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Genetic characterization and genotype-phenotype associations in a large cohort of patients with hypertrophic cardiomyopathy – An ancillary study of the Portuguese registry of hypertrophic cardiomyopathy☆

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ABSTRACT

Background: We present an ancillary study of the Portuguese Registry of Hypertrophic Cardiomyopathy (PRo-HCM). This is one of the largest HCM genetic studies based on a registry.
Methods and results: Collected genetic variants were re-analysed for pathogenicity. Demographic, clinical, imaging and outcome data were analysed for associations with genotype, focusing on comparisons between patients with (G+) *vs* without (G−) a pathogenic/likely pathogenic (P/LP) variant in one the 9 main causal sarcomeric genes. From the 1042 patients in the registry, 528 (51%) had genetic testing. 152 (28%) were G+ and 98 pts. (19%) had variants of unknown significance. From the patients with the 9 mentioned genes sequenced (424 pts), 14.6% had P/LP variants in *MYBPC3*, 8.7% *MYH7*, 4.5% *TNNT2*, 1.7% *TNNI3*. Patients were 51 ± 16 years-old, 59% males. Genotype was associated with the following: birthplace ($p = 0.005$); age ($p < 0.001$); family history of HCM ($p < 0.0005$); hypertension ($p < 0.0005$); chest pain ($p = 0.015$); pattern of hypertrophy ($p = 0.006$); left ventricular hypertrophy on the ECG ($p < 0.0005$); family history of sudden cardiac death (SCD) ($p = 0.002$). G+ patients more frequently had more than one risk factor for SCD ($p = 0.002$) and a higher ESC-SCD risk score ($p = 0.003$). In survival analysis, G+ was associated with SCD ($p = 0.017$) and *MYH7*+ with LV systolic dysfunction ($p = 0.038$).
Conclusion: Half of the registry patients had genetic testing. Sarcomere-positive patients had distinct demographics, ECG, imaging characteristics and family history and are at increased risk of SCD. The presence of a *MYH7* mutation was associated with evolution towards LV systolic dysfunction.

1. Introduction

The Portuguese Registry of Hypertrophic Cardiomyopathy (PRo-HCM) registry provides a detailed contemporary assessment of the clinical profile, management strategies and outcomes of HCM. The overall results were recently published [1]. The main article focused on a description of the clinical and outcome characteristics of the cohort. The collected genetic data were *a priori* planned to be analysed in a separate publication. We hereby present one of the largest genetic characterization of HCM patients enrolled in a registry, as an ancillary analysis of the PRo-HCM genotype data.

Hypertrophic cardiomyopathy (HCM) genotype-phenotype associations described in the literature are almost exclusively based in single-centre data [2]. Furthermore, the published national and international registries of HCM did not describe genotype findings in detail and did not analyse genotype-phenotype associations [3] [4,5], with the exception of a German registry [6]. Some genotype-phenotype correlations in

☆ All authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

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HCM have been replicated in recent work on large populations, mostly focusing on a comparison between individuals with and without a mutation in one of the main sarcomere protein causal genes [2,7]. These have described an association of a genotype-positive status with wall thickness, family history of the disease, family history of sudden death, age and sudden death [2,7]. Gene-specific or variant-specific associations, although potentially more useful for decision-making, are more inconsistent and more difficult to replicate [2,7]. This has limited the clinical use of genetics for prognostication in HCM [8]. However, its role in diagnosis and family screening is well established and cascade screening (predictive testing) can identify or exclude individuals at risk of developing the condition [9].

In this work, our main aim was to characterize the status of the clinical use of genetic testing in HCM patients and, after a thorough revision of the variants reported by the co-investigators, to describe the main genetic findings regarding sarcomere genes and explore genotype-phenotype associations in this large registry population.

2. Methods

2.1. The Portuguese Registry of Hypertrophic Cardiomyopathy and phenotype data collection

PRo-HCM was a national multicentre registry designed to collect information on the current approach to HCM in Portugal and to facilitate future improvements regarding diagnosis and therapeutic management of the condition [1].

Please see supplementary material for detailed methods.

2.2. Genetic data collection and analysis

One of the seven sections of the CRF collected data on genetic testing, family screening and genetic counseling. Genetic testing was performed at the discretion of the participating centres and was done in accredited clinical laboratories. The classification of identified genetic variants was assigned by the investigators, as pathogenic/likely pathogenic (P/LP), unknown significance or benign/likely benign, according to the available knowledge of pathogenicity at the moment of recruitment, as provided by clinical genetic laboratories. These data were not initially centrally reviewed by the coordinators of the registry.

For the current study and the purpose of the genotype-phenotype analyses, all collected individual variants were re-analysed and classified for pathogenicity following stringent criteria based on current guidelines [10]. Pathogenic and likely pathogenic (P/LP) variants were those classified as such on ClinVar [11], with a minor allele frequency (MAF) $< 1 \times 10^{-4}$ (as accessed Oct 2017) in gnomAD [12].

Demographic, clinical, imaging and outcome data, including survival, were analysed for associations with genotype. Endpoints considered for survival analysis were: all-cause mortality, cardiovascular mortality or cardiac transplant, sudden cardiac death and equivalents (appropriated shocks or aborted sudden cardiac arrest), non-fatal stroke, evolution to LV systolic dysfunction during follow-up, and a composite end-point of all of the previous.

Analyses focused on comparisons between patients with *vs* without a pathogenic/likely pathogenic (P/LP) variant in one or any of the 9 main HCM causal sarcomeric genes (*MYH7*, *MYBPC3*, *TNNT2*, *TNNI3*, *MYL2*, *MYL3*, *ACTC1*, *TPM1*, *CSRP3*) and in comparisons between patients carrying a P/LP in one of the 3 more prevalent causal genes (*i.e. MYBPC3 vs MYH7 vs TNNT2*).

2.3. Statistical analysis

Continuous variables are presented as mean and standard deviation. Categorical variables are given as absolute and relative frequencies. Chi-square or Fisher tests were used for comparisons of categorical variables and Student's *t*-tests or the non-parametric equivalent for continuous variables. Survival curves were constructed according to the Kaplan-Meier method, and comparisons were performed using the log-rank test for univariate analysis. All *p*-values were two-sided and considered significant when <0.05. In addition, we performed a multiple testing correction strategy, correcting the analyses for the number of gene tests. We performed five tests (one per sarcomeric gene (*MYH7*, *MYBPC3*, *TNNT2* and *TNNI3*), plus an additional test for all sarcomeric mutations combined (pathogenic/likely pathogenic – any main sarcomere). Therefore, we used $p < 0.01$, which is 0.05/5, and signaled this threshold on the different tables. All analyses were performed using SPSS version 19.0.

3. Results

3.1. Study population. Demographic, geographic and centre volume analysis

Of the 62 institutions contacted, 37 accepted to participate. The final number of participating centers was 29 [1]. From the 1042 HCM patients recruited to the registry, 528 (51%) had genetic testing; 436 (83%) were probands and 92 (17%) relatives. The most commonly reported cause for not having genetic testing was the absence of a request by the cardiologist/geneticist in 64%; 8% of the patients declined genetic testing. There was not a significant difference between the frequency of tested males (309 in 613, 50%) compared to females (219 in 429, 51%). Sixty-nine percent of the relatives had genetic testing, in contrast to 48% of the probands ($p < 0{,}001$).

Thirty-percent from the total of probands or affected relatives enrolled in lower volume centres (recruiting ≤ 50 cases) had genetic testing, compared to 58% in higher volume centres ($p < 0.001$). Regarding different geographic regions, 56% of patients from the South were tested, 64% from the Centre and 37% from the North ($p < 0.001$).

The genotype-phenotype analyses focused on the 424 patients in whom enough detail was provided to allow assessment of the pathogenicity of the reported variants. The demographic and clinical characteristics of the genetically tested patients are described in Table 1 and Supplementary Tables 1,4 and 5. In summary, these were 51 ± 16 years-old, 309 (59%) males and 97% Caucasians. Regarding the geographic distribution, the birthplace of the patients was 31% North, 28% Centre and 41% South. These characteristics are similar to the previously published overall population of the registry [1].

3.2. Overall genetic testing results and re-assessment of the pathogenicity of the reported variants

After systematically reviewing the variants submitted by the investigators, 152 patients (28%) were re-classified as genotype-positive (G+) and 98 patients (19%) as having variants of unknown significance (VUS). Supplementary Fig. 1. In the previously published registry data [1], 210 (40%) patients were considered as carrying P/LP mutations and 40 (8%) patients were considered as having VUS. All P/LP variants and VUS are listed in Supplementary Table 3.

From the patients with at least the 9 mentioned genes sequenced (424), 14.6% had P/LP variants in *MYBPC3*, 8.7% in *MYH7*, 4.5% in *TNNT2*, 1.7% in *TNNI3*. Supplementary Fig. 2.

3.3. Genotype associations with baseline demographic and clinical parameters

Genotype status was associated with the following demographic and clinical parameters, as presented in Table 1, Supplementary Tables 1,4,5 and Fig. 1.

Birthplace was also correlated with genotype, with a lower percentage of positive cases in the North (22%) compared to the South (39%) and Centre (44%), $p = 0.005$. A higher yield of genetic testing was found in relatives (69%) compared to probands (20%), $p < 0.001$. This last association was also present when analysing *MYBPC3* and *MYH7* individually, compared to sarcomere-negatives and other genes.

Age at first evaluation was lower in genotype-positive (46 ± 17 *vs* 53 ± 15 years-old, $p < 0.001$; 48 ± 19 *vs* 54 ± 15 years-old for probands, $p = 0.011$) patients. There was no statistically significant difference between genders. Individuals presenting with symptoms were less frequently genotype-positive (25% *vs* 41% in asymptomatic patients, $p = 0.001$). More specifically, angina at presentation was associated with a lower prevalence of a genotype-positive status (21% compared to 33% in patients without angina, $p = 0.015$). These were not significant when analysing index-cases only (Supplementary Table 4). No significant associations were found for other individual symptoms or between genotype and reason for diagnosis (Supplementary Table 5).

A genotype-positive status was also more prevalent in patients with a family history of HCM (54% *vs* 12%, $p < 0.0005$), a family history of SCD (42% *vs* 26%, $p = 0.002$) and no history of hypertension (39% *vs* 16%, $p < 0.0005$). These associations were also significant when only analysing probands. No other past medical history or co-morbidities were associated with the genotype status. Similar associations with age, symptomatic status at presentation, family history and hypertension

Table 1
Associations between genotype and demographic and clinical presentation at baseline.

Parameters	Pathogenic/likely pathogenic variant - any main sarcomere gene			Pathogenic/likely pathogenic variant - *MYH7*			Pathogenic/likely pathogenic variant - *MYBPC3*			Pathogenic/likely pathogenic variant - *TNNT2*			Pathogenic/likely pathogenic variant - *TNNI3*		
	No	Yes	p[a]	No	Yes	p[a]	No	Yes	p[a]	No	Yes	p[a]	No	Yes	p[a]
Demographics															
Birthplace, n (%)															
North	73 (77,7)	21 (22,3)	**0,005***	88 (93,6)	6 (6,4)	0,155	84 (89,4)	10 (10,6)	**0,039**	89 (94,7)	5 (5,3)	0,951	93 (98,9)	1 (1,1)	0,591
Centre	52 (56,5)	40 (43,5)		82 (89,1)	10 (10,9)		69 (75,0)	23 (25,0)		88 (95,7)	4 (4,3)		89 (96,7)	3 (3,3)	
South/Islands	84 (60,9)	54 (39,1)		118 (85,5)	20 (14,5)		112 (81,2)	26 (18,8)		130 (94,2)	8 (5,8)		136 (98,6)	2 (1,4)	
Type of patient, n (%)															
Proband	278 (80,1)	69 (19,9)	<**0,001***	331 (95,4)	16 (4,6)	<**0,001***	313 (90,2)	34 (9,8)	<**0,001***	334 (96,3)	13 (3,7)	0,130	341 (98,3)	6 (1,7)	1000
Relative with +phenotype	24 (31,2)	53 (68,8)		56 (72,7)	21 (27,3)		49 (63,6)	28 (36,4)		71 (92,2)	6 (7,8)		76 (98,7)	1 (1,3)	
Age at diagnosis (years), n (%)															
≤35	44 (53,7)	38 (46,3)	<**0,001***	66 (80,5)	16 (19,5)	<**0,001***	68 (82,9)	14 (17,1)	0,591	77 (93,9)	5 (6,1)	0,224	79 (96,3)	3 (3,7)	0,436
36–50	82 (67,8)	39 (32,2)		108 (89,3)	13 (10,7)		100 (82,6)	21 (17,4)		117 (96,7)	4 (3,3)		119 (98,3)	2 (1,7)	
51–65	94 (81,0)	22 (19,0)		112 (96,6)	4 (3,4)		103 (88,8)	13 (11,2)		112 (96,6)	4 (3,4)		114 (98,3)	2 (1,7)	
66–75	68 (82,9)	14 (17,1)		80 (97,6)	2 (2,4)		72 (87,8)	10 (12,2)		79 (96,3)	3 (3,7)		82 (100,0)	0 (0,0)	
>75	13 (59,1)	9 (40,9)		20 (90,9)	2 (9,1)		18 (81,8)	4 (18,2)		19 (86,4)	3 (13,6)		22 (100,0)	0 (0,0)	
Mean ± SD	53 ± 16	46 ± 18	<**0,001***	52 ± 16	41 ± 17	<**0,001***	51 ± 16	49 ± 17	0,409	51 ± 16	51 ± 20	0,963	51 ± 16	49 ± 19	0,065
Presentation															
Chest pain, n (%)															
No	189 (66,8)	94 (33,2)	**0,015**	251 (88,7)	32 (11,3)	**0,021**	235 (83)	48 (17)	0,161	270 (95,4)	13 (4,6)	1000	278 (98,2)	5 (1,8)	1000
Yes	85 (79,4)	22 (20,6)		103 (96,3)	4 (3,7)		95 (88,8)	12 (11,2)		103 (96,3)	4 (3,7)		106 (99,1)	1 (0,9)	
Asymptomatic, n (%)															
No	200 (75,5)	65 (24,5)	**0,001***	243 (91,7)	22 (8,3)	0,356	233 (87,9)	32 (12,1)	**0,008***	255 (96,2)	10 (3,8)	0,410	261 (98,5)	4 (1,5)	1000
Yes	74 (59,2)	51 (40,8)		111 (88,8)	14 (11,2)		97 (77,6)	28 (22,4)		118 (94,4)	7 (5,6)		123 (98,4)	2 (1,6)	
Family history of HCM, n (%)															
No	172 (88,2)	23 (11,8)	<**0,001***	189 (96,9)	6 (3,1)	<**0,001***	184 (94,4)	11 (5,6)	<**0,001***	191 (97,9)	4 (2,1)	**0,004***	193 (99,0)	2 (1,0)	0,423
Yes	79 (46,5)	91 (53,5)		142 (83,5)	28 (16,5)		123 (72,4)	47 (27,6)		155 (91,2)	15 (8,8)		166 (97,6)	4 (2,4)	
Hypertension, n (%)															
No	140 (60,6)	91 (39,4)	<**0,001***	200 (86,6)	31 (13,4)	<**0,001***	186 (80,5)	45 (19,5)	**0,002***	219 (94,8)	12 (5,2)	0,457	226 (97,8)	5 (2,2)	0,465
Yes	160 (84,2)	30 (15,8)		185 (97,4)	5 (2,6)		173 (91,1)	17 (8,9)		183 (96,3)	7 (3,7)		188 (98,9)	2 (1,1)	

Bold font signals statistically significant associations; "*" signals statistically significant associations after applying multiple testing correction.
[a] Chi-squared test or Student's t-test for two independent groups. HCM: hypertrophic cardiomyopathy.

were found for *MYH7* and *MYBPC3* individually, compared to sarcomere-negatives and other genes, with the exception of age for *MYBPC3*. A positive association with family history of the disease was also found for *TNNT2*. When directly comparing the main causal genes, *MYH7* patients were younger at diagnosis compared to *MYBPC3* (38.7 ± 16.6 *vs* 48.9 ± 16.8, $p = 0.005$).

3.4. Genotype associations with baseline electrocardiogram, exercise test and cardiac imaging (Supplementary Table 2, Supplementary Table 6 for probands and Fig. 1)

A higher prevalence of left ventricular hypertrophy criteria on the ECG was observed in genotype-negative patients (69% *vs* 43%, $p < 0.0005$; 71 *vs* 56%, $p = 0.029$ for probands). A similar association was found when individually analysing *MYBPC3* compared to sarcomere-negatives and other genes. *MYH7* patients had more frequently an abnormal ECG compared to *MYBPC3* (83.9% *vs* 59.6%, $p = 0.020$). The presence of a *MYH7* mutation was associated with non-specific intraventricular conduction abnormalities (36% *vs* 13% other genes and negatives, $p = 0.001$; 40% *vs* 14% in direct comparison with *MYBPC3*, $p = 0.006$). No significant associations with other ECG features were detected.

No significant associations were found between any of the baseline 24 h ECG monitoring (Holter) parameters and genotype.

Regarding the exercise test data, an abnormal blood pressure response to exercise was more frequent in genotype-positive patients (23% *vs* 9.4%, $p = 0.019$; a similar trend was present when only analysing probands, 24.1% *vs* 9.6%, $p = 0.05$). The presence of a mutation in *TNNI3* was associated with increased prevalence of ventricular ectopy (50% *vs* 5%, $p = 0.022$).

In terms of the echocardiographic parameters, ventricular dimensions were lower in genotype-positive patients (LVEED 45.6 ± 6.1 *vs* 47.3 mm, $p = 0.017$; 45 ± 7 *vs* 47 ± 7 mm, $p = 0.016$ for probands) and posterior wall thickness was higher in genotype-negative patients (11.1 ± 2.5 *vs* 10.3 ± 2.8 mm, $p < 0.001$). Whilst the last association lost significance when analysing probands-only, there was a significant difference in index patients for septal wall thickness (20 ± 5 mm in genotype-positive *vs* 18 ± 5 mm in genotype-negatives, $p = 0.016$). A lower prevalence of an apical pattern of hypertrophy (2% in *vs* 16%, $p = 0.006$; similar for only probands, $p = 0.014$) was observed in genotype-positive patients. *MYH7* patients had higher septal wall thickness (21 ± 5 *vs* 18 ± 5 mm, $p = 0.002$), compared to sarcomere-negatives and other genes. *TNNT2* patients had higher left atrial volumes (67.6 ± 25.9 *vs* 37.7 ± 17.6 ml, $p = 0.01$). No other echocardiographic parameters were significantly associated with genotype-status. No significant associations with exercise echocardiography or cardiac MRI (magnetic resonance imaging) parameters were detected.

3.5. Genotype associations with follow-up profiles and outcomes (Table 2, Supplementary Table 7 for probands and Fig. 2)

The mean time of follow-up since diagnosis was 5.7 ± 7.2 years (median 3 years).

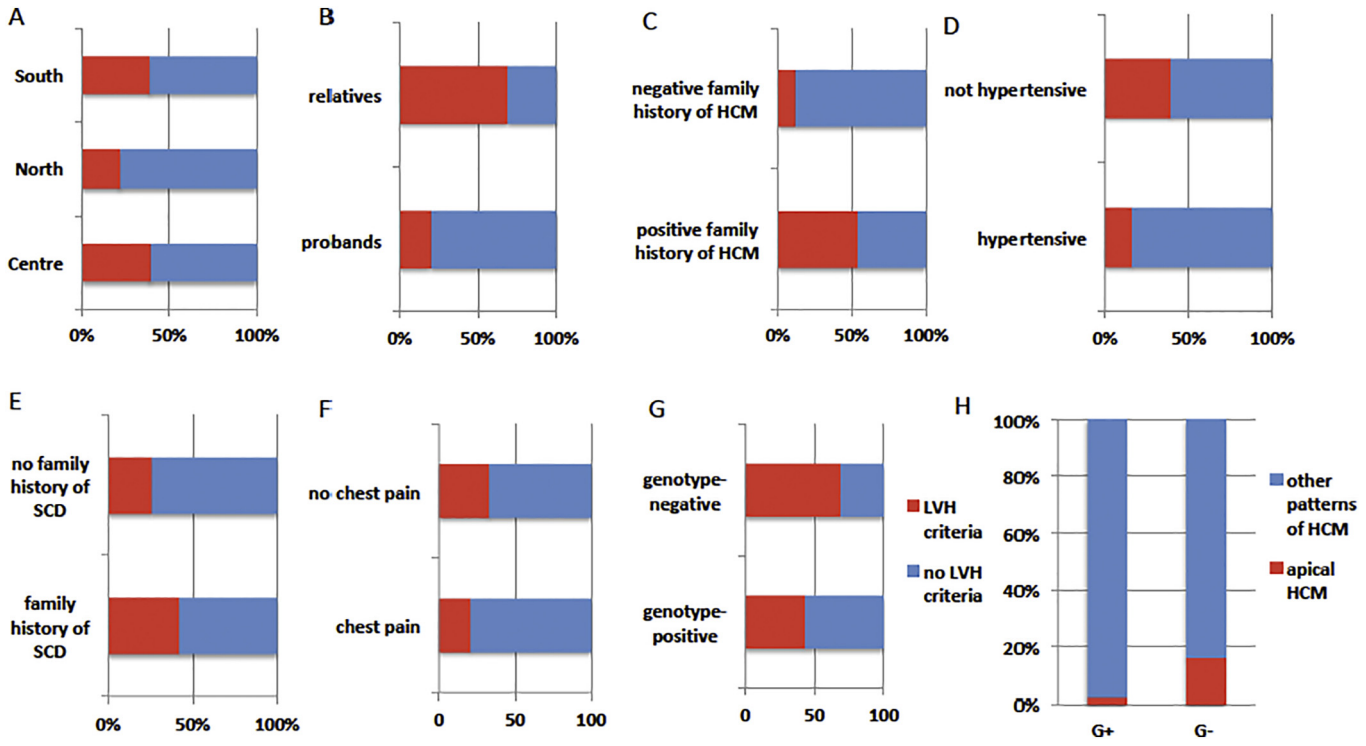

Fig. 1. Genotype-phenotype associations with: A. place of birth ($p = 0.005$), B: type of patient ($p < 0.001$), D: family history of HCM ($p < 0.0005$), E: hypertension ($p < 0.0005$), F: family history of SCD ($p = 0.002$), F: presence of chest pain at presentation ($p = 0.015$), G: LVH criteria on the ECG ($p < 0.0005$). A to G: Red: genotype-positive; blue: genotype-negative individuals. H: pattern of hypertrophy on echocardiography ($p = 0.006$). "G+": genotype-positive; "G−": genotype-negative.

With regard to the pre-established follow-up profiles [1], a significant association was detected for an asymptomatic *vs* symptomatic profile for genotype-positive (60.2% *vs* 40.2%, $p = 0.001$; in probands-only, 57.4% *vs* 39.8%, $p = 0.019$) and *MYBPC3*-positive (59.3% *vs* 43.7%, $p = 0.035$) patients.

Genotype-positive patients more frequently had any of the individual major American Heart Association/American College of Cardiology (AHA/ACC) [13] risk factors for SCD compared to genotype-negative patients (66.4% *vs* 50%, $p = 0.033$; 71% *vs* 49.3%, $p = 0.019$ for probands). These patients also had a higher median European Society of Cardiology (ESC) SCD risk score [9] (4.6 *vs* 2.7%/5 years, $p = 0.003$; 4.8 *vs* 2.7%, $p = 0.001$ for probands) and a lower prevalence of a low-risk category score (*i.e.* <4%/5 years) - 39% *vs* 69%, $p = 0.002$ (30% *vs* 69.7%, $p = 0.001$ for probands). The same associations were present in patients with a *MYBPC3* mutation, compared to sarcomere-negatives and other genes, which in addition had an increased prevalence of non-sustained ventricular tachycardia on Holter (38% *vs* 23% all other genes and negatives, $p = 0.014$; 40.4% *vs* 13.8% in direct comparison with *MYH7*, $p = 0.013$).

In survival analysis, a genotype-positive status was associated with SCD (log-rank $p = 0.017$) but with no other mortality endpoint including overall mortality or cardiovascular mortality (Fig. 2). *TNNT2* was also associated with SCD on survival analysis (log-rank $p = 0.01$).

The presence of a *MYH7* mutation was associated with LV systolic dysfunction during follow-up (log-rank $p = 0.038$). (Fig. 2).

When directly comparing the endpoints between patients with different causal genes, no significant differences in outcome were detected.

4. Discussion

We hereby present the largest and most comprehensive study on HCM genetics ever performed in a European HCM registry population, taking advantage of the data collected in the context of the Portuguese Registry of Hypertrophic Cardiomyopathy (Pro-HCM) [1]. We have characterized the status of the clinical use of genetic testing, described the main genetic findings regarding sarcomere genes and have found novel genotype-phenotype associations.

4.1. Prevalence of genetic testing and regional differences

In the previously published European registries on cardiomyopathies, the information regarding genetics is very scarce. Regarding the Italian registry of HCM [3], <1% of patients had genetic testing, which reflects the almost absent availability of clinical genetic testing at the time; furthermore, no detailed information was published regarding the results. In the much more contemporary ESC – EORP pilot registry on cardiomyopathies [5], 41.4% (462) patients underwent genetic testing, with a causative mutation reported in 51.1% (236) of all cardiomyopathy patients. In the more recently published ESC long-term registry on cardiomyopathies [4], 755/1627 (46.4%) had genetic testing for any cardiomyopathy, with more prevalent testing in relatives (60.9%) compared to probands (33.8%). This expected difference was also observed in our study, as the recruited relatives were often tested in the context of predictive testing. Regional differences were observed in the ESC registry, with *e.g.* 48.8% patients tested in the South compared to 10.4% in the East. However, the results of the ESC registry are reported overall for all cardiomyopathies and no detailed information is provided regarding the genetic testing results for individual types of cardiomyopathy or genotype-phenotype associations, including survival. In a recently published Japanese registry on HCM, no genetics data were reported [14].

The proportion of patients that had genetic testing in the Portuguese registry, around 50%, was slightly higher but similar to the reported in the ESC registry, although again a direct comparison is difficult, as the European registry did not report figures on HCM in isolation. In any case, this is an interesting result, considering the known challenges

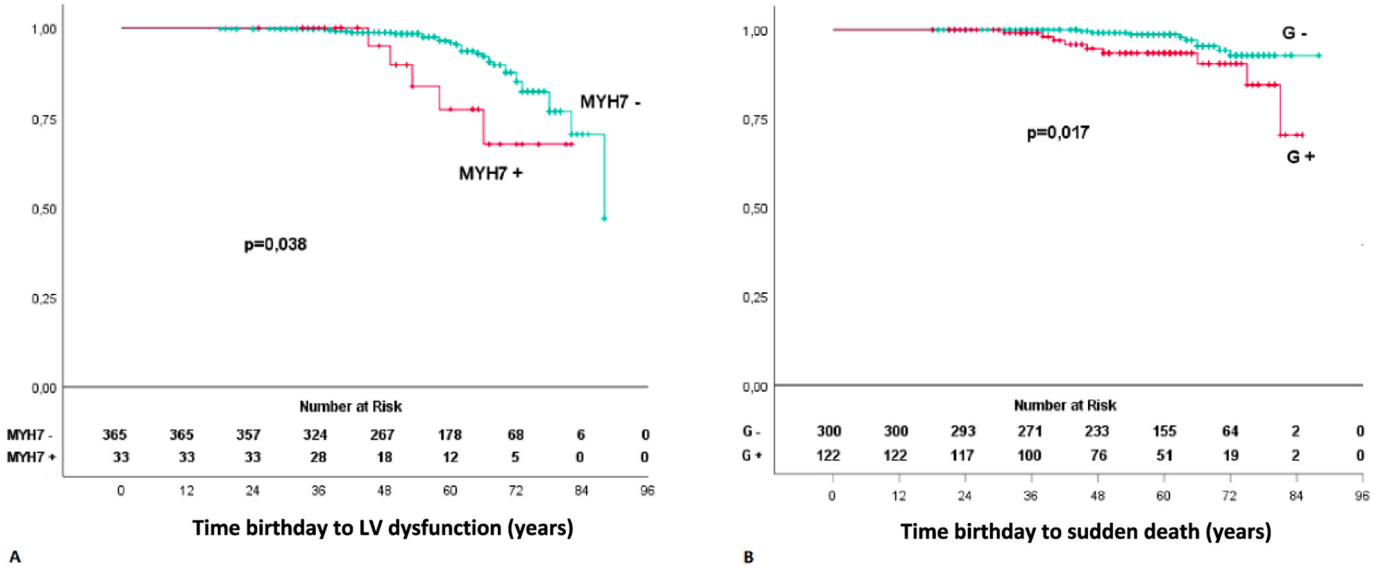

Fig. 2. Survival curves reflecting Kaplan-Meyer analysis of survival for: A: LV (left ventricle) systolic dysfunction; B: Sudden cardiac death.

regarding funding for genetic testing within national health services and the relatively limited economical resources in Portugal compared to some of the other European countries.

The extent of testing in Portugal was significantly different depending on the volume of patients recruited to the registry by a given centre, which might reflect the availability of dedicated clinics and possibly

Table 2
Associations between genotype and risk factors for sudden cardiac death.

Parameters	Pathogenic/likely pathogenic variant - any main sarcomere gene			Pathogenic/likely pathogenic variant - *MYH7*			Pathogenic/likely pathogenic variant - *MYBPC3*			Pathogenic/likely pathogenic variant - *TNNT2*			Pathogenic/likely pathogenic variant - *TNNI3*		
	No	Yes	p[a]	No	Yes	p[a]	No	Yes	p[a]	No	Yes	p[a]	No	Yes	p[a]
SCD risk factors according to ESC 2003 guidelines and ACC/AHA 2011 guidelines															
Syncope[b]	39 (13,1)	18 (14,9)	0,628	48 (13)	9 (17,6)	0,369	42 (12,8)	15 (16,5)	0,365	54 (13,6)	3 (13,6)	1000	54 (13,2)	3 (33,3)	0,110
Family history of SCD[b]	75 (26,0)	49 (41,5)	**0,002** *	104 (29,2)	20 (39,2)	0,147	85 (26,6)	39 (44,8)	**0,001** *	117 (30,4)	7 (31,8)	0,887	120 (30,2)	4 (44,4)	0,464
NSVT[b]	64 (23,6)	31 (28,2)	0,351	88 (26,3)	7 (14,9)	0,089	68 (22,6)	27 (33,8)	**0,040**	91 (25,3)	4 (19,0)	0,521	92 (24,7)	3 (33,3)	0,696
MLVWT ≥ 30 mm[b]	20 (6,7)	9 (7,5)	0,761	24 (6,5)	5 (9,8)	0,557	22 (6,7)	7 (7,7)	0,738	27 (6,8)	2 (9,1)	1000	29 (7,1)	0 (0,0)	0,644
ABPRE[b]	15 (9,3)	4 (7,3)	0,787	17 (8,8)	2 (9,1)	1000	16 (8,9)	3 (8,1)	1000	18 (8,8)	1 (9,1)	1000	18 (8,6)	1 (16,7)	1000
Number of risk factors[b]															
0	151 (50,0)	41 (33,6)	**0,033**	170 (45,7)	22 (42,3)	0,777	161 (48,5)	31 (33,7)	0,064	185 (46,0)	7 (31,8)	0,279	191 (46,0)	1 (11,1)	0,128
1	103 (34,1)	58 (47,5)		142 (38,2)	19 (36,5)		123 (37,0)	38 (41,3)		148 (36,8)	13 (59,1)		156 (37,6)	5 (55,6)	
2	35 (11,6)	17 (13,9)		43 (11,6)	9 (17,3)		35 (10,5)	17 (18,5)		50 (12,4)	2 (9,1)		49 (11,8)	3 (33,3)	
3	12 (4,0)	5 (4,1)		15 (4,0)	2 (3,8)		12 (3,6)	5 (5,4)		17 (4,2)	0 (0,0)		17 (4,1)	0 (0,0)	
4	1 (0,3)	1 (0,8)		2 (0,5)	0 (0,0)		1 (0,3)	1 (1,1)		2 (0,5)	0 (0,0)		2 (0,5)	0 (0,0)	
Other potential risk modifiers															
LAD bridging[b]	7 (5,3)	3 (9,7)	0,405	9 (6,1)	1 (6,7)	1000	7 (5,2)	3 (10,7)	0,379	10 (6,4)	0 (0,0)	1000	9 (5,7)	1 (20,0)	0,276
Apical aneurysm[b]	12 (4,2)	2 (1,7)	0,254	12 (3,4)	2 (4,2)	1000	12 (3,8)	2 (2,3)	0,547	13 (3,4)	1 (4,5)	1000	14 (3,6)	0 (0,0)	1000
LV systolic dysfunction[b]	16 (5,4)	6 (5,0)	0,875	20 (5,5)	2 (4,1)	0,760	16 (5,0)	6 (6,6)	0,596	21 (5,4)	1 (4,5)	1000	21 (5,2)	1 (11,1)	0,391
LVOTO[b]	112 (38,8)	26 (22,6)	**0,002** *	123 (34,5)	15 (31,9)	0,730	119 (37,8)	19 (21,3)	**0,004** *	133 (34,6)	5 (25,0)	0,376	137 (34,7)	1 (11,1)	0,175
ESC SCD risk score 2014															
SCD score (value)[c]	4,3 ± 3,9	6,6 ± 6,7	**0,003** *	4,8 ± 4,8	5,6 ± 5,1	0,266	4,5 ± 3,9	6,3 ± 7,0	0,271	4,9 ± 5,0	4,5 ± 2,0	0,350	4,9 ± 4,9	–	n.a.
SCD score (risk category)[b]															
≤4%	59 (69,4)	12 (38,7)	**0,002** *	61 (61,6)	10 (58,8)	0,620	58 (65,2)	13 (48,1)	0,261	69 (62,7)	2 (33,3)	0,097	70 (60,9)	1 (100,0)	1000
4%–6%	9 (10,6)	11 (35,5)		18 (18,2)	2 (11,8)		13 (14,6)	7 (25,9)		17 (15,5)	3 (50,0)		20 (17,4)	0 (0,0)	
≥6%	17 (20)	8 (25,8)		20 (20,2)	5 (29,4)		18 (20,2)	7 (25,9)		24 (21,8)	1 (16,7)		25 (21,7)	0 (0,0)	

SCD: sudden cardiac death; NSVT: non-sustained ventricular tachycardia; MLVWT: maximal left ventricular wall thickness; ABPRE: abnormal blood pressure response to exercise; LAD: left anterior descending; LVOTO: left ventricular outflow tract obstruction. ESC: European Society of Cardiology; ACC: American College of Cardiology; AHA: American Heart Association. Bold font signals statistically significant associations. "*" signals statistically significant associations after applying multiple testing correction.

[a] Chi-squared test or Student's *t*-test for two independent groups or Mann–Whitney test.

[b] n (%).

[c] Mean ± std. deviation; n.a. = not available.

an easier access to testing within the context of research funding in University and affiliated Hospitals.

4.2. Classification of genetic variants and yield of genetic testing

A clinical need for higher stringency and caution when classifying a variant as likely pathogenic/pathogenic - and hence attributing causality - has recently been advocated and demonstrated in cardiomyopathies [15]. In particular, the availability of data from large scale genetic projects like Exac and GnomAD [12], has led to the identification of previously considered LP/P variants with a minor allele frequency that would be incompatible with the known (relatively rare) prevalence of diseases such as HCM or long QT, leading to the reclassification of many of these variants as variants of unknown significance or even likely benign [15,16]. Public databases such as Clinvar [11] have also latterly become available, facilitating sharing of variant (re)classification amongst clinical laboratories and researchers. Recently published criteria from the ACMG reflect this stringency [10]. In view of these current concepts, for homogenization of results collected through a long timespan and from different laboratories and also stimulated by the fact that one of our main aims was to explore genotype-phenotype associations on our cohort, we have reanalyzed all the variants reported by the study investigators on the CRF. This lead to a decrease in the yield of a positive genetic result in the registry, from 40% in the original report to 28% in the present study; VUS's increased in prevalence from 8 to 19%.

4.3. Genotype-phenotype associations with demographics, clinical and imaging traits and prognosis

As expected, more than two thirds of relatives were positive compared to only one fifth of probands. The difference in yield amongst different regions of birthplace is interesting, with the Centre having twice the prevalence compared to the North. This finding might reflect a difference in the genetic background of the populations. Analysis of putative founder mutations is one of the possible future directions of research from the data collected in the registry. More stringent referral criteria for testing in some regions compared to others can also justify a higher testing yield (*i.e.* some centers might predominantly test younger patients, with clear family history).

In this unprecedented large cohort of Portuguese HCM patients, we have confirmed previously reported associations of genotype-positivity – for the whole cohort and when analysing only probands - with a younger age, family history of HCM and SCD, absence of hypertension, increased prevalence of asymmetric compared to apical HCM and wall thickness. These correlations were already described in previous publications, either for sarcomere gene mutation presence in general or some of the most prevalent genes. [2,17] [18] [7,19] In this registry study, these associations were confirmed in a “real-world” population including older and lower risk patients, which is a different setting from the cohorts where these genotype-phenotype correlations have been described.

Our analyses revealed other associations, some of them novel. Genotype-negative patients were more symptomatic at presentation, specifically with more angina than the genotype-positive patients. Possible causes of chest pain in HCM, when epicardial coronary artery disease is absent, include LVOT obstruction, mismatch between hypertrophied myocardium and coronary flow, diastolic dysfunction and small vessel disease. We have not been able to detect a significant difference in obstruction profile or exercise test results that could justify the aforementioned association and further work is needed to fully understand this finding, which could have relevant management implications. One possible explanation is the presence of an increased percentage of genotype-positive cases in relatives - who tended to be more asymptomatic - which could have influenced this association. However, while baseline data on probands-only showed an equal distribution of angina and symptoms overall between genotype-negatives and positives, the analysis of the follow-up profile in index-cases intriguingly revealed an increased proportion of asymptomatic profiles in genotype-positive compared to genotype-negative patients.

We also found interesting associations with electrocardiographic parameters. Namely, patients with *MYH7* mutations had a higher prevalence of non-specific intraventricular conduction abnormalities. This last finding is potentially in keeping with a recent meta-analysis [7] showing an increased prevalence of conduction disease in *MYH7* patients. Genotype-negative patients had higher voltages on the ECG. This finding is difficult to interpret and could be eventually explained by a lower amount of fibrosis in genotype-negative HCM patients. Another possible explanation is the relation with a higher prevalence of hypertension present in genotype-negative patients, which might contribute to higher voltages as an environmental modifier of this specific trait. Our data were probably underpowered to detect correlations with cardiac MRI parameters, which could contribute to explain the genotype-voltage association.

Genotype-positive patients had a higher SCD risk profile and accordingly, the presence of any sarcomere mutation was associated with increased SCD in the survival analysis. This association is similar to what was described in other recent studies [7], and suggests that the subset of HCM patients where a sarcomere mutation is identified are at increased risk of sudden death compared to the others. Interestingly, a number of risk factors used for stratification are in common to the ones recently shown to cluster in sarcomere-positive patients [7]. However, in order to translate these observations for the purpose of clinical risk stratification, larger studies looking into an independent effect of genotype in prognosis are needed.

We have additionally found that *MYH7* was associated with an increased incidence of LV systolic dysfunction during lifetime, which suggests a possible contribution of genetic background towards a “burn-out” profile, known to occur in 5–10% of HCM patients and associated with a poor prognosis [20].

When applying stringent criteria for pathogenicity, no patient carried multiple variants, precluding an analysis of increased variant burden on prognosis.

4.4. Limitations

As a registry, this study suffered from the inherent limitations and potential biases of data collection heterogeneity, retrospective and observational analysis. Patients were enrolled on a voluntary basis.

Missing data precluded further multivariate analysis and more advanced prognostic modeling. Similarly to the great majority of publications in HCM, this fact together with the low number of events limited the ability to test for an independent effect of the genotype.

The authors recognize the challenge of assigning pathogenicity to genetic variants. For uniformization purposes of results generated from a wide variety of centres and genetic testing laboratories, generated throughout a long timespan, an option was made to apply very stringent criteria. These may justify a low yield of positive results. Furthermore, variants of unknown significance might in the meantime have been proven in each centre to be pathogenic (*e.g.* through co-segregation analysis). A direct question regarding co-segregation was not present in the CRF.

As with other similar registries, and despite the fact that metabolic or multi-organ syndromes associated with LVH were considered exclusion criteria, exclusion of phenocopies was dependent on each individual investigator. Another exclusion criteria dependent on the individual investigator judgment was the presence of secondary LVH (at least stage 2 systemic hypertension). We expect “hypertensive heart disease” as an explanation for LVH to account for only an insignificant proportion of the population, even amongst genotype-negative individuals (in this regard, an average maximal wall thickness of 18 mm in genotype-negative patients is particularly reassuring).

Finally, and again in common with other similar work, there is a potential bias resulting from performing multiple testing, in that some of the associations identified may be incidental findings. To account for this we have signaled in the various tables a more stringent significance threshold, resulting from a multiple testing correction strategy.

5. Conclusion

Half of the recruited Portuguese HCM patients had genetic testing. This is very similar to the recently published European Registry [4]. The percentage of positive results was relatively low when using current strict criteria, but the total yield and the distribution amongst causal genes are similar to published populations. Importantly, the genotype-positive patient cohort has distinct demographics, imaging characteristics and family history, and seems to be at increased risk for sudden cardiac death. Associations with individual genes include a novel correlation between *MYH7* mutations and LV systolic dysfunction.

Please see supplementary material for a full list of investigators and centres. Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijcard.2018.12.012.

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Conflicts of interest

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