Presence of Hypertrophic Cardiomyopathy Related Gene Mutations and Clinical Manifestations in Vietnamese Patients With Hypertrophic Cardiomyopathy

Minh Thu Tran Vu, MD; Thuy Vy Nguyen, PhD; Nha Van Huynh; Hoang Tam Nguyen Thai; Vinh Pham Nguyen, MD, PhD; Thuy Duong Ho Huynh, PhD

Background: Hypertrophic cardiomyopathy (HCM) is associated primarily with pathogenic mutations in sarcomeric genes. The aim of this study was to identify the prevalence and distribution of disease-causing mutations in HCM-associated genes and the genotype-phenotype relationship in Vietnamese patients with HCM.

Methods and Results: Genetic testing was performed by next-generation sequencing in 104 unrelated probands for 23 HCM-related genes and in 57 family members for the mutation(s) detected. Clinical manifestations were recorded for genotype-phenotype correlation analysis. Mutation detection rate was 43.4%. Mutations in MYBPC3 accounted for 38.6%, followed by TPM1 (20.5%), MYH7 (18.2%), TNNT2 (9.1%), TNNT3 (4.5%) and MYL2 (2.3%). A mutation in GLA associated with Fabry disease was found in 1 patient. A mutation in TPM1 (c.842T>C, p.Met281Thr) was identified in 8 unrelated probands (18.2%) and 8 family members from 5 probands. Genotype-positive status related to MYH7, TPM1, and TNNT2 mutations was associated with severe clinical manifestations. MYH7-positive patients displayed worse prognosis compared with MYBPC3-positive patients. Interestingly, TPM1 c.842T>C mutation was associated with high penetrance and severe HCM phenotype.

Conclusions: We report for the first time the prevalence of HCM-related gene variants in Vietnamese patients with HCM. MYH7, TPM1, and TNNT2 mutations were associated with unfavorable prognosis.

Key Words: Genetic mutations; Hypertrophic cardiomyopathy; Next-generation sequencing

HCM is the most common genetic heart disease, with a prevalence of 1/500. It is a clinically heterogeneous disease with incomplete penetrance and variable expression. HCM can be sporadic, but is mostly inherited as an autosomal dominant trait caused by mutations in sarcomeric genes. Additionally, in a small percentage of cases HCM is the consequence of mutations in some non-sarcomeric genes. More than 1,400 mutations in HCM-associated genes have been identified to date. MYBPC3 and MYH7 are the universally predominant HCM-associated genes, whose mutations account for 50–70% of positive-tested patients, followed far behind by TNNT2, TNNT3, MYL2, MYL3, TPM1, and ACTC1. However, differences in the prevalence of HCM genes and mutation types are reported for different populations.

Identification of HCM genetic determinants can be efficiently performed thanks to the development of next-generation sequencing (NGS). The massive parallel sequencing approach has prompted analysis of a large number of the genes involved, as well as the detection of possible modifier elements that underlie the high variability of the HCM phenotype.

Previous reports on systematic screening of mutations in HCM-related genes are mostly based on American and European populations. Information on Asian populations is limited, and nearly non-existent in the Vietnamese population.

In this study we used a targeted NGS approach to identify the mutational profiles of several HCM-related sarcomeric and non-sarcomeric genes and their possible relationship with cardiac clinical outcomes in Vietnamese patients.

Methods

Study Subjects

The study population comprised 104 unrelated subjects presenting at Tam Duc Heart Hospital and Ho Chi Minh...
City Heart Institute with familial or sporadic HCM during 2015–17 and 57 of their family members. All subjects gave written informed consent and received genetic counseling before providing blood samples. The study was reviewed and approved by the ethical committees of Tam Duc Heart Hospital and Ho Chi Minh City Heart Institute according to local ethics regulations. HCM was diagnosed when the maximum left ventricular (LV) wall thickness (LVWT) was ≥15 mm, in the absence of other diseases that can cause secondary hypertrophy. 8

For all patients, clinical history registration and physical examination, as well as adverse events including sudden cardiac death (SCD), heart failure, atrial fibrillation (AF), stroke, implantation of pacemaker or implantable cardioverter-defibrillator, were recorded. A 3-generation family history related to HCM was documented. 8 All patients underwent chest X ray, supine 12-lead ECG, M-mode, 2D and Doppler transthoracic echocardiography and 48-h ambulatory ECG monitoring.

LV morphology was classified as eccentric, apical, sigmoid interventricular septal, concentric, and other. 9 During ambulatory ECG monitoring, nonsustained ventricular tachycardia (NSVT) was defined as ≥3 consecutive ventricular ectopic beats at a rate of >120 beats/min lasting <30 s. LV hypertrophy (LHV) on 12-lead ECG was determined using the Sokolow-Lyon criteria.11 Family histories of SCD, and syncope episodes were defined as previously described. 8 Familial HCM was defined if at least 1 family member of the proband was diagnosed with HCM. 12

Genetic Analysis
A total of 23 genes related to HCM and HCM phenocopies was selected for analysis. 7,9 The gene panel included 10 sarcomeric genes (MYH7, MYBPC3, TNN2, TNN3, TPM1, MYL2, MYL3, ACTC1, TNN1, MYH6), 9 genes encodingZ-disk components and Ca2+ homeostasis factors (LDB3, CSRP3, TAP, VCL, ACTN2, MYOZ2, NEXN, JPH2, PLN), and 4 genes involved in HCM phenocopies (GLA, LAMP2, PRKAG2, TTR). The coding sequence (CDS) region, flanking sequences (paddin +15 base pairs), and untranslated regions (UTR) of each targeted gene were enriched by long-range PCR (LR-PCR). Primer sequences for 53 LR-PCR amplicons that range in size from 2.0 to 15.1 kb were listed in Supplementary Table 1. The coordinates of genomic regions were based on NCBI build 38 (UCSC hg38).

Genomic DNA was extracted from peripheral blood using QIAamp DNA Blood Mini Kit (Qiagen) according to the manufacturer’s instructions. All regions to be sequenced were amplified using PrimeSTAR GXL DNA polymerase (Takara). Amplicons were pooled at an equal molar ratio and purified by GeneJET PCR Purification Kit (Thermo Fisher Scientific). The pooled amplicons were processed using Nextera XT DNA Sample Preparation Kit (Illumina) to create indexed, paired-end libraries for sequencing on the Illumina MiSeq (2×150 or 2×250 base reads) or MiniSeq (2×150 base reads) platform, according to the manufacturer’s protocol (Illumina).

For a second testing in probands expressing a clear HCM phenotype but negative with the 23-gene panel, the TruSight Cardio Sequencing Kit (Illumina) was used to perform targeted enrichment. This panel includes 174 genes with known associations to 17 different inherited cardiac conditions. Captured libraries prepared by Trusight Rapid Capture kit were loaded onto a MiniSeq (2×150 base reads) sequencing platform, according to the manufacturer’s protocol (Illumina).

Variant Calling, Filtering, and Classification
Variants (single-nucleotide polymorphisms (SNPs) and indels) were called using the Genome Analysis ToolKit (Broad Institute, Cambridge, MA, USA) according to guidelines,13 and human reference genome hg38. The read depth and coverage of each BAM file were calculated using BEDtools.14 Identified variants were annotated using ANNOVAR.15 Variants with at least 20-fold coverage were further analyzed using Alamut Visual (Interactive Biosoftware). Synonymous variants, intronic variants outside of the flanking regions, and variants with a minor allelic frequency (MAF) ≤5% in the 1000 Genomes Project, dbSNP, Exome Aggregation Consortium (ExAC), and Genome Aggregation (gnomAD) databases were excluded based on the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP) recommendations for variant frequency in control populations.16 For disease-specific refinement of the ACMG/AMP guidelines, we adopted CardioClassifier, a disease- and gene-specific computational decision support tool that defines more specific thresholds for inherited cardiac disorders. According to CardioClassifier, the maximum credible population allele frequency for any HCM causative variant was set at 0.004%. Therefore, in this study variants with a frequency less and greater than 0.004% were categorized as PM2 and BS1, respectively.17 Various in silico prediction programs, including SIFT, PolyPhen-2, AlignGVGD, MutationTaster, MutationAssessor, LRT, and FATHMM, were used to analyze missense variants.18 The analysis of intrinsic changes was performed with MaxEntScan (MES), and Splice Site Finder-like (SSF);18 and GERP++ was used to explore nucleotide-specific estimates of evolutionary constraint.21 Rules PP5/BP6 were removed.18,22 All pathogenic (P) and likely pathogenic (LP) variants detected through NGS were confirmed by Sanger sequencing.

Statistical Analysis
Statistical analyses were performed with Stata 11.1. Clinical and molecular data of each patient were tabulated. Phenotypic data were expressed as mean±SD for continuous variables, and percentages for categorical variables. Genotypic data were displayed as number and type of nonsynonymous single-nucleotide variants and small insertion-deletion identified in each gene. Student’s t test was used to compare continuous data and Fisher’s exact test was used for categorical data. Values of p<0.05 were considered significant.

Results
Study Population Characteristics
During the period of December 2015 to November 2017, 104 consecutive HCM patients were enrolled in this study. Patients suffered from symptoms including chest pain (50%), shortness of breath (47.1%), palpitations (33.7%), and syncope (6.7%). An important rate of asymptomatic patients (21.2%) was identified through regular health checkups and familial screening. Mean age at diagnosis was 48.5±16 years, and 66 patients (63.5%) were male. A family history of HCM was present in 19 patients (18.3%) and 11 patients (10.6%) had a family history of SCD. LVH on ECG was observed in 67 patients (64.4%). The echocardiography studies revealed maximal LVWT ranging from 15 mm to 39 mm (mean, 22.5±4.8 mm), including 9 patients (8.7%) with extreme LVH
(LVWT ≥30 mm). Eccentric hypertrophy was observed in 77 patients (74%). LV outflow tract obstruction (LVOTO: LV outflow gradient ≥30 mmHg) was detected in 17 patients (16.3%), and 6 patients (5.8%) had LV apical aneurysm. AF and NSVT were found in 11 patients (10.7%) and 10 patients (9.7%), respectively. Hypertension was present in 42 patients (40.4%). There were 5 patients (4.8%) with chronic ischemic cardiomyopathy.

**Genetic Profiles**
Analyses of 23 HCM-related genes in the 104 patients revealed a total of 60 non-benign variants, including 24 P and LP variants and 36 VUS in 72 patients (69.2%) (Supplementary Table 2). A total of 13/72 patients (18.06%) harbored 2 variants each; 1 P/LP variant together with 1 VUS was identified in each of 5/13 patients; 2 VUS were found in each of the other 8 patients. No mutation was found in 32 patients (30.8%). Of the variants, 8 were novel and 12 were identified in each of 5/13 patients; 2 VUS were found in each of 2 probands. In this study, patients with P or LP variants were considered as genotype-positive; in contrast, patients harboring benign, likely benign variants, VUS or no mutation were classified as genotype-negative. Genotype-positive and genotype-negative patients accounted for 42.3% and 57.5% of the study population, respectively. Familial and sporadic HCM accounted for 29.5% and 70.5%, respectively. Of all genotype-positive subjects.

In the genotype-positive cases, MYBPC3 variants were predominant, with a frequency of 38.6%, followed by variants of TPM1 (20.5%), MYH7 (18.2%), and TNNT2 (9.1%). Other P variants present at low frequency in the study population were identified in TCAP, TNNB3, GLA, MYL2 (Figure). All, except TCAP encoding a Z-disk protein and GLA associated with Fabry disease, were sarcomeric genes. No HCM-related P mutations were found in MYL3, ACTC1, MYH6, TNNC1, LDB3, CSRPS, VCL, ATCN2, MYOZ2, NEXN, JPH2, PLN, LAMP2, PRKAG2, or TTR.

The 6 P and LP variants were non-private (Table 1). The highly recurrent MYBPC3 variant c.1504C>T (p.Arg502Trp) was found in 3 unrelated probands. Interestingly, variants TPM1 c.842T>C (p.Met281Thr) and MYBPC3 c.2308G>A (p.Asp770Asn) were respectively identified in 8 and 6 unrelated subjects. One patient tested positive with a previously reported P variant in GLA causing Fabry disease. Two novel P and LP variants were identified, including MYBPC3 c.1420G>T (p.Glu474Ter) and TPM1 c.422T>A (p.Met141Lys).

We performed a second screening in 5 genotype-negative probands exhibiting an apparent HCM phenotype, using the TruSight Cardio Sequencing Panel (Illumina). NGS data were analyzed for an additional of 20 DCM–HCM-related genes, because end-stage HCM patients display a DCM-like phenotype. Results obtained with the TruSight Cardio Sequencing Panel on the 5 probands confirmed the absence of all P/LP variants and VUS in the targeted genes analyzed by the 23-gene panel. Several VUS were found in other genes analyzed by the commercial panel (Supplementary Table 3).

Subsequent screening of family members for the mutation identified in the probands was performed in 57 family members of 23 probands who gave informed consent to participate in the study. We identified 18 genotype-positive carriers for TNNT2 (3), TPM1 (8), MYBPC3 (5), MYH7 (2) (Supplementary Table 4).

**Clinical Characteristics of HCM-Associated Mutations**
We compared the clinical characteristics of genotype-negative and -positive patients. The comparison was also made between patients who tested positive with the 4 most prevalent HCM genes in our study, MYH7, MYBPC3, TPM1 and TNNT2, and patients from the genotype-negative group. Genotype-positive patients were younger at diagnosis than genotype-negative subjects (42.7±15.2 vs. 52.7±15.4 years; p=0.008), had a family history of HCM and a higher percentage of eccentric LVH (86.4% vs. 65%; p=0.01). The genotype-positive group had significantly higher risk factors, including family history of SCD (22.7%), NSVT (18.2%), and apical aneurysm (13.6%) compared with 1.7% (p=0.001), 3.3% (p=0.02), and 0% (p=0.005) in the genotype-negative group, respectively (Table 2). Family history of HCM was mostly observed in patients who were TNNT2- (75%) and TPM1-positive (55.6%). A family history of SCD was recorded in MYH7 (50%), TNNT2 (50%) and TPM1 (33.3%) -positive subjects. MYH7-positive patients were younger at diagnosis, and had apical aneurysm (37.5%) and extreme LVWT (37.5%). Those positive for TPM1 exhibited eccentric LVH (100%) and NSVT (33.3%).

Genetic testing of 57 family members revealed 18 genotype-positive carriers (31.6%). HCM phenotype was observed in 12/18 (66.7%) carriers. The most common HCM pattern was eccentric LVH (9/12 carriers, 75%). We observed a highly recurrent TPM1 P variant c.842T>C present in 8 unrelated patients. Although the number of patients was insufficient for statistical analysis, they displayed some common clinical characteristics: 4/8 had a family history of HCM, 3/8 had a family history of SCD, 2/8 manifested NSVT (Table 3). Subsequent analysis of 10 family members of the TPM1-positive probands showed genotype-positive results in 8 members (80%), including 6 who displayed HCM symptoms such as shortness of breath, eccentric LVH, and NSVT (Table 3).

**Discussion**
Screening of 23 HCM-related genes in 104 probands identified disease-causing mutations in 44 patients (42.3%), a detection rate similar to a previous study,23 and higher than in others.24,25

**Figure.** Genetic contribution to hypertrophic cardiomyopathy in a Vietnamese population.
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<td>38</td>
<td>−</td>
<td>LP</td>
<td>HCM088, HCM095</td>
<td>—</td>
</tr>
<tr>
<td>TNNI3**</td>
<td>0.00E+00</td>
<td>33</td>
<td>−6.62</td>
<td>P</td>
<td>HCM121, HCM127</td>
<td>Mogensen et al, 2003**</td>
</tr>
<tr>
<td>TNNT2**</td>
<td>0.00E+00</td>
<td>25.6</td>
<td>−2.83</td>
<td>P</td>
<td>HCM002</td>
<td>Thierfelder et al, 1994**</td>
</tr>
<tr>
<td>TNNT2**</td>
<td>0.00E+00</td>
<td>35</td>
<td>−4.66</td>
<td>P</td>
<td>HCM055</td>
<td>Ho et al, 2009**</td>
</tr>
<tr>
<td>TNNT2**</td>
<td>0.00E+00</td>
<td>−</td>
<td>−</td>
<td>LP</td>
<td>HCM099, HCM101</td>
<td>Watkins et al, 1995**</td>
</tr>
<tr>
<td>TPM1</td>
<td>0.00E+00</td>
<td>29</td>
<td>−5.35</td>
<td>LP</td>
<td>HCM145</td>
<td>—</td>
</tr>
<tr>
<td>TPM1**</td>
<td>0.00E+00</td>
<td>20.3</td>
<td>−2.57</td>
<td>P</td>
<td>HCM035, HCM071, HCM079, HCM104, HCM106, HCM107, HCM110, HCM111, HCM119</td>
<td>Van Driest et al, 2003**</td>
</tr>
</tbody>
</table>

*Novel variants, **cases with family information in Supplementary Table 4. −, no information. ACMG, American College of Medical Genetics; HCM, hypertrophic cardiomyopathy; Het, heterozygous; LP, likely pathogenic; P, pathogenic; SNP, single-nucleotide polymorphism.
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and MYH7 are commonly reported as the 2 most prevalent HCM-related genes in Caucasian and some non-Caucasian populations.\textsuperscript{26,28,29} The highest prevalence of MYBPC3 mutations observed in this study was consistent with previous reports.\textsuperscript{26,29} However, instead of MYH7, the second most prevalent HCM-related gene in this study was TPM1. TPM1 disease-causing variants were previously identified at a very low prevalence in South Asian and Japanese people.\textsuperscript{28,30} Because mutations in TPM1 are considered a very rare

We identified a total of 30 HCM-associated mutations, including 5 novel ones; 12/30 were non-private mutations. In earlier reports, HCM-associated variants were mostly described as “private”. However, wider implementation of genetic testing with larger gene panels has led to a decrease in the number of novel variants discovered. We observed a high rate of recurrent P (67.8%) and LP variants that were present in more than 2 unrelated subjects, results that are in line with those reported for large cohorts.\textsuperscript{24,26,27} MYBPC3

\begin{table}[h]
\centering
\begin{tabular}{|l|l|l|l|l|}
\hline
\textbf{Patients, n (%)} & \textbf{MYBPC3} & \textbf{MYH7} & \textbf{TPM1} & \textbf{TNNT2} & \textbf{Negative} \\
\hline
44 (42.3) & 17 (16.3) & 8 (7.7) & 9 (8.7) & 4 (3.8) & 60 (57.7) \\
\hline
26 (59.1) & 12 (70.6) & 5 (62.5) & 4 (44.4) & 2 (50) & 40 (66.7) \\
\hline
42.7±15.2 & 45.4±13.4 & 36±11.6 & 46.2±20.2 & 34.8±10.4 & 52.7±15.4 \\
\hline
23±5 & 20.7±3.1 & 27.4±4.8 & 21.9±4 & 23.2±5.9 & 22.3±4.7 \\
\hline
3 (6.8) & 1 (5.9) & 0 (0) & 0 (0) & 1 (25) & 8 (13.3) \\
\hline
13 (29.6) & 3 (17.7) & 2 (25) & 5 (55.6) & 3 (75) & 6 (10) \\
\hline
2 (4.6) & 1 (5.9) & 0 (0) & 0 (0) & 1 (25) & 6 (10) \\
\hline
23 (52.3) & 13 (76.5) & 1 (12.5) & 5 (55.6) & 1 (25) & 32 (53.3) \\
\hline
20 (45.5) & 4 (23.5) & 7 (87.5) & 4 (44.4) & 3 (75) & 28 (46.7) \\
\hline
1 (2.2) & 0 (0) & 0 (0) & 0 (0) & 0 (0) & 0 (0) \\
\hline
10 (22.7) & 1 (5.6) & 4 (50) & 3 (33.3) & 2 (50) & 1 (1.7) \\
\hline
8 (18.2) & 0 (0) & 0 (0) & 3 (33.3) & 1 (25) & 2 (3.3) \\
\hline
2 (4.6) & 0 (0) & 1 (12.5) & 0 (0) & 1 (25) & 5 (8.3) \\
\hline
6 (13.6) & 0 & 3 (37.5) & 0 (0) & 0 (0) & 0 (0) \\
\hline
6 (13.6) & 0 & 3 (37.5) & 1 (11.1) & 1 (25) & 3 (5) \\
\hline
38 (86.4) & 15 (88.2) & 8 (100) & 9 (100) & 4 (100) & 39 (65) \\
\hline
2 (4.6) & 1 (5.9) & 0 (0) & 0 (0) & 0 (0) & 14 (23.3) \\
\hline
\end{tabular}
\caption{Clinical Characteristics of Genotype-Positive Patients, Especially Patients With Mutations in MYH7, MYBPC3, TPM1, and TNN2 Compared With Genotype-Negative Patients}
\end{table}

\begin{table}[h]
\centering
\begin{tabular}{|l|l|l|l|l|l|}
\hline
\textbf{Proband / Relatives} & \textbf{Age (years)} & \textbf{Family history of HCM} & \textbf{Family history of SCD} & \textbf{Clinical manifestation} \\
\hline
HCM035 & 35 & + & + & NSVT \\
HCM035.1 & 9 & & & Eccentric LVH, shortness of breath \\
HCM035.2 & 19 & & & No symptoms \\
HCM035.3 & 25 & & & Eccentric LVH \\
HCM071 & 29 & + & & Shortness of breath, eccentric LVH, NSVT, HCM (age 28 years) \\
HCM071.1 & 36 & & & No symptoms \\
HCM071.2 & 2 & & & \\
HCM079 & 23 & & & \\
HCM104 & 64 & & & \\
HCM106 & 29 & + & & Eccentric LVH, ICD, HCM (age 51 years) \\
HCM106.1 & 53 & + & & NSVT, apical aneurysm \\
HCM107 & 54 & & & Shortness of breath, eccentric LVH \\
HCM107.1 & 38 & & & Shortness of breath, eccentric LVH \\
HCM107.2 & 32 & & & \\
HCM111 & & & & \\
HCM111.1 & 41 & & & \\
HCM119 & & & & \\
HCM119.1 & 56 & + & + & NSVT \\
\hline
\end{tabular}
\caption{Clinical Characteristics of TPM1 Genotype-Positive Proband and Relatives}
\end{table}

ICD, implantable cardioverter-defibrillator; LVH, left ventricular hypertrophy; NSVT, nonsustained ventricular tachycardia; NYHA, New York Heart Association; SCD, sudden cardiac death.
cause of HCM, its prevalence of 7.7% is an unusually high detection rate. However, an HCM-causing mutation in \( \text{TMP1} \), c.523G>A (p.Asp175Asn), was previously identified in a Finnish population with a similar prevalence (6.5%) as a founder mutation showing regional clustering. Interestingly, in this study the c.842T>C variant accounted for 88.9% of the \( \text{TMP1} \) mutations, suggesting a mutation with founder effect. Unfortunately, we were unable to perform haplotype analysis for confirmation. \( \text{TNNT2} \) (9.1%) and \( \text{TNNT3} \) (4.5%) were identified as the next 2 most common causes of HCM after \( \text{MYBPC3} \) and \( \text{MYH7} \), in agreement with previous studies in several European and Asian populations.

Few mutations were found in non-sarcomeric genes. Except for P mutations identified in \( \text{TCAP} \), the other variants were mostly classified as VUS, consistent with previous studies. Variant c.835C>T (p.Arg279Cys) of the non-sarcomeric gene \( \text{NEXN} \) was classified as VUS in this study (Supplementary Table 2). Interestingly, based on in silico algorithms, SIFT, Mutation Taster and Polyphen-2, this variant identified in 2 probands was predicted to be a disease-causing mutation. Both patients with this missense mutation were diagnosed at a young age; 1 had a family history of HCM and exhibited mitral valve regurgitation. This same variant was previously reported as segregating with HCM phenotype in a Chinese family. We observed clinical manifestations of renal failure besides marked thickening of the LV in the patient harboring a GLA mutation; but unfortunately were unable to perform a kidney biopsy to confirm the diagnosis. Testing with a larger gene panel in 5 patients exhibiting clear HCM phenotypes exclusively resulted in VUS. The concordant sequencing data obtained with a large commercial and a small in-house gene panel showed that the in-house gene panel design, as well as the NGS validation criteria established for this study, gave reproducible and reliable results. More importantly, these results confirmed the suggestion that broader gene panels with more than 11 core sarcomeric and metabolic cardiomyopathy genes did not significantly increase test yield and sensitivity.

Among the novel P and LP variants identified, \( \text{MYBPC3} \) c.1420G>T resulted in premature interruption of the reading frame, suggesting functional impairment. Family screening for the P variant identified 3 family members harboring the same mutation. None of the 3 genotype-positive members expressed clinical manifestations, even in middle-age, which suggested a low penetrance or very late onset of disease. Further studies are required to establish the genotype–phenotype correlation of this variant. The \( \text{TPM1} \) LP mutation c.422T>A was found in the protein coding region and possibly affects protein function. Unfortunately, there were no family member screening data for this mutation.

Genotype-positive patients were younger at diagnosis, had a higher rate of eccentric LVH, apical aneurysm and NSVT, had family histories of HCM and SCD, and a 5-year risk for SCD ≥ 6%, in line with a previous report showing higher rates of heart failure outcomes in genotype-positive compared with genotype-negative patients. The younger age at diagnosis, and family histories of HCM and SCD observed in patients in this study were consistent with a study of a larger HCM population. However, we did not record any difference in the maximum LVWT between patients with HCM-positive or -negative genotype.

Comparative phenotype of the 2 most commonly predominant HCM-associated genes, \( \text{MYBPC3} \) and \( \text{MYH7} \), is controversial. In this study, we showed that compared with \( \text{MYBPC3} \), \( \text{MYH7} \) mutations were associated with a worse prognosis including New York Heart Association (NYHA) class II, family history of SCD, extreme LVWT and apical aneurysm. These findings were in agreement with previous reports but differed from studies showing indistinguishable phenotypes expressed by the 2 genes. We observed a later age of onset in patients with \( \text{MYBPC3} \) than in those with \( \text{MYH7} \) mutations, but the difference was not statistically significant, as previously reported.

Furthermore, in our study \( \text{TNNT2} \) and \( \text{TPM1} \) positive status was significantly associated with a family history of HCM and SCD. Risk of SCD related to \( \text{TNNT2} \) mutations was previously reported in Japanese patients with HCM.

Study of recurrent mutations related to clinical manifestations in HCM patients is an effective approach to exploring the genotype-phenotype relationship. In our study, \( \text{TPM1} \) c.842T>C mutation was associated with incomplete, thus high, penetrance and unfavorable prognosis. All 8 probands with \( \text{TPM1} \) c.842T>C variant exhibited a clear HCM phenotype, including eccentric LVH, maximal LVWT ≥ 20 mm, and a family history of HCM and/or SCD. Furthermore, a high percentage of family members harboring the same mutation also displayed severe HCM clinical manifestations. This finding revealed the important disease-causing role of a \( \text{TPM1} \) mutation, at least in the Vietnamese population. This was different from the results obtained in a study of Finnish patients with HCM showing relatively favorable prognosis of a founder mutation in \( \text{TMP1} \).

Study Limitations

First, our study population was relatively small; a larger sample size is necessary to confirm our conclusion. Second, genetic analyses were focused on a 23-gene panel, and testing with a larger panel was only performed in a few individuals; therefore, we cannot exclude the role of other genes and mutations in the clinical phenotype of genotype-negative patients in this study. However, the frequency of other HCM-related genes is generally low and we believe they will not radically affect the results. Third, the survey of mutation segregation in family members of genotype-positive patients was incomplete because of refusal to participate; several mutations classified as VUS in this study could have been classified differently with a more thorough segregation study. Lastly, long-term follow-up data regarding the prognosis of the patients engaged in this study were lacking. Despite these limitations, our study provided the first data based on genetic and clinical analyses of HCM in Vietnamese patients, confirming several recurrent mutations found in other study populations. We also identified a \( \text{TPM1} \) variant as a possible founder mutation in Vietnamese HCM patients.

Conclusions

We reported for the first time the prevalence of 23 HCM-related genes in Vietnamese HCM patients. HCM-associated mutations were identified in 9/23 genes tested (\( \text{MYBPC3} \), \( \text{MYH7} \), \( \text{TPM1} \), \( \text{TNNT2} \), \( \text{TNNT3} \), \( \text{MYL2} \), \( \text{MYH6} \), \( \text{TCAP} \), \( \text{GLA} \)). Genotype-positive status was associated with a family history of HCM and the presence of eccentric LVH. \( \text{MYH7} \), \( \text{TNNT2} \) and \( \text{TPM1} \) mutations were associated with a worse prognosis. We found a high proportion of genotype-negative cases (29.8%) with substantial HCM phenotype that requires more understanding of additional genetic,
epigenetic as well as environmental factors involved in the HCM etiology. Interestingly, 1 pathogenic mutation in TPM1 was detected in 8 unrelated probands and in 8 family members from 5 probands.

These results might have some implications for genetic testing of HCM in Vietnamese people. A first approach should include sarcomeric genes and genes involved in HCM phenocopies such as GLA, LAMP2 and PRKAG2. An expanded HCM gene panel should be reserved for patients with atypical clinical manifestations.

Acknowledgments

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Disclosures

We declare no conflicts of interest.

References


