

# Mutations in Sarcomeric Genes *MYH7*, *MYBPC3*, *TNNT2*, *TNNI3*, and *TPM1* in Patients With Hypertrophic Cardiomyopathy

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**Introduction and objectives.** Mutation of a sarcomeric gene is the most frequent cause of hypertrophic cardiomyopathy. For each such gene, however, previous studies have reported a range of different mutation frequencies, and clinical manifestations have been highly heterogeneous, both of which limit the use of genetic information in clinical practice. Our aim was to determine the frequency of mutations in the sarcomeric genes *MYH7*, *MYBPC3*, *TNNT2*, *TNNI3*, and *TPM1* in a cohort of Spanish patients with hypertrophic cardiomyopathy.

**Methods.** We used sequencing to analyze the coding regions of these five genes in 120 patients (29% with a family history) and investigated how the patient phenotype varied with the gene mutated.

**Results.** In total, 32 patients were found to have mutations: 10 in *MYH7* (8%), 20 in *MYBPC3* (16%), 2 in *TNNT2*, 1 in *TPM1*, and none in *TNNI3*. Overall, 61% of mutations had not been described before. Two patients had 2 mutations (ie, double mutants). There was no difference in the mean age at diagnosis or the extent of the hypertrophy between those with *MYH7* mutations and those with *MYBPC3* mutations.

**Conclusions.** Some 26% of patients had a mutation in one of the five sarcomeric genes investigated. More than half of the mutations had not been described before. The *MYBPC3* gene was the most frequently mutated, followed

by *MYH7*. No phenotypic differences were observed between carriers of the various mutations, which makes it difficult to use genetic information to stratify risk in these patients.

**Key words:** Hypertrophic cardiomyopathy. Sudden cardiac death. Mutations. Sarcomeric genes.

## Espectro mutacional de los genes sarcoméricos *MYH7*, *MYBPC3*, *TNNT2*, *TNNI3* y *TPM1* en pacientes con miocardiopatía hipertrófica

**Introducción y objetivos.** Las mutaciones en los genes sarcoméricos son la causa más frecuente de miocardiopatía hipertrófica. Para cada gen, la frecuencia de mutaciones varía entre los estudios, y las manifestaciones clínicas son muy heterogéneas, lo que dificulta el empleo de la información genética en la práctica clínica. Nuestro objetivo es determinar la frecuencia de mutaciones en los genes sarcoméricos *MYH7*, *MYBPC3*, *TNNT2*, *TNNI3* y *TPM1* en una serie de pacientes con miocardiopatía hipertrófica.

**Métodos.** Se analizaron las regiones codificantes de estos cinco genes mediante secuenciación en 120 pacientes (el 29% con antecedentes familiares), comparando el fenotipo según el gen mutado.

**Resultados.** Se hallaron mutaciones en 32 pacientes; 10 y 20 tenían mutaciones en *MYH7* (8%) y *MYBPC3* (16%). Se hallaron mutaciones de *TNNT2* y *TPM1* en 2 y 1 pacientes, y ninguna de *TNNI3*. Dos pacientes tenían dos mutaciones (dobles mutantes). El 61% de las mutaciones no habían sido descritas previamente. No hallamos diferencias en la media de edad al diagnóstico o el tamaño de la hipertrofia entre los portadores de mutaciones en *MYH7* y los de *MYBPC3*.

**Conclusiones.** El 26% de los pacientes tenían mutaciones en alguno de los cinco genes estudiados. Más de la mitad de las mutaciones no habían sido descritas. El gen *MYBPC3* fue el más mutado, seguido de *MYH7*. No se hallaron diferencias fenotípicas entre los pacientes según el gen mutado, lo que dificultaría el empleo de la información genética para estratificar el riesgo en estos pacientes.

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**Palabras clave:** *Miocardiopatía hipertrófica. Muerte súbita cardíaca. Mutaciones. Genes sarcoméricos.*

## ABBREVIATIONS

ANOVA: analysis of variance  
 HCM: hypertrophic cardiomyopathy  
 SCD: sudden cardiac death  
 SSCA: single strand conformation analysis

## INTRODUCTION

Hypertrophic cardiomyopathy (HCM) is the most common reason for sudden cardiac death in young adults, and a major cause of morbidity and mortality in the elderly. The associated clinical picture is highly variable and ranges from incapacitating symptoms to no symptoms at all. Many patients remain asymptomatic for long periods, although the percentage of patients with severe symptoms increases with age.<sup>1</sup>

It has been estimated that 0.2% of individuals have a wall thickness  $\geq 15$  mm.<sup>2</sup> The pathophysiological basis of HCM is mutation of genes encoding the sarcomeric proteins. Around 30% to 40% of patients are diagnosed as sporadic cases, although the incomplete penetration of some mutations could mean that the percentage of true familial cases is underestimated.<sup>3</sup> The clinical heterogeneity is primarily due to the fact that at least 12 genes can mutate. The first mutations were found in the *MYH7* gene, which encodes cardiac beta myosin heavy chain.<sup>4,7</sup> Mutations were later identified in other genes, such as *TNNT2* (troponin-T) and *MYBPC3* (cardiac myosin-binding protein C).<sup>8-14</sup>

Most of the mutations have been found in a single family, which makes it difficult to obtain conclusive data on the phenotype associated with each mutation. Relevant data on the genotype-phenotype correlation have only been obtained in mutations found in many patients. The initial studies indicated that mutations in *MYH7* would result in severe forms of hypertrophy, and mutations in *TNN2* cause a less severe hypertrophy, but a high risk of SCD. Individuals who carry mutations in *MYBPC3* would present less severe forms and a lower risk of SCD.<sup>3,8,12-25</sup> However, mutations with a poor prognosis have been described in genes initially related to less aggressive forms, a fact that illustrates the difficulty to predict phenotype from genotype. The purpose of our study was to identify the prevalence and phenotypic characteristics of mutations in 5

sarcomeric genes (*MYH7*, *MYBPC3*, *TNNT2*, *TNNI3*, and *TPMI*) observed in patients in the Spanish regions of Asturias and Cantabria.

## METHODS

### Patients

We studied 120 unrelated patients who had been diagnosed between 2002 and 2007 by cardiologists at the Hospital Universitario Central de Asturias and the Hospital Universitario Marqués de Valdecilla in Santander. The diagnosis was performed following the American College of Cardiology/European Society of Cardiology (ACC/ESC) criteria, using as an inclusion criteria a left wall thickness  $>15$  mm on echocardiography when no other cause explained the hypertrophy.<sup>26</sup> The clinical characteristics of the patients are shown in Table 1.

Patients with any relative diagnosed with HCM were considered familial cases and patients with no record of any relatives with the condition were considered sporadic cases. In patients with a mutation, the presence of the mutation in all relatives who agreed to participate in the study was determined, regardless of whether they had symptoms of the disease or not; those found to have the mutation underwent an echocardiographic study.

The study was approved by the Clinical Research Ethics Committee of the Hospital Universitario Central de Asturias and all participants gave written informed consent to be included in the study.

### Genetic Testing

The polymerase chain reaction was used to amplify the exons and flanking intronic bases of the *MYH7* (38 exons), *MYBPC3* (34 exons), *TNNT2* (15 exons), *TNNI3* (9 exons), and *TPMI* (9 exons) genes. The primers used for the polymerase chain reaction were designed using reference sequences deposited in the GenBank database. Each fragment of the polymerase chain reaction product was purified and sequenced by BigDye chemistry in an ABI310 unit (Applied Biosystems; Foster City, California, United States) (Figure 1). The mutations and polymorphisms found in the 5 sarcomeric genes were named by following the criteria of the Cardiogenomics database ([www.cardiogenomics.org](http://www.cardiogenomics.org)). Information on the primers and amplification conditions can be obtained from the authors at the correspondence address.

The mutations in the *TNNT2* (15 exons) and *TNNI3* (9 exons) genes in 115 of the 120 cases, and in selected exons of *MYH7* and *MYBPC3* in some patients have already been reported.<sup>27-29</sup>

**TABLE 1. Clinical and Echocardiographic Characteristics and Treatment of the 120 Patients Included in the Study, Patients With MYH7 or MYBPC3 Mutations, and Patients Without Mutations in Any of the 5 Genes**

	Patients (n=120)	MYH7 (n=10)	MYBPC3 (n=18)	No Mutation (n=88)
Men/women (% men)	78/42 (65)	5/5 (50)	13/5 (72)	58/30 (67)
Age at onset of symptoms, y	43 (17, 3-76)	35 (17, 3-51)	42 (18, 8-72)	44 (17, 8-76)
Family history <sup>a,b</sup>	35 (29)	7 (70)	7 (39)	18 (20)
SCD	21 (17)	3 (30)	4 (22)	12 (13)
Sporadic	85 (71)	3 (30)	11 (61)	70 (79)
Presentation (clinical symptoms)				
Dyspnea <sup>c</sup>	83 (69)	8 (80)	9 (50)	64 (73)
NYHA index, n (%)				
Class I-II	60 (49)	3 (30)	5 (28)	52 (56)
Class III-IV	21 (18)	4 (40)	4 (22)	13 (15)
Angina	42 (35)	2 (20)	4 (22)	33 (35)
Syncope	22 (18)	2 (20)	2 (11)	18 (19)
Atrial fibrillation	23 (18)	2 (20)	4 (22)	17 (18)
Arrhythmia (Holter monitoring)	28 (22)	1 (10)	1 (6)	26 (28)
Echocardiographic data				
Interventricular septum, mm	20 (6, 13-35)	21 (5, 16-29)	22 (5, 17-35)	19 (6, 13-35)
Gradient, mm Hg	56 (47)	6 (60)	7 (39)	47 (53)
LVOT >30 mm Hg	35 (29)	4 (40)	6 (33)	25 (28)
Treatment				
Pharmacologic	84 (70)	6 (60)	11 (61)	65 (74)
Pacemaker	7 (6)	1 (10)	0	6 (7)
Cardiac defibrillator	3 (2)	0	0	3 (3)
Myectomy	1 (1)	1 (10)	0	0
Heart transplant	5 (4)	2 (20)	0	3 (3)

HCM indicates hypertrophic cardiomyopathy; IVS/PW, interventricular septum/posterior wall; LVOT, left ventricular outflow tract gradient; NYHA, New York Heart Association functional class; SCD, sudden cardiac death (before age 50).

<sup>a</sup>First-degree relatives diagnosed with HCM and/or SCD.

<sup>b</sup> $P = .05$ , patients with MYH7 versus no mutation.

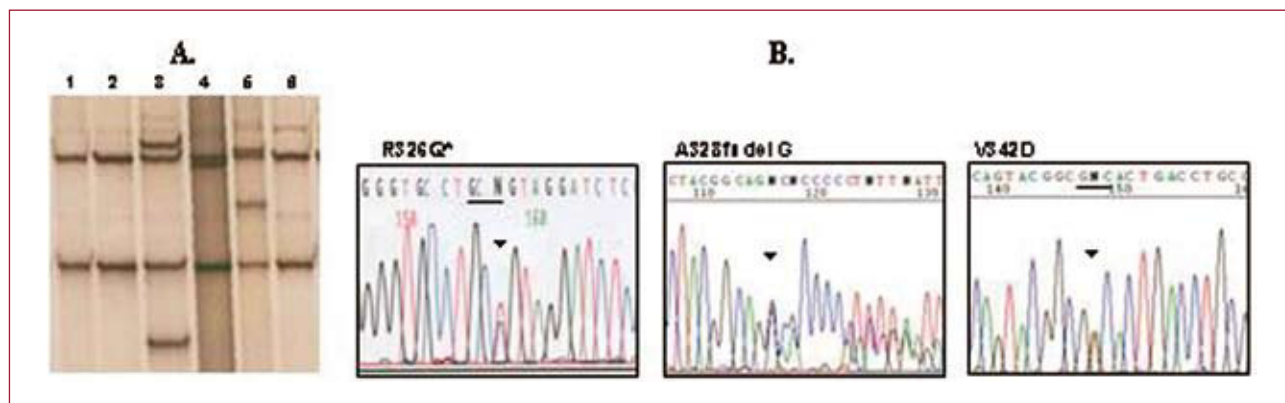
<sup>c</sup> $P = .02$ , patients with MYH7 versus MYBPC3.

Values are expressed as the mean (SD, interval) or n (%). Among patients with MYBPC3, 2 who had mutations have not been included. The arrhythmias observed during Holter monitoring included atrial fibrillation, sustained and nonsustained ventricular tachycardias, supraventricular and sustained tachycardia, and atrioventricular block.

### Study of Control Subjects

Several conditions must be met to consider a nucleotide change in a gene as a mutation associated with the development of a disease. First, the change

must alter the amino acid sequence of the protein. Second, the mutation must be found in all the affected persons of a single family; thus, we can exclude the pathogenic effect of a change if an affected person has not inherited it. Moreover,



**Figure 1.** A: electrophoretic patterns in single-strand conformation analysis gels for the exon 13 fragment of the MYBPC3 gene. Lanes 1, 2, and 6 correspond to normal (unmutated) sequences. In lane 3, the pattern corresponds to the V342D change (mutation); in lane 4, to the R326Q change (polymorphism), and in lane 5, to the A328fs deletion (mutation). B: sequences of fragments with the 3 nucleotide variants of exon 13 of MYBPC3 (\*reverse strand sequence).

**TABLE 2. Characteristics of the Patients With Mutations in MYH7, TNNT2, and TPM1**

Gene	Exon	Mutation	Position <sup>a</sup>	Sex/Age, y	Family History of HCM/SCD	Presentation	IVS/PW, mm	Carriers/Studied	Hypertrophy	Reference
MYH7	3	S4L	c.c10t	M/40	No	Syncopal	16/16	0/2	0	This study
MYH7	9	F247L	c.c1739c	M/35	Yes (HCM)	Syncopal, CA, AF	19/17	1/1	1	This study
MYH7	14	R453C	c.c1357t	F/25	Yes (HCM + SCD)	Dyspnea, AF, HT	17/12	1/1	1	33
MYH7	16	A583V	c.c1748t	M/16	Yes (HCM + SCD)	Dyspnea, angina	23/12	2/4	2	This study
MYH7	18	R663H	c.g1988a	M/51	Yes (HCM + SCD)	Dyspnea, angina	29/12	2/3	2	30,33
MYH7	20	R723G	c.c2167g	M/31	Yes (HCM)	Dyspnea	23/15	0/2	0	This study
MYH7	21	R787C	c.c2359t	M/51	Yes (HCM)	Dyspnea	19/13	0/4	0	This study
MYH7	22	M822V	c.g2464a	F/3	No	Dyspnea, MR, HT	18/18	0/3	0	27,33
MYH7	22	R870H	c.g2609a	F/40	No	Dyspnea, myectomy	24/22	1/1	1	30,33
MYH7	32	K1459N	c.g4377t	F/56	Yes (HCM)	Dyspnea	19/19	0/2	0	30
TNNT2	9	R92Q	c.g275a	M/40	Yes (SCD)	Dyspnea	19/10	1/1	0	33
TNNT2	16	R278C	c.c832t	F/49	No	Dyspnea, angina	22/12	3/7	1	33
TPM1	5	D175N	c.g522a	F/41	Yes (HCM)	Dyspnea, angina, SVT	32/13	2/4	2	33

AF indicates atrial fibrillation; CA, cardiac arrest; F, female; HT, heart transplantation; IVS/PW, interventricular septum/posterior wall; M, male; MR, mitral regurgitation; SCD, sudden cardiac death; SVT, supraventricular tachycardia.

<sup>a</sup>We considered A on the first encoding codon (ATG, Met) as nucleotide 1.

The carrier/studied column indicates the number of carriers and persons genetically tested in each family (excluding the index case). The hypertrophy column indicates the number of these carriers with hypertrophy.

carriers of the mutation would be more likely to develop the disease and, therefore, should not be found among people with no symptoms of the disease.

All variants found in the patients and not listed in the databases as polymorphisms were analyzed in 200 healthy subjects who had given written informed consent to participate in the study. All were older than 18 years of age and had no symptoms of cardiovascular disease, but had not undergone an echocardiographic study; therefore, asymptomatic hypertrophy cannot be ruled out. Each fragment found to have a nucleotide change that could be a mutation was amplified in the patient who presented it and in the 200 control subjects. The electrophoretic migration pattern was then analyzed in nondenaturing polyacrylamide gels using the single strand conformation analysis (SSCA) technique and following a previously described protocol (Figure 1).<sup>27,28</sup>

### Degree of Amino Acid Conservation Between Species

The mutations affect amino acids that are important for the structure and function of the protein, which would limit its evolutionary divergence. All nucleotide changes that modified the protein sequence and were not found in the control subjects were considered possible pathogenic mutations. As an additional criterion of involvement in the disease, we determined the degree of conservation between humans, chimpanzees, and mice, comparing the sequences of the 3 species

deposited in the ENSEMBL database ([www.ensembl.org](http://www.ensembl.org)).

### Statistical Analysis

The SPSS<sup>TM</sup> statistical program, version 11.0, was used for the statistical analyses. Analysis of variance (ANOVA) and the Mann-Whitney *U* test were used to compare continuous variables, and the  $\chi^2$  test was used for the discrete variables. A *P* value less than .05 was considered significant for all analyses.

## RESULTS

In 109 patients, HCM was suspected on the basis of clinical manifestations (exertional dyspnea, palpitations, angina, or syncope) and in 11, because electrocardiographic abnormalities were observed during a routine medical examination. Of the 120 patients, 35 (29%) had at least 1 relative who also had experienced SCD. Thirty-one mutations were found in 32 patients: 10 with mutation in *MYH7*, 20 in *MYBPC3*, 2 in *TNNT2*, and 1 in *TPM1* (Tables 2 and 3). Two *MYBPC3* mutations (G263X and E542Q) were found in more than 1 patient, 1 had 2 mutations (R278C-TNNT2 and R733H-MYBPC3), and another was homozygous for the A627V mutation in *MYBPC3*.

### Mutations in MYH7

Of the 120 patients, 10 (8%) had gene mutations for beta myosin heavy chain (Table 2). Nine mutations were located in the first 22 exons of the

**TABLE 3. Characteristics of the Patients With Mutations in MYBPC3**

Exon	Mutation	Position <sup>a</sup>	Sex/Age, y	Family History of HCM	Presentation	IVS/PW, mm	Carriers/ Studied	Hypertrophy	Reference
7	Y237C	c.a710g	M/33	Yes (HCM)	Aortic murmur	35/30	2/4	1	This study
8	G263X	c.g787t	M/32	No	Incidental diagnosis, preexcitation syndrome	19/16	3/3	0	This study
8	G263X	c.g787t	M/49	Yes (HCM + SCD)	Asymptomatic	17/11	0/1	0	This study
13	A328fs del G	c.g982c	M/30	No	Dyspnea, mitral murmur	24/17	2/4	0	This study
13	V342D	c.t1025a	M/43	No	Incidental diagnosis	17/13	2/3	1	33
14	Q404fs del C	c.c1210a	F/31	Yes (HCM + SCD)	Dyspnea, angina	23/10	0/2	0	This study
18	R495W	c.c1483t	M/17	No	Incidental diagnosis	27/10	3/3	1	This study
18	G531R	c.c1591a	F/72	No	Dyspnea, mitral murmur, AF	20/13	1/1	0	33
18	G532fs del G	c.g1595c	F/59	No	Dyspnea, AF, angina	17/10	0/2	0	This study
18	E542Q	c.g1624c	M/43	No	Fatigue	18/13	1/2	0	33
18	E542Q	c.g1624c	M/57	No	Angina, murmur	20/14	0/0	0	33
20	A627V	c.c1880t	M/16	Yes (SCD)	Dyspnea, angina, HT	28/17	3/5	1	33
24	R726C	c.c2176t	F/68	No	Asymptomatic	24/12	0/0	0	This study
24	R733H	c.g2190a	F/49	No	Dyspnea, angina	22/12	4/7	0	This study
25	V771M	c.g2311a	M/8	Yes (HCM)	Dyspnea, syncope	18/11	2/5	1	33
26	M844fs ins GA	c.t2531g	F/60	Yes (SCD)	Dyspnea, fatigue	17/17	8/9	3	This study
27	R891fs ins G	c.c2671g	M/44	No	Dyspnea	18/15	1/1	1	This study
29	Q998E	c.c2992g	M/37	Yes (SCD)	Syncope, palpitations	22/14	0/0	0	33
30	R1022S	c.c3064a	M/31	Yes (HCM)	Dyspnea, AF	25/21	1/3	1	This study
32	R1138H	c.g3413a	M/60	No	Dyspnea, AF, angina	19/19	0/1	0	This study

AF indicates atrial fibrillation; CA, cardiac arrest; F, female; HT, heart transplantation; IVS/PW, interventricular septum/posterior wall; M, male; MR, mitral regurgitation; SCD, sudden cardiac death; SVT, supraventricular tachycardia.

<sup>a</sup>We considered A on the first encoding codon (ATG, Met) as nucleotide 1.

The index case is included among the carriers. The carrier/studied column indicates the number of carriers and persons genetically tested in each family (excluding the index case). The hypertrophy column indicates the number of these carriers with hypertrophy.

gene, and the K1459N change was found in the protein tail. Four of the mutations have already been described. The mean age at diagnosis of these 10 patients was 35 years (Table 1), and 7 (70%) had a family history of the disease. Patients with the R453C, A583V, and R663H mutation had relatives who had experienced early SCD (before age 50). The V822M mutation was found in a woman diagnosed at 3 years of age. The mutation was not found in either parent and, therefore, would be considered de novo. The patients with R453C and V822M had received transplants at age 43 and 22, respectively.

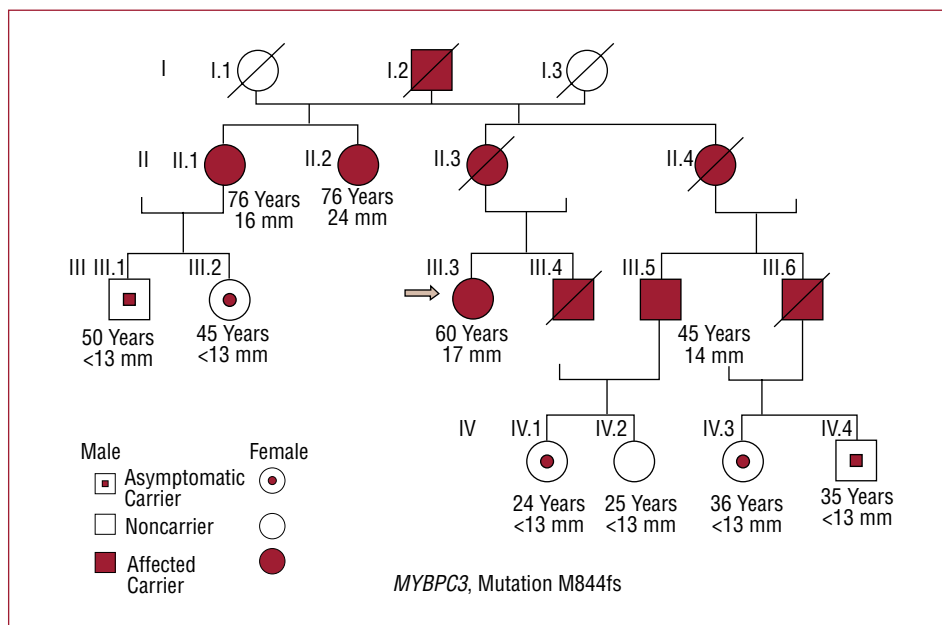
### Mutations in MYBPC3

We found 18 mutations in *MYBPC3* in 20 of the 120 (16%) patients (Table 3). Only 6 (33%) of the 18 were known. All *MYBPC3* mutations affected amino acid residues that were conserved between species. Five were frameshift changes due to nucleotide insertion/deletion (A328fs del G, Q404fs del C, G532fs del G, M844fs ins GA, and R891fs ins G): 1 was a stop codon and 13 were amino acid changes. The mean age at diagnosis in these patients was 42 years, and 8 (40%) had a family history of HCM and/or SCD (Table 1). In 10 of the sporadic

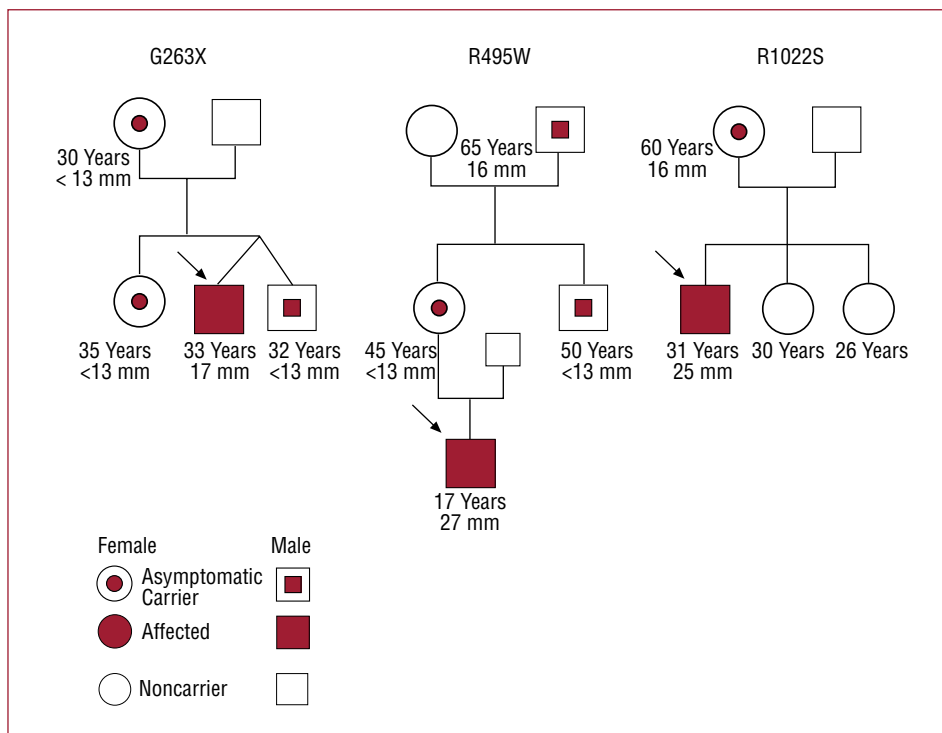
cases, we were able to study some of the relatives and found several asymptomatic carriers (Table 3).

One patient, who was homozygous for the mutation, was the only patient with a mutation in *MYBPC3* who had received a heart transplant. Two relatives who carried the mutation were clinically asymptomatic and had no hypertrophy. M844fs was identified in 1 patient with 8 relatives who were also carriers, although only 3 had clinical symptoms of the disease (Figure 2). A patient with the R773H mutation also had the TNNT2-R278C mutation and is described below.

Three young patients experienced exertional dyspnea while engaging in sports (Figure 3). In these cases, mutations G263X, R495W, and R1022S were identified. Patient G263X was 32 years old and had hypertrophy of 19 mm; his mother, sister, and a twin brother also had the mutation, but were clinically asymptomatic and had no hypertrophy. Patient R495W was 17 years old and had a septal wall thickness of 27 mm; his mother, uncle, and grandfather were also carriers, but only the grandfather had hypertrophy (18 mm) at age 85. Patient R1022S was 31 years old and had a septum of 25 mm; his mother also had the mutation, but showed no clinical symptoms, except for a septum of 18 mm.



**Figure 2.** Family tree with M844fs mutation in the *MYBPC3* gene. The index case is indicated with an arrow. Three of this patient's relatives were also carriers of the mutation, had clinical symptoms of the disease, and hypertrophy of 14, 17, and 24 mm. Another 5 carriers had no clinical symptoms or hypertrophy.



**Figure 3.** Families of 3 sporadic cases with mutations in *MYBPC3*. The index cases are indicated with an arrow. All 3 were regularly involved in sports and were diagnosed after presenting symptoms associated with physical exercise. In the families of the R495WS and R1022S patients, there was another carrier without symptoms of the disease, but with cardiac hypertrophy.

### Mutations in *TNNT2*, *TNNI3*, and *TPM1*

In 2 (1.66%) of the 120 patients, we found the R92Q and R278C mutations in *TNNT2* (Table 2). The case with R278C also had the R733H mutation in *MYBPC3*. In the *TPM1* gene, the D175N mutation was found in 1 patient diagnosed at age 41 with severe hypertrophy (32 mm) (Table 2). His son and his brother had this mutation and a hypertrophy of 27 and 20 mm.

### Double Mutants

A woman diagnosed at age 49 had 2 mutations: R278C in *TNNT2* and R733H in *MYBPC3*. A 40-year-old daughter and a 6-year-old granddaughter were double carriers, but were asymptomatic; 2 daughters age 47 and 42 were carriers of the R733H mutation, but also had no clinical symptoms or hypertrophy. A 52-year-old sister with the R278C mutation had mild symptoms and a septal thickness of 13 mm.

## Genotype-Phenotype Correlation

The clinical and echocardiographic characteristics were compared according to mutated gene (Table 1). The mean age at diagnosis was lower in patients with mutations in *MYH7* compared with *MYBPC3*, although the difference was not significant. Patients with no mutations were older on average than patients with mutations, although the differences were not significant. Hypertrophy was 21 (5) mm in the *MYH7* patients, 22 (5) mm in the *MYBPC3* patients, and 19 (6) mm in patients without a mutation. A history of the disease was reported by 70% of *MYH7* patients, compared with 40% of *MYBPC3* patients and 18% of patients with no mutation.

## Polymorphisms

In addition to the mutations, various changes unrelated to the disease (polymorphisms) were found in the 5 genes. All these nucleotide changes were also identified in the control subjects. In *MYH7*, 27 polymorphisms were found; 23 were in the exons and only 1 involved an amino acid change (S1491C). In *MYBPC3*, 24 polymorphisms were found, 11 of them in exons, of which 5 showed amino acid changes: R17Q, S236G, R326Q, W382R, and V896M. Information on the changes in these genes can be requested from the authors at the correspondence address.

## DISCUSSION

Our study is the first to analyze the complete sequence of the 5 most commonly mutated sarcomeric genes in HCM in a large series of Spanish patients. Previous reports have investigated all the *MYH7* exons in large series and several exons of various genes in small case studies.<sup>27-32</sup>

The most commonly mutated gene was *MYBPC3* (16% of cases), followed by *MYH7* (8%), and *TNNT2* and *TPMI* (<2%). We found a lower incidence of mutations, compared to those described by other authors. Most of those studies were conducted at referral hospitals that had received patients with severe forms of HCM who would have been more likely to have a family history of the disease. In our study, only 29% of cases had a history of HCM and/or SCD, whereas other studies had up to 90% of familial forms.<sup>8,12</sup> The frequency of sarcomeric mutations would be higher among patients with a family history of the disease, and the lower number of mutations identified in our study could be due to a higher frequency of sporadic cases. Additionally, no mutations were found in 43% of patients with a family history of HCM, which indicates that other

genes could explain the familial segregation in these cases.

The low frequency of mutations in *MYH7* (8%) has been described by other authors, such as Laredo et al<sup>30</sup> in patients from Galicia. Of the patients with *MYBPC3* mutations, 61% were sporadic, compared with only 30% of those with a *MYH7* mutation. Higher penetration has been reported<sup>3,8,12,15,33</sup> for *MYH7* mutations, which would increase the probability that the disease will manifest in the carriers of each family. Patients with *MYH7* mutations would manifest the disease at an earlier age and would have a higher degree of hypertrophy, a more malignant phenotype, and a poorer prognosis.<sup>8</sup> Although we found a younger age at onset of the disease among *MYH7* patients, the difference with *MYBPC3* patients and cases with no mutation was not statistically significant, probably because of the smaller number of patients with mutations. Moreover, no differences were found in the interventricular septal thickness between the 3 groups. The low frequency of mutations in the thin-filament encoding genes *TNNT2* and *TPMI* (<2%) is similar to that described by other groups.<sup>8</sup>

The initial classification of the mutations as “malignant” or “benign” has been refined in more recent studies that have shown the difficulty of stratifying the prognosis for most mutations.<sup>8,12-14</sup> Two of our cases illustrate this clinical heterogeneity, even among carriers in the same family. A woman with 2 mutations in *TNN2* and *MYBPC3* had been diagnosed due to angina and dyspnea at age 49 and had hypertrophy of 22 mm. A daughter and a granddaughter were also double carriers, but were clinically asymptomatic and had no hypertrophy. This indicates that the presence of 2 sarcomeric mutations would not necessarily be associated with an early, severe manifestation of the disease. In another family with the M844fs mutation in *MYBPC3*, 9 carriers were identified, of which only 3 had hypertrophy. Some carriers remained asymptomatic into an advanced age, but 2 had suffered sudden cardiac death before age 50. These cases indicate that the clinical manifestations are a result of genetic and nongenetic risk factors and, therefore, the genetic information of each patient should not be used as the only basis to establish the prognosis. Nevertheless, individuals with no heart disease but carriers of sarcomeric gene mutations could manifest symptoms at a later date and, therefore, should undergo periodic assessments to avoid the adverse effects of HCM.

Three patients were diagnosed by ultrasound, performed to investigate fatigue associated with physical exercise. The genetic study identified 3 mutations in *MYBPC3* in their 3 families, in which we found asymptomatic carriers. These 3 cases

indicate that some mutations, particularly in *MYBPC3*, could have low penetration, but that physical exercise would accelerate the development of symptoms and hypertrophy among the carriers. In athletes with no history of the disease, HCM may indicate the presence of a sarcomeric mutation, probably in *MYBPC3*.

All mutations in *MYH7* would translate into changes in a single amino acid, whereas *MYBPC3* also had frameshift changes. Moreover, polymorphisms with an amino acid change were less common in *MYH7*. This indicates selective pressure against mutations that modify various amino acids of the beta myosin heavy chain that would lead to a high risk of early death; hence, they would not be observed in adults with HCM.<sup>8</sup>

Finally, half the mutations in *MYH7* (5/10) and most of those found in *MYBPC3* (13/18) have not been previously described.<sup>33</sup> Only 2 of the 11 mutations found by Laredo et al<sup>30</sup> (R663H and K1459N) were also found in our patients. This indicates that direct analyses of known mutations would not be highly useful because they do not identify mutations in true carrier cases.<sup>34</sup> Complete sequencing of the sarcomeric genes is necessary in these cases to definitively exclude the presence of any mutation.

## CONCLUSIONS

In an analysis of the 5 most commonly mutated sarcomeric genes in HCM in a series of 120 patients in the regions of Asturias and Cantabria, mutations were found in 26% of cases. The most commonly mutated gene was *MYBPC3*, followed by *MYH7*, *TNNT2*, and *TPMI*. More than half the mutations have not been described. We found no mutations in *TNNI3*. There were no differences in the mean age at diagnosis or in the interventricular septal thickness between the *MYH7* and *MYBPC3* carriers. Our study illustrates the difficulty to define the prognosis in carriers with mutations in these genes.

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