Next-generation sequencing (NGS) as a molecular diagnostic tool for hypertrophic cardiomyopathy in a Chinese boy due to novel compound heterozygous mutations in the MYBPC3 gene

A case report

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Abstract

Rationale: Hypertrophic cardiomyopathy (HCM) is mainly caused by mutations in genes encoding sarcomeric proteins. One of the most commonly mutated HCM genes is the MYBPC3 gene. Mutations in this gene lead mainly to truncation of the protein, which gives rise to a relatively severe phenotype. Analyses of gene mutations associated with HCM are valuable for molecular diagnosis, genetic counseling, and management of familial HCM.

Patient concerns: A 12-year-old boy presented with palpitations and dyspnea after exercise for 1 year. Echocardiography showed myocardial asymmetric hypertrophy of the ventricular septum, the anterior wall, and the lateral wall of the left ventricle. The thickness of the interventricular septum was estimated to be 33 mm. ECG showed left ventricular high voltage and ST-T changes. He had been diagnosed with HCM 3 months previously.

Diagnoses: Due to his clinical presentation, he was determined to have HCM via a molecular analysis, revealing compound heterozygotes (p.R597W and p.Q1012Sfs*8) in the MYBPC3 gene.

Interventions: The patient was prescribed metoprolol to slow the heart rate and increase diastolic filling time.

Outcomes: The boy was treated with metoprolol 6.75 mg b.i.d. Approximately 3 months later, review of the echocardiography showed that the peak velocity across the LVOT dropped to 2.3 m/seconds and that the pressure gradient dropped to 21 mm Hg.

Lessons: A custom next-generation sequencing (NGS) technology for the HCM panel allowed us to identify compound heterozygous mutations in the MYBPC3 gene, confirming NGS as a molecular diagnostic tool.

Abbreviations: cMyBP-C = cardiac myosin-binding protein C, HCM = hypertrophic cardiomyopathy, HGVS = Human Genome Variation Society, LVOT = left ventricular outflow tract, MYBPC3 = cardiac myosin-binding protein C, MYH7 = cardiac myosin heavy chain, NGS = next-generation sequencing, SAM = systolic anterior motion.

Keywords: genetics, hypertrophic cardiomyopathy, MYBPC3 gene

1. Introduction

Hypertrophic cardiomyopathy (HCM) is a familial, genetically determined, primary cardiomyopathy (HCM1 to HCM26; www.ncbi.nlm.nih.gov/omim) caused by mutations in genes encoding sarcomere or sarcomere-associated cardiac muscle proteins, which typically lead to myofibrillar disorganization, myocyte hypertrophy, and interstitial fibrosis. Classic (sarcomic) HCM is predominantly inherited in an autosomal-dominant manner, and few HCM patients present with Mendelian autosomal recessive disease.[1] To date, more than 1000 different pathogenic mutations have been found in more than 25 genes,[2,3] such as the cardiac myosin heavy chain (MYH7) and cardiac myosin-binding protein C (MYBPC3) genes, which are responsible for approximately 80% of cases.[4]

Presently described is a case of a 12-year-old boy from a Chinese family with HCM in whom a mutation in the MYBPC3 gene was identified for the first time in our hospital as a cause of this disease. Molecular analysis was performed using a next-generation sequencing (NGS) strategy that enabled the identification of compound heterozygous pathogenic mutations in the MYBPC3 gene: c.1789C>T (p.R597W) and c.3033delG (p.Q1012Sfs*8).

2. Case report

The subject was a 12-year-old boy who presented to our hospital with syncope after exercise and palpitations for 1 year.

Cardiac
auscultation revealed a systolic ejection murmur at the apex, and no other positive signs were found. No other family members were diagnosed with heart disease. Echocardiography showed myocardial asymmetric hypertrophy of the ventricular septum, the anterior wall and the lateral wall of the left ventricle. The interventricular septum was spindle shaped, and the greatest thickness was 33 mm (Fig. 1A–D). The interventricular septum-to-posterior wall thickness ratio was 5:1, which was much greater than the normal value of 1.3:1. The echocardiogram of the myocardial hypertrophy region was irregularly intensified on the 2D image. The systolic anterior motion (SAM) of the anterior mitral leaflet was observed. The diameter of the left ventricular outflow tract was narrow, and the systolic blood flow velocity and pressure difference were significantly increased. The peak velocity across the left ventricular outflow tract (LVOT) was 3.2 m/s, and the pressure gradient was 41 mm Hg. There were no problems with the heart valves. The ascending aorta, aortic arch, and descending aorta were normally developed. The thickened ventricular wall motion was within normal range. The left ventricular systolic function was normal (71%). The left ventricular diastolic dysfunction was assessed by E/e’ and E/A. The E/A was 1.2, and the E/e’ was 5.8. The left ventricular diastolic function was normal. Myocardial longitudinal strain and deformation parameters were obviously reduced on the base.

Figure 1. Electrocardiogram and echocardiogram of the patient. (A) The interventricular septum was spindle shaped and thickened to 33 mm. (B–D) Different left ventricular short-axis views showing asymmetry of ventricular hypertrophy. (E) The electrocardiograph showed sinus arrhythmia, left ventricular high voltage and ST-T changes.
and middle segment of the left ventricle. The electrocardiogram showed sinus arrhythmia, high left ventricular voltage, and ST-T changes (Fig. 1E).

Combined with patient history and examinations, we could easily verify that the hypertrophy of the myocardium was not caused by aortic valve disease, that is, subvalvular or supravalvular aortic stenosis or hypertension. Additionally, the patient had no skeletal muscle disease or mental retardation. The diagnosis of glycogen storage disease was also excluded. Thus, we diagnosed the patient with classic hypertrophic cardiomyopathy. He was treated with metoprolol 6.75 mg b.i.d. Approximately 3 months later, review of the echocardiography showed that the peak velocity across the LVOT dropped to 2.3 m/seconds and that the pressure gradient dropped to 21 mm Hg.

Informed written consent was obtained from the patient for publication of this case report and accompanying images. Genomic DNA for the patient was tested by NGS using a custom-designed hypertrophic cardiomyopathy panel based on the Roche Nimblegen SeqCap EZ Choice XL Library (Roche; http://sequencing.roche.com/en/products-solutions/by-category/target-enrichment/hybridization.html). A custom Perl script was used to produce the reads and coverage statistics for 26 genes (MYH7, MYLK2, CAV3, TNNT2, TPM1, MYBPC3, PRKAG2, TNNI3, MYL3, TTN, MYL2, ACTC1, CSRP3, TNNT1, MYH6, VCL, MYOZ2, JPH2, PLN, CALR, NEXN, MYBN, ACTN2, LDB3, TCAP, and FLNC) in the hypertrophic cardiomyopathy panel. All identified variants were annotated according to the guidelines published by the Human Genome Variation Society (HGVS). Two mutations (p.R597W and p.Q1012Sfs*8) were tested as validation controls to verify the reliability of our custom NGS strategy (Table 1). Sanger sequencing was performed to confirm all the deleterious mutations and potentially pathogenic variants and to segregate them in the families (Fig. 2). Primer sequences and annealing temperatures are available from the authors upon request. The PCR products were resolved on an automated sequencer (ABI 3130xl Genetic Analyzer, Applied Biosystems). The results were analyzed by SeqMan software by assembling and visualizing the aligned sequences compared with the reference sequence (UCSC Genome Browser).

3. Discussion

Classic HCM is generally diagnosed during adulthood, and therefore, presentation during infancy or childhood is very rare (annual incidence of approximately 0.47 per 100,000 children). More often, childhood HCM is seen in association with other underlying conditions, such as Noonan’s syndrome, metabolic disorders (Pompe’s disease, fatty acid oxidation defects, mucopolysaccharidosis (Hurler’s syndrome)), or endocrinological disorders (maternal diabetes and hyperthyroidism).[7,8]

This study provides clinical features and mutational analysis of a patient with severe HCM and his family. The boy carried compound heterozygous mutations in the MYBPC3 gene: 1 allele mutation (p.R597W) in exon 16, inherited from his father, and an allele deletion (p.Q1012Sfs*8) in exon 27, inherited from his mother. Neither of these mutations had been observed in the 1000 Genomes database, indicating that these variants are very rare. The mutations identified in highly conserved amino acids among many species may have influenced the structure and function of the proteins (Fig. 3). To further assess whether these 2 mutations are indeed potential pathogenic factors, 4 bioinformatic analyses were performed, using MutationTaster, PolyPhen-2, PROVEAN, and SIFT software, and indicated that they were disease-causing or damaging mutations (Table 1). Four other family members (I.2, I.4, II.1, and II.2) containing a heterozygous MYBPC3 pathogenic mutation associated with HCM carriers were asymptomatic, indicating that a single HCM-heterozygous mutation is insufficient to affect myocardial function and lead to hypertrophy in our autosomal recessive pedigree (Fig. 2).

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Table 1

| Mutation | PolyPhen2 | MutationTaster | SIFT | PROVEAN |
|----------|-----------|----------------|------|---------|
| MYBPC3   |           |                |      |         |
| NM_000256 | –         | –              | disease causing | 1 |
| Exon27   | c.303delG |                | –    | –       |
| p.Q1012Sfs*8 |   |                | –    | –       |
| MYBPC3   |           |                |      |         |
| NM_000256 |           | 1.000          | disease causing | 0.999 |
| Exon16   | c.1789C>T |                |      |         |
| p.R597W  |           | 0.001          | damaging | 0.001 |
|          |           |                |       |         |

PolyPhen Prediction Score: benign (cutoff <0.5), probably damaging (0.5-0.6), disease causing (0.6-1.0). MutationTaster: polymorphism or disease causing, SIFT PREDICTION (cutoff = 0.05) - tolerated or damaging. PROVEAN Prediction: (cutoff = –2.5) - deleterious or neutral.
In contrast to truncating mutations, missense mutations lead to stable mutant cMyBP-C that exert a more potent effect in disrupting sarcomere function. The MYBPC3 variant described here from the paternal mutation leads to the substitution of a positively charged residue (R) with a nonpolar residue (W). It occurs in the Immunoglobulin I-set domain of the protein and could interfere with the protein incorporation in the A-band of the sarcomere. The "poison peptide" hypothesis proposes that mutant sarcomeric proteins incorporate into myofibrils and act as dominant-negative proteins. The c.3033delG (p. Q1012Sfs*8) deletion was found in our patient and is located in exon 27, leading to a premature stop codon and truncated protein beyond the C-terminal peptide of 372 amino acids (consisting of motifs VII to X) of MYBPC3. This deletion is thought to lead to reduced expression of MYBPC3, due to protein instability and/or loss of the C-terminal of MYBPC3 that binds myosin thick filaments and titin, which specify correct incorporation of cMyBP-C into the A-band of the sarcomere.

Molecular diagnosis using an NGS strategy represents a significant medical challenge for these cases. In fact, the identification of 2 mutations in our patient permits us to propose a screening test and an adequate cardiology follow-up not only for the parents and their children but also for the other members of the family. Furthermore, as different modes of inheritance are described in HCM, identification of mutations, particularly in sporadic cases with no familial history, gives parents the opportunity to obtain appropriate genetic counseling for future reproduction. Preimplantation or prenatal genetic screening should be adopted, as this type of genotype leads to potentially lethal developmental malformations. This new approach using an NGS strategy allows a rapid molecular diagnosis for families presenting with cardiomyopathies with a broad coverage of the known disease-causing genes at a reasonable cost. Gene panels that include a large number of genes could identify gene variants that could explain more HCM cases than those currently explained.
Author contributions

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Investigation: Xu Chen.
Manuscript drafting: Xu Chen, Weiliang Zhu, and Jun Jiang.
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