Cardiology in the Young

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Cite this article: Tong W, Liu W, Guo H, Wang J, Yu S, Zhang J, Liu C, Chen J, and Zhao X (2020) A novel *MYBPC3* c.2737+1 (IVS26) G>T mutation responsible for high-risk hypertrophic cardiomyopathy. *Cardiology in the Young* **30**: 100–106. doi: 10.1017/S1047951119002701

Received: 5 August 2019
Revised: 8 October 2019
Accepted: 15 October 2019
First published online: 21 November 2019

Keywords:
Hypertrophic cardiomyopathy; *MYBPC3* gene; genotype; phenotype

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A novel *MYBPC3* c.2737+1 (IVS26) G>T mutation responsible for high-risk hypertrophic cardiomyopathy

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Abstract

Background: Hypertrophic cardiomyopathy is an autosomal dominant hereditary disease characterised by left ventricular asymmetry hypertrophy. However, our knowledge of the genetic background in hypertrophic cardiomyopathy cases is limited. Here, we aimed to evaluate pathogenic gene mutations in a family with high-risk hypertrophic cardiomyopathy and analyse the genotype/phenotype relationships in this family. *Methods:* The proband, her parents, and her niece underwent whole-exome sequencing, and the genotypes of family members were identified using Sanger sequencing. mRNA expression was detected using reverse transcription sequencing. Structural impairments were predicted by homologous modelling. A family survey was conducted for patients with positive results to obtain information on general clinical symptoms, electrocardiography, ambulatory electrocardiography, echocardiography, and 3.0T cardiac magnetic resonance findings. Regular follow-up was performed for up to 6 months. *Results:* Five family members, including the proband, carried a cleavage site mutation in the *MYBPC3* gene (c.2737+1 (IVS26) G>T), causing exon 26 of the *MYBPC3* gene transcript to be skipped and leading to truncation of cardiac myosin-binding protein C. Family survey showed that the earliest onset age was 13 years old, and three people had died suddenly at less than 40 years old. Three pathogenic gene carriers were diagnosed with hypertrophic cardiomyopathy, and all showed severe ventricular septal hypertrophy. *Conclusion:* The c.2737+1 (IVS26) G>T mutation in the *MYBPC3* gene led to exon 26 skipping, thereby affecting the structure and function of cardiac myosin-binding protein C and leading to severe ventricular hypertrophy and sudden death.

Hypertrophic cardiomyopathy is an autosomal dominant hereditary disease characterised by left ventricular asymmetry hypertrophy and myofibrillar disorders. It is the primary cause of sudden cardiac death among young individuals, with an incidence of 0.5–1% per year.[1–3] Several echocardiography-based epidemiological studies have indicated that 1 in every 500 people in the general population has cardiac abnormalities; however, the prevalence rate is increasing (1 case per 200) with advancements in genetic testing and the development of more sensitive cardiac diagnostic imaging techniques.[4] To date, 27 genes have been found to be associated with hypertrophic cardiomyopathy, 9 of which have been definitively implicated in hypertrophic cardiomyopathy.

MYBPC3, which encodes cardiac myosin-binding protein C, is the most commonly mutated gene in patients with hypertrophic cardiomyopathy, accounting for 35–40% of pathogenic mutations.[5–8] However, we still have limited knowledge concerning the genetic backgrounds of approximately 40% of hypertrophic cardiomyopathy cases, emphasising the need for further exploration of the genetic pathogenesis of the disease.[9] With the development of next-generation sequencing technology, genome sequencing in individuals is now feasible, making this tool essential for detecting the genetic causes of Mendelian diseases.[10] This is particularly true for whole-exome sequencing, a high-throughput sequencing technology in which coding regions from total genomic DNA are enriched by exon capture and processed for next-generation sequencing. Whole-exome sequencing has shown remarkable efficiency in identifying common and rare variants in an individual's genes.[11,12] Because hypertrophic cardiomyopathy shows high heterogeneity, the differences between clinical phenotypes and genotypes have not been clarified, and the effects of variations in the nature of pathogenic mutations (i.e., position, type, and dose) on the structure and function of the encoded protein may contribute to the observed heterogeneity.[13,14]

In this study, we performed trio-whole-exome sequencing, that is, exome sequencing of up to three or more family members, including the patient, with the goal of identifying novel

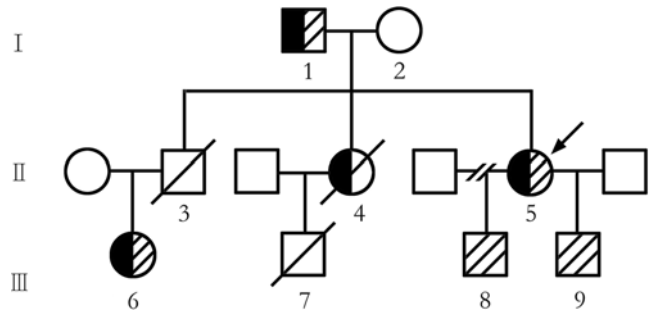

Figure 1. Pedigree of the family with hypertrophic cardiomyopathy (I, II, and III refer to the first, second, and third generations of the family, respectively). Black represents clinically diagnosed patients with hypertrophic cardiomyopathy, shadows represent carriers of pathogenic mutations, slashes represent sudden death, and the arrow indicates the proband.

cleavage site mutations mapped to *MYBPC3* c.2737+1 (IVS26) G>T. The new cleavage site mutation was thought to be associated with myocardial hypertrophy and had not been previously identified. We also evaluated the possible effects of this mutation on specific protein functions and explored the relationships between genotype and clinical phenotype.

Methods

Ethics statement

The study was approved by local ethics committees as per the revised Declaration of Helsinki (2004). Patients and members of the control group were recruited in the Institute of Cardiovascular Diseases, Xinqiao Hospital. Consent was obtained from all the patients being informed of the purposes and risks of this study.

Objectives and clinical data collection

Nine individuals (three people died and six people survived) were enrolled for clinical and genetic studies from a hypertrophic cardiomyopathy family treated in the Institute of Cardiovascular Diseases, Xinqiao Hospital in December 2018 (Fig 1). Medical history, physical examination, 12-lead electrocardiogram, ambulatory electrocardiographic, echocardiography, and 3.0T cardiac magnetic resonance were collected from them, and surviving patients were regularly follow-up by outpatient or telephone. The diagnosis of hypertrophic cardiomyopathy was mainly based on echocardiographic demonstration of a hypertrophied and non-dilated left ventricle, with a wall thickness ≥15 mm or ≥13 mm for people with a family history of hypertrophic cardiomyopathy, in the absence of another cardiac or systemic disease capable of producing a similar magnitude of hypertrophy.[15]

Extraction of genomic DNA

Under the premise of informed consent, 2 ml whole blood (Ethylenediaminetetraacetic acid anticoagulation) was collected from the family members and extracted their genome-wide DNA using BloodGen MidiKit (CWBIO, Beijing, China), the procedure was carried out according to the kit instructions.

Next-generation sequencing and DNA sequence analysis

Genomic DNA samples were sheared by sonication. The sheared genomic DNA was then hybridised with NimbleGen 2.0 probe sequence capture array of Roche, (http://www.nimblegen.com/products/seqcap/ez/v2/index.html) to enrich the exonic DNA (Joy Orient, Beijing, China). The libraries were first tested for enrichment by quantitative polymerase chain reaction and for size distribution and concentration using the Agilent Bioanalyzer 2100 (Agilent, Beijing, China). The samples were then sequenced on an Illumina Hiseq2500 (Illumina, Beijing, China). Two parallel reactions were done for each sample.

Data filtering, mapping, and variant detection

Exon-enriched DNA was sequenced by the Illumina hiseq2500 platform following the manufacturer's instructions (Illumina). Raw image files were processed by the BclToFastq (Illumina) for base calling and generating the raw data. The low-quality variations were filtered out using the quality score ≥ 20 (Q20). The sequencing reads were aligned to the National Center for Biotechnology Information human reference genome (hg19) using Burrows-Wheeler Alignment tool. Samtools and Pindel were used to analyse single nucleotide polymorphism and indel of the sequence.

Data analysis

Data analysis was applied as below: Step 1. Synonymous changes and single nucleotide polymorphisms that minor allele frequency was higher than 5% were removed (http://www.ncbi.nlm.nih.gov/projects/SNP). Step 2. Nonsynonymous changes were filtered using SIFT software (http://sift.jcvi.org). Step 3. Analysed the function of mutated genes and its relation of disease.

Mutation confirmed by Sanger-method sequencing

Sanger sequencing was used to confirm the mutation in the proband, her parents, and niece. The polymerase chain reaction primers and length of polymerase chain reaction product are shown in Supplementary table S1. Polymerase chain reaction product was sequenced by ABI 3730XL and analysed by DNASTAR software and compared with mRNA template (MYBPC3: NM_ 000256).

cDNA sequencing (reverse transcription sequencing) verification of single splice sites

To verify whether the c.2737+1 (IVS26) G>T mutation affects cDNA splicing, we extracted the mRNA of the proband (Kit: RiboPure™-Blood Kit Invitrogen by Thermo Fisher Scientific, Shanghai, China), reverse transcribed into cDNA as a template, and amplified the exon around the target site, Exon24-28, and performed Sanger sequencing. The polymerase chain reaction primers and length of polymerase chain reaction product are shown in Supplementary table S1.

Prediction of structural impairments

A predicted model of the C7 domain in cardiac myosin-binding protein C (residues 867–964) was generated using the webserver of *I-TASSER* (Zhang Y 2008). A bundle of homologous structures available in protein data bank were used as templates in structure prediction utilising a hierarchical approach integrating multiple threading methods and iterative template-based fragment assembly simulations. The top model with good confidence (C-score = 0.90) was used for further analysis. All structure representations were generated using the molecular visualisation program *PyMOL* (Schrodinger, LLC 2015).

https://doi.org/10.1017/S1047951119002701 Published online by Cambridge University Press

Table 1. Proband (II-5) clinical data

	Echocardiography								
Time of inspection	IVS, mm	LVPW, mm	LAD, mm	LVEF, %	LVOTG, mmHg	12-lead ECG	Holter	Duration of NSVT (S)	CMR
January, 2015	17.7	8.1	29	77	0	Abnormal Q wave	–	–	–
June, 2015	23.7	7.1	29	72	9	Abnormal Q wave	NSVT	5.7	–
April, 2018	30	8	30	69	14.7	Abnormal Q wave	–	–	–
December, 2018	27	8.2	30	67	7.6	Abnormal Q wave	NSVT	0.7	LGE

Holter = ambulatory electrocardiographic; IVS = interventricular septum diameter; LAD = Left atrial diameter; LGE = late gadolinium enhancement; LVEF = left ventricle ejection fraction; LVOTG = left ventricular outflow tract gradient; LVPW = Left ventricular posterior wall thickness; NSVT = Non-sustained ventricular tachycardia

Results

Proband clinical data

The proband (II-5) was a 39-year-old woman who came to the clinic because many relatives in the family had suddenly died for unknown reasons, and the psychological burden of this was affecting her quality of life. She was first found to have cardiac hypertrophy by echocardiography during an examination in January 2015 and during regular follow-up at a local clinic. Follow-up data are shown in Table 1. The 12-lead electrocardiogram, ambulatory electrocardiography, echocardiography, and cardiac magnetic resonance images are shown in Figure 2. The proband felt mild dizziness occasionally, but no other specific discomforts were reported in the medical history survey. Physical examination showed that blood pressure was 98/60 mmHg, heart rate was 70 bpm, and there were no pathological noises in valves.

Clinical data for family members

The proband's niece (III-6) was the first to be found to have cardiac hypertrophy (interventricular septum 16.6 mm) in the family because of repeated syncope when she was 13 years old (in 2009). Syncope occurred frequently in the next 10 years, about 1–2 times per year. The proband's brother (II-3), nephew (III-7), and sister (II-4) died at 24 (in 1999), 18 (in 2014), and 40 (in 2018) years of age, respectively, for unknown reasons. None of these relatives had any relevant symptoms before death. Clinical data were collected from nine family members (Table 2).

Exome analysis and mutation data

We performed exome sequencing for four family members (the proband, her biological parents, and her niece) as trio analysis including DNA samples of both biological parents, and whole-exome sequencing data were analysed as described above. We found that all but one (*MYBPC3*) of the variants that were identified in the affected individual segregated with the phenotype within the family (Supplementary table S2). Applying American College of Medical Genetics and Genomics and the Association for Molecular Pathology criteria PVS1, PM2, PP1, and PP3, we classified the *MYBPC3* gene (c.2737+1 (IVS26) G>T) heterozygous variant as pathogenic.[16] These findings provided strong genetic evidence that the identified *MYBPC3* gene (c.2737+1 (IVS26) G>T) variant was the disease-causing variant in this family. Unfortunately, the two young sons of the proband (III-8, III-9) were also confirmed to carry the pathogenic gene by Sanger sequencing (Fig 3a)

cDNA sequencing (reverse transcription sequencing) verification data

Nucleotide sequencing of the amplicon from the venous blood of the proband confirmed that exon 26 was skipped during splicing of the mutant allele (Fig 3b). Exon 26 contained 135 bases from c.2603 to c.2737, and the mutation therefore resulted in creation of a stop codon without introduction of novel sequence because the missing bases were in multiple of three.

Prediction of structural impairments and functional loss in the cardiac myosin-binding protein C protein

Next, we used data from the National Center for Biotechnology Information database and found that c.2602/c.2603/c.2604 encoded glycine (p.868), whereas c.2737/c.2738/c.2739 encoded cysteine (p.912). After c.2603–c.2737 were skipped, c.2602/c.2738/c.2739 formed the codon GGC (guanine, guanine, cytosine), which encoded glycine. This resulted in deletion of 44 amino acids (p.869–p.912; Fig 3c).

An ensemble of 3D structures of the C7 domain in cardiac myosin-binding protein C (residues 867–964) was built by means of homologous modelling using the *I-TASSER* server (Zhang Y 2008). The top model with C-score = 0.90 (a value indicating modelling confidence, usually in the range of −5 to 2 and the higher value, the higher confidence) showed similar topology with the nuclear magnetic resonance structure of the 3rd fibronectin-type three domain of human MYBPC1 protein (protein data bank entry 2YUX) and a typical β-barrel architecture (Fig 3c). The peptide region of residues 869–912 (red box and red ribbon shown in Fig 3c) that would be deleted upon the mutation found in this study covers three β-strands: β1–β3. Apparently, the absence of these strands would definitely prevent formation of the β-barrel at the structure core and leads to collapse of the overall architecture.

From structural point of view, the severe disruption of the correct fold in the C7 domain would produce cascade effects in the overall structure of cardiac myosin-binding protein C, including orientation and position changes of the following domains (C8-Cx), which would further disturb the insertion of them into the A-band and gives rise to marked functional loss of the intact protein. Schematic representation of MYBPC3 gene, mRNA, protein structure, and its interaction partners are shown in Supplementary figure S1.[17]

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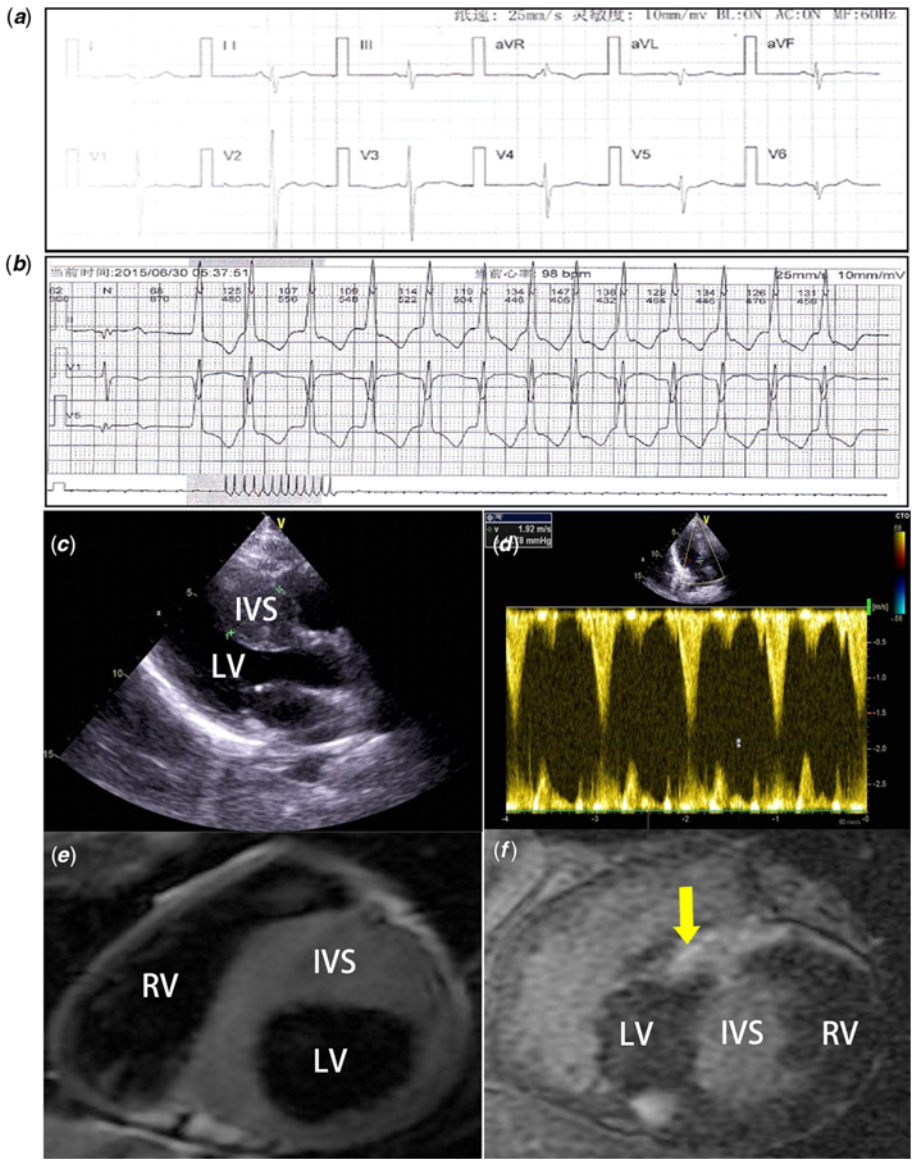

Figure 2. The 12-lead ECG, ambulatory electrocardiography, echocardiography, and cardiac magnetic resonance images of proband. (***a***) 12-lead ECG showed an abnormal Q wave. (***b***) Ambulatory electrocardiography showed non-sustained ventricular tachycardia (NSVT) lasted up to 5.7 seconds. (***c*** and ***e***) Echocardiograph and CMR images showed massive hypertrophy of the interventricular septum (IVS). (***d***) Echocardiograph image showed left ventricular outflow tract gradient (LVOTG) increased. (***f***) Enhanced CMR showed late gadolinium enhancement (LGE) (arrow).

Discussion

The c.2737+1 (IVS26) G>T heterozygous mutation caused hypertrophic cardiomyopathy with a high degree of penetrance, high incidence of sudden death, and severe myocardial hypertrophy

From the pedigree of the family, we speculated that the proband's brother (II-3), sister (II-4), and nephew (III-7) were all likely to carry the pathogenic gene and may have hypertrophic cardiomyopathy. And if so, six of eight carriers in this family showed myocardial hypertrophy, with the earliest onset age being 13 years old. Another two were still young, and we predict that they may also develop hypertrophic cardiomyopathy in the future. Three of six individuals with hypertrophic cardiomyopathy in this family died suddenly at less than 40 years of age, with the youngest being 18 years old. Late gadolinium enhancement observed in cardiac magnetic resonance and non-sustained ventricular tachycardia indicated fibrosis of the myocardium and instability of myocardial electrical activity, which could result in high risk of sudden death.[18] Traditionally, the clinical phenotypes of hypertrophic cardiomyopathy caused by *MYBPC3* heterozygous mutation include late onset, low penetrance, and favourable clinical course.[19,20] However, severe phenotypes were observed in carriers of the novel *MYBPC3* heterozygous mutation. Differences in disease severity in patients with various mutations in *MYBPC3* have not been fully clarified to date. Erdmann et al indicated that severe hypertrophy and life-threatening arrhythmias were more common in patients with truncation rather than missense mutations in *MYBPC3*,[21] consistent with our observations.

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Table 2. Clinical and genetic data of family members with hypertrophic cardiomyopathy

Family member	Mutation gene	Clinical diagnosis	Symptoms or adverse event	Sex/age (years)	Echocardiography					12-lead ECG	Holter	CMR
					IVS, mm	LVPW, mm	LAD, mm	LVEF, %	LVOTG, mmHg			
I-1	Positive	HCM	–	M/60	23.5	11	35.5	72	4	ST-T change	NSVT	LGE
I-2	Negative	Normal	–	F/70	11.9	11	27	63	0	ST-T change	Normal	–
II-3	–	–	SCD	M/24	–	–	–	–	–	–	–	–
II-4	–	HCM	SCD	F/40	15.2	9.1	33	63	0	Abnormal Q wave	–	–
II-5	Positive	HCM	Dizziness	F/39	27	8.2	30	67	7.6	Abnormal Q wave	NSVT	LGE
III-6	Positive	HCM	Syncope	F/23	23.4	9	26	70	57	Abnormal Q wave	PVC	–
III-7	–	–	SCD	M/18	–	–	–	–	–	–	–	–
III-8	Positive	Normal	–	M/16	10	10	28	70	0	Normal	Normal	–
III-9	Positive	Normal	–	M/4	6	6	22	69	0	Normal	–	–

F = female; Holter = ambulatory electrocardiographic; IVS = interventricular septum diameter; LAD = Left atrial diameter; LGE = late gadolinium enhancement; LVEF = left ventricle ejection fraction; LVOTG = left ventricular outflow tract gradient; LVPW = Left ventricular posterior wall thickness; M = male; NSVT = Non-sustained ventricular tachycardia; PVC = premature ventricular contraction; SCD = sudden cardiac death

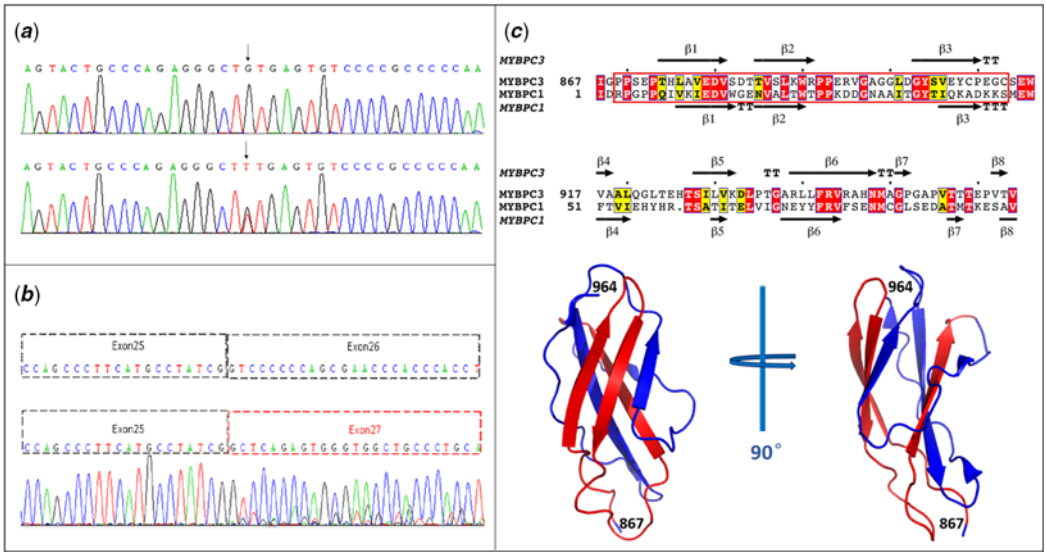

Figure 3. Images of DNA, cDNA sequence analysis, and three-dimensional structures of protein. (***a***) Mutation analysis of the *MYBPC3* gene showed a single bp sequence variant (G>T) on the allele from the donor splice sequences (arrow). The National Center for Biotechnology Information reference sequence is shown in the upper panel, and the mutated sequence is shown in the lower panel. (***b***) Sequence analysis of the cloned reverse transcription polymerase chain reaction product derived from RNA of proband and a control showed the in-frame skipping of exon 26 caused by the c.2737+1 (IVS26) G>T mutation. The National Center for Biotechnology Information reference sequence is shown in the upper panel, and the mutated mRNA sequence is shown in the lower panel. (***c***) Structure-based sequence alignment of the C7 domain in MYBPC3 and MYBPC1 (PDB entry 2YUX) with secondary structure elements is shown in the upper panel, and the residues in the red box are the deleted ones upon the mutation found in this study. The predicted structure of the C7 domain (residues 867–964) in cMyBP-C obtained from homologous modelling is shown in the lower panel, and the deleted region is coloured in red while the remaining in blue.

The novel deletion mutation resulted in structural collapse and functional disruption of the C7 domain, in turn preventing the cardiac myosin-binding protein C molecule from being correctly inserted into the A-band

Molecular genetic data may contribute to interpretation of the clinical manifestations of hypertrophic cardiomyopathy observed in the family. Gilbert et al demonstrated that the final four domains (C7–C10) of cardiac myosin-binding protein C are necessary for proper localisation of these proteins in the A-band. Although the last three modules of the titin-binding domain (C8–C10) are contained in this region, these domains are not sufficient for proper insertion of the molecule into the A-band, and an additional FnIII module (C7) is needed.[22–24]

https://doi.org/10.1017/S1047951119002701 Published online by Cambridge University Press

Hypertrophic cardiomyopathy-specific human embryonic stem cell-derived cardiomyocytes carrying the mutation encoding the C7 domain displayed hallmark features of hypertrophic cardiomyopathy

Da Rocha et al used a human embryonic stem cell line carrying a naturally occurring mutation in *MYPBC3* (c.2905+1G>A) to study hypertrophic cardiomyopathy pathogenesis. This mutation causes exon 27 to be skipped without frameshift, thereby eliminating the C7 domain. Human embryonic stem cell-derived cardiomyocytes carrying the mutation displayed hallmark features of hypertrophic cardiomyopathy, including sarcomere disarray, hypertrophy, and impaired calcium impulse propagation, and patients carrying this mutation also showed severe myocardial hypertrophy and a high incidence of sudden death. Functional data from this study may, in part, provide an explanation for the clinical data observed in the family we studied.[25]

Exon skipping strategies in MYBPC3-targeting therapy are limited

In the last decade, several strategies have been developed to combat genetic defects.[26] Exon skipping strategies have been proposed to prevent frameshifts in truncating mutations in patients with hypertrophic cardiomyopathy, accounting for most known mutations in *MYBPC3* (>60%).[17,27] Additionally, in MYBPC3-targeted knock-in mice,[28] skipping of exons 5 and 6 was induced, resulting in an in-frame deletion. Newborn mice abolished cardiac dysfunction and prevented left ventricular hypertrophy.[28] The family in this study illustrated that skipping of exon 26 did not likely prevent cardiac hypertrophy.

Significance of genetic testing

Currently, the predominant reason for genetic testing in clinical practice is to identify family members who do not express the phenotype but may be at risk of developing disease. For relatives who do not show a phenotype but carry a known pathogenic mutation, for example, the proband's two young sons (III-8, III-9), it is necessary to follow up and evaluate changes in their condition regularly. However, if a phenotype-negative relative carries no pathogenic mutation, regular evaluation is unnecessary. A negative genetic test not only alleviates psychological concerns and the economic burden of further cardiovascular surveillance but also eliminates restrictions with regard to lifestyle and competitive sports. The role of genetic testing in predicting prognosis is limited based on clinical statistical analysis of superficial genes and clinical phenotypes, although emerging data have suggested that deep analysis of the mechanisms through which mutations affect the structure and function of proteins or interactions with other proteins may contribute to prediction of prognosis.[14,29] Consistent with early onset, severe myocardial hypertrophy and a high incidence of sudden death were observed in the family members who carried the novel mutation. Further in-depth analyses of the mutant protein structure may also improve our ability to predict prognosis in such patients.

In conclusion, in this study, we performed trio-whole-exome sequencing to identify a novel cleavage site mutation mapped to *MYBPC3* c.2737+1 (IVS26) G>T, which was thought to be associated with myocardial hypertrophy in a family with high-risk hypertrophic cardiomyopathy and had not been previously identified. Exon 26 skipping during splicing of the mutant allele was verified by cDNA sequencing and led to structural collapse and functional disruption of the C7 domain, thereby preventing the cardiac myosin-binding protein C molecule from being correctly inserted into the A-band and causing myocardial hypertrophy in the family. These observations may provide important prognostic information for clinicians managing patients with hypertrophic cardiomyopathy. Further epidemiological studies of the mutation c.2737+1 (IVS26) G>T and model verification using hESC-CMs carrying the c.2737+1 (IVS26) G>T mutation are necessary.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/S1047951119002701

Acknowledgements. We express thanks to the Joy Orient Translational Medicine Research Centre, Beijing, China for the work of gene amplification and sequence analysis, and Editage (www.editage.cn) for English language editing.

Financial Support. This study was supported by grant from the National Natural Science Foundation of China (81670428, 81570217).

Conflicts of Interest. The authors declare that there are no conflicts of interest.

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