Asn391Thr Mutation of β-Myosin Heavy Chain in a Hypertrophic Cardiomyopathy Family
From Genotype to Phenotype
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Summary
The present study was performed to identify the genetic abnormalities in a family with familial hypertrophic cardiomyopathy. Peripheral blood samples were collected from 22 members of a Chinese family with hypertrophic cardiomyopathy and 307 healthy controls. A total of 26 candidate pathogenic genes were analyzed in the proband using targeted capture sequencing. Identified mutations were analyzed using Sanger sequencing in all family members and healthy controls.

A missense mutation (c.1172A>C, p. Asn391Thr) in exon 12 of MYH7 was identified in eight family members, among which six of them were hypertrophic cardiomyopathy carriers. Three carriers presented with cardiac dysfunction. Four members of this pedigree died suddenly, three of whom were diagnosed with hypertrophic cardiomyopathy.

From the results of this study, we concluded that the Asn391Thr mutation of MYH7 is a malignant mutation for HCM and that mutation carriers should get effective treatment to prevent sudden death.

Key words: MYH7, High-throughput Sequence, Genotype-phenotype correlation

Hypertrophic cardiomyopathy (HCM) is a myocardial disease of unknown cause with left ventricular wall thickening as the main indicator, and patients usually don’t have performance about aggravation of heart load. In November 2011, the American College of Cardiology Foundation/American Heart Association (ACCF/AHA) jointly issued guidelines in HCM, which refers to unknown causes left ventricular hypertrophy without ventricular cavity expansion and can’t found other cardiac disease or systemic disease that can lead to ventricular hypertrophy, and clinical echocardiographic hint that the left ventricular thickness ≥15 mm or more. HCM is one of the main causes of sudden cardiac death among young people. The estimated prevalence of HCM in the general population is approximately 0.2%. The risk of atrial fibrillation and heart failure is often present in patients for a lifetime.

HCM is the first monogenic heart disease whose pathogenic gene has been identified. It is mainly caused by a mutation in the gene that codes for the sarcomere structural protein. The first missense mutation gene associated with HCM was identified in the cardiac-myosin heavy chain gene (MYH7) by linkage analysis in 1990. Since then, researchers have found > 1400 mutations in > 20 genes associated with the disease. However, according to a recent study, there has been no significant progress in the past decade from a clinical perspective. So far, MYH7 mutation is the main genetic factor that occupies 30%-50% of all HCM pathogenic genes. In this study, we identified an Asn391Thr mutation in MYH7 of a Han Chinese family with HCM, and we analyzed the clinical characteristics of the mutation.

Methods
Patients: This study included three generations of a Han family with HCM, with a total of 22 participants (Figure 1). Clinical data include data from physical, electrocardiographic, and two-dimensional and Doppler echocardiographic examinations. The diagnostic criterion of HCM in the general population is approximately 0.2%. The risk of atrial fibrillation and heart failure is often present in patients for a lifetime.

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**Mutation sequencing:** To extract genomic DNA, we collected peripheral venous blood from the participants and used EDTA as an anticoagulant. We then used the blood extraction kit (Cat. # DP349-02, Tiangen, Beijing) to extract genomic DNA from leukocytes, according to the manufacturer's instructions.

We used a high-throughput sequencing method to sequence 26 disease genes coding exons and 5-base pair of the upstream and downstream intron sequences of the proband. Gene sequencing for the proband was performed as follows. There are 26 pathogenic genes for detection: MYH7, MYBPC3, TNNT2, TNNI3, MYL2, MYL3, MYH6, TTN, TPM1, ACTC1, TNNC1, PRKAG2, LAMP2, GLA, CSRP3, MYOZ2, JPH2, ACTN2, CAV3, LDB3, VCL, PLN, MYPN, NEXN, CALR3, and ANKR51. Genomic DNA was broken into about 250-bp fragments by ultrasound. We used AMPureXP magnetic beads (Agencourt, Beckman Coulter, CA, USA) to enrich the 200-300-bp fragments. After the universal connection, polymerase chain reaction (PCR) was performed for eight cycles and the customized Agilent liquid capture library (Agilent Technologies, Santa Clara, CA, USA) to enrich the target area. After 10 cycles amplification of the genome, which had been enriched, the Illumina GA IIx (Illumina Inc., CA, USA) to enrich the target area. After 10 cycles amplification of the genome, which had been enriched, the Illumina GA IIx (Illumina Inc., CA, USA) sequenced paired ends, with each side reading 120 bp. After removing the repeated sequencing data by PICARD, we used CLC Genomics Workbench (CLC-bio, Aarhus, Denmark) to compare the reference sequence of the human genome (GRCh37/hg19) and analyze the depth and variation of the target area of 26 pathogenic genes. Experimentation confirmed that different sequence depths have different gene detection rates, but the two factors are not positively correlated, and the cost is higher with more comprehensive sequencing. Therefore, we selected 25×, 50×, 75× three related, and the cost is higher with more comprehensive gene detection rates, but the two factors are not positively confirmed that different sequence depths have different target area of 26 pathogenic genes. Experimentation and analyze the depth and variation of the target area of 26 pathogenic genes coding exons and 5-base pair of the upstream and downstream intron sequences of the proband. Gene sequencing for the proband was performed as follows. There are 26 pathogenic genes for detection: MYH7, MYBPC3, TNNT2, TNNI3, MYL2, MYL3, MYH6, TTN, TPM1, ACTC1, TNNC1, PRKAG2, LAMP2, GLA, CSRP3, MYOZ2, JPH2, ACTN2, CAV3, LDB3, VCL, PLN, MYPN, NEXN, CALR3, and ANKR51. Genomic DNA was broken into about 250-bp fragments by ultrasound. We used AMPureXP magnetic beads (Agencourt, Beckman Coulter, CA, USA) to enrich the 200-300-bp fragments. After the universal connection, polymerase chain reaction (PCR) was performed for eight cycles and the customized Agilent liquid capture library (Agilent Technologies, Santa Clara, CA, USA) to enrich the target area. After 10 cycles amplification of the genome, which had been enriched, the Illumina GA IIx (Illumina Inc., CA, USA) sequenced paired ends, with each side reading 120 bp. After removing the repeated sequencing data by PICARD, we used CLC Genomics Workbench (CLC-bio, Aarhus, Denmark) to compare the reference sequence of the human genome (GRCh37/hg19) and analyze the depth and variation of the target area of 26 pathogenic genes. Experimentation confirmed that different sequence depths have different gene detection rates, but the two factors are not positively correlated, and the cost is higher with more comprehensive sequencing. Therefore, we selected 25×, 50×, 75× three sequencing depth to detection. By comparison, we find that these three conditions can meet the requirements of the experiment and 25× cost is low. The mutation is not the variation to be satisfied, and the proportion of base variation must be <20%. So, the loci sequencing depth must be ≥25× for the variation to be satisfied, and the proportion of base variation must be ≥20%.

PCR amplified MYH7 extra 12 exons. The upstream sequence of the primer was 5′-GCC AGC AGT CAT CTC TTTACC A-3′ and the downstream sequence was 5′-GGG AGC GAG TGA GTT ATT GTT C-3′. The amount of PCR reaction volume was 50 μL, including genomic DNA 20 ng, upstream and downstream primers 10 pmol, 2 × Taq Master Mix (Beijing KangWei century biological technology Co., Ltd.) 25 μL. The amplification condition of the PCR was predegeneration: 95°C, 10 minutes; denaturation: 95°C, 30 seconds; annealing: 54°C, 30 seconds; extension: 72°C, 40 seconds (35 cycles); and final extension, 72°C, 10 minutes. After product purification, sequencing was performed with double DNA termination method.

**Bioinformation analysis:** The frequency of mutations in the general population was confirmed using dbSNP (http://www.ncbi.nlm.nih.gov/SNP/) and 1000 Genomes (http://www.1000genomes.org). The pathogenicity of the mutation was evaluated and tested through PolyPhen-HCM (http://www.genetics.bwh.harvard.edu/hcm).

**Results**

**Genetic monitoring:** Through comprehensive targeted sequencing for 26 HCM-causing genes, c.1172A>C (Asn391Thr) mutation, which is located in exon 12 of MYH7 was identified in the proband and encodes an amino acid of 391-bit asparagine threonine (Figure 2A). No pathogenic mutations were found in the other 25 genes in the proband. In addition, this mutation was not found in the other family members or in the 307 healthy controls. The Asn391Thr mutation of MYH7 was not reported in the SNP database. Homology comparison found that MYH7 Asn391 residues were highly conserved between different species (Figure 2B). Then we sequenced the family members through mutation detection, and eight family members were found to be carriers of the mutation, of which six suffered from HCM. The other two carriers were relatively young, IV 1 and IV 4, and had not been confirmed. Therefore, the occurrence of disease is not consistent with the occurrence of mutation. (Figure 1).

**Data analysis:** The proband (III 4) was a 46-year-old middle-aged man who was diagnosed with HCM at the age of 18. The main symptoms were exertional dyspnea and chest pain. Electrocardiographic examination showed abnormal Q wave, ST segment depression, T wave inversion, and atrium expansion accompanied by complete left bundle branch block. Echocardiography showed that the ventricular septum was approximately 13 mm thick, left ventricular posterior wall was 10 mm, left ventricular end-diastolic diameter was 52 mm, left ventricular ejection fraction was 55%, and second, tricuspid regurgitation was

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**Figure 1.** The family pedigree. Annotation, □: Normal male, ○: Normal female, ■: Male patient, ●: Female patient, †: Proband, +: Carrier with mutation, —: Carrier with no mutation, ?: Death unknown, †: Dead.
in small quantities.

**Family genotype-phenotype analysis:** A total of eight family members including the proband were mutation carriers, among which six showed HCM-related signs (Figure 1). The clinical data of the family members are shown in the Table. The onset age of one patient (II 9) was 8 and of three patients (III 3, III 15, and III 16) were < 16. All patients had symptoms of cardiac insufficiency, such as exertional dyspnea, palpitations, amaurosis. In another family member (III 11), onset age was unknown and no obvious clinical symptoms were apparent. At the age of 32, the proband was confirmed and received the screening tests. The electrocardiographic examination showed that all patients had sinus rhythm, four patients (II 9, III 3, III 15, and III 16) had abnormal Q wave and ST segment depression, three patients (II 9, III 3, and III 11) had the change of T wave inversion, and one patient (II 9) had wide QRS wave and the complete left bundle branch block. The ultrasound cardiology showed a 13-mm-thick ventricular septum (II 9) and left atrial and right ventricular enlargement (47 and 16 mm, respectively) and have ventricular septal echogenicity; in the other four patients (III 3, III 11, III 15, and III 16), ventricular septal thickening was ≥ 15 mm (20, 16, 15, and 21 mm, respectively).

Four members of the family died. They were the proband’s grandfather (I 1, 49 years), uncle (II 1, 49 years), father (II 3, 40 years), and uncle (II 12, 40 years). The proband’s grandfather died of heart attack, but it was not confirmed whether this was due to HCM, and the remain-
ing three patients were diagnosed with HCM.

Two asymptomatic carriers (IV 1 and IV 4) in the family had no remarkable clinical data. Because of their young age, we could not rule out sickness in the future and whether they should be followed up. Other members did not carry this mutation.

Discussion

Myofibril is composed of thick and thin myofilaments. The thick myofilament is made up of myosin and myosin-binding protein C. Myosin is an important myocardial contractile protein needed during the contraction of the myocardium.\(^\text{10,11}\) The length of MYH7 on chromosome 14q11-12 is 23 kb with 26,213 bases, including 41 exons and 38 exons encoding 1935 amino acids. The β-myosin heavy chain of these amino acids is a part of the myosin. The curvy spiral rod of heavy chain dimers makes up the valve stem of the myosin and the dimers unfold makes up the head of myosin. Between the two is the head rod anastomose. At the end of the myosin head C, each heavy chain makes up the leverage area and combines with the light chain and necessary light chain.\(^\text{12-13}\)

MYH7 mutation is the most common missense mutation, and the mutation mainly focuses on exons 3-23 and mostly takes place in the head and head joint of the myosin.\(^\text{14}\) As the earliest discovered HCM-related mutation, MYH7 mutation shows extensive, explicit, serious degree of hypertrophy and is highly malignant.\(^\text{15}\) In early time, for regard to the mechanism of MYH7 mutations leading to HCM some scholars considered that it was determined by the position of the mutations. They commonly thought that the mutation which can change the amino acid charge located in the myosin head, especially the mutation that can affect the myosin head and actin-binding site. And the HCM patients who had this mutation had higher penetration rates and more severe symptoms.\(^\text{16}\) But this theory has been challenged by clinical genetic screening. Now some scholars have put forward a “toxic peptides” mechanism as follows: transcription of the genetic mutation can lead to translation of the mutant protein body and it can weaken and cover the function of the wild-type protein body which is encoded by normal genes and expressed. This mutation affects the structure of the myocardium, reduces the contractility of the myocardium and cause compensatory hypertrophy, eventually leading to illness.\(^\text{17}\)

Most studies have reported MYH7 mutations in HCM. Xie, et al. found R663H and E924K mutations for five FHC families by screening.\(^\text{18}\) Song, et al. found Arg663Cys and Arg663His mutations and explained the genetic heterogeneity of the two mutations.\(^\text{19}\) Wang found a malignant phenotype of Val606Met in a Chinese pedigree with HCM.\(^\text{20}\) Our study found that MYH7 c.1172A>C (Asn391Thr) mutation is in the 391 codon of exon 12 of MYH7 and is the first mutation discovered in Chinese individuals and that the mutation is malignant. This mutation is in the ATP enzyme active site of the actin head and changes the charge of amino acids, which can change asparagine to threonine. This change makes β-myosin heavy chain cannot play enzyme activity and hydrolysis ATP, so that can’t provide necessary energy for myocardial contraction and reduce myocardial contraction force, at the end they will lead to disease. For HCM genetic testing, if there is a genetic mutation with a clinical phenotype, we would determine that the genetic mutation may be the pathogenic gene of HCM. But Kircher, et al. suggested that pathogenicity should be calculated using not only PolyPhen-HCM but also CADD score.\(^\text{21}\) However, this view has not been included in the research criteria, so a detailed explanation is not required here. Here we hope to conduct corresponding research in the future.

In this study, eight persons were found to be carriers of MYH7 c.1172A>C (Asn391Thr) mutation, of whom six had HCM. Their clinical characteristics were as follows: (1) Onset age was early and was < 20 years, except for one patient (III 11) whose onset age is unknown; (2) Excluding III 11, five patients all had clinical manifestations of cardiac insufficiency, such as exertional dyspnea, palpitations, and amaurosis; (3) Electrocardiography showed that all patients had more than one of the following two conditions: low ST segment, T wave inversion, or abnormal Q wave, and one patient (II 9) also had ventricular arrhythmias such as widened QRS wave; (4) Echocardiography showed that all patients had ventricular septal thickening, and one of the patients (II 9) had left heart hypertrophy and another patient (III 16) had outflow tract obstruction; (5) Four people in this family died before they reached 50 years old, and three of them had been diagnosed with HCM. According to the heart function of the patient’s family, we calculated that the dead family members had associations with heart failure or malignant arrhythmia. The mutation was not detected in the 307 subjects and don’t have contact with the disease. So, the mutation is a pathogenic mutation rather than an SNP of HCM.

For the first time, we detected MYH7 c.1172A>C (Asn391Thr) mutation in a FHC family in China. The penetration is higher of these people who carry the mutation, and onset age is early (≤ 20 years). They have varying degrees of cardiac insufficiency, high mortality, and earlier age at death (≤ 50 years old). Therefore, the mutation is a deleterious mutation. In the known mutation, most of the mutations are malignant mutations. Carriers of this mutation should be diagnosed early and given treatment to prevent the occurrence of heart failure and sudden death.

Disclosures

Conflicts of interest: None.

References


