



A Novel Truncating Variant in MYBPC3 Causes Hypertrophic Cardiomyopathy

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Abstract

Background: Familial Hypertrophic Cardiomyopathy (HCM) is the most common genetic cardiovascular disease. Related mutations contributing to hypercontractility and poor relaxation in HCM have been incompletely understood. The purpose of this study was to explore and verify a novel variant in cardiac Myosin-Binding Protein C3 (MYBPC3) in a HCM family.

Methods: Clinical information was collected and cardiac evaluation was performed in the pedigree. Second-generation sequencing technology was used to investigate the proband and his family. Computational prediction of mutation effects at genomic level and 3D visualization of the mutated protein were achieved by in silico analysis.

Results: Typical interventricular septal thickening was detected in all the four HCM patients. A c.1042_1043insCGGCA mutation of MYBPC3 was verified in the proband and family members. Mild phenotype associated with delayed onset and relative favorable prognosis were observed in the pedigree. In silico analysis of the mutation revealed that c.1042_1043insCGGCA led to an early termination of MYBPC protein synthesis at C2 domain, losing the domains that are essential for myosin- and titin- binding.

Conclusion: The novel c.1042_1043insCGGCA mutation of MYBPC3 was a genetic basis for HCM. Our gene sequence based computational analysis predicted the pathogenicity of the mutation by correlating MYBPC3 genotypes with clinical phenotypes.

Introduction

Hypertrophic cardiomyopathy is an autosomal genetic disease occurred in absence of common triggers such as hypertension and aortic stenosis [1]. The prevalence of HCM is 1:500 in the general population [2]. Striking cardiomegaly and stunning asymmetry with disproportionate involvement of interventricular septum was observed in many HCM cases [3]. Postmortem examination in hearts of HCM victims show myocyte disarray accompanied with significantly increased myocardial fibrosis [4].

Many patients with HCM experience adverse clinical outcomes including Heart Failure (HF), arrhythmias, and sudden cardiac death. The mortality of patients with HCM was shown to be about 3-fold higher than that of the general population at similar ages [5]. It is the most common cause of sudden cardiac death among young athletes without precedent symptoms.

Many efforts have been made to gain an accurate and comprehensive understanding of the molecular basis and clinical course of the disease. Pathogenic variants in genes encoding sarcomere proteins are involved in hypertrophic cardiomyopathy [6]. More than 64 genes encoding thick and thin filaments have been claimed to be causative in HCM with varying levels of supportive evidence [7].

Here we report a novel mutation in the myosin binding protein from a sporadic pedigree. We use DNA sequencing technology and bioinformatic tools to illustrate the genetic background and possible influence of the mutated protein. We aim to gain evidence upon the relationship between genotype and clinical manifestation, underscoring the value of genomics in elucidating the

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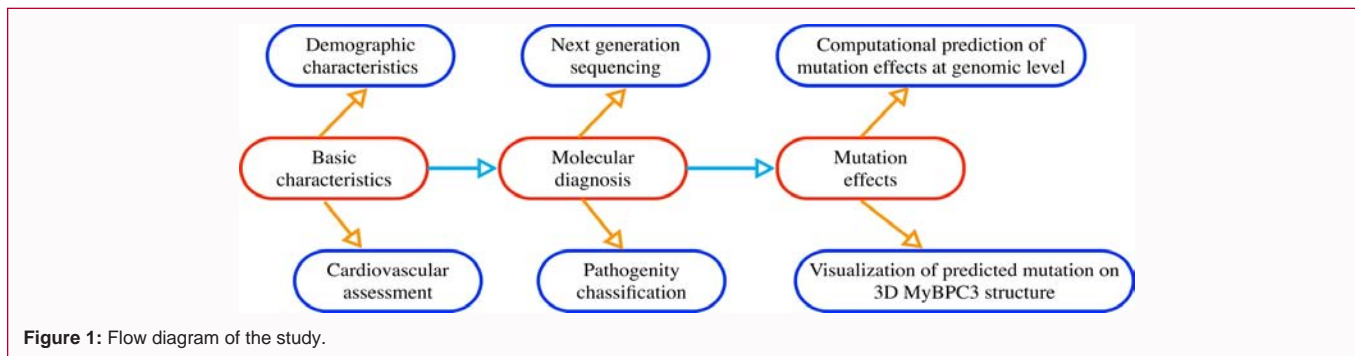


Figure 1: Flow diagram of the study.

underlying mechanism of HCM.

Materials and Methods

Ethical approval

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the First Affiliated Hospital of Zhejiang University. Informed consent was obtained from all individual participants in the study. A flowchart of the study was shown in Figure 1.

Index family

Demographic data was collected for every patient at the day of admission. All patients underwent a complete cardiovascular evaluation, including cardiac physical examination, blood test of brain natriuretic peptide, 12-lead Electrocardiogram (ECG) and echocardiography. The diagnostic criteria for hypertrophic cardiomyopathy were according to the recommendations by the American College of Cardiology/American Heart Association Joint Committee [8].

Sample collection and DNA isolation

Genomic DNA from blood samples collected in Ethylenediaminetetraacetic Acid (EDTA) tubes was extracted using the DNeasy Blood & Tissue Kit (Cat No. 69504, Qiagen, Germany) according to the manufacturer's protocol. The concentration of genomic DNA was determined with an UV-Vis/florescence spectrophotometer (ES-2, MALCOM, Japan).

Genome sequencing

Genomic DNA extracted from peripheral blood was fragmented to an average size of ~350 bp and subjected to DNA library creation using established Illumina paired-end protocols. The Illumina Novaseq 6000 platform (Illumina Inc., San Diego, CA, USA) was utilized for genomic DNA sequencing in Novogene Bioinformatics Technology Co., Ltd (Beijing, China) to generate 150-bp paired-end reads with a minimum coverage of 10x for ~99% of the genome (mean coverage of 30x). After sequencing, basecall files conversion and demultiplexing were performed with bcl2fastq software (Illumina). The resulting FASTQ data were submitted to in-house quality control software for removing low quality reads, and then were aligned to the reference human genome (hs37d5) using the Burrows-Wheeler Aligner (BWA) [9], and duplicate reads were marked using Sambamba tools [10]. Genomic variations were annotated using ANNOVAR software [11].

Computational prediction of mutation effects at genomic and protein level

To study the mutation effects on gene regulation at genomic level,

DNAMAN V6.0 software was used to carry out multiple sequence alignments. To illustrate the stability and conformational effects on protein level, 3D model was created using online automated protein structure server SWISS-MODEL (<https://swissmodel.expasy.org>).

Results

Basic characteristics of the HCM family

Pedigree of the family was shown in Figure 2 and main clinical features was shown in Table 1. Briefly, the proband was a 93-year-old man who was diagnosed with HCM at the age of 52-year-old. He suffered from repeated episodes of heart failure and was given diuretic drugs. When hearing the death of her spouse, individual II-1 ran down from the 5th floor, causing a sudden death. Individual II-2 was diagnosed with HCM at the age of 51 due to chest discomfort and palpitation. He was given beta-blocker to relieve symptoms. Of note, individual II-3 and II-4 remained asymptomatic thus far, without any episodes of palpitations, arrhythmia, or syncope.

Cardiovascular evaluation of the family

Typical echocardiographic images, along with ECG results were shown in Figure 3 and Figure S1, respectively. Notably, septal thickness increased significantly in all the four patients with Inter-Ventricular Septum dimension (IVSd)/Left Ventricular Posterior Wall dimension (LVPWd) ratio >1.3, indicating the diagnosis of HCM [8,12]. No left ventricular out flow obstruction was detected in the affected members. For echocardiographic details see Table 2. ECG shows significant alterations of the hypertrophic heart. The index case shows increased QRS voltages as SV1 + RV5 and RV6 both are larger than 35 mm. Meanwhile, arrhythmia including atrial premature beat and intraventricular block occurred in the proband. T-wave inversion

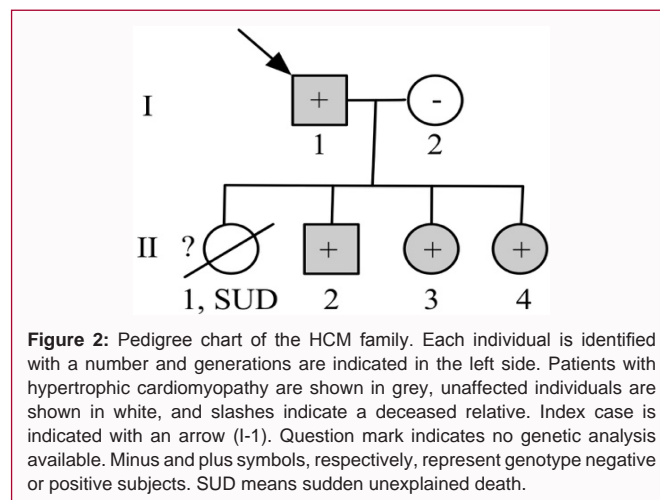


Figure 2: Pedigree chart of the HCM family. Each individual is identified with a number and generations are indicated in the left side. Patients with hypertrophic cardiomyopathy are shown in grey, unaffected individuals are shown in white, and slashes indicate a deceased relative. Index case is indicated with an arrow (I-1). Question mark indicates no genetic analysis available. Minus and plus symbols, respectively, represent genotype negative or positive subjects. SUD means sudden unexplained death.

Table 1: Baseline characteristics of the HCM patients.

Demographic characteristics	I-1	II-2	II-3	II-4
Age, y	93	55	52	48
Sex	Male	Male	Female	Female
Age at diagnosis, y	52	51	52	48
Medical history				
Current smoker	No	No	No	No
Diabetes	No	No	No	No
Hypertension	Yes	Yes	No	No
Dyspnea or shortness of breath	Yes	No	No	No
Angina pectoris (chest pain)	No	No	No	No
Syncope	No	No	No	No
Cardiac arrest	No	No	No	No
Surgical myectomy	No	No	No	No
Heart failure episodes	Yes	No	No	No
NYHA class	II	I	I	I
Medications				
Beta blockers	Yes	Yes	Yes	No
Calcium antagonists	No	No	No	No
Diuretics	Yes	No	No	No
Warfarin	No	No	No	No
Biomarkers				
BNP (pg/ml)	434.97	<35	<35	<35

Abbreviation: BNP: Brain Natriuretic Peptide; HCM: Hypertrophic Cardiomyopathy; NYHA: New York Heart Association

Table 2: Variable of transthoracic echocardiography of the pedigree.

Echocardiographic analysis	I-1	II-2	II-3	II-4
IVSd (mm)	39	26	26	14
IVSs (mm)	41	30	29	18
LVPWd (mm)	10	10	8	9
LVPWs (mm)	14	14	12	13
IVSd/LVPWd ratio	3.9	2.6	3.3	1.6
EF (%)	60	70	72	65
FS (%)	30	40	41	35

Abbreviations: EF: Ejection Fraction; FS: Fractional Shortening; IVSd: Interventricular Septal end-diastolic thickness; IVSs: Interventricular Septal end-systolic thickness; LVPWd: Left Ventricular Posterior Wall end-diastolic thickness; LVPWs: Left Ventricular Posterior Wall end-systolic thickness

in at least two adjacent precordial leads was detected in individual II-2, II-3 and II-4.

Molecular diagnosis and pathogenicity classification

Mutation screening revealed a candidate mutation in MYBPC3 from all the four patients. A heterozygous mutation c.1042_1043insCGGCA located in exon 12 occurred in the proband and his offspring. Base sequence of exon 12 before and after mutation was shown in the supplementary material. The c.1042_1043insCGGCA mutation causes a shift in the reading frame beginning with Proline, changing it to an Arginine, and creates a premature stop codon at the new reading frame. The amino acid sequence prior and post mutation was shown in the supplementary material. This mutation is expected to result in an abnormal, truncated protein or loss of protein from this allele due to nonsense-mediated mRNA decay.

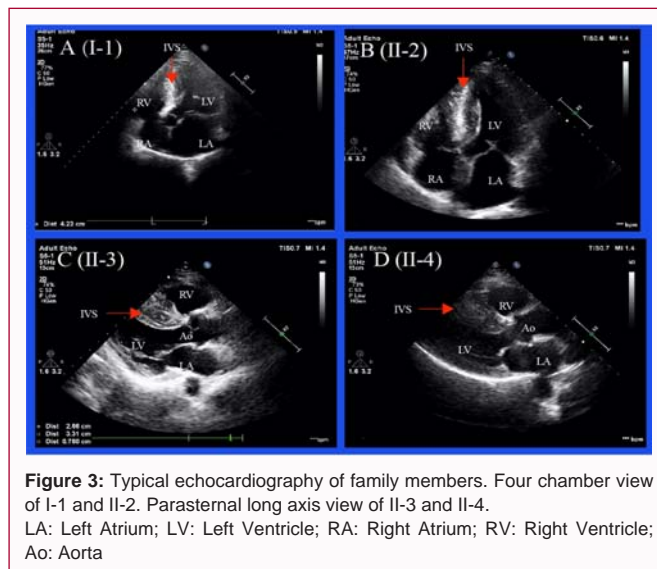


Figure 3: Typical echocardiography of family members. Four chamber view of I-1 and II-2. Parasternal long axis view of II-3 and II-4. LA: Left Atrium; LV: Left Ventricle; RA: Right Atrium; RV: Right Ventricle; Ao: Aorta

According to the American College of Medical Genetics (ACMG) framework, the c.1042_1043insCGGCA in the MYBPC3 gene is interpreted as a pathogenic mutation based on the following criteria: PS4: The prevalence of the variant in affected individuals is significantly increased compared to the prevalence in population controls; PM1: The variant located in a mutational hot spot without benign variation; PM2: The variant is at extremely low frequency in the wider population; PM4: Protein length changes due to insertions in a non-repeat region [13] (Figure 4).

Prediction of mutant effects on gene regulation and visualization of mutated MYBPC3 protein

In silico analysis of the mutation revealed that c.1042_1043insCGGCA led to an early termination of MYBPC3 protein synthesis at C2 domain in homo sapiens. The truncated protein ended with aa.350, losing the domains that are essential for myosin- and titin- binding (Figure 5).

Discussion

Main findings

In this study, we described a novel mutation in the encoding region of MYBPC3 from a HCM family. All the four patients turned out to be compound heterozygote carriers of MYBPC3 gene. The mutation led to truncated MYBPC3 protein that lack the C-terminus myosin and titin binding domain. Although prior studies have reported HCM cases with MYBPC3 mutation [14], to the best of our knowledge, our study is the first to report the c.1042_1043insCGGCA mutation in exon 12.

Role of mutated MYBPC3 in hypertrophic cardiomyopathy

Variants in sarcomere and sarcomere-associated protein genes including MYBPC3, Myosin Heavy chain [MYH7], cardiac troponin T [TNNT2], cardiac troponin I [TNNI3], α -Tropomyosin [TPM1], myosin essential and regulatory light chains [MLY2, MYL3], and Actin [ACTC] are identified as the most common cause of HCM [15]. Mutations in MYBPC3 often lead to reduced expression of full-length cardiac Myosin Binding Protein-C (cMYBP-C), which is composed of a chain of eleven globular immunoglobulin and fibronectin domains (C0-C10) and an extensible M-domain between C1 and C2 [16]. As in the present study, the frame shift mutation led to an early

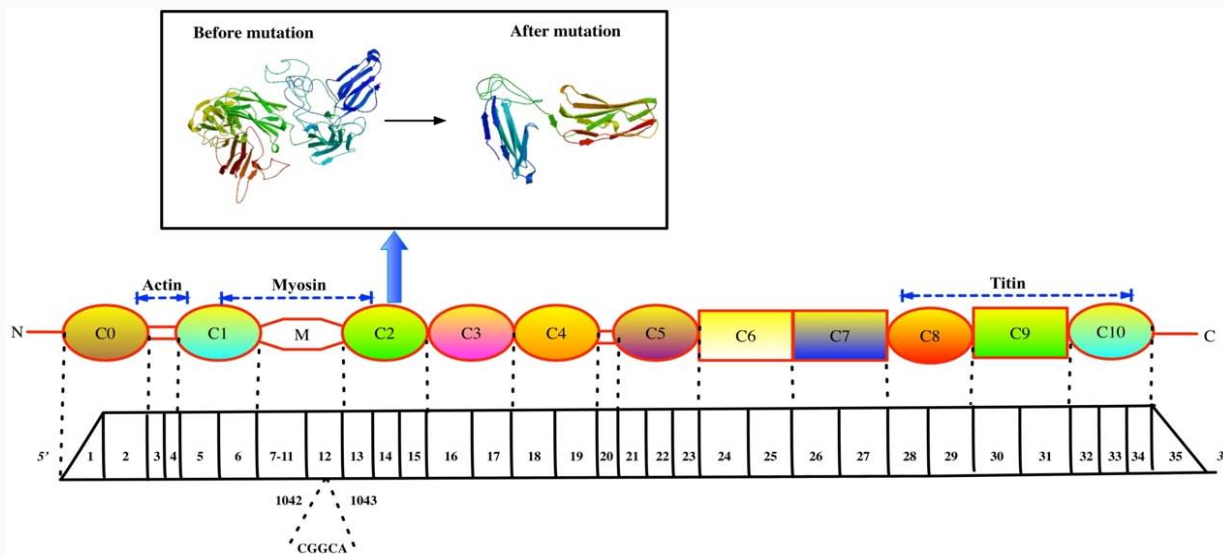


Figure 4: Structural representation of full-length cMyBP-C protein and MYBPC3 exons. Cartoon 3D model of cMyBP-C before and after mutation is shown in the rectangle. cMyBP-C is composed of 11 domains, being numbered C0 to C10 from the N-terminus to C-terminus. It includes 8 Immunoglobulin (Ig) domains (circles) and 3 Fibronectin type III (FNIII) domains (rectangles). Four specific phosphorylation sites located between C1 and C2, known as MyBP-C motif. The regions of interaction with other sarcomeric proteins (actin, myosin, and titin) is shown with dotted arrows. Relationship between the 35 exons of MYBPC3 cDNA and cMyBP-C domains is indicated by dashed lines.



Figure S1: Typical ECG results show significant alterations of the hypertrophic heart.

termination of cMyBP-C protein synthesis at C2 domain, disturbing its normal structural and regulatory functions.

Relationship between genotype and phenotype

Mutations in genes encoding contractile proteins result in contractile dysfunction of the cardiac fibers, leading to compensatory hypertrophy and decreased diastolic function [17]. Meanwhile, some gene mutation can interfere with calcium circulation and sensitivity of calcium ion in the myocardium, thus affecting its energy metabolism and leading to myocardial hypertrophy [17-19]. For different gene mutations, the functional mode or dominant effect of the gene products may be various, causing diverse phenotypes [15,20,21].

As in the present study, variant in MYBPC3 is the main contributor to the development of HCM. Studies have shown that

mutations in MYBPC3 are relatively more benign when comparing with those in MYH7 [15,22]. In addition, it was observed that patients with MYBPC3 variants often have an older age of onset, along with slower progression and thus better prognosis [23,24]. The proband in the current pedigree is consistent with a normal life expectancy. However, as in the case of individual II-1, sudden death may occur under extreme circumstances. Furthermore, when MYBPC3 is combined with other mutations, the phenotype can be much more serious.

Value of genetic testing

Genetic testing enables an early diagnosis among relatives of patients with hypertrophic cardiomyopathy [25]. It is of great significance for the differential diagnosis of diseases with similar

clinical phenotypes and it may aid in risk stratification and helpful to establish individualized therapeutic strategies [26]. Physicians can provide accurate genetic counseling and prenatal testing with a genetic diagnosis [27]. As in the current study, HCM family members can benefit from the above-mentioned advantages of genetic testing. The development of sequencing technology contributes significantly to the improvement of screening, genetic counseling and risk stratification of HCM, holding promise for the prevention and early detection of the disease.

Conclusion

In conclusion, we performed next generation sequencing to identify a novel frameshift mutation mapped to MYBPC3 c.1042_1043insCGGCA, which was thought to be associated with late-onset myocardial hypertrophy and had not been reported before. Premature termination at C2 domain was predicted by computational tools and structural and functional disruption of cMyBP-C occurs, thereby leading to an abnormal, truncated protein or loss of protein from this allele due to nonsense-mediated mRNA decay. These features may provide important information for physicians in clinical management of hypertrophic cardiomyopathy. Further researches of the mutation c.1042_1043insCGGCA are needed.

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References

1. Maron BJ, Desai MY, Nishimura RA, Spirito P, Rakowski H, Towbin JA, et al. Management of hypertrophic cardiomyopathy: JACC State-of-the-art review. *J Am Coll Cardiol*. 2022;79:390-414.
2. Matthia EL, Setteducato ML, Elzeneini M, Vernace N, Salerno M, Kramer CM, et al. Circulating biomarkers in hypertrophic cardiomyopathy. *J Am Heart Assoc*. 2022;11:e027618.
3. Litt MJ, Ali A and Reza N. Familial hypertrophic cardiomyopathy: Diagnosis and management. *Vasc Health Risk Manag*. 2023;19:211-21.
4. Grassi S, Campuzano O, Coll M, Cazzato F, Sarquella-Brugada G, Rossi R, et al. Update on the diagnostic pitfalls of autopsy and post-mortem genetic testing in cardiomyopathies. *Int J Mol Sci*. 2021;22:4124.
5. Sebastian SA, Panthangi V, Singh K, Rayaroth S, Gupta A, Shantharam D, et al. Hypertrophic cardiomyopathy: Current treatment and future options. *Curr Probl Cardiol*. 2023;48:101552.
6. Yamada T, Nomura S. Recent findings related to cardiomyopathy and genetics. *Int J Mol Sci*. 2021;22:12522.
7. Melas M, Beltsios ET, Adamou A, Koumarelas K, McBride KL. Molecular Diagnosis of Hypertrophic Cardiomyopathy (HCM): In the heart of cardiac disease. *J Clin Med*. 2022;12:225.
8. Ommen SR, Mital S, Burke MA, Day SM, Deswal A, Elliott P, et al. 2020 AHA/ACC Guideline for the diagnosis and treatment of patients with hypertrophic cardiomyopathy: Executive summary: A report of the American College of Cardiology/American Heart Association Joint Committee on clinical practice guidelines. *J Am Coll Cardiol*. 2020;76:3022-55.
9. Kumar S, Agarwal S, Ranvijay. Fast and memory efficient approach for mapping NGS reads to a reference genome. *J Bioinform Comput Biol*. 2019;17(2):1950008.
10. Tarasov A, Vilella AJ, Cuppen E, Nijman IJ, Prins P. Sambamba: Fast processing of NGS alignment formats. *Bioinformatics*. 2015;31(12):2032-34.
11. Wang K, Li M, Hakonarson H. ANNOVAR: Functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Res*. 2010;38(16):e164.
12. Ommen SR, Semsarian C. Hypertrophic cardiomyopathy: A practical approach to guideline directed management. *Lancet*. 2021;398:2102-8.
13. Miller DT, Lee K, Abul-Husn NS, Amendola LM, Brothers K, Chung WK, et al. ACMG SF v3.1 list for reporting of secondary findings in clinical exome and genome sequencing: A policy statement of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*. 2022;24:1407-14.
14. Marian AJ. Molecular genetic basis of hypertrophic cardiomyopathy. *Circ Res*. 2021;128:1533-53.
15. Gao J, Collyer J, Wang M, Sun F, Xu F. Genetic dissection of hypertrophic cardiomyopathy with myocardial RNA-Seq. *Int J Mol Sci*. 2020;21:3040.
16. Toepfer CN, Wakimoto H, Garfinkel AC, McDonough B, Liao D, Jiang J, et al. Hypertrophic cardiomyopathy mutations in MYBPC3 dysregulate myosin. *Sci Transl Med*. 2019;11(476):eaat1199.
17. Suay-Corredera C, Pricolo MR, Herrero-Galán E, Velázquez-Carreras D, Sánchez-Ortiz D, García-Giustiniani D, et al. Protein haploinsufficiency drivers identify MYBPC3 variants that cause hypertrophic cardiomyopathy. *J Biol Chem*. 2021;297:100854.
18. Moore J, Ewoldt J, Venturini G, Pereira AC, Padilha K, Lawton M, et al. Multi-omics profiling of hypertrophic cardiomyopathy reveals altered mechanisms in mitochondrial dynamics and excitation-contraction coupling. *Int J Mol Sci*. 2023;24:4724.
19. Viola HM, Richworth C, Solomon T, Chin IL, Szappanos HC, Sundararaj S, et al. A maladaptive feedback mechanism between the extracellular matrix and cytoskeleton contributes to hypertrophic cardiomyopathy pathophysiology. *Commun Biol*. 2023;6:4.
20. Wijnker PJM, Sequeira V, Kuster DWD, Velden JV. Hypertrophic cardiomyopathy: A vicious cycle triggered by sarcomere mutations and secondary disease hits. *Antioxid Redox Signal*. 2019;31:318-58.
21. Chou C, Chin MT. Pathogenic mechanisms of hypertrophic cardiomyopathy beyond sarcomere dysfunction. *Int J Mol Sci*. 2021;22:8933.
22. Helms AS, Thompson AD, Glazier AA, Hafeez N, Kabani S, Rodriguez J, et al. Spatial and functional distribution of MYBPC3 pathogenic variants and clinical outcomes in patients with hypertrophic cardiomyopathy. *Circ Genom Precis Med*. 2020;13:396-405.
23. Suay-Corredera C and Alegre-Cebollada J. The mechanics of the heart: Zooming in on hypertrophic cardiomyopathy and cMyBP-C. *FEBS Lett*. 2022;596:703-46.
24. Holler V, Seebacher H, Zach D, Schwegel N, Ablasser K, Kolesnik E, et al. Myocardial deformation analysis in MYBPC3 and MYH7 related sarcomeric hypertrophic cardiomyopathy-the Graz hypertrophic cardiomyopathy registry. *Genes (Basel)*. 2021;12:1469.
25. Burkart V, Kowalski K, Aldag-Niebling D, Beck J, Frick DA, Holler T, et al. Transcriptional bursts and heterogeneity among cardiomyocytes in hypertrophic cardiomyopathy. *Front Cardiovasc Med*. 2022;9:987889.
26. Velicki L, Jakovljevic DG, Preveden A, Golubovic M, Bjelobrck M, Ilic A, et al. Genetic determinants of clinical phenotype in hypertrophic cardiomyopathy. *BMC Cardiovasc Disord*. 2020;20:516.
27. Walsh R, Offerhaus JA, Tadros R, Bezzina CR. Minor hypertrophic cardiomyopathy genes, major insights into the genetics of cardiomyopathies. *Nat Rev Cardiol*. 2022;19:151-67.