



# Genetic basis of end-stage hypertrophic cardiomyopathy

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Received 22 March 2011; revised 5 June 2011; accepted 21 June 2011

## Aims

Hypertrophic cardiomyopathy (HCM) is characterized by a heterogeneous presentation and clinical course. A minority of HCM patients develop end-stage HCM and require cardiac transplantation. The genetic basis of end-stage HCM is unknown but small series, isolated case reports and animal models have related the most aggressive heart failure course with the presence of multiple mutations.

## Methods and results

Twenty-six patients (age  $40.4 \pm 14.5$  years; 46% male) transplanted for end-stage HCM underwent genetic screening of 10 HCM-related genes (*MYH7*, *MYBPC3*, *TNNT2*, *TNNI3*, *TPM1*, *TNNC1*, *MYL3*, *MYL2*, *ACTC*, *LDB3*). Additional genetic screening of *LAMP2/PRKAG2* and mitochondrial DNA (*mtDNA*) was performed in four and three cases, respectively. Findings were correlated with clinical and histological features. Pathogenic mutations were identified in 15 patients (58%). Thirteen patients (50%) had mutations in sarcomeric genes (six in *MYH7*, three in *MYBPC3*, two in *MYL2*, one in *TNNI3*, and one in *MYL3*) and two patients had mutations in *LAMP2*. Only three patients (13%) had double mutations and all in homozygosis. Except for a more frequent family history of HCM, patients with mutations in sarcomeric genes did not show any clinical feature that distinguished them from patients without mutations in these genes. Evaluation of 44 relatives from 12 families identified 13 mutation carriers, 9 of whom had an overt HCM phenotype.

## Conclusion

Heart transplanted HCM has a heterogeneous genetic background where multiple mutations are uncommon. The clinical course of HCM is not primarily dependent on the presence of multiple sarcomeric mutations. Clinical and genetic evaluation of relatives does not support differential clinical management in HCM based on genetics.

## Keywords

Hypertrophic cardiomyopathy • Heart failure • Mutations • Sarcomeric proteins • Cardiac transplant

## Introduction

Hypertrophic cardiomyopathy (HCM) is the most common genetic cardiac disease with a prevalence of 1:500 in the general population.<sup>1,2</sup> Although most patients have a good prognosis, a significant minority suffers from life-threatening complications, primarily sudden cardiac death (SCD) and end-stage heart failure.<sup>2–5</sup> The variable clinical presentation and course in HCM may relate to its

heterogeneous genetic nature. Mutations in >10 genes encoding for the myofilament contractile proteins of the cardiac sarcomere are present in 30–65% of HCM patients,<sup>6–10</sup> but other non-sarcomeric genetic defects have also been associated with the condition.<sup>11,12</sup>

Previous genotype–phenotype association studies in patients with HCM have shown that the genotype has limited value in predicting the clinical course. Nonetheless, small observational cohort

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studies, isolated case reports, and animal models have suggested that the presence of multiple mutations is associated with earlier disease expression and a more severe phenotype.<sup>6,9,13</sup> Hypertrophic cardiomyopathy patients undergoing heart transplantation for end-stage heart failure are a subgroup with severe disease expression, but the association of the cardiac phenotype with the genotype and with the presence of multiple or compound heterozygosity has not been systematically examined.

The primary aim of this study was to determine the genotype and prevalence of pathogenic mutations in HCM heart transplant recipients and to compare the histopathological characteristics of explanted organs and clinical features from mutation carriers with those of non-carriers.

## Methods

### Study population and inclusion criteria

The study cohort comprised all adult HCM patients who underwent heart transplantation for end-stage heart failure at our institution between April 1984 and December 2008, except for four patients from whom no genetic material was available. Data collection was retrospective. The study was approved by the ethics committee of Hospital Universitario Puerta de Hierro, Madrid, Spain, and complies with the principles of the declaration of Helsinki.

Pre-transplant diagnosis of HCM was based on echocardiographic demonstration of increased left ventricular (LV) wall thickness ( $\geq 15$  mm), in the absence of another cardiac or systemic disease of sufficient severity to account for the observed magnitude of LV hypertrophy.<sup>14</sup> Patients with Friedreich's ataxia, Noonan's syndrome, and metabolic disorders were excluded.

### Baseline clinical evaluation

The baseline pre-transplant assessment comprised physical examination, 12-lead electrocardiogram (ECG), echocardiography, 6 min walk test, upright exercise testing, biventricular radionuclide ventriculography, and cardiac catheterization. Additional studies including muscle/endomyocardial biopsies, electrophysiological study and cardiac magnetic resonance imaging were performed only if there was a specific clinical indication.

Based on patient history and family pedigree analysis, HCM was defined as familial if one or more relatives (in addition to the proband) had HCM during life or at post-mortem examination. A family history of sudden death was defined as SCD in a first-degree relative <55 years old.

Patient records were independently reviewed by two investigators who were blinded to the genetic results. Clinical data from first pre-transplant evaluation at our unit were collected.

### Genetic evaluation

Since September 1993, all patients placed on the heart transplant waiting list at our centre are asked to provide a blood sample for genetic analysis. Hypertrophic cardiomyopathy patients transplanted before September 1993 were invited to participate in the study.

DNA was extracted from blood samples and stored at  $-70^{\circ}\text{C}$ . DNA was amplified by PCR using primers designed to amplify the coding exons and the flanking intronic sequences of 10 HCM-related genes: myosin binding protein C (*MYBPC3*), beta-myosin heavy chain (*MYH7*), regulatory and essential light chains (*MYL2* and *MYL3*), troponin-T (*TNNT2*), troponin-I (*TNNI3*), troponin-C

(*TNNC1*), alpha-tropomyosin (*TPM1*), alpha-actin (*ACTC*), and ZASP/Cypher (*LDB3*).

Additional genetic analysis was carried out in selected patients based on additional criteria. Based on clinical data (existence of pre-excitation on ECG, conduction disturbances requiring pacemaker implantation, etc.); biochemical data (depressed mitochondrial respiratory chain activity); or histological findings (absence of disarray, presence of vacuoles, nuclear alterations, ragged red fibres, etc.), coding exons and the flanking intronic sequences of *LAMP2* and *PRKAG2* genes (related with glycogen metabolism) or mitochondrial DNA were also examined.

Following PCR amplification, direct sequencing of amplicons was performed on an ABI PRISM 3130 DNA analyser using BigDye Terminator chemistry (v3.1, Applied Biosystems). Primer sequences and PCR conditions are available on request.

For every sequence variant detected, a cohort of 200 ethnically matched control subjects was screened using the same methods.

Patients were classified as carriers of pathogenic mutations if they had: a genetic variant not found in controls that was previously reported to be associated with HCM, a novel sequence variant not found in controls that predicts a premature truncation, frameshift or abnormal splicing, or a novel missense mutation not found in controls that affects a conserved amino-acid residue.

Conservation of amino-acid residues was determined by Homologene (<http://www.ncbi.nlm.nih.gov/homologene>) by multiple alignment of orthologues in various species, including *Homo sapiens*, *Mus musculus*, *Rattus norvegicus*, *Gallus gallus*, and *Xenopus tropicalis*.

### Family screening

All relatives of probands with mutations were offered clinical and genetic evaluation after genetic counselling. In accordance with our unit's genetic testing policy, genetic screening was not offered to relatives <16 years of age if they were asymptomatic and clinical evaluation (including ECG and echocardiogram) was normal.

Family screening was considered positive if one or more relatives had HCM and the same genetic defect as the proband.

### Pathological examination of explanted hearts

Gross and microscopic examinations of explanted hearts were performed as previously described,<sup>4,15</sup> by an experienced histopathologist blinded to the genetic and clinical data. Blocks of the free wall of the LV, right ventricle (RV), and interventricular septum were examined. Tissue specimens were embedded in paraffin, sectioned at  $6\ \mu\text{m}$ , and stained with haematoxylin-eosin and Masson's trichrome. Blocks were examined microscopically to assess myocyte disarray, interstitial and replacement fibrosis, and intramural small vessel disease. Fibrosis was defined as interstitial when myocytes were encircled by collagen matrix and replacement type when myocytes were substituted by connective tissue. Disarray and fibrosis were graded 0 to 3+ as previously described.<sup>4</sup>

### Statistical analysis

Continuous variables are expressed as mean value  $\pm$  standard deviation. Discrete variables are shown as percentages. Differences between means were compared using the Student *t*-test and the Mann-Whitney *U* test for normally distributed and non-normally distributed continuous data, respectively.  $\chi^2$  with Yates' correction and Fisher exact analysis were used to test for associations between dichotomous variables. Probability values reported are two sided, and values <0.05 were considered statistically significant. All data were analysed using the SPSS software (version 15.0).

## Results

During the study period 727 patients had a heart transplant at our institution. End-stage HCM was the indication for heart transplantation in 30 patients (4.1%). Genetic material was not available in four patients who were excluded from the study. The study cohort consisted of 26 adult transplant recipients (mean age at transplantation  $40.4 \pm 14.5$  years; range 18.2–65, 46% male). All patients underwent genetic screening for nine sarcomeric genes and *LDB3*. Their clinical characteristics and histopathological findings are summarized in supplementary online *Tables SA* and *SB*. Four patients were related (Patients H6 and H15 were sisters and Patients H8 and H29 were aunt and nephew, respectively). Sixteen patients (62%) had known family history of HCM and eight (31%) had family history of SCD.

Based on the predominant pathophysiological disease component: 19 patients (73%) had systolic dysfunction (burnt-out HCM; left ventricle ejection fraction [LVEF] < 50%) and 7 (27%) heart failure with preserved systolic function in the absence of LV outflow tract obstruction.

Three patients (12%) had undergone previous myectomy and five (19%) had a pacemaker implanted. Five (19%) had an implantable cardioverter-defibrillator (ICD) (four patients for primary prevention of SCD and one after an aborted SCD) and three have had appropriate interventions during follow-up.

Eighteen (69%) patients had paroxysmal or chronic atrial fibrillation (AF).

## Genetic analysis

Thirteen patients (50%) had pathogenic mutations in at least one sarcomeric gene and two patients had a mutation in *LAMP2* (supplementary online *Table SA*). No mutations were identified in 11 patients.

### Sarcomeric and *LDB3* genetic screening

Ten disease-causing sarcomeric protein mutations were identified in 13 patients. Six patients had mutations in *MYH7*, three had mutations in *MYBPC3*, two in *MYL2*, one in *TNNI3*, and one in *MYL3* (supplementary online *Table SA*).

Three patients (H6, H15, and H26) from two families (#6 and #26) harboured a genetic defect in homozygosity (consanguinity was present in both families—see supplementary online figure for family trees). No other patients had multiple or compound heterozygosity ( $\geq 2$  distinct mutations in the same or in different sarcomeric genes).

Six mutations (including the two found in homozygosity) have been described previously in patients with HCM;<sup>10,16</sup> two were novel mutations that predicted premature truncation of the transcribed protein (Y79X-*MYBPC3*, previously described in the same family by our group<sup>17</sup>) or affected an acceptor splice site (IVS23-1 G>A-*MYBPC3*; <http://bioinfo.itb.cnr.it/oriel/splice-view.html>); and, two were novel missense mutations (M849T-*MYH7* and G128C-*MYL3*) that were not found in controls and affected a conserved amino-acid residue.

### *LAMP2*, *PRKAG2*, and mitochondrial DNA genetic screening

Four patients (H5, H18, H23, and H28) underwent genetic screening of *LAMP2* and *PRKAG2* genes. Three of them (H5, H23, and H28) had pre-excitation in pre-transplant ECG and did not show mutations in analysed sarcomeric genes. Although a sarcomeric mutation had been found in Patient H18, *LAMP2* and *PRKAG2* were screened based on the presence of vacuoles in the explanted heart.

Patient H5 had a new insertion mutation that predicted premature truncation in *LAMP2* and H23 had a previously described stop-codon mutation in the same gene. None of them showed skeletal muscle myopathy, mental retardation, or other signs of Danon's disease.

Three other patients (H2, H4, and H26) were tested for mutations in mitochondrial DNA based on histological findings in explanted hearts (H2 and H26) or depressed activities of Complexes I and IV of the mitochondrial respiratory chain in muscle biopsies (H2 and H4). On sarcomeric genetic analysis Patients H2 and H4 did not have any abnormality while H26 was homozygous for D778E mutation in *MYH7*. None of the patients had mutations in mitochondrial DNA.

## Family screening

Members of the 13 families with genetic abnormalities were invited for family and genetic screening. Twelve families (92%) agreed to participate in the study. From a total of 47 relatives contacted, 44 (94%) agreed to be clinically and genetically screened.

Thirteen relatives (30% of screened) had mutations and nine (69% of mutation carriers) were clinically affected.

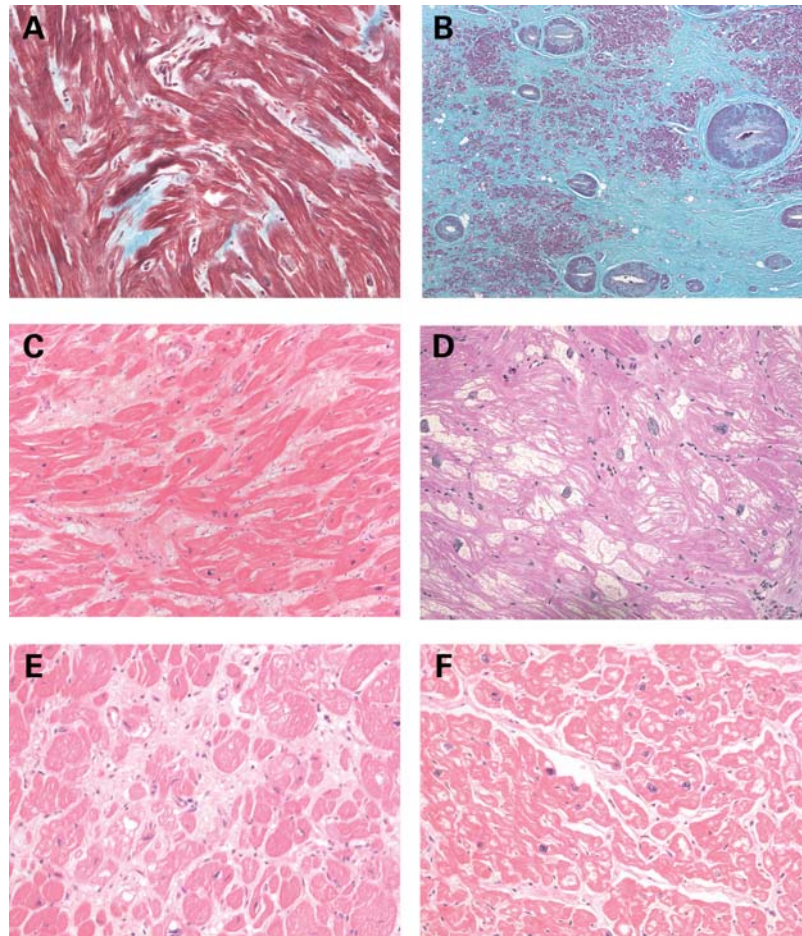
Cosegregation of mutations with HCM in another relative was demonstrated in six families (#6, #8, #11, #13, #26, and #27—see supplementary online figure for family trees).

## Patients with sarcomeric mutations vs. patients without mutations

The clinical features of patients with mutations in sarcomeric genes were indistinguishable from those observed in patients without sarcomeric mutations with the exception of the family history of HCM that was more common among patients with sarcomeric mutations (*Table 1*). The difference in mean LV ejection fraction, which was more depressed in patients without mutations in sarcomeric genes, was of borderline significance ( $33 \pm 11$  vs.  $46 \pm 21\%$ ;  $P = 0.051$ ) (*Table 1*).

On histopathological examination (supplementary online *Table SB*) the mean heart weight was higher in patients without sarcomeric gene mutations (*Table 1*). Both groups had similar amount of interstitial and replacement fibrosis, myocyte disarray, and small vessel disease (*Table 1*). Although the amount of fibrosis was almost identical in both groups, there was a trend towards more presence of small vessel disease and higher degree of disarray among patients with mutations (*Table 1*).

However, we must point out that these differences were not maintained universally among patients from both groups: some patients without sarcomeric mutations exhibited the so-called HCM histological hallmark signs (myocyte disarray, small vessel



**Figure 1** Microscopic examinations from Patients H27 (A), H30 (B), H20 (C), H28 (D), H23 (E), and H2 (F). (A) Masson's trichrome stain ( $\times 200$ ) shows typical disarray of sarcomeric hypertrophic cardiomyopathy. Patient had a mutation in *MYBPC3*. (B) Masson's trichrome stain ( $\times 25$ ) shows extensive replacement fibrosis and small vessel disease. Mutation in *MYH7*. (C) Haematoxylin–eosin stain ( $\times 25$ ) shows disarray and interstitial fibrosis. No gene defect identified. (D) Haematoxylin–eosin stain ( $\times 100$ ) shows myocyte vacuolization, abnormal nucleus, and complete disorganization of fibres. No gene defect was identified. (E) Haematoxylin–eosin stain ( $\times 100$ ) shows myocyte vacuolization and severe interstitial and replacement fibrosis. Mutation in *LAMP2*. (F) Haematoxylin–eosin stain ( $\times 100$ ) shows myocyte vacuolization, abnormal nucleus, and some interstitial fibrosis. No gene defect was identified.

disease), whereas several patients with sarcomeric mutations did not (Figure 1, Supplementary material online Table SB).

## Discussion

This study is the largest cohort of genetically screened end-stage HCM patients and the largest cohort of heart transplanted HCM described in detail. Pathogenic mutations were identified in almost 60% of transplanted patients, and the majority had sarcomeric gene mutations. A minority of patients were homozygous for a mutation, while none had two different mutations. The phenotype of patients with sarcomeric mutations was indistinguishable from that observed in patients without mutations.

The characteristic clinical heterogeneity of HCM has been extensively related to its heterogeneous genetic background, as mutations in more than 20 different genes have been related with the condition.<sup>1,2,18</sup>

The influence of genetics on clinical course is controversial as initial genotype–phenotype studies linking clinical course with defects in certain genes have proved to be of limited value.<sup>18,19</sup> Nonetheless, previous reports based on isolated case descriptions or small series along with animal studies have suggested that multiple or compound heterozygosity in sarcomeric genes is frequent among HCM patients with the most severe clinical course who develop end-stage heart failure.<sup>6,9,12</sup> As a consequence of improvement in genetic screening techniques, HCM patients with double or even triple heterozygosity are increasingly being recognized.<sup>6–10</sup> Several series have established the frequency of double mutations to be around 5% (Table 2) and some authors have suggested a closer clinical follow-up with prompt initiation of pharmacological therapies in these patients based on their complex genotype.<sup>9</sup>

Our analysis shows that multiple mutations could have a deleterious effect (as seen in family #6 where homozygotes had an



**Table 1** Clinical, electrocardiographic, echocardiographic, haemodynamic, and histological characteristics of patients with and without sarcomeric mutations

Variable	Patients with sarcomeric mutations (n = 13)	Patients without sarcomeric mutations (n = 13)	P-value
Mean age (years)	41.2 ± 15.2	39.5 ± 14.3	0.77
Sex, n (%)			0.69
Male	5 (39%)	7 (54%)	
Female	8 (61%)	6 (46%)	
Previous myectomy, n (%)	3 (23%)	0 (0%)	0.22
Family history			
HCM, n (%)	11 (85%)	6 (46%)	0.01
SCD, n (%)	6 (46%)	3 (23%)	0.41
ECG			
AF, n (%)	11 (85%)	8 (62%)	0.38
Mean QRS duration (ms)	112 ± 24	110 ± 18	0.78
LV hypertrophy, n (%) <sup>a</sup>	6 (46%)	8 (66%)	0.53
Echocardiography			
Right atria (mm)	52.8 ± 9.3	51.7 ± 11.8	0.81
Max wall thickness (mm)	18.4 ± 5.7	18.2 ± 5.9	0.92
LVEF (%)	46 ± 21	33 ± 11	0.05
LV end-diastolic dimension (mm)	49 ± 12	56 ± 10	0.17
RVEF on isotopic ventriculography (%) <sup>b</sup>	32 ± 9	32 ± 8	0.95
Right catheterization			
Wedge (mmHg)	24.9 ± 8.5	22.4 ± 8.9	0.48
Cardiac index (L/min/m <sup>2</sup> ) <sup>c</sup>	1.8 ± 0.25	1.92 ± 0.53	0.46
Pulmonary vascular resistance (WU)	3 ± 1.6	2.6 ± 1.4	0.50
Histopathology			
Heart weight (g)	386 ± 92	497 ± 99	0.007
Max LVFW/IVS wall thickness (mm)	19 ± 5.4	20.9 ± 5.5	0.39
LVFW replacement fibrosis <sup>d</sup>	1.62 ± 1.12	1.69 ± 1.25	0.87
Interstitial fibrosis <sup>d</sup>	1.69 ± 0.63	1.54 ± 0.88	0.61
Small vessel disease, n (%)	9 (69%)	4 (31%)	0.12
Myocyte disarray <sup>d</sup>	1.38 ± 1.33	0.69 ± 1.03	0.15

WU indicates woods units; IVS, ventricular septum; LVEF, left ventricle ejection fraction; LVFW, left ventricular free wall.

<sup>a</sup>By Sokolow–Lyon criteria.<sup>33</sup> One patient without mutations excluded due to paced ventricular rhythm.

<sup>b</sup>Six patients (46%) from the sarcomeric group and five (38%) from the non-sarcomeric group underwent RV isotopic ventriculography.

<sup>c</sup>One patient with sarcomeric mutation excluded due to concomitant right-left cardiac shunt.

<sup>d</sup>Mean value for myocyte disarray and myocardial replacement/interstitial fibrosis graded on histological sections semi-quantitatively in each patient from 0 to 3+ (0, absent; 1+, mild; 2+, moderate; 3+, severe);

aggressive course whereas heterozygotes did not express the condition, Figure 2) but, we have found also families with only one mutation and a highly severe clinical course (Figure 2).

Our findings therefore reflect that the severity of phenotypic expression in HCM is probably more dependent on the interplay between the genetic defect, environmental factors, and other non-sarcomeric genetic factors, rather than on the modifying effects of additional sarcomeric mutations.

Four of the seven mutations already described (E22K-MYL2, R719W-MYH7, R719Q-MYH7, and R293X-LAMP2) have been previously associated with heart failure/heart transplantation.<sup>18,20,21</sup> This finding appears to stress the concept of ‘malignant’ mutations that confer a bad prognosis but, unfortunately, controversy about this concept is likely to continue<sup>18,22</sup> as there

are other publications where these genetic variants have shown a ‘benign’ course among several families.<sup>18,23,24</sup>

Of the 10 patients with a family history of SCD included in our study, only 3 have exhibited a family history of heart failure (supplementary online Table SA) underscoring again the clinical heterogeneity of HCM.

This study confirms that HCM genetic variability is maintained in aggressive forms of the condition. The prevalence of patients with sarcomeric mutations in this study was 50%, which is similar to the prevalence reported in unselected cohorts of adults and children with HCM (Table 2). Although multiple mutations were only found in a minority of patients from our series (11.5%), the prevalence of multiple mutations was greater than in unselected cohorts (Table 2).

**Table 2** Distribution of sarcomere-protein gene mutations in different hypertrophic cardiomyopathy populations

Variable	Adult HCM <sup>a</sup>	Paediatric HCM <sup>b</sup>	Spanish HCM <sup>c</sup>	Transplanted HCM
Gene, %				
MYH7	22.8	24.9	9.2	23.1
MYBPC3	30	21.4	16	11.5
TNNT2	4	3.5	1.5 <sup>d</sup>	0
TNNI3	2.3	1.7	0 <sup>d</sup>	3.8
TPM1	0.5	1.2	0.8 <sup>d</sup>	0
MYL2	3	0	NA	7.7
MYL3	0.3	1.7	NA	3.8
ACT	0.3	2.3	NA	0
Mutation detection, %	54.7	50.9	25.2 <sup>e</sup>	50
Probands, no.	685	173	250	26
Probands with multiple mutations, %	5	6.4	1.2 <sup>e</sup>	11.5

<sup>a</sup>This category refers to the percentage of unrelated adult probands with a mutation in each sarcomere-protein gene. Distribution of mutations among genes only available in 400 patients. Data are from Richard et al.,<sup>6</sup> Olivotto et al.,<sup>7</sup> and Girolami et al.<sup>9</sup>

<sup>b</sup>This category refers to the percentage of unrelated paediatric ( $\leq 15$  years old) probands with a mutation in each sarcomere-protein gene. Data are from Morita et al.<sup>8</sup> and Kaski et al.<sup>10</sup>

<sup>c</sup>This category refers to the percentage of unrelated Spanish adult probands with a mutation in each sarcomere-protein gene. Data are from Garcia-Castro et al.,<sup>25</sup> Laredo et al.,<sup>26</sup> and Rodriguez-Garcia et al.<sup>27</sup> NA denotes not available.

<sup>d</sup>Calculated over 130 patients from Garcia-Castro et al.<sup>25</sup>

<sup>e</sup>Calculated only with data from MYH7 and MYBPC3.

Of note, the prevalence of mutations found in beta-myosin heavy chain (*MYH7*) in our cohort doubled the number of mutations found in myosin binding protein C (*MYBPC3*). Although the number of patients in our cohort is small, this finding may reflect association of *MYH7* mutations with progression to heart failure, especially if we take into account that *MYBPC3* is the gene more frequently affected among Spanish HCM patients (Table 2).<sup>24–27</sup>

### Clinical characteristics of end-stage heart failure in hypertrophic cardiomyopathy

Heart failure HCM has been described in between 5 and 17% of HCM cohort series<sup>3,4,9</sup> with an annual incidence of 1%.<sup>24</sup> Although SCD has been traditionally the main complication studied in HCM, disability and death related to heart failure is also important and has gained little attention until recently.<sup>3,4,28</sup>

Heart failure in HCM does not occur in a single unique clinical setting, but under a variety of circumstances due to different pathophysiological mechanisms including: (a) systolic dysfunction (burn-out HCM); (b) LV outflow obstruction; and (c) non-obstructive heart failure with preserved systolic function (usually with restrictive physiology). Although LV outflow obstruction can usually be managed with a wide range of drugs and procedures, the treatment of severe heart failure with either systolic dysfunction or restrictive physiology has few therapeutic alternatives. Our cohort of heart transplanted HCM patients confirms this, as it was completely composed of patients from the former two groups. Also, in our cohort of 30 heart transplanted HCM patients there were similar numbers of women (15; 50%) and men, in contrast with general HCM cohorts which are largely dominated by male patients.<sup>29,30</sup> The association between female gender and development of heart failure in HCM has also been reported by

other authors<sup>30</sup> and has been related to a higher trend towards restrictive physiology in females as a consequence of smaller cavities for similar amounts of wall thickness as males. Atrial fibrillation was very common among our patients (69%), showing that its appearance confers an adverse prognosis. Some of our patients with paroxysmal AF became highly symptomatic and required cardiac transplant when AF became permanent, suggesting that efforts should be attempted to maintain sinus rhythm in HCM patients.

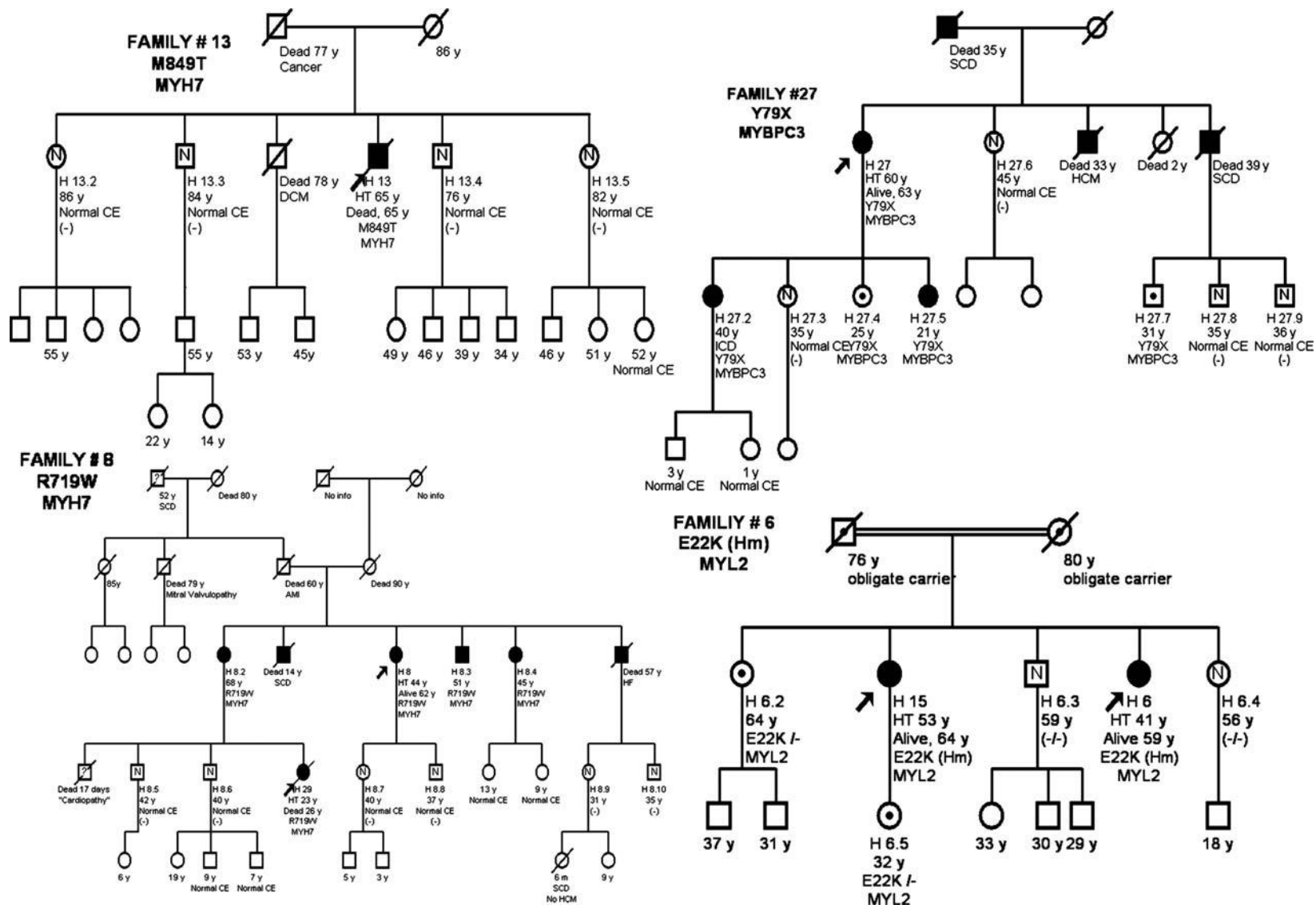
Finally, the clinical course of heart transplanted HCM was extremely quick with a mean time of  $6.5 \pm 6$  years from onset of symptoms to development of New York Heart Association Class III/IV and  $1 \pm 0.7$  years from that point to heart transplant (Figure 3). These data are consistent with previous reports<sup>3,4</sup> and should be taken into account by the physician attending patients who reach these milestones, in order to refer them promptly to a heart transplant centre.

### Previous studies in transplant populations and implications for relatives

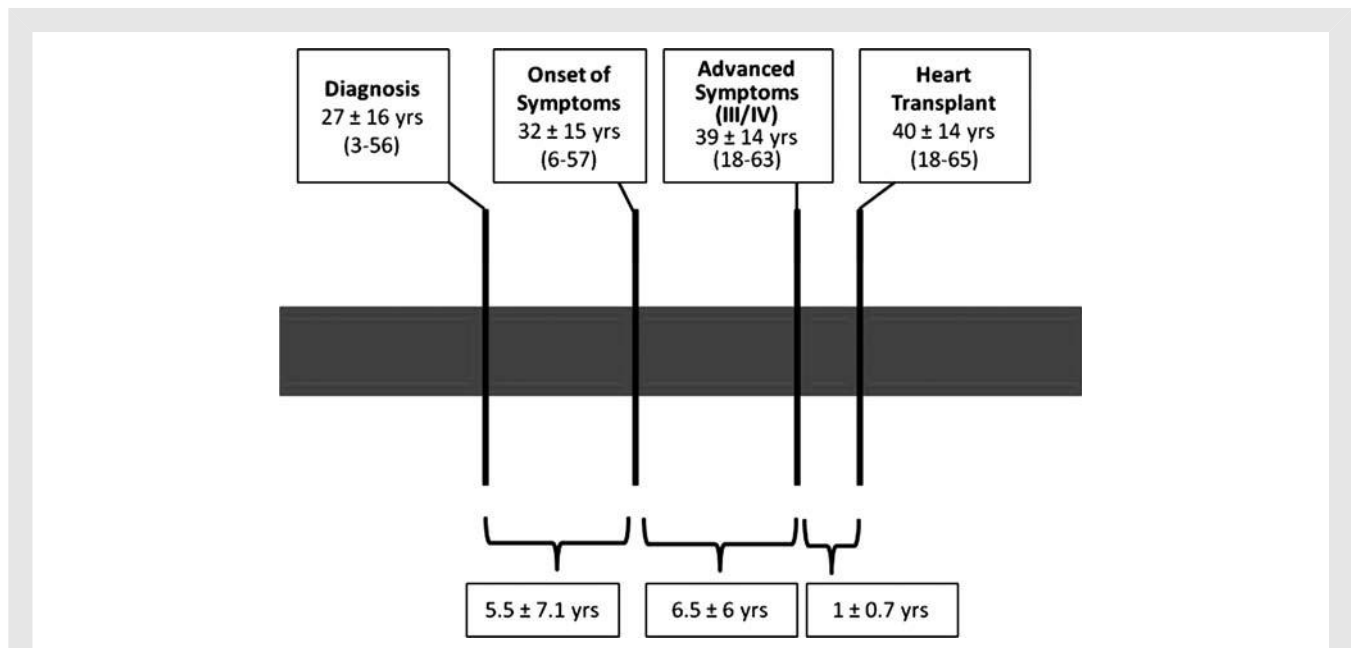
Very few studies have examined genetic characteristics in heart transplant recipients and have been circumscribed to patients with dilated cardiomyopathy.<sup>31,32</sup>

End-stage heart failure HCM has been proposed as a 'model' for clarifying the role of genetics, and molecular, biochemical, biophysical, cellular, and physiological processes in the evolution of HCM, dilated cardiomyopathy, and heart failure.<sup>28</sup> Analysis of explanted hearts from transplanted HCM patients provides a unique opportunity to do this.

Besides its research interest, the histopathological analysis of the explanted heart could also provide important information to guide genetic analysis that may benefit relatives.



**Figure 2** Family trees from four selected families (#13, top left; #27, top right; #8, bottom left; #6, bottom right) of heart transplanted hypertrophic cardiomyopathy patients. Squares and circles indicate male and female family members, respectively. Consanguinity is indicated by double horizontal lines. Symbols with a single slash mark are deceased family members. Arrows indicate probands. Solid symbols are affected individuals. Symbols containing a dot are unaffected carriers. Symbols containing an 'N' are unaffected non-carriers. The ages stated refer to age at the time of death for deceased family members and current age for living family members. HT indicates heart transplant; y, years; CE, clinical evaluation (including electrocardiogram and echocardiogram); SCD, sudden cardiac death; ICD, implantable cardioverter-defibrillator; HF, heart failure; AMI, acute myocardial infarction; DCM, dilated cardiomyopathy.



**Figure 3** Time line with patient ages describing evolution of 26 hypertrophic cardiomyopathy patients to heart transplant.

Family screening in our study revealed 13 mutation carriers (9 clinically affected) among 44 relatives (30%) from 12 families. Comprehensive genetic screening in these families led to the identification of 31 non-carriers (and their descendents) who will not require additional clinical evaluation in the future. This, along with the possibility of giving appropriate genetic counselling to carriers (including reproductive and life plans), illustrates the benefits of genetic screening tests in patients with end-stage HCM (see Families #8 and #13 from Figure 2).

Interestingly, as shown in supplementary online Table SA, 9 of the 17 patients (53%) with a family history of HCM also had a family history of heart failure (9 of 26; 35% in overall cohort) and 4 of the 24 families included in the study had at least 2 members transplanted (Families #2, #4, #6, and #8). The prevalence of familial HCM-related heart failure in our cohort is much higher than the prevalence of heart failure among unselected HCM cohorts (5–17%) highlighting the familial association of this type of clinical course and stressing the need for intensive clinical care in such families.

Despite this clear association, the results of family evaluation in this study underscore the profound clinical heterogeneity of HCM and do not support the concept of prompt initiation of pharmacological therapies based solely on genetics, as some relatives showed the same genetic findings as transplanted HCM patients but did not express the disease or had a non-aggressive course of the condition (see Families #8 and #27 from Figure 2).

## Conclusions

This study confirms that HCM has a heterogeneous genetic background even in patients with a severe clinical course. Although multiple mutations occur more frequently among end-stage heart

failure HCM patients than in general series of HCM, they affect only a minority of these patients. Patients with certain single mutations could also have a very aggressive course. Phenotypic expression of HCM does not seem to be primarily dependent on the combined effect of multiple sarcomeric mutations and is likely to be influenced by environmental factors as well as other non-sarcomeric genetic factors.

Heart transplanted HCM is characterized by a high prevalence of AF, absence of LV outflow tract obstruction, increased number of women than in the general HCM population, accelerated clinical course, and heterogeneous histopathology. Outcomes of family screening emphasize the importance of offering appropriate genetic screening strategies to the relatives of HCM patients.

## Supplementary material

Supplementary material is available at *European Journal of Heart Failure* online.

## Acknowledgements

We are grateful to the families for their participation in the study and to Carmen Prior, PhD; Mrs Ana Briceño; and Mrs Macarena Orejudo for their excellent technical assistance. We are indebted to Dr Costas O'Mahony for critical review of the manuscript.

## Funding

This work was supported by the Instituto de Salud Carlos III (grants PI08/0978 and PI06/0205); Spanish Society of Cardiology (2008 to J.S.); Fundación Investigación Biomédica Hospital Puerta de Hierro (2008 to J.S.); and the Spanish Ministry of Health (Red Cooperativa de Insuficiencia Cardíaca (REDINSCOR) RD06/03/



0018). P.A. received funding as a research fellow from Fundación Carolina-BBVA.

**Conflict of interest:** none declared.

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