Asn391Thr Mutation of β -Myosin Heavy Chain in a Hypertrophic Cardiomyopathy Family From Genotype to Phenotype

Xiaotong Feng,¹ MD, Tingting He,¹ MD, Ji-Gang Wang,² MD and Peng Zhao,² MD

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.

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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.¹⁾ 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%.²⁾ The risk of atrial fibrillation and heart failure is often present in patients for a lifetime.³⁾

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.^{4,5)} Since then, researchers have found > 1400 mutations in > 20 genes associated with the disease.⁶⁾ However, accord-

ing to a recent study, there has been no significant progress in the past decade from a clinical perspective.⁷⁾ So far, *MYH7* mutation is the main genetic factor that occupies 30%-50% of all HCM pathogenic genes.⁸⁾ 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 was an interventricular septum or ventricular wall thickness of ≥ 15 mm in adults, as shown by echocardiography, and other cardiovascular system and systemic diseases, such as aortic stenosis and difficult-to-control hypertension over a long time that may lead to myocardial hypertrophy were ruled out. The control group included 307 healthy volunteers. All subjects signed informed consent forms. This study was implemented strictly in accordance with the Declaration of Helsinki, and it was approved by the ethics committee of the Peking Union

From the ¹Department of Forensic Medicine, School of Basic Medicine, Qingdao University, Qingdao, China and ²Department of Pathology, the Affiliated Hospital of Qingdao University, Qingdao, China.

Address for correspondence: Peng Zhao, MD, Department of Pathology, The Affiliated Hospital of Qingdao University, No. 16 Jiangsu Road, Qingdao, 266003, China. E-mail: qyzaopeng@sina.com

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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 GTG ATT GTT C-3'. The amount of PCR reaction volume was 50 μ L, including genomic DNA

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

Figure 2. Sequencing results of *MYH7* Asn391Thr mutation and conservative analysis among species.

Table. The Clinical Data of Patients and Asymptomatic Carriers in the Family

No	Sex	Age	Onset age	Clinical manifestation	Electrocardiogram				Ultrasound cardiogram		
					ST segment depression	Abnormal T wave	Abnormal Q wave	Other abnormalities	Interventricular septum	Posterior wall of left ventricle	Outflow obstruction
Patients											
III-4	Male	46	18	Exertional dyspnea, Chest pain	Yes	Yes	Yes	Atrial enlargement, Complete left bundle branch block	13	10	No
III-11	Male	32	unknown	No	No	Yes	No	No	16	10	No
III-16	Male	17		16	Exertional dyspnea, Chest pain, Amaurosis	Yes	No	Yes	No	21	10
II-9	Female	46	14	Exertional dyspnea, Chest pain	Yes	Yes	Yes	Wide QRS Complete left bundle branch block	13	9	No
III-15	Male	18	16	No	Yes	No	Yes	No	15	9	No
III-3	Male	27	16	Exertional dyspnea, Chest pain	Yes	Yes	Yes	No	20	7	No
Carrier with no manifestation											
IV-1	Female	10 months		No	No	No	No	No	4	4	No
IV-4	Female	20 months		No	No	No	No	No	4	4	No

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

down, 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 cardiography 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.^{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 curly 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.¹¹⁻¹³⁾

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.¹⁴⁾ As the earliest discovered HCM-related mutation, *MYH7* mutation shows extensive, explicit, serious degree of hypertrophy and is highly malignant.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.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.¹⁷⁾

Most studies have reported *MYH7* mutations in HCM. Xie, *et al.* found R663H and E924K mutations for five FHC families by screening.¹⁸⁾ Song, *et al.* found Arg 663Cys and Arg663His mutations and explained the genetic heterogeneity of the two mutations.¹⁹⁾ Wang found a malignant phenotype of Val606Met in a Chinese pedigree with HCM.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.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.

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