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ORIGINAL RESEARCH

A Case Study Identified a New Mutation in the *TTN* Gene for Inherited Hypertrophic Cardiomyopathy

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Background: Hypertrophic cardiomyopathy (HCM) is the most common hereditary cardiomyopathy, with variable pathogenesis, clinical presentation, and prognosis. Although mutations in genes encoding sarcomere proteins have been reported to explain the genetic etiology of 40%-60% of HCM patients, the etiology of approximately 1/3 of HCM patients remains unknown. Whole-exome sequencing (WES) is an effective method for identifying the genes that cause genetic diseases. In the present study, WES and systematic genetic screening were performed to determine the genetic causes of HCM in Chinese HCM family.

Materials and Methods: Peripheral blood genomic DNA was collected from 9 family members of a Chinese Han HCM pedigree, including an HCM proband. Candidate variants obtained by WES were verified using Sanger sequencing, pathogenic mutation screening was conducted among family members, and the mutations were systematically analyzed using bioinformatics.

Results: WES revealed a novel heterozygous missense mutation, c.20233 C>T (p.R6745C), located in exon 80 of the HCM-related gene *TTN*, which may be a pathogenic mutation in the family. In addition, this mutation was predicted to damage protein function. WES combined with Sanger sequencing results showed that the other two HCM patients in this family carried this *TTN* mutation, while none of the healthy family members carried the mutation except for a 3 years old girl.

Conclusion: In this study, a new pathogenic mutation of *TTN* was found in a Chinese family with HCM, and disease-causing gene carriers in the family members were identified through pedigree screening. These findings have theoretical and application value for understanding the genetic basis of HCM, as well as for early risk stratification and early prevention and intervention of patients, and highlight the important role of genetic testing in the diagnosis and treatment of genetic diseases.

Keywords: cardiomyopathy, HCM, whole exome sequencing, *TTN*, genetic testing

Introduction

Hypertrophic cardiomyopathy (HCM) is a hereditary condition characterized by cardiac hypertrophy. It is clinically characterized by asymmetrical septal hypertrophy with or without left ventricular outflow tract obstruction.[1] Common clinical manifestations of HCM include dyspnea, precardiac pain, dizziness and syncope, fatigue, palpitations, heart failure, and sudden death. The diagnosis of HCM requires a comprehensive analysis of history, clinical manifestations, and laboratory and imaging tests. Epidemiological studies based on echocardiography have shown that the incidence of HCM in the general population is approximately 1 in 500.[2–4] HCM has obvious heterogeneity, and the age of onset, severity, and prognosis of patients vary greatly. Most patients have no symptoms or mild symptoms throughout their

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lives. Some patients in the early stages (infancy) show obvious cardiac hypertrophy and serious symptoms, need drugs and other means to intervene, and even for some patients, the first clinical symptom is sudden death. Furthermore, even in patients with the same genetic mutation in the same family, symptoms can be heterogeneous. Untreated patients with HCM experience progressive exacerbations due to left ventricular diastolic dysfunction, left ventricular outflow tract obstruction, and mitral valve insufficiency, and a small percentage of patients with HCM develop end-stage heart failure due to impaired left ventricular systolic function.

HCM is one of the most common monogenically inherited cardiomyopathies. It has no significant regional, ethnic, or gender differences in onset and manifests in an autosomal dominant inheritance pattern.[4] Approximately 60% of patients with HCM have a distinct familial disease, and although autosomal recessive and X-linked inheritance patterns have been described, they are very rare.[5] HCM is often described as a sarcomere disease, because pathogenic gene mutations have been detected in almost all sarcomere proteins. According to the latest international guidelines for the diagnosis and treatment of HCM, approximately 40%-60% of adolescent and adult HCM cases are caused by mutations in the heart muscle-associated protein gene, and only 5%-10% of adult patients are caused by other genetic or non-genetic factors, including congenital metabolic abnormalities, neuromuscular diseases, mitochondrial diseases, and aging amyloidosis.[6,7] Although HCM has been discovered in humans for a long time, the discovery of HCM pathogenic genes began in the 1990s,[8] and more than 2,000 HCM-related gene mutations have been found in at least 28 genes (including 11 sarcoma-globin genes) in the past 30 years.[1]

MYH7 (myosin heavy chain 7) *and MYBPC3* (myosin-binding protein C) are the most common pathogenic genes for HCM, and mutations in these two genes are the genetic basis for nearly half of familial HCM morbidity.[9,10] Mutations in *TNNT2, TNNT3* (cardiac troponin I), and *TPM1* (alpha-tropomyosin) are relatively rare, occurring in less than 10% of HCM cases.[11–13] Although rare, mutations in the genes *ACTC1* (alpha-myocardial actin), *MYL2* (myosin regulated light chain), *MYL3* (myosin essential light chain), and *CSRP3* (cysteine and glycine-enriched protein 3) can cause HCM.[14–16] In sporadic cases and small family studies, mutations in the genes *TTN* (titin), *TCAP* (titin-cap), *MYOZ2* (myozenin 2), *TRIM63* (tripartite motif containing 63), and *FHL1* (four and a half LIM Domains 1) were associated with HCM.[17–21] In addition, mutations in genes such as *TNNC1* (cardiac troponin C), *MYH6* (myosin heavy chain 6), *PLN* (phosphoreceptor protein), *CAV3* (fossa protein 3), *ALPK3* (alpha-kinase 3), *JPH2* (proteinophile 2) were also found in some HCM patients.[22–27]

After several years of research, significant progress has been made some important progress. People's understanding of HCM has gradually entered the gene level from the clinical level, and thousands of HCM-related gene mutations have been found, which provides a deeper understanding of the pathogenesis of HCM so that the diagnosis and treatment are more accurate. However, one-third of patients with familial HCM still have an unknown cause, and this proportion is higher in sporadic cases, suggesting that there are many more HCM-related genetic mutations that have not yet been discovered.[6,7] In this study, we report a family including 3 patients with HCM, diagnosed by medical history collection, electrocardiography, and echocardiography. A new genetic mutation, c.20233 C>T (p.R6745C) on the gene *TTN* encoding titin, was found to be a possible pathogenic mutation in this family, and an asymptomatic carrier of the pathogenic gene mutation was found in this family through genetic screening. Existing studies have shown that some HCM is caused by heterozygous mutation in the *TTN* gene, such as mutation p.Arg740Leu, p.Lys23480fs and p. Ser3799Tyr.[17,28–30] This study not only provide important genetic reference materials for genetic counseling of this HCM family but also enrich the mutation spectrum of *TTN* gene and lay a foundation for future HCM genetic research and gene therapy, which has important practical and theoretical significance.

Materials and Methods

Study Object

The subjects of this study were from a HCM family of 3 generations and 9 people admitted to the Union Hospital, Tongji Medical College, Huazhong University of Science and Technology. The clinical diagnosis of HCM was based on HCM guideline.[31] The family consisted of 6 males and 3 females, among whom the oldest was 59 years old and the youngest was 3 years old. After obtaining the informed consent of all 9 family members, 5–10mL of peripheral blood from all

family members were collected using disposable blood collection needles and sodium citrate collection vessels, and stored in a 4°C refrigerator for subsequent genomic DNA extraction. This study was approved by the hospital Ethics Committee and complied with the Declaration of Helsinki.

Clinical Evaluation and Laboratory Assessment

We inquired about the basic information of each individual family member, including age, sex, occupation, clinical symptoms, past medical history, and family medical history. At the same time, basic auxiliary examinations such as blood pressure measurement, routine electrocardiogram and cardiac ultrasound were conducted for family members. In addition, the proband underwent a series of blood tests for lipid, aspartate aminotransferase (AST), CK/CK-MB, TNI, BNP, LDH, TSH, FT3, and FT4 levels.

DNA Extraction and Whole-Exome Sequencing

Genomic DNA was extracted from the peripheral blood using a TIANamp Blood Genomic DNA Extraction Kit (Tiangen Company). In this study, WES was performed on peripheral blood genomic DNA of patients II1 and III1 with clinical symptoms. Using an Agilent Sureselect human exon sequence capture V6 combined with Illumina sequencing.

Sequence Analysis

Using WES, tens of thousands of gene mutations were found in the genome of each test subject, most of which were unrelated to the disease. To identify pathogenic gene mutations as soon as possible and reduce the burden of subsequent analysis and verification, the obtained mutations should be screened based on known information combined with previous research screening procedures.[32] The main screening criteria were as follows:

(1) Sequencing depth ≥30;
(2) The frequency of mutation in the 1000 Genomes, ESP6500, and ExAC databases is less than 0.1%, and has never been reported.
(3) Mutations located in the exon or splice site region of the gene.
(4) Single-nucleotide variations must be non-synonymous variants, stop-gained/loss variants, or protein-truncating variants.
(5) Mutations present in both HCM patients (II1 and III1);
(6) Mutations located in the gene that has been reported to be related to inherited HCM (Supplementary Table S1).
(7) It is predicted to be pathogenic/deleterious variant by mutation function prediction software.

Sanger Sequencing Validation

Because Sanger sequencing is recognized as the "gold standard" with extremely high accuracy of close to 100%, it has been routinely used to confirm detected variants before reporting results. Using Sanger sequencing, candidate mutations obtained by WES can be screened for among family members. On one hand, the pathogenicity of the mutation can be further defined in the family; on the other hand, early diagnosis can be made for individuals carrying disease-causing gene mutations in the family but with a normal phenotype. DNA was amplified in a 25μL volume of PCR reaction system with 12.5μL 2×TSINGKE Master Mix, 0.5μL forward primer (5'-CCGAAATGAGTGACAAAG-3'), 0.5μL reverse primer (5'-CACGTCCACATGAAAGAG-3'), 2μL DNA and 9.5μL double steaming water with ABI Veriti PCR Amplifier (Applied Biosystem, America). The amplified products were sequenced using Sanger sequencing, and the sequencing results were analyzed using the GeneTool.

Function Prediction

First, we screened for mutations in various large databases to determine its frequency, such as the 1000 Genomes Project (http://www.internationalgenome.org/), ExAC (http://exac.broadinstitute.org/), NHLBI GO Exome Sequencing Project (http://evs.gs.washington.edu/EVS/), Known VARiants (Kaviar) (http://db.systemsbiology.net/kaviar/), and ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/).

Second, we used bioinformatics to predict the effects of the discovered mutations on protein structure and function and then predicted the pathogenicity of the mutation. The mutation risk prediction was conducted by SIFT (http://sift.jcvi.org/), the predicted results were divided into D: Deleterious (SIFT≤0.05) and T: Tolerated (SIFT>0.05); Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/), the predicted results were divided into D: Probably damaging (Predicted score≥0.909); P: Possibly damaging (0.447≤Predicted score≤0.909) and B: Benign (Predicted score≤0.446) and Mutation Taster (http://www.mutationtaster.org/), according to the predicted results, genetic variants can be divided into two categories: D (disease-causing) and N (polymorphism).The mutation site conservative prediction was used by ClustalX (http://www.clustal.org/).

Clinical Interpretation

We used the American College of Medical Genetics and Genomics (ACMG) classification standards and guidelines for genetic variation for clinical evaluation and interpretation of the discovered mutation.[33] Based on "supporting/moderate/strong evidence", variants were rated as "pathogenic, "likely pathogenic, "uncertain significance, "likely benign, and "benign" in this classification standards.

Results

Clinical Case Description

This study aimed to identify an autosomal dominant family with HCM. Through medical history inquiry and clinical screening, such as electrocardiogram and cardiac ultrasound, three HCM patients were diagnosed in this family, including proband II1 and his two sons (III1 and III3). The two sons of the proband showed no other clinical symptoms except for obvious myocardial hypertrophy on ultrasonography. A pedigree diagram of this family is shown in Figure 1A. The electrocardiograms of some family members are shown in Figure 1B. Table 1 presents the basic information on some family members. Table 2 shows the ultrasound examination results of the HCM members.

Proband II1, male, 59 years old, was hospitalized due to "subxiphoid pain without inducement for a week, continued to not relieveIII, accompanied by palpitation, chest tightness, dyspnea and other symptoms at admission, without nausea, vomiting, dizziness, and dark. Physical examination revealed a body temperature 36.6°C, heart rate of 76 beats/min, and blood pressure of 127/87 mmHg. He was conscious and there was no swelling of the superficial lymph nodes. Heart sounds were normal, heart rhythm was consistent, systolic murmurs were audible on the lower left side of the sternum, no tremor was touched, respiratory sounds were clear in both lungs, and dry and wet rales and pleural friction sounds were not heard. Electrocardiogram results showed partial ST segment changes and left anterior branch block. Echocardiography revealed an uneven thickening of the left ventricular wall. The measured results for the left ventricular wall were as follows: the thickness of the basal interventricular septum was approximately 32 mm, middle interventricular septum was approximately 34 mm, and apical interventricular septum was approximately 24 mm. No significant segmental wall motion abnormalities were observed in the left ventricle wall. The ascending aorta was not wide and the aortic valve was okay in shape and activity. The pulmonary artery was not wide and the pulmonary valve shape and activity were acceptable The left ventricle was enlarged; however, the remaining ventricles were not significantly enlarged. The morphology and activity of the mitral and tricuspid valves were normal and the atrioventricular septum was not discontinuous. Further, left heart contrast-enhanced ultrasonography showed asymmetric hypertrophy of the left ventricular wall, which was evident in the previous septum and apex of the heart, consistent with the above findings (Table 3). The patient was diagnosed with asymmetric HCM (occult obstruction). Table 4 presents the main auxiliary examination items and results for the proband.

Patient III1, male, 35 years old, the oldest son of the proband, a construction worker, reported no discomfort during ordinary times, and no abnormalities were found on physical examination. Electrocardiography revealed a non-specific conduction delay and the possibility of anterior subepicardial myocardial injury. Further echocardiographic examination showed obvious ventricular septal thickening, and the mitral valve showed the SAM sign, which could be opened and closed poorly. The shapes and activities of the ascending aorta, pulmonary aorta, and valves were normal. The left ventricular wall was thickened and no abnormal segmental wall motion was observed. No continuous middle section was

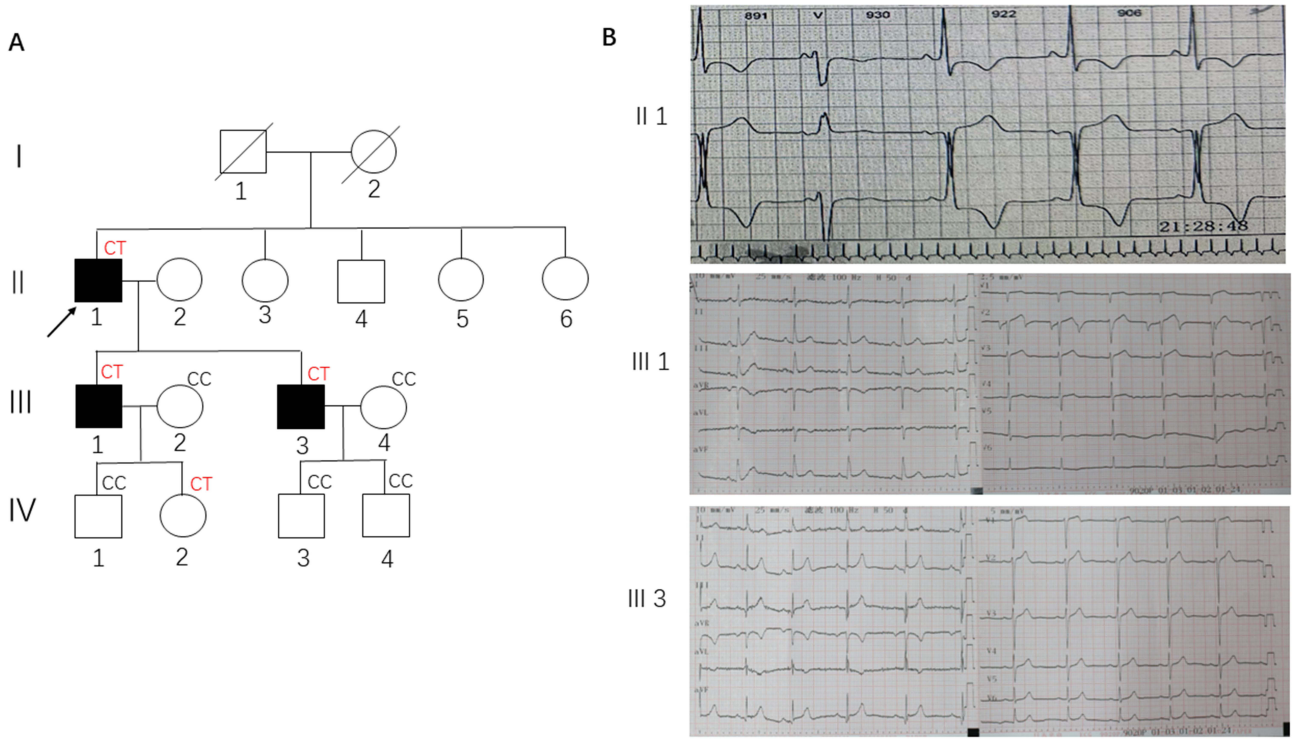

Figure 1 Pedigree map of hypertrophic cardiomyopathy and electrocardiogram of family members. (**A**). Pedigree map of hypertrophic cardiomyopathy; (**B**). Electrocardiogram of family members. Squares represent males, circles represent females, black represent patients, arrows represent probands, and oblique lines represent deceased.

observed in the atrial or interventricular septa. Combined with further left echocardiography, the patient was diagnosed with obstructive HCM (prewall and interventricular septum) with mild mitral valve insufficiency.

Patient III3, male, 30 years old, the youngest son of the proband, a construction worker, reported good health without any symptoms. The patient had no history of hypertension or diabetes mellitus. Electrocardiographic screening revealed a sinus arrhythmia. Echocardiography showed ventricular septal thickening, septal thickness of 14 mm, the ascending aorta and pulmonary aorta were not wide, the valve shape and activity were normal, the mitral and tricuspid valves were normal, no obvious enlargement of the atrioventricular cavity was observed, and no obvious abnormal segmental wall motion was observed in the left ventricular wall. Based on the previous history, examination results, and diagnostic criteria, the patient was diagnosed with HCM.

No abnormalities were found in the other family members after clinical examination, physical examination, electrocardiography, or echocardiography.

Table 1 Basic Information of Family Members

Member	Gender	Age/Year Old	Cardiac Uhrasonography	Clinical Symptoms
II1	Male	59	Interventricular septum thickening, 34mm	Chest pain, chest tightness
III1	Male	35	Interventricular septum thickening, 17mm	None
III2	Female	37	N/A	None
III3	Male	30	Interventricular septum thickening, 14mm	None
III4	Female	27	N/A	None
IV1	Male	12	No abnormalities	None
IV2	Female	3	No abnormalities	None
IV3	Male	7	No abnormalities	None
IV4	Male	4	No abnormalities	None

Note: N/A indicates that no data is obtained.

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Table 2 Summary of Ultrasound Examination Results of HCM Patients

Measuring Position	Measurements (cm)			Normal Adult Reference Value (cm)
	III	IIII	III3	
Internal diameter of ascending aorta (AAO)	3.3	2.8	2.6	2.5–3.3
Left atrial diameter (LA)	4.2	3.2	2.6	2.7–3.5
Left ventricular diameter (LV)	4.4	4.3	4	3.5–5.3
Interventricular septal thickness (IVS)	3.4	1.5	1.4	0.8–1.1
Right atrial diameter (RA)	4.1	3.7	3.5	3.2–4.5
Right ventricular diameter (RV)	3.5	3.4	3.1	3.2–4.4
Main pulmonary artery diameter (PA)	2.5	2.4	2	2.4–2.8
Shortening fraction (FS)	43%	40%	35%	>25%
Ejection fraction (EF)	74%	70%	65%	50%-70%

Table 3 Measurement Results of Left Ventricular Wall Thickness by Contrast-Enhanced Ultrasound in Proband Heart

Measuring Position	Basal Segment (cm)	Intermediate Segment (cm)	Apical Segment of Heart (cm)
Anterior wall	1.8	1.9	1.7
Anteroseptum	3.2	3	–
Paries anterolateral	1.1	1.4	–
Paries posterolateral	1	1.1	–
Inferior wall	1.1	1	1.8
Posterior septum	1.7	1.8	–
Ventricular septum	–	–	2
Lateral wall	–	–	2
Apex of heart cap	–	–	1.5

Table 4 Part of Supplementary Inspection Items and Results for the Proband

Inspection Item	Testing Result	Reference Range	Unit
AST	45	8–40	U/L
CK-MB	5.8	<6.6	Ng/mL
CK	55	38–174	U/L
LDH	183	109–245	U/L
TNI	128.7	<262	Pg/mL
BNP	291.4	<100	Pg/mL
TSH	1.274	0.35–4.94	Uiu/mL
FT3	3.5	2.63–5.7	Pmol/L
FT4	12.2	9–19.8	Pmol/L
CHOL	2.37	<5.2	Mmol/L
HDL-C	0.85	1.16–1.42	Mmol/L
LDL-C	1.15	2.7–3.1	Mmol/L
TG	0.76	<1.7	Mmol/L

Genetic Screening and Validation

Through WES sequencing, an average of approximately 10G of sequencing data were obtained for each sample, and the data quality was good, meeting the requirements for subsequent data analysis. The types of variants detected by WES were summarized in the Supplementary Table S2. After a series of screening analyses, we identified *TTN* gene mutation (c.20233 C>T, p.R6745C) as a candidate gene mutation. The candidate gene mutation was verified by Sanger sequencing in another patient III3 and in healthy adult members III2 and III4 of the family. *TTN* (c.20233 C>T, p.R6745C) heterozygous mutation was carried by patient III3 but not by the healthy members III2 and III4 in the family; that is, *TTN* mutation was co-isolated from the disease in the family. In addition, except for member IV2, none of the phenotypically normal individuals in the family carried a *TTN* (c.20233 C>T, p.R6745C) gene mutation. The Sanger sequencing map of some family members is shown in Figure 2.

Functional Analysis of Pathogenic Mutation

TTN (c. 20233 C>T, p.R6745C) is a missense mutation caused by a single base substitution located in exon 80, which causes cytosine to be replaced by thymine in exon 20233 of *TTN* gene (transcript NM_133378). This resulted in the replacement of arginine with cysteine at position 6745 in the protein sequence (p.R6745C). After a systematic search, no related records of *TTN* gene mutation (c. 20233 C>T) were found in multiple databases such as HGMD, and no related literature reports of this mutation were found in the PubMed database, suggesting that this mutation is a newly discovered rare mutation. The multiple functional prediction results showed that the mutation was harmful: the mutation predicted by SIFT is deleterious, by Polyphen2 is probably damaging, and by the Mutation Taster is disease-causing (Figure 3A). *TTN* gene mutation (c. 20233 C>T, p.R6745C) was located in the immunoglobulin (Ig) 49 domain of the titin-containing protein in sarcomere I. Conservative sequence analysis showed that the sequence in which the mutation was located was highly conserved (Figure 3B). Gene mutations can adversely affect the structure and function of proteins. Missense mutations in the Ig domain can cause incomplete titin folding and affect its stability. *TTN* expression profile showed that *TTN* was highly expressed in the heart and skeletal muscles (Figure 4A). Mutations that cause structural and functional

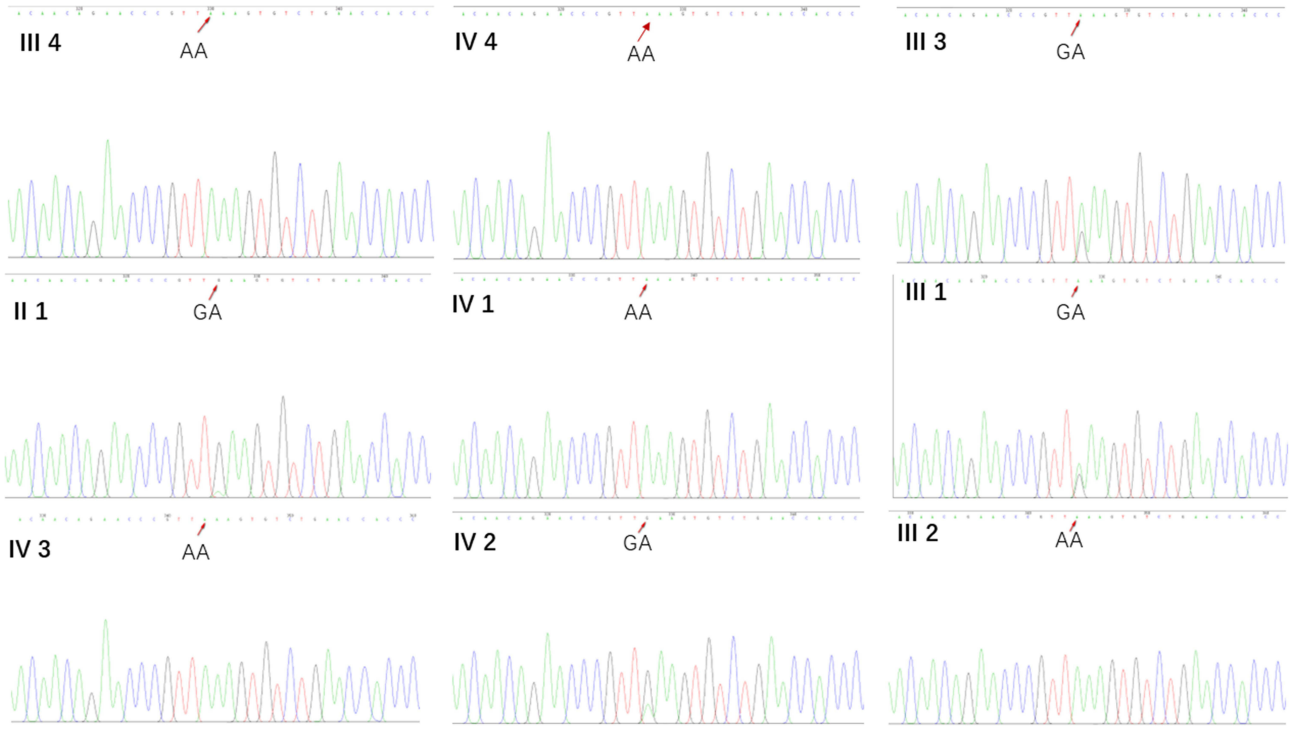

Figure 2 The Sanger sequencing map of family members. The red arrow indicates the mutation location.

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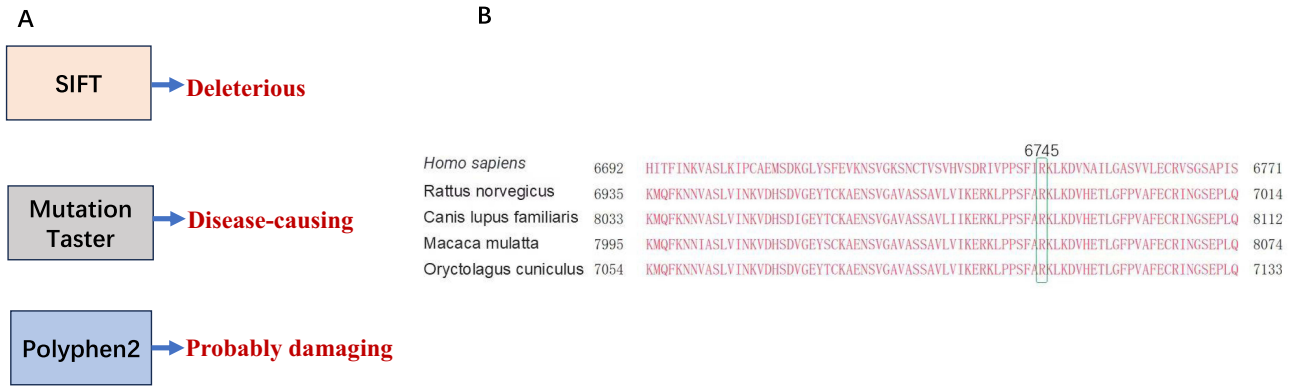

Figure 3 Prediction of the mutation. **(A)**. Prediction the harmfulness of mutation; **(B)**. Prediction the conservation of the mutation site.

changes in *TTN* may affect its normal function in the heart and skeletal muscles, resulting in a range of phenotypes through a variety of signaling pathways (Figure 4B).

Clinical Interpretation of Pathogenic Mutation

According to the ACMG classification criteria of genetic variation, mutation *TTN* (c. 20233 C>T, p.R6745C) was rated as likely pathogenic, and the rating evidence was moderate evidence PM1, PM2, and supporting evidence PP1, PP2, PP3, and PP4.

Discussion

In this study, we identified a new gene mutation *TTN* (c. 20233 C>T, p.R6745C) in a Chinese Han family with HCM, which changed the highly conserved site 6745 of titin protein from arginine to cysteine (p.R6745C), and multiple functional predictions indicated that the mutation was harmful. This gene mutation was only found in all 3 HCM patients but not in healthy adults of this family, that is, genotype phenotypic co-segregation, suggesting that this mutation is closely related to familial HCM. According to phenotypic and genetic screening results, the mutation is autosomal dominant in this family. In addition, Sanger sequencing revealed that a 3 years old child in the family carried the mutation.

HCM, the most common hereditary cardiomyopathy, has significant heterogeneity in the population and incomplete penetrance (penetrance refers to the percentage of individuals with a known genotype in a population exhibiting the corresponding phenotype under different environmental conditions).[34] The degree of myocardial involvement and time of onset cannot be predicted for individuals carrying the pathogenic gene, but the proportion of HCM pathogenic gene carriers exhibiting phenotypes such as myocardial hypertrophy tends to increase with age. The diagnosis and genetic cause of the disease can be further clarified through genetic testing. Second, through genetic screening, the possibility of disease in some family members can be excluded and the economic and psychological burden of the family can be reduced. Asymptomatic and phenotypic carriers of the disease-causing gene in the family, especially young children and adolescents who carry the disease-causing gene, often do not show myocardial hypertrophy or other symptoms or signs.[35] In this study, we found that the 3 years old girl IV2 in this HCM family carried the familial pathogenic gene mutation, and the screening of this patient is helpful to monitor the progression of HCM. According to the European Society of Cardiology (ESC) diagnostic guidelines for HCM, mutation carriers without morphological abnormalities may develop non-specific electrocardiographic abnormalities, which can be regarded as early or mild manifestations of HCM in family members with a family history of hereditary HCM. Regular ultrasound and electrocardiographic examinations were performed on mutation carriers. Early intervention is possible before the disease progresses.[6,7] In addition, for HCM patients with disease-causing genes who have fertility needs, genetic block can be performed by preimplantation genetic diagnosis (PGD) or amniotic fluid puncture embryo gene testing to achieve eugenics and good fertility, so that the offspring do not carry disease-causing gene mutations and avoid the risk of disease.*TTN* is located on chromosome 2q31 and contains 364 exons throughout its length. *TTN* coding protein titin, also known as fibroin, is a giant sarcomal protein with a total length of 27,000–33,000 amino acids (the average length of human protein is 375 amino acids), an average molecular mass of 3,800kDa, and is the largest polypeptide chain in the human body.[36,37] Titin acts as a skeleton in the

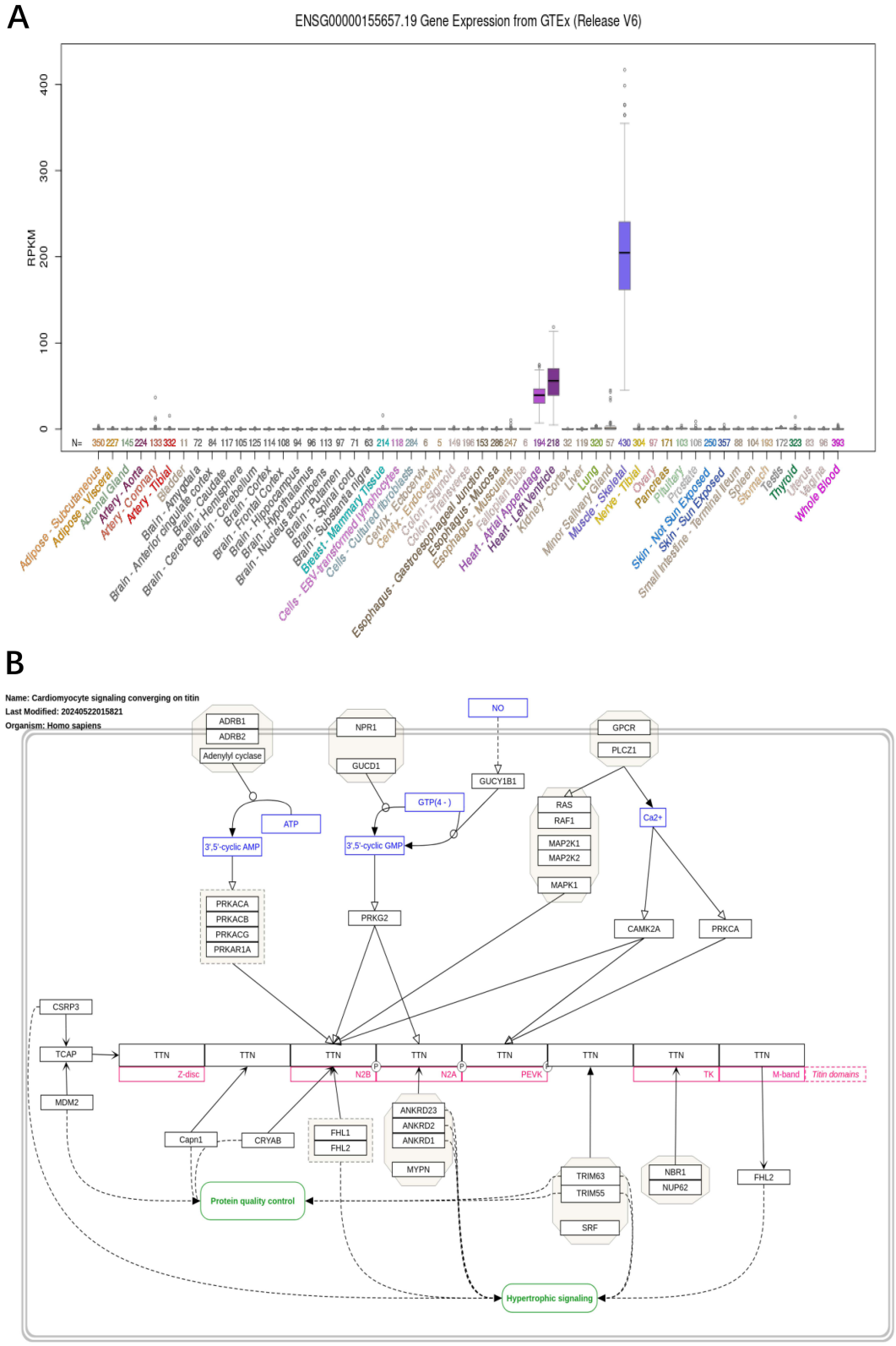

Figure 4 Expression and structure of the *TTN* gene. **(A)**. Expression of the *TTN* gene in individual tissues (https://www.gtexportal.org/home/); (**B**). Structure of the *TTN* gene and its signaling pathway in cardiomyopathy (https://www.wikipathways.org/pathways/WP5344.html).

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sarcomere and is more than 1um in length, accounting for 1/2 of the length of the sarcomere,[38] is the main source of passive elasticity of the sarcomere and promotes the assembly of myofilaments and the formation of the sarcomere. It acts as an external support point for the interaction of the coarse and fine myofilaments and fuses with the coarse and fine myofilaments in the sarcomere M-line and Z disk, respectively.[39] Titin contains binding sites for many other structural proteins, a large number of repetitive immunoglobulin-like domains, fibronectin-like domains (Fn), PEVK domains, N2B-specific sequences (N2-bus), and binding sites for various ligands, such as kinase domains at the carboxyl terminus.

Mutations in *TTN* are associated with several genetic diseases, such as dilated cardiomyopathy, arrhythmogenic right ventricular cardiomyopathy, restrictive cardiomyopathy and perinatal cardiomyopathy. Since 1999, Satoh et al[17] first found that *TTN* missense mutation, Arg740Leu, can cause HCM, only a few HCM-related *TTN* mutations have been reported.[30,39] Although the new mutation we found came from a small family, it can provide a new locus reference for *TTN* gene screening in the future. In the future, it could be included in extensive screening for HCM genetic testing to gain a deeper understanding of the effect of this mutation on HCM and strengthen the link between this mutation or *TTN* and HCM.

The pathogenic mechanism of *TTN* gene mutation varies; the most common is *TTN* truncation mutation (TTN_{TV}), which can cause meaningless degradation of transcripts, and finally cause the protein to produce insufficiency or produce non-functional truncation protein, that is, we often say haploinsufficiency. The substitution of a single amino acid by a missense mutation can have different effects depending on the location of the mutation. For example, the *TTN* mutation Arg740Leu can increase the affinity of the titin protein for alpha-coactin by approximately 40%.[17] The mutation Ser3799Tyr in the N2B domain of *TTN* gene increases the affinity of titin protein to the FHL2 protein.[30] The researchers theorized that the increased affinity between proteins caused by the mutation may prevent cardiomyocytes from maintaining the energy levels required for normal contraction and Ca^{2+} reuptake into the sarcoplasmic reticulum, which in turn triggers cardiac hypertrophy.[40] Mutations in the N2A domain of *TTN* genes Arg8500His and Arg8604Gln increase the affinity of titin to the Z-disk protein-cardiac anchor protein (CARP), and mutations can cause abnormal localization of the CARP protein in cardiomyocytes.[41] Gene mutation Thr2896IIe located in the 10th immunoglobulin-like region (Ig10 domain) of *TTN*, can promote proteolytic hydrolysis of the Ig10 domain, making the mutant protein unstable under physiological conditions.[39] The pathogenic mechanisms underlying most *TTN* missense mutations remain unclear. The new mutation p.R6745C found in this study in the HCM family was located in exon 80 and was a missense mutation caused by a single-base substitution. The mutation is located in the Ig49 domain of the titin-bearing protein in sarcomere I. Bioinformatic analysis showed that the mutation is harmful. Gene mutations adversely affect protein structure and function. Previous studies have shown that there is no protein binding site near the Ig49 domain,[38] but according to Anderson[42] and Hastings et al[43] the Ig domain missense mutation causes incomplete folding of the titin protein, affecting its stability. At present, there is a lack of studies on Ig49 domain, and whether missense mutations in Ig49 domain affect protein function by affecting protein folding and stability is still unclear. Due to the complex structure of *TTN* and the large protein molecules, it is difficult to explore how mutations affect protein's structure and function, which is also our future research direction. In the future, we will verify this mutation in a larger cohort and carry out corresponding functional studies to explore the specific mechanism of this mutation leading to disease, especially its impact on *TTN* function, so as to make our study more complete.With the continuous development of science and technology, genome editing may become a new means for treating HCM. In 2017, Chinese and American scientists collaborated on gene editing of human embryos using CRISPR/Cas9 technology and successfully repaired HCM disease-causing gene mutations carried by embryos for the first time.[44] The experimental results were safe and effective, and there were no off-target mutations, suggesting that this genome-editing treatment may be used in the future together with PGD to correct inherited disease-causing gene variants in human embryos.

Conclusion

This study identified a new heterozygous pathogenic gene mutation, c. 20233 C>T (p.R6745C) in the gene *TTN* for inherited HCM highlighting the important role of genetic testing in the diagnosis and treatment of hereditary diseases such as HCM.

Ethics Approval and Consent to Participate

This study followed the guidelines set forth by the Declaration of Helsinki and was approved by Union Hospital, Tongji Medical College, Huazhong University of Science and Technology (UHCT-IEC-SOP-016-02-01). Informed consent was obtained from all participants. Minors under the age of 18 will have their parents provide informed consent.

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Consent to Publish

Consent for publication was obtained from all participants in this study.

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Disclosure

The authors report no conflicts of interest in this work.

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