Clinical Case Report

Medicine®

OPEN

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

Xu Chen, MD[a], Jun Jiang, MD[b], Weiliang Zhu, MD[a], Yuan Wu, MD[a], Maolong Su, MD[a,*]

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 (sarcomeric) 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

Editor: N/A.

This work was supported by grants from The Medical Innovation Project of Fujian Province Fund (grant nos. 2016-CXB-13) and the Science and Technology Planning Project of Xiamen (grant nos. 3502Z20164052).

The authors have no conflicts of interest to disclose.

[a] Department of Echocardiography, Xiamen Cardiovascular Hospital, Xiamen University, Xiamen, Fujian Province., [b] Macro & Micro-Test Bio-Tech Co., Ltd., No. 28 Yuhua Road, Airport High-Tech Park, Shunyi District, Beijing, China.

[*] Correspondence: Maolong Su, Department of Echocardiography, Xiamen Cardiovascular Hospital, Xiamen University, Hubinnan Road 205, Xiamen City, Fujian Province, China (e-mail: sumaolong@xmu.edu.cn).

Medicine (2019) 98:12(e14676)

Received: 10 October 2018 / Received in final form: 17 January 2019 / Accepted: 30 January 2019

http://dx.doi.org/10.1097/MD.0000000000014676

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

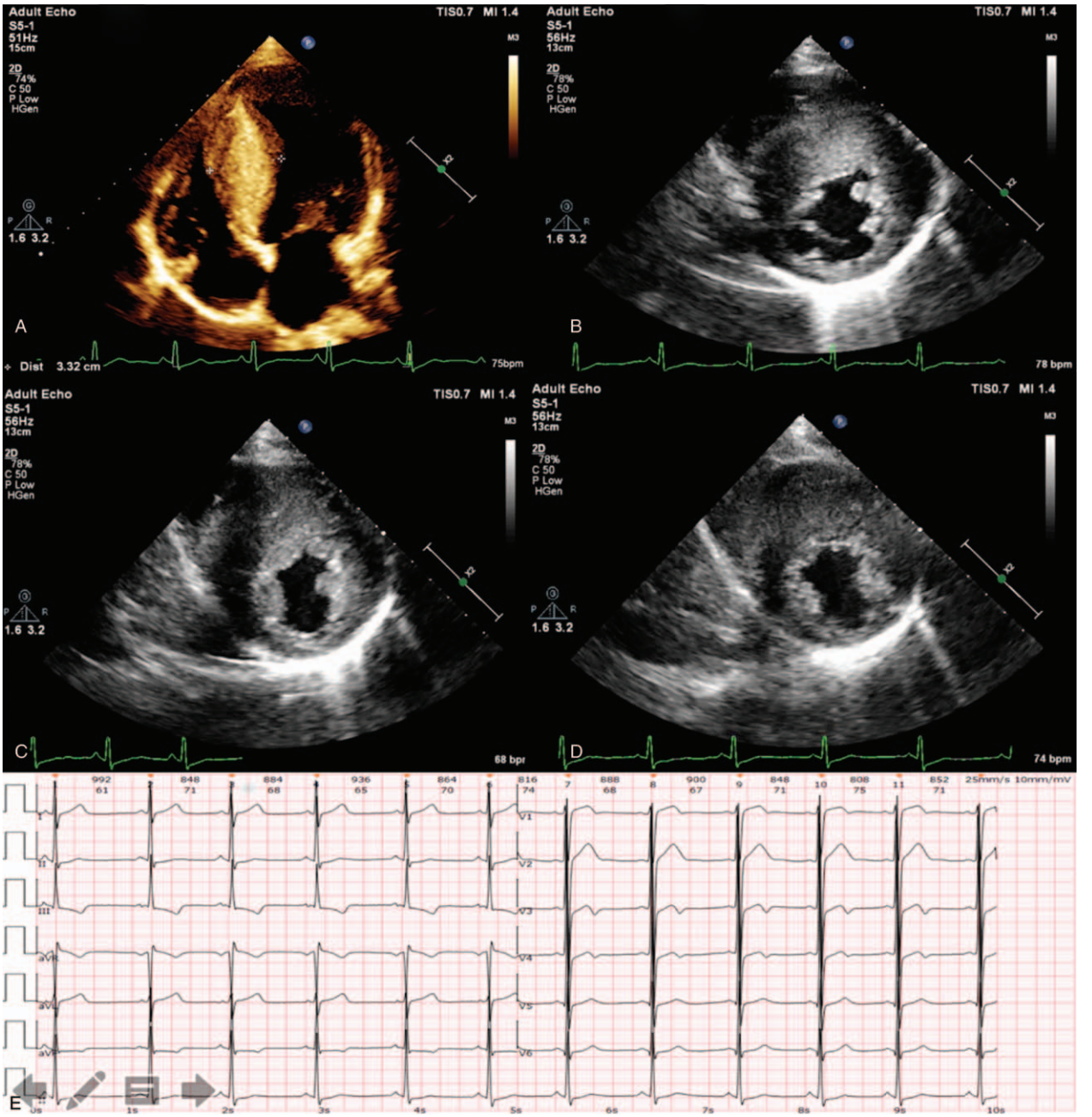

Figure 1. Electrocardiogram and echocardiogram of the patient. (A) The interventricular septum was spindle shaped and thickened to 33 mm. (B–D) Different left ventricular short-axis views showing asymmetry of ventricular hypertrophy. (E) The electrocardiograph showed sinus arrhythmia, left ventricular 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*, *TNNC1*, *MYH6*, *VCL*, *MYOZ2*, *JPH2*, *PLN*, *CALR3*, *NEXN*, *MYPN*, *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).[5,6] 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, mucopolysaccharidoses (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).

MYBPC3 encodes a thick filament-associated cardiac myosin-binding protein C (cMyBP-C), a signaling node in cardiac myocytes that contributes to the maintenance of sarcomeric structure and regulation of contraction and relaxation. MYBPC3 mutations represent the most prevalent cause of inherited HCM (40%). Studies have reported that 70% of the mutations in MYBPC3 are truncating variants, which cause a more severe HCM phenotype than those associated with missense mutations.[9] In contrast to heterozygous pathogenic mutations, homozygous or compound heterozygous truncating pathogenic MYBPC3 variants cause severe cardiomyopathy, leading to heart failure and death within the first year of life.[10] Wijnker et al[11] compared the pathomechanisms of a truncating mutation and a missense mutation in MYBPC3 in engineered heart tissues. The widely accepted hypothesis is that truncating MYBPC3 mutations cause haploinsufficiency, in contrast to missense mutations, which incorporate into the sarcomere and act in a dominant-negative manner. Some studies have provided evidence that truncated cMyBP-C are not detectable in human patient samples, as they seem to be susceptible to degradation by nonsense-mediated RNA

Table 1

Bioinformatic analysis of the mutations.

Mutation	Bioinformatic analysis							
	PolyPhen2		MutationTaster		SIFT		PROVEAN	
	Prediction	Score	Prediction	Score	Prediction	Score	Prediction	Score
MYBPC3 (NM_000256) Exon27 c.3033delG p.Q1012Sfs*8	–	–	disease causing	1	–	–	–	–
MYBPC3 (NM_000256) Exon16 c.1789C>T p.R597W	probably damaging	1.000	disease causing	0.999	damaging	0.001	deleterious	−6.80

PolyPhen Prediction Score: benign ≤0.5; probably damaging (>0.5), MutationTaster Prediction: polymorphism or disease causing, SIFT PREDICTION (cutoff = 0.05) - tolerated or damaging, PROVEAN Prediction: (cutoff = −2.5) - deleterious or neutral.

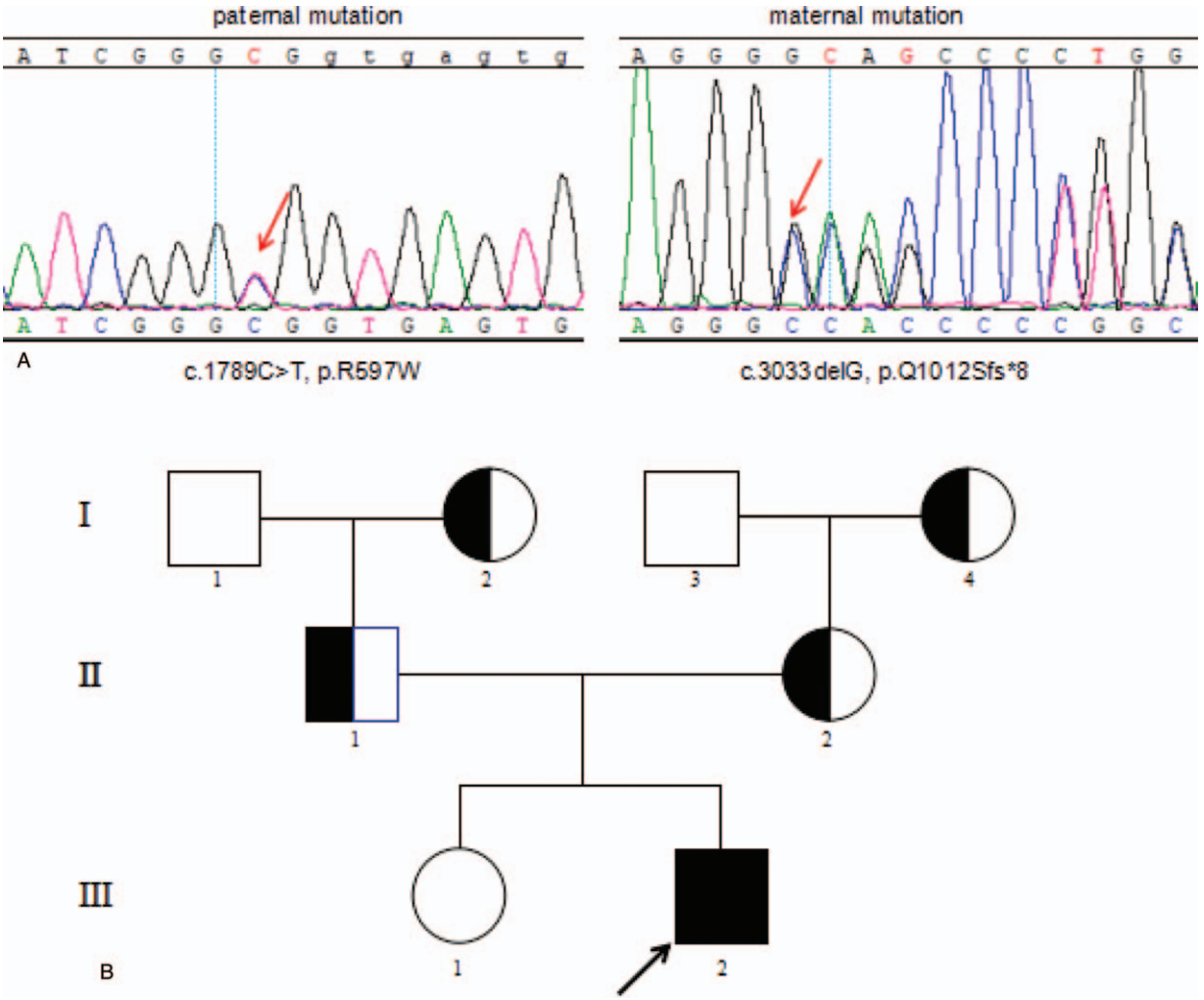

Figure 2. Family pedigree and sequence electropherograms showing the *MYBPC3* gene mutation in a 12-year-old boy and his family members. (A) Sequence analysis of the *MYBPC3* gene revealed compound heterozygous mutations for c.1789C>T(p.R597W) and c.3033delG(p.Q1012Sfs*8). The red arrows indicate the heterozygotes from the patient's family members. B, Pedigree illustrating the segregation of the mutant alleles to the index patient (III.2). The father (II.1) and the patient (III.2) are heterozygous for the c.1789C>T(p.R597W) mutation inherited from the paternal grandmother, whereas the mother (II.2) and his sister (III.1) show the wild-type sequence at this position. The indel mutation c.3033delG(p.Q1012Sfs*8), leading to the loss of the original stop codon, results in a truncated protein. The mutation, which was inherited from the maternal grandmother, was found in the heterozygous state in the patient (III.2) and the mother (II.2). The father and the sister carry the homozygous wild-type allele at this position.

decay.[12,13] In contrast to truncating mutations, missense mutations lead to stable mutant cMyBP-C that exert a more potent effect in disrupting sarcomere function.[9] 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.[14] 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.[15]

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 cardiologic 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.

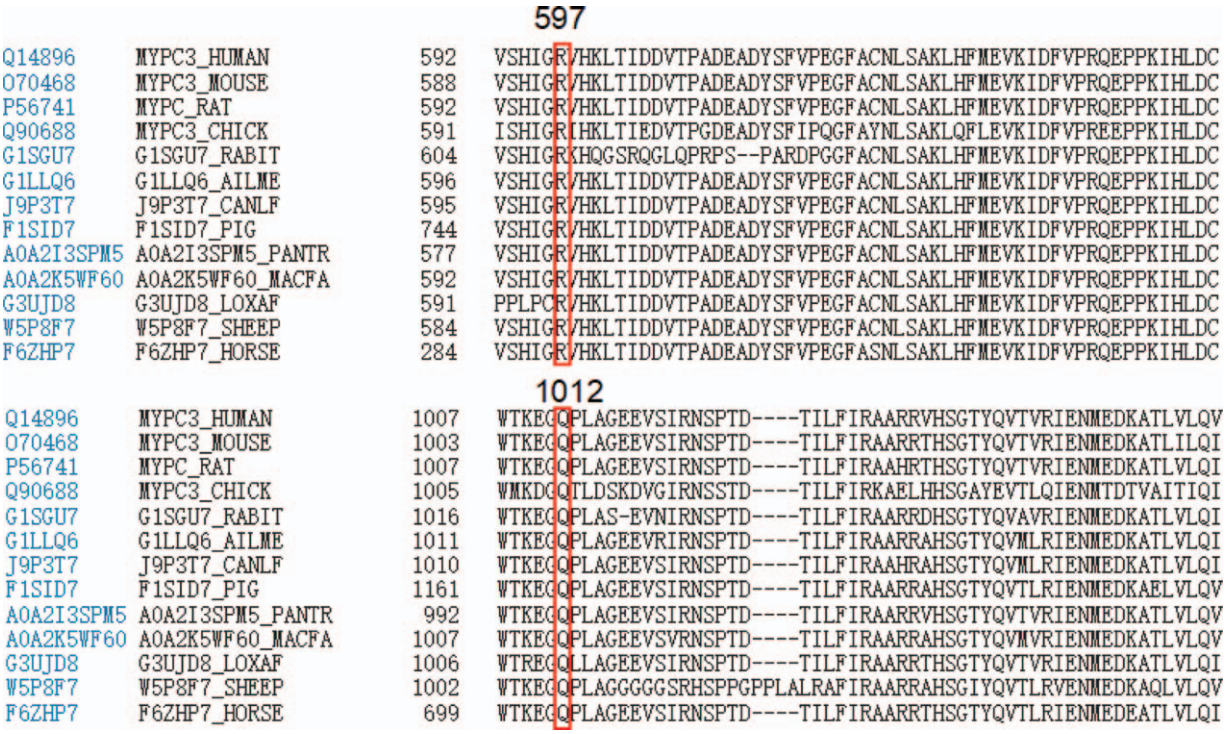

Figure 3. As determined using Clustal W, the mutations p.R597W and p.Q1012Sfs*8 involved amino acids in the *MYBPC3* gene that were highly conserved across many species.

Author contributions

Conceptualization: Xu Chen and Jun Jiang.
Data curation: Jun Jiang.
Formal analysis: Xu Chen, Jun Jiang.
Investigation: Xu Chen.
Manuscript drafting: Xu Chen, Weiliang Zhu, and Jun Jiang.
Manuscript review & editing: Maolong Su and Jun Jiang.
Resources: Yuan Wu.
Writing – original draft: Xu Chen, Jun Jiang, Weiliang Zhu.
Writing – review & editing: Jun Jiang, Maolong Su.

References

[1] Zahka K, Kalidas K, Simpson MA, et al. Homozygous mutation of MYBPC3 associated with severe infantile hypertrophic cardiomyopathy at high frequency among the Amish. Heart 2008;94:1326–30.
[2] Pinto YM, Wilde AA, van Rijsingen IA, et al. Clinical utility gene card for: hypertrophic cardiomyopathy (type 1-14). Eur J Hum Genet 2011;19:19.
[3] Seidman CE, Seidman JG. Identifying sarcomere gene mutations in hypertrophic cardiomyopathy: a personal history. Circ Res 2011; 108:743–50.
[4] Semsarian C. Guidelines for the diagnosis and management of hypertrophic cardiomyopathy. Heart Lung Circ 2011;20:688–90.
[5] Lipschultz SE, Sleeper LA, Towbin JA, et al. The incidence of pediatric cardiomyopathy in two regions of the United States. New Engl J Med 2003;348:1647–55.
[6] Nugent AW, Daubeney PEF, Chondros P, et al. The epidemiology of childhood cardiomyopathy in Australia. N Engl J Med 2003; 348:1639–46.
[7] Maron BJ, Tajik AJ, Ruttenberg HD, et al. Hypertrophic cardiomyopathy in infants: clinical features and natural history. Circulation 1982;65:7–17.
[8] Towbin JA, Lipschultz SE. Genetics of neonatal cardiomyopathy. Curr Opin Cardiol 1999;14:250–62.
[9] Marian AJ, Braunwald E. Hypertrophic cardiomyopathy: genetics, pathogenesis, clinical manifestations, diagnosis, and therapy. Circ Res 2017;121:749–70.
[10] Wessels MW, Herkert JC, Frohn-Mulder IM, et al. Compound heterozygous or homozygous truncating MYBPC3 mutations cause lethal cardiomyopathy with features of noncompaction and septal defects. EJHG 2015;23:922–8.
[11] Wijnker PJ, Friedrich FW, Dutsch A, et al. Comparison of the effects of a truncating and a missense MYBPC3 mutation on contractile parameters of engineered heart tissue. J Mol Cell Cardiol 2016;97:82–92.
[12] Helms AS, Davis FM, Coleman D, et al. Sarcomere mutationspecific expression patterns in human hypertrophic cardiomyopathy. Circ Cardiovasc Genet 2014;7:434–43.
[13] Mohamed IA, Krishnamoorthy NT, Nasrallah GK, et al. The role of cardiac myosin binding protein C3 in hypertrophic cardiomyopathy-progress and novel therapeutic opportunities. J Cell Physiol 2017; 232:1650–9.
[14] Geisterfer-Lowrance AA, Kass S, Tanigawa G, et al. A molecular basis for familial hypertrophic cardiomyopathy: a beta cardiac myosin heavy chain gene missense mutation. Cell 1990;62:999–1006.
[15] Gilbert R, Kelly MG, Mikawa T, et al. The carboxyl terminus of myosin binding protein C (MyBP-C, C-protein) specifies incorporation into the A-band of striated muscle. J Cell Sci 1996;109(Pt 1):101–11.