

# Identification of a novel hypertrophic cardiomyopathy-associated mutation using targeted next-generation sequencing

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**Abstract.** Hypertrophic cardiomyopathy (HCM), one of the most common forms of myocardial diseases, is the major cause of sudden cardiac death in young adults and competitive athletes. Analyses of gene mutations associated with HCM are valuable for its molecular diagnosis, genetic counseling, and management of familial HCM. To dissect the relationship between the clinical presentation and gene mutations of HCM, the genetic characterizations of 19 HCM-related genes in 18 patients (8 cases from 6 pedigrees with familial HCM and 10 cases without familial HCM) were detected using next-generation sequencing (NGS). As a result, 12 disease-related mutations were identified in the 18 subjects, including 6 single mutations and 3 double mutations [MYBPC3 (p.Gln998Glu) plus TNNI3 (p.Arg145Gly), PRKAG2 (p.Gly100Ser) plus MYBPC3 (p.Lys1209Serfs\*28) and TNNI3 (p.Glu124Gln) plus GLA (p.Trp47\*)]. The 3 heterozygous double mutations were discovered for the first time in the malignant familial HCM patients. Of the 6 single mutations, a novel mutation was found in tafazzin (TAZ, p.Ile208Val), and a mutation in  $\beta$ -myosin heavy chain gene (MYH7, p.Arg54Gln), which was reported as rare in the general population, was firstly found in one HCM patient. Identification of novel and rare mutations in HCM patients have added new data to the spectrum of gene mutations associated with this disease. These findings provide an essential basis for the molecular diagnosis and better management of family members at risk of familial HCM.

## Introduction

Hypertrophic cardiomyopathy (HCM: OMIM 192600) is one of the most common inherited cardiac diseases characterized by marked thickening of the left ventricle or/and interventricular septum. HCM may affect groups of all ages and ethnicity, with an incidence of 1 in 500 individuals in the general population worldwide (1). In China, at least one million individuals are expected to be diagnosed with this disease (2). It has been considered as the major cause of sudden cardiac death in young adults and competitive athletes. Most HCM patients experience obvious clinical symptoms, including shortness of breath, palpitations, angina and syncope. However, HCM also presents with high variability in clinical presentation due to the genetic heterogeneity of the patients. It is predominantly inherited in an autosomal dominant pattern, but fewer HCM patients present with X-linked inheritance or Mendelian autosomal recessive disease. The first mutation highly associated with HCM was discovered in the  $\beta$ -myosin heavy chain gene (*MYH7*) in 1990 (3). To date, over 1,400 responsible mutations have been documented in more than 25 genes (4,5). Genetic testing can provide more accurate information for clinical diagnosis, especially for those ambiguous HCM cases, and for evaluation of the risk of disease occurrence of individuals with familial HCM (6).

Compared to the traditional genetic testing method, Sanger sequencing, which is costly and time-consuming, recently developed next-generation sequencing (NGS) technologies can improve cost effectiveness. NGS is highly feasible for massive parallel sequencing and is suitable for inherited disease testing (7-9), including cardiomyopathies (10-12). Yunnan Province, located in southwestern China, consists of diverse ethnic groups (13). However, the genetic characterizations of HCM in this region have been poorly studied (14,15). In the present study, we used NGS technology to perform genetic screening of the entire exon sequence and the flanking regions of 19 most common HCM causative genes in a Yunnan population. One novel mutation in tafazzin (TAZ, p.Ile208Val), a single rare mutation in MYH7 (p.Arg54Gln), and three double mutations responsible for HCM were respectively detected in our HCM

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patients. The prevalence and spectrum of gene mutations associated with HCM in Yunnan were systematically described.

## Materials and methods

**Subjects and clinical evaluation.** From September 2013 to December 2015, 18 patients with HCM, including 8 cases from 6 pedigrees with familial HCM and 10 cases without familial HCM, and 100 healthy controls were recruited at the Department of Cardiology, The First People's Hospital of Yunnan Province. Written informed consent was obtained from all subjects. The demographic data including age, gender, and history of cardiovascular diseases and other familial diseases were recorded simultaneously with sample collection. According to the 2011 American College of Cardiology Foundation (ACCF) and the American Heart Association (AHA) guideline for the diagnosis of HCM (16), a left ventricular septum (LVS) or/and interventricular septal thickness (IVST)  $\geq 15$  mm in the absence of any other condition that could explain the hypertrophy was diagnosed as HCM. This research project was approved by the Ethics Committee of the First People's Hospital of Yunnan Province, and performed in compliance with the principles of the Declaration of Helsinki.

**DNA isolation and sequencing.** Genomic DNA was isolated from the peripheral whole blood of 18 HCM patients and 100 healthy controls using a genomic DNA Miniprep kit (AxyPrep; Axygen, Union City, CA, USA) following the manufacturer's protocol. OD<sub>260/280</sub> of DNA samples was  $\sim 1.8$  after purification with AMPure XP reagents (Beckman Coulter, Fullerton, CA, USA). The DNA concentration was measured using the Qubit dsDNA HS assay kit (Life Technologies, Carlsbad, CA, USA). Whole coding exons and in exon-intron boundaries of 19 HCM-related genes were amplified and then sequenced on Ion Torrent PGM (Life Technologies). These genes were *MYH7*, myosin binding protein C (*MYBPC3*),  $\alpha$ -actinin 2 (*ACTN2*), desmin (*DES*),  $\alpha$ -galactosidase A (*GLA*), lysosome-associated membrane protein 2 (*LAMP2*), LIM domain-binding 3 (*LDB3*),  $\alpha$ -myosin heavy chain (*MYH6*), regulatory myosine light chain 2 (*MYL2*), regulatory myosine light chain 3 (*MYL3*), *TAZ*, myopalladin (*MYPN*), AMP-activated protein kinase (*PRKAG2*), sodium channel, voltage-gated type V  $\alpha$ -subunit (*SCN5A*), Titin-cap (*TCAP*), troponin I 3 (*TNNI3*), troponin T 2 (*TNNT2*),  $\alpha$ -tropomyosin 1 (*TPMI*), and vinculin (*VCL*).

**Molecular genetic analysis.** Low quality reads of which the read depth was less than 30x were discarded (17,18), and qualified sequences were mapped to human reference genome (hg19). The variants of the 19 genes in each sample were annotated using online software Ion Reporter (<https://ionreporter.lifetechnologies.com/ir/secure/home.html>). The missense variant was considered to be possibly related with HCM on the basis of the following criteria (19,20): i) the variant has been reported as an HCM-related mutation according to the documents or Human Gene Mutation Database (HGMD, <http://www.hgmd.cf.ac.uk/ac/index.php>), and/or ii) the predicted amino acid showed a change in a highly conserved evolution site across many species; iii) the missense variant was absent in the 100 healthy controls and its minor allele frequency (MAF) was  $< 5\%$  in

the 1000 Genomes Project (<http://www.1000genomes.org/>), HapMap (<http://hapmap.ncbi.nlm.nih.gov/>) and/or Exome Aggregation Consortium (ExAC) databases (<http://exac.broadinstitute.org/>); iv) it was predicted as a disease-related mutation by Mutation Taster (21).

All mutations potentially related with HCM were further confirmed by conventional dideoxy sequencing using the BigDye Terminator v.3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA), and the obtained sequences were analyzed using ABI 3130 Genetic Analyzer (Life Technologies). Specific primers were applied to amplify the fragments of genomic DNA containing identified candidate variations (Table II).

Evolutionary conservation analysis of the rare and novel mutations, which were performed in many species (including *Macaca mulatta*, *Mus musculus*, *Danio rerio*, *Drosophila melanogaster*, *Xenopus tropicalis*, *Bos taurus* and *Loxodonta*), was conducted using Clustal W (<http://www.genome.jp/tools/clustalw/>) and the Weblogo (<http://weblogo.berkeley.edu/logo.cgi>) (15). Based on the lowest energy theory, the structure of the proteins with a rare mutation found in this study was predicted by using Robetta (<http://rosetta.bakerlab.org/>) and SWISS-MODEL (<http://www.swissmodel.expasy.org/>) online program, and the result was visualized using the Visual Molecular Dynamics (VMD) software package (version 1.9.2, <http://www.ks.uiuc.edu/Research/vmd/index.html>).

## Results

**Demographic and clinical characteristics.** From September 2013 to December 2015, a total of 18 HCM patients were recruited, including 11 (61%) males and 7 (39%) females. Among these, 8 patients were from 6 pedigrees with familial HCM, and the remaining 10 patients did not have familial HCM. Their mean age at diagnosis was 45 years (range 23-79) with a standard deviation (SD) of 16 years. The detailed demographic and clinical characteristics of the patients are shown in Table I. All the investigated patients presented typical clinical manifestations of HCM. The mean left ventricular ejection fraction (LVEF) was increased to 67.1% (SD=9.9), and the IVST was  $18.8 \pm 2.7$  mm, the left ventricular septum thickness (LVST) was  $26.3 \pm 9.7$  mm, and the median left ventricular posterior wall (LVPWT) was  $11.7 \pm 1.9$  mm as measured by doppler echocardiography.

**Sequence alignment and annotation analysis.** Exons and in exon-intron boundaries of 19 HCM-related genes were sequenced using Ion Torrent PGM (Life Technologies). There were 383 variants obtained from qualified reads, with single nucleotide polymorphisms, insertions or/and deletions. The mean depth of coverage over all missense variants was 165.4-fold (ranged from 30 to 498) as shown in Fig. 1. Those variants were annotated and the synonymous variants were filtered out using Ion Reporter online software (<https://ionreporter.lifetechnologies.com/ir/secure/home.html>). As a result, 86 non-synonymous variants were detected in the 18 HCM patients (data available upon request).

**Screening for variants with higher potential association with HCM.** We next focused on the variants which were potentially related with HCM. Based on the potentially pathogenic criteria

Table I. Clinical characteristics of the HCM patients as determined by echocardiography.

Patient no.	Gender	Age (years)	Family history	IVST (mm)	LVPWT (mm)	LVED (mm)	LVST (mm)	LVEF (%)
A14	F	44	N	17.6	8.8	42.7	36.6	68
8	F	58	N	17.3	15.9	60.7	45.3	50
9	M	34	N	NA	NA	NA	NA	NA
15	M	50	N	15.6	12.1	44.7	27.0	70
16	M	57	N	16.3	13.5	49.6	26.2	78
24	M	44	N	15.8	9.1	41.5	30.3	53
25	F	58	N	19.0	14.1	43.6	22.9	79
26	F	79	N	NA	NA	NA	NA	NA
56	M	77	N	NA	NA	NA	NA	NA
57	F	57	N	21.0	9.0	35.0	NA	65
Family A, III:2	F	42	Y (HCM)	22.6	11.2	40.9	28.3	NA
Family A, III:4	F	36	Y (HCM)	22.1	12.0	42.4	28.0	NA
Family A, III:5	M	34	Y (HCM)	22.0	10.9	44.2	31.7	NA
Family B, II:1	M	23	N	15.6	12.1	NA	NA	NA
Family C, III:1	M	27	Y (HCM)	22.8	11.5	47.7	12.6	NA
Family D, III:1	M	26	Y (SCD)	21.1	12.0	40.3	16.0	74
Family E, II:1	M	47	Y (HCM)	16.4	11.3	53.0	13.5	73
Family F, II:1	M	28	Y (SCD)	22.0	12.6	43.1	15.1	61
Mean $\pm$ SD	NA	45 $\pm$ 16	NA	18.8 $\pm$ 2.7	11.7 $\pm$ 1.9	44.9 $\pm$ 6.2	26.3 $\pm$ 9.7	67.1 $\pm$ 9.9

F, female; M, male; Y, Yes; N, No; HCM, hypertrophic cardiomyopathy; IVST, interventricular septal thickness; LVED, left ventricular end-diastolic diameter; LVPWT, left ventricular posterior wall thickness; LVEF, left ventricular ejection fraction; LVST, left ventricular septal thickness; SCD, sudden cardiac death; NA, not applicable.

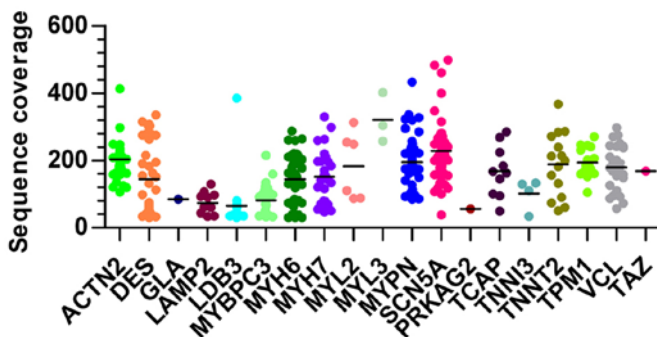


Figure 1. Sequencing coverage of mutation sites in the coding region of 19 hypertrophic cardiomyopathy (HCM)-related genes. The sequence coverages for each gene ranged from 30 to 498x in 16 HCM patients, and the mean coverage was 165.4x for overall selected target genes.

(mentioned in Materials and methods), 12 mutations were selected from 13 HCM patients and further confirmed by Sanger sequencing (Table II). There were 6 single mutations and 3 double mutations which were most frequently found in *MYBPC3* and *MYH7* with a prevalence of 38.5% (5/13) and 23.1% (3/13), respectively. Other variants, including *TNNI3*, *SCN5A*, *GLA*, *TAZ*, *PRKAG2* and *MYH6*, were found in 53.8% (7/13) of the patients (Figs. 2 and 4). One novel single mutation in *TAZ* (p.Ile208Val) was found neither in the 1000 Genomes Project databases (<http://www.1000genomes.org/>) nor in the 100 healthy control chromosomes. One reported single mutation in *MYH7*

(p.Arg54Gln), considered as rare in the general population, was firstly found in a patient without familial HCM and did not exist in the databases and the control group mentioned above. The mutations identified in highly conserved amino acids among many species may have influenced the structure and function of the proteins (Fig. 5). Therefore, the predicted protein structure of a mutant *MYH7* (p.Arg54Gln) was compared to the corresponding wild-type. The homology modeling analysis showed that the amino acid change resulted in the structural differences between the wild-type and mutant *MYH7* (Figs. 5 and 6). In addition, the 3 double mutations were found in *MYBPC3* (p.Gln998Glu) plus *TNNI3* (p.Arg145Gly), *PRKAG2* (p.Gly100Ser) plus *MYBPC3* (p.Lys1209Serfs\*28), and *TNNI3* (p.Glu124Gln) plus *GLA* (p.Trp47\*) (Table III and Fig. 2).

**Mutations and phenotypes of patients with familial HCM.** Family A, B and F were of the Han ethnic group; family C, D and E were of the Yi, Naxi and Pumi ethnic groups, respectively. Our results showed that 3 single mutations and 3 double mutations were found in 6 pedigrees with familial HCM (Fig. 2). A 28-year-old male HCM patient, experiencing palpitation and chest tightness, was found to carry a novel mutation (*TAZ*, p.Ile208Val). A mutation of p.Gln998Glu in *MYBPC3* was detected in a proband patient (family A, III:5) who was diagnosed as having HCM at age 34, with intermittent chest tightness and shortness of breath, and a typical thick IVST (22 mm). His 2-year-old sisters were respectively diagnosed as HCM patients at age 36 and 42 years,

Table II. Sequences of primers used for validation of target genes.

Gene symbol	Transcript name	Exon	Nucleotide changes	Primer (5' to 3')	PCR fragment (bp)
<i>MYH7</i>	NM_000257.2	3	c.161G>A	Sense: CCAAAGCCAGCCTATGGA ACTCT Antisense: GGTCCCAATGGCTGCAATAAC	2,348
<i>MYH7</i>	NM_000257.2	8	c. [730T>C, 732C>T]	Sense: CTTGCTGGTCTCCAGTAGTATTGT Antisense: GGCTGAGCCTAGCAGATTCAT	536
<i>MYH6</i>	NM_002471	12	c.1087A>T	Sense: CTGGAGGTGGATGGAGGATGA Antisense: GGTGAGGAGTTGGGATTGTGGT	2,155
<i>SCN5A</i>	<i>SCN5A</i>	21	c.3575G>A	Sense: CATCTCTTCAACCATCCAACCTTCTGC Antisense: TCCCTGCCACAACCCTGCATC	275
<i>TNNI3</i>	NM_000363.4	6	c.370G>C	Sense: AGGTCTCCCTGTTTTTGGTTCC Antisense: GGACCTTCATGTACCTCTTTGCTCT	1,076
<i>GLA</i>	NM_000169	1	c.140G>A	Sense: CTGGTATGGAAATAGGGCGGGTC Antisense: CCTGATTGCGGACAGTTTGCTGG	682
<i>MYBPC3</i>	NM_000256.3	27	c.2992C>G	Sense: TATGTGACCAGTGGGCAGTTC Antisense: GGGTCTTGTGACTGCACAAAG	1,093
<i>MYH7</i>	NM_000257.2	22	c.2572C>T	Sense: GCTAATCAGTGACAAAGCCAGGATC Antisense: AGGGTGGAAGAGCCAACAGTAGC	1,434
<i>TNNI3</i>	NM_000363.4	6	c.433C>G	Sense: AGGTCTCCCTGTTTTTGGTTCC Antisense: GGACCTTCATGTACCTCTTTGCTC	1,076
<i>PRKAG2</i>	NM_000116.4	3	c.298G>A	Sense: CAGTCCTGTGTGGTCAGAACTTGG Antisense: GGACCAGAAGGATTACGCTTTGAT	907
<i>MYBPC3</i>	NM_000256.3	31	c.3624delC	Sense: AGAGGCTCTCGGCATCAGGAAG Antisense: ACATAGATGCCCCCGTCAAAG	906
<i>TAZ</i>	NM_000116.4	8	c.622A>G	Sense: TCAGGGCCCAGCTTATGCTAAC Antisense: TTTAATGTCTCGGTGCCAGGAAG	441

*MYH7*,  $\beta$ -myosin heavy chain; *SCN5A*, sodium channel, voltage-gated type V  $\alpha$ -subunit; *TNNI3*, troponin I 3; *GLA*,  $\alpha$ -galactosidase A; *MYBPC3*, myosin binding protein C; *PRKAG2*, AMP-activated protein kinase; *TAZ*, tafazzin.

Table III. Mutations identified in target genes of the patients with HCM.

Subjects	Gene name	Amino acid change	Mutation type	Protein location	Frequency
A14	<i>MYH7</i>	p.Arg54Gln	Missense	Myosin N-terminal SH3-like domain	1
9	<i>MYH6</i>	p.Met363Leu	Missense	Class II myosins, motor domain	1
25, 26	<i>SCN5A</i>	p.Arg1192Gln	Missense	Sodium ion transport-associated region	2
8	<i>MYH7</i>	p.Phe244Leu	Missense	Myosin motor domain	1
Family A, III:2, III:4 and III:5; family C, III:1	<i>MYBPC3</i>	p.Gln998Glu	Missense	Ig-like C2-type 6	4
Family B, II:1	<i>MYH7</i>	p.Arg858Cys	Missense	Tropomyosin	1
Family C, III:1	<i>TNNI3</i>	p.Arg145Gly	Missense	Troponin	1
Family F, II:1	<i>TAZ</i>	p.Ile208Val	Missense	LPLAT_AGPAT-like	1
Family D, III:1	<i>MYBPC3</i>	p.Lys1209Serfs*28	Frame shift	Ig-like C2-type 7	1
Family D, III:1	<i>PRKAG2</i>	p.Gly100Ser	Missense	N-terminal binding	1
Family E, II:1	<i>GLA</i>	p.Trp47*	Termination	$\alpha$ -galactohydrolase activity	1
Family E, II:1	<i>TNNI3</i>	p.Glu124Gln	Missense	Cardiac troponin C-binding domain	1

*MYH7*,  $\beta$ -myosin heavy chain gene; *SCN5A*, sodium channel, voltage-gated type V  $\alpha$ -subunit gene; *TNNI3*, troponin I 3 gene; *GLA*,  $\alpha$ -galactosidase A gene; *MYBPC3*, myosin binding protein C gene; *PRKAG2*, AMP-activated protein kinase; *TAZ*, tafazzin.

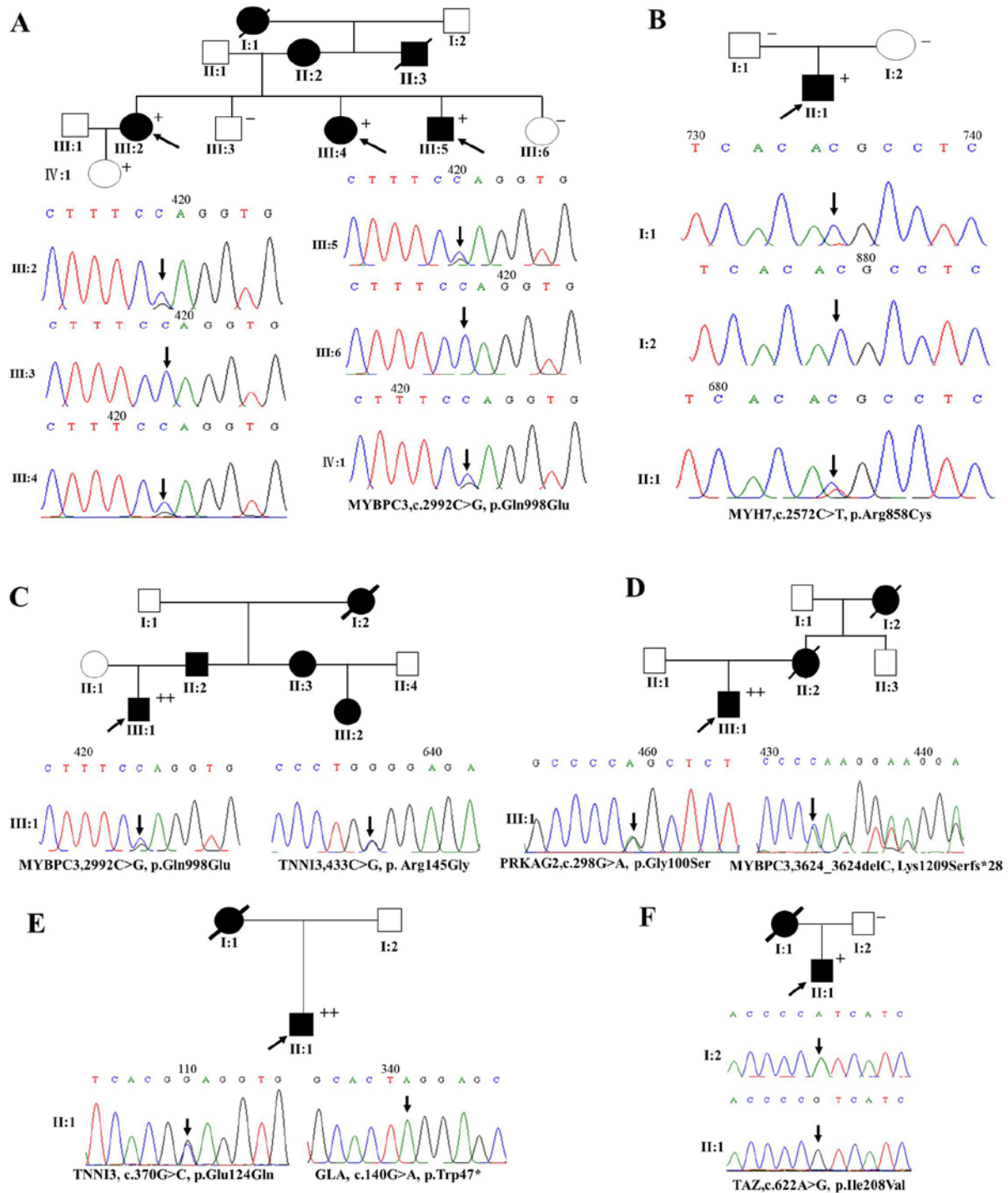


Figure 2. The genetic analyses of target gene mutations in patients from 6 pedigrees with familial hypertrophic cardiomyopathy (HCM) (families A, B, C, D, E and F). Squares, male family members; circles, female family members; open symbols, normal individuals; solid symbols, affected individuals; black arrow, proband; plus (+) signs, the presence of disease mutation; minus (-) signs, the absence of disease mutation. Both (+) and (-) indicate members for whom the PCR and Sanger sequencing validation were carried out.

and his father and grandmother had undergone sudden death. Another proband (family B, II:1) having MYH7 p.Arg858Cys was diagnosed as HCM at age 23 years and experienced chest pain, but his father and mother did not have disease phenotype. More importantly, the third proband (family C, III:1) carrying a double mutation (MYBPC3, p.Gln998Glu plus TNNI3, p.Arg145Gly) had a greater IVST (18.8; >15 mm), and his father, aunt and female cousin were diagnosed with

HCM a few years ago. A proband (family D, III:1) carrying a double mutation (PRKAG2, p.Gly100Ser plus MYBPC3, p.Lys1209Serfs\*28) was diagnosed with HCM at age 26, and his mother and grandmother had undergone sudden cardiac death. A proband (family E, II:1) carrying a double mutation (TNNI3, p.Glu124Gln plus GLA, p.Trp47\*) presented with heart failure at age 47 years, and the echocardiography showed that he had a high IVST (Figs. 2 and 3A).

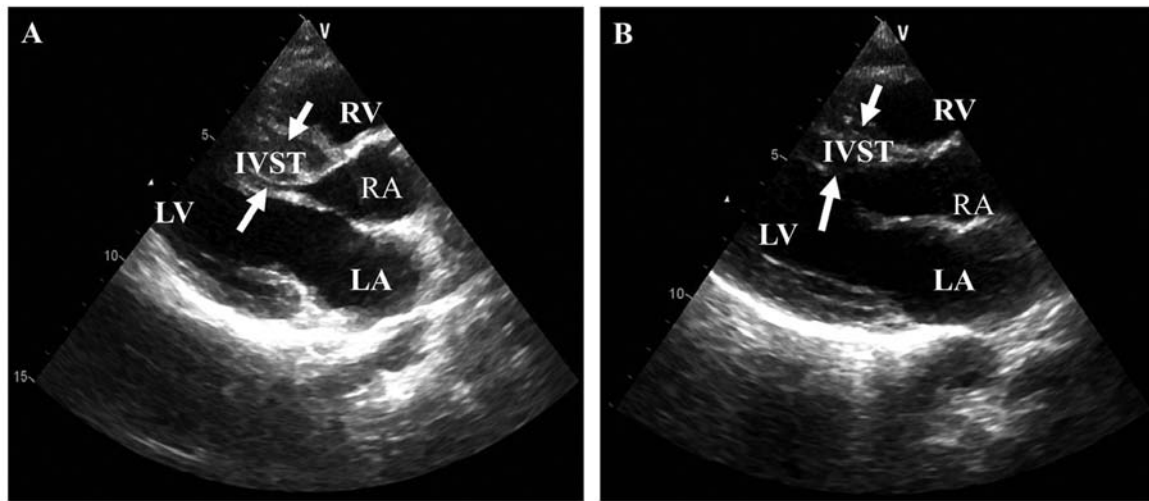


Figure 3. Two-dimensional echocardiography of hypertrophic cardiomyopathy (HCM) patients. (A and B) The echocardiogram of the four chambers of proband family F (patient II: 1) and patient A14, respectively. White arrows indicate areas of hypertrophy.

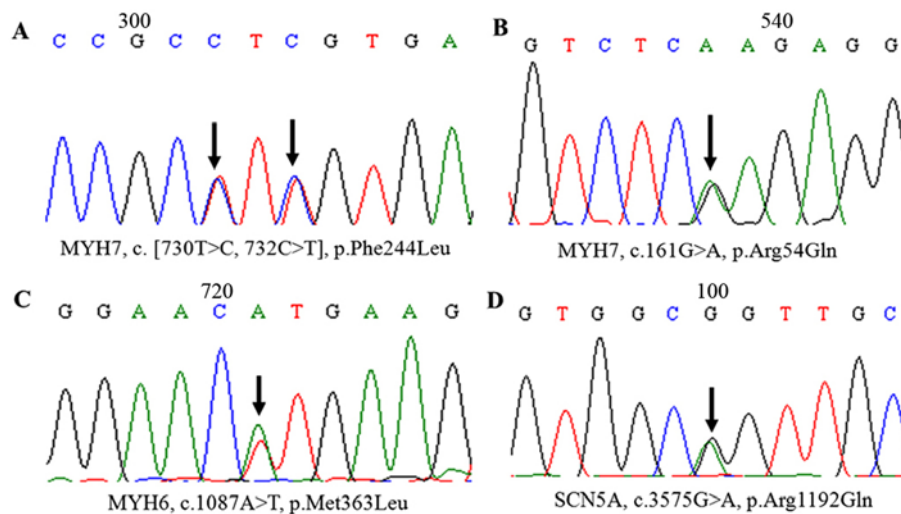


Figure 4. Identification of potential disease mutations in hypertrophic cardiomyopathy (HCM). Nucleotide mutation sites are shown with arrows. (A) A double heterozygous mutation at the nucleotide position c. (730T>C, 732C>T) of the  $\beta$ -myosin heavy chain gene (*MYH7*) gene in HCM patient 8. (B) A mutation at the nucleotide position c.161G>A of the *MYH7* gene in HCM patient A14. (C) A heterozygous mutation at the nucleotide position c.1087A>T of the  $\alpha$ -myosin heavy chain (*MYH6*) gene in patient 9. (D) A heterozygous mutation at the nucleotide position c.3575G>A of the sodium channel, voltage-gated type V  $\alpha$ -subunit (*SCN5A*) gene in patient 25 and 26.

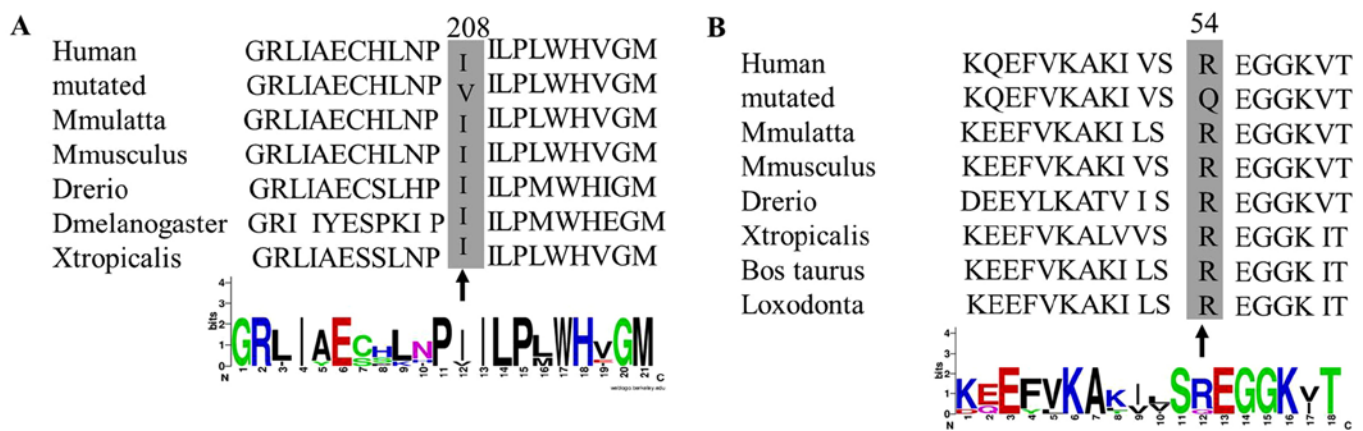


Figure 5. As determined using Clustal W, (A and B) the synonymous mutations -p.Ile208Val and p.Arg54Gln involved an amino acid in tafazzin (*TAZ*) and  $\beta$ -myosin heavy chain (*MYH7*) genes that were highly conserved across many species, respectively.



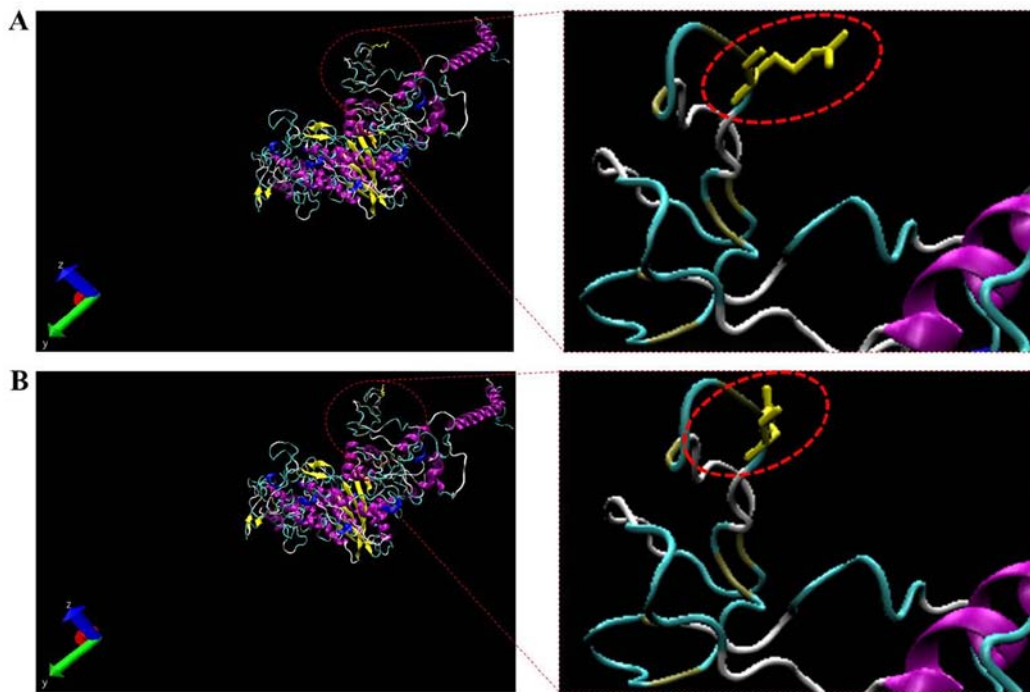


Figure 6. The structure modeling of predicted  $\beta$ -myosin heavy chain gene (MYH7) with wild-type and mutant. (A and B) Wild-type MYH7 (codon 1 to 841) and mutant p.Arg54Gln, respectively. The wild-type and mutated site are emphasized by a red circle and are locally zoomed.

## Discussion

In the present study, we described the molecular characterization of 18 HCM patients in Yunnan Province via targeted NGS technology. A total of 383 variants were identified with an average read depth of 165.4-fold (Fig. 1). Generally, a read of a targeted nucleotide with read depth  $\geq 30\times$  reads was considered as qualified (17,18). By filtering out synonymous variations of the targeted genes, 86 qualified non-synonymous variations were obtained (data available upon request). This indicated the feasibility of NGS for genetic testing of 19 HCM-targeted genes.

There were 12 mutations identified in 13 HCM patients (Table III). The overall genetic diagnostic rate was 72.2% (13/18) and the frequency of disease-causing mutations in the HCM cohort were much higher than previous documentations (22-24), including an American cohort (54.2%), French cohort (60.6%) and Japanese cohort (67%) (25-27). Mutations in the *MYBPC3* gene were the most prevalent that were detected in 5 of the 13 patients (38.5%). It has been reported that mutations in the *MYBPC3* gene are present in close to 20-30% of HCM cases (4,20,28), and our results were consistent with these previous studies. The second most prevalent mutations were found in *MYH7* in 3 patients (3/13, 23.1%), which were followed by mutations in *TNNI3* (15.4%). The respective mutation rate of *SCN5A*, *PRKAG2*, *MYH6*, *TAZ* and *GLA* genes was close to 7.7%.

It is noteworthy that two single mutations were found for the first time in HCM patients. One in *TAZ* (p.Ile208Val) was novel and was found in a familial HCM male patient of 28 years. The other single mutation was in *MYH7* (p.Arg54Gln), which has been rarely reported in the general population but had not been previously found in HCM patients. This mutation had a

very low minor allele frequency (1/60,659) in ExAC browser Beta database (<http://exac.broadinstitute.org/>). Both *TAZ* (p.Ile208Val) and *MYH7* (p.Arg54Gln) mutations resulted in polarity changes in the altered amino acids and these 2 mutations were not detected in 200 normal chromosomes. It has been speculated that these mutations may have a significant impact on the structure and function of the corresponding proteins. Indeed, the homology modeling analysis showed that, compared to wild-type MYH7, the structure of mutant MYH7 was altered at the mutated site. More detailed data are needed to detect whether these mutations influence the pathogenicity of HCM.

In the 6 pedigrees with familial HCM, 3 of them (50%) were found to carry double mutations, which were firstly discovered in this study. These double mutations were in *MYBPC3* (p.Gln998Glu) plus *TNNI3* (p.Arg145Gly), *PRKAG2* (p.Gly100Ser) plus *MYBPC3* (p.Lys1209Serfs\*28), and *TNNI3* (p.Glu124Gln) plus *GLA* (p.Trp47\*), respectively. According to previous literature, ~15% of familial HCM patients carry a double heterozygous mutation (29), which was far less than our results. This implies that special molecular genetic mechanisms exist in Yunnan patients with familial HCM. There are 26 ethnic groups in Yunnan Province and consanguineous marriages are widely acceptable. The higher consanguineous marriage percentage than that of the same groups in developed regions of China (30) has led to the accumulation of defective gene mutations making the offspring at higher disease risks.

Of these 18 patients, the relationships between the phenotype and the genetics of HCM in familial HCM patients carrying double mutations were further analyzed. The 27-year-old patient (family C, III:1) showed a severe phenotype (IVST=22.8 mm), and had double mutations in *MYBPC3*

(p.Gln998Glu) plus TNNI3 (p.Arg145Gly). A 'double dose effect' of gene mutation (15) was speculated to lead to a more malignant clinical phenotype and an early onset of HCM. The patient (family D, III:1) with the PRKAG2 (p.Gly100Ser) plus MYBPC3 (p.Lys1209Serfs\*28) mutations was characterized as having relatively severe hypertrophy and an early onset of HCM, the same feature of the patient from family E (TNNI3, p.Glu124Gln plus GLA, p.Trp47\*). The TNNI3 (p.Glu124Gln) mutation was originally reported in Taiwanese patients with familial HCM (29). The Taiwanese are the descendants of early settlers from the southeast coast of China during the last few centuries (31). The present study showed its second appearance in Chinese HCM patient. Moreover, the children of HCM probands (family C, III:1; family D, III:1; and family E, II:1) would have a higher risk rate of this disease and similar mutations. According to our results, it is recommended that these probands should implement prenatal screening for the mutations of MYBPC3, p.Gln998Glu plus TNNI3, p.Arg145Gly; PRKAG2, p.Gly100Ser plus MYBPC3, p.Lys1209Serfs\*28; and TNNI3, p.Glu124Gln plus GLA, p.Trp47\*, respectively. For family B, the proband (II:1, 23 years of age) with the MYH7, p.Arg858Cys mutation did not show a severe phenotype, but his parents had no disease phenotype and the genetic testing was negative. This suggests that MYH7 (p.Arg858Cys) is a *de novo* mutation.

This study described the mutational spectrum of patients with HCM in Yunnan, China. However, the number of HCM patients was limited and the obtained clinical data were not consistent for all subjects. A large-scale study of HCM patients in Yunnan is needed to further confirm our results.

In conclusion, in the present study, 2 single and 3 double mutations were firstly found in HCM patients. The 3 double mutations were detected in different ethnic groups and a novel single mutation was found in TAZ (p.Ile208Val). The mutation in MYH7 (p.Arg54Gln), previously reported as being rare in the general population and having a very low minor allele frequency, was found in a female patient without familial HCM. Our results add new data to the mutational spectrum of Yunnan HCM patients. It provides useful information for the presymptomatic intervention of HCM and the management of patients with familial HCM.

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