# Clinical Case Reports

CASE REPORT

# Familial hypertrophic cardiomyopathy associated with a new mutation in gene MYBPC3

Esther Aurensanz Clemente[1] (iD), Ariadna Ayerza Casas[1], Cecilia García Lasheras[1], Feliciano Ramos Fuentes[1], Ines Bueno Martínez[1], Juana Pelegrín Díaz[2], Pablo Ruiz Frontera[3] & Lorenzo Montserrat Iglesias[4]

[1]Department of Pediatrics, HCU Lozano Blesa, Zaragoza, Spain
[2]Department of Cardiology, HCU Lozano Blesa, Zaragoza, Spain
[3]Intensive Care Unit, HU Miguel Servet, Zaragoza, Spain
[4]Scientific Department, Health in Code, A Coruña, Spain

## Correspondence

Esther Aurensanz Clemente, Department of Pediatrics, HCU Lozano Blesa, San Juan Bosco. 50012 Zaragoza, Spain.
Tel: +34622210110;
E-mail: estheraurensanz@gmail.com

## Key Clinical Message

We think that the main interests of this study are the report of a new mutation in gene MYBPC3 as a cause of Hypertrophic cardiomyopathy (HMC), and the verification of the fact that not always is the number of mutations related to the severity of the disease.

## Background

Hypertrophic cardiomyopathy is a primary disease of the myocardium caused mainly by mutations in the genes that encode sarcomeric proteins. From a pathological point of view, HMC is characterized by the presence of myocardial hypertrophy, myocyte disorganization, and fibrosis, which contribute to the development of a broad spectrum of functional disorders [1, 2].

Hypertrophic cardiomyopathy is the most common cause of sudden cardiac death for young adults and is a significant cause of morbidity and mortality for the elderly, with an estimated prevalence of 1 in every 500 individuals [3]. The associated clinical condition varies significantly and extends from incapacitating symptoms to a lack of these symptoms. The clinical heterogeneity of the disease reflects the considerable variety of its genetic causes. HMC can be secondary to mutations in more than 30 different genes, and the most common genes are

those that encode the main sarcomeric proteins. To date, more than 1000 mutations in these genes have been reported to be associated with the disease (https://www.ncbi.nlm.nih.gov/clinvar/). The two most commonly involved genes in this disease are *MYBPC3*, which encodes myosin-binding protein C, and *MYH7*, which encodes the beta-myosin heavy chain. These genes are responsible for approximately 40% of cases of HMC and 80% of identified pathogenic sarcomeric mutations [3, 4].

The management of this disease has classically involved the long-term systematic assessment of first-degree relatives of the affected individual. Understanding the genetic defect allows us to provide patients and relatives with effective genetic counseling [3, 4, 13].

Early detection of the mutation in relatives enables us to narrow the follow-up and detect potential complications sooner. Lastly, relatives who do not carry the mutation can be discharged, thereby avoiding unnecessary follow-up. There is controversy, however, concerning the

prognostic utility of genetic studies for HMC. For example, mutations in *MYBPC3* are believed to be associated with less severe forms, later onset, and a better prognosis than those caused by mutations in *MYH7* and in other sarcomeric genes [2, 10, 15]. However, there have been reports of mutations with poor prognoses in genes initially related to less aggressive forms, and there are numerous nonpathogenic variants in genes that are considered of greater risk [6, 11, 16, 17]. This situation illustrates the difficulty in predicting the phenotype based on the genotype if we only consider the affected gene, as well as the need to assess each identified mutation individually. It has recently been proposed that the only genetic marker with prognostic value is the presence of complex genotypes, with more than one mutation or mutations in homozygosis [7, 8, 9]. In this study, we describe a family that illustrates the limitations of this concept.

## Case Report

Index patient: A 51-year-old Spanish man was followed up in cardiology for preexcitation in Wolff–Parkinson–White syndrome since the age of 18, with an electrocardiogram demonstrating a short PR interval, a positive delta wave in diaphragmatic and lateral side, negative in V1, and positive in V2-V6. The Holter monitor showed no rhythm disorders, maintaining the preexcitation throughout the tracing. The patient's echocardiogram showed moderate concentric hypertrophy. Annual follow-ups were conducted during cardiology appointments, and the patient remained asymptomatic until the age of 46 when he began to experience palpitations and exertional dyspnea. The echocardiogram showed asymmetric left ventricular hypertrophy, with a maximum thickness of 23 mm in the posterior median septum, with no obstruction in the left ventricular outflow tract. The decision was made to perform ablation of the accessory pathway, which had good results. The patient continued feeling palpitations despite the ablation, with no electrocardiographic signs of preexcitation. However, supraventricular and ventricular extrasystoles were observed in the Holter. The patient is currently on treatment with 5 mg/day of bisoprolol, with no symptoms in recent months but with electrocardiographic signs of left ventricular hypertrophy with no preexcitation. The echocardiogram shows ventricular hypertrophy, with pronounced septal growth.

Family history: The index patient's parents died at an early age; the mother died at age 53 (diagnosed with mitral stenosis), and the father died suddenly at age 48. The family tree is shown in Figure 1.

The child of the index patient (III-4, Fig. 1) was followed up in pediatric visits for a systolic murmur with normal echocardiogram. During a routine echocardiography check-up at 15 years of age, the child was observed to have severe septal hypertrophy (Fig. 2), with no obstruction of the left ventricular outflow tract. As the condition progressed, the size of the interventricular septum increased; an implantable cardioverter defibrillator (ICD) was therefore placed at the age of 18 years. The child is currently asymptomatic. The echocardiogram shows asymmetric median septal and apical hypertrophic cardiomyopathy, with a nonobstructive maximum thickness of 33–35 mm and preserved ejection fraction, with no defibrillator discharges.

At the age of 32 years, a brother of the index patient (II-2, Fig. 1) experienced an episode of atypical chest pain with palpitations and no clear trigger. He was therefore hospitalized for study. The electrocardiogram showed a sinus rhythm, with deep Q waves lasting 0.04 sec in II, III, and aVF. An echocardiogram, stress testing, propafenone test, cardiac scintigraphy, and Holter monitoring were performed, the results of which were normal. Despite treatment with beta-blockers, the brother's palpitations continued, and the electrocardiographic pattern observed during admission remained. At the age of 41, the brother underwent an annual follow-up echocardiogram, which showed a pattern of nonobstructive hypertrophic cardiomyopathy, with a septal thickness of 19 mm.

The other family members studied (II-1, III-1, III-2, and III-3) (Fig. 1) remained asymptomatic and had normal electrocardiograms and echocardiograms.

A genetic analysis of *PRKAG2* and *LAMP2* was performed for the index patient. These genes associate hypertrophic cardiomyopathy with Wolff–Parkinson–White syndrome. The results of the analysis were negative. We
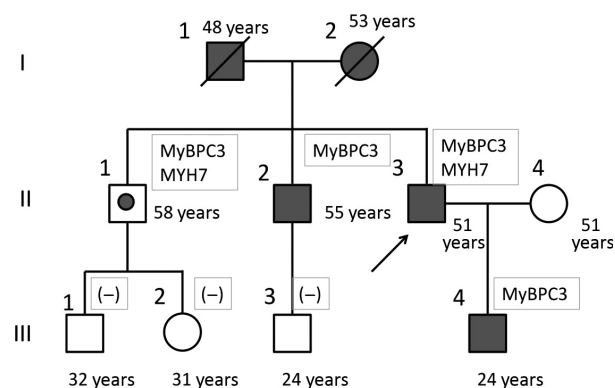


**Figure 1.** The family tree. Circle: female. Square: male. Symbols in gray: affected members of the family of disease/sudden death. Symbols in white: family members without heart disease. Symbols enclosing a circle: carrying members of the mutation without heart disease today. Symbols with diagonal line: deceased members. Arrow: index patient.
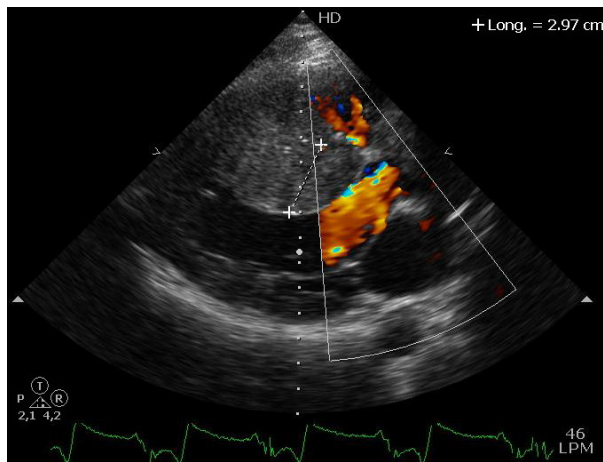
**Figure 2.** The echocardiogram showed asymmetric left ventricular hypertrophy, with a maximum thickness of 29.7 mm in the posterior median septum, with no obstruction in the left ventricular outflow tract.

therefore proceeded with the sequencing in both directions of the exons and flanking intronic regions of the genes most commonly associated with HMC: *MYBPC3*, *MYH7*, *TNNT2*, *TNNI3*, and *TPM1*. We verified that this patient with HMC was heterozygous for two missense mutations: the first in gene *MYBPC3*: g.47363647G>A, Ala562Val in exon 18. This mutation affects a highly conserved residue (Ala562) located in domain C4, which is not known to interact with any protein. However, it might be necessary for the flexibility of the N-terminal region, thus important for making its interactions with the S2 region of the myosin possible or with the actin filament [5]. To date, this variant has not been reported and does not appear in public databases that record thousands of control individuals (Exome Variant Server and dbSNP). Bioinformatic analysis with three softwares suggests that the mutation has a high probability of producing a deleterious effect: Polyphen-2 (=Polymorphism Phenotyping), Sorting Intolerant From Tolerant (SIFT), and PMut. Prediction provided by Polyphen-2 is benign, with a score of 0.354 (score range: 0–1); SIFT predicts that the substitution affects the protein function with a score of 0 and low confidence (score < 0.05 deleterious). Prediction provided by PMut is neutral with a reliability of 2 (reliability range: 0–9).

The second genetic variant affects gene *MYH7*: g.23892910A>G, Met982Thr. The variant changes a highly conserved residue in the evolution (Met982), located in the myosin neck (Leu839-Lys1216) between the *cMYBPC3* binding region (Leu839-Lys964) and the functional domain of the hinge region (Phe1125-Asn1217). This variant has been associated with the development of cardiomyopathies [1, 12, 14, 18] but has also been identified in control

populations. In silico studies were performed to determine the effect of aminoacid substitution M for T at residue 982 using three softwares: Polyphen-2 (=Polymorphism Phenotyping), Sorting Intolerant From Tolerant (SIFT), and PMut. Prediction provided by Polyphen-2 is probably harmful with a score of 0.948 (Rango de score range: 0–1); SIFT predicts that the substitution affects the protein function with a score of 0 (score < 0.05 deleterious) and low confidence. Prediction provided by PMut is pathological with a reliability of 5 (reliability range: 0–9).

In the Exome Variant Server database (5000 Genome project), this variant has been identified in 19 of 4300 Americans of European descent (0.4%) and in three of 2203 African Americans (0.1%). The ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/) classifies this variant as having uncertain significance.

After these findings, we proceeded with the family study, identifying the same mutation in gene *MYBPC3* (but not the variant in *MYH7*) in the two family members with HMC (child and brother of the index patient; II-2 and III-4, respectively) (Fig. 1). We also observed the presence of both variants (genes *MYBPC3* and *MYH7*) in the asymptomatic brother (II-1) of the index patient and with no HMC shown in the echocardiogram to date (Fig. 1). No mutations were detected in the other relatives. Given the considerable difference in clinical expression between the siblings who carried the two variants, we conducted a more extensive study for the index patient and his 15-year-old child (with earlier expression). The study consisted of massive parallel sequencing (next-generation sequencing) of all coding exons and flanking intronic regions of the 214 genes related to familial heart disease (http://www.healthincode.com), including 56 genes previously associated with or candidates for the development of HMC, without identifying additional pathogenic variants.

This study presents the first description of the Ala562-Val mutation. For the study of its pathogenicity, we therefore only have the information provided by bioinformatic studies and the family study. Within the same region, more than a dozen missense mutations have been reported and associated with the development of hypertrophic cardiomyopathy and dilated cardiomyopathy. More than half of these mutations have been identified in probands that showed an additional mutation. In these cases, the phenotype was more serious and the presentation was earlier. In contrast, the relatives who showed only one mutation in this region generally developed mild and late phenotypes.

The bioinformatic analysis suggests that this mutation has a high probability of being deleterious, but these results do not allow us to definitively establish its pathogenicity. The results of the family study are also highly suggestive but insufficient to confirm the

cosegregation of the disease with the mutation. The index patient was diagnosed at 46 years of age. He has two siblings who have the same mutation, but only one of them has developed HMC (at age 32), while the other is a healthy carrier of the mutation. The index patient's child is a carrier of the mutation and was diagnosed with HMC at the age of 15 years, the earliest presentation in this family. The only adverse event recorded for the family is the death of the index patient's father who died at the age of 48 of unknown causes and for whom we have no genetic study.

With this data, we can conclude that the Ala562Val mutation is probably associated with the development of the disease but can have a late expression or incomplete penetrance (not all carriers develop the phenotype). The mutation might also be insufficient for developing cardiomyopathy. Therefore, more data are needed to confirm its pathogenicity.

The Met982Thr variant in gene MYH7 has been associated with HMC, noncompaction cardiomyopathy, and dilated cardiomyopathy. This mutation has been described in several articles and was first identified in an American patient with left ventricular hypertrophy belonging to the Framingham cohort. This study did not report additional clinical or family data [14]. This mutation has also been identified in two of 4078 controls, although one of them had ventricular dilation and left atrial dilation. The second control had a mildly dilated left atrium and high ECG voltages. Therefore, these disorders suggest that these "healthy controls" could actually be carriers of subclinical heart disease. Millat et al. described this mutation in two index cases of a cohort of patients diagnosed with HMC. However, in both cases, the mutation was associated with a second mutation. One of the patients had been diagnosed at 13 years of age and was also a carrier of the Asn696Ser mutation in MYH7 (compound heterozygosity), a mutation that has been reported in pediatric patients. The second patient was diagnosed at 50 years of age and was also a carrier of an unreported Val219Phe mutation in MYBPC3 (double heterozygosity) [12]. Met982Thr has also been reported in association with dilated cardiomyopathy, although more phenotypic details were not provided [18]. The mutation has also been identified in a sample from Mallorca from a 61-year-old patient with a family history of HMC and diagnosed with left ventricular noncompaction. The patient had a maximum thickness of 15 mm, systolic dysfunction with an ejection fraction of 40%, and nonsustained ventricular tachycardia in the Holter [1]. The total number of controls published for this mutation to date is more than 4500 and has been identified in two of these controls (0.04%). However, in the 5000 Genome Project database, the frequency of this mutation is 0.4% among individuals of European descent and 0.1% in African Americans. Although any of these controls could be affected by the disease, this information suggests that this variant is not a sufficient cause of the development of HMC and could even be nonpathogenic. It is possible that this variant is associated with a mild phenotype and/or late expression, which requires the presence of an additional factor (mainly genetic) to express itself clinically.

It is important to analyze the implication of the combination of variants, given that both are potentially associated with the patient's phenotype. It is believed that the combination of the two mutations (double heterozygosity) could additively contribute to the development and form of expression of the phenotype presented by the index patient (HMC).

In the study family, we can observe how patient III-4 (Fig. 1), who has the most symptoms, an earlier age of onset and greater involvement of the HMC requiring defibrillator implantation, presented a mutation only in gene MYBPC3. However, patient II-1 (Fig. 1) had mutations in both genes and remained asymptomatic with normal echocardiograms. The index patient (II-3) had both mutations and symptoms and echocardiogram compatible with HMC and preexcitation, while patient II-2 (Fig. 1) had similar symptoms and HMC pattern, with a single mutation in gene MYBPC3. With these findings, we cannot confirm that the combination of the two mutations has a more severe phenotype, given that one of the patients with both mutations is asymptomatic while the one who has the most symptoms and earliest onset only presented the MYBPC3 mutation.

It is important to consider several possibilities when interpreting these findings:

• That the only truly pathogenic variant is the Ala562Val mutation, which will have a variable clinical expression determined by the presence of additional unidentified genetic, epigenetic, and environmental factors.

• That both variants are pathogenic, but they do not necessarily have a synergistic effect. This would explain the apparently healthy carrier of both variants, who might develop the phenotype later in life. It would also explain the fact that a carrier of one mutation can have a more severe phenotype than a carrier of both variants.

• That neither of the two mutations is pathogenic, and the disease has another cause in the family: It is important to continue considering this possibility given that the variant in MYH7 has been identified at a low rate in controls (although a number of these controls have subsequently been considered potentially affected) and that there is still insufficient information to confirm the pathogenicity of the MYBPC3 mutation. For this reason, we performed a more in-depth study using

massive parallel sequencing, with negative results, which decreases but does not eliminate the possibility of an additional mutation responsible for the disease.

In general, the identification of the causal mutation in asymptomatic relatives clearly identifies patients who are likely to develop HMC in the future and those who, by virtue of not being carriers of the mutation, do not require specific follow-up [3, 4, 13]. In this study, we have shown the difficulty involved in [19] establishing the causal mutation or whether there is more than one mutation. In this family, we cannot be sure that we have identified all causes of the disease. Therefore, in this case, it is prudent to continue with the periodic follow-up of first-degree relatives of the affected patients even though they do not present any of the two mentioned variants.

Various authors have suggested that the most relevant genetic datum for the prognosis of patients with HMC is the number of identified mutations and that the individual prognosis and implications of each mutation are not relevant. The study of this family shows that this criterion has significant limitations, and the presence of more than one mutation might not be equivalent to high risk.

## Authorship

EAC, AAC, and CGL: initiated the human studies. FRF, IBM, and JPD: identified, characterized, and provided patient data. EAC, AAC, and PRF: prepared the figures. FRF and LMI performed the molecular genetic diagnoses. EAC, AAC, PRF, and LMI: drafted and reviewed the manuscript. All authors analyzed the data, discussed the results, and were provided the opportunity to comment on the manuscript. All coauthors have read and approved the submission of this MS to the journal.

## Conflict of Interest

The authors declare that they have no competing interests.

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