BRIEF REPORT

Hypertrophic Cardiomyopathy. A Study of the Troponin-T Gene in 127 Spanish Families

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The information available on the correlation between genotype and phenotype and the prognostic implications of different troponin-T gene mutations is sparse and, at times, contradictory. We studied the TNNT2 gene in 127 patients with hypertrophic cardiomyopathy and identified three mutations in patients from four families (3.1%): the Phe87Leu mutation, which has not been previously reported, the Arg278Cys mutation (two families) and the Asp271Ile mutation. Seven carriers of the Phe87Leu mutation (aged 29 to 52 years) were found to have mild hypertrophy (i.e., a wall thickness <16 mm). There were 11 deaths associated with the condition (seven sudden deaths), and four of those who died were aged between 14 and 16 years. No sudden deaths occurred in the other three families. In conclusion, troponin-T mutations were responsible for 3% of the hypertrophic cardiomyopathy cases in our study population. The Phe87Leu mutation was associated with only mild hypertrophy but with a high risk of sudden death.

Key words: *Hypertrophic cardiomyopathy. Genetics. Troponin T. Sudden death.*

Miocardiopatía hipertrófica. Estudio del gen de la troponina T en 127 familias españolas

La información sobre las relaciones genotipo-fenotipo y el pronóstico de las diferentes mutaciones en el gen de la troponina T es escasa y en ocasiones contradictoria.

Se realizó estudio del gen *TNNT2* en 127 pacientes con miocardiopatía hipertrófica (MCH), identificándose 3 mutaciones en 4 familias (3,1%): Phe87Leu, no descrita, Arg 278Cys (2 familias) y Asp271Ile. Se identificaron 7 portadores de Phe87Leu (29 a 52 años) con hipertrofia leve (grosor < 16 mm). Hubo 11 muertes relacionadas con la enfermedad (7 de ellas súbitas), con 4 de los fallecidos entre 14 y 16 años de edad. No hubo muertes súbitas en las otras 3 familias. En conclusión, las mutaciones en el gen de la troponina T son responsables de un 3% de casos de MCH en nuestra población. La mutación Phe-87Leu se asocia a hipertrofia de grado leve y alto riesgo de muerte súbita.

Palabras clave: *Miocardiopatía hipertrófica. Genética. Troponina T. Muerte súbita.*

INTRODUCTION

Hypertrophic cardiomyopathy (HCM) is an autosomal dominant hereditary disease caused by over 500 mutations in 11 different genes.[1-4] Information about the relationship between genotype and phenotype and the prognosis associated with various mutations in the troponin T gene *(TNNT2)* is limited and at times contradictory.[4,5-13] The aim of this study was to identify mutations in the *TNNT2* gene and study the correlations between the genotype and the phenotype.

METHODS

Blood samples were studied from 127 consecutive patients with HCM (mean age, 50 [16] years; 66% men; maximum wall thickness, 18 [5] mm; 28% with obstruction). The patients came from 3 centers: the Hospital Universitario in A Coruña (60 patients), the Hospital Virgen de la Arrixaca (37 patients),

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TABLE 1. **Results of the Genetic Study of the *TNNT2* Gene**

Exon/Intron	Nucleotide Change	Amino Acid Change	Carriers, No.	Comment
Intron 3	Del5pb	Intronic variant	35	Does not affect protein
Exon 8	TCT>TCC	Ser69Ser	15	Does not affect protein
Exon 9	AAG>AAA	Lys97Lys	1	Does not affect protein
Exon 9	ATC>ATT	Ile106Ile	46	Does not affect protein
Exon 14	AAG>AGG	Lys253Arg	6	Polymorphism described
Exon 8	TTT>CTT	Phe87Leu	1	New mutation
Exon 15	AAC>ATC	Asp271Ile	1	Mutation described
Exon 16	CGC>TGC	Arg278Cys	2	Mutation described

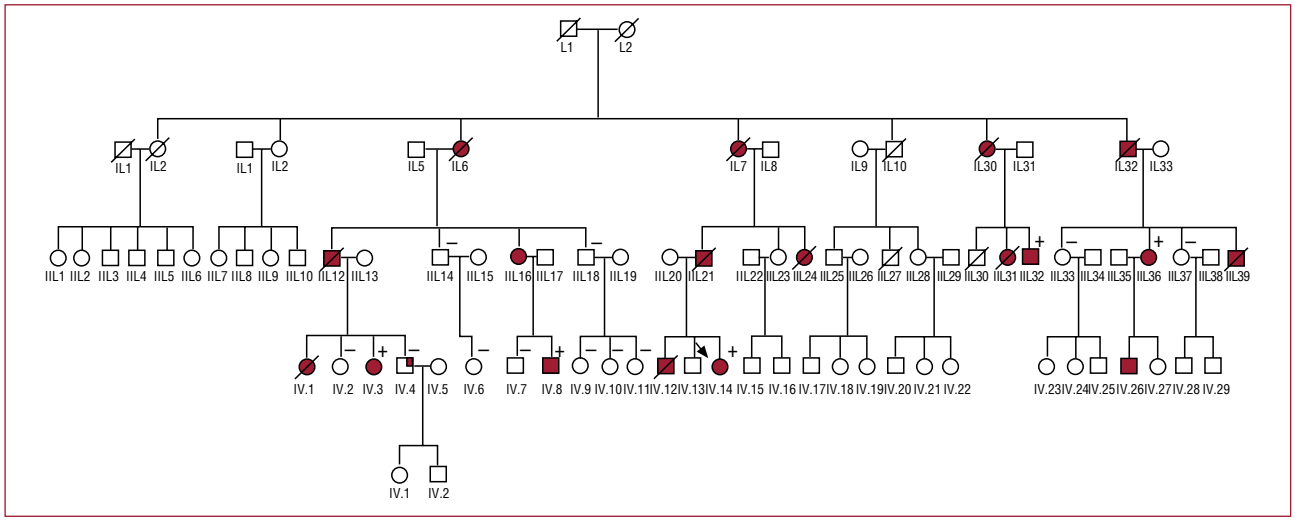

Figure 1. Family tree of A8 with Phe87Leu mutation in *TNNT2*.

and the Hospital General in Alicante (30 patients). Cardiac evaluation included ECG, echocardiogram, Holter, and an exercise stress test. All the patients gave written informed consent.

Each index patient provided 4 mL of blood, drawn into an EDTA tube. The DNA was extracted and amplified, and *TNNT2* exons were analysed by screening with denaturing high-performance liquid chromatography (DHPLC). In the event of an anomalous profile, the exon was sequenced (sequencer: ABI310/ABI3130). Specific primers were designed for the amplification of the fragments of interest.

RESULTS

Eight different alterations were found in 107 exons. Three variants were considered to be causal mutations: Phe87Leu (not previously reported), Arg278Cys and Asp271Ile (Table 1). The Asp271Ile mutation was identified in a Galician family and the Arg278Cys mutation in one family from Murcia and another from Alicante. Both these mutations had already been associated with HCM. The Phe87Leu mutation was considered to be causative as it segregated with the disease, affected a highly conserved residue and was not identified in 140 healthy controls. Table 2 summarizes the clinical characteristics of the carriers.

Clinical Characteristics and Genotype-Phenotype Correlation

Phe87Leu

This mutation was identified in a family with history of sudden death (SD) (Figure 1). In total, 18 persons were affected, with 11 related deaths: 7 SD, 3 due to heart failure and 1 from stroke. Four of those who died were aged between 14 and 16 years and were asymptomatic ([IV.1], [IV.12], [III.31], and [III.39]). Of the 10 patients for whom an echocardiogram was available, 7 had mild hypertrophy (<16 mm); in 2 of these the echocardiogram did not show the hypertrophy and the diagnosis was made at autopsy. Two of the patients with mild hypertrophy had a restrictive pattern. Three patients had a wall thickness >20 mm (maximum, 27 mm [IV.8]). Only the index case [IV.14] had a gradient. Systolic function was conserved, except in one case (ejection fraction, 40% [III.21]). One of the affected members had recurrent

TABLE 2. **Clinical Characteristics of the Patients Carrying Mutations in the *TNNT2* Gene**

Fam/ID	Age/Sex	Age at Diagnosis	Symptoms	NYHA	ECG	LVH, mm	OTO	Systemic Failure	FHSD	NSVT	APR	Gadolinium Uptake	Treatment	Mutation
M92/I.1[a,b]	55/F	50	Dyspnea	II	Diagnostic	22	No	Normal	No	Yes	NP	Yes	Verapamil, amiodarone, anticoagulants	Arg278Cys
M92/I.2[b]	59/M	18	Palpitations, Dyspnea	II	Diagnostic	22	No	EF, 45%	No	Yes	No	Yes	Sota, diuretics	Arg278Cys
M92/II.2[b]	27/M	27	No	I	Not diagnostic	12	No	Normal	No	No	No	No	No	Arg278Cys
M92/II.3	30/M	30	No	I	Normal	10	No	Normal	No	—	—	—	No	Arg278Cys
M92/II.4	29/M	29	No	I	Normal	10	No	Normal	No	—	—	—	No	Arg278Cys
M92/II.5[b]	21/M	18	Presyncope	II	Diagnostic	40	No	Normal	No	Yes	Hypotension	NP	IAD, no medical treatment	Arg278Cys
A16/II.1[a]	64/M	59	Palpitations, pAF	I	Diagnostic	26	50 mm Hg	Normal	No	Yesc	Flat	Yes	Amiodarone, bisoprolol, anticoagulants	Arg278Cys
A16/III.1	33/M	33	No	I	Brugada	11	No	Normal	No	No	Normal	—	No	Arg278Cys
A8/III.16	52/F	24	Dyspnea	III	Diagnostic	14	No	Normal	Yes	No	Normal	Yes	Amiodarone, diuretics, bisoprolol	Phe87Leu
A8/III.32	39/M	39	Dyspnea	II	Diagnostic	13	No	EF, 40%	Yes	—	—	—	ACE inhibitors	Phe87Leu
A8/III.36	40/F	8	Dyspnea, asthenia	II	Diagnostic	14	No	Normal	Yes	No	Flat	Yes	IAD, ACE inhibitors	Phe87Leu
A8/IV.3	30/F	30	Syncope, Dyspnea	II	Diagnostic	18	No	Normal	Yes	No	Normal	—	IAD, ACE inhibitors, diuretics	Phe87Leu
A8/IV.8	30/M	8	Presyncope	I	Diagnostic	27	No	Normal	Yes	No	No	No	No	Phe87Leu
A8/IV.14[a]	29/F	14	Palpitations, PAF	II	Diagnostic	25	30 mm Hg	Normal	Yes	No	Hypotension	Yes	Amiodarone, bisoprolol	Phe87Leu
A8/IV.26	9/M	9	No	I	Diagnostic	12	No	Normal	Yes	—	—	—	No	Phe87Leu
C5/II.2[a]	62/M	44	Palpitations, chest pain, PAF	I	Diagnostic	22	No	Normal	No	No	Hypotension	No	Amiodarone, beta blockers	Asp271Ile
C5/III.1	36/M	33	Chest pain	I	Diagnostic	14	No	Normal	No	No	Normal	No	No	Asp271Ile
C5/III.2[d]	31/F	26	No	I	Not diagnostic	9	No	Normal	No	No	Normal	No	No	Asp271Ile

— indicates not available or not done; APR, abnormal pressure response; F, female; FHSD, family history of sudden death; IAD, implantable automatic defibrillator; LVH, left ventricular hypertrophy, maximum wall thickness; M, male; NP, not possible; NSVT, non-sustained ventricular tachycardia; OTO, outflow tract obstruction; PAF, paroxysmal atrial fibrillation; pAF, persistent atrial fibrillation.

[a]Proband.

[b]Carriers of a double in MYH7 and D928N.

[c]NSVT at peak exercise on ergometry.

[d]Presented ostium secundum type atrial septal defect.

syncope [IV.3]. In general, the younger affected members of the family were asymptomatic and over half the affected members aged between 40 and 50 years had dyspnea (NYHA II-III). The family and genetic study led to implantation of automated defibrillators for primary prevention in 2 affected members [III:36 and IV:3]. The mutation was not identified in 6 members with a normal phenotype.

Arg278Cys

This mutation was identified in two families, M92 and A16 (Figure 2 and Table 2). Of the 8 carriers, 4 fulfilled the diagnostic criteria for HCM (A16: II:1; M92: I:1, I:2, II:5), 1 had a doubtful phenotype (M92: II:2) and 3 had a normal echocardiogram and ECG (M92: II:3 and II:4, A16: III:1) (Table 2). These families had no history of adverse events related with the disease.

One of the carriers in family M92 had very marked hypertrophy (40 mm) (II:5), which contrasted with the normal phenotype of this patient's siblings who were carriers. His father (I:2) had moderate hypertrophy and his mother (I:3) had mild hypertrophy. In order to attempt to clarify the relationship between genotype and phenotype in family M92, sequencing was made of another 8 sarcomeric genes (*MYH7, MYBPC3,* alpha tropomyosin, actin, *TNNI2, TNNC1,* and the myosin light chains). The patient with severe hypertrophy (II:5) carried an Asp 928Asn mutation in the beta myosin heavy chain (MYH7), which was also present in his father (I:2), aunt (I:1) and cousins (II:1) and (II:2). Three of the 4 carriers of the 2 mutations had a moderate or severe phenotype (2 adults and 1 young person). The mother of the case with severe hypertrophy (I:3) was negative for both mutations.

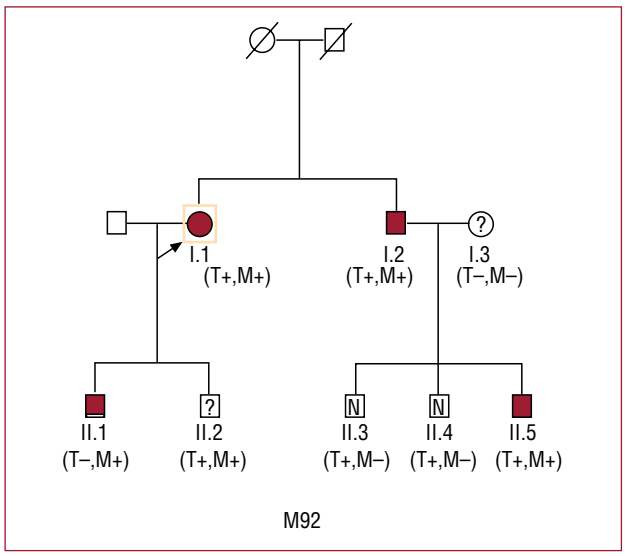

Fig. 2. Family tree of M92 with Arg278Cys mutation in *TNNT2*. M indicates *MYH7*; T, *TNNT2*.

Asp271Ile

This mutation was identified in a family with no history of SD. The index case was diagnosed at the age of 44 years because of atypical pain and paroxysmal atrial fibrillation (II:2). His son had mild hypertrophy and an abnormal electrocardiogram at the age of 33 years (III:1). His daughter had an ostium secundum type atrial septal defect with no ventricular hypertrophy (III:2).

DISCUSSION

Hypertrophic cardiomyopathy is a heterogeneous disease in both its clinical presentation and its molecular basis. Only a few families have been reported with each of these mutations, and it is consequently difficult to draw conclusions about the relationship between genotype and phenotype that could enable their risk profile to be established.

We know that certain mutations are associated with a worse prognosis.[4] The *TNNT2* gene harbors some of the so-called malignant mutations. The prevalence of mutations in the *TNNT2* gene in this sample of the Spanish population was 3%, similar to that found by other authors.[3,6,7]

In this report we describe for the first time the Phe87Leu mutation, identified in a family from Alicante. This mutation affects the binding site of troponin with alpha tropomyosin. The phenotype is characterized by mild hypertrophy, on occasions absent, and a very poor prognosis. A few cases posed serious diagnostic problems, which were able to be resolved from the genetic information.

The Arg278Cys mutation was identified in 2 families. This mutation has been reported in 11 families from various countries.[4,6,7,10] The mutation affects an amino acid located at the binding site of tropomyosin and troponins I and C. Including our two families, there are now 36 carriers. The penetrance is incomplete, and hypertrophy, which becomes evident at advanced ages (50 years), is usually mild (17 mm). Reports exist of 11 SD, most in persons older than 60 years of age, although 2 cases occurred in young persons without ventricular hypertrophy.[4] Six of the 11 SD happened in the same family, so there might have been some additional unidentified risk factor.[6]

We identified a family with the Asp271Ile mutation; only one case has been previously reported, and we do not have the clinical data.[3] Three carriers in our family had a mild phenotype, and in one of these there was atrial communication. The data currently available do not allow the risk profile to be established.

Our study confirms that carriers of the mutations in *TNNT2* can develop mild phenotypes, as has been indicated previously,[4,5] and that mutations exist that are associated with a very high risk of SD, as is the case of the Phe87Leu mutation. In these cases, genetic analysis is a very useful clinical tool.

A few patients with severe phenotypes might carry double mutations, or the phenotype could be the consequence of the involvement of unknown modulating agents.[3,13]

We have also confirmed that some mutations in *TNNT2,* such as Arg278Cys, can cause late onset HCM. In these cases, the risk of SD may be greater in patients of a certain age.

In conclusion, mutations in the *TNNT2* gene caused 3% of cases of HCM in our population. The Phe87Leu mutation is associated with mild hypertrophy and a high risk of SD. The Arg278Cys mutation is associated with mild hypertrophy, incomplete penetrance and a high risk in older carriers. The presence of double mutations should be suspected in persons with severe forms of the disease.

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