Rev Esp Cardiol. 2016;69(2):149–158

Original article

Clinical and Prognostic Profiles of Cardiomyopathies Caused by Mutations in the Troponin T Gene

Tomás Ripoll-Vera,[a,*] José María Gámez,[a] Nancy Govea,[b] Yolanda Gómez,[a] Juana Núñez,[a] Lorenzo Socías,[a] Ángela Escandell,[a] and Jorge Rosell[b]

[a] Servicio de Cardiología, Hospital Son Llàtzer, IdISPa, Ciberobn, Palma de Mallorca, Balearic Islands, Spain
[b] Sección de Genética, Hospital Son Espases, Palma de Mallorca, Balearic Islands, Spain

Article history:
Received 23 March 2015
Accepted 29 June 2015
Available online 24 October 2015

Keywords:
Cardiomyopathy
Genetics
Genetic mutation
Troponin
Hypertrophy
Heart failure
Sudden death

ABSTRACT

Introduction and aims: Mutations in the troponin T gene (*TTNT2*) have been associated in small studies with the development of hypertrophic cardiomyopathy characterized by a high risk of sudden death and mild hypertrophy. We describe the clinical course of patients carrying mutations in this gene.

Methods: We analyzed the clinical characteristics and prognosis of patients with mutations in the *TNNT2* gene who were seen in an inherited cardiac disease unit.

Results: Of 180 families with genetically studied cardiomyopathies, 21 families (11.7%) were identified as having mutations in *TNNT2*: 10 families had Arg92Gln, 5 had Arg286His, 3 had Arg278Cys, 1 had Arg92Trp, 1 had Arg94His, and 1 had Ile221Thr. Thirty-three additional genetic carriers were identified through family assessment. The study included 54 genetic carriers: 56% were male, and the mean average age was 41 ± 17 years. There were 33 cases of hypertrophic cardiomyopathy, 9 of dilated cardiomyopathy, and 1 of noncompaction cardiomyopathy, and maximal myocardial thickness was 18.5 ± 6 mm. Ventricular dysfunction was present in 30% of individuals and a history of sudden death in 62%. During follow-up, 4 patients died and 14 (33%) received a defibrillator (8 probands, 6 relatives). Mean survival was 54 years. Carriers of Arg92Gln had early disease development, high penetrance, a high risk of sudden death, a high rate of defibrillator implantation, and a high frequency of mixed phenotype.

Conclusions: Mutations in the *TNNT2* gene were more common in this series than in previous studies. The clinical and prognostic profiles depended on the mutation present. Carriers of the Arg92Gln mutation developed hypertrophic or dilated cardiomyopathy and had a significantly worse prognosis than those with other mutations in *TNNT2* or other sarcomeric genes.

Perfil clínico y pronóstico de las miocardiopatías causadas por mutaciones en el gen de la troponina T

Palabras clave:
Miocardiopatía
Genética
Mutación génica
Troponina
Hipertrofia
Insuficiencia cardiaca
Muerte súbita

RESUMEN

Introducción y objetivos: Las mutaciones en el gen de la troponina T *(TNNT2)* se han asociado en pequeños estudios al desarrollo de miocardiopatía hipertrófica caracterizada por alto riesgo de muerte súbita e hipertrofia leve. Se describe el curso clínico de los pacientes portadores de mutaciones en este gen.

Métodos: Se analizaron las características clínicas y el pronóstico de los sujetos con mutaciones en el gen *TNNT2* atendidos en una unidad de cardiopatías familiares.

Resultados: A partir de 180 familias con miocardiopatías estudiadas genéticamente, se identificó a 21 (11,7%) con mutaciones en *TNNT2:* 10 familias Arg92Gln, 5 Arg286His, 3 Arg278Cys, 1 Arg92Trp, 1 Arg94His y 1 Ile221Thr. A través de la evaluación familiar se identificó a 33 portadores genéticos adicionales. El estudio incluyó a 54 portadores genéticos: el 56% varones con una media de edad de 41 ± 17 años; 33 miocardiopatías hipertróficas, 9 dilatadas y 1 no compactada, con grosor máximo de 18,5 ± 6 mm; con disfunción ventricular el 30% y antecedentes de muerte súbita el 62%. En el seguimiento 4 fallecieron y 14 (33%) recibieron un desfibrilador (8 probandos, 6 familiares). La supervivencia media fue de 54 años. Los portadores de Arg92Gln tuvieron desarrollo precoz, alta penetrancia, alto riesgo de muerte súbita, alta tasa de implante de desfibrilador y alta frecuencia de fenotipo mixto.

* Corresponding author: Servicio de Cardiología, Hospital Son Llàtzer, Ctra. Manacor Km. 4, 07198 Palma de Mallorca, Balearic Islands, Spain.
E-mail address: tripoll@hsll.es (T. Ripoll-Vera).

http://dx.doi.org/10.1016/j.rec.2015.06.025
1885-5857/

Conclusiones: Las mutaciones en el gen *TNNT2* fueron más frecuentes en esta serie. Su perfil clínico y pronóstico depende de la mutación hallada. Los portadores de la mutación Arg92Gln desarrollaron miocardiopatía hipertrófica o dilatada y tuvieron un pronóstico significativamente peor que con otras mutaciones en *TNNT2* u otros genes sarcoméricos.

Abbreviations

DCM: dilated cardiomyopathy
HCM: hypertrophic cardiomyopathy
ICD: implantable cardioverter-defibrillator
MMT: maximal myocardial thickness
SCD: sudden cardiac death

INTRODUCTION

Hypertrophic cardiomyopathy (HCM) is an inherited autosomal-dominant disease with a heterogeneous clinical presentation and natural history,[1] and is a frequent cause of sudden cardiac death (SCD) in young people[2–4]; it is associated with mutations in genes coding for sarcomere proteins.[5–7] In the literature, debate surrounds the genotype-phenotype correlation of individual mutations,[7,8] concerning establishing a prognosis according to the mutation present, which could help stratify the disease and allow appropriate genetic counselling to families. Mutations in the troponin T gene (*TNNT2*) were described years ago in several publications with few families, and researchers postulated a high prevalence of SCD in young carriers[5,6,9,10] who, in addition, had a phenotype of mild left ventricular hypertrophy.[6,11]

This study aimed to describe the clinical course of a series of patients and relatives—a relatively large series considering the low prevalence of the disease—who were carriers of mutations in *TNNT2* and to expand existing knowledge on their prognosis.

METHODS

The cohort was made up of apparently unrelated probands with cardiomyopathy, most with an HCM phenotype. They were assessed in a familial heart disease clinic in *Hospital Son Llàtzer* (Palma de Mallorca, Balearic Islands, Spain) over a 7-year period, undergoing genetic study of mutations in the *TNNT2* gene (and 4 other sarcomeric genes: *MYBPC3, MYH7, TNNI3*, and *TPM1*, as well as lamin A/C if the proband had a dilated cardiomyopathy [DCM] phenotype). All relatives of carriers were offered clinical and genetic assessment.

Hypertrophic cardiomyopathy was diagnosed when the maximal myocardial thickness (MMT) was $\geq$ 15 mm in at least 1 segment in the absence of other diseases to explain the hypertrophy.[12,13] In probands with SCD as the first clinical manifestation, the diagnosis of HCM was confirmed at autopsy whenever possible. Relatives were considered affected when they met the HCM familial criteria ($\geq$ 13 mm).[14]

All patients and relatives underwent electrocardiography, echocardiography, stress testing, and 24 hours Holter monitoring, as per the methods described,[15] as well as cardiac magnetic resonance whenever possible.

The main risk factors for SCD were defined as a family history of SCD, syncopal episode of arrhythmic origin or unknown etiology, nonsustained ventricular tachycardia $\geq$ 120 bpm, MMT $\geq$ 30 mm and abnormal blood pressure response to exercise (in those younger than 40 years).[16]

The *TNNT2* gene was sequenced with Sanger sequencing or next generation sequencing (NGS). Of 21 probands, 19 were studied using Sanger (the 5 main sarcomeric genes were sequenced: *MYBPC3, MYH7, TNNT2, TNNI3*, and *TPM1*) and 2 were studied with NGS (in one patient, 12 genes: the 5 sarcomeric genes plus *ACTC1, GLA, MYL2, MYL3, PRKAG2, PTPN11*, and *TNNC1*, and in the other patient, 27 genes: the previous 12 plus *CASQ2, DMD, DTNA, FKBP1A, KCNH2, LDB3, LMNA, MIB1, MYH6, NOTCH1, PLN, RYR2, SCN5A, TAZ*, and *TTN*, the latter combination because of the noncompaction phenotype). A change in the amino acid sequence compared with the reference sequence was considered a pathogenic mutation when it met the following criteria: it segregated in the affected family members, it was not present in 200 chromosomes from healthy unrelated individuals, it had not yet been identified in populations of thousands of individuals from different ethnic groups included in the 5000 Genomes Project (Exome Variant Server), the 1000 Genomes Project, or dbSNP (Short Genetic Variations database), and it affected a residue that is phylogenetically conserved between troponin T species and isoforms. An allelic variant was considered rare when segregation could not be demonstrated and the variant was not present in controls, and polymorphic when it was not associated with the disease and it was present in controls. The previously described variants were reviewed to assess their pathogenicity, and new mutations were studied with *in silico* tools.

Informed consent for DNA extraction was obtained from each individual. The study adhered to the principles of the Declaration of Helsinki, the Council of Europe Convention on Human Rights and Biomedicine, and the UNESCO Universal Declaration on the Human Genome and Human Rights.

Statistical analysis was performed using the SPSS application (v.15.0, SPSS Inc.; Chicago, Illinois, United States). Data with normal distribution are expressed as means (95% confidence interval). The differences between the means were compared using an unpaired 2-tailed Student *t* test. Categorical data were compared using a chi-square test. Continuous data with abnormal distribution were analyzed with a Mann-Whitney *U* test. The predefined outcomes for survival analysis were as follows: SCD, first appropriate shock from implantable cardioverter-defibrillator (ICD), death due to heart failure, cardiac transplant, and other cardiovascular death. The cumulative probability for the occurrence of an event was calculated using the Kaplan-Meier method. A comparison was performed with previously published data. Survival analysis from birth was also performed for individual mutations. A probability value of $P < .05$ was considered statistically significant.

RESULTS

We studied 180 consecutive unrelated probands with cardiomyopathy (15 HCM, 15 DCM, and 10 noncompacted) looking for

mutations in the *TNNT2* gene and in the 4 other main sarcomere genes (*MYBPC3, MHY7, TNNI3*, and *TPM1*); 21 probands (11.7%) had pathogenic mutations in *TNNT2*. Ninety-eight relatives gave consent for clinical examination (mean, 4.7 relatives/family), and 78 gave consent for genetic analysis of *TNNT2*; a mutation was found in 33 individuals (42%). The total number of probands plus relatives carrying *TNNT2* mutations was 54. Assuming that some first-degree relatives who did not undergo genetic study but who did have HCM had the same *TNNT2* mutation, the total was 68 patients: 57 affected individuals and 11 asymptomatic carriers.

In 21 families, 6 different mutations were identified: Arg92Gln in 10 families, Arg286His in 5, Arg278Cys in 3, and Arg92Trp, Arg94His, and Ile221Thr in 1 family each. All these variants except 1 (Ile221Thr) had already been published as causes of HCM.[8,14,15,17–19]

Double mutations were found in 4 probands (19%): 2 with the Arg278Cys mutation (1 with the Arg502Gln mutation in *MYBPC3* and 1 with Arg723Cys in *MHY7*), 1 proband with the Arg286His mutation and the Arg326Gln mutation in *MYBPC3*, and 1 proband with the Arg92Gln mutation, who was a carrier of a variant in *MYBPC3* that could have acted as a genetic modifier. No relatives were carriers of double mutations. The distribution of mutations and patients was as follows (Figure 1): 30 individuals had Arg92Gln (55.6%), 8 had Arg278Cys (14.8%), 8 had Arg286His (14.8%), 4 had Arg92Trp (7.4%), 3 had Arg94His (5.6%), and 1 had Ile221Thr (1.8%).

All affected patients underwent risk stratification for SCD. Thirteen probands (62%), and 100% of families (n = 10) with the Arg92Gln mutation had a history of SCD.

Electrocardiograms and echocardiograms were studied at the first assessment of all mutation carriers, except for 3 patients (because of SCD being the clinical presentation). The electrocardiograms were generally abnormal (voltage criteria for left ventricular hypertrophy with negative T-waves in precordial and inferior leads), sometimes with only mild abnormalities on echocardiography.

At the first assessment, 9 of the patients with the Arg92Gln mutation had a DCM phenotype with severe left ventricular dysfunction. One affected individual with Arg92Gln had a noncompaction cardiomyopathy phenotype. The others had HCM (Tables 1 and 2).

In the analysis of the 57 individuals with cardiomyopathy, the mean age of presentation was 37 ± 17 years; 30 (56%) were male. The initial symptoms were dyspnea in 10 individuals (24%), SCD in 5 (12%), chest pain in 2 (5%), and syncope in 1 (2%); the others were asymptomatic and diagnosis was made following familial screening in 17 (40%) and routine electrocardiography in 7 (17%). Complications during follow-up consisted of ventricular tachycardia or ventricular fibrillation in 3 individuals (7%), heart failure in 17 (40%), syncope in 6 (14%), chest pain in 5 (12%), stroke in 3 (7%), and atrial fibrillation in 11 (24%).

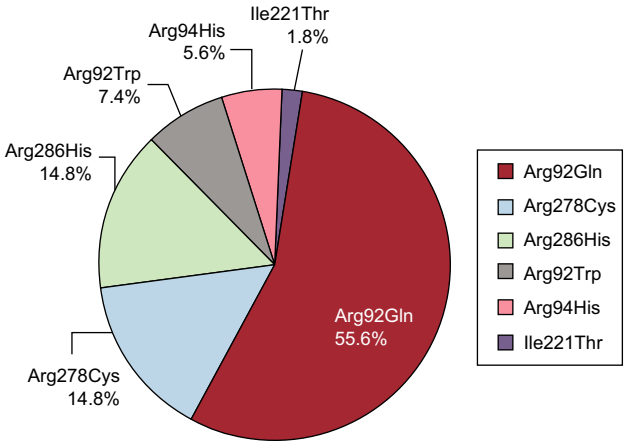

Figure 1. Distribution of mutations and percentage of patients.

The mean MMT was 18.4 ± 6 (8-35) mm. Patients with the Arg92Gln mutation had a mean MMT of 15.8 ± 4 mm. Only 3 (7%) had left ventricular outflow tract obstruction > 30 mmHg (1 patient each with the Arg278Cys, Arg286His and Ile221Thr mutations). There was a high prevalence of left ventricular systolic dysfunction, which was present in 12 patients (31%): 10 with the Arg92Gln mutation, 1 with Arg92Trp, and 1 with Arg94His. The mean left atrial diameter was 43 ± 9 mm. Cardiac magnetic resonance was performed in 17 patients, 15 of whom (88%) had extensive late gadolinium enhancement.

All patients were followed up (mean, 5 ± 2.5 years). No significant changes were observed in cardiac dimensions or systolic function, independently of cardiac phenotype (HCM, DCM, or noncompaction).

Fourteen individuals (33%) had an ICD implanted: 8 probands (6 as primary prevention and 2 as secondary prevention) and 6 relatives (5 as primary prevention and 1 as secondary prevention). Of 14 patients with ICD, 11 had the Arg92Gln mutation; 1 had Arg92Trp; 1 had Arg278Cys (double mutation in *MYBPC3*), and 1 had Arg94His.

Three patients with Arg92Gln required a biventricular device for congestive heart failure, and 2 required a dual chamber pacemaker due to sinus dysfunction or atrioventricular block. No patients required myectomy or cardiac transplantation.

During follow-up, 3 patients died: 1 due to heart failure at 60 years (patient with severe left ventricular dysfunction and dilatation, Arg92Gln), another due to stroke (Arg94His), and the third of unknown cause (patient with chronic heart failure and ventricular dysfunction, Arg92Gln phenotype and relatives with Arg92Gln mutation, but no genetic confirmation).

Three patients had at least 1 appropriate ICD shock: 2 with Arg92Gln and 1 with Arg278Cys (this patient had a double mutation in *MYBPC3).*

Regarding the 11 deaths related to *TNNT2* mutations—the total of SCDs from the pedigree analysis (n = 6), SCDs as first presentation of the disease (n = 3), and the deaths during follow-up (n = 2)—6 (54.5%) were related to sport, 9 (81.8%) were male, and the mean age was 21.7 ± 10.9 years. Seven (63.6%) had an HCM phenotype, 2 (18.2%) had a DCM phenotype, and 2 (18.2%) had an unknown phenotype. The mean MMT was 14.6 ± 5 mm. Two of the patients who died had had an episode of atrial fibrillation. In the Arg92Gln group, 100% of the group had a family history of SCD vs 28.6% of the group with other *TNNT2* mutations distinct from Arg92Gln ($P = .008$). Sudden cardiac death was the first presentation of disease in 6 patients from the Arg92Gln group; no patients in the group without Arg92Gln had SCD as the first presentation of disease. Also, 3 recoveries from SCD were documented in the group with Arg92Gln and none were documented in the group without. Regarding appropriate ICD therapies, these occurred in 3 patients from the group with Arg92Gln and 1 in the group without. During follow up, 2 patients from the Arg92Gln group died; there were no deaths related to other mutations.

Regarding penetrance, 50% of individuals in the Arg92Gln group had a positive phenotype at 37 years (Figure 2).

Survival from birth was calculated for carriers of *TNNT2* mutations, including relatives identified from a family tree who had HCM or were obligate carriers. The Arg92Gln mutation was associated with a higher rate of SCD at a young age. The mean survival of patients with Arg92Gln was 54 years (95% confidence interval, 46-62 years), but decreased to 48 years if patients with

Table 1
Clinical and Genetic Data on Patients and Families Included in the Study

Family	*TNNT2* mutation	Age/ sex	Phenotype	Age at diagnosis, years	FHSD	Presentation	NYHA functionnal class	AF	VT	MMT, mm	LVOT obstruction	LA diameter, mm	LVEF	LGE	PM/ICD (age, years)	Appropriate ICD therapy	Died
1/Proband	Arg92Gln	22/M	DCM	22	Yes	SCD	I	No	No	12	No				No		Yes
1/Mother	Arg92Gln	70/F	DCM	60		Dyspnea	II	Yes	No	11	No	44	40		PM (60)		No
1/Sister	Arg92Gln	42/F	DCM	42		SCD	I	Yes	No	8	No				No		Yes
2/Proband	Arg92Gln	31/M	HCM	13	Yes	SCD	I	No	Yes	20	No	48	60		ICD (13)	Yes	No
2/Father	Arg92Gln	53/M	HCM	37		Family screening	II	Yes	Yes	20	No	52	55		ICD (38)	Yes	No
2/Aunt	Arg92Gln	45/F	HCM	28		Family screening	III	Yes	No	22	No	50	60	Yes	No		No
3/Proband	Arg92Gln	64/F	DCM	50	Yes	Dyspnea	II	Yes	No	14	No	50	45	Yes	No		No
3/Sister	Arg92Gln	40/F	HCM	34		Family screening	I	No	No	14	No	46	60	Yes	No		No
3/Grandson*	Arg92Gln	8/M	—			—	I	No	No	7	No	25	65		No		No
3/Brother	Arg92Gln	76/M	DCM	69		Dyspnea	IV	Yes	Yes		No		30		No		Yes
3/Nephew	Arg92Gln	45/M	HCM	35		Family screening	I	No	No	17	No	33	48	Yes	No		No
3/Niece	Arg92Gln	52/F	HCM	43		Family screening	I	No	No	14	No	45	60		No		No
3/Grand-nephew	Arg92Gln	21/M	HCM	20		Family screening	I	No	No	18	No		65	Yes	No		Yes
3/Grand-niece	Arg92Gln	19/F	HCM	19		Family screening	I	No	No	14	No		62	Yes	ICD (19)	No	No
4/Proband	Arg92Gln	72/M	DCM	67	Yes	Dyspnea	III	Yes	No	11	No	55	30	Yes	CRT (69)		Yes
4/Sister	Arg92Gln	65/F	DCM	55		Dyspnea	III	Yes	Yes	15	No	60	33		ICD (63)	?	No
4/Nephew	Arg92Gln	54/M	DCM	50		Dyspnea	III	No	No		No		30		CRT (52)		No
4/Grand-nephew	Arg92Gln	19/M	HCM	11		SCD	I	No	Yes	13	No	30	65		ICD (11)	?	No
5/Proband	Arg92Gln	66/M	DCM	40	Yes	Chest pain	II	Yes	Yes	20	No	50	30		CRT + ICD (62)	?	No
6/Proband	Arg92Gln	49/M	HCM	35	Yes	Family screening	I	No	Yes	15	No	43	60	Yes	ICD (45)	No	No
6/Sister	Arg92Gln	54/F	HCM	24		Family screening	II	Yes	Yes	19	No	48	30		ICD	?	No
6/Daughter*	Arg92Gln	7/F	—			—	I	No	No	7	No	27	70		No		No
7/Proband	Arg92Gln	40/M	HCM	36	Yes	Family screening	I	No	No	22	No	47	60	Yes	ICD (38)	No	No
7/Nephew*	Arg92Gln	12/M	—			—	I	No	No	8	No	28	65		No		No
7/Niece*	Arg92Gln	6/F	—			—	I	No	No		No		60		No		No
8/Proband	Arg92Gln	36/M	HCM	17	Yes	SCD	I	No	Yes	20	No	33	62		ICD (17)	?	No
8/Son*	Arg92Gln	8/M	—			—	I	No	No	9	No	27	70		No		No
9/Proband	Arg92Gln	52/F	HCM	51	Yes	Dyspnea	II	No	No	15	No	31	58	No	No		No
9/Daughter	Arg92Gln	27/F	HCM	26		Family screening	I	No	No	20	No	29	65	Yes	No		No
10/Proband	Arg92Gln	46/M	NCCM	40	Yes	Routine ECG	III	No	Yes	10	No	60	21		ICD (44)	No	No
11/Proband	Arg92Trp	69/F	HCM	57	Yes	Family screening	II	No	No	25	No	42	33		ICD (69)	No	No
11/Son	Arg92Trp	32/M	HCM	28		Family screening	I	No	No	15	No	40	65		No		No
11/Daughter	Arg92Trp	25/F	HCM	20		Family screening	I	No	No	13	No	40	74	Yes	No		No
11/Daughter*	Arg92Trp	36/F	—			—	I	No	No	10	No	33	65		No		No
12/Proband	Arg278Cys	47/M	HCM	18	No	Routine ECG	I	No	No	23	No	38	60	Yes	No		No
12/Father	Arg278Cys	71/M	HCM	57		Routine ECG	I	No	No	35	Yes	58	58		No		No
12/Cousin	Arg278Cys	40/M	HCM	37		Family screening	I	No	No	16	No	36	62		No		No
13/Proband	Arg278Cys	41/F	HCM	37	No	Dyspnea	I	No	No	18	No	35	65	Yes	No		No
13/Brother	Arg278Cys	44/M	HCM	42		Family screening	I	No	No	22	No	36	70	Yes	No		No

Table 1 *(Continued)*
Clinical and Genetic Data on Patients and Families Included in the Study

Family	*TNNT2* mutation	Age/ sex	Phenotype	Age at diagnosis, years	FHSD	Presentation	NYHA functionnal class	AF	VT	MMT, mm	LVOT obstruction	LA diameter, mm	LVEF	LGE	PM/ICD (age, years)	Appropriate ICD therapy	Died
13/Mother*	Arg278Cys	75/F	—			—	I	No	No	10	No	32	60		No		No
14/Proband	Arg278Cys	68/M	HCM	55	No	Chest pain	II	No	Yes	30	No	44	68		ICD (62)	Yes	No
14/Daughter*	Arg278Cys	29/F	—			—	I	No	No	9	No	31	62		No		No
15/Proband	Arg286His	80/F	HCM	77	Yes	Dyspnea	II	No	No	21	Yes	51	70		PM (79)		No
15/Son*	Arg286His	48/M	—			—	I	No	No	10	No	35	65		No		No
16/Proband	Arg286His	35/M	HCM	25	No	Routine ECG	I	No	No	21	No	34	60		No		No
17/Proband	Arg286His	36/F	HCM	34	No	Routine ECG	I	No	No						No		No
18/Proband	Arg286His	16/M	HCM	15	No	Routine ECG	I	No	No	21	No	28	65		No		No
18/Mother*	Arg286His	40/F	—			—	I	No	No	10	No	35	60		No		No
18/Cousin*	Arg286His	6/M	—			—	I	No	No		No		75		No		No
19/Proband	Arg286His	53/M	HCM	52	No	Routine ECG	I	No	No	21	No	32	67	Yes	No		No
20/Proband	Arg94His	15/M	HCM	14	Yes	Family screening	I	No	No	33	No	41	64	No	No		No
20/Father	Arg94His	39/M	HCM	34		Syncope	II	No	No		No		40		ICD (34)	?	No
20/Aunt	Arg94His	36/F	HCM	22											No		Yes
21/Proband	Ile221Thr	75/F	HCM	62	No	Dyspnea	III	Yes	No	22	Yes	50	60		No		No

AF, atrial fibrillation; CRT, cardiac resynchronization therapy; DCM, dilated cardiomyopathy; ECG, electrocardiogram; F, female; FHSD, family history of sudden death; HCM, hypertrophic cardiomyopathy; ICD, implantable cardioverter-defibrillator; LA, left atrium; LGE, late gadolinium enhancement; LVEF, left ventricular ejection fraction; LVOT, left ventricular outflow tract; M, male; MMT, maximal myocardial thickness; NCCM, noncompaction cardiomyopathy; NYHA, New York Heart Association; PM, pacemaker; SCD, sudden cardiac death; VT, sustained or nonsustained ventricular tachycardia.

* Carriers (genotype +, phenotype –).

Table 2
Clinical, Echocardiographic, and Prognostic Characteristics of Patients with Cardiomyopathy According to the *TNNT2* Mutation

	Arg92Gln (n = 25)	Arg278Cys (n = 6)	Arg286His (n = 5)	Arg92Trp (n = 3)	Arg94His (n = 3)
Age, years	46.4 ± 17	52 ± 14	44 ± 24	42 ± 23	30 ± 13
Male, No. (%)	14 (56)	5 (83)	3 (60)	1 (33)	2 (66.6)
Phenotype, No. (%)	HCM, 15 (60); DCM, 9 (36); NCCM, 1 (4)	HCM	HCM	HCM	HCM
Age at diagnosis, years	37 ± 16	41 ± 14	40.6 ± 24	35 ± 19	23 ± 10
FHSD, %	100 (n = 21)	0 (n = 3)	20 (n = 5)	100 (n = 1)	0 (n = 1)
Presentation, %	Family screening, 44; dyspnea, 28; SCD, 20	Family screening, 33; routine ECG, 33; dyspnea, 16; chest pain, 16	Routine ECG, 80; dyspnea, 20	Family screening, 100	Family screening, 33; syncope, 33
NYHA average	1.76	1.2	1.2	1.3	1.5
AF, No. (%)	(40)	0	0	0	0
VT, No. (%)	(40)	1 (16.6)	0	0	0
MMT, mm	15.8 ± 4	24 ± 7	21 ± 0	17.7 ± 6	
LVOT obstruction, No. (%)	0	1 (16.6)	1 (25)	0	0
LA diameter, mm	45 ± 9.6	41 ± 9	36 ± 10	40.6 ± 1.6	
LVEF, %	49 ± 15	64 ± 5	65 ± 4	57 ± 21	52 ± 17
LGE, No. (%)	10 (91)	3 (100)			
ICD, No. (%)	11 (44)	1 (16.6)	0	1 (33)	1 (33)
Died, No. (%)	5 (20)	0	0	0	1 (33)

AF, atrial fibrillation; DCM, dilated cardiomyopathy; ECG, electrocardiogram; FHSD, family history of sudden death; HCM, hypertrophic cardiomyopathy; ICD, implantable cardioverter-defibrillator; LA, left atrium; LGE, late gadolinium enhancement; LVEF, left ventricular ejection fraction; LVOT, left ventricular outflow tract; MMT, maximal myocardial thickness; NCCM, noncompaction cardiomyopathy; NYHA, New York Heart Association; SCD, sudden cardiac death; VT, sustained or nonsustained ventricular tachycardia.

recovery from SCD or appropriate ICD shock were included (Figure 3). In the group with Arg92Gln, survival at 55 years was only 50% (95% confidence interval, 41%-58%).

The low rate of SCD hampered subgroup analysis. However, when probands were compared with relatives, there was no difference in cardiac mortality (Kaplan-Meier log rank test, $P = .62$). Survival curves of the study population were compared: the Arg92Gln mutation was compared against the 5 other *TNNT2* mutations (due to the different prognostic profile previously mentioned), against other sarcomeric genetic mutations, and against patients with no identified mutation (Figure 4). Analysis of the survival curve showed that patients with the Arg92Gln mutation had a much lower survival rate than other groups (other *TNNT2* mutations, other sarcomeric gene mutations, and patients with no identified mutation). There were statistically significant differences from the group with other *TNNT2* mutations (log rank test, 11.71; $P = .0006$). Also, the group of patients with *TNNT2* mutation generally had a worse survival rate than the group with other gene mutations or no identified mutations, but this was due to the patients with the Arg92Gln mutation. Patients with double mutations were not analyzed together with other individual mutations.

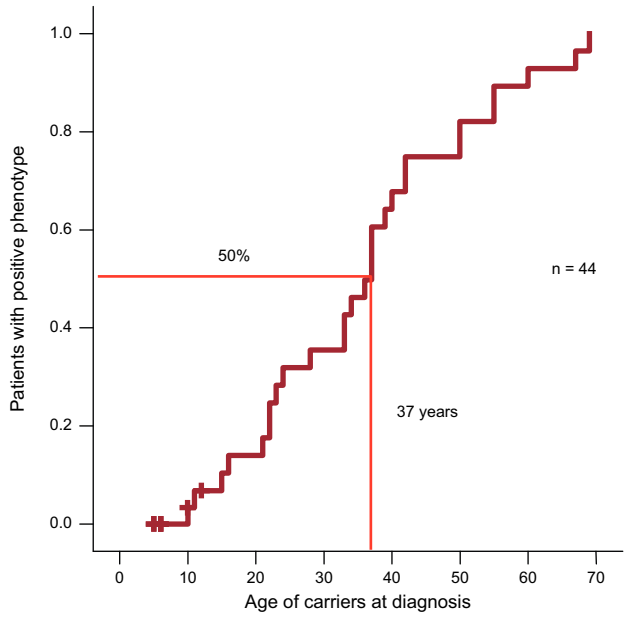

Figure 2. Disease penetrance in carriers of the Arg92Gln mutation: 25% at 23 years, 50% at 37 years, and 75% at 50 years.

DISCUSSION

Mutations in *TNNT2* are considered an infrequent cause of HCM (5%).[8,9,20,21] In this study, the prevalence of *TNNT2* mutations was higher than in previous series,[9,10,17,18,22–24] partly because of a probable founder effect. This study had the highest number of families, and one of the broadest longitudinal cohorts of *TNNT* mutations (Table 3) since the first published study to show a high incidence of SCD in such patients.[9] Our results confirm that SCD is common in young people with some mutations of this gene, but this finding cannot be extrapolated to all known mutations.

Electrocardiography was almost always abnormal, sometimes with only mild abnormalities on echocardiography, which underscores the role of both investigations in detecting this disease, something which has previously been expressed, but is important to reiterate.

In comparison with previous studies,[9,10,17,18,22–24] there was a lower prevalence of left ventricular outflow tract obstruction at rest, which was present in only 7% of patients, somewhat similar to the most recent published study.[23]

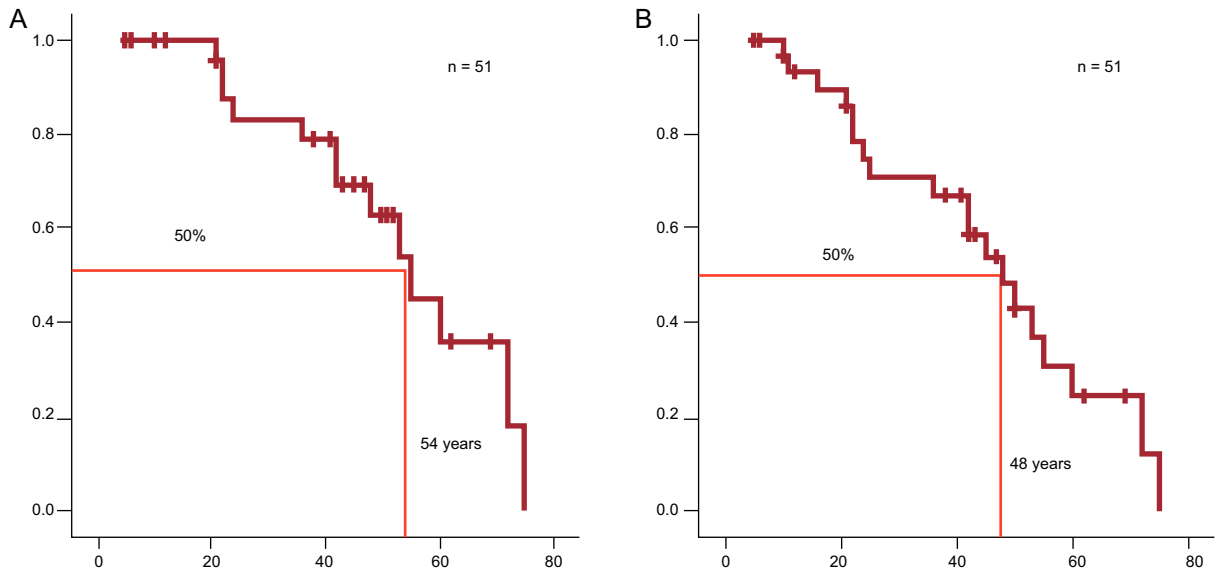

Figure 3. A: Sudden cardiac death-free survival of all individual carriers of the Arg92Gln mutation (including relatives with hypertrophic cardiomyopathy and obligate carriers). B: As in A, but including recovery from sudden cardiac death and patients with appropriate therapies from implantable cardioverter defibrillator.

We identified 6 studies on mutations in *TNNT2* that analyzed survival.[9,10,17,18,22,23] They comprised a total of 258 carriers and 68 deaths apparently of cardiovascular causes, mostly sudden, but 50 of those deaths were from only 1 publication. Therefore, most of those studies had a low number of SCDs, which coincides with the prospective data from this study (Table 3).

Family tree analysis (Figure 5) showed a high prevalence of SCD in affected families, but only with some mutations (mainly Arg92Gln, but also Arg92Trp and Arg94His). The SCD rate was too small to perform adequate statistical analysis of subgroups, as was the case in previous studies.[23] The different prognoses in families with the same mutations indicates that other mechanisms (genetics, epigenetics, or environmental factors) could have had an influence.

Previous studies[24] have already documented cases of SCD with little or even no hypertrophy in carriers of *TNNT2* mutations. This

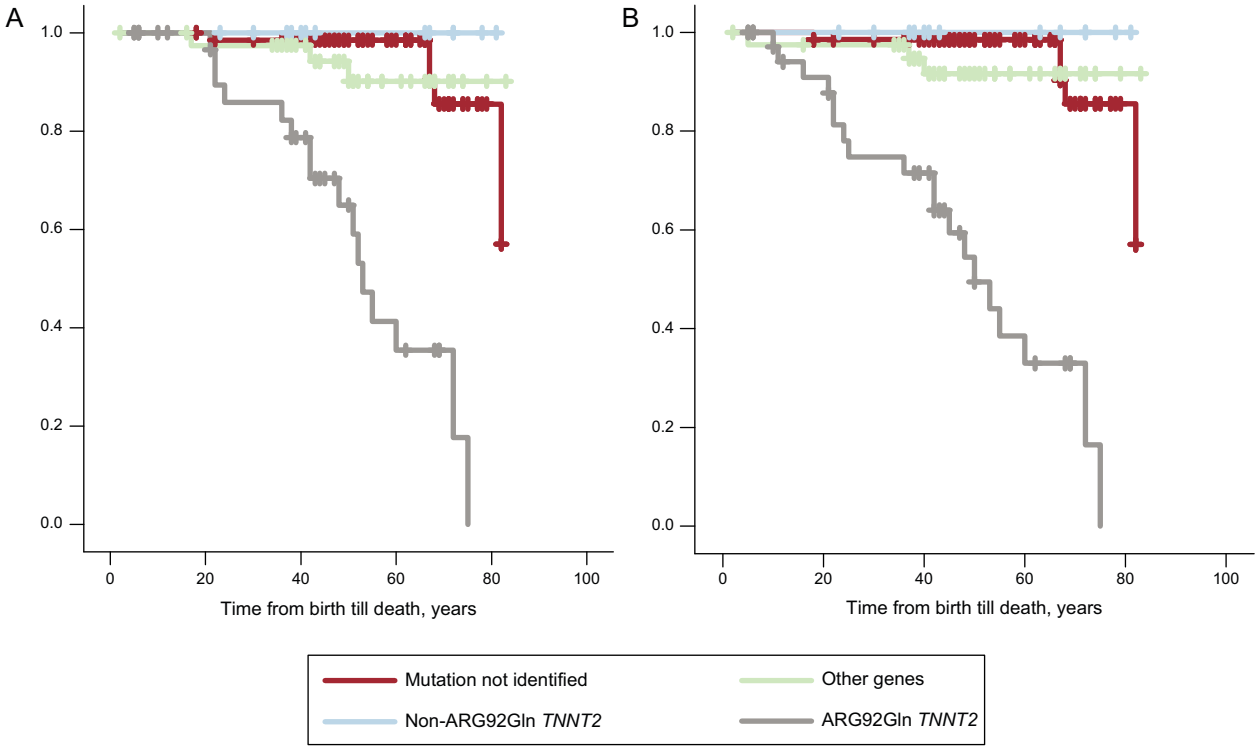

Figure 4. A: Sudden cardiac death-free survival in the 4 groups of patients with hypertrophic cardiomyopathy according to genetic result. B: Sudden cardiac death-free survival including recovery from sudden cardiac death and patients with appropriate implantable cardioverter-defibrillator therapies.

Table 3
Published Studies on Survival With Mutations in the *TNNT2* Gene

Study	No. of families	No. of patients	No. of cardiac deaths	No. of sudden deaths	Mutations
Watkins et al[9]	11	112	50	39	Ile79Asn Arg92Gln Phe110Ile ΔGlu160 Glu163Lys Glu244Asp Intron 15 G>A Arg278Cys
Nakajima-Tanaguchi et al[22]	1	4	2	2	Ala104Val
Moolman et al[10]	2	22	7	7	Arg92Trp
Anan et al[17]	6	18	2	2	Phe110Ile
Torricelli et al[18]	5	10	0	0	Phe110Ile Arg130Cys ΔGlu160 Arg92Gln Arg278Cys
Pasquale et al[23]	20	92	?	7	Arg278Cys Arg92Leu Arg92Trp ΔGlu163 IVS15+1G>A Ala104Val Arg278His Arg92Gln Arg94Leu Glu163Lys Glu83Lys Ile79Asn
Ripoll-Vera et al, 2015*	21	54	11	6	Arg92Gln Arg92Trp Arg286His Arg278Cys Arg94His Ile221Thr

* Current study results.

was also observed in some of our patients, but only with the Arg92Gln mutation. In patients who had SCD, HCM was more common than DCM, and MMT was only 14.6 ± 6 mm. Because of the relative lack of events in the present cohort, the relative risk of SCD could not be determined in mutation carriers who had normal echocardiograms.

This study has detected important prognostic differences between patients with 1 of the *TNNT2* mutations (Arg92Gln) and those with the 5 other *TNNT2* mutations, other sarcomeric gene mutations, and no identified mutation. Compared with other groups, patients with the Arg92Gln mutation had an earlier presentation and worse prognosis. They had a high incidence of SCD, a mixed phenotype (HCM with mild hypertrophy, DCM with ventricular dysfunction, noncompaction cardiomyopathy), absence of obstruction, significant fibrosis, and frequent need for ICD or resynchronization therapy.

Progressive thinning of the myocardium and deterioration in contractile function are a well-known phenomenon of HCM.[3,4] In this study, 9 patients already had ventricular dilatation at presentation (age, 50.6 ± 14.6 years, all with Arg92Gln) and 2 died of heart failure during follow-up, indicating that progression of heart failure could be relatively common in such patients. In fact, the patients with HCM were younger (28.8 ± 11.8 years) than those with DCM. However, there were no cases of progressive ventricular dilatation. This could have been due to the short follow-up, and therefore we cannot conclude with complete certainty that patients with DCM at diagnosis really were patients with HCM in burn-out phase.

One proband with Arg92Gln had noncompaction cardiomyopathy, an association that had not been described until now. It is important to remember that the *TNNT2* gene should always be included in genetic study in patients with HCM and DCM, and possibly also, based on our findings, in cases of noncompaction cardiomyopathy.

The Arg92Trp mutation affects the same amino acid as Arg92Gln, and therefore it behaves similarly. It is associated with HCM, mild hypertrophy, and a high incidence of SCD.[5,19]

The Arg278Cys mutation is also associated with late-presentation HCM and mild-to-moderate hypertophy.[9,18,25–27] Sudden cardiac death is uncommon in young people but is common in patients of advanced age. It is often associated with other pathogenic variants.[27] From the 3 families with the Arg278Cys mutation, 2 probands had double mutations. There was no history of SCD, and the MMT was highly variable (14-35 mm).

The Arg286His mutation has been associated with HCM.[19] The 3 families with this mutation had HCM with an MMT of 21 mm, without a high risk of SCD.

The Arg94His mutation is also a known cause of HCM. The first symptoms can manifest in childhood and patients may also have severe arrhythmic events with an apparently mild phenotype.[28]

Finally, the Ile221Thr mutation had not previously been published. It was considered a rare genetic variant affecting 1 relevant functional region. It could only be studied in the index case (with clear HCM), and therefore it was not possible to study its segregation in the family to confirm its pathogenicity.

Double mutations could confer a worse prognosis. Other studies on mutations in the *TNNT2* gene did not offer data on additional mutations on other genes. In this series, double mutations were found in 4 probands (19%), but no double mutations were found in the relatives studied. In the literature, double mutations are found in only 5% of cases of HCM.[7,23] Only 1 of our 4 patients with a double mutation had an unfavorable risk stratification, and had ICD implantation, but there were insufficient data to establish a prognosis relating to double mutations.

A probable founder effect was demonstrated for the Arg92Gln mutation. Of the 10 families, 9 originated from the same village. Using extensive genealogical study (parish archives and population census), we found that 6 of these families had a common ancestor born in 1784. Haplotype study was not performed as it was not available in our setting.

Limitations

As with previous studies, there could have been selection bias because the study was carried out in a specialized unit. Patients with genetic and clinical (or pathological) confirmation were included, as were relatives with a positive phenotype and those who had sudden death, who were also presumed to be affected despite not having genetic confirmation.

The phenotypic differences found between some patients with the same mutations shows that the prognosis of these individuals is influenced by many factors other than the mutation itself.

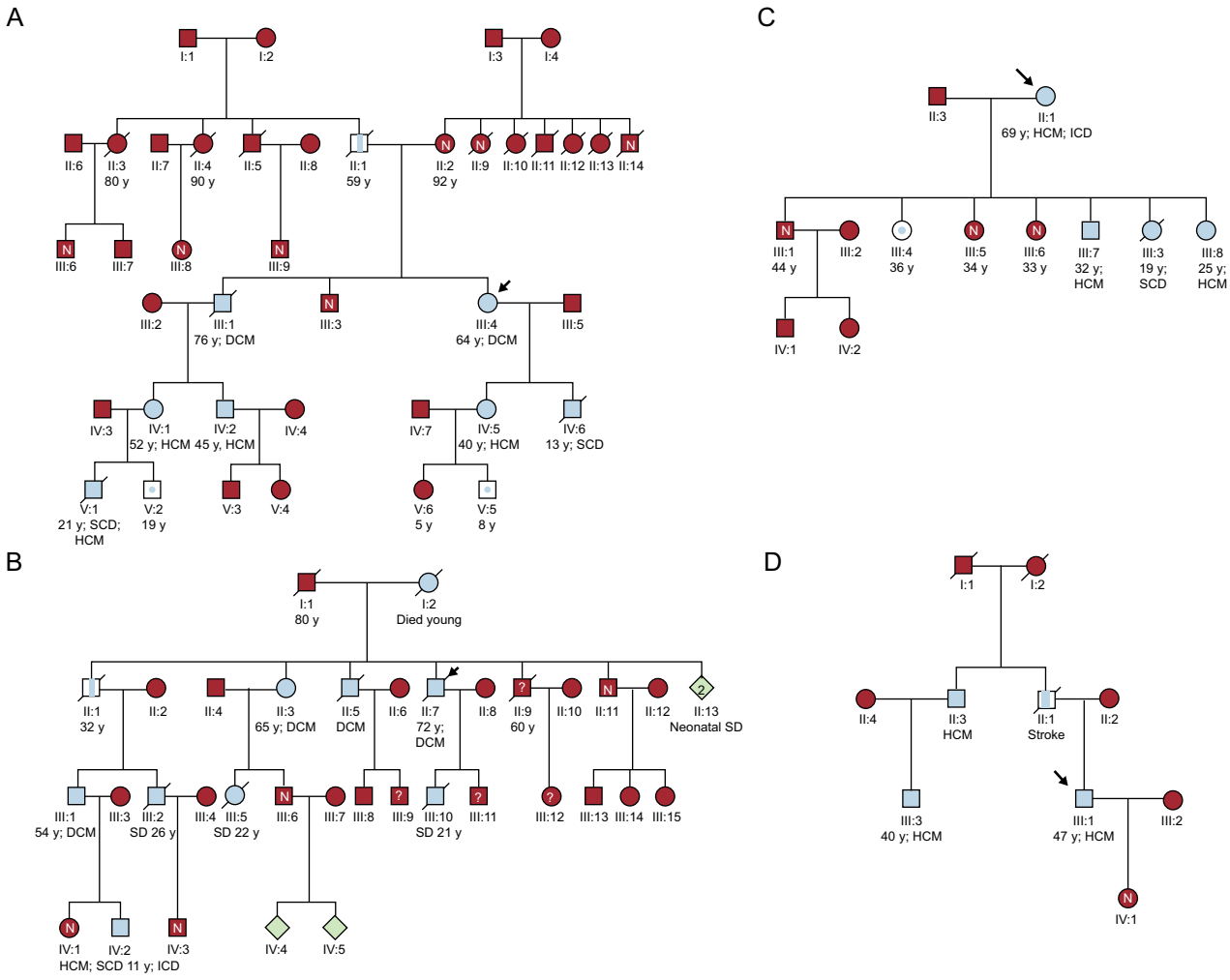

Figure 5. Family trees of the 3-Arg92Gln- (A), 4-Arg92Gln- (B), 11-Arg92Trp- (C) and 12-Arg278Cys- (D) families. DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; ICD, implantable cardioverter-defibrillator; SCD, sudden cardiac death; SD, sudden death; y, years.

CONCLUSIONS

Investigation of the genotype-phenotype correlation in HCM remains a challenge. Mutations in the *TNNT2* gene were more common in our series than in previous studies, partly because of a probable founder effect. The clinical and prognostic profiles depended greatly on the mutation. Risk profile was significantly worse in carriers of Arg92Gln than in other patients. Sudden cardiac death was a frequent complication and can occur in young individuals with little or no hypertrophy. Dilated cardiomyopathy with ventricular dysfunction was fairly common among carriers of some mutations (Arg92Gln).

Overall, these findings have important implications for the clinical and genetic study of families with cardiomyopathy, above all the finding of the Arg92Gln mutation, which, given its demonstrated malignancy, should cause a change in the management of individuals in SCD prevention.

FUNDING

Red de Investigación Cardiovascular del Instituto de Salud Carlos III (RD12/004/0069) and CIBEROBN *(Centro de Investigación Biomédica en Red de la Fisiopatología de la Obesidad y la Nutrición)* (CB12/03/30038), Madrid, Spain.

CONFLICTS OF INTEREST

None declared.

REFERENCES

1. Maron BJ, Gardin JM, Flack JM, Gidding SS, Kurosaki TT, Bild DE. Prevalence of hypertrophic cardiomyopathy in a general population of young adults: echocardiographic analysis of 4144 subjects in the CARDIA study: Coronary Artery Risk Development in (Young) Adults. Circulation. 1995;92:785–9.
2. Corrado D, Basso C, Pavei A, Michieli P, Schiavon M, Thiene G. Trends in sudden cardiovascular death in young competitive athletes after implementation of a preparticipation screening program. JAMA. 2006;296:1593–601.
3. Spirito P, Maron BJ, Bonow RO, Epstein SE. Occurrence and significance of progressive left ventricular wall thinning and relative cavity dilatation in hypertrophic cardiomyopathy. Am J Cardiol. 1987;60:123–9.
4. Biagini E, Coccolo F, Ferlito M, Perugini E, Rocchi G, Bacchi-Reggiani L, et al. Dilated-hypokinetic evolution of hypertrophic cardiomyopathy: prevalence, incidence, risk factors, and prognostic implications in pediatric and adult patients. J Am Coll Cardiol. 2005;46:1543–50.
5. Ackerman MJ, VanDriest SL, Ommen SR, Will ML, Nishimura RA, Tajik AJ, et al. Prevalence and age-dependence of malignant mutations in the beta-myosin heavy chain and troponin T genes in hypertrophic cardiomyopathy: a comprehensive outpatient perspective. J Am Coll Cardiol. 2002;39:2042–8.
6. Varnava A, Baboonian C, Davison F, De Cruz L, Elliott PM, Davies MJ, et al. A new mutation of the cardiac troponin T gene causing familial hypertrophic cardiomyopathy without left ventricular hypertrophy. Heart. 1999;82:621–4.

7. Elliott PM, Gimeno JR, Thaman R, Shah J, Ward D, Dickie S, et al. Historical trends in reported survival rates in patients with hypertrophic cardiomyopathy. Heart. 2006;92:785–91.
8. Van Driest SL, Ackerman MJ, Ommen SR, Shakur R, Will ML, Nishimura RA, et al. Prevalence and severity of «benign» mutations in the beta-myosin heavy chain, cardiac troponin T, and alpha-tropomyosin genes in hypertrophic cardiomyopathy. Circulation. 2002;106:3085–90.
9. Watkins H, McKenna WJ, Thierfelder L, Suk HJ, Anan R, O'Donoghue A, et al. Mutations in the genes for cardiac troponin T and α-tropomyosin in hypertrophic cardiomyopathy. N Engl J Med. 1995;332:1058–64.
10. Moolman JC, Corfield VA, Posen B, Ngumbela K, Seidman C, Brink PA, et al. Sudden death due to troponin T mutations. J Am Coll Cardiol. 1997;29:549–55.
11. Spirito P, Bellone P, Harris KM, Bernabo P, Bruzzi P, Maron BJ. Magnitude of left ventricular hypertrophy and risk of sudden death in hypertrophic cardiomyopathy. N Engl J Med. 2000;342:1778–85.
12. Maron BJ, Spirito P, Wesley YE, Arce J. Development and progression of left ventricular hypertrophy in children with hypertrophic cardiomyopathy. N Engl J Med. 1986;315:610–4.
13. Shapiro LM, McKenna WJ. Distribution of left ventricular hypertrophy in hypertrophic cardiomyopathy: a two-dimensional echocardiographic study. J Am Coll Cardiol. 1983;2:437–44.
14. McKenna WJ, Spirito P, Desnos M, Dubourg O, Komajda M. Experience from clinical genetics in hypertrophic cardiomyopathy: proposal for new diagnostic criteria in adult members of affected families. Heart. 1997;77:130–2.
15. Monserrat L, Elliott PM, Gimeno JR, Sharma S, Penas-Lado M, McKenna WJ. Non-sustained ventricular tachycardia in hypertrophic cardiomyopathy: an independent marker of sudden death risk in young patients. J Am Coll Cardiol. 2003;42:873–9.
16. Maron BJ, McKenna WJ, Danielson GK, Kappenberger LJ, Kuhn HJ, Seidman CE, et al.; Task Force on Clinical Expert Consensus Documents. American College of Cardiology; Committee for Practice Guidelines. European Society of Cardiology. American College of Cardiology/European Society of Cardiology clinical expert consensus document on hypertrophic cardiomyopathy: a report of the American College of Cardiology Foundation Task Force on Clinical Expert Consensus Documents and the European Society of Cardiology Committee for Practice Guidelines. J Am Coll Cardiol. 2003;42:1687–713.
17. Anan R, Shono H, Kisanuki A, Arima S, Nakao S, Tanaka H. Patients with familial hypertrophic cardiomyopathy caused by a Phe110Ile missense mutation in the cardiac troponin T gene have variable cardiac morphologies and a favorable prognosis. Circulation. 1998;98:391–7.
18. Torricelli F, Girolami F, Olivotto I, Passerini I, Frusconi S, Vargiu D, et al. Prevalence and clinical profile of troponin T mutations among patients with hypertrophic cardiomyopathy in Tuscany. Am J Cardiol. 2003;92:1358–62.
19. Van Driest S, Ellsworth EG, Ommen SR, Tajik AJ, Gersh BJ, Ackerman MJ. Prevalence and spectrum of thin filament mutations in an outpatient referral population with hypertrophic cardiomyopathy. Circulation. 2003;108:445–51.
20. Mogensen J, Bahl A, Kubo T, Elanko N, Taylor R, McKenna WJ. Comparison of fluorescent SSCP and denaturing HPLC analysis with direct sequencing for mutation screening in hypertrophic cardiomyopathy. J Med Genet. 2003;40:e59.
21. Forissier JF, Carrier L, Farza H, Bonne G, Bercovici J, Richard P, et al. Codon 102 of the cardiac troponin T gene is a putative hot spot for mutations in familial hypertrophic cardiomyopathy. Circulation. 1996;94:3069–73.
22. Nakajima-Taniguchi C, Matsui H, Fujio Y, Nagata S, Kishimoto T, Yamauchi-Takihara K. Novel missense mutation in cardiac troponin T gene found in Japanese patient with hypertrophic cardiomyopathy. J Mol Cell Cardiol. 1997;29:839–43.
23. Pasquale F, Syrris P, Kaski JP, Mogensen J, McKenna WJ, Elliott P. Long-term outcomes in hypertrophic cardiomyopathy caused by mutations in the cardiac troponin T gene. Circ Cardiovasc Genet. 2012;5:10–7.
24. Gimeno JR, Monserrat L, Pérez-Sánchez I, Marín F, Caballero L, Hermida-Prieto M, et al. Miocardiopatía hipertrófica. Estudio del gen de la troponina T en 127 familias españolas. Rev Esp Cardiol. 2009;62:1473–7.
25. Theopistou A, Anastasakis A, Miliou A, Rigopoulos A, Toutouzas P, Stefanadis C. Clinical features of hypertrophic cardiomyopathy caused by an Arg278Cys missense mutation in the cardiac troponin T gene. Am J Cardiol. 2004;94:246–9.
26. Olivotto I, Girolami F, Ackerman MJ, Nistri S, Bos JM, Zachara E, et al. Myofilament protein gene mutation screening and outcome of patients with hypertrophic cardiomyopathy. Mayo ClinProc. 2008;83:630–8.
27. García-Castro M, Coto E, Reguero JR, Berrazueta JR, Álvarez V, Alonso B, et al. Espectro mutacional de los genes sarcoméricos *MYH7, MYBPC3, TNNT2, TNNI3 y TPM1* en pacientes con miocardiopatía hipertrófica. Rev Esp Cardiol. 2009;62:48–56.
28. Millat G, Bouvagnet P, Chevalier P, Dauphin C, Jouk PS, Da Costa A, et al. Prevalence and spectrum of mutations in a cohort of 192 unrelated patients with hypertrophic cardiomyopathy. Eur J Med Genet. 2010;53:261–7.