Variation p.R1045H in MYH7 correlated with hypertrophic cardiomyopathy in a Chinese pedigree

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Research Article

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Abstract

Objective: Inherited hypertrophic cardiomyopathy (HCM) is a fatal disease that damages heart function and may cause the heart to stop beating suddenly. The genetic factors play an important role in HCM. Pedigree analysis is a good way to identify the genetic defects that cause disease. Methods: A HCM pedigree was found in Yunnan of China. Whole-exome sequencings were performed for finding the genetic variant of HCM. Another 30 HCM patients and 200 health controls were also used to investigate the frequency of variation by TaqMan-MGB method.

Results: The variation NM_000257.4:c.3134G>A (NP_000248.2:p.Arg1045His, rs397516178, short as c.3134G>A) was found to co-segregate with the symptoms of HCM. Meanwhile, the variation was not found in 200 controls. After genotyping the variation in 30 HCM patients, one patient carried the variation and had a family history.

Conclusion: Our findings support that this variation may be closely related to the occurrence of the disease. According the ACMG guideline, the c. 3134G>A should be classified as "Likely pathogenic".

Background

Inherited cardiomyopathy (ICM) is a disease with abnormal cardiac structure and function, which is mainly caused by genetic factors. Inherited hypertrophic cardiomyopathy (HCM) is the common type of ICM\(^1,2\). The main pathological manifestation of inherited HCM is left ventricle and ventricular septal asymmetry hypertrophy, which is also the main cardiogenic cause of sudden death in this disease\(^2,3\). The latest epidemiological investigation shows that most hereditary modes of HCM are autosomal dominant with an incidence rate of about 200/100,000, and there is no difference in genders\(^4\). Most of the patients show occasional syncope or even no specific clinical manifestations, which results in being unable to find the disease at the first time and delays the best treatment opportunity for the patients\(^5\). At present, surgery is the main treatment method to relieve the potential risks of hypertrophic cardiomyopathy, therefore, early diagnosis of the disease becomes very important. Genetic diagnosis is an effective application for early prediction of disease occurrence\(^6,7\), so it is valuable to study the mutations of pathogenic genes in HCM.

Previous reports confirmed that the major pathogenic genes of hypertrophic cardiomyopathy are related genes encoding sarcomere proteins, of which the genes encoding cardiac β-myosin heavy chain (MYH7) are the most important one\(^8,9\). MYH7 gene, located on chromosome 14q12, is one of the most important sub-units of myosin and plays a very important role in the energy supply of myocardial cells and the maintenance of Ca\(^{2+}\) concentration inside and outside myocardial cells. MYH7 is mainly expressed in the ventricular muscle of the heart and expressed in a small amount in skeletal muscle. This gene is the first discovered pathogenic gene related to the pathogenesis of HCM. About 30%-50% of HCM patients are mainly caused by MYH7 gene mutation\(^10,11\).
Although there has been much research on HCM molecular genetics in recent years, research on the correlation between MYH7 gene variations and HCM is still insufficient in China\(^8,12\). In this study, we found a pedigree with an "uncertain significance" variation on the MYH7. All the patients carried the variation at the same time the other family members did not carry the variation. The incidence of this variation was investigated in the Chinese HCM patients, and its characteristics were evaluated using American College of Medical Genetics (ACMG) criteria\(^13\).

**Materials And Methods**

**Samples information:**

A family with HCM was found (Figure 1a). The proband, her big brother and their mother were diagnosed with HCM. In this family, HCM is an autosomal dominant inheritance. These patients, father of proband and little brother of proband were all enrolled in this study. Meanwhile, 200 healthy people were selected as the control group, 30 patients with HCM were enrolled too. Through the inquiry survey, only two of these 30 patients had a family history of heart disease. All the patients provided written informed consent, and the study was approved by the Ethics Committee of The First People's Hospital of Yunnan Province. All research subjects are Chinese Han people.

The clinical diagnosis of HCM referred to the 2011 American Heart Association standard\(^14\): echocardiography indicates that the left ventricular wall thickness or interventricular septum thickness is more than 15mm; other causes of myocardial hypertrophy such as hypertension, rheumatic heart disease, mitral valve disease, congenital heart disease (atrial septum, ventricular septal defect) and myocardial hypertrophy accompanied by metabolic diseases were excluded.

**Blood samples and DNA extraction**

A 3mL of peripheral blood were collected from five pedigree members, HCM patients and 200 control with EDTA anti-coagulation tube. The samples were kept in a refrigerator at -80°C for later use. The genome DNA extraction kit (No.9765, TaKaRa, Japan) was used to extract DNA from 250μl peripheral blood.

**Next generation sequencing of HCM pedigree members**

The whole-exome sequencing was performed on the 5 members of HCM pedigree (Figure1a). Their genomic DNA were fragmented to an average size of 180~280bp and subjected to a library creation using the Agilent SureSelect Human All ExonV6 Kit (Agilent Technologies, Santa Clara, CA, USA ). The Illumina Novaseq 6000 platform (Illumina Inc., San Diego, CA, USA) was utilized for genomic DNA sequencing in Novogene Bioinformatics Technology Co., Ltd (Beijing, China).

After sequencing, the bcl2fastq software (Illumina) was used to basecalling. After removing low quality reads, the fastaq files were aligned to the reference human genome (hs37d5) using the Burrows-Wheeler
Aligner (bwa)\textsuperscript{15}. Single nucleotide variants (SNVs) and indels were called with samtools to generate gVCF files\textsuperscript{16}.

**Sanger sequencing and TaqMan-MGB**

The variation found in the HCM pedigree was re-sequenced by sanger sequencing in all pedigree members. The PCR primers were MYH7F: GCTGTCTTGGGTCTGCTTG and MYH7R: GGTTCCTGAAGTCTGAACA. PCRs were performed using the condition: 94°C for 3min; 94°C for 30s; annealing at 54°C for 30s; extension at 72°C for 30s; for a total of 40 cycles. PCR products were sequencing at 3130 Sequencer (Applied Biosystems, USA) following the protocol of sequence kit (Y-411-1002, Applied Biosystems, USA). TaqMan-MGB was used to analyze the genotype of the variation found in the HCM pedigree in 200 controls and 30 HCM patients. The typing method was followed the protocol of TaqMan-MGB kit (Applied Biosystems, USA).

**Results**

**Symptoms of patients**

In the HCM pedigree of this study, proband (III-2) had dyspnea, shortness of breath and syncope in severe cases, and her apical four-chamber showed markedly thickened ventricular septum by echocardiography (Figure 1b). The echocardiographs shown the big brother and the mother of proband all have the thickened ventricular septums more than15mm. They had dyspnea, shortness of breath too. According to the description of the proband, her grandmother died of sudden cardiac stop. Her father and her brother had no clinical symptoms.

The ventricular wall thickness or interventricular septum thickness of 30 patients with HCM are all more than 15mm. Most of these 30 patients have dyspnea and shortness of breath. Some of them had syncope in severe cases. At the same time the 200 normal controls showed a normal hearts by echocardiography and no clinical symptoms.

**Variation identification in HCM pedigree**

The whole-exome sequencing was performed in the five people in Figure 1a. About 50G data was gotten. The Q30 of these five samples are all larger than 92%. After performing an analysis of variation segregation based on family genetic model, we found a variation located on the MHY7 which only can be found in the patients but not in the normal people of the pedigree. It is NM_000257.4:c.3134G>A (NP_000248.2:p.Arg1045His, rs397516178, short as c.3134G>A). We re-sequenced this SNP in the five family members by sanger sequencing, and the results were list in the Figure 1c

**Frequency investigation of c.3134G>A by TaqMan-MGB**

We investigated the frequency of c.3134G>A in 200 normal controls and 30 HCM patients. The variant was not found in 200 normal people and only found in a HCM patient of these 30 HCM patients. This
patient has dyspnea and shortness of breath, and his ventricular wall thickened (17mm). According to his dictation, his mother had symptoms of shortness of breath before she died.

**ACMG Evaluation of Variant Sites**

According the American College of Medical Genetics and Genomics (ACMG) guidelines, the c.3134G>A was evaluated. The variant was absent from our controls. Its frequency is 0.000048 (6/125568) in the TOPMED database and 0.00003 (1/31380) in the GnomAD database and was not found in asia people based on the information of these databases. It meets the criteria PM2 of ACMG. The functional effect of this variant was predicted by the SIFT and PROVEAN. The score of SIFT was 0.000 (Damaging), and the score of PROVEAN was -3.96 (Deleterious). Multiple computational predictions support a deleterious effect of this variant (criteria PP3 of ACMG). The variation in was marked as pathogenic in ClinVar database (ClinVar Accession: RCV000629019.2; criteria PP5 of ACMG). This variant co-segregates with the disease phenotype and affected two family members in our pedigree (criteria PP1 of ACMG).

**Discussion**

Since Jareho et al. had completed sequencing analysis of a family of patients with hypertrophic cardiomyopathy at the end of last century, and successive studies had confirmed that missense mutation of MYH7 gene can lead to the development of hypertrophic cardiomyopathy. After that, many variations were found in the MYH7. There are 32 pathogenic variations were found in this gene, at the same time many conflicting interpretations appeared. So it is important found more evidence for these variations. Determination of the relationship between variations and the diseases is very important for molecular genetic diagnosis, prognosis, and risk assessment for patients. In this study, we found a family that the carrier of c.3134G>A co-segregated with the HCM. The variation was reported in several Italy HCM patients. Finding c. 3134G>A in more HCM families, especially families with different genetic backgrounds, co-segregation with the disease is very important to determine the pathogenicity of the variation. According the PP1 of ACMG, if there more co-segregations were found from diverse ethnic background. The criterion can be taken as moderate or strong evidence. Therefore, the PP1 may taken as a moderate evidence PM because the c. 3134G>A were found in different pedigree with different ethnic genetic background. At last, the evidence PM2, PP3, PP5 and PM (Upgrade from PP1) supported the c. 3134G>A is pathogenic. Based the ACMG the c. 3134G>A should be classified as "Likely pathogenic ".

MYH7 is expressed predominantly in human ventricle, and more than 10 times that in skeletal muscle. The gene encodes beta heavy chain subunit of muscle myosin. Muscle myosin contained 2 heavy chain subunits, 2 alkali light chain subunits, and 2 regulatory light chain subunits. The MYH7 may encode most beta heavy chain subunits in human heart. Mutations in the gene maybe reduce the ability of myosin to slide along actin filament, and impair the function of the heart muscle. The variation, p.Arg1045His, is located in the SMC (structural maintenance of chromosomes) N terminal domain of cardiac β-myosin, and the function of SMC protein is binding DNA and organizing and segregating chromosomes for partition. However, little is known about how domain SMC affects the function of myosin. Research
based on the function of the mutant protein is still very worthwhile. These studies can not only further clarify what kind of role of the SMC domain plays a role in muscle contraction, but also provide an important basis for determined relevance between c. 3134G>A and HCM.

**Abbreviations**

HCM: Inherited hypertrophic cardiomyopathy  
ICM: Inherited cardiomyopathy  
MYH7: cardiac β-myosin heavy chain  
ACMG: American College of Medical Genetics  
SMC: Structural maintenance of chromosomes

**Declarations**

**Ethics approval**: The study was performed according to the Helsinki Declaration and the study was approved by the Ethics Committee of The First People's Hospital of Yunnan Province, and signed informed consent from all study subjects was obtained.

**Availability of data and materials**: All data generated or analysed during this study are included in this published article.

**Competing Interests**: All the authors have approved the manuscript and agree with submission, and have no conflicts of interest to declare.

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**Authors' contributions**: YZ and YYS contributed to the conception or design of the work. MJP, LL, XXD and HYW contributed to the acquisition, analysis or interpretation of data for the work. HYW and LJL drafted the manuscript. MJP critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of work ensuring integrity and accuracy.

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**Consent for publication**: Not Applicable.

**References**
1. Hata Y, Hirono K, Yamaguchi Y, Ichida F, Oku Y, Nishida N. Minimal inflammatory foci of unknown etiology may be a tentative sign of early stage inherited cardiomyopathy. *Modern pathology: an official journal of the United States and Canadian Academy of Pathology, Inc*. 2019; 32: 1281-90.

2. Kuster DWD, Lynch TL, Barefield DY, Sivaguru M, Kuffel G, Zilliox MJ, et al. Altered C10 domain in cardiac myosin binding protein-C results in hypertrophic cardiomyopathy. *Cardiovascular research*. 2019; 115: 1986-97.

3. Limongelli G, Monda E, Tramonte S, Gragnano F, Masarone D, Frisso G, et al. Prevalence and clinical significance of red flags in patients with hypertrophic cardiomyopathy. *International journal of cardiology*. 2020; 299: 186-91.

4. Maron BJ, Rowin EJ, Maron MS. Global Burden of Hypertrophic Cardiomyopathy. *JACC Heart failure*. 2018; 6: 376-78.

5. Adamczak DM, Oko-Sarnowska Z. Sudden Cardiac Death in Hypertrophic Cardiomyopathy. *Cardiology in review*. 2018; 26: 145-51.

6. Imori Y, Takano H, Mase H, Matsuda J, Sangen H, Izumi Y, et al. Bisoprolol transdermal patch for perioperative care of non-cardiac surgery in patients with hypertrophic obstructive cardiomyopathy. *BMC cardiovascular disorders*. 2019; 19: 316.

7. Zhu C, Wang S, Ma Y, Wang S, Zhou Z, Song Y, et al. Childhood Hypertrophic Obstructive Cardiomyopathy and Its Relevant Surgical Outcome. *The Annals of thoracic surgery*. 2020; 110: 207-13.

8. Liu HT, Ji FF, Wei L, Zuo AJ, Gao YX, Qi L, et al. Screening of MYH7 gene mutation sites in hypertrophic cardiomyopathy and its significance. *Chinese medical journal*. 2019; 132: 2835-41.

9. Rose J, Kraft T, Brenner B, Montag J. Hypertrophic cardiomyopathy MYH7 mutation R723G alters mRNA secondary structure. *Physiological genomics*. 2020; 52: 15-19.

10. Du Y, Wang Y, Han X, Feng Z, Ma A. MYH7 Gene-Related Mutation p.V878L Identified in a Chinese Family with Hypertrophic Cardiomyopathy. *International heart journal*. 2019; 60: 1415-20.

11. Wang B, Wang J, Wang LF, Yang F, Xu L, Li WX, et al. Genetic analysis of monoallelic double MYH7 mutations responsible for familial hypertrophic cardiomyopathy. *Molecular medicine reports*. 2019; 20: 5229-38.

12. Wang B, Guo RQ, Wang J, Yang F, Zuo L, Liu Y, 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.

13. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of
Medical Genetics and Genomics and the Association for Molecular Pathology. *Genetics in medicine: official journal of the American College of Medical Genetics*. 2015; **17**: 405-24.

14 Gersh BJ, Maron BJ, Bonow RO, Dearani JA, Fifer MA, Link MS, *et al.* 2011 ACCF/AHA guideline for the diagnosis and treatment of hypertrophic cardiomyopathy: executive summary: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *Journal of the American College of Cardiology*. 2011; **58**: 2703-38.

15 Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics*. 2009; **25**: 1754-60.

16 Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, *et al.* The Sequence Alignment/Map format and SAMtools. *Bioinformatics*. 2009; **25**: 2078-9.

17 Choi Y, Chan AP. PROVEAN web server: a tool to predict the functional effect of amino acid substitutions and indels. *Bioinformatics*. 2015; **31**: 2745-7.

18 Montag J, Syring M, Rose J, Weber AL, Ernstberger P, Mayer AK, *et al.* Intrinsic MYH7 expression regulation contributes to tissue level allelic imbalance in hypertrophic cardiomyopathy. *Journal of muscle research and cell motility*. 2017; **38**: 291-302.

19 Olivotto I, Girolami F, Ackerman MJ, Nistri S, Bos JM, Zachara E, *et al.* Myofilament protein gene mutation screening and outcome of patients with hypertrophic cardiomyopathy. *Mayo Clinic proceedings*. 2008; **83**: 630-8.

20 Frisso G, Limongelli G, Pacileo G, Del Giudice A, Forgione L, Calabro P, *et al.* A child cohort study from southern Italy enlarges the genetic spectrum of hypertrophic cardiomyopathy. *Clinical genetics*. 2009; **76**: 91-101.

**Figures**
Figure 1

The information of the HCM pedigree. a.) The pedigree map of HCM family in this study. II-2 is a proband. b.) II-2 apical four-chamber view showing markedly thickened ventricular septum. RV: right ventricle, LV: left ventricle, IVS: ventricular septum, RA: right atrium, LA: left atrium. c.) The results of sanger sequencing for verifying the variation (NM_000257.4:c.3134G>A, p.Arg1045His) finding by whole-exome sequencing. I-01 was sequenced too, and his did not carry the variation. For beautiful image, it was not shown in this picture.