ORIGINAL ARTICLE

A Preliminary Study of the Phenotype-Genotype Correlation in Cardiomyopathies in Patients Referred to a Tertiary Healthcare Center in the Suburbs of Buenos Aires

Estudio preliminar de correlación fenotipo-genotipo en miocardiopatías de pacientes derivados a un centro de alta complejidad del conurbano bonaerense

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ABSTRACT

Background: Cardiomyopathies are defined as a disorder of the myocardium in which the heart muscle is structurally and functionally abnormal, in the absence of coronary artery disease, hypertension (HT), valvular heart disease and congenital heart disease. These diseases are relatively common and a major cause of morbidity and mortality worldwide.
Although genetic testing is recommended for family screening, lack of solid data on specific genotype-phenotype associations has reduced its impact on clinical management.
Objectives: This study aims to analyze the frequency of mutations in a population of patients with cardiomyopathy referred to a tertiary healthcare center and to analyze the genotype-phenotype correlation of the identified mutations.
Methods: We prospectively included 102 patients with suspected familial hypertrophic cardiomyopathy (HCM), 70 of which were index cases, from an ambispective cohort of patients with cardiomyopathies treated in a tertiary healthcare public hospital in the province of Buenos Aires, from January 2012 to August 30, 2022.
Results: Of 102 patients, 83 were considered affected. Of these, 31 were HCM and 52 were phenocopies, with no difference in prognosis. A genetic study was carried out in 77 patients, of whom 57 presented recognizable mutations, in 80% of the cases coinciding with a Mayo Score ≥3. Twenty-eight variants of uncertain significance were detected.
Conclusions: It was confirmed that molecular testing guided by the Mayo Score provided high probability of detecting mutations. Molecular testing proved to be important due to the phenotypic and genotypic overlap in cardiomyopathies. Understanding the causative genetic variant, nowadays, does not affect the clinical management of most HCM patients, but is helpful in a small group of genes with treatment options.

Key words: Cardiomyopathies - Cardiomyopathy Hypertrophic/genetics - Sarcomeres - Genetic Association Studies - Genetic Testing

RESUMEN

Introducción: Las miocardiopatías se definen como un trastorno del miocardio en el que el músculo cardíaco es estructural y funcionalmente anormal, en ausencia de enfermedad arterial coronaria, hipertensión arterial (HTA), enfermedad valvular y enfermedad cardíaca congénita. Estas enfermedades son relativamente frecuentes, y suponen una importante causa de morbimortalidad a nivel global.
Aunque el estudio genético se recomienda para el cribado familiar, la falta de datos robustos sobre asociaciones genotipo-fenotipo específicas ha reducido su impacto en el manejo clínico.
Objetivos: El objetivo de este estudio es analizar la frecuencia de mutaciones en una población de pacientes con miocardiopatía derivados a un centro de alta complejidad y el análisis de la correlación genotipo-fenotipo en las mutaciones identificadas.
Material y métodos: Se estudiaron en forma prospectiva 102 pacientes con sospecha de miocardiopatía hipertrófica (MCH) familiar, de los cuales 70 constituían casos índices, de una cohorte ambispectiva de pacientes con miocardiopatías controladas en un hospital público de alta Complejidad de tercer nivel de atención de la provincia de Buenos Aires, desde enero 2012 al 30 agosto 2022.
Resultados: De 102 pacientes 83 fueron considerados afectados. De eelos, 31 eran MCH y 52 fenocopias, sin diferencia en el pronóstico. Se realizó estudio genético en 77 pacientes, de los cuales 57 presentaron mutaciones reconocibles, en el 80% de los casos coincidentes con un Score de Mayo ≥3. Se detectaron 28 variantes de significado incierto.
Conclusiones: Se comprobó que realizar estudio molecular guiado por el Score de Mayo permitió obtener un alto grado de probabilidad de detectar mutaciones. Se evidenció la importancia del estudio molecular debido a la existencia de solapamiento fenotípico y genotípico de las miocardiopatías. El conocimiento de la variante genética causal actualmente no afecta el manejo clínico de la mayoría de los pacientes con MCH, pero es de ayuda ante un pequeño grupo de genes que tienen opciones de tratamiento.

Palabras clave: Cardiomiopatías - Cardiomiopatía Hipertrófica - Sarcómeros - Estudio de Asociación Genética - Pruebas Genética

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INTRODUCTION

Cardiomyopathies are defined as a disorder of the myocardium in which the heart muscle is structurally and functionally abnormal, in the absence of coronary artery disease, hypertension (HT), valvular heart disease and congenital heart disease. These diseases are relatively common and a major cause of morbidity and mortality worldwide. (1)

There are several classifications which are intended to differentiate some cardiomyopathies from others, although in many cases they are more confusing than helpful. (2,3)

Hypertrophic cardiomyopathy (HCM) is a primary myocardial disease affecting both sexes, caused by mutations of genes encoding sarcomere proteins, with an estimated prevalence of up to 1/200-500 people, often inherited, with a complex genetic and phenotypic expression and a natural history. (4-7)

Thousands of mutations in more than 50 genes have been described to be associated with HCM; however, the frequency of the mutations identified may vary in different studies, and data available are scarce. (4-7)

The mechanisms by which sarcomere variants result in the clinical phenotype have not yet been elucidated. Sarcomere genes trigger changes in the myocardium which lead to hypertrophy and fibrosis, a small and stiff ventricle with impaired systolic and diastolic function despite the preserved left ventricular ejection fraction (LVEF). Several features, such as abnormal intramural coronary arteries responsible for ischemia and mitral valve abnormalities, appear to have no direct association with the sarcomere variants. (4-7)

Patients lacking a pathogenic variant are believed to have non-Mendelian HCM and probably better prognosis than patients with sarcomere pathogenic mutations. Identifying the genetic basis of HCM creates opportunities to understand how the disease develops and how to disrupt its progression. (5)

Although genetic testing is recommended for family screening, lack of solid data on specific genotype-phenotype associations has reduced its impact on clinical management. (5)

This study aims to analyze the frequency of mutations in a population of patients with cardiomyopathy referred to a tertiary healthcare center and the genotype-phenotype correlation of the identified mutations.

METHODS

Study population

We prospectively included 102 patients with suspected familial HCM, 70 of which were index cases, from an ambispective cohort of patients with cardiomyopathies treated in a tertiary healthcare public hospital in the province of Buenos Aires from January 2012 to August 30, 2022.

The diagnosis of HCM was obtained according to the criteria of the WHO and the European Society of Cardiology (ESC) Working Group on Myocardial and Pericardial Diseases. (1)

The patients diagnosed with HCM were those presenting 1 major electrocardiographic (ECG) or transthoracic echocardiographic (TTE) criterion or 2 minor TTE criteria plus 1 minor ECG criterion, or 2 minor ECG criteria plus 1 minor TTE criterion. (7)

Patients diagnosed with cardiomyopathy were asked about relevant medical and family history considering three generations, underwent a physical examination, a genogram, an ECG, a TTE, a Holter ECG (in affected patients), an exercise stress test or a cardiopulmonary exercise test (in affected patients), a complete blood count with NT-proBNP and troponin counts (in affected patients).

Echocardiographic parameters

The studies were performed with an Epiq 7 CVx 3D echocardiograph (Philips Medical Systems) using an S5-1 transducer. Measurements of LVEF and diastolic function were obtained according to the recommendations of the American Society of Echocardiography. (8,9)

Values of LVEF <52% in men and <54% in women were considered depressed.

The Speckle-tracking analysis was performed according to current EACI/ASE consensus recommendations. (10) Cine loops from three standard LV apical views (four, two and three chambers) were recorded using grey-scale harmonic imaging at the highest possible frame rate (55-90 frames/s). The analysis of the recorded files was performed off-line by an experienced echocardiographer unblinded to the patient's diagnosis.

The 2D global longitudinal strain (GLS) was evaluated in 16 LV segments on average (Strain post-processing software: TOMTEC. Dynamic Heart Model). The operator manually adjusted the region of interest in segments that could not be correctly traced. Normal value of global GLS Philips: -21±2%.

Cardiac magnetic resonance (CMR)

A Philips Medical Systems Achieva X Series 3T machine was used. Anatomical images of the heart were obtained with dark-blood and bright-blood sequences. A functional study with triggered cine imaging was performed. Images were acquired by enhaced T1- and T2-weighted sequences, fat suppression, sequences with variable TE and tagging. First-pass (0.1 mmol/kg) and late images (late enhancement) sequences were obtained with gadolinium injection (total dose: 0.2 mmol/kg), which were then post-processed and assessed with Extended MR Space 2.6.3.3 software.

Mutational analysis

The Mayo HCM genotype predictor score (Mayo Score) was used to predict the diagnostic yield of genetic testing and guide the use of next generation sequencing (NGS) method. (11,12)

Molecular testing was performed on those with Mayo Score ≥3 (range -1 to 5) or relatives of patients with positive mutations. First-degree relatives underwent a physical examination, an ECG and a TTE to identify those who were affected. They were offered to undergo genetic testing.

The technical component of the confirmatory sequencing was performed by Invitae Corporation from saliva samples collected by oral swabbing. The classification of identified variants was carried out pursuant to the guidelines of the American College of Medical Genetics and Genomics. (13)

All patients signed an informed consent form, and the study was approved by the institution's ethics committee.

Study of the genotype-phenotype correlation

For the description of the phenotypic characteristics, the classical four phenotypes of Maron´s classification based on the location and degree of hypertrophy was used. (14)

To correlate phenotype (F) with genotype (G), the Lever's classification was used to evaluate the pretest probability based on the anatomical subtype. (15)

HCM was defined as obstructive when it had a significant intraventricular pressure gradient (≥30 mmHg) at rest, as latent obstructive when the gradient was evident following provocation maneuvers (Valsalva/standing/exercise) and as non-obstructive when the gradient was <30 mmHg.

The definitions are shown in the Appendix.

Cardiovascular events

Cardiovascular (CV) events were defined as the presence or absence of the following:

- Need for implantable cardioverter defibrillator (ICD) or pacemaker implantation.
- Sudden death
- Cardiovascular hospitalization
- Septal myectomy or alcohol septal ablation

The follow-up lasted 3 years following the diagnosis by means of outpatient clinic visits or telephone calls.

Statistical analysis

The statistical softwares Epi Info for PC version 7.2.4.0 and Statistix 7 were used.

Qualitative variables were described using numbers and percentages. Quantitative variables were described using mean and standard deviation or median and interquartile range (IQR), depending on normal or non-normal distribution, respectively.

For comparisons between groups, Student's t test was used for continuous variables with normal distribution, and non-parametric tests, such as Mann-Whitney U test for continuous variables with non-normal distribution and Chi-square test (χ^2) or Fisher's exact test for categorical variables. A p-value <0.05 was considered statistically significant.

RESULTS

A total of 102 patients were evaluated. The diagnostic flow diagram is shown in Figure 1.

The presence of a phenotype compatible with HCM was determined based on electrocardiographic and echocardiographic criteria. Patients were classified into two groups: "affected" (n= 83, 81.4%, 95% CI 72.4-88.4%) and "non-affected" (n=19, 18.6%, 95% CI 11.6-27.5%) (Table 1).

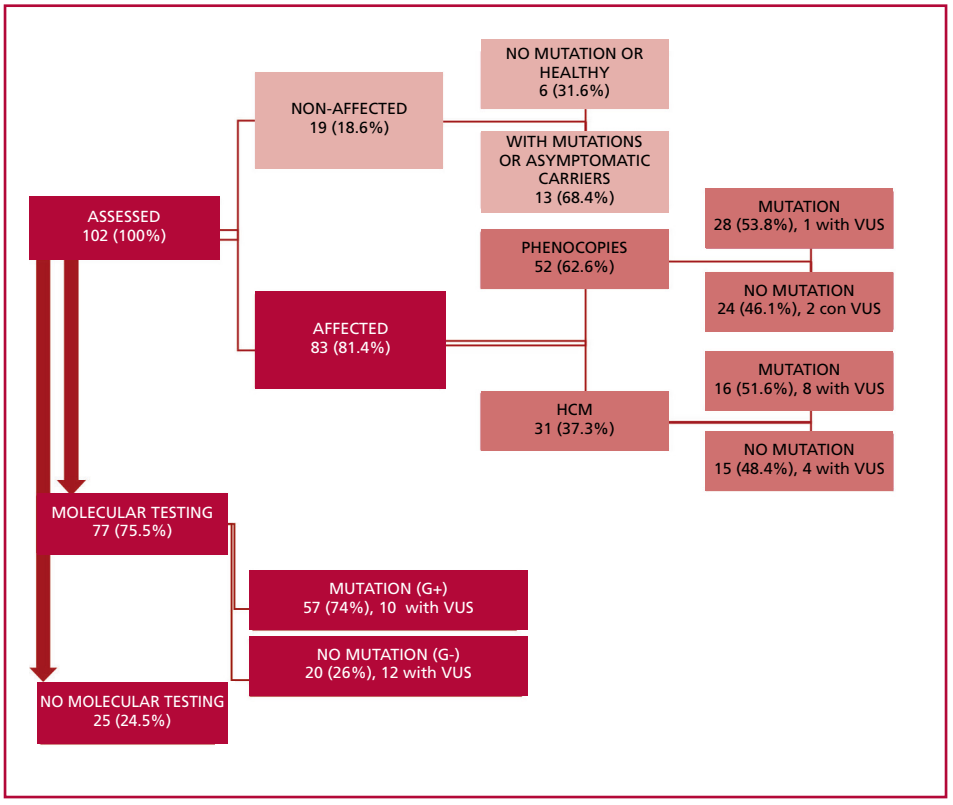
The mean age of symptom diagnosis was 39±16.7 years, with earlier onset in women (34.7±15 years; p<0.001), those with family history (36.7±16 years; p<0.001) and pathogenic TNNT2 variants.

Among those affected, the presence of symptoms was more frequent in men (n=43) than women (n=40), and dyspnea was the most frequent symptom.

ECG and TTE were performed in 100% of patients and CMR in 64 (62.7%). CMR was not performed in some patients due to claustrophobia, denial, or cardiac devices.

Table 1B shows the characteristics of the 83 "affected" patients. A total of 71% (n=59) had preserved

Fig. 1. Diagnostic flowchart



HCM: hypertrophic cardiomyopathy; VUS: variant of uncertain significance

Table 1. Studied population characteristics

A. Clinical and electrocardiographic parameters

Clinical parameters	Patients (N=102)	Affected (N=83)	Non-affected (n=19	p
Current age (years)	45 ± 16	47.6 ± 16	33.7 ± 10	<0.001
Age at diagnosis (years)	39 ± 16.7	41.9 ± 16	28.8 ± 14	0.001
Symptomatic	83 (97.6%)	81 (97.6%)	2 (10.5%)	<0.001
Female	56 (54.9%)	40 (48.2%)	16 (84.2%)	0.003
Family history	71 (69.6%)	52 (62.6%)	19 (100%)	<0.001
Weight (kg)	70 ± 18	70 ± 18	69 ± 18	0.9
Hypertension	24 (23.5%)	23 (27.7%)	1 (5.3%)	0.02
Systolic blood pressure (mmHg)	107 ± 16.8	105 ± 16	117 ± 14	0.005
Obesity	22 (21.5%)	18 (21.7%)	4 (21%)	0.6
Heart rate (beats/minute)	71.6 ± 15	71.3 ± 16	73.2 ± 8.3	0.62
Diabetes	8 (7.8%)	7 (8.4%)	1 (5.2%)	0.53
Dyslipidemia	10 (9.8%)	10 (12%)	0	0.11
Dyspnea NYHA Functional Classification ≥II	80 (78.4%)	80 (96.4%)	0	<0.001
Angina	38 (37.2%)	37 (44.5%)	1 (5.3%)	<0.001
Syncope	50 (49%)	48 (57.8%)	2 (10.5%)	< 0.001
Coronary artery disease	4 (3.9%)	4 (4.8%)	0	0.43
BNP value (pg./mL)		986 (122-3237)		
Troponin I		12.5 (4-59.5)		
Electrocardiogram				
Abnormal	88 (86.3%)	82 (98.8%)	6 (31.6%)	<0.001
Negative T waves	34 (33.3%)	33 (39.7%)	1 (5.3%)	< 0.001
LVH signs	54 (52.9%)	51 (61.4%)	3 (15.8%)	< 0.001
CLBBB	12 (11.7%)	12 (14.4%)	0	0.07
CRBBB	4 (3.9%)	4 (4.8%)	0	0.43
LAHB	23(22.5%)	23 (27.7%)	0	<0.001
QS pattern	52 (50.9%)	51 (61.4%)	1 (5.3%)	<0.001
Microvoltage	25 (24.5%)	25 (30.1%)	0	0.01
Sinus rhythm	92 (90.2%)	73 (87.9%)	19 (100%)	0.24
Paroxysmal or permanent AF	18 (17.6%)	18 (21.7%)	0	0.01
Ventricular tachycardia	12 (11.7%)	12 (14.4%)	0	0.07
Echocardiography				
Normal LVEF	78 (76.5%)	59 (71%)	19 (100%)	<0.001
LVEF (%)	62 (55-66)	60 (52-67)	66 (61-70)	0.03
LV hypertrophy	61 (59.8%)	61 (73.5%)	0	<0.001
LA dilatation	79 (77.4%)	76 (91.5%)	3 (15.8%)	<0.001
Diastolic dysfunction	82 (80.4%)	80 (96.4%)	2 (10.5%)	<0.001
E/e´ ratio	11.6 ± 4.6	12.7 ± 4.2	6.6 ± 1.7	<0.001
Average GLS (%)	17 (12-20)	16 (10-19)	22 (20-22)	<0.001

AF: atrial fibrillation; CLBBB: complete left bundle branch block; CRBBB: complete right bundle branch block; GLS: global longitudinal strain; LA: left atrium ; LAHB: left anterior hemiblock; LVEF: left ventricular ejection fraction; LVH: left ventricular hypertrophy; LVSF: left ventricular systolic function; SBP: systolic blood pressure.

Qualitative variables are expressed as n (%) and quantitative variables as mean ± standard deviation or median and interquartile range.

LVEF compared to 100% in those non-affected; p<0.001. A total of 37.4% (n=31) had HCM and 62.6% (n=52) were phenocopies. In approximately half of the patients, the HCM was classified as obstructive (51.6%), and most of them (25) had preserved LVEF (80%). The mean GLS of patients with amyloidosis was 14% (9-17) and statistically different (p=0.01) from that of patients with HCM, and the LVEF/GLS index with a cut-off value ≥4.3±1.6 allowed to differentiate amyloidosis from HCM (p<0.001), as in previous studies. (16)

Identified mutations

A molecular testing was performed in 77 patients (75.5%), 57 of which (74%) had mutations (G+) and 20 (26%) did not have (G-). Forty-six (80.7%) out of the 57 G+ patients had a Mayo Score ≥3 , p<0.001 vs. G- patients. Twenty-two (28.5%) variants of uncertain significance (VUS) were detected (Figures 2 and 3). Two patients had double heterozygosity pathogenic variants and 10 had VUS in addition to the pathogenic mutation.

Among the 19 non-affected patients, disease could be ruled out in 6 (31.6%), while 13 (68.4%) were asymptomatic carriers. Disease penetrance ("affected patients with mutations") was 77.2% (44 out of the 57 G+ patients); 16 (36.4%) out of the 44 had HCM.

Genotype-phenotype correlation

According to Maron's classification, the most frequent forms of presentation were type 1 and type 3 (septal and anterolateral involvement, respectively), with

Table 1. Studied population characteristics

B. Echocardiographic and cardiac magnetic resonance characteristics of "affected" population

Echocardiographic parameters	Affected n=83	HCM n=31	Phenocopy n=52	p
Normal LVEF	59 (71%)	25 (80.6%)	34 (65.4%)	0.10
LVEF (%)	60 (52-67)	64 (55-69)	58 (44-66)	0.09
IVS thickness (mm)	13 (9-17)	18 (15-28)	12 (9.5-15)	<0.001
LVMI (g/m²)	109 (78-141)	138 (119-185)	109 (86-133)	0.001
LVOTO	16 (19.3%)	16 (51.6%)	0	<0.001
LA dilatation	76 (91.9%)	30 (96.7%)	46 (88.4%)	0.18
LAVI (ml/m²)	41 (32-56)	51.5 (43-82.5)	40 (36-55)	0.003
Diastolic dysfunction	80 (96.4%)	31 (100%)	49 (94.2%)	0.24
1- Prolonged relaxation	8 (9.6%)	0	8 (15.4%)	0.1
2- Pseudonormal	57 (67.5%)	24 (77.4%)	32 (61.5%)	0.1
3- Restrictive	11 (13.2%)	3 (9.7%)	8 (15.4%)	0.1
4- Monophasic	8 (9.6%)	4 (12.9%)	3 (5.7%)	0.1
E/e´ ratio	12.7 ± 4.2	13.4 ± 3.9	12.3 ± 4.4	0.27
Subaortic membrane	2 (2.4%)	2(6.4%)	0	0.13
Bicuspid AV	2 (2.4%)	2(6.4%)	0	0.13
Aortic regurgitation	16 (19.3%)	10 (32.2%)	6 (11.5%)	0.02
Mitral regurgitation	63 (76%)	26 (83.8%)	37 (71.1%)	0.19
Tricuspid regurgitation	68 (82%)	28 (90.3%)	40 (77%)	0.10
sPAP	27 (0-39)	33 (25-43)	30 (0-40)	0.15
Average GLS (%)	16 (10-19)	17 (13-19)	15 (9-17)	<0.001
LVEF/GLS	3.7 (3.3-4.7)	3.6 (3.2-4.2)	3.9 (3.4-5.3)	0.27
CARDIAC MAGNETIC RESONANCE	63 (75.9%)	25 (80.6%)	38 (73%)	0.43
	80 (62-96)	85 (62-122)	73 (62-90)	0.24
LVEF (%)	61 (44-71)	70 (59-73)	60 (40-67)	<0.001
RVEF (%)	72 (61-78)	71 (64-81)	72 (60-77)	0.34
LGE	44 (72.1%)	18 (85.7%)	26 (65%)	0.07

GLS: global longitudinal strain; HCM: hypertrophic cardiomyopathy; IVS: interventricular septum; LA: left atrium; LAVI: left atrial volume index; LGE: late gadolinium enhancement; LVEF: left ventricular ejection fraction; LVMI: left ventricular mass index; LVOTO: left ventricular outflow tract obstruction; RVEF: right ventricular ejection fraction;;; sPAP: systolic pulmonary artery pressure

Qualitative variables are expressed as n (%) and quantitative variables as mean ± standard deviation or median and interquartile range.

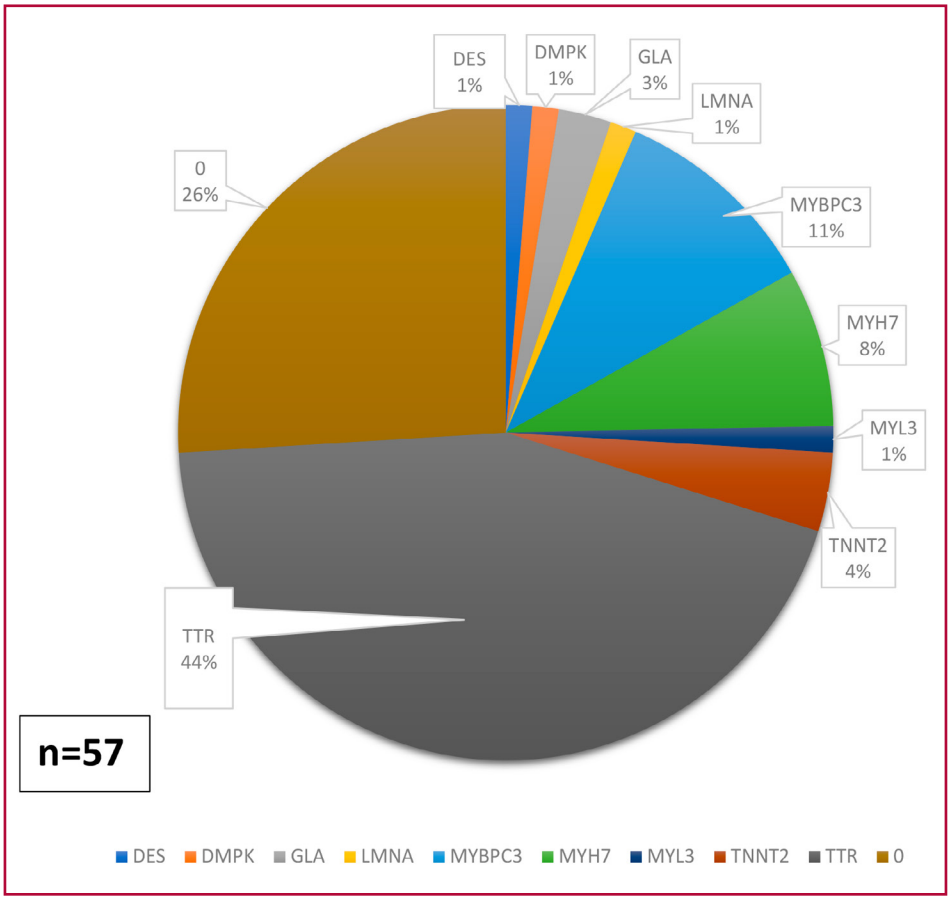


Fig. 2. Identified genetic variants

Gene	Variant	n
DES	c.1360C>T - p.(Arg454Trp)	1
DMPK	605377: 19q13.32	1
GLA	c.1244T>C p.(Leu415Pro)	1
LMNA	c.205del (p.Val69Trpfs*27)	1
MYBPC3	c.1808_1821del (p.Ile603Thrfs*6)	4
	c.3192dup (p.Lys1065Glnfs*12)	1
	c.1624G>C (p.Glu542Gln)	1
	c.1877 C>G (p.Ser626)	1
MYH7	c.485A>G (p.Tyr162Cys)	1
	c.788T>C (p.Ile263Thr)	1
	c.2770G>A (p.Glu924Lys)	2
	c.1208G>A (p.Arg403Gln)	3
MYL3	c.454G>A - p.(Glu152Lys)	1
TNNT2	c.812A>T (p.Asn271Ile)	2
	c.487_489del (p.Glu163del)	1
TTR	Thr60Ala	1
	Val50Met	27
	Val122Ile	6
N	TOTAL	57

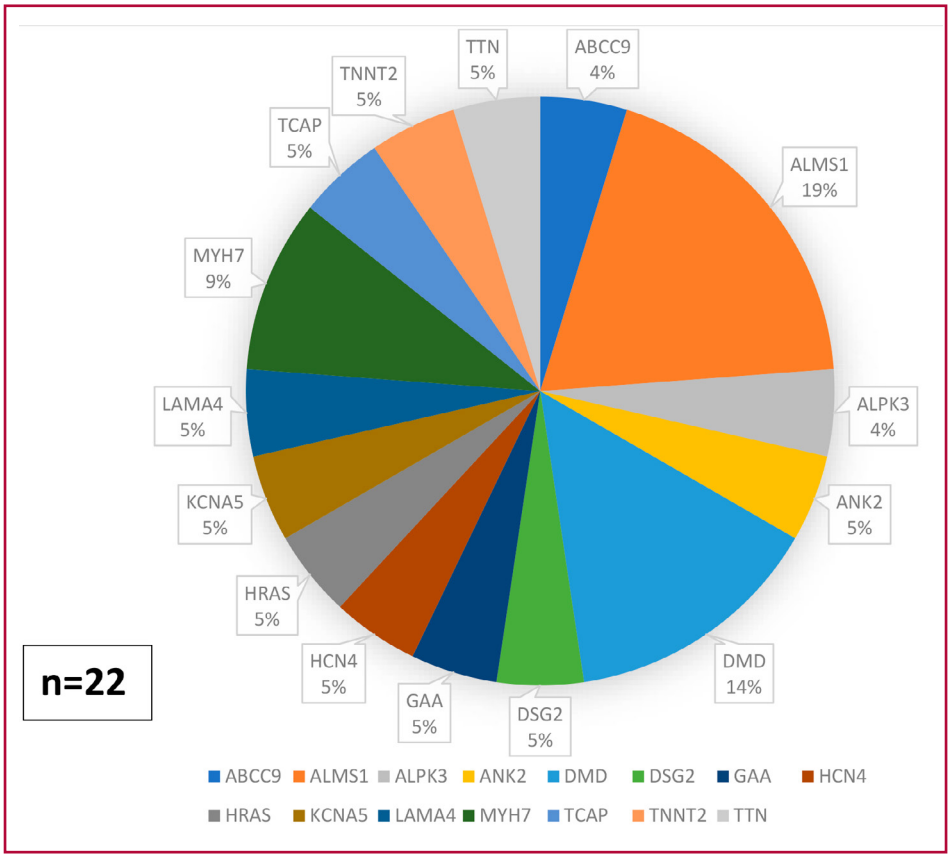


Fig. 3. Variants of uncertain significance (VUS)

more G+ detected in type 1 in HCM (9, 75%) and in type 3 in phenocopies (13, 68.4%), p<0.001.

Lever´s classification into 2 and 4 (reverse curvature septum and neutral septum, respectively) was useful when assessing the likelihood of having G+ based on the anatomical phenotype expressed by the patient (Table 2 and Figure 4).

There were no significant differences in CV events, medical treatments or procedures performed between G+ and G- patients (Figure 5).

The median time from the cardiomyopathy onset to the CV event was 2.4 years. Patients with phenocopies (29, 55.7%) experienced the same number of CV events as those with HCM (17, 54.8%), p=0.93. More cardiovascular deaths occurred in men (10, 21.7%) than women (3, 5.3%); p=0.01.

DISCUSSION

Cardiomyopathies are a heterogeneous group of myocardial diseases associated with mechanical and/or electrical dysfunction that usually present with inappropriate ventricular hypertrophy or dilatation and are due to a variety of causes, frequently genetic. (1)

For genetics to become useful in clinical decision-making, it is necessary to get detailed information about the clinical and morphological characteristics of the carriers of different mutations, such as that provided by this study.

The Mayo Score enabled a better selection of probands and a cost-effective molecular testing, with a clear financial limitation.

Hypertrophic remodeling also occurs in disorders that clinically mimic HCM, including Fabry's disease (mutations in GLA) and transthyretin (TTR) amyloidosis, among others. More than 1500 mutations in at least 8 sarcomere protein genes have been reported in HCM, although most (80%) mutations alter the -myosin heavy chain (MYH7) or the myosin-binding protein C gene (MYBPC3). The diverse molecular origin combined with the background genomic variability and lifestyle differences between patients have made it difficult to definitively understand the genotypic and phenotypic relationships. (4)

Previous studies suggest that mutations in MYH7 cause about 15-30% of HCM cases. (17,18) In our patients, mutations in this gene are less frequent and appear in 7.5% of the studied families. This difference may be related to the degree of selection of the studied population.

Although we found no significant differences in age at diagnosis, the mean age in patients with G+ was 37.4±15 years compared to 42.4±18 years in patients with G-, similar to other series. (19,20)

An interesting finding in our study was the higher frequency of mutations identified in women (36, 63%; p=0.06), without statistical significance; but as HCM is usually inherited in an autosomal dominant manner, it would be expected that 50% of patients were female. However, in nearly all series described, the proportion of women is about 30-40%, and they are usually older at the time of diagnosis. In our study, it was the opposite (age at diagnosis in women: 37±15 years compared to 46.5±15 years in men; p<0.01). (17-20)

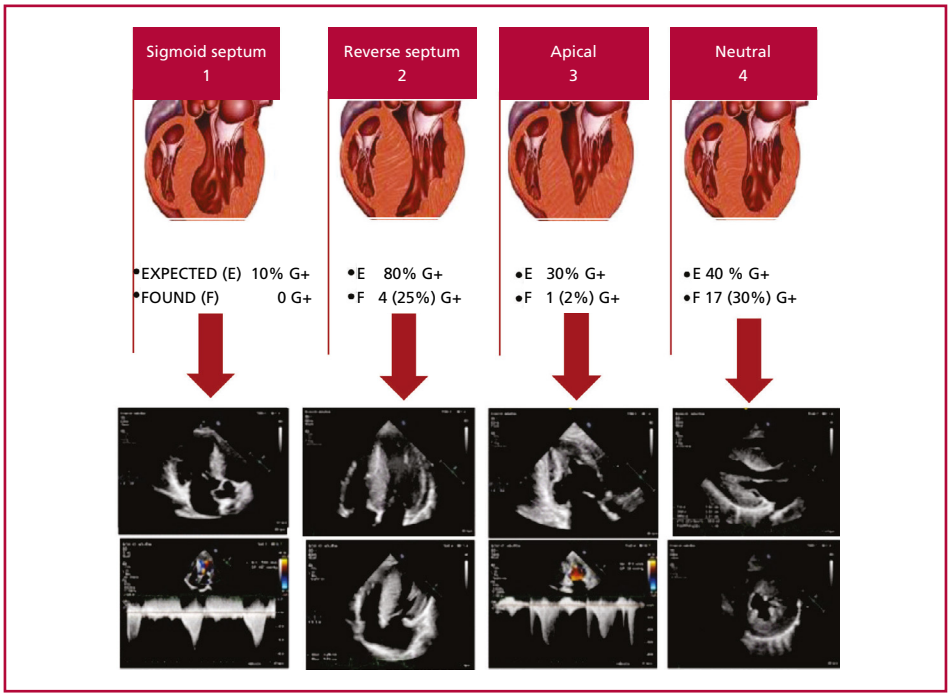
Women had a higher prevalence of the obstructive phe-

Table 1. Studied population characteristics

B. Echocardiographic and cardiac magnetic resonance characteristics of "affected" population

Phenotypes	Patients n=77	With mutations (G+) n=57	Without mutations (G-) n=20	p
Phenocopies	42 (54.5%)	33 (57.8%)	9 (45%)	0.46
HCM	21 (27.3%)	16 (28%)	5 (25%)	0.52
MARON Maron 1	17 (22%)	13 (23%)	4 (20%)	0.53
MARON Maron 2	6 (7.8%)	5 (8.7%)	1 (5%)	0.88
MARON Maron 3	21 (27.3%)	14 (24.6%)	7 (35%)	0.26
MARON Maron 4	6 (7.8%)	5 (8.7%)	1 (5%)	0.88
LEVER Lever 1	1 (1.3%)	0	1 (5%)	0.39
Lever 2	18 (23.4%)	14 (24.5%)	4 (20%)	0.46
Lever 3	1 (1.3%)	1 (1.75%)	0	0.39
Lever 4	25 (32.4%)	17 (29.8%)	8 (40%)	0.39
Age at diagnosis	38 (29-48)	36 (28-44)	45.5 (37.5-51.5)	0.05
Female	46 (59.7%)	36 (63.1%)	10 (50%)	0.44
HT	15 (19.5%)	8 (14%)	7 (35%)	0.04
CV events	33 (42.8%)	22 (38.6%)	11 (55%)	0.31
Heart transplant	1 (4%)	1 (2.2%)	0	0.44
Ventricular tachycardia	14 (13.8%)	5 (8.7%)	3 (15%)	0.34
ICD	16 (20.8%)	11 (19.3%)	5 (25%)	0.40
Pacemaker	12 (15.6%)	8 (14%)	4 (20%)	0.37
Myectomy	6 (7.8%)	4 (7%)	2 (10%)	0.49
CV hospitalization	26 (33.7%)	16 (28%)	10 (50%)	0.06
CV death	11 (14.3%)	8 (14%)	3 (15%)	0.58

CV: cardiovascular; HT: hypertension; ICD: implantable cardioverter defibrillator;



Color Doppler echocardiography image from own source. Figure modified from Lever HM, Karam RF, Currie PJ, Healy BP. Hypertrophic cardiomyopathy in the elderly. Distinctions from the Young based on cardiac shape. Circulation 1989; 79(3):580-9.

Fig. 4. Hypertrophy patterns according to the classification of Lever et al. Pretest probability for a positive genetic testing result according to the anatomical subtype.

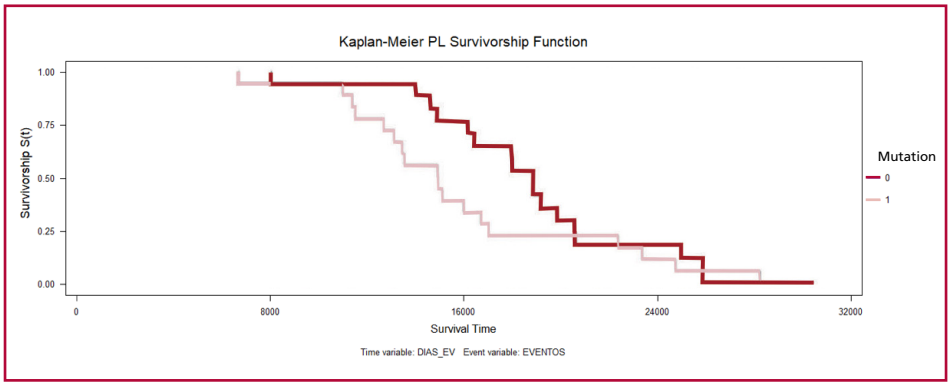


Fig. 5. Kaplan-Meier event-free survival curve. Patients with G+ have a mortality hazard ratio (HR) of 1.67 compared to those with G-, p=0.14.

notype, more severe symptoms requiring septal reduction therapy and one patient even underwent a transplant. However, there was higher mortality in men than women.

The identification of mutations in different families allows a more accurate assessment of the genotype-phenotype correlation and the proper interpretation of the pathogenic role of each mutation. Several findings from our study emphasize the importance of a complete family study. While in some mutations, such as TNNT2 c.812A>T (p.Asn271Ile), the phenotype is reproduced similarly in most carriers, in others, such as MYBPC3 c.1808_1821del (p.Ile603Thrfs*6), there is a remarkable difference between the phenotype of index cases (severe hypertrophy in young patients) and family carriers with mild hypertrophy, in spite of having similar or older ages. In these cases, it should be considered that additional genetic or environmental factors may account for the large difference in expression. Several studies have shown that HCM patients may have more than one mutation and that the presence of double mutations is associated with a more severe expression of the disease, as was the case in two patients in our study. (6, 17-20)

In clinical practice, HCM frequently coexists with hypertension (8 patients with HCM in our study, 25.8%) due to the high prevalence of both diseases. This hemodynamic situation inevitably modifies the HCM phenotype as well as exercise (athlete's heart) and other comorbidities (diabetes mellitus, obesity, and chronic renal failure). It is clear that it is often impossible to recognize the real cause or the main modifier of LV hypertrophy.

Nowadays, it is believed that wild-type (wt) TTR amyloidosis (wtTTR), which has been intensively studied and underdiagnosed, has relatively high prevalence. In our study we detected 46 patients with cardiac amyloidosis (45%), 60.7% of which were TTR amyloidosis variant (TTRv), 26% wtTTR, and the rest other types of amyloidosis. We believe that amyloidosis was a contributor to higher mortality in the "phenocopies" group in comparison with the HCM group.

Limitations

It was a single-center study conducted at a tertiary healthcare center in individuals who were likely to be more severely ill and symptomatic.

There may be some additional mutations that have not been identified because of the use of predetermined gene panels.

Samples are impossible to be collected in deceased subjects or in those who declined to participate in the study or were not notified to be an index case.

Conclusions

It was confirmed that molecular testing guided by the Mayo Score provided high probability of detecting mutations. Molecular testing proved to be important due to the phenotypic and genotypic overlap in cardiomyopathies.

Understanding the causative genetic variant does not currently affect the clinical management of most HCM patients, but it is helpful in a small group of genes, such as GAA, GLA, LAMP2, PRKAG2 and TTR, which are undoubtedly associated with diseases that mimic HCM and have different clinical profiles, inheritance patterns and treatment options; therefore, in those cases, molecular testing represents a significant step towards customized approaches.

Conflicts of interest

None declared.

(See authors conflicts of interest forms in the website/ Supplementary material)

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ANNEX 1

Diagnostic criteria for HCM. World Health Organization/International Society and Federation of Cardiology 1997

Major Criteria	Minor Criteria
Echocardiographic (TTE)	- Anterior septum or posterior wall ≥12 mm
- Anterior septum or posterior wall ≥13 mm	- Posterior septum or free wall ≥ 14 mm
- Posterior septum or free wall ≥ 15 mm	- Moderate SAM
- Severe SAM	- Redundant mitral valve leaflets
Electrocardiographic (ECG)	
- LVH + repolarization changes	- CLBBB or intraventricular conduction defect in left ventricular leads
- T wave inversion in leads I and aVL, V3-V6 (≥3 mm), or II, III, aVF (≥5 mm)	- Minor repolarization changes in left ventricular leads
- Abnormal Q waves (>40 ms or >25% R wave) in at least 2 leads from II, III, aVF, V1-4 or I aVL or V5-6	- Deep S waves in V2 (>25 mm)
Clinical	Unexplained syncope, dyspnea, or precordial pain
Diagnosis of hypertrophic cardiomyopathy	
1 major criterion, or	2 minor TTE criteria + 1 minor ECG criterion

SAM: systolic anterior motion; LVH: left ventricular hypertrophy; CLBBB: complete left bundle branch block.
Table modified from 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(2):130-132Heart 1997;77(2):130-132

ESC Diagnostic Criteria for HCM 2008

Adults

Wall thickness ≥15 mm in one or more LV segments – determined by any imaging technique: echocardiography, cardiac magnetic resonance (CMR) or computed tomography (CT)– that may not be explained by loading conditions only.

Children

LV wall thickening with a Z score >2 standard deviations from the expected mean.

Family members

Unexplained presence of an increase in LV thickness ≥13 mm in one or more LV segments, determined by any imaging technique (echocardiography, CMR or CT).

From Elliott P, Andersson B, Arbustini E, Bilinska Z, Cecchi F, Charron P, et al. Classification of the cardiomyopathies: a position statement from the European Society Of Cardiology Working Group on Myocardial and Pericardial Diseases. Eur Heart J 2008;29(2):270-6

- **HCM phenocopies (imitations)**": Cardiac or systemic diseases capable of producing LVH that should not be labelled as HCM. The use of HCM to describe increased LV wall thickness associated with systemic disorders or secondary causes of LV hypertrophy (LVH) may be confusing. Systemic disorders include metabolic and multi-organ syndromes, such as the RASopathies (variants in several genes involved in RAS-MAPK signaling pathway), mitochondrial myopathies, glycogen/lysosomal storage diseases in children, and Fabry's cardiomyopathy, amyloidosis, sarcoidosis, hemochromatosis and Danon's cardiomyopathy in adults. In these diseases, although the magnitude and distribution of LV wall thickening may be similar to those of the isolated HCM caused by variants in sarcomere genes, the pathophysiological mechanisms responsible for the hypertrophy, the natural history and the treatment strategies are different.
- Echocardiographic criteria for amyloidosis were defined by the presence of LVH with a cut-off point ≥12 mm at the septal level or posterior wall according to the 10th International Symposium on Amyloidosis, 2004.

Clinical variable	Points
Age <45 years	1
Left ventricular wall thickness >20 mm	1
Family history of HCM	1
Family history of sudden cardiac death	1
Reverse septal curvature	1
Hypertension (HT)	-1

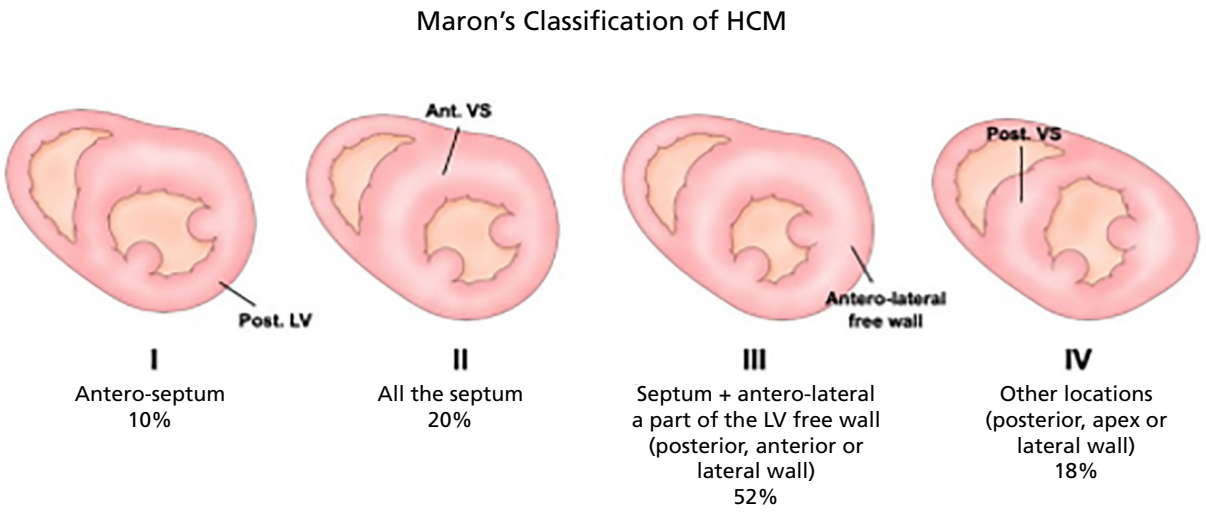
Mayo HCM Genotype Predictor Score

NGS results were compared to the Mayo Score (range -1 to 5) according to clinical and echocardiographic variables. One patient with a Mayo Score of 5 had a pathogenic mutation (100% yield). Patients with a Mayo Score of 4 had a pathogenic mutation in 71% of the cases. Patients with a Mayo score of 3 or 2 had a pathogenic mutation in 50% and 35% of the cases, respectively. The yield of genetic testing with a score of -1 to 1 was low (6-21%).

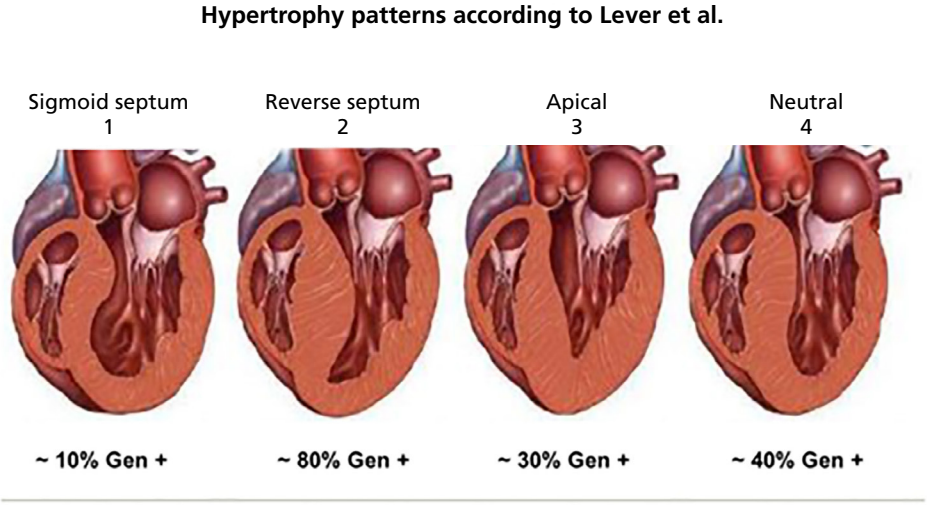
Bonaventura J, Norambuena P, Tomašov P, Jindrová D, Šedivá H, Macek M Jr, Veselka J. The utility of the Mayo Score for predicting the yield of genetic testing in patients with hypertrophic cardiomyopathy. Arch Med Sci. 2019 May;15(3):641-649. doi: 10.5114/aoms.2018.78767 Epub 2018 Oct 8. PMID: 31110529; PMCID: PMC6524174.

ANNEX 2

Maron et al.9 have established a morphological classification into four types: type I, septal-anterior hypertrophy; type II, septal-anterior and septal-posterior hypertrophy; type III, septal and anterolateral hypertrophy;



and type IV, septal-posterior and/or anterolateral hypertrophy.
Modified from Maron BJ. Hypertrophic cardiomyopathy. Curr Probl Cardiol. 1993 Nov;18(11):639-704. doi: 10.1016/0146-2806(93)90025-w. PMID: 7903919.

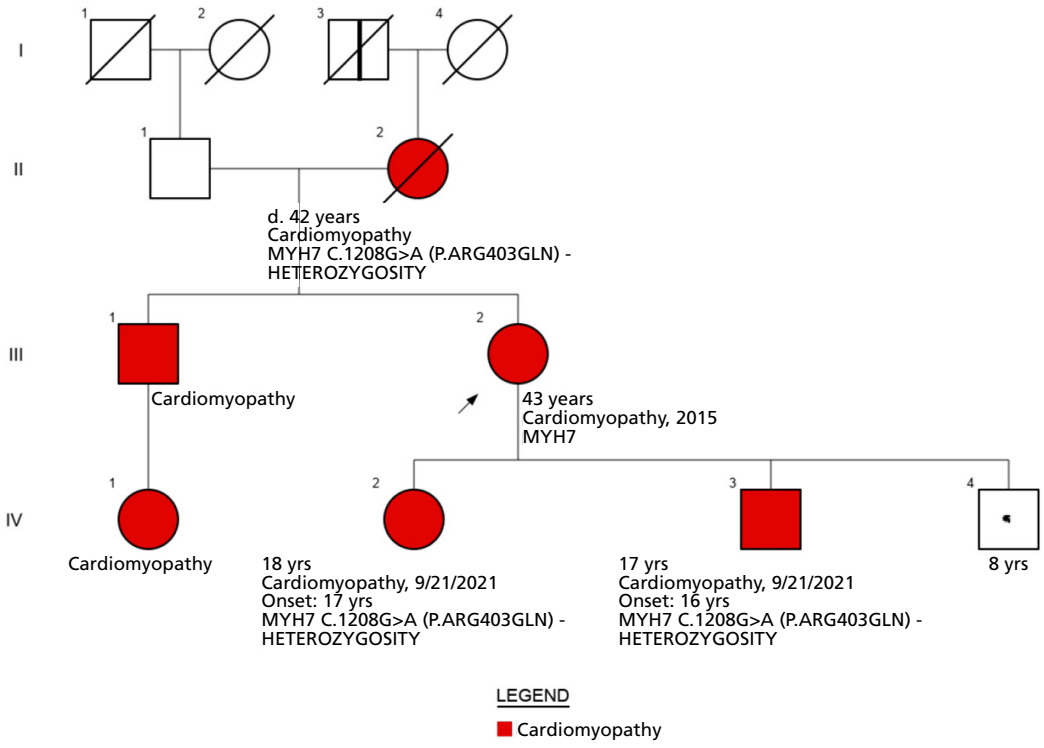


Pretest probability for a positive genetic testing result according to the anatomical subtype

This classification has proved to be very useful when considering the probability of presenting a positive genetic testing based on the anatomical phenotype expressed by the patient, the so-called echocardiography-guided genetic testing.
Modified from Lever HM, Karam RF, Currie PJ, Healy BP. Hypertrophic cardiomyopathy in the elderly. Distinctions from the young based on cardiac shape. Circulation 1989 Mar; 79(3):580-9.

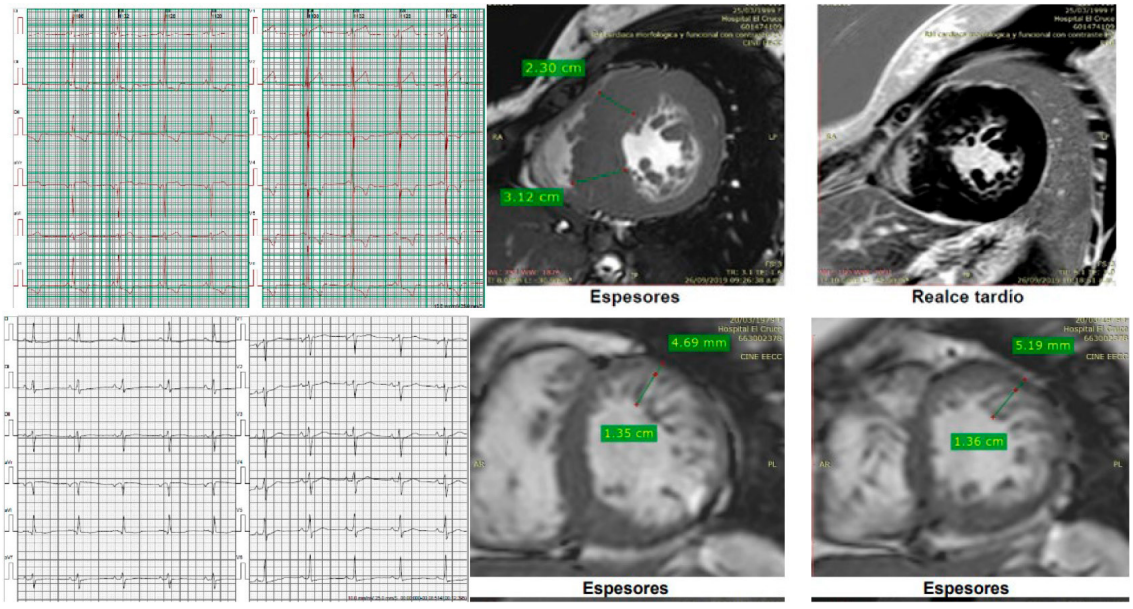
ANNEX 3

Examples of clinical cases:

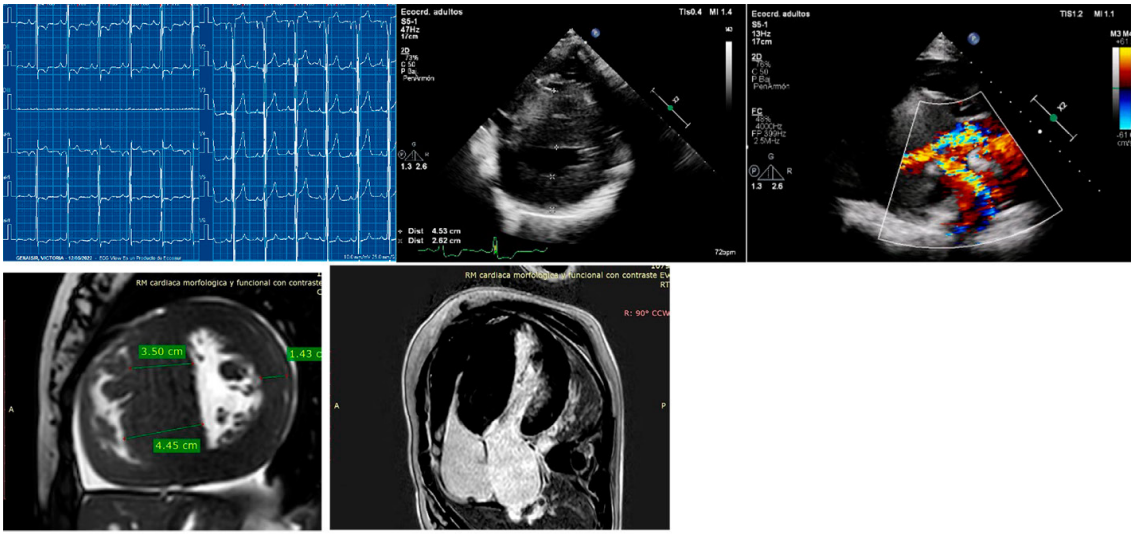


Example of a studied family's genogram. References: squares: men, circles: women, red: patients with clinical diagnosis of HCM, white: patients without HCM or mutation or not assessed, symbols with central black dot:

mutation carriers without HCM phenotype, symbols with vertical black bar: subjects with possible HCM by medical history (not proven). Diagonal line: deceased patients, arrow: index case.
The index case is a woman with severe hypertrophy diagnosed at the age of 37, with ICD implantation for syncopal sustained monomorphic ventricular tachycardia (VT) at the age of 47. The CMR showed non-compacted myocardium (NCM) and G+ (MYH7: c.1208G>A (p. Arg403Gln). Her 3 sons have G+, two of them with F+, pathological ECG (left ventricular hypertrophy and negative T waves on the anterolateral side). Above: ECG and CMR of one of her sons. Below: patient's ECG and CMR.



Case 2. A 17-year-old adolescent with effort angina (F+ and G-) and VUS+.



ANNEX 4

Mutational analysis

On the basis of the saliva samples collected by oral swabbing, the targeted regions of genomic DNA obtained from the sample are enriched by hybridization-based protocol and sequenced by Illumina technology. All targeted regions are sequenced with a depth ≥50x or supplemented with additional analyses.

Reads are aligned to a reference sequence (GRCh37) and sequence changes are identified and interpreted in the context of a single clinically relevant transcript, as listed below.

Enrichment and analysis focus on the coding sequence of the indicated transcripts, 20 bp of flanking intronic sequence and other specific genomic regions shown to be responsible for the disease at the time of assay design. Promoters, untranslated regions, and other non-coding regions are not otherwise interrogated. For some genes, only targeted loci (as indicated in the table above) are analyzed. Exonic deletions and duplications are called by an internal algorithm that determines the number of copies at each target by comparing the read depth for each target in the proband sequence with the mean read depth and the read depth distribution, obtained from a clinical dataset. Markers in the X and Y chromosomes are analyzed for quality control purposes and may detect deviations from the expected sex chromosome complement. Such deviations may be included in the report in accordance with internal guidelines. Confirmation of the presence and location of reportable variants is performed according to strict criteria established by Invitae (1400 16th Street, San Francisco, CA 94103, # 05D2040778), as necessary, using one of several validated orthogonal approaches (PubMed ID 30610921). The following analyses are performed if relevant to the request. For PMS2 exons 12-15, the reference genome was modified to force all sequence reads derived from PMS2 and the PMS2CL pseudogene to align to PMS2, and variant calling algorithms were modified to admit an expectation of 4 alleles. If a rare SNP or indel variant is identified by this method, both PMS2 and the PMS2CL pseudogene are amplified by long-range PCR and the location of the variant is determined by Pacific Biosciences (PacBio) SMRT sequencing of the relevant exon in both long-range amplicons. If a CNV is identified, MLPA or MLPA-seq is run to confirm the variant. If confirmed, both PMS2 and PMS2CL are amplified by long-range PCR, and PacBio sequences the identity of the fixed differences between PMS2 and PMS2CL from the long-range amplicon to disambiguate the location of the CNV.

The technical component of the confirmatory sequencing is performed by Invitae Corporation (1400 16th Street, San Francisco, CA 94103, #05D2040778). For the C9orf72 repeat expansion testing, hexanucleotide repeat units are detected by repeat primed PCR (RP-PCR) with fluorescently labelled primers, followed by capillary electrophoresis. Interpretation reference ranges: benign (normal range): <25 repeat units, uncertain: 25-30 repeated units, pathogenic (full mutation): ≥31 repeated units. A second round of RP-PCR using a non-overlapping primer set is used to confirm the initial call in the case of suspected allele sizes of 22 or more repeats. For RNA analysis of the genes listed in the Genes Analyzed table, complementary DNA is synthesized by reverse transcription from RNA derived from a blood sample and enriched with specific gene sequences using capture hybridization. After high-throughput sequencing with Illumina technology, the output reads are aligned to a reference sequence (genome build GRCh37; custom derivative of the RefSeq transcriptome) to identify the locations of exon junctions through the detection of split reads. The relative usage of exon junctions in a test sample is quantitatively assessed and compared to the usage observed in control samples. Abnormal exonic junction usage is evaluated as evidence in the Sherloc variant interpretation framework. If an abnormal splicing pattern is predicted based on a DNA variant outside the typical reportable range, as described above, the presence of the variant is confirmed by targeted DNA sequencing. RNA sequencing is performed by Invitae Corporation (1400 16th Street, San Francisco, CA 94103, #05D2094793). Invitae Corporation (5 Technology Drive, Irvine CA 92618, #05D1052995) performs the technical component of fibroblast cell culture and gDNA extraction from a skin punch biopsy.

A PMID is a unique identifier that refers to a published scientific article. Search by PMID at http://www.ncbi.nlm.nih.gov/pubmed.

An rsID is a unique identifier that refers to a single genomic position and is used to associate population frequency information with sequence changes at that position. The reported population frequencies are derived from several public sites that aggregate data from large-scale population sequencing projects, including ExAC (http://exac.broadinstitute.org), gnomAD (http://gnomad.broadinstitute.org) and dbSNP (http://ncbi.nlm.nih.gov/SNP).

A MedGen ID is a unique identifier that refers to an article in MedGen, NCBI's centralized database of information on genetic disorders and phenotypes.

Search by MedGen ID at http://www.ncbi.nlm.nih.gov/medgen. An OMIM number is a unique identifier that refers to a complete entry in the Online Mendelian Inheritance of Man (OMIM). Search by OMIM number at http://omim.org/. Invitae uses information from individuals undergoing testing to inform variant interpretation. If "Invitae" is cited as a reference in the variant details, this may refer to the individual in this request and/or to historical internal observations.