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ORIGINAL ARTICLE

Usefulness of Genetic Testing in Hypertrophic Cardiomyopathy: an Analysis Using Real-World Data

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Abstract This study sought to determine the usefulness of genetic testing to predict evolution in hypertrophic cardiomyopathy (HCM) and to assess the role of genetic testing in clinical practice. Genetic results of 100 HCM patients tested for mutations in ≥10 HCM-causing genes were evaluated. Patients were classified as with poor (group A) or favourable (group B) clinical course.

Forty-five pathogenic mutations (PM) were identified in 28 patients (56 %) from group A and in 23 (46 %) from group B ($p = 0.317$). Only 40 patients (40 %) exhibited PM that had been previously reported and only 15 (15 %) had PM reported in ≥10 individuals. PM associated with poor prognosis were identified in just five patients from group A (10 %). Genetic findings are

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Clinical relevance of the manuscript
Hypertrophic cardiomyopathy (HCM) is a heterogeneous disease with a broad clinical spectrum related to its diverse genetic profile. Although genetic findings are not currently recommended to predict prognosis, very few studies have analysed this issue and controversy remains about the usefulness of genetic testing to predict disease progression. The current study provides comprehensive data to support the view that genetic findings are not useful to predict prognosis in HCM patients. Our results indicate that it is not appropriate to perform genetic testing in HCM patients to predict patients' and relatives' clinical course. By contrast, real-world data obtained in this study reinforces the usefulness of genetic testing to provide genetic counselling and to enable familial evaluation.

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not useful to predict prognosis in most HCM patients. By contrast, real-world data reinforce the usefulness of genetic testing to provide genetic counselling and to enable cascade genetic screening.

Keywords Hypertrophic cardiomyopathy · Mutations · Genetics · Prognosis · Family screening

Abbreviations

HCM	Hypertrophic cardiomyopathy
HT	Heart transplant
ICD	Implantable cardioverter defibrillator
NGS	Next-generation sequencing
PM	Pathogenic mutation
SCD	Sudden cardiac death
VUS	Variant of uncertain significance

Introduction

Hypertrophic cardiomyopathy (HCM) is the most frequent inherited cardiac disease [1, 2]. Although most HCM patients have a good prognosis, a significant number suffer from life-threatening complications, primarily sudden cardiac death (SCD) and end-stage heart failure [3, 4].

The heterogeneous phenotypic expression of HCM has been related to its diverse genetic profile [5] and, to date, more than 1400 pathogenic mutations (PM) in >10 genes have been described to cause HCM [1, 5].

The impact of genetics in the clinical course of HCM is controversial [6–8]. Although several studies suggest that some mutations associate with a poor clinical course [8–10], findings are inconsistent and large variations in clinical course are often seen in individuals harbouring the same genetic defect [11–13].

Current clinical guidelines advocate genetic testing in HCM to facilitate identification of relatives at risk of developing the disease but not for establishing prognosis [14]. While the notion that genetic findings are not useful to predict prognosis in HCM is the predominant position amongst cardiomyopathy experts, it is important to state that very few studies have analysed this issue in depth and, consequently, there is little scientific evidence to support this accepted viewpoint. Furthermore, some studies have recently described several phenotype–genotype associations for some mutations supporting the opposite viewpoint (that genetic findings allow prediction of clinical course) [9, 10, 15], and the American Heart Association/American College of Cardiology HCM guidelines include genetic findings as a modifier factor to predict SCD and guide implantable cardioverter defibrillator (ICD) implantation [16]. As such, controversy remains in the field.

The impact of genetic testing in familial management and its ability to provide genetic counselling and reduce costs associated with periodic familial surveillance in real-world clinical practice has not been fully investigated. The aims of this study were twofold: first, to determine if genetic testing is useful to predict prognosis in HCM patients with poor clinical course, and second, to analyse the usefulness of genetic testing in the real world.

Methods

Patient Population

We retrospectively identified 100 unrelated HCM patients followed up at two inherited cardiac diseases units in Spain. Patients were selected after merging both units' databases. For all patients, detailed clinical data at baseline (defined as their first presentation to participating units) and follow-up visits, as well as detailed genetic, family history, and echocardiographic data, were collected. Major adverse cardiac events were considered from birth to the last follow-up.

Two groups of 50 individuals each were constructed by selecting the first 50 consecutive HCM index cases evaluated who had either "poor" or "favourable" clinical course and had undergone complete genetic evaluation (tested for ≥10 HCM-associated genes). Patients were genetically tested during the period 2008–2014. Patients were considered to have a poor clinical course if they had a SCD event, an appropriate ICD discharge and/or had required a heart transplant (HT) for end-stage heart failure. Patients with a "favourable clinical course" were those who had none of the above events during follow-up. The study was approved by the ethics committee of participant centres and conformed to the ethical guidelines of the Declaration of Helsinki. Informed written consent was obtained from all patients.

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Genetic Evaluation

Genetic testing was performed as part of the clinical service at both centres either by Sanger sequencing (29 cases) or by next-generation sequencing (NGS) with a panel of genes associated with HCM (71 cases). The genetic testing technology used in each case depended on local practice. Generally, all patients examined during the period 2008–2012 were tested by Sanger sequencing and NGS was used from 2013 onwards.

Sanger sequencing included all coding exons and flanking intronic regions of the 10 most frequently mutated sarcomeric genes associated with HCM: *MYH7*, *MYBPC3*, *TNNI3*, *TNNT2*, *TNNC1*, *TPM1*, *MYL2*, *MYL3*, *ACTC1* and *LDB3*. Based on clinical data, *LAMP2* and *PRKAG2* were also examined in four individuals. DNA was sequenced on both strands.

NGS analysis was undertaken at certified genetic testing laboratories using an inherited cardiac disease gene panel including 56 genes associated with HCM (Supplemental material). Overall mean coverage of the samples was 1558 reads and 99.46 % of the fragments had coverage >30. Sanger sequencing was used to confirm the genetic variants found and to evaluate fragments with low coverage in HCM-related genes.

Genetic variants and allelic frequencies were scored based on dbSNP (www.ncbi.nlm.nih.gov/SNP), Exome Variant Server (EVS) (evs.gs.washington.edu), 1000 Genomes (www.1000genomes.org), Ensembl (www.ensembl.org), Human Gene Mutation Database (HGMD® Professional) (http://www.hgmd.cf.ac.uk) and Exome Aggregation Consortium (ExAC) (exac.broadinstitute.org) databases. Only non-common variants (minor allele frequency < 0.01 % in ExAC) were used in this study. In silico pathogenicity prediction of novel genetic variations was performed using Polyphen-2, PROVEAN and Mutation Taster.

Sequence variants were classified as PM or variants of uncertain significance (VUS). Variants were considered PM if (i) they had been reported previously as disease-causing mutations associated with HCM in the literature or in online international databases such as ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/) and HGMD showing a minor allele frequency (MAF) <0.01 % in the ExAC population; (ii) they were novel (not reported before as pathogenic in the literature) sequence variants in a previously HCM-associated gene, with a MAF <0.01 % that predicted a premature truncation, frameshift or abnormal splicing of the protein; (iii) they were novel missense variants in a previously HCM-associated gene, with a MAF <0.01 % that cosegregated with the disease in the family; or (iv) that affects a highly conserved protein residue amongst species and with a predicted functional alteration in ≥2 of the 3 in silico software tools used, with a MAF <0.01 % if familial genetic screening information is unavailable. Genetic variants were classified as VUS if they were novel missense variants in a previously HCM-associated gene, with unclear predicted in silico functional alteration and without evidence of familial cosegregation. Variants previously described in the literature as VUS were classified as such.

Conservation of amino acid residues affected by genetic variants was determined using AlaMut (version 2.4.5; Interactive Biosoftware, Rouen, France) as previously reported [17].

Review of Literature

After the identification of the PM in both groups, we reviewed all articles in which they had been reported (Supplemental material, no limitations to number of genes/patients applied) and the available information from PM carriers was extracted. By internal consensus, a PM was considered as "associated with poor prognosis" when it had been described in the literature in ≥10 individuals and when ≥20 % of the reported gene carriers had had an adverse event (SCD, appropriate ICD discharge or HT). The last search was performed on March 10, 2015. Additionally, to summarise the current information about genetic findings in different HCM populations, we undertook a search in PubMed for all studies published in the last 10 years analysing ≥8 genes in ≥50 HCM individuals. The flow chart of the study selection process for this second literature review can be found in Supplemental material (Fig. S1).

Family Screening

First-degree relatives of probands with identified genetic variants were offered clinical (including ECG and echocardiography) and genetic evaluation. All relatives signed an informed consent. Cascade clinical and genetic screening was performed in additional relatives of first-degree relatives who were found to be carriers of PM. Genetic screening was not offered to relatives <16 years if they were asymptomatic and clinical evaluation was normal.

Impact of Genetic Testing for Reproductive/Professional Counselling

We evaluated the clinical utility (reproductive, professional and sports counselling) derived from genetic testing and cascade genetic screening. For this purpose, we identified index patients and relatives who were ≤40 years old and had received reproductive counselling. Regarding the impact of genetic testing in the selection of a profession/job amongst genotype positive-phenotype negative and non-carriers, the cut-off age was set at 30 years. We considered that all individuals below this age might have benefited from knowing their genetic carrier status when electing a professional career.

Impact of Genetic Testing in Cost Savings

We reviewed the economic impact of genetic analysis, estimating the savings from cessation of monitoring of genotype-negative

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relatives. The cost of clinical monitoring in Spain was estimated as 303 Euro per each follow-up visit based on official healthcare cost lists (Table S6, Supplementary material). Savings derived from the avoidance of lifetime clinical screenings were estimated for genotype-negative relatives according to their ages at the time of genetic testing and the expected total number of avoided evaluations until age 75 based on guidelines [14, 16]. No clinical screens were assumed for individuals older than 75 years.

Statistical Analysis

Continuous variables are expressed as mean value ± standard deviation. Discrete variables are shown as percentages. Differences between means were compared using Student's t test and Mann–Whitney U test for normally distributed and non-normally distributed continuous data, respectively. Chi-squared with Yates' correction and Fisher exact analyses were used to test for associations between dichotomous variables. Probability values reported were two-sided, and values <0.05 were considered statistically significant. All data were analysed using SPSS software version 16 (SPSS Inc., Chicago, IL, USA).

Results

A total of 100 HCM patients were studied (52 ± 15 years, 73 % male). Fifty patients (52 ± 16 years, 68 % male) were considered to have a "poor clinical course" [29 (58 %) had a HT, 10 (20 %) an aborted SCD and 15 (30 %) an appropriate ICD discharge]. In this group, a total of 20 patients (40 %) had family history of HCM and 14 (28 %) had family history of unexplained SCD. Twenty-two patients (44 %) had impaired left ventricular ejection fraction, and 30 (60 %) had severe heart failure symptoms (NYHA III-IV) at the time of the adverse event. The mean maximal left ventricular wall thickness was 21 ± 7 mm; 10 patients (20 %) had left ventricular outflow tract obstruction and 3 patients (6 %) had undergone surgical myectomy.

The group of HCM patients with "favourable clinical course" comprised also 50 individuals (53 ± 13 years, 78 % male). Twenty-two patients (44 %) had known family history of HCM and 17 (34 %) had family history of SCD. All except three patients had a normal ejection fraction and all were in NYHA class I–II. Nineteen patients (38 %) presented left ventricular outflow tract obstruction, and five (10 %) had undergone myectomy. Nineteen patients (38 %) had an ICD implanted for SCD primary prevention.

Patients with poor clinical course were significantly younger at diagnosis (34 ± 19 vs 43 ± 15 years; $p = 0.014$) and presented more frequently left bundle branch block than patients with favourable clinical course (24 vs 6 %; $p = 0.012$). Table 1 summarises the phenotype of the study cohort.

Genetic Findings

A total of 45 pathogenic mutations were identified in 51 HCM patients (51 %). Amongst those with poor clinical course, 28 (56 %) presented 24 PM in sarcomeric genes and 2 in *LAMP2* (Table S2, Supplementary material). Genes most frequently mutated were *MYBPC3* and *MYH7* (nine and eight PM, respectively). A total of 17 PM (65 %) had been previously reported as pathogenic, whereas 9 mutations (34 %) were novel variants. Five patients (10 %) carried multiple PM: three patients harboured a genetic defect in homozygosis ($MYL2^{E22K}$, $MYH7^{I144T}$ and $MYH7^{D778E}$) and two patients showed combined heterozygosis ($MYBPC3^{IVS23+1G>A}$ with $ACTC1^{L10M}$ and $MYH7^{A797T}$ with $TNNI3^{R162Q}$). In patients with favourable clinical course, 22 PM were identified in 23 (46 %) patients (Table S3, Supplementary material). A total of 21 PM were found in sarcomeric genes and 1 in a non-sarcomeric gene (*GLA*). The most frequently affected gene was *MYBPC3* (14 PM). Five mutations (24 %) were novel variants, while 17 had previously been described as pathogenic. Only two patients (4 %) harboured multiple PM, both in double heterozygosis ($TPM1^{S215L}$ and $MYH7^{Q1215Hi}$; $MYBPC3^{I160N}$ and GLA^{R301Q}).

The likelihood of identifying an HCM-associated mutation did not differ between the two groups (56 vs 46 %; $p = 0.317$); the gene distribution of PM in sarcomeric genes was also similar between both groups. Only three PM were identified in more than one individual and only two were found in >3 unrelated patients: $MYBPC3^{IVS23\ dsG-A+1}$ and $MYBPC3^{IVS22-1G>A}$ (five patients for each). The prevalence of complex genotypes did not differ between both groups (10 vs 4 %; $p = 0.240$).

Overall, a higher frequency of family history of HCM was observed in genotype-positive patients vs genotype-negative patients (57 vs 33 %; $p = 0.020$), but no differences were observed between genotype-positive and genotype-negative individuals regarding family history of SCD (39 vs 23 %; $p = 0.107$) or age at HCM diagnosis (35.8 ± 18.9 vs. 41.9 ± 16 years, $p = 0.087$).

Review of the Literature

A total of 14 of the 45 PM identified (31 %) had not been previously reported and so no information was available in the literature. Information on the remaining 34 PM (17 in the poor and 17 in the favourable group) revealed that data from ≥10 genetic carriers were available only for 10 variants (8 present in the poor clinical course group and 4 in the favourable group) affecting 15 individuals included in the study (8 and 7 subjects from each group). Adverse events were reported in ≥20 % of carriers in four of these PM: $MYBPC3^{IVS23dsG-A+1}$, $MYH7^{D778E}$, $MYH7^{R719W}$ and $MYH7^{R719Q}$, implying that 4 out of the 46 PM identified (8 %) could have been considered

Table 1 Phenotype of the entire HCM cohort (n = 100)

	Overall (n = 100)	Poor clinical course (n = 50)	Favourable clinical course (n = 50)	p value
Male sex	73 (73 %)	34 (68 %)	39 (78 %)	0.260
Age at HCM diagnosis, years	38 ± 18	34 ± 19	43 ± 15	0.014
Age at genetic testing, years	52 ± 15	52 ± 16	53 ± 13.5	0.931
Positive genotype	51 (51 %)	28 (56 %)	23 (46 %)	0.423
Multiple mutations	7 (7 %)	5 (10 %)	2 (3.7)	0.240
Heart transplant	29 (29 %)	29 (58 %)	0	<0.001
Myectomy	8 (8 %)	3 (6 %)	5 (10 %)	0.443
Pacemaker	12 (12 %)	9 (18 %)	3 (6 %)	0.070
ICD for primary prevention	35 (35 %)	16 (32 %)	19 (38 %)	0.087
ICD for secondary prevention	11 (11 %)	11 (22 %)	0	<0.001
Sudden death before ICD implant	10 (10 %)	10 (20 %)	0	0.004
Appropriate ICD discharge	15 (15 %)	15 (30 %)	0	<0.001
Syncope	20 (20 %)	13 (26 %)	7 (14 %)	0.379
NSVT	32	18 (36 %)	14 (28 %)	0.057
Family history of HCM	42 (42 %)	20 (40 %)	22 (44 %)	0.877
Family history of SCD	31 (31 %)	14 (28 %)	17 (34 %)	0.388
History of AF	48 (48 %)	27 (54 %)	21 (42 %)	0.267
LBBB	15 (15 %)	12 (24 %)	3 (6 %)	0.012
NYHA I/II/III/IV	44/23/15/18	11/3/12/18	31/16/3/0	
Echocardiographic data				
LVEF >50 %/40–50 %/ 30–40 %/<30 %	70/7/7/16	23/5/6/16	47/2/1/0	
LVOTO	29 (29 %)	10 (20 %)	19 (38 %)	0.555
Maximal wall thickness, mm	21.7 ± 6.5	21 ± 7.5	22 ± 5.5	0.652
Wall thickness ≥ 30 mm	17 (17 %)	9 (18 %)	8 (16 %)	0.695

HCM hypertrophic cardiomyopathy, *AF* atrial fibrillation, *LVEF* left ventricular ejection fraction. *ICD* implantable cardioverter defibrillator, *LBBB* left bundle branch block, *LVOTO* left ventricular outflow tract obstruction, *NSVT* non-sustained ventricular tachycardia

as associated with poor prognosis based on the published information (Tables 2 and 3). Conversely, six PM identified had been described in ≥10 genetic carriers in the literature with <20 % of those carriers showing adverse events. These PM theoretically could have been considered as conferring "benign prognosis" based on the available information.

Only five patients with poor clinical course had PM that could have predicted an adverse clinical course. Moreover, three patients (6 %) of the poor clinical course group had PM thought to confer good prognosis (Figure 1). On the other hand, three patients with favourable clinical course had PM associated with poor prognosis and only four (8 %) presented mutations that could have been considered as associated with benign prognosis (Fig. 1).

Impact of Genetic Testing

A total of 119 relatives of HCM patients with PM were genetically studied (79 were relatives of patients with adverse clinical course and 40 of patients with favourable clinical course). Genetic study led to the identification of 57 PM carriers and 62 non-carriers. Non-carriers were released from future clinical surveillance, which was also not necessary in their offspring.

Twenty-seven genetic carriers (45 %) were found to have HCM, while 30 genetic carriers were genotype positive-phenotype negative.

As a result of cascade genetic testing, the definitive genetic status was known for 56 individuals ≤40 years (14 index patients and 42 relatives). All of these individuals might benefit

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Table 2 Pathogenic mutations found in HCM patients with "poor clinical course" with the prognostic information available in the literature

Gene	Mutation	Patient no.	ExAC frequencies	Family carriers/ expression[b]	Family members with poor clinical course	Proband carriers/ expression[c]	Clinical adverse events[d]	Clinical adverse events[d] (%)	Number of articles	References
LAMP2	R293X	1	0.0001027	0/0	–	1/1	1	100	1	W1
MYBPC3	R589H	1	0.000000	2/0	0	2/2	–	–	2	W2, W3
MYBPC3	E258K	1	0.00003903	0/0	–	53/24	3	5	16	13, W4, W5, W6, W7, W8, W9, W10, W11, W12, W13, W14, W15, W16, W17, W18
MYBPC3	IVS22-1G>A	4	0.000000	3/3	2	1/1	0	0	1	W14
MYBPC3	IVS23 ds G-A+1	2	0.000000	0/0	–	96/67	22	23	7	18, W5, W9, W19, W20, W21, W22
MYBPC3	N1023frfX1050	1	0.000000	0/0	–	1/1	–	–	1	W23
MYBPC3	IVS30+5G>C	1	0.00003052	0/0	–	1/1	–	–	1	W24
MYH7	A797T	1[e]	0.00003296	1/1	0	66/32	9	13.6	14	W4, W6, W8, W14, W17, W18, W25, W26, W27, W28, W29, W30, W31, W32
MYH7	K847del	1	0.000000	0/0	–	5/5	1	20	5	W6, W8, W14, W17, W29
MYH7	D239N	1	0.000000	2/2	0	1/1	–	–	2	W25, W33
MYH7	D778E	1	0.000000	2/2	0	10/10	3	30	3	W5, W34, W35
MYH7	R719W	1	0.000000	4/4	1	64/64	26	40	3	W5, W36, W37
MYH7	R719Q	1	0.000000	0/0	–	21/21	9	43	10	W5, W6, W8, W22, W25, W38, W39, W40, W41, W42
MYL2	E22K	1	0.00000827	3/1	0	10/7	0	0	2	W43, W44
TNNI3	R162Q	1[e]	0.00002498	3/2	0	27/15	2	74	8	W22, W45, W46, W47, W48, W49, W50, W51
TNNI3	R162W	1	0.00003328	0/0	–	–	–	–	1	W28
ACTC	L10M	1	0.00002517	0/0	–	1/1	–	–	1	W11

[a] Number of patients with a mutation found in this study

[b] Number of family members with a mutation found in this study/number of family members with disease expression

[c] Probands with this mutation found in the literature/probands with disease expression

[d] Clinical adverse events defined as sudden death, ICD discharge or heart transplant, reported in the literature

[e] Index patient with double heterozygosity

Table 3 Mutations found in HCM patients with "favourable clinical course" with the prognostic information available in the literature

Gene	Mutation	ExAC frequency	Patient no.[a]	Family carriers/ expression[b]	Family members with poor clinical course	Proband carriers/ expression[c]	Clinical adverse events[d]	Clinical adverse events[d] (%)	Number of articles	References
GLA	R301Q	0.000000	1	1/NA	0	22/13	1	–	21	W52–W73
MYBPC3	K505del	0.000000	1	2/0	0	3/3	–	–	1	W8
MYBPC3	G263X[b]	0.000000	1	2/2	0	9/6	0	0	2	W74, W75
MYBPC3	D770N	0.00000830	1			4/4	–	–	4	W4, W6, W18, W76
MYBPC3	R495W	0.000000	1			6/3	0	0	2	W74, W75
MYBPC3	M844R fs*36	0.000000	1	1/0	0	1/1	–	–	1	W19
MYBPC3	R502W	0.00002486	1			115/73	10	8	12	W5, W6, W12, W22, W25, W28, W37, W40, W42, W77, W78, W79
MYBPC3	IVS23 ds G-A+1	0.000000	3	1/0	0	96/67	22	23	7	18, W5, W9, W19, W20, W21, W22
MYBPC3	c.1224-19G>A	0.000000	1	2/0	0	3/3	0	0	1	W80
MYBPC3	IVS22-1G>A	0.000000	1	1/0	0	1/1	–	–	1	W14
MYBPC3	IVS29+5G>A	0.00001696	1	1/0	0	5/4	0	0	3	W17, W81, W82
MYBPC3	R502Q	0.000000	1			69/44	2	3	12	W4, W9, W16, W18, W29, W35, W40, W81, W83, W84, W85, W86
MYH7	R1382W	0.000000	1	1/1	0	1/1	–	–	1	W5
MYH7	A797T	0.00003296	2			66/32	9	13.6	14	W4, W6, W8, W14, W17, W18, W25, W26, W27, W28, W29, W30, W31, W32
MYH7	Q1215H	0.000000	1			1/1	–	–	1	W86
TNNT2	R286H	0.00007882	1	3/0		5/3	0	0	3	W45, W87, W88
TPM1	S215L	0.00000825	1			2/2	–	–	2	W40, W89

NA not available

[a] Number of patients with a mutation found in this study

[b] Number of family members with a mutation found in the literature/number of family members with disease expression

[c] Probands with this mutation found in the literature/probands with disease expression

[d] Clinical adverse events defined as sudden death, ICD discharge or heart transplant, reported in the literature
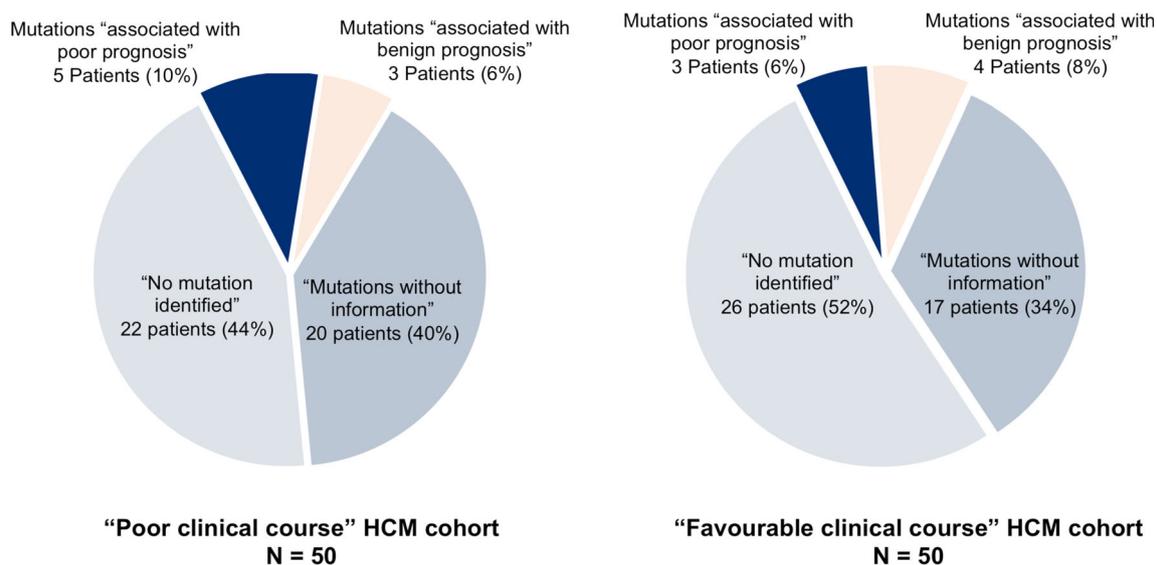
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Fig. 1 Pie charts reflecting predictive information based on genetic findings in 100 HCM index cases with poor (**a**) or favourable (**b**) clinical course. Mutations without information are those described in <10 individuals previously

from knowing their genetic status in order to make informed reproductive decisions.

Finally, 7 of the 30 genotype positive-phenotype negative relatives (23 %) and 3 of the 60 non-carriers (5 %) were younger than 30 years and might therefore benefit from knowing their genetic status in selecting a professional career.

The estimated savings derived from the cessation of clinical screening of genotype-negative relatives was 83,022 Euro (1339.06 Euro per genoype-negative relative). If we consider the cost of genetic screening for a single mutation (approximately 100 Euro) and compare it with the estimated avoided costs of serial clinical monitoring as recommended by guidelines [14, 16], genetic screening saved approximately 76,822 Euro in this group of patients.

Discussion

This study examines the usefulness of genetic findings to predict prognosis in HCM by studying two different cohorts of HCM patients: one with "poor clinical course" and one with "favourable clinical course". After comprehensive genetic screening, our results are suggestive of a lack of usefulness of gene testing to predict prognosis in most HCM patients. In the present study, only a minority of HCM patients with a poor course presented PM that had been described previously in a substantial number of individuals and were known to be associated with a bad outcome. Furthermore, some HCM patients with poor clinical course presented PM that were associated with a benign clinical course and some HCM individuals with a favourable clinical course presented PM associated with poor prognosis. By contrast, real-world data obtained in this study

reinforces the usefulness of genetic testing to provide genetic counselling and to enable familial evaluation.

Genetic Testing to Predict Prognosis in HCM

The ability of genetic findings to predict prognosis in HCM has been a matter of debate for over 20 years [2, 5–7]. Initial attempts to demonstrate a relationship between a specific mutation and the resulting phenotype linked certain HCM-causing genes either with a favourable (*MYBPC3*) [18] or a negative (*TNNT2*) [8] outcome; however, later studies with a larger number of nonrelated patients showed that those gene associations were inconsistent [11–13].

Although it has been demonstrated that HCM patients with a positive genetic test have a more severe disease phenotype than those with a negative genetic test [4], a recent meta-analysis has shown that it is not possible to establish an accurate gene-based genotype–phenotype relationship [19]. Along this line, our findings emphasise the genetic and clinical heterogeneity that characterises HCM with similar genetic background across both groups of patients studied. In fact, the overall prevalence and gene distribution of mutations have been shown to be similar between different HCM populations (Table 4). In the present study, we also found that the distribution of affected genes was very similar between both studied groups; however, it should be noted that the number of patients with mutations in *MYH7* was higher in the poor clinical course cohort. This trend is in accord with a higher prevalence of mutations in *MYH7* in the paediatric population with HCM (Table 4) and with the younger presentation of *MYH7* carriers amongst end-stage HCM patients reported by Biagini and coworkers [20]. Our findings and these suggest that *MYH7* mutations could promote an earlier presentation of the disease compared with mutations in other genes.

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Table 4 Distribution of sarcomere-protein gene mutations in different populations

Present study

Cohort	Adult population with HCM[a]	Paediatric population with HCM[b]	End-stage HCM[c]	With "poor clinical course"	With "favourable clinical course"
Gene *n*, (%)					
MYBPC3	719 (19)	48 (29.4)	38 (24)	13 (26)	16 (32)
MYH7	509 (13)	57 (34)	26 (16.6)	8 (16)	4 (8)
TNNT2	81 (2)	6 (3.6)	3 (2)	0	1 (2)
TNNI3	54 (1.4)	3 (1.8)	9 (5.7)	2 (4)	0
MYL2	26 (0.7)	0	2 (1.2)	1 (2)	0
MYL3	17 (0.4)	3 (1.8)	1 (0.6)	1 (2)	0
ACTC1	19 (0.5)	4 (2.4)	0	1 (2)	0
TPM1	28 (0.7)	2 (1.2)	3	1 (2)	2 (4)
Probands with multiple mutations *n*, (%)	122 (3.2)	11 (6.7)	21 (13)	5 (10)	2 (4)
Probands, *n*	3826	163	156	50	50
Mutation detection rate *n*, (%)	1453 (38)	87 (53)	103 (66)	28 (56)	24 (48)
Non-sarcomeric positive *n*, (%)	4 (0.1)	1 (0.1)	1 (0.6)	2 (4)	1 (2)

[a] Information included from studies that evaluate ≥8 sarcomeric genes. Data are from ref. [4], W17, W18, W35, W88, W90, W91, W92 and W93

[b] Information included from studies that evaluate ≥8 sarcomeric genes. Data are from W40 and W79

[c] Data from ref. [21]. This study evaluated the presence of mutations in eight sarcomeric genes, PRGAG2 and LAMP2, in patients with HCM and LVEF <50 %

Previous studies have suggested that double or compound PM affect 3–5 % of HCM individuals and are associated with a more severe clinical course [21–24]. Indeed, this was observed recently in a cohort of end-stage HCM patients where complex genotypes were twice more frequent than in the reference cohort [20]. Our results show a higher (although not statistically significant) frequency of complex genotypes in the poor prognosis group, which strengthens the hypothesis that the mutational load might have a deleterious effect; however, although the 10 % prevalence of double PM in the adverse course group is considerable, the number of individuals with multiple PM remain a minority in this group, highlighting that clinical course of HCM is not primarily dependent on the presence of multiple PM. It is important to highlight the three patients with poor clinical course harbouring a genetic defect in homozygosis (no cases in the favourable clinical course group): two patients (*MYL2*[E22K] and *MYH7*[D778E]) had been reported previously [3, 25], whereas a patient with the homozygous mutation *MYH7*[I144T] was reported for the first time. Unfortunately, cosegregation of this mutation with the disease was not confirmed.

Therefore, the severity of phenotypic expression in HCM seems to be modulated by multiple factors, including environmental and patients' intrinsic factors [25, 26]. Recent data suggest that factors that modulate disease phenotype such as hypertension might explain the extreme clinical heterogeneity of HCM amongst patients with the same mutation [25].

Our findings do not question the usefulness of genetic testing to help in the identification of patients with phenocopies of sarcomeric HCM. Conditions including transthyretin familial amyloid cardiomyopathy, mitochondrial disorders and Danon's and Fabry's disease have a different underlying pathophysiology and a particular natural history, and so genetic results can help to predict prognosis and guide appropriate management in these individuals [5, 7].

Individual Mutations Are Not Useful to Predict Prognosis

Our study shows that identifying a particular PM has little impact on predicting prognosis. This conclusion is based on three facts: (i) almost all PM were identified just in one individual (were not repeated); (ii) one third of PM identified were "novel" and the available information for most of the remaining PM was scarce; and (iii) a minority of PM could be classified as associated with "poor" or "benign" prognosis, with inconsistent behaviour in the patients of the two cohorts.

To date, more than 1400 mutations have been described in association with HCM and the majority are "private" mutations; consequently, it is extremely difficult to demonstrate genotype–phenotype correlations. Moreover, very few genotype–phenotype relationships have been shown to be reproducible because of the variable expression of the same or similar mutations [6, 12,

27]. We identified PM in two cohorts of patients with different prognosis and extracted from the literature all available information about each one in order to predict clinical course. According to the information retrieved, five patients (10 %) from the poor clinical course group carried a PM associated with poor prognosis, meaning that if it had been identified in advance it might have predicted the phenotypic expression. Therefore, in the best scenario, current genetic knowledge can be used to predict prognosis only in one of each 10 HCM individuals that will develop serious adverse events. In the remaining individuals of our study with severe adverse outcomes, genetic study would have not helped in predicting the aggressive phenotype either because no PM would have been identified or because, even though a mutation had been identified, the information currently available is insufficient to test its clinical significance. In accordance with this lack of usefulness, three patients (6 %) from the poor clinical course group carried PM associated with a good clinical profile and three patients without clinical events harboured PM theoretically associated with poor prognosis.

Supporting these findings, 14 patients with poor clinical course and 17 with favourable clinical course had a family history of SCD, underscoring the variation in clinical course between relatives hosting the same PM.

One of the limitations of this study is its relatively small sample size. However, it is difficult to recruit a large number of nonrelated patients who share some specific clinical features. In the present study, we genotyped 50 unrelated patients with a poor clinical course and compared their genetic background with that from a group of individuals with a good clinical profile. The principal objective of this study was to provide data about the usefulness of genetic study results in predicting patient's clinical course. The available literature of the genetic variants found in both groups provided useful data to correctly predict the clinical evolution of HCM patients in just nine (9 %) individuals (five from the "poor" and four from the "favourable" group). Despite the limited sample size of the study, these results support the view that the identification of a causal mutation is currently not clinically useful in predicting prognosis in HCM.

Family Screening and Implications for Relatives

Current guidelines recommend genetic testing in HCM patients to facilitate identification of relatives at risk of developing the disease and to provide genetic counselling [14, 16]. Genotype determination can be used to precisely identify relatives at risk for developing disease at an early stage and focus longitudinal follow-up or, conversely, to discharge relatives who have not inherited the PM and are not at risk for disease development. Although this gene-based diagnostic strategy in families has proven to be cost-effective in theoretical economic models [28], its clinical impact for reproductive and sports/professional career counselling has not been evaluated.

In this study with real-world patients, family screening revealed 57 mutation carriers (27 with HCM) and 62 non-carriers. Relatives not carrying a PM and their offspring were reassured and did not require clinical evaluation or lifestyle restrictions. The recommended periodic monitoring for relatives of HCM patients in whom the genetic status is unknown includes medical consultation, electrocardiogram and echocardiography [14, 29]. This translates approximately to a cost of 303 Euros per visit in Spain [30, 31]. Over time, the serial clinical follow-up of relatives would represent a considerable cost to the health system. Current NGS genetic testing for HCM in an index patient is commercially available for less than 1000 Euros, and testing of relatives for individual mutations costs less than 100 Euro. These costs will continue to decrease in the coming years, but even with current costs, the estimated savings derived from cessation of follow-ups are relevant.

In our study, we also estimated the benefit derived from offering reproductive, professional and sports counselling. Forty-two relatives ≤40 years old knew their genetic status and could receive reproductive advice. Relatives found to be carriers of a PM were informed about reproductive methods to prevent PM transmission and those with a negative genotype were informed that their descendants would not have the disease.

Clinical practice guidelines recommend patients with a PM without phenotype to participate in recreational sport activities, avoid competitive sports and choose the activity on an individual basis [14]. However, recommendations for professional activities are not established. It seems reasonable to advise young people with a disease-related mutation on these issues. Ten relatives ≤30 years old underwent genetic testing and benefited from professional and sports counselling in our study.

A recent study from the Mayo Clinic has shown that only a minority of HCM patients at this institution chose to be genetically tested [32]. Although the reasons for declining or accepting genetic testing are complex and are dependent on individual patient characteristics and circumstances, we believe that the benefits found in our work could be used to support genetic testing in HCM patients.

Conclusions

Our results indicate that it is currently not feasible to predict a patient's clinical course based solely on the genetic defect identified. The clinical value of genetic testing is currently limited to familial genetic counselling, in particular for relatives who are considering predictive genetic testing or for parents seeking reproductive advice.

Compliance with Ethical Standards

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