

Beta-Myosin Heavy Chain Gene Mutations in Patients With Hypertrophic Cardiomyopathy

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Introduction and objectives. To determine the frequency of mutations in the beta-myosin heavy-chain gene (*MYH7*) in a cohort of patients with hypertrophic cardiomyopathy (HCM) and their families, and to investigate correlations between genotype and phenotype.

Methods. Single-strand conformation polymorphism analysis and sequencing of fragments with abnormal *MYH7* gene mobility were carried out in 128 consecutive index patients with HCM. The phenotypes of patients with and without mutations were compared and the phenotypes of identified families were recorded.

Results. A total of 11 mutations were found in 13 families (10%); 7/11 had been previously described. The I736T mutation was found in 3 families and the A797T in 2. One patient had 2 mutations (i.e., I736T and R787H). Mutations were more frequent in patients with a family history of sudden death (31%) and in those with severe hypertrophy (39% had a thickness ≥ 30 mm). Mutations were found in 29 of 42 members of the 13 families, including 6 family members (20%) who were healthy carriers and aged ≤ 36 years. Sudden death had occurred in 8 members of 4 families: four in 2 families with the I736T mutation, 1 in a family with A797T, 1 in a family with R870H, and 2 in a family with A901P.

Conclusions. *MYH7* mutations were present in 10% of our families. Mutations were more frequent in patients with a family history of sudden death and in those with severe hypertrophy. Most mutations had been described previously. Some appeared in several families. For some mutations, the correlation between genotype and phenotype was stable, while for others, there were marked differences between the phenotypes of the index

patients and their relatives, suggesting the presence of additional genetic factors that have yet to be identified.

Key words: *Cardiomyopathy. Hypertrophy. Genetics. MYH7.*

Mutaciones en el gen de la cadena pesada de la betamiosina en pacientes con miocardiopatía hipertrófica

Introducción y objetivos. Determinar la frecuencia de mutaciones en el gen de la cadena pesada de la beta-miosina (*MYH7*) en una cohorte de pacientes con miocardiopatía hipertrófica (MCH) y en sus familiares, y analizar la correlación entre genotipo y fenotipo.

Métodos. Detección de polimorfismo en la conformación de hebras monocatenarias y secuenciación de fragmentos con movilidad anormal del gen *MYH7* en 128 casos índice consecutivos con MCH. Comparación de fenotipo entre pacientes con y sin mutaciones y descripción del fenotipo de las familias identificadas.

Resultados. Identificamos 11 mutaciones en 13 familias (10%), 7/11 previamente descritas. La mutación I736T se identificó en 3 familias y la A797T en 2. Un caso presentó 2 mutaciones (I736T y R787H). Las mutaciones fueron más frecuentes en pacientes con antecedentes familiares de muerte súbita (31%) y con hipertrofia severa (39% con grosor ≥ 30 mm). Había mutación en 29 de 42 miembros de las 13 familias, incluidos 6 (20%) portadores sanos (edad ≤ 36 años). Había antecedentes de muerte súbita en 9 familiares de 4 familias (4 en 2 familias con I736T, uno con A797T, uno con R870H y 2 con A901P).

Conclusiones. Las mutaciones en *MYH7* aparecen en un 10% de nuestras familias y son más frecuentes cuando hay antecedentes familiares de muerte súbita o hipertrofia severa. La mayor parte había sido descrita previamente y algunas se repiten en varias familias. Ciertas mutaciones muestran una correlación genotipo-fenotipo estable, mientras que en otras, las marcadas diferencias entre casos índice y familiares hacen sospechar la presencia de factores genéticos adicionales que debemos identificar.

Palabras clave: *Miocardiopatía. Hipertrofia. Genética. MYH7.*

SEE EDITORIAL ON PAGES 994-6

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ABBREVIATIONS

HCM: hypertrophic cardiomyopathy.
SSCP: single strand conformation polymorphism.
LVW: left ventricular wall.

INTRODUCTION

Hypertrophic cardiomyopathy (HCM) is a primary disease of the myocardium caused by mutations in the genes that code for sarcomeric proteins.¹⁻⁷ It is believed that mutations in the gene for the beta-myosin heavy chain (*MYH7*) cause between 15% and 30% of all cases of HCM.⁸⁻¹² The reported frequency of mutations identified in *MYH7* has, however, varied from study to study, and very little information is available regarding the Spanish population.^{13,14} In addition, knowledge on the correlations between the genotypes for the different mutations and their associated phenotypes is very limited. The aim of the present study was to analyze the frequency of mutations in the gene coding for the beta-myosin heavy chain in a large population of patients with HCM, and to determine the genotype-phenotype relationships for those identified.

METHODS

Study Subjects

The study subjects were 128 consecutive index patients with HCM. These patients belonged to a cohort of more than 400 with HCM who were monitored at a cardiomyopathy clinic belonging to a tertiary hospital serving a population of 500 000. Nearly all the patients diagnosed with HCM in this population are monitored at this clinic, which also monitors patients referred from other centers in the Autonomous Region of Galicia (northwestern Spain; total population 2 500 000). Hypertrophic cardiomyopathy was diagnosed according to the criteria of the WHO and the Myocardial and Pericardial Disease Working Group of the European Society of Cardiology.^{1,2} The clinical study of the patients and their family members included an inspection of their clinical background, a physical examination, an electrocardiogram (ECG), an echocardiogram, Holter monitoring (in affected patients), ergometric testing (in affected patients), a hemogram, a complete blood test, and the collection of peripheral blood samples for DNA extraction and the separation of plasma and serum. All patients gave their informed consent to be included in the study, which was approved by the ethics committee of the participating institutions.

Analysis of Mutations

DNA was isolated from samples of peripheral blood using the GFX Genomic Blood DNA Purification Kit (Amersham Biosciences, Sweden).¹⁵ The coding sequence of the *MYH7* gene was amplified (between exons and 3 and 40).¹⁶⁻¹⁸ The primers used were based on the intronic flanking regions, using the GenBank sequence with accession number AJ238393 as a reference. The exons were analyzed by single strand conformation polymorphism (SSCP) analysis using the GeneGel SSCP Starter Kit (Amersham Pharmacia Biotech, Sweden). Samples with abnormal mobility patterns were reamplified, purified and sequenced. A change in the amino acid sequence compared to the reference sequence¹⁶⁻¹⁹ was considered a pathogenic mutation when the following three criteria were met^{8,20}: when the mutation was carried only by family members with HCM and not by healthy relatives, when it was not present in 200 chromosomes from non-related healthy people (100 healthy, non-related individuals), and when the affected amino acid was conserved between species and myosin isoforms. Rare allelic forms were considered to be those changes that did not segregate with the disease and which were not found in the control population. Polymorphisms considered not to be associated with HCM were those changes in the sequence that were present in the control population. Mutations were independently confirmed by restriction length fragment polymorphism (RFLP) or amplified refractory mutation system (ARMS) analysis.

Genotype-Phenotype Correlations

The demographic, clinical and echocardiographic characteristics of the index case individuals with and without mutations of *MYH7* were studied. The family members of the patients in whom mutations were identified were invited to undergo a clinical study, an ECG, an echocardiographic analysis, and genetic analysis. The phenotypic characteristics of the mutation carriers in each family were recorded.

Statistical Analysis

All statistical operations were performed with SPSS v.12 software. The differences between patients with and without mutations of *MYH7* were analyzed using the χ^2 test for categorical variables, and the non-parametric Mann-Whitney U test for continuous variables. Significance was set at $P < .05$ (two-tailed) for all tests.

RESULTS

Mutations Identified

Eleven mutations were detected (Table 1), 7 of which have been previously described.^{8,9,11,21-32} These

TABLE 1. Mutations Identified*

Mutation	Position	Exon	Location in <i>MYHC</i>	Confirmation	Status	No. of Families
M388T	t8772c	13	Head	NlaIII	New	1
R442C	c9057t	14	Head	HhaI	New	1
R663H	g11252a	18	Head	HhaI	Described	1
I736T	t12315c	20	Head	Hpy8I	Described	3
G768R	g12684a	21	Head	MspI	Described	1
R787H	g12742a	21	Head	MluI	Described	1
A797P	g127716	21	Head	ARMS I	New	1
A797T	g12771a	21	Head	HphI	Described	2
R870H	g13248a	22	Neck	Acil	Described	1
A901P	g13961c	23	Neck	Bpu10I	New	1
K1459N	g20750t	32	Tail	Tsp509I	Described	1

*A indicates alanine; C, cysteine; G, glycine; H, histidine; I, isoleucine; K, lysine; M, methionine; N, asparagine; P, proline; R, arginine; T, threonine.

TABLE 2. Polymorphisms Found in *MYH7*

Exon	Sequence	Amino Acid
Exon 3	gtc-gtg	Val52Val
	acc-act	Thr63Thr
Exon 5	ctc-ctg	Leu120Leu
Exon 7	gca-gcg	Ala199Ala
Exon 8	ttt-ttc	Phe244Phe
Exon 9	acc-aca	Thr265Thr
Exon 11	gac-gat	Asp325Asp
Exon 12	ggc-ggt	Gly354Gly
	gcc-gct	Ala355Ala
	aag-aaa	Lys365Lys
	gag-gaa	Glu374Glu
	cca-ccg	Pro375Pro
	gac-gat	Asp376Asp
Exon 14	gca-gct	Ala426Ala
Exon 15	atc-att	Ile524Ile
Exon 16	gaa-gag	Glu535Glu
Exon 19	ggc-ggg	Gly682Gly
Exon 23	gct-gcc	Ala917Ala
	ttg-ctg	Leu957Leu
Exon 24	att-atc	Ile989Ile
	gtg-gtc	Val1025Val
	caa-cag	Gln1030Gln
Exon 25	ctg-ctc	Leu1038Leu
Exon 26	gca-gct	Ala1092Ala
	cag-caa	Gln1112Gln
Exon 27	ctg-cta	Leu1135Leu
Exon 30	tgc-tgt	Cys1340Cys
Exon 32	tcc-tgc	Ser1491Cys
Exon 33	acc-act	Thr1522Thr
Exon 35	gcg-gcc	Ala1702Ala
Exon 37	gaa-gag	Glu1787Glu

mutations affected 13 of the 128 families studied (10.2%). Mutation I736T was detected in 3 families and A797T in 2. One patient had 2 mutations: I736T and R787H. One rare allelic variant (R1781H, exon 37) was detected that affected a residue in the tail domain of the beta-myosin heavy chain, as well as 32 polymorphisms not associated with HCM (i.e., they were found in the control population). Only one of the latter was associated with an amino acid

TABLE 3. Characteristics of Index Cases With and Without an *MYH7* Mutation*

	With (n=13)	Without (n=115)	P
Age at diagnosis, y	44 (19)	51 (16)	.17
Age at start of monitoring, y	50 (18)	53 (16)	.4
Male sex	38%	65%	.07
Family background of HCM	62%	25%	.01
Family background of SD	31%	7%	.02
HBP	23%	39%	.4
Initial	NYHA		
I-II	92%	89%	.4
III-IV	8%	115%	
NYHA III-IV at some time	46%	30%	.3
Angina	62%	41%	.4
Syncope	15%	17%	1
Non-sustained VT	46%	30%	.3
Abnormal BP response	67%	35%	.08
Maximum thickness LVW, mm	27 (8)	22 (6)	.08
Maximum thickness LVW/BSA, mm/m ²	15 (5)	12 (3)	.009
Maximum thickness ≥ 30 mm	39%	9%	.009
LVTD/SC, mm/m ²	23 (3)	25 (5)	.2
LVSV/SC, mm/m ²	12 (3)	15 (4)	.006
Ejection fraction, %	80 (8)	70 (12)	.0004
Left atrium, mm	50 (14)	47 (8)	.7
Gradient ≥ 30 mm Hg	31%	28%	1

*LVTD indicates left ventricular end-diastolic volume; LVSV, left ventricular end-systolic volume; HBP, high blood pressure; HCM, hypertrophic cardiomyopathy; SD, sudden death; NYHA, New York Heart Association functional class; BSA, body surface area; BP, blood pressure; VT, ventricular tachycardia; LVW, left ventricular wall.

change compared to the reference sequence (Table 2).

Clinical Factors Associated With the Presence of Mutations in *MYH7*

Table 3 shows the characteristics of the patients with and without *MYH7* mutations. Mutations were identified in 22% of the patients with a family

TABLE 4. Genotype Phenotype Correlations: I736T and R787H*

Family	Case	Phenotype	Genotype	Age at Diagnosis, y	Sex	Maximum Thickness, mm	Morphology	Obstruction	Treatment	Comment, age
A	II:1	+	(?)	(?)	F	(?)	(?)	(?)	PM	SD(57)
A	II:3	+	I736Ta	–	M	–	–	(?)	PM	SD(52)
A	II:5	+	I736T	52	M	32	ASH	+	Medical	NYHAIII(60)
A	II:7	+	(?)	–	M	–	–	(?)	–	CVA
A	III:1	+	I736T	32	M	18b	ASH	+	Myectomy	MCD.NYHAIII(45)
A	III:4	+	(?)	<30	F	28	ASH	+	Medical	NYHAII(32)
A	III:6	–	–	–	M	12	–	–	No	–
A	III:7	–	–	–	M	11	–	–	No	–
A	III:9	–	(?)	–	M	9	–	–	No	Normal(21)
A	IV:1	–	I736T	16	F	10	–	–	No	Healthycarrier(16)
A	IV:2	–	–	–	M	–	–	–	No	–
B	II:1	(?)	(?)	–	M	–	–	(?)	No	SD(57)
B	II:2	(?)	(?)	–	M	–	–	(?)	No	SD(52)
B	II:4	+	I736T	76	F	35	ASH	+	Medical	NYHAIII(80)
B	III:7	–	–	–	F	–	–	(?)	No	–
C	I:2	+	(?)	43	F	17a	ASH	+	Myectomy andPM	DeathHF(69)
C	II:3	+	I736T+R787H	43	F	30	ASH	–	Medical	NYHAII(47)
C	III:1	+/-	I736T	21	M	11	–	–	–	Asymptomatic(22)
C	III:2	+	I736T	16	M	13	ASH	–	–	Asymptomatic(17)

*CVA indicates cerebrovascular accident; ASH, asymmetric septal hypertrophy; HF, heart failure; I736T, isoleucine736threonine; PM, pacemaker; SD, sudden death.

†Obligate carrier.

††Post myectomy.

background of HCM, and in 33% of those with a family background of sudden death. One in every 3 patients with a left ventricular wall (LVW) thickness of ≥ 30 mm had a *MYH7* mutation. Patients with mutations had more severe hypertrophy, a smaller left ventricular end-systolic diameter, and a larger left ventricular ejection fraction. No significant differences

were seen in the age of patients with and without mutations, nor in the percentage of patients with high blood pressure. A trend was seen towards mutations being more common among women (Table 3). No significant differences were seen in terms of symptoms, medical treatment or the interventions performed.

TABLE 5. Genotype-Phenotype Correlation: A797P and A797T*

Family	Case	Phenotype	Genotype	Age at Diagnosis, y	Sex	Maximum Thickness, mm	Morphology	Obstruction	Treatment	Comment, Age
D	I:1	(?)	(?)	–	M	(?)	–	–	(?)	Cardiopathy (55)
D	I:2	(?)	(?)	–	F	(?)	–	–	(?)	Arrhythmias (65)
D	II:1	–	–	–	F	(?)	–	–	–	–
D	II:3	+	A797P	55	M	18	ASH	–	Medical	MF NYHA I (59)
D	II:5	+	A797P	34	F	19	ASH	–	Medical	NYHA I (36)
D	III:6	+	A797P	22	M	20	ASH	–	No	NYHA I (25)
D	III:7	+	A797P	15	F	8	–	–	No	NYHA I (22)
E	II:1	(?)	(?)	–	M	(?)	–	–	–	SD (22)
E	II:2	+	A797T	28	M	40	ASH	–	Medical	NYHA II (40)
F	I:1	(?)	(?)	–	M	–	–	–	–	Embolism (52)
F	I:2	–	(?)	–	F	–	–	–	–	–
F	II:1	+	A797T	45	F	14	ASH	–	No	NYHA I (46)
F	II:2	+	A797T	30	M	36	ASH	–	Medical	NYHA II (44)
F	II:4	–	–	–	F	–	–	–	–	–
F	II:5	–	A797T	36	M	10	–	–	No	Healthy carrier (37)
F	II:6	–	–	–	F	–	–	–	–	–
F	II:7	–	–	–	F	–	–	–	–	–
F	III:1	+	A797T	21	M	15	ASH	–	No	NYHA I (22)

*A797P indicates alanine797proline; A797T, alanine797tryptophan; ASH, asymmetric septal hypertrophy; MF, mitral failure; SD, sudden death.

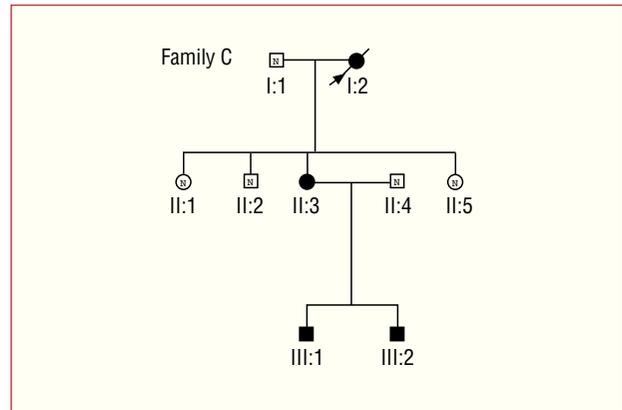
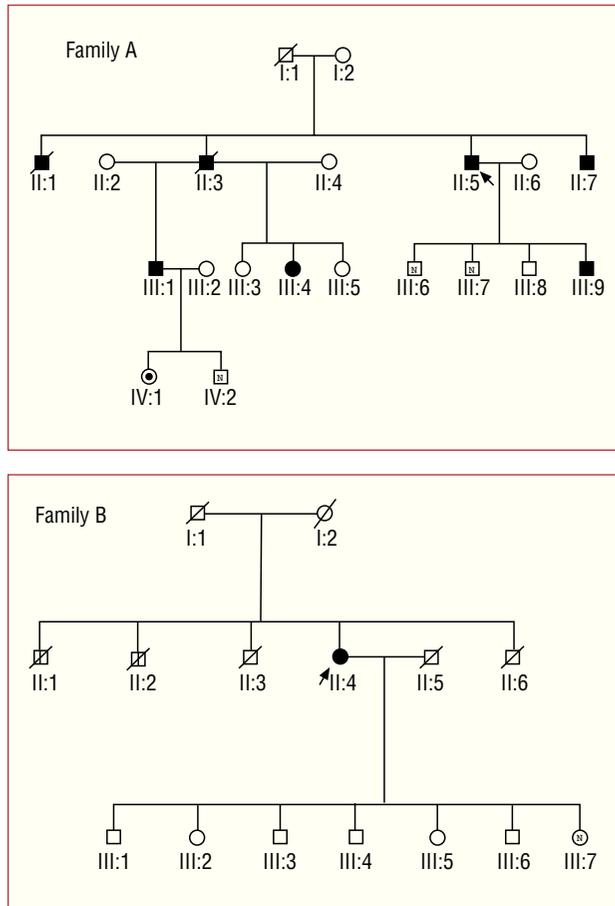


Figure 1. Trees for families A, B, and C, corresponding to Table 4. The squares are males, the circles females. Patients diagnosed with HCM are in black. Symbols marked with an N are individuals with no sign of HCM and who have no mutation. The symbols with a black dot at the center are carriers of a mutation but with no HCM phenotype. The symbols with a black vertical bar are subjects whose clinical backgrounds suggest HCM (not confirmed). The white symbols are subjects without HCM who were not subjected to genetic study. A diagonal line indicates that a subject is deceased. Arrows mark the index case patients. HCM indicates: hypertrophic cardiomyopathy.

Genotype-Phenotype Correlation

Tables 4-7 summarize the main clinical data of the index case patients and relatives belonging to the 13 families in which a mutation was identified. Genetic study results were available for 42 members of these 13 families: 29 carried mutations of the studied gene and

13 did not. Of the former, 6 (20%) were carriers with no positive diagnostic criteria for HCM (healthy carriers): 2 (a 16 and a 21 year-old) had the mutation I736T, 1 (aged 36 years) carried A797T, and 3 (aged 30, 33, and 34 years) carried the mutation K1459N. Eight members of 4 families died suddenly (4 sudden deaths in 2 families with mutation I736T, 1 with A797T, 1 with R870H, and 2 in 1 family with mutation A901P; none of these individuals had undergone genetic studies).

Mutation I736T (Table 4; Figure 1)

This mutation was found in 8 carriers belonging to 3 families. The majority of carriers over 30 years of age

TABLE 6. Genotype-Phenotype Correlation: M388T, R442C, R663H, and G768R*

Family	Case	Phenotype	Genotype	Age at Diagnosis, y	Sex	Maximum Thickness, mm	Morphology	Obstruction	Treatment	Comment, Age
G	II:2	-	-	-	F	-	-	-	-	-
G	III:1	+	M388T	38	M	13	ASH	-	No	NYHA I (42)
G	IV:1	-	-	-	M	-	-	-	-	-
H	II:1	+	G768R	28	M	26	ASH	-	Medical	Death restrictive HF (50)
H	II:4	(?)	(?)	(?)	M	(?)	(?)	(?)	Surgery	Heart surgery (<40)
H	III:3	-	-	-	M	-	-	-	-	-
I	II:3	+	R442C	50	F	22	Atypical	-	Medical	Angina-AF (53)
I	III:1	+	(?)	29	M	13	Conc.	-	-	Asymptomatic (29)
J	IV:1	+	R663H	23	F	42	ASH	+	Medical	Asymptomatic (25)
J	III:1	+	(?)	55	F	15	ASH	-	Medical	NYHA II (57)

*AF indicates atrial fibrillation; G768R, glutamine768arginine; M388T, methionine388threonine; R442C, arginine442cysteine; R663H, arginine663histidine; ASH, asymmetric septal hypertrophy; HF, heart failure; Conc., concentric.

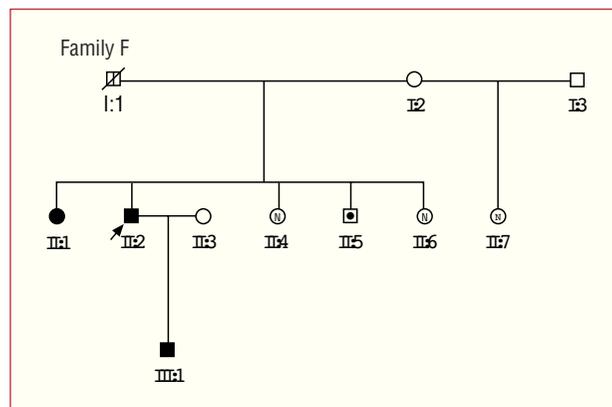
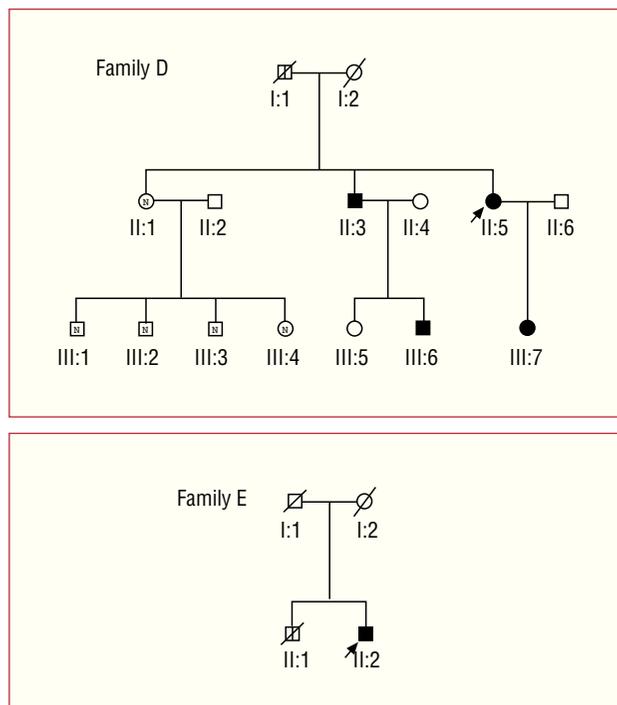


Figure 2. Trees for families D, E, and F, corresponding to Table 5. The squares are males, the circles females. Patients diagnosed with HCM are in black. Symbols marked with an N are individuals with no sign of HCM and who have no mutation. The symbols with a black dot at the center are carriers of a mutation but with no HCM phenotype. The symbols with a black vertical bar are subjects whose clinical backgrounds suggest HCM (not confirmed). The white symbols are subjects without HCM who were not subjected to genetic study. A diagonal line indicates that a subject is deceased. Arrows mark the index case patients. HCM indicates hypertrophic cardiomyopathy.

showed severe LVW hypertrophy; 2 of these individuals had undergone myectomy (1 of these required a heart transplant due to systolic dysfunction 13 years later, and 1 died at 69 years of age due to heart failure). In families A and B there were 4 sudden deaths in men aged 50-60 years who had not previously been diagnosed with HCM.

Mutation R787H (Table 4; Figure 1)

This mutation was identified in a woman who also had a I736T mutation. She suffered severe LVW hypertrophy; her mother had undergone a myectomy.

Mutations A797P and A797T (Table 5; Figure 2)

Mutation A797T, which has been associated with HCM, was identified in 2 families. The carriers of this mutation showed a very variable degree of disease severity: 2 carriers (aged 28 and 30 years—the index case patients of families E and F) showed a maximum LVW thickness of 36-40 mm, 2 (aged 21 and 45 years) showed thicknesses of 14 and 15 mm respectively, and 1 carrier (36 years old) showed no signs of HCM. One member of family E (with no diagnosis of HCM) had died suddenly at 22 years of age; 1 member of family F died of an embolism at age 52.

Mutation A797P was identified in 4 patients in family D. The echocardiograph of a 15 year-old carrier was normal but the ECG was diagnostic of HCM (pathological Q waves associated with the anterolateral wall and a high QRS complex voltage).

In the remaining carriers, moderate hypertrophy affected the septum and the basal and mid anterior LVW. The oldest patient showed dilation of the left atrium and mitral ring, and required interventionist surgery for severe symptoms of mitral regurgitation accompanied by systolic dysfunction.

Mutation M388T (Table 6; Figure 3)

The index case patient was a woman who at 54 was diagnosed with severe LVW hypertrophy. She was implanted with a defibrillator for sustained monomorphic ventricular tachycardia with syncope at age 62. At the age of 40 her son presented with mild LVW hypertrophy and an ECG confirming a pathological process (hypertrophy of the left ventricle plus negative T waves associated with the anterolateral wall).

Mutation G768R (Table 6; Figure 3)

This mutation was identified in a patient with non-obstructive HCM but with restrictive behavior who died in hepatic coma while awaiting a heart-liver transplant.

Mutation R442C (Table 6; Figure 3)

This mutation was identified in a woman with hypertrophy localized to the lower LVW wall (an atypical distribution) who suffered thoracic pain in relation with atrial fibrillation. Her son, a sportsman,

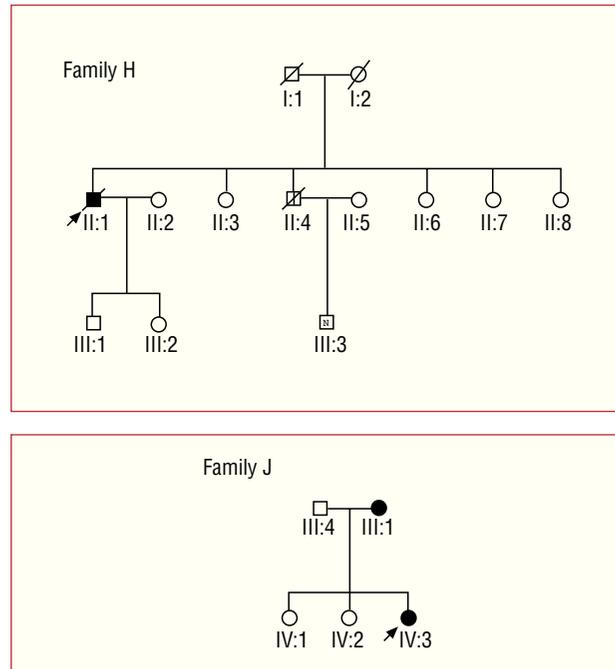
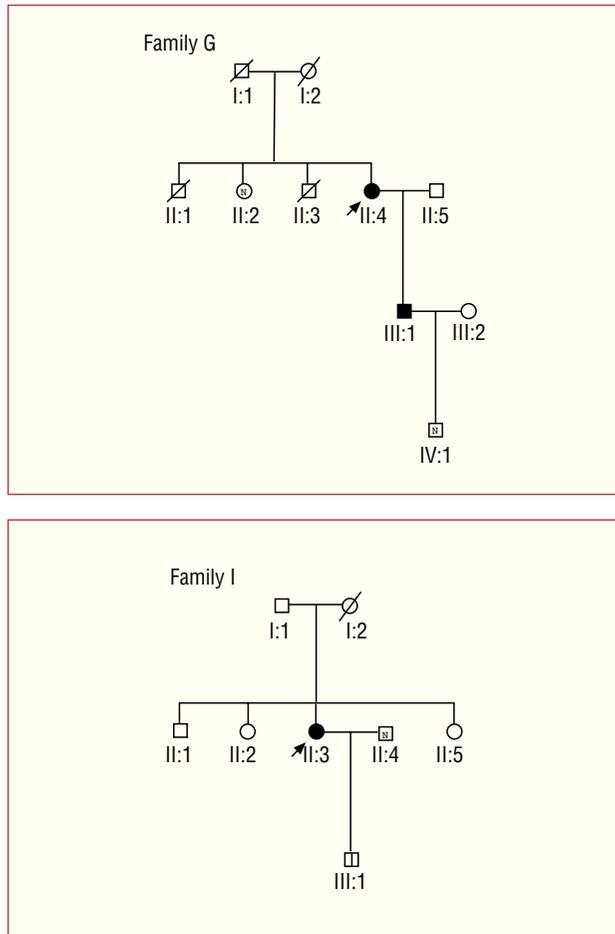


Figure 3. Trees for families G, H, I, and J, corresponding to Table 6. The squares are males, the circles females. Patients diagnosed with HCM are in black. Symbols marked with an N are individuals with no sign of HCM and who have no mutation. The symbols with a black dot at the center are carriers of a mutation but with no HCM phenotype. The symbols with a black vertical bar are subjects whose clinical backgrounds suggest HCM (not confirmed). The white symbols are subjects without HCM who were not subjected to genetic study. A diagonal line indicates that a subject is deceased. Arrows mark the index case patients. HCM indicates hypertrophic cardiomyopathy.

showed mild concentric LVW hypertrophy; his ECG showed negative T waves.

Mutation R663H (Table 6; Figure 3)

This mutation was identified in a woman of 23 years with severe LVW hypertrophy (42 mm) and severe subaortic obstruction. The study of her family led to her mother being diagnosed with HCM with mild septal hypertrophy (15 mm).

Mutations in the Neck and Tail Domains of the Beta-Myosin Heavy Chain (Table 7; Figure 4)

This mutation was identified in a woman aged 59 years who suffered moderate, non-obstructive LVW hypertrophy but who had preserved systolic function. One of her brothers had non-obstructive HCM with moderate LVW hypertrophy which, after 12 years of monitoring, developed into severe systolic dysfunction, parietal thinning, and sudden death. The mutation A901P was detected in a woman with severe LVW hypertrophy (27 mm); 2 of her family members (aged 15 and 45 years) had died suddenly, and her

cousin (female) with HCM died of unclear reasons at age 35. The patient also experienced bouts of non-sustained ventricular tachycardia during Holter monitoring and had abnormal blood pressure during a stress test; it was recommended she be implanted with a defibrillator.

Mutation K1459N was detected in 5 members of a single family. Three of these individuals (aged 30, 31, and 33 years) showed a normal phenotype. The index case patient was a woman diagnosed at the age of 76 with a maximum LVW thickness of 27 mm. One of her daughters had HCM with mild LVW hypertrophy (15 mm) but was asymptomatic. Another daughter died at age 53; she suffered heart failure and had been diagnosed with HCM.

DISCUSSION

Earlier studies have suggested that mutations of MYH7 cause between 15% and 30% of all cases of hypertrophic cardiomyopathy.^{2,8,9} In the present patients, the mutations of this gene were less common, and appeared in 10% of the families studied. This may have several explanations. Firstly, the mutation

TABLE 7. Genotype-Phenotype Correlation: R870H, A901P, and K1459N*

Family	Case	Phenotype	Genotype	Age at Diagnosis, y	Sex	Maximum Thickness, mm	Morphology	Obstruction	Treatment	Comment, Age
K	II:2	+	R870H	59	F	20	ASH	-	Medical	NYHA II (65)
K	II:6	+	(?)	38	M	22	ASH	-	Medical	SD; systolic dysfunction (50)
L	II:2	+	(?)	(?)	F	(?)	(?)	(?)	(?)	Cardiac death (35)
L	II:3	+	(?)	(?)	M	15	(?)	(?)	(?)	Alive (<60)
L	III:1	+	(?)	(?)	M	(?)	(?)	(?)	(?)	-
L	III:5	+	(?)	(?)	M	(?)	(?)	(?)	(?)	MS (45)
L	III:7	+	A901P	30	F	27	ASH	+	Medical	NYHA II (47)
L	IV:1	+	(?)	(?)	F	(?)	(?)	(?)	(?)	MS (15)
M	I:2	+	K1459N	76	F	27	ASH	-	Medical	NYHA II (81)
M	II:2	+	(?)	(?)	F	(?)	(?)	(?)	(?)	Death HF (53)
M	II:3	+	K1459N	48	F	15	ASH	-	No	NYHA I (55)
M	III:1	-	K1459N	34	F	9	-	-	No	Healthy carrier (34)
M	III:2	-	K1459N	33	F	7	-	-	No	Healthy carrier (33)
M	III:3	-	-	31	M	11	-	-	-	-
M	III:4	-	K1459N	30	F	11	-	-	No	Healthy carrier (30)
M	III:5	-	-	-	F	10	-	-	-	-
M	III:6	-	-	-	M	-	-	-	-	-

*A901P indicates alanine901proline; ASH, asymmetric septal hypertrophy; HF, heart failure; K1459N, lysine1495asparagine; SD, sudden death; R870H, arginine870histidine.

frequency depends on the population selection bias. The present work shows that when subgroups of patients with severe LVW hypertrophy who have a family background of hypertrophic cardiomyopathy (which implies a greater penetrance of these mutations

or earlier clinical manifestations of disease) or who have a family background of sudden death are studied, the mutation frequency for *MYH7* is greater, reaching as high as 30% (Table 3). Therefore, it is to be expected that studies performed in large reference

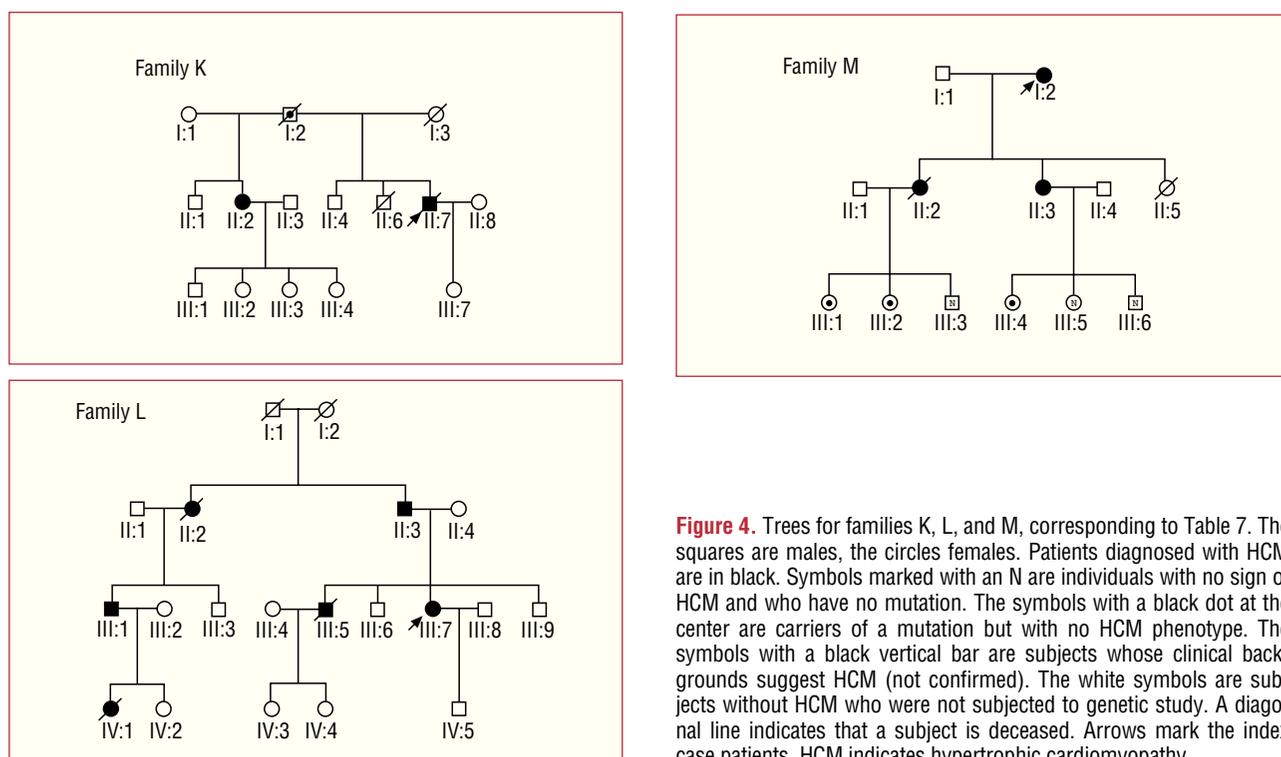


Figure 4. Trees for families K, L, and M, corresponding to Table 7. The squares are males, the circles females. Patients diagnosed with HCM are in black. Symbols marked with an N are individuals with no sign of HCM and who have no mutation. The symbols with a black dot at the center are carriers of a mutation but with no HCM phenotype. The symbols with a black vertical bar are subjects whose clinical backgrounds suggest HCM (not confirmed). The white symbols are subjects without HCM who were not subjected to genetic study. A diagonal line indicates that a subject is deceased. Arrows mark the index case patients. HCM indicates hypertrophic cardiomyopathy.

centers that receive referred patients with serious disease will detect the highest mutation frequencies. The present cohort of patients, however, represented a regional, much less selected population, most of whose members came from the health area of our hospital. Secondly, although no significant differences were found between patients with and without *MYH7* mutations in terms of age at the time of diagnosis, the mean age of the former was 44 years and that of the latter 51 years. Other authors have shown that the mutation frequency of this gene is very low in patients who are of advanced age when they are diagnosed,³³ and it should be borne in mind that the mean age at diagnosis in the present patients was 10 years older than in other studies. Thirdly, the mutation frequency of a gene can vary in different populations, and in some areas the high frequency of certain mutations renders the proportion of mutations identified in other genes much reduced.^{34,35}

An interesting finding from the present study was the higher mutation frequency among women. The normal pattern of inheritance of HCM is autosomal dominant, and it would therefore be expected that 50% of the patients be women. However, in the present study, and indeed in all others, the number of affected women only amounted to 30% or 40% of the total number of individuals—yet they showed more symptomatic disease.³⁶ The identification of a greater number of mutations among women, plus the finding that these mutations appear with greater frequency in patients with severe LVW hypertrophy, confirms that the difference in prevalence in HCM between the sexes is due to the fact that the disease is only noticed in women when it is more severe.

Currently, many authors insist that most of the mutations identified in patients with HCM are “private mutations” or new mutations. The present work, a systematic study of the *MYH7* gene, confirms that a high percentage of the mutations usually identified have already been described (7 out of 11 in the present study). Two of the mutations identified in the present work appeared in several supposedly non-related families. The identification of mutations in different families allows a more precise evaluation of the correlation between genotype and phenotype, and a more adequate interpretation of the pathogenic role of each mutation. Several findings in the present study indicate the importance of undertaking complete family studies. For some mutations, such as 1736T, the phenotype was reproduced similarly in the majority of carriers, while the phenotypes of the index patients carrying A797T or R663H (severe LVW hypertrophy in young patients) and their carrier family members (mild hypertrophy) were quite different, despite the latter’s similar or even greater ages. Additional genetic or environmental factors may explain this difference in expression. Some studies

have reported that patients with HCM can possess more than 1 mutation, and that double mutations are associated with a more severe expression of the disease.⁸ In the present work, which focuses on *MYH7* alone, 1 patient was found to have 2 mutations known to be associated with the development of HCM; mutations in other genes might, therefore, also be expected in other patients.

Most studies on the presence of mutations in *MYH7* have been limited to an analysis of the regions coding for the head and neck domains of the beta-myosin heavy chain. Recent investigations have also shown that mutations affecting the tail domain of the protein may also be associated with HCM. In the present work, the mutation K1459N was identified and found to affect this domain, but it appears not to hinder the formation of supercoiled helices. This may explain its association with a benign phenotype, its incomplete penetrance, and its late expression.

Of the mutations identified in the neck domain, A901P had not been previously described. This mutation was not found in 200 chromosomes from control individuals; it was therefore not a variant of this population. The alpha-helical supercoiled structure that characterizes the neck domain of the beta-myosin heavy chain is completely altered due to the presence of a proline residue—which is incompatible with this type of structure. This serious modification of the protein structure can, in part, explain the severity of the phenotype in the family studied, which had seen many sudden deaths among its members.

For the 13 families studied, a total of eight sudden deaths were recorded. Only for one of these patients was a genetic diagnosis available (an obligate carrier). Such a lack of genetic information is normal, and represents an important limitation when trying to interpret the prognostic significance of the different mutations identified in family members. It is important to remember that patients who experience sudden death may have different characteristics, for example they may have additional mutations. The systematic collection of samples from patients with HCM for genetic analysis, the clinical monitoring of these individuals, and the most complete study of their families possible, are essential if solid conclusions are to be drawn on the prognostic implications of the different mutations.

Limitations

The study involved PCMS and the sequencing of fragments with abnormal motility. The sensitivity of PCMS is some 84%-89%,^{37,38} meaning some mutations could have remained undetected.

Although the study of the families of patients with mutations was as large as possible, it was not

complete. No samples were available for their deceased members, some people declined to take part, and some were not informed of the study by the index case patient.

The study focused on the gene *MYH7*; the possible influence of the phenotypes arising from the mutation of other genes related to HCM was not assessed.

Mutations of the gene *MYH7* were found to be a relatively common cause of HCM. The chances of finding mutations in this gene is greater in patients with a family background of sudden death and severe LVW hypertrophy. The majority of the mutations identified (7 of 11) had already been described in other populations; the same mutations were seen in a number of families. The present work thus identifies four new mutations associated with HCM. Some mutations showed a relatively stable genotype-phenotype correlation, while others were associated with marked differences in phenotype between the index case patient and his/her family members. This suggests that other genetic or environmental factors are also involved.

Genetic studies are essential for understanding the great clinical and prognostic heterogeneity of HCM. For genetics to be useful in clinical decision-making, it is essential that detailed information on the clinical and morphological characteristics of the carriers of different mutations be known.

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