outcomes in patients with HCM.[22,23] A possible mechanism is that low LA compliance with diastolic dysfunction predisposes to an increase in pulmonary capillary wedge pressure, exertional dyspnea, or HF. Therefore, LA reservoir strain measurements may help predict prognosis in HCM patients.

This study has several limitations. First, there may be bias because not all patients with suspected HCM underwent genetic testing, as the test was conducted only on patients who consented. Second, since patient enrollment was conducted over a long period, genetic testing technology was further developed in between, and the AGMS criteria may have changed. Third, the number of patients with a family history of HCM or SCD could have been underestimated because this study is retrospective. Also, family screening was not entirely done prior to the commencement of the study. Furthermore, this was a single-center retrospective study, so a large-scale multicenter prospective study is required.

Genetic testing is informative for the diagnosis of HCM, especially in patients highly suspected of having other cardiomyopathies. Sarcomere gene mutation in HCM was significantly associated with poor prognosis.

SUPPLEMENTARY MATERIALS

Supplementary Table 1

List of 369 genes in the NGS panel

Supplementary Table 2

Types and frequencies of gene mutation in HCM patients

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