**EXPERIMENTAL STUDY**

**MYH7 Gene-Related Mutation p.V878L Identified in a Chinese Family with Hypertrophic Cardiomyopathy**

Yuan Du, MD, Ya Wang, MD, Xiu Han, MD, Zhanbin Feng, MD and Aiqun Ma, MD

**Summary**

Hypertrophic cardiomyopathy (HCM) is one of the most common inherited cardiovascular diseases and possesses a high risk for sudden cardiac death. Although mutations in more than 20 genes have been reported to be associated with HCM thus far, the genetic backgrounds of most HCM patients are not fully understood. We performed a genetic analysis in a Chinese family that presented with HCM using next-generation sequencing (NGS). Clinical data, family histories, and blood samples were collected from the proband and family members. Five patients showed typical clinical symptoms of HCM. One subject was the victim of sudden cardiac death. By NGS, we determined that these subjects with HCM symptoms carried a missense heterozygous genetic mutation c.2632C>A (p.V878L) in the myosin heavy chain 7 (MYH7) gene with an autosomal dominant pattern of inheritance. Individuals without this mutation showed no symptoms or cardiac structural abnormalities related to HCM. Bioinformatics evaluation predicted this mutant as “damaging” and “disease causing”. Additionally, sequence alignment showed that this mutant is located in an evolutionarily conserved region of MYH 7 in multiple species. Our results describe a potentially pathogenic mutation associated with HCM, which may extend the spectrum of HCM phenotypes related to MYH7 gene mutations.

(Key words: Next generation sequencing, Bioinformatic)

Hypertrophic cardiomyopathy (HCM) is a primary myocardial disease characterized by left ventricular (LV) hypertrophy, accompanied by myofibrillar disarrays and diastolic dysfunction of cardiac ventricles, which occurs in the absence of other cardiovascular or systemic causes. The prevalence of HCM is 1:500 in the general population and about 0.16% in China. Currently, HCM appears to be the leading cause of sudden death in young people, with particular predilection for children and young adults (age < 30 years). HCM is the first cardiac disease associated with a genetic background and it is one of the most common inherited cardiovascular diseases. Until now, pathogenic causative mutations have been identified in 25~30 genes. About 90% of pathogenic mutations are missense mutations in which one highly conserved DNA nucleotide is replaced by a different nucleotide resulting in a different codon, leading to alterations of the physical and functional properties of proteins. HCM is an autosomal dominant hereditary disease, therefore, each first-degree relative of an affected patient has a 50% chance of carrying the mutation and potentially developing HCM. HCM-related mutations lead to sarcomeric and/or regulatory protein abnormalities in cardiomyocytes, which result in myofibrillar disorganization, myocyte hypertrophy, and interstitial fibrosis.

In the above-mentioned genes, myosin heavy chain 7 (MYH7) and myosin binding protein C3 (MYBPC3) are the two most frequent disease-causing genes, which are estimated to account for approximately half of the patients with familial HCM; while other genes including troponin T (TNNT2), troponin I (TNNI3), α-tropomyosin (TPM1), and α-actin (ACTC) each account for a small proportion of patients (1% to 5%). However, other genetic alterations associated with HCM are under exploration. Recently, striking scientific advances in molecular genetics have resulted in the availability of comprehensive genetic testing and enabled us to seek out more genetic mutations in families with HCM.

Traditionally, genetic studies focus on the most likely HCM-related genes using conventional Sanger sequencing, despite the practical difficulties of keeping up with the ever-increasing number of test requests and disease-associated genes. In recent years, next-generation sequencing (NGS) has emerged as a revolutionary technology which enables the generation of a high amount of genetic information.
netic data.\(^8\) This massive amount of information has triggered the development of potent bioinformatic tools to help interpret potential causality implications.\(^8\) In this report, taking advantage of the NGS assay combined with clinical assessments, we identified a \(MYH7\) mutation (c.2632C>A [p.V878L]) in a Chinese family affected by HCM. This report extends the spectrum of HCM phenotypes associated with \(MYH7\) gene mutations.

**Methods**

**Clinical assessment:** Informed consent was obtained from all affected family members and control subjects. Detailed clinical evaluation, including accurate medical history, physical examination, serum creatinine kinase (CK), 12-lead electrocardiogram (ECG), and transthoracic echocardiography were performed in the proband and her relatives. Due to the long distance to the hospital, several members did not present to the hospital for clinical examination. One hundred unrelated blood donors with normal ECGs and echocardiography served as controls. This study was approved by the Ethics Committee of the First Affiliated Hospital of Xi’an Jiaotong University (Xi’an, Shaanxi) and conforms to the principles outlined in the Declaration of Helsinki.

Echocardiographic diagnostic criteria for HCM include an unexplained maximal wall thickness (measured at end-diastole) ≥ 15 mm (or > 2 standard deviation for age, height and gender) in any myocardial segment or septal/posterior wall thickness ratio of > 1.3 in a nondilated ventricle and > 1.5 in the setting of systemic hypertension.\(^10\)

**Samples:** Blood samples were collected from the proband and her family members. RNA-free high-molecular-weight DNA was prepared using a Tiangen Blood DNA Extraction Kit (TIANGEN[DP318-03(200)], China). The quality and concentration of genomic DNA samples were assessed by agarose gel electrophoresis and a NanoDrop spectrophotometer.

**Next generation sequencing and analysis:** For genes from nucleus genome, 1,000 ng of genomic DNA was fragmented into an average size of 250 bp, and then the fragmented genomic DNA was used to prepare sequencing library. 8 bp barcode sequenced adaptors were then ligated with the DNA fragments before final hybridization with a customer-made gene panel focused exome of genes related to cardiovascular disease (NimbleGen, Roche). This NGS gene panel not only includes cardiomyopathy-related genes, but also includes all the genes associated with cardiovascular diseases (1876 genes, detailed information in Supplemental Table). The quality and quantity were measured by both an AATI fragment analyzer and qPCR. Purified sequencing libraries were pooled together and were massively parallel sequenced by Illumina HiSeq X ten platform to produce an average of 2.5 Gb per sample, resulting in cardiovascular disease nucleus genome with average sequencing depth around 150×. These data were then aligned to the hg19 genome using NextGene (V 2.3.4, Softgenetics, USA) which is an alignment method that is similar to BLAST methodology to align sequence reads to the reference. NextGene uses a preloaded index alignment algorithm that employs a suffix array that is represented by the Burrows-Wheeler Transform (BWT). All the mutations were compared with databases such as dbSNP, ExAC or 1000 genome projects. All the mutations found that can potentially cause HCM were subject to Sanger sequencing (ABI3130) for verification.

**Conservation analysis and bioinformatics prediction:** The homologs (similarity attributable to descent from a common ancestor) of the region including Val878 in homo sapiens were detected (HomoloGene, http://www.ncbi.nlm.nih.gov/homologene). The potential pathogenicity of the identified missense mutations was evaluated by combining different methods: PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2), SIFT (http://sift.jcvi.org), and Mutation Taster (http://www.mutationtaster.org). The reference protein ID of \(MYH7\) was ‘P12883’ and the Ensembl transcript ID was ‘ENST00000355349’.

**Results**

**Clinical features of the proband:** The proband of this family was a 48-year-old female (III-6, Figure 1 and Table). She had recurrent paroxysmal palpitations and chest distress for about 6 months, without angina pectoris, dyspnea or syncope. Physical examination revealed a grade III/VI harsh long systolic murmur heard best over the left lower sternal border. An ECG revealed sinus rhythm and LV hypertrophy. There were nonspecific ST-T wave changes in the anterolateral leads and Q wave in the inferior leads (Figure 2A). An echocardiogram showed septal hypertrophy of 23 mm (Figure 2B) and a left ventricle outflow tract (LVOT) gradient of 43 mmHg at rest. Cardiac catheterization revealed systolic obliteration of the left ventricle with hypertrophied septum further impinging into the cavity (Figure 2C) and a pressure gradient of 31 mmHg across the LVOT. Coronary angiography revealed 30% stenosis of the proximal left circumflex coronary artery (LCX). The left anterior descending (LAD) and right coronary artery (RCA) were normal.

**Pedigree analysis:** During the proband’s clinical visits, we were informed that her father (II-1, Figure 1) died suddenly at the age of 54, most likely from sudden cardiac death (SCD), and her older brother (III-1) was diagnosed with HCM at a different hospital one year previously. Unfortunately, the records of her father were not available to us. Her mother (II-2) had a history of hypertension and conforms to the principles outlined in the Declaration of Helsinki.
I n t H e a r t J
Advance Publication 3

Figure 1. Pedigree structure of the family. Family members are identified by generations and numbers. Squares, male family members; circles, female members.

### Table. Clinical Characteristics of the Family Members

| Echocardiography |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
|------------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ID   | Age/Gender | Gene mutation | LAD (mm) | LVEDD (mm) | IVST (mm) | LVPWT (mm) | EF (%) | ECG | Symptoms | Comments |
| I-1  | 24/M       | -             | -        | -          | -          | -          | -      | - | - | Died |
| I-2  | 27/F       | -             | -        | -          | -          | -          | -      | - | - | Died |
| II-1 | 25/F       | -             | -        | -          | -          | -          | -      | - | - | Sudden death |
| II-2 | 72/F       | No            | 40       | 59         | 10         | 9          | 48     | Sinus rhythm and complete RBBB | Slight dyspnea | Hypertension for 20 years |
| II-3 | 60/M       | c.2632C>A     | 43       | 53         | 20         | 9          | 67     | Sinus rhythm and LV hypertrophy | Angina and dyspnea | No |
| II-5 | 47/M       | c.2632C>A     | 38       | 49         | 21         | 8          | 72     | Sinus rhythm and LV hypertrophy | Angina and dyspnea | No |
| III-1| 53/M       | c.2632C>A     | 37       | 47         | 15         | 8          | 60     | Sinus rhythm and LV hypertrophy | Angina and dyspnea | Hypertension for 4 years |
| III-4| 50/F       | No            | 35       | 48         | 9          | 9          | 68     | Sinus rhythm and LV hypertrophy | Normal | No |
| III-6| 48/F       | c.2632C>A     | 37       | 49         | 23         | 11         | 68     | Sinus rhythm and LV hypertrophy | Palpitation and chest distress | No |
| III-8| 47/F       | c.2632C>A     | 37       | 48         | 22         | 8          | 70     | Sinus rhythm and LV hypertrophy | Angina and dyspnea | No |
| III-10| 43/F      | No            | 32       | 49         | 8          | 9          | 65     | Normal | No | No |
| III-11| 40/M      | -             | -        | -          | -          | -          | -      | - | - | - |
| III-13| 30/F      | No            | 28       | 45         | 8          | 8          | 70     | Normal | No | No |
| III-14| 20/M      | -             | -        | -          | -          | -          | -      | - | - | - |
| IV-1 | 24/M       | No            | 29       | 47         | 7          | 8          | 68     | Normal | No | No |
| IV-2 | 27/F       | -             | -        | -          | -          | -          | -      | - | - | - |
| IV-3 | 25/F       | -             | -        | -          | -          | -          | -      | - | - | - |
| IV-5 | 23/F       | c.2632C>A     | 27       | 46         | 10         | 9          | 70     | Normal | No | No |
| IV-6 | 20/M       | No            | 28       | 46         | 9          | 10         | 65     | Normal | No | No |
| IV-7 | 15/M       | No            | 27       | 45         | 7          | 7          | 72     | Normal | No | No |

* Subject II-1 died at the age of 54. ECG indicates electrocardiogram; LAD, left atrial diameter; LVEDD, left ventricular end diastolic diameter; IVST, interventricular septum thickness; LVPWT, left ventricular posterior wall thickness; EF, ejection fraction; LV, left ventricle; and RBBB, right bundle branch block.

1) investigated were asymptomatic and had normal echocardiogram and ECG results. There were no manifestations of skeletal muscle disease in any family members, and the levels of CK were all normal during our interviews.

**Mutation identification:** To identify the genetic basis of the HCM suffered by this family, a custom-made NGS panel consisting of 1876 genes previously associated with cardiomyopathies and related striated muscle disorders was used (Supplemental Table). Genetic sequencing was performed in the proband using this NGS panel. A mutation c.2632C>A was found in MYH7 (RefSeq: NM_000257.2), corresponding to a nonsynonymous amino acid change from valine to leucine at position 878 (p.V878L, Figure 3A). Next, MYH7 was sequenced in all family members and 100 controls. The mutation c.2632C>A was
confirmed in the subjects with HCM manifestations (II-3, II-5, III-1 and III-8) and was not seen in other asymptomatic family members (III-4, III-10, III-13, IV-1, IV-6 and IV-7) and 100 unrelated control subjects. The proband’s daughter (IV-5) who had no clinical features of HCM was also found to carry the same mutation.

**Functional prediction of p.V878L mutation:** The mutant p.V878L in the MYH7 protein was located in the neck of
S2 domain of cardiac muscle β-myosin heavy chain (MHC-β). This area is a functionally important conserved region, which possesses high sequence consistency and has been conserved in the evolutionary process (Figure 3 B, C). To predict the pathogenic consequence of this mutation, bioinformatic analysis was performed. The predictive score of this p.V878L mutant of MYH7 by ‘PolyPhen-2’, and ‘Mutation Taster’ algorithms was 0.992 and 0.9999, which means ‘most probably damaging’, and ‘disease causing’, respectively. Therefore, the mutant is likely to lead to alterations in the functions of the encoded proteins and have a potential pathogenic effect.

**Discussion**

In the present study, we performed next-generation sequencing on a typical Chinese HCM family and identified a missense heterozygous genetic mutation in MYH7 (c.2632C>A [p.V878L]) which might be the potentially pathogenic mutation.

The MYH7 gene encodes myosin heavy chain 7 (MYH7, also known as MHC-β), which is located on chromosome 14q11.2 and contains 40 exons. MHC-β is the major protein comprising the thick filament in cardiac muscle and plays a major role in cardiac muscle contraction. Typical diseases correlated with mutations of MYH7 include HCM, dilated cardiomyopathy (DCM), scapuloperoneal myopathy, Laing early-onset distal myopathy, and myosin storage myopathy. MYH7 was the first HCM gene to be identified and it is one of the most prevalent HCM disease genes. MYH7 mutations are responsible for approximately 40% of all genotyped HCM cases. Until now, more than 1,600 mutations in the MYH7 gene have been identified (http://www.hgmd.cf.ac.uk). Here we report a missense mutation of the MYH7 gene, c.2632C>A (p.V878L) in exon 22. In this Chinese family, the MHC-β associated diseases manifest as HCM, without signs of skeletal muscular dysfunction.

MHC-β is composed of N-terminal globular heads and alpha helical tails that dimerize and multimerize into a coiled-coil motif to form the light meromyosin (LMM), thick filament rod. Each head, or subfragment-1 (S1), contains the actin and ATP binding regions and is responsible for the force transduction properties of myosin. The N-terminal region of the rod, termed subfragment-2 (S2), joins the heads at the neck region. LMM mediates filament assembly and also provides sites for the binding of myosin-associated proteins such as myosin binding protein C (MyBP-C) and titin. Missense mutations of the MYH7 gene affect different structural and functional domains of the MHC-β protein, leading to the incorporation of abnormal polypeptides, which eventually result in contractile dysfunction and an alteration in sarcromeric and myocyte structure and organization. The location of the mutation influences the severity and prognosis of the disease in some kindreds. For example, the non-conservative missense mutations, p.R403Q (in actin binding region) and p. R719C (light chain binding interface) are linked to a high incidence of sudden cardiac death and decreased life expectancy. In contrast, patients carrying p.L908V, a conservative mutation occurring in the head-rod junction, are characterized by a lower incidence of sudden death and a more benign course. In our study, the p.V878L missense mutation in MYH7 is a conservative missense mutation located in the neck of S2 domain. Wang, et al. have reported a different mutation at this site, p.V878A, in a Chinese family with HCM. The subjects carrying this p.V878A mutation showed moderate or mild HCM phenotypes. The p.V878L variant also has been reported by GeneDx (https://www.ncbi.nlm.nih.gov/clinvar/variation/181197), and the database says it is a variant of unknown significance. We found that it is a deleterious variant.

Our study indicated that except for one SCD victim (II-1), the most HCM survivors were between 40 and 60 years old. The present Chinese family members are characterized by mild to moderate hypertrophy without malignant arrhythmia. It seems that they have a relatively good prognosis and longer life span. However, the mechanisms of how this missense mutation interferes with the MHC-β function and then leads to HCM are not clear yet. Further biochemical and cell biological studies are needed to determine the consequence of this missense mutation.

The most devastating complication of HCM is sudden cardiac death (SCD), with an annual rate of approximately 1.3%. The proband’s father (II-1) died of SCD, and the proband’s mother (II-2) did not carry the p.V878L mutation. The proband’s two uncles (II-3 and II-5) manifesting with HCM carried the mutation. Thus, we concluded that the proband’s father may have suffered from HCM and carried the p.V878L mutation.

Environmental factors, including gender, age, lifestyle, and genetic factors (so-called modifier genes) have been suggested to modulate clinical presentation in patients harboring the same mutation. In the present study, the penetrance of the MYH7 gene mutation is high: 83.8% (5/6) of the mutation carriers manifested with HCM. Our predictive genetic testing revealed one asymptomatic individual (IV-5) who was 23 years old. It is well known that the absence of clinical expression does not reduce the risk for HCM-related complications. Therefore, on the basis of our genetic diagnosis of HCM in this family, we have been able to identify this young mutation carrier without phenotypic expression, which will facilitate better management before the onset of symptoms.

Bioinformatic prediction can give us some useful information about the pathogenicity of the MYH7 p.V878L mutant, however, it cannot reflect the real pathology of the mutant in the cardiac myocytes. Due to the inaccessibility of human heart tissue, we could not obtain a sufficient amount of the patient’s heart tissues. Using the CRISPR/Cas9 gene editing method to create mutant mice or using patient-specific induced pluripotent stem (iPS) cells-derived cardiomyocytes are state-of-the-art methods to investigate the pathogenicity of human mutant directly and repetitively. In the future, we will further study the pathology effect of MYH7 p.V878L mutant using patient-specific iPSCs-derived cardiomyocytes.

In conclusion, we report a missense heterozygous genetic mutation in the MYH7 gene (c.2632C>A [p.V878L]) in a family that presented with HCM. The present study confirms the genotype-phenotype correlation of the p.V878L mutation and provides further insight into genetic
contributions to HCM pathology. Moreover, our findings indicated that NGS assay is a useful approach for the identification of pathogenic mutations associated with inherited cardiomyopathy.

Acknowledgment

The authors are very thankful to the patients for their cooperation.

Disclosure

Conflicts of interest: None.

References

1. Kittleson MD, Meurs KM, Harris SP. The genetic basis of hypertrophic cardiomyopathy in cats and humans. J Vet Cardiol 2015; 17: S53-73.
2. Semsarian C, Ingle J, Maron MS, et al. New perspectives on the prevalence of hypertrophic cardiomyopathy. J Am Coll Cardiol 2015; 65: 1249-54.
3. Zou Y, Song L, Wang Z, et al. Prevalence of idiopathic hypertrophic cardiomyopathy in China: a population-based echocardiographic analysis of 8080 adults. Am J Med 2004; 116: 14-8.
4. Maron BJ, Maron MS. Hypertrophic cardiomyopathy. Lancet 2013; 381: 242-55.
5. Papoularides C. Challenges and controversies in hypertrophic cardiomyopathy: clinical, genomic and basic science perspectives. Rev Esp Cardiol 2018; 71: 132-7.
6. Maron BJ, Maron MS, Semsarian C. Genetics of hypertrophic cardiomyopathy after 20 years: clinical perspectives. J Am Coll Cardiol 2012; 60: 705-15.
7. Ho CY. Genetic considerations in hypertrophic cardiomyopathy. Prog Cardiovasc Dis 2012; 54: 456-60.
8. Meyer T, Pankuweit S, Richter A, et al. Detection of a large duplication mutation in the myosin-binding protein C3 gene in a case of hypertrophic cardiomyopathy. Gene 2013; 527: 416-20.
9. Marian AJ, Braunwald E. Hypertrophic cardiomyopathy: genetics, pathogenesis, clinical manifestations, diagnosis, and therapy. Circ Res 2002; 90: 263-9.
10. Weissler-Snir A, Crean A, Rakowski H. The role of imaging in the diagnosis and management of hypertrophic cardiomyopathy. Expert Rev Cardiovasc Ther 2016; 14: 51-74.
11. Greber-Platzer S, Marx M, Fleischmann C, et al. Beta-myosin heavy chain gene mutations and hypertrophic cardiomyopathy in Austrian children. J Mol Cell Cardiol 2001; 33: 141-8.
12. Arad M, Penas-Lado M, Monserrat L, et al. Gene mutations in apical hypertrophic cardiomyopathy. Circulation 2005; 112: 2805-11.
13. Tanjore RR, Srikinaliparam AD, Calambur N, et al. Genotype-phenotype correlation of R870H mutation in hypertrophic cardiomyopathy. Clin Genet 2006; 69: 434-6.
14. Hougs L, Havndrup O, Bundgaard H, et al. One third of Danish hypertrophic cardiomyopathy patients with MYH7 mutations have mutations [corrected] in MYH7 rod region. Eur J Hum Genet 2005; 13: 161-5.
15. Frazier A, Judge DP, Schulman SP, et al. Familial hypertrophic cardiomyopathy associated with cardiac beta-myosin heavy chain and troponin I mutations. Pediatr Cardiol 2008; 29: 846-50.
16. Lee DD, Veith RL, Dimmock DP, et al. Hypertrophic cardiomyopathy: a new mutation illustrates the need for family-centered care. Pediatr Cardiol 2014; 35: 1474-7.
17. Daehnlow S, Erdmann J, Kneppel T, et al. Novel mutations in sarcomeric protein genes in dilated cardiomyopathy. Biochem Biophys Res Commun 2002; 298: 116-20.
18. Villard E, Duboscq-Bidot L, Charron P, et al. Mutation screening in dilated cardiomyopathy: prominent role of the beta myosin heavy chain gene. Eur Heart J 2005; 26: 794-803.
19. Watkins H, Rosenzweig A, Hwang DS, et al. Characteristics and prognostic implications of myosin missense mutations in familial hypertrophic cardiomyopathy. N Engl J Med 1992; 326: 1108-14.
20. Pegoraro E, Gavassini BF, Borsato C, et al. MYH7 gene mutation in myosin storage myopathy and scapulo-peroneal myopathy. Neuromuscular Disord 2007; 17: 321-9.
21. Meredith C, Herrmann R, Parry C, et al. Mutations in the slow skeletal muscle fiber myosin heavy chain gene (MYH7) cause Laing early-onset distal myopathy (MPD1). Am J Hum Genet 2004; 75: 703-8.
22. Brand P, Dyck PJ, Liu J, et al. Distal myopathy with coexisting heterozygous TIA1 and MYH7 Variants. Neuromuscular Disord 2016; 26: 511-5.
23. Sabater-Molina M, Pérez-Sánchez I, Hernández Del, Rincón JP, et al. Genetics of hypertrophic cardiomyopathy: a review of current state. Clin Genet 2018; 93: 3-14.
24. Blair E, Redwood C, de Jesus, Oliveira M, et al. Distal myopathy with coexisting heterozygous TIA1 and MYH7 Variants. Neuromuscular Disord 2016; 26: 511-5.
25. Wu A, Rakowski H, Liew JC, et al. Mutations of the beta myosin heavy chain gene in hypertrophic cardiomyopathy: critical functional sites determine prognosis. Heart 2003; 89: 1179-85.
26. Epstein ND, Cohn GM, Cyran F, et al. Differences in clinical expression of hypertrophic cardiomyopathy associated with two distinct mutations in the beta-myosin heavy chain gene. A 908 Leu——Val mutation and a 403Arg——Gln mutation. Circulation 1992; 86: 345-52.
27. Wang B, Guo RQ, Wang J, et al. The Cumulative Effects of the MYH7-V878A and CACNA1C-A1594V Mutations in a Chinese Family with Hypertrophic Cardiomyopathy. Cardiology 2017; 138: 228-37.
28. Maron BJ. Hypertrophic cardiomyopathy: a systematic review. JAMA 2004; 292: 1308-20.
29. Wang S, Zou Y, Fu C, et al. Worse prognosis with gene mutations of beta-myosin heavy chain than myosin-binding protein C in Chinese patients with hypertrophic cardiomyopathy. Clin Cardiol 2008; 31: 114-8.

Supplemental Files

Supplemental Table

Please see supplemental files; https://doi.org/10.1536/ihj.19-146