Case Report: Identification of the First Synonymous Variant of Myosin Binding Protein C3 (c.24A>C, p.P8P) Altering RNA Splicing in a Cardiomyopathy and Sudden Cardiac Death Case

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Background: Sudden cardiac death (SCD), based on sudden cardiac ejection cessation, is an unexpected death. Primary cardiomyopathies, including dilated cardiomyopathy (DCM), are one of main causes of SCD. The DCM is characterized by a cardiac dilatation and a reduced systolic function with a prevalence of 1/250 in adults. The DCM has been reported with more than 60 disease-causing genes, and MYBPC3 variants are one of the most common and well-known causes of DCM.

Methods: We identified a 29-year-old female who died of SCD. We performed a whole-exome sequencing (WES) to detect her genetic etiology and used minigene modeling and immunohistochemistry staining to verify the pathogenicity.

Results: We determined that the woman died of SCD caused by DCM due to an identified novel synonymous variant of MYBPC3 (NM_000256.3: c.24A>C, p.P8P) in the deceased. The variant can result in abnormal splicing, which was confirmed by minigene models and immunohistochemistry staining.

Conclusion: We may have identified the first deleterious synonymous variant of MYBPC3 in an SCD case and verified its significant impact on RNA splicing. Our description enriched the spectrum of MYBPC3 variants and emphasized the significance of synonymous variants that are always disregarded in genetic screening.

Keywords: MYBPC3, sudden cardiac death, dilated cardiomyopathy, synonymous variant, splicing

INTRODUCTION

Sudden cardiac death (SCD) is an unexpected death based on sudden cardiac ejection cessation. It accounts for 15–20% of unnatural death in developed countries (1, 2). The prominent symptoms of SCD include chest pain, dyspnea, palpitations, presyncope, and syncope. Primary electrical disorders, atherosclerosis, congenital cardiovascular diseases, and cardiomyopathies are the four main causes of SCD (3).
Cardiomyopathies can be divided into dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy (HCM), restrictive cardiomyopathy (RCM), arrhythmogenic right ventricular cardiomyopathy (ARVC), and myocarditis (4). Among these cardiomyopathies, DCM is the most frequent, with a prevalence in adults of at least 1/250 (5). The DCM is characterized by cardiac dilatation and reduced systolic function. A heritable pattern is present in 20–35% of cases (6). Most of inherited DCMs show an autosomal dominant pattern and are usually present in the second or third decade of life (5).

Dilated cardiomyopathy (DCM) has been reported with more than 60 disease-causing genes, involving the sarcomere, Z disc, sarcoglycans, cytoskeletal complex, nuclear envelope, potassium and sodium channels, heat shock proteins, transcription factors, and mitochondria proteins (5).

The MYBPC3 variants are one of the most common and well-known causes of DCM. The MYBPC3 encodes the cardiac isoform of myosin-binding protein C and is located in the cross-bridge-bearing zone of A bands in striated muscle (isoform of myosin-binding protein C and is located in the well-known causes of DCM. The MYBPC3 usually present in the second or third decade of life (5).

In the study, we identified a 29-year-old female who died of SCD caused by DCM. We detected a synonymous variant of MYBPC3 (NM_000256.3: c.24A>C, p.P8P) in the deceased. The variant occurred in the penultimate base of exon 1 and resulted in abnormal splicing. Minigene and immunohistochemistry verified its pathogenicity. To the best of our knowledge, the variant may be the first reported deleterious synonymous variant of MYBPC3. Our identification enriched the spectrum of MYBPC3 variants and emphasized the significance of synonymous variants which are always disregarded in genetic screening.

MATERIALS AND METHODS
Subjects
The study was approved by the Institutional Review Board of Changsha Forensic Appraisal Center. Written informed consent was obtained from the legal guardian and/or next of kin of the deceased for the publication of any potentially identifiable images or data included in this article. The sample was evaluated according to postmortem measures of the International Society and Federation of Cardiology. The anatomical assessment was performed by an expert forensic pathologist and confirmed by a second independent pathologist.

DNA/RNA Extraction and Reverse Transcription
Genomic DNA was extracted by the DNeasy Blood and Tissue Kit (Qiagen, Valencia, USA), and RNA was extracted by the RNeasy Mini Kit (Qiagen, Valencia, USA). Reverse transcription was performed using the ReverTaid First Strand cDNA Synthesis Kit (Thermo, Vilnius, Lithuania).

Whole-Exome Sequencing
Berry Genomics Company Limited (Chengdu, China) performed an exome capture, a high-throughput sequencing, and a common filtering, as described in a previous article (9). In this study, we retained all synonymous variants. After common filtering, we predicted the influence of these variants on splicing using NetGene2-2.42 (https://services.healthtech.dtu.dk/service.php?NetGene2-2.42). Causative variants were screened by the list of cardiopathy-related genes (Supplementary Table S1) and were classified based on the American College of Medical Genetics and Genomics (ACMG) Standards and Guidelines (10).
injury or disease was observed. We concluded that she died of SCD that might have been triggered by DCM. According to a description from her mother (I:2), we speculated that her father (I:1) might have died of a heart attack at 37. The mother and sister (II:2) were unaffected.

Genetic Analysis

The mother hoped to know whether her other daughter had a risk of SCD and turned to us for help. Blood from the heart of the deceased was collected and stored in a refrigerator at −80°C. We extracted a DNA from the blood and preformed a whole-exome sequencing (WES). We eliminated common variants using Genome Aggregation Database (GnomAD; http://gnomad.broadinstitute.org) and Chinese Millionome Database (CMDB; http://cmdb.bgi.com/cmdb/) and predicted the pathogenicity of variants using MutationTaster (http://www.mutationtaster.org), Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/), and SIFT (http://provean.jcvi.org/index.php). Five variants in cardiopathy-related genes were identified in the deceased (Table 1). Based on the ACMG classification of these variants, we highly suspected that MYBPC3 variant (NM_000256.3:c.24A>C, p.P8P) was the genetic etiology of the deceased. The variant was classified as “Likely pathogenic” following the ACMG evidence PS3, PM2, PP1, and PP3. Although the variant was synonymous, it occurred in the penultimate base of exon 1, which may affect the RNA splicing. Sanger sequencing verified the MYBPC3 variant in the deceased (Figure 1C). Her mother and sister did not harbor this variant.

Minigene Modeling and Immunohistochemistry Staining

To investigate whether the synonymous variant altered the RNA splicing, we constructed minigene models (Figure 2A). Sanger sequencing validated the mutagenesis of c.10G>A and c.24A>C in the minigene (Figure 2B). The pcDNA3.1-MYBPC3-c.10G>A plasmid was used to exclude the possibility that the mutagenesis, itself, broke the splicing site. The HEK293 cells were used in transfection. Agarose gel electrophoresis indicated that pcDNA3.1-MYBPC3-minigene model and pcDNA3.1-MYBPC3-c.10G>A model had a minigene full-length band (approximately 1,500 bp) and a spliceosome band (approximately 300 bp), while the pcDNA3.1-MYBPC3-c.24A>C model did not produce the spliceosome band, suggesting that c.24A>C caused abnormal splicing (Figure 2C). The abnormal splicing may lead to intron 1 retention and frame shift. Immunohistochemistry staining showed that compared with the deceased without SCD, the MYBPC3 expression was decreased in the left ventricle tissue of II:1 (Figure 2D). Therefore, we inferred that the synonymous variant damaged the...
splicing, reduced MYBPC3, and was one genetic precipitating factor of her DCM and SCD.

**DISCUSSION**

Sudden cardiac death (SCD) is defined as “sudden and unexpected death occurring within an hour of the onset of symptoms, or occurring in patients found dead within 24 h of being asymptomatic, presumably due to a cardiac arrhythmia or hemodynamic catastrophe” (12). In the study, through postmortem and disease history talking, we confirmed that the deceased had DCM through negative pathological and toxicological assessment, without other fatal defects. The DCM could have triggered the hemodynamic anomaly and had threatened her life. Hence, we postulated that SCD was her cause of death. In addition, her father had a sudden death despite a momentarily struggle, supporting our speculation that the father also died of SCD.

Dilated cardiomyopathy (DCM) is one of the most common causes of heart failure and accounts for around 60% of childhood cardiomyopathies (6). Common causes of DCM include infection, inflammation, autoimmunity, chemical and toxin exposure, and genetic variants (13). Genetic causes are important at all ages as approximately 2% of family DCM harbor MYBPC3 variants (6). In the study, the deceased carried a synonymous variant of MYBPC3 (c.24A>C, p.P8P). In this variant, the penultimate base of exon 1, A, was substituted by C, which was predicted to change the splicing site. Her mother and sister, without the MYBPC3 variant, was not found to have a DCM or other cardiovascular diseases.

According to the ACMG guideline, we listed the following evidence and determined that the synonymous variant of MYBPC3 was “Likely pathogenic.” (1) We performed a minigene modeling and an immunohistochemistry staining and verified the damaging effect of the variant (PS3). (2) The variant was absent from controls in GnomAD and CMDB (PM2). (3) The variant was not identified in the unaffected family members (PP1). (4) MutationTaster predicted the synonymous variant to be disease-causing as a result of splice site alteration (PP4) (10). The MYBPC3 splicing variants (c.24 + 1G>A and c.24 + 3A>C) and our variant (c.24A>C) impacted on the same splicing site. Notably, these two splicing variants were associated with HCM (14, 15). Therefore, we considered that the synonymous variant was the genetic etiology of the deceased.

To confirm the pathogenicity of our variant, we established minigene models. Generally, minigene should be constituted by the affected exon and the adjacent introns and exons at its upstream and downstream (16). In the present case, the synonymous variant was located in the first coding exon. Thus, we constructed the minigene lack of upstream sequences as it may reduce the splicing efficiency. The minigene full-length band was prominently brighter than the spliceosome band in the pcDNA3.1-MYBPC3-minigene model. In the pcDNA3.1-MYBPC3-c.24A>C model, the spliceosome band was absent, suggesting that the variant damaged the RNA splicing. We constructed the pcDNA3.1-MYBPC3-c.10G>A model to be a negative control, while variant c.10G>A promoted
TABLE 1 | Variants identified in the deceased by whole-exome sequencing (WES) in combination with the filtration of cardiopathy-related genes.

| Gene      | Variant                          | MutationTaster | PolyPhen-2 | SIFT | GnomAD | CMDB | OMIM clinical phenotype                                      | American college of medical genetics classification |
|-----------|----------------------------------|----------------|------------|------|--------|------|------------------------------------------------------------|-----------------------------------------------------|
| LDB3      | NM_007078.2: c.2131T>C, p.S711P | D              | D          | D    | -      | -    | AD, Cardiomyopathy, dilated, 1C, with or without LVNC; AD, Cardiomyopathy, hypertrophic, 24; AD, Left ventricular non-compaction 3; AD, Myopathy, myofilbrillar, 4. | Uncertain significance (PM2, PP3, PP5, BP5) |
| MYBPC3    | NM_000256.3: c.24A>C, p.P8P     | D              | -          | -    | -      | -    | AD, Cardiomyopathy, dilated, 1MM; AD/AR, Cardiomyopathy, hypertrophic, 4; AD, Left ventricular non-compaction 10. | Likely pathogenic (PS3, PM2, PP1, PP3) |
| BBS2      | NM_031885.3: c.422A>G, p.N141S  | D              | D          | T    | 0.00014| 0.00091| AR, Bardet-Biedl syndrome 2; AR, Retinitis pigmentosa 74. | Likely benign (PM2, PP3, BS4, BP5) |
| EVC2      | NM_147127.4: c.2643G>C, p.Q881H | D              | B          | D    | 0.00007| -    | AR, Ellis-van Creveld syndrome; AD, Weyers acrofacial dysostosis. | Likely benign (PM2, PP3, BS4, BP5) |
| BBS4      | NM_033028.4: c.1548_1549del, p.S711P     | D              | -          | -    | 0.00037| -    | AR, Bardet-Biedl syndrome 4. | Likely benign (PM2, PP3, BS4, BP5) |

D, disease causing; T, tolerant; B, benign; AR, recessive dominant; AD, autosomal dominant.

the splicing capacity. Mutant models exhibited the different influences of the exonic variants on the splicing and verified the pathogenicity of variant c.24A>C. The variant c.24A>C caused intron retention and frame shift, which may lead to a premature termination of translation and/or protein degradation. As per our expectation, MYBPC3 expression was decreased in the left ventricle tissue of the deceased. Although Ito et al. predicted and summarized the MYBPC3 variants that alter RNA splicing, which included several missense variants, the variant (c.24A>C, p.P8P) may be the first known synonymous variant in MYBPC3 that causes disease. This, therefore, reminded us of the significance of synonymous variants (16).

The MYBPC3 is mapped to 11p11.2, spans more than 21 kb, and contains 35 exons (17, 18). MYBPC3 is transversely arrayed in sarcomere A-bands, binds myosin heavy chain in thick filaments, binds titin in elastic filaments, and modulates contraction. An MYBPC3 defect may affect the actin-myosin interactions, break sarcomere stabilities, alter calcium handling in myocardial cells, and cause DCM, HCM, or left ventricular non-compaction (8). In the study, the MYBPC3 variant (c.24A>C, p.P8P) reduced the MYBPC3 expression and was associated with DCM and SCD.

Sudden cardiac death (SCD) is a major public health problem worldwide. It is responsible for approximately 540,000 deaths per year in China and 170,000 to 450,000 deaths per year in the United States (12). Nearly 80% of patients with SCD had preexisting heart disease. Notably, patients presented SCD at the first clinical manifestation (19). Genetic screening of cardiopathy-related genes, such as MYBPC3, can contribute to assessing the risk of cardiopathy and SCD and help prevent and treat related diseases. Ehlermann et al. suggested that MYBPC3 variants are not generally associated with a good prognosis and might cause sudden death even in asymptomatic individuals (20).

CONCLUSIONS

In summary, we reported a young woman who died of SCD. Using WES, we identified a novel variant of MYBPC3 (NM_000256.3: c.24A>C, p.P8P) in the deceased, which may be the first known synonymous variant of MYBPC3 that can cause disease. It damaged the splicing and resulted in the reduced MYBPC3 expression. Our findings expanded the genetic spectrum of SCD, confirmed the pathogenicity of the MYBPC3 variant (c.24A>C, p.P8P), emphasized the significance of synonymous variants, and contributed to the clinical diagnosis of cardiomyopathy and SCD.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

Written informed consent was obtained from the deceased’s legal guardian/next of kin for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

J-YJ contributed to conception and design and carried out the analysis and interpretation of data. JX performed acquisition and analysis and interpretation of data. JD contributed in the analysis and interpretation of data. YD and YS assisted in conception and design and wrote the original draft. RX revised
the draft and approved the revisions. All authors contributed to the article and approved the submitted version.

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**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.806977/full#supplementary-material

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