INTRODUCTION

Hypertrophic cardiomyopathy (HCM) is a genetic cardiac disease characterized by left ventricular hypertrophy (Gersh et al., 2011). HCM is diagnosed in patients with left ventricular wall thickness of more than 15 mm. HCM is a common disorder and affects more than 0.2% of the general population (Semsarian, Ingles, Maron, & Maron, 2015). Genetic factors contribute to approximately 50% of all cases of HCM, which are thought to be inherited in an autosomal dominant manner. HCM is usually caused by variants in sarcomere-related genes, including MYBPC3, MYH7, TNNT2, TNNI3, TPM1, ACTC1, MYL2, MYL3, and other genes (Ingles et al., 2019; Liew, Vassiliou, Cooper, & Raphael, 2017). Most HCM patients are found to carry one pathogenic allele; however, a minority harbor more than one variant in one gene or two/three distinct genes. This may result in a more severe clinical phenotype with a higher incidence of heart failure or sudden death (Ingles et al., 2019; Liew, Vassiliou, Cooper, & Raphael, 2017).
et al., 2005; Maron, Maron, & Semsarian, 2012; Zheng et al., 2016). Recently, the oligogenic inheritance of congenital heart disease has been proved by the experimental model (Gifford et al., 2019). Although a panel of 7–10 HCM-associated genes have been used for HCM patient screening (Das, Ingles, Bagnall, & Semsarian, 2014), structural variations cannot be easily detected by Sanger sequencing or targeted sequence capture methods. Thus, it is likely that important genetic variants in certain HCM patients might be overlooked.

In this study, we recruited two patients from a family with history of HCM. Whole-exome sequencing (WES) and pedigree analyses revealed the existence of two variants in each patient. One identified variant included the whole-gene deletion of troponin I3 (TNNI3), which traditional Sanger sequencing might be unable to detect. Therefore, we conclude that WES is likely a powerful tool to identify both the point mutations and larger deletions/insertions involved in HCM.

2 MATERIALS AND METHODS

2.1 Patients

A Chinese family with two diagnosed HCM members was recruited for this study. The proband (Figure 1, II:1) and his mother (Figure 1, I:2) were diagnosed with HCM based on the HCM diagnostic criteria (Gersh et al., 2011). The thickness of the left ventricular wall of the proband’s heart was 19.2 mm. The proband was 36-year-old male, and his mother was 62-year-old. The proband has a history of syncope after sports (playing basketball), and has no other special symptoms in daily life.

Detailed ultrasound description of the proband’s heart is described below: the left ventricle diameter was in the normal range and the interventricular septum was thickened with the thickest part located in the anterior septum measuring approximately 19.2 mm. There were no obvious abnormalities in the motion of left ventricular wall. There was no obvious abnormality in the overall contraction or synergy, and the motion score of the left ventricular wall was 16 points. The size of the left atrial was normal with good cavity echo; the size of right heart was normal, no obvious abnormalities were observed in wall motion; no obvious interrupted echo was detected in the atrial septum. CDFI showed small blood flow from left to right with an approximate width of flow beam of 2.5 mm. There was no obvious interrupted echo in the interventricular septum, and no obvious blood flow was detected by Doppler. There was no obvious abnormality in the mitral valve echo. Mitral regurgitation (MR) was detected by CDFI during systole; no obvious abnormality of the aortic valve echo was observed and the opening function was normal, similar to the reflux and tricuspid in diastolic CDFI. CDFI trachea probe showed a small amount of return flow, which led to inaccurately determining the pressure difference; the pulmonary valve echo was normal with normal opening, the blood flow velocity of the systolic valve was not fast and no abnormal blood flow was found during diastole. The morphology of the ascending aorta and the inner diameter of the pulmonary artery were normal and no abnormal blood flow was detected. There was no apparent abnormality in pericardial echo. Therefore, the ultrasound results were suggestive of hypertrophic cardiomyopathy, normal left ventricular function, and patent foramen ovale.

This work has been approved by the Ethics Committee of Beijing Obstetrics and Gynecology Hospital. Written consents were obtained from all participants before samples were collected. Five milliliters of peripheral blood were collected from each patient.

2.2 Whole-exome sequencing analysis

Whole-exome sequencing (WES) was performed on DNA samples from the proband and his mother. WES was performed by a commercial sequencing service (Annoroad Gene Technology). All exons were captured and enriched using SureSelect Human All Exon V6 kit (Agilent Technologies). After the DNA libraries were prepared, they were sequenced with the HiSeq™ PE150 system. Clean reads were obtained by removing the contaminant reads from the raw reads. Then, clean reads were aligned to the UCSC hg19 reference genome by Burrows-Wheeler Aligner. High-quality BAM files were generated by using Samtools and Picard, and variants were called from BAM files by GATK. Single-nucleotide polymorphisms (SNPs) and insertion-deletions (InDels) were annotated by ANNOVAR. Structural variations (SVs) were firstly detected by DELLY2 software and then were annotated by ANNOVAR. SVs referred to five different genetic variation types including deletions, duplications, insertions, inversions, and transversions. The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

3 RESULTS

DNA samples from both the proband (II:1) and his mother (I:2) were subjected to WES analysis. Pedigree analysis suggested a dominant mode of inheritance (Figure 1a). Previous studies have shown that mutations in 57 genes can cause or be associated with HCM, including 8 definitive genes (MYBPC3, MYH7, TNNI2, TNNI3, TPM1, ACTC1, MYL2, and MYL3), 3 moderate evidenced genes (CSRP3, TNNC1, and JPH2) and other limited or no evidenced genes (such as TTN, KLF10, MYPN, ANKRD1, MYLK2, MYOZ2, NEXN, VCL, TRIM63, RYR2, MYH6, OBSCN, PDLIM3, TCAP,
MYOM1, and CALR3) (Das et al., 2014; Green et al., 2013; Ingles et al., 2019; Liew et al., 2017). Therefore, we focused on the genetic variations (SNPs, InDels, and SVs) occurring in any of the above-mentioned genes. Moreover, we considered only the genetic variations that existed in both patients.

Based on the above-mentioned strategy for variation analysis, a known pathogenic heterozygous missense variant in the myosin heavy chain 7 gene, MYH7 (NM_000257:exon20:c.2207T>C:p.Ile736Thr) was identified in both patients and was further validated by Sanger sequencing (Figure 1b). The p.Ile736Thr variant was very conserved from human to zebrafish (Figure 1c). The allele frequency of c.2207T>C in MYH7 was 0 in gnomAD, ExAC, 1,000 Genomes and ESP6500 exome or genome sequencing databases (Table 1). In silico analysis by Polyphen-2, SIFT, PROVEAN, MutationTaster, SNPs&GO and FATHMM-MKL suggested that p.Ile736Thr variant was a disease-causing variant (Table 1). This variant was also interpreted as a pathogenic variant by ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/variation/164342/).

Besides SNPs, SVs were also examined and analyzed. A heterozygous deletion in chromosome 19 was detected in both patients (Figure 2a,b). This large region in chromosome 19 contains the whole-gene of TNNI3. Therefore, both HCM patients harbored a MYH7 variant and a TNNI3 whole-gene deletion.

**FIGURE 1** Pedigree analysis and sequence alignment analysis of MYH7 protein. (a) The family tree shows two patients with HCM. The black cycle and black square indicate the patients. (b) Sanger sequencing validated the MYH7 variant in this family. Sanger sequencing confirmed the heterozygous MYH7 variant in the two patients. The red arrow points to the mutational site (c.2207T>C). (c) Sequence alignment of MYH7 protein in different species. The red arrow indicates the p.Ile736 site, which is 100% conserved in different species.

| Species     | Sequence Alignment |
|-------------|--------------------|
| Human       | AA PEGQFDI        |
| Cow         | AA PEGQFDI        |
| Pig         | AA PEGQFDI        |
| Dog         | AA PEGQFDI        |
| Norway Rat  | AA PEGQFDI        |
| House Mouse | AA PEGQFDI        |
| Mouse       | AA PEGQFDI        |
| Chicken     | TA PEGQFDI        |
| Zebrafish   | AA PEGQFDI        |

**DISCUSSION**

In this study we detected two variants in all HCM patients studied and having the same pedigree. One variant was the whole gene deletion of TNNI3. The TNNI3 is located on
chromosome 19q13.42; its encoded protein is expressed in cardiac muscle tissues and is one of three subunits forming the troponin complex. \textit{TNNI3} is the seventh HCM-associated gene to be discovered (Kimura et al., 1997), and contributes to 4%–8% of all HCM patients (Liew et al., 2017). The \textit{TNNI3} is highly constrained and intolerant to loss-of-function variations (Marian & Braunwald, 2017). To date, about 30 different \textit{TNNI3} variants have been linked to HCM (Zhao et al., 2015), however, as far as we are aware no whole \textit{TNNI3} deletion has been associated with HCM. Therefore, our study is the first to report a heterozygous \textit{TNNI3} genomic locus deletion in HCM patients. Previous studies have identified \textit{TNNI3} missense variants by Sanger sequencing or targeted next-generation sequencing for specific genes, thus increasing the likelihood of larger genomic deletions or insertions to be overlooked. Whole-exome sequencing technology can detect both SNPs and structural variations. Thus, from the cases in our study we recommend that WES is a more efficient tool for the identification of genetic variations in the patients with HCM.

The second variant we identified in both HCM patients was located in the \textit{MYH7} (NM_000257:exon20:c.2207T>C:p.Ile736Thr). Approximately 30% of all HCM cases are associated with variants in the \textit{MYH7} (Richard et al., 2003). The p.Ile736Thr \textit{MYH7} variant is a known pathogenic allele and has been reported by several studies (Barriales-Villa et al., 2010; Erdmann et al., 2003; Perrot et al., 2005). Nevertheless, the p.Ile736Thr variant has also been considered a benign variant, due to its association with close to normal life expectancy (Tripathi et al., 2011).

Recently, Gifford et al., identified three missense variants in \textit{MKL2} (Gln670His), \textit{MYH7} (Leu387Phe), and \textit{NKX2-5} (Ala119Ser) in three offspring with childhood-onset cardiomyopathy (Gifford et al., 2019). By using CRISPR-Cas9 technology the authors generated mice encoding the orthologous variants and found that triple-compound heterozygous mice recapitulated the human disease phenotype (Gifford et al., 2019). In our study, we found two distinct variants, \textit{MYH7} missense variant (p.Ile736Thr) and \textit{TNNI3} deletion. So in order to confirm the digenic inheritance pattern, further functional studies such as using CRISPR-Cas9 technology to generate the compound heterozygous mice are needed.

Even if our study found that HCM patients might potentially harbor \textit{TNNI3} deletion, we still need to realize that, given that these two patients have a known pathogenic \textit{MYH7} variant, the clinical consequence of the \textit{TNNI3} deletion is uncertain, in the absence of further family members that may have only one of the two variants. Therefore, we did not rule out that \textit{TNNI3} deletion might be a nonpathogenic factor.

In summary, our study identified the first HCM case with whole \textit{TNNI3} deletion, and we further provide evidence that WES is a powerful tool to comprehensively analyze genetic variants in HCM patients. Therefore, our study added the notion that digenic inheritance may contribute to HCM pathogenesis.
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CONFLICT OF INTEREST
The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS
MBR, XRC, and LL were involved in data analysis of the whole-exome sequencing. MBR, XW, and XRC were involved in clinical data collection. LL and CY were involved in manuscript preparation.

REFERENCES
Barriales-Villa, R., Centurión-Inda, R., Fernández-Fernández, X., Ortiz, M. F., Pérez-Alvarez, L., Rodríguez García, I., … Monserrat, L. (2010). Severe cardiac conduction disturbances and pacemaker implantation in patients with hypertrophic cardiomyopathy. Revista Española De Cardiología, 63, 985–988. https://doi.org/10.1016/s1885-5857(10)70192-4
Das, K. J., Ingles, J., Bagnall, R. D., & Semsarian, C. (2014). Determining pathogenicity of genetic variants in hypertrophic cardiomyopathy: Importance of periodic reassessment. Genetics in Medicine, 16, 286–293. https://doi.org/10.1038/gim.2013.138
Erdmann, J., Daehmlow, S., Wischke, S., Senyuva, M., Werner, U., Raible, J., … Regitz-Zagrosek, V. (2003). Mutation spectrum...
in a large cohort of unrelated consecutive patients with hypertrophic cardiomyopathy. Clinical Genetics, 64, 339–349. https://doi.org/10.1034/j.1399-0004.2003.00151.x

Gersh, B. J., Maron, B. J., Bonow, R. O., Dearani, J. A., Fifer, M. A., Link, M. S., … Yancy, C. W. (2011). 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. Circulation, 124, 2761–2796. https://doi.org/10.1161/CIR.0b013e318223e230

Gifford, C. A., Ranade, S. S., Samarakoon, R., Salunga, H. T., De Soysa, T. Y., Huang, Y., … Srivastava, D. (2019). Oligogenic inheritance of a human heart disease involving a genetic modifier. Science, 364, 865–870. https://doi.org/10.1126/science.aat5056

Green, R. C., Berg, J. S., Grody, W. W., Kalia, S. S., Korf, B. R., Martin, C. L., … Biesecker, L. G. (2013). ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing. Genetics in Medicine, 15, 565–574. https://doi.org/10.1038/gim.2013.73

Ingles, J., Doolan, A., Chiu, C., Seidman, J., Seidman, C., & Semsarian, C. (2005). Compound and double mutations in patients with hypertrophic cardiomyopathy: Implications for genetic testing and counselling. Journal of Medical Genetics, 42, e59. https://doi.org/10.1136/jmg.2005.033886

Ingles, J., Goldstein, J., Thaxton, C., Caleshu, C., Corty, E. W., Crowley, S. B., … Funke, B. (2019). Evaluating the clinical validity of hypertrophic cardiomyopathy genes. Circulation-Genomic and Precision Medicine, 12, e002460. https://doi.org/10.1161/CIRCGEN.119.002460

Kimura, A., Harada, H., Park, J. E., Nishi, H., Satoh, M., Takahashi, M., … Sasazuki, T. (1997). Mutations in the cardiac troponin I gene associated with hypertrophic cardiomyopathy. Nature Genetics, 16, 379–382. https://doi.org/10.1038/ng0897-379

Liew, A. C., Vassiliou, V. S., Cooper, R., & Raphael, C. E. (2017). Hypertrophic cardiomyopathy-past, present and future. Journal of Clinical Medicine, 6(12), 118. https://doi.org/10.3390/jcm6120118

Marian, A. J., & Braunwald, E. (2017). Hypertrophic cardiomyopathy: Genetics, pathogenesis, clinical manifestations, diagnosis, and therapy. Circulation Research, 121, 749–770. https://doi.org/10.1161/CIRCRESAHA.117.311059

Maron, B. J., Maron, M. S., & Semsarian, C. (2012). Double or compound sarcomere mutations in hypertrophic cardiomyopathy: A potential link to sudden death in the absence of conventional risk factors. Heart Rhythm: the Official Journal of the Heart Rhythm Society, 9, 57–63. https://doi.org/10.1016/j.hrthm.2011.08.009

Perrot, A., Schmidt-Traub, H., Hoffmann, B., Prager, M., Bit-Avragim, N., Rudenko, R. I., … Osterziel, K. J. (2005). Prevalence of cardiac beta-myosin heavy chain gene mutations in patients with hypertrophic cardiomyopathy. Journal of Molecular Medicine (Berlin), 83, 468–477. https://doi.org/10.1007/s00109-005-0635-7

Richard, P., Charron, P., Carrier, L., Ledeuil, C., Cheav, T., Pichereau, C., … Komajda, M. (2003). Hypertrophic cardiomyopathy: Distribution of disease genes, spectrum of mutations, and implications for a molecular diagnosis strategy. Circulation, 107, 2227–2232. https://doi.org/10.1161/01.CIR.0000066323.15244.54

Semsarian, C., Ingles, J., Maron, M. S., & Maron, B. J. (2015). New perspectives on the prevalence of hypertrophic cardiomyopathy. Journal of the American College of Cardiology, 65, 1249–1254.

Tripathi, S., Schultz, I., Becker, E., Montag, J., Borchert, B., Francino, A., … Kraft, T. (2011). Unequal allelic expression of wild-type and mutated beta-myosin in familial hypertrophic cardiomyopathy. Basic Research in Cardiology, 106, 1041–1055. https://doi.org/10.1007/s00395-011-0205-9

Zhao, Y., Feng, Y., Zhang, Y. M., Ding, X. X., Song, Y. Z., Zhang, A. M., … Xia, X. S. (2015). Targeted next-generation sequencing reveals hot spots and doubly heterozygous mutations in Chinese patients with familial cardiomyopathy. BioMed Research International, 2015, 561819. https://doi.org/10.1155/2015/561819

Zheng, H., Huang, H., Ji, Z., Yang, Q., Yu, Q., Shen, F., … Xiong, F. (2016). A double heterozygous mutation of TNNI3 causes hypertrophic cardiomyopathy in a han chinese family. Cardiology, 133, 91–96. https://doi.org/10.1159/000440877

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