Predictors of Fabry disease in patients with hypertrophic cardiomyopathy: How to guide the diagnostic strategy?

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Background Fabry disease (FD) is a treatable cause of hypertrophic cardiomyopathy (HCM). We aimed to determine the independent predictors of FD and to define a clinically useful strategy to discriminate FD among HCM.

Methods Multicenter study including 780 patients with the ESC definition of HCM. FD screening was performed by enzymatic assay in males and genetic testing in females. Multivariate regression analysis identified independent predictors of FD in HCM. A discriminant function analysis defined a score based on the weighted combination of these predictors.

Results FD was found in 37 of 780 patients with HCM (4.7%): 31 with p.F113L mutation due to a founder effect; and 6 with other variants (p.C94S; p.M96V; p.G183V; p.E203X; p.M290I; p.R356Q/p.G360R). FD prevalence in HCM adjusted for the founder effect was 0.9%. Symmetric HCM (OR 3.464, CI95% 1.151-10.430), basal inferolateral late gadolinium enhancement (LGE) (OR 10.677, CI95% 3.633-31.380), bifascicular block (OR 10.909, CI95% 2.377-50.059) and ST-segment depression (OR 4.401, CI95% 1.431-13.533) were independent predictors of FD in HCM. The score ID FABRY-HCM [*–0.729 + (2.781xBifascicular block) + (0.590xST depression) + (0.831xSymmetric HCM) + (2.130xbasal inferolateral LGE)*] had a negative predictive value of 95.8% for FD, with a cut-off of 1.0, meaning that, in the absence of both bifascicular block and basal inferolateral LGE, FD is a less probable cause of HCM, being more appropriate to perform HCM gene panel than targeted FD screening.

Conclusion FD prevalence in HCM was 0.9%. Bifascicular block and basal inferolateral LGE were the most powerful predictors of FD in HCM. In their absence, HCM gene panel is the most appropriate step in etiological study of HCM. (Am Heart J 2020;226:114-26.)

Fabry disease (FD) (OMIM 301500) is an X-linked lysosomal storage disorder caused by mutations in the *GLA* gene, which codes for the enzyme α-galactosidase A. The deficiency of the enzymatic activity of α-galactosidase A leads to the lysosomal accumulation of globotriaosylceramide (GB3) and other related glycosphingolipids, causing multiorgan damage. Hypertrophic cardiomyopathy (HCM) is the main cardiac manifestation of FD, occurring more commonly in males (43% vs 26%) and nearly one decade later in females (mean age of onset 39 ± 10 vs 50 ± 11 years).[1]

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The prevalence of FD in patients with HCM has widely varied from 0% to 12% in previous studies (Supplementary Table I), due to different inclusion criteria, study designs and screening methods, although the latest studies, based on larger cohorts of patients and improved screening methods, have suggested a narrower prevalence range of 0% to 4%.[2-18] Moreover, the common finding of genetic variants of unknown significance (GVUS) in screenings of FD[19] mandates that prevalence results must be continuously re-evaluated as growing knowledge on Genetics comes to establish the pathogenic or benign nature of these variants.

FD is a treatable cause of HCM. Enzyme replacement therapy (ERT) has demonstrated to improve, stabilize or slow the progression of left ventricular (LV) mass,[20-22] wall thickness,[20,21] mid-wall fractional shortening[22] and strain[21] as well as NYHA class[20] and exercise tolerance.[21] However, early diagnosis and treatment are crucial, as the presence of cardiac fibrosis at the timing of ERT initiation may hamper long-term improvement of LV morphology and function and exercise capacity.[21] More recently, migalastat has also shown to improve LV mass and wall thickness.[23]

However, despite increasing awareness on the diagnostic red-flags,[24] FD remains a late diagnosis, being the median delay from symptom onset to diagnosis of 10.5 years (95% CI: 8-13).[25] Therefore, it is essential to accurately determine independent predictors of FD in patients with HCM and to create clinically useful tools that could lead to the early diagnosis of this treatable disease. Herein, we present the prevalence of FD in patients with HCM in a large multicenter study. To the best of our knowledge, this is the first study providing the independent predictors of FD in a large cohort of patients with HCM and a score to identify Fabry cases in patients with HCM.

Methods

FD screening in patients with HCM

Between January 2008 and March 2018, 780 consecutive patients with HCM were recruited from the Cardiology consultation of 12 Portuguese hospital centers (Supplementary Figure 1) and underwent FD screening. The inclusion criteria were adult patients (≥18 years) presenting HCM according to the ESC,[26] ie, left ventricular hypertrophy (LVH) with increased LV wall thickness (≥ 15 mm), by echocardiography, that was not explained by hypertension, valve disease or other cardiac overload conditions.

Enzymatic activity of α-galactosidase A was measured in all patients in dried blood spot (DBS) samples, as described by Gaspar et al.[27] The molecular analysis of the *GLA* gene was performed by PCR sequencing of all exons and their flanking intronic regions, as described by Shabbeer et al,[28] in all females and in males with reduced α-galactosidase A enzymatic activity (<0.3 nmol/h/spot).

Genealogy study

We published in 2013, for the first time, the founder effect of FD due to the mutation p.F113L in the Portuguese region of Guimarães, based on genealogy and haplotype analysis.[29,30] In this study, we also performed a genealogy research of all Fabry index patients that were found with the p.F113L mutation, in order to confirm the familial connection between them and a common ancestor. Historians searched for and analyzed birth, marriage and death certificates, the most recent in the Citizen Records and the most remote, dating to the 17th century, in the Parish Records currently filed in the City Archives.

Predictors of FD in patients with HCM

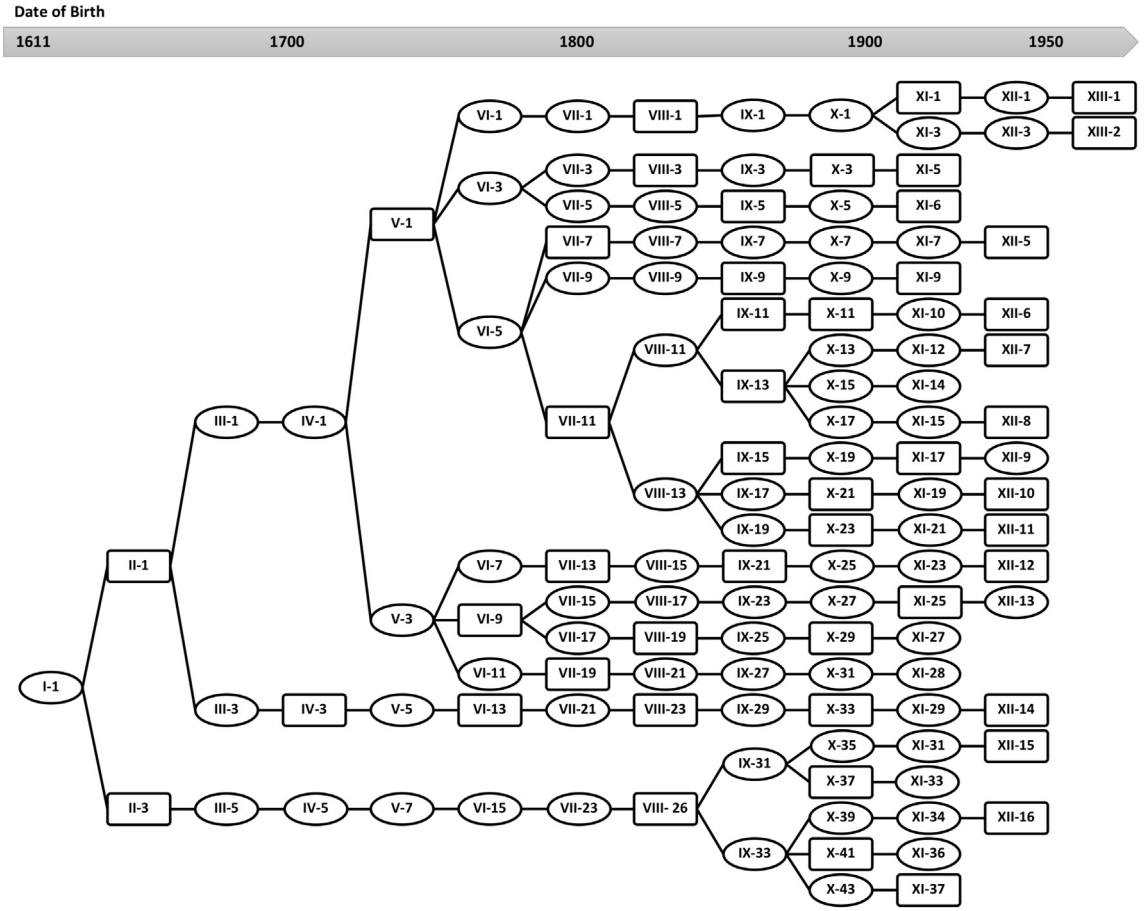
We collected clinical, electrocardiographic, cardiac imaging, laboratory and genetic data. Categorical variables were expressed as percentage. According to the Kolmogorov-Smirnov test, continuous variables were all non-normally distributed and therefore expressed as median with interquartile range (IQR). Comparison of variables between Fabry and non-Fabry patients was performed by χ^2 test for categorical variables and Mann-Whitney U test for continuous variables. Patients with *GLA* variants of uncertain clinical significance were excluded from this statistical analysis. In order to identify independent predictors of FD among patients with HCM, we performed a multivariate binary logistic regression analysis, including the cardiac categorical variables that were readily available on ECG, echocardiogram and cardiac MRI and showed statistically significant differences between FD and non-FD patients. We performed a Discriminant Function Analysis for FD in patients with HCM. We used the Wilk's lambda to calculate the unstandardized coefficients for each of the FD predictors that have been previously identified by multivariate regression analysis and then determined a discriminant score based on the weighted combination of the independent predictors of FD. We calculated the mean discriminant scores for Fabry and non-Fabry patients and determined the best discriminant cut-off for the score. We used the maximum likelihood technique to assign each case to a group according to the specified cut-off, in order to determine the sensitivity, specificity and positive and negative predictive values of the score in the prediction of FD in patients with HCM.

Statistical significance was set at $P < .05$.

Ethical issues

This research project was approved by the Ethics Committee of all the hospital centers included in this study. All patients provided written informed consent.

Figure 1



Family pedigree of FD patients with the mutation p.F113L, demonstrating a genealogical connection to a common ancestor who was born in 1611 in the Portuguese region of Guimarães. The individuals are coded by generation number in roman numerals-Individual number within the generation. Males are represented as squares and females as circles. For simplification, only Fabry patients are depicted and respective spouses were removed from the pedigree. From the 31 p.F113L patients, 27 were found and born in the region of Guimarães, three were found in other regions but were born <10 km from Guimarães and one was found 375 km from Guimarães. The genealogical connection was found for 23 patients (21 from Guimarães and two that were born near Guimarães). The family connection of the remaining p.F113L patients was not found due to lack of archive data.

Results

Prevalence of FD in HCM

The study included 780 patients with HCM, predominantly males (59.7%), with mean age of 66.0 ± 14.3 years, 22.1% of them with family history of HCM. LVH was asymmetrical in 65.0% of the cases. Mean interventricular septum thickness was 17.6 ± 4.3 mm. Mean LV ejection fraction was 64.4 ± 10.1%. LV outflow tract (LVOT) obstruction occurred at rest in 28.3%. Cardiac MRI was performed in 475 patients (60.9%) and revealed LGE in 274 cases (57.7%), mostly intramyocardial (81.5%) and in the septum (basal (50.0%) and mid (48.2%) antero-septal and basal (44.9%) and mid (53.6%) infero-septal segments). 24 h-Holter monitoring was performed in 684 patients (87.7%) and detected ventricular tachycardia (VT) in 15.0% (Supplementary Table II). A sarcomere gene panel had been performed in 497 patients (63.7%). Sarcomeric variants were found in 124 patients (24.9%), mainly in the *MYBPC3* (11.7%), *MYH7* (6.4%), *TNNT2* (2.4%) and *TPM1* (2.0%) genes. Phenocopies were found in 20 patients (2.6%): amyloidosis (15 cases), mitochondrial disease (2 cases), *PRKAG2* cardiomyopathy (1 case), hemochromatosis (1 case) and sarcoglycanopathy (1 case).

FD was diagnosed in 37 of the 780 patients with HCM (4.7%). The pathogenic mutation p.F113L was identified in 31 patients (4.0%) (Supplementary Table III), due to the founder effect previously documented in the Portuguese region of Guimarães[29,30] (Figure 1).

Table I. Characteristics of the patients with non-F113L *GLA* gene variants

Gender, age	*GLA* gene variant	Enzymatic activity of α-GAL A in plasma (nmol/h/mL) / leukocytes (nmol/h/mg)	Urinary GB3 (μg/mmol creatinine) / Plasma GB3 (nmol/mL)	Plasma Lyso-GB3 (ng/mL)	IVS / PW thickness (mm)	LV mass (g/m2)	LVH characteristics	Other clinical manifestations
Pathogenic *GLA* gene variants								
M, 50	p.C94S	1.0 / 0	NA / 17.77	107.19	21 / 18	277	Symmetrical Non-obstructive EF 58% DD grade II	Non-sustained VT Renal failure Microalbuminuria Proteinuria Stroke Deafness Acroparesthesias Anhidrosis Cornea verticillata Angiokeratomas
F, 56	p.M96V	2.2 / 5.6	124 / NA	6.73	22 / 17	167	Symmetrical Non-obstructive EF 70% DD grade II Diffuse LGE	Short PR interval Atrial fibrillation Microalbuminuria Cornea verticillata Deafness
F, 50	p.G183V	3.9 / 21.0	76 / n.d.	4.34	9 / 15	98	PW LVH Non-obstructive EF 80% LGE on basal inferolateral segment	Short PR interval Brain white matter lesions
F, 56	p.E203X	6.7 / 7.1	81 / 12.86	11.64	20 / 12	196	Asymmetrical Non-obstructive EF 68% DD grade I LGE on inferolateral wall	Renal failure Microalbuminuria Proteinuria Stroke Brain white matter lesions Deafness Cornea verticillata
F, 43	p.M290I	14.0 / 6.0	NA / n.d.	n.d.	23 / 12	212	Asymmetrical Non-obstructive EF 78% DD grade II LGE on inferior and inferoseptal walls, apical segments and basal inferolateral segment	Proteinuria Brain white matter lesions
M, 63	p.R356Q p.G360R	1.0 / 0	251 / NA	71.1	20 / 15	129	Symmetrical Non-obstructive DD grade II LGE in the apical segments and mid-septal segments	Myocardial infarct Stroke Brain white matter lesions Acroparesthesias Hypohidrosis Abdominal pain and diarrhea Angiokeratomas Cornea verticillata Deafness
Other *GLA* gene variants								
M, 28	p.A143T	NA / NA	NA / NA	NA	23 / 13	209	Asymmetrical Obstructive DD grade II LGE in the basal septal and mid anteroseptal and inferolateral segments *Pathogenic mutation on the *MYBPC3* gene (p.R943X)	Non-sustained VT

(continued on next page)

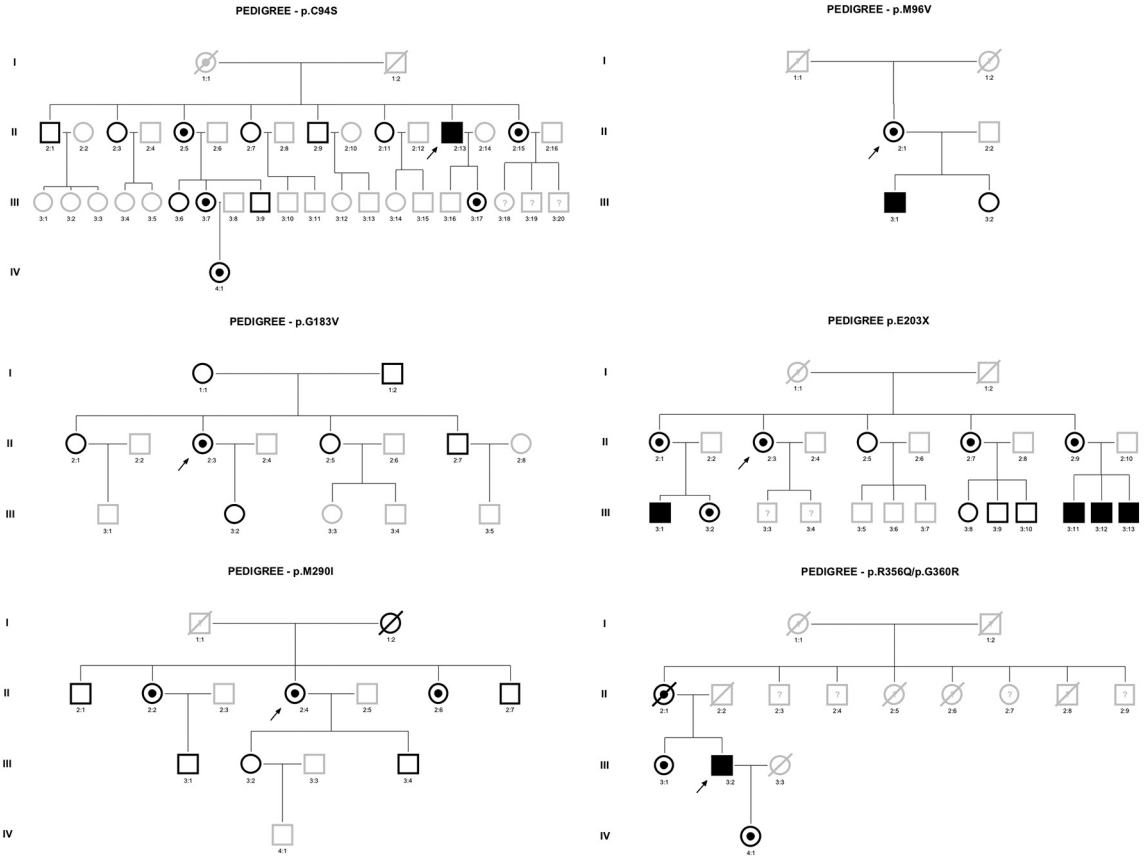
Table I (*continued*)

Gender, age	*GLA*gene variant	Enzymatic activity of α-GAL A in plasma (nmol/h/mL) / leukocytes (nmol/h/mg)	Urinary GB3 (μg/mmol creatinine) / Plasma GB3 (nmol/mL)	Plasma Lyso-GB3 (ng/mL)	IVS / PW thickness (mm)	LV mass (g/m2)	LVH characteristics	Other clinical manifestations
M, 73	p.R118C	NA / NA	NA / NA	NA	21 / 10	197	Asymmetrical Obstructive DD grade II LGE in septum *GVUS on *MYBPC3* gene (p.D610H)	1st degree AV block Complete RBBB Non-sustained VT
F, 69	p.R118C	NA / NA	NA / NA	NA	11 / 9	214	Apical HCM EF 52% DD grade II No LGE	None
F, 75	p.R118C	NA / NA	NA / NA	NA	15 / 13	133	Symmetrical Non-obstructive EF 70% DD grade I No LGE *GVUS on *MYH7* gene (p.R1781H)	None
M, 36	p.D313Y	NA / NA	NA / NA	1.0	18 / 10	89	Asymmetrical Non-obstructive EF 71% DD grade I LGE in antero-septal segments	None
F, 70	p.D313Y	NA / NA	NA / NA	1.6	20 / 20	234	Symmetrical Obstructive EF 70% DD grade II No LGE	Short PR interval Complete LBBB Paroxysmal atrial fibrillation
F, 70	p.D313Y	NA / NA	NA / NA	1.2	24 / 6	112	Asymmetrical Non-obstructive EF 81% DD grade I No LGE	Atrial fibrillation
F, 76	p.D313Y	NA / NA	NA / NA	NA	15 / 12	177	Symmetrical Non-obstructive DD grade I No LGE	None
F, 83	p.D175E	NA / NA	NA / NA	NA	16 / 10	185	Asymmetrical Non-obstructive EF 68% LGE in the septal and basal inferolateral segments	Renal failure Microalbuminuria Proteinuria Stroke
F, 61	p.V22A p.A73S	9.0 / 31.0	NA / NA	NA	16 / 10	98	Asymmetrical Non-obstructive EF 60% DD grade II LGE on septum *Pathogenic mutation on the *MYBPC3* gene (p.R502W)	1st degree AV block Non-sustained VT Acroparesthesias

α-GAL A, α-galactosidase A; AV, atrioventricular; DD, diastolic dysfunction; EF, ejection fraction; GVUS, genetic variant of unknown significance; IVS, interventricular septum; LBBB, left bundle branch block; LGE, Late gadolinium enhancement; LV, left ventricular; LVH, left ventricular hypertrophy; NA, non-available; n.d., non-detectable; PW, posterior wall; RBBB, right bundle branch block; VT, ventricular tachycardia.

Reference values: Enzymatic activity of α-galactosidase A on plasma 6–19 nmol/h/mL, and on leukocytes 36–80 nmol/h/mg; urinary GB3 0.87–13 μg/mmol creatinine; plasma GB3 0.8–4.52 nmol/mL; plasma Lyso-GB3 0–1.9 ng/mL.

Figure 2



Pedigrees of the Fabry families with non-F113L *GLA* gene variants.

Considering this founder effect, the prevalence of FD in patients with HCM would be 0.9%.

The other six Fabry patients presented respectively the following *GLA* gene variants: p.C94S; p.M96V; p.G183V; p.E203X; p.M290I; and p.R356Q/p.G360R (Table I). Lysosomal inclusions were demonstrated in the myocardium of the p.M290I patient and in the skin of the p.R356Q/p.G360R patient.

Their pedigrees are illustrated in Figure 2. Relevant family history of these *GLA* gene variants include: (i) p.C94S: Affected relatives presented clinical manifestations compatible with FD (renal failure in one sister; and proteinuria and acroparesthesias in one daughter, one niece and one great-niece) and increased plasma lysoGB3; (ii) p.M96V: The affected 19-year old son presented renal failure, proteinuria, cornea *verticillata*, angiokeratomas, acroparesthesias, hypohidrosis, abdominal pain and diarrhea as well as increased urinary GB3 (182 μg/mmol creatinine) and plasma lyso-GB3 (10.82 ng/mL). Skin biopsy showed lysosomal inclusions compatible with FD; (iii) p.G183V: All her relatives tested negative for this *GLA* variant, being impossible to determine if this case results from a sporadic "de novo" mutation or an illegitimate father; (iv) p.E203X: All the affected relatives exhibited a classical phenotype with increased FD biomarkers; (v): p.M290I: Her two affected sisters have been so far asymptomatic; (vi) p.R356Q/p.G360R: His daughter and sister had the same *GLA* gene variants. The daughter has been so far asymptomatic and his sister had HCM and microalbuminuria.

The screening also found two males with residual enzymatic activity of α-galactosidase A, carrying respectively the p.A143T (0.15 nmol/h/spot) and p.R118C (0.23 nmol/h/spot) variants; two females with the p.R118C variant; four patients with the p.D313Y variant; an elderly female with diabetes, hypertension and dyslipidemia, no family history suggestive of FD and the GVUS p.D175E; and a female with the novel variants p.V22A/p.A73S, who presented a pathogenic mutation in the *MYBPC3* gene and in whom pedigree analysis did not support the

Table II. Comparison of baseline characteristics between the patients with HCM secondary to FD vs non-FD

Baseline characteristics	Fabry patients (n = 37)	Non-Fabry patients (n = 733)	*P*
Male gender (%)	24 (64.9%)	439 (59.9%)	.547
Age (years) (median, IQR)	64.0 (57.5, 75.0)	68.0 (57.0, 77.0)	.453
Family history of LVH (%)	23 (65.7%)	134 (19.8%)	<.001
Symptoms (%)	32 (86.5%)	559 (76.5%)	.158
Dyspnea (%)	28 (75.7%)	401 (54.9%)	.013
Chest pain (%)	11 (29.7%)	182 (24.9%)	.509
Palpitations (%)	9 (24.3%)	132 (18.1%)	.339
Pre-syncope (%)	9 (24.3%)	64 (8.8%)	.002
Syncope (%)	4 (10.8%)	92 (12.6%)	.750
NYHA class			
I (%)	18 (62.1%)	197 (38.6%)	
II (%)	11 (37.9%)	265 (52.0%)	.056
III (%)	0 (0.0%)	46 (9.0%)	
IV (%)	0 (0.0%)	2 (0.4%)	
Echocardiogram (n = 38 vs 742)			
LVH pattern			
Asymmetrical (%)	17 (45.9%)	483 (66.0%)	
Symmetrical (%)	20 (54.1%)	145 (19.8%)	<.001
Apical (%)	0 (0.0%)	93 (12.7%)	
Other (%)	0 (0.0%)	11 (1.5%)	
LV outflow tract obstruction at rest (%)	3 (8.1%)	181 (24.8%)	.020
IVS thickness (mm) (median, IQR)	18.0 (17.0, 21.0)	17.0 (15.0, 20.0)	.087
PW thickness (mm) (median, IQR)	13.0 (12.0, 17.0)	11.0 (9.0, 13.0)	<.001
LV end-diastolic diameter (mm) (median, IQR)	43.0 (38.0, 46.5)	47.0 (43.0, 52.0)	<.001
LV mass (g/m^2) (median, IQR)	154.5 (129.0, 195.0)	153.0 (116.0, 194.0)	.422
LV ejection fraction (%) (median, IQR)	67.0 (61.0, 74.0)	65.0 (58.0, 71.0)	.139
Septal Sa (cm/s) (median, IQR)	5.9 (5.0, 6.0)	7.0 (5.0, 9.0)	<.001
Lateral Sa (cm/s) (median, IQR)	6.0 (6.0, 7.0)	8.0 (6.0, 10.0)	<.001
LV Diastolic dysfunction (%)	32 (86.5%)	586 (84.8%)	.781
Grade I (%)	17 (53.1%)	304 (52.3%)	
Grade II (%)	15 (46.9%)	252 (43.4%)	.480
Grade III (%)	0 (0.0%)	25 (4.3%)	
Undetermined grade (%)	0 (0.0%)	5 (0.7%)	
Septal Ea (cm/s) (median, IQR)	5.0 (4.0, 6.0)	5.4 (4.0, 7.0)	.098
Lateral Ea (cm/s) (median, IQR)	6.0 (5.0, 9.0)	7.0 (5.6, 9.0)	.328
LA volume (mL/m2) (median, IQR)	33.0 (28.0, 39.0)	35.4 (27.0, 48.0)	.185
Cardiac MRI (n = 28 vs 442)			
Late gadolinium enhancement (%)	23 (85.2%)	244 (55.8%)	.003
Electrocardiogram (n = 37 vs 732)			
Sinus rhythm (%)	36 (97.3%)	609 (83.2%)	.023
Atrial fibrillation (%)	1 (2.7%)	89 (12.2%)	.081
Atrial flutter (%)	0 (0.0%)	11 (1.5%)	.453
Pacemaker rhythm (%)	2 (5.4%)	41 (5.6%)	.960
Short PR interval (%)	2 (7.1%)	13 (1.9%)	.059
Atrioventricular block (%)	4 (11.4%)	67 (9.3%)	.670
Left bundle branch block (%)	2 (5.7%)	54 (7.5%)	.697
Right bundle branch block (%)	4 (11.4%)	48 (6.7%)	.276
Bifascicular block (%)	13 (37.1%)	25 (3.5%)	<.001
T wave inversion (%)	22 (62.9%)	348 (48.7%)	.103
ST segment depression (%)	19 (54.3%)	197 (27.6%)	.001
Sokolow-Lyon Index (mm) (median, IQR)	30.0 (18.0, 51.0)	32.0 (25.0, 41.0)	.933
24 h Holter (n = 36 vs 638)			
Sinus rhythm (%)	35 (97.2%)	539 (84.4%)	.035
Atrial fibrillation (%)	3 (8.3%)	88 (13.8%)	.353
Atrial flutter (%)	1 (2.8%)	8 (1.3%)	.437
Pacemaker rhythm (%)	3 (8.3%)	32 (5.0%)	.381
Atrioventricular block (%)	4 (11.4%)	51 (8.0%)	.476
Left bundle branch block (%)	2 (5.7%)	35 (5.5%)	.959
Right bundle branch block (%)	5 (14.3%)	29 (4.6%)	.011
Bifascicular block (%)	13 (37.1%)	14 (2.2%)	<.001
Supraventricular tachycardia (%)	18 (50.0%)	157 (24.6%)	.001
Ventricular tachycardia (%)	6 (16.7%)	108 (17.0%)	.964
Devices			

Table II (*continued*)

Baseline characteristics	Fabry patients (n = 37)	Non-Fabry patients (n = 733)	*P*
Pacemaker (%)	5 (13.5%)	54 (7.4%)	.171
Implantable cardioverter-defibrillator (%)	0 (0%)	69 (9.4%)	.050
Diagnostic red-flags for Fabry disease			
Stroke (%)	4 (10.8%)	66 (9.1%)	.730
Renal failure (%)	4 (10.8%)	89 (12.7%)	.739
Microalbuminuria >30 mg/24 h (%) (n = 34 vs 295)	19 (55.9%)	90 (30.9%)	.004
Proteinuria >300 mg/24 h (%) (n = 32 vs 288)	9 (28.1%)	39 (13.7%)	.032
Other comorbidities			
Hypertension (%)	22 (59.5%)	500 (68.2%)	.266
Diabetes mellitus (%)	7 (18.9%)	158 (21.6%)	.694
Dyslipidemia (%)	24 (64.9%)	386 (52.9%)	.154
Obesity (%)	4 (10.8%)	166 (22.6%)	.090
Smoking (%)	5 (13.5%)	89 (12.2%)	.809
Coronary heart disease (%)	5 (13.5%)	62 (8.5%)	.290

pathogenicity of these *GLA* variants (Table I). In fact, this p.V22A/p.A73S female had normal enzymatic activity of alpha-galactosidase A in plasma (9 nmol/h/mL) and mildly reduced in leukocytes (31 nmol/h/mg). She did not have children. Her mother also presented HCM as well as the *MYBPC3* mutation, but her *GLA* status is unclear, as the HCM genetic testing did not include the *GLA* gene. Her brother carried only one of these variants (p.A73S), but had no clinical manifestations and presented normal enzymatic activity of alpha-galactosidase A in plasma (10 nmol/h/mL) and leukocytes (37 nmol/h/mg). He also has no children.

Predictors of FD in patients with HCM

Compared to non-Fabry patients, Fabry patients reported more commonly family history of LVH and complained more often of dyspnea and pre-syncope. LVH was mainly symmetrical, with higher posterior wall thickness and lower LV end-diastolic diameter. LVOT obstruction at rest was less common. Septal and lateral Sa were lower. LGE was more common, particularly in the basal and mid inferolateral segments (63.6% vs 9.6%, $P < .001$; 54.5% vs 8.1%, $P < .001$), basal and mid inferior segments (40.9% vs 9.6%, $P < .001$; 40.9% vs 13.5%, $P < .001$) and apical lateral, anterior and septal segments (31.8% vs 13.5%, $P = .017$; 36.4% vs 18.4%, $P = .037$; 36.4% vs 19.1% $P = .049$). Fabry patients also presented more commonly bifascicular block and ST-segment depression on ECG and microalbuminuria and proteinuria (Table II). Of note, nine of the 37 Fabry patients did not perform cardiac MRI, for the following reasons: nonconditional pacemaker (n = 2), severe renal failure (n = 2), nonconditional pacemaker and severe renal failure (n = 1), other medical device/foreign body incompatible with MRI (n = 3) and patient refusal (n = 1).

On binary logistic multivariate regression analysis, we included the cardiac categorical variables that were readily available from ECG, echocardiogram and cardiac MRI and showed statistically significant differences between FD and non-FD patients (Table II): bifascicular block, ST-segment depression, symmetrical pattern of LVH, LV outflow tract obstruction at rest and basal inferolateral LGE. The following variables were identified as predictors of FD: symmetric LVH (OR 3.464, CI 95% 1.151-10.430, $P = .027$), basal inferolateral LGE (OR 10.677, CI 95% 3.633-31.380, $P < .001$), bifascicular block (OR 10.909, CI 95% 2.377-50.059, $P = .002$) and ST-segment depression on ECG (OR 4.401, CI 95% 1.431-13.533, $P = .032$). The most powerful predictors were bifascicular block and basal inferolateral LGE.

Based on the identified predictors of FD, we created the score ID FABRY-HCM to help to identify FD cases among patients with HCM:

ID FABRY-HCM = *−0.729 + (2.781 x Bifascicular block on ECG) + (0.590 x ST depression on ECG) + (0.831 x Symmetric LVH) + (2.130 x basal inferolateral LGE).*

If these variables are present, its respective value in the score formula will be 1; if they are absent, its value will be 0. In the ID FABRY-HCM score, the cut-off value of 1.0 has a sensitivity of 63.4%, specificity of 97.2%, positive predictive value of 68.3% and a negative predictive value of 95.8%. From the analysis of this score, we can conclude that neither the presence of symmetrical HCM or ST-segment depression alone, or the combination of the two, is enough to obtain a score in favor of FD. However, the presence of either bifascicular block or basal inferolateral LGE alone achieves a favorable score for FD. More importantly, in the absence of both bifascicular block and basal inferolateral LGE, the negative predictive value for FD is 95.8%.

Discussion

This study found a FD prevalence of 0.9% in patients with HCM and identified bifascicular block, basal inferolateral LGE, symmetrical HCM and ST-segment depression as independent predictors of FD among patients with HCM. Moreover, this study provides a score to identify FD cases in patients with HCM. According to this score, HCM in the absence of both bifascicular block and basal inferolateral LGE has a negative predictive value of 95.8% for FD.

Prevalence of FD in HCM

Compared to previous studies on the prevalence of FD in HCM,[2-18] our study presents several strengths: (i) large sample size; (ii) inclusion of males and females; (iii) well-defined diagnostic criteria of HCM; (iv) combined enzymatic and genetic approach as a screening method; (v) solid evidence of the pathogenicity of *GLA* variants that were included for prevalence calculation.

To the best of our knowledge, this is the second largest study on the prevalence of FD in HCM.[2-18] In previous studies, inclusion criteria widely ranged (i) from extremely restrictive (patients submitted to myectomy[4] or endomyocardial biopsy[5]); (ii) to extremely broad (LVH ≥12 mm,[16] ≥13 mm,[3,6,12,13] >13 mm[7] or ≥13 mm without exclusion of hypertension and valve disease[2,14]); and (iii) from more elaborated, combining LV thickness with several factors (no obstruction,[15] negative genetic test for sarcomeric HCM[6,9,18] or exclusion of family history of sudden death in >2 relatives, inheritance pattern inconsistent with X-linked pattern and histology compatible with sarcomeric HCM[18]); (iv) to more straightforward (ESC definition of HCM[8,10,11,17]). Like in these last studies, our study inclusion criteria were based on the ESC definition of HCM and, therefore, as described in the literature, LVH was more commonly asymmetrical with preferential septal involvement and LGE was more commonly found in the septum. LV diastolic dysfunction with preserved ejection fraction was found in most patients. Obstruction at rest (23.8%), atrial fibrillation (11.7%) and VT (15.0%) were, however, less frequent than reported in the literature (33.3%, 22.5% and 25.0%, respectively).[26]

Some of the previous studies included only males and used enzymatic assay as screening method.[2,3,12,13,17] One study included only females and used electron microscopy of endomyocardial biopsies as a screening method.[5] Other studies included both genders and used urinary GB3,[16] electron microscopy of myectomy specimens,[4] the enzymatic assay alone,[10] the genetic testing[6,7,9,11] or a combined enzymatic and genetic approach.[8,14,15,18] Similarly to these last studies, we included males and females and used a combined enzymatic and genetic approach that allows FD diagnosis in both genders.[31]

GVUS are commonly found on FD screenings.[19] In our study, most of the reported *GLA* variants were already described as pathogenic mutations. The mutation c.337 T > C (p.F113L) leads to enzyme misfolding and consequent degradation in the endoplasmic reticulum, causing a late-onset phenotype characterized by predominant cardiac involvement.[30] The mutation c.281G > C (p.C94S) affects an enzyme disulfide bond and causes a severe classical phenotype.[32] The mutation c.548G > T (p.G183V) occurs in a highly conserved residue, buried in the enzyme 3D structure of the catalytic domain, and is predicted to cause enzyme instability and a classical phenotype.[33] The mutation c.607G > T (p.E203X) affects the enzyme active site and causes a classical phenotype of FD.[34] The mutation c.870G > A (p.M290I) leads to enzyme destabilization and misfolding and was associated to a classical phenotype.[33,35] Our patient presents a similar missense mutation (c.870G > C), described as pathogenic in ClinVar database,[36] resulting in the same protein abnormality (p.M290I). The variant c.1067G > A (p.R356Q) is most likely benign, but the mutation c.1078G > C (p.G360R) occurs in the enzyme dimer interface, causing a classical phenotype of FD.[37]

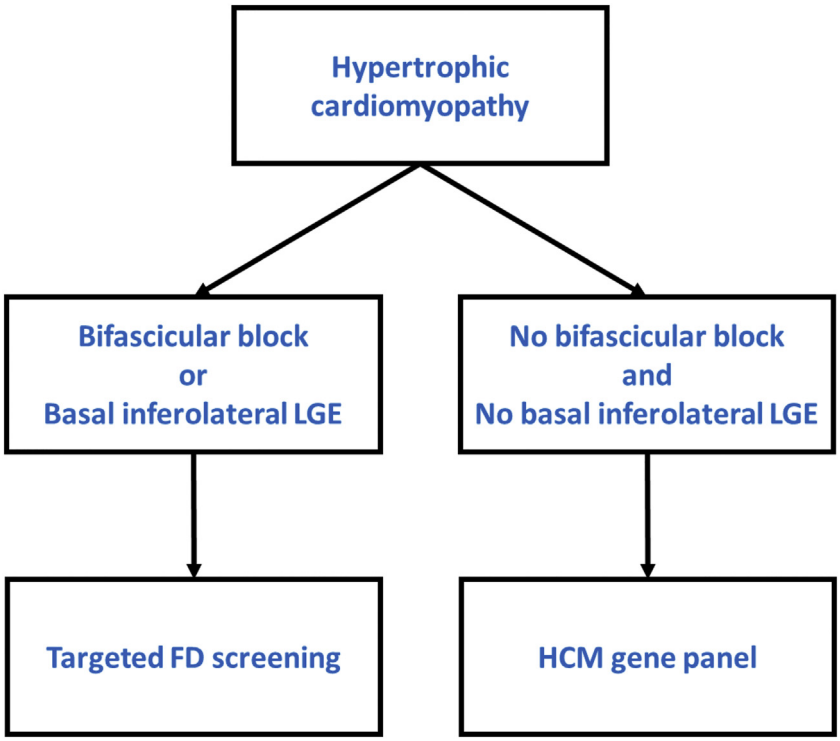
To the best of our knowledge, the *GLA* variants p.V22A and p.A73S were never reported before. According to in-silico models Polyphen-2 and MutationTaster, p.V22A is predicted to be a polymorphism, while p.A73S is predicted to be a disease causing variant.[38,39] The variant p.A73E, which affects the same amino acid as p.A73S, was already reported in a 58-year old female with LVH, end-stage renal disease and stroke and reduced enzymatic activity of α-galactosidase A.[40] Furthermore, p.A73V, another variant at the same codon, was associated to reduced enzymatic activity of α-galactosidase A in a male newborn.[41] However, the documentation of the p.A73S variant in her brother with normal enzymatic activity of alpha-galactosidase A on plasma and leukocytes, together with the finding of a pathogenic mutation on the *MYBPC3* gene, also present in her mother with HCM, made us classify this HCM as sarcomeric and this *GLA* variant as non-disease causing.

To our knowledge, the variant c.286A > G (p.M96V) has also never been reported, but indisputable evidence of its pathogenic role was based on the severe classical phenotype of FD exhibited by the patient and her son, both with increased urinary GB3 and plasma lyso-GB3, together with the demonstration of lysosomal inclusions on skin biopsy.

Patients with the genetic variants p.A143T and p.R118C were not classified as Fabry patients, as the pathogenic role of these variants has recently been questioned.[42,43]

Terryn et al reported p.A143T cases with clinical manifestations that could be attributable to FD, but without GB3 deposits in organ biopsies and in which biopsies revealed alternative diagnoses.[42] Recently, Valtola et al reported elevated plasma lysoGB3 levels

Figure 3



Proposed diagnostic strategy to the identification of Fabry cases during the etiological study of HCM.

and GB3 deposits in p.A143T males with LVH, who had a relatively high residual enzymatic activity (25%-40%) and a negative test for an HCM panel with 59 genes, suggesting that p.A143T is very likely a late-onset FD-causing variant.[44] Nevertheless, our p.A143T patient presented severe HCM at the age of 28 years old, which is too young for an HCM due to late-onset FD, even for a male, which means that even if we considered p.A143T variant as pathogenic, this variant alone could not explain HCM at this age neither its severity. Moreover, he had a documented pathogenic mutation in the *MYBPC3* gene that could fully explain the HCM phenotype. Therefore, we did not classify this HCM case as caused by FD, although we cannot exclude a contributing role of this *GLA* variant to the progression of HCM with advancing age.

Besides the relatively high residual enzymatic activity in leukocytes in p.R118C males (32%-45%), Ferreira et al reported the case of a 56-year old male with this *GLA* variant, who presented mild LVH and myelin figures within rare cardiomyocytes, but had normal levels of plasma lyso-Gb3 and urinary GB3 measured at the age of 64 years and no significant progression of the cardiac disease from the age of 54 to 64 years.[43] In our 73-year-old male with asymmetrical obstructive HCM with LGE in the septum, it is reasonable to hypothesize that it could be a sarcomeric HCM, given the pattern of LGE and LVH which is more typical of a sarcomeric cause than FD, the presence of obstruction which is rare in FD and the fact that the genetic testing, besides revealing a GVUS in the *MYBPC3* gene, included only 11 HCM genes. In our 69-year old female with apical HCM, although genetic testing was not performed, a sarcomeric cause is also possible, as apical HCM is rare in FD. In the 75-year old female with moderate symmetrical non-obstructive HCM with no LGE, besides the GVUS on the *MYH7* gene, we should also consider the possibility of isolated basal septal hypertrophy of the elderly. Although we cannot exclude that the p.R118C variant may be associated to a very attenuated and late-onset FD, the unclear role of this *GLA* variant and the possibility of alternative diagnoses that could explain the HCM phenotype in our p.R118C patients made us exclude these patients from the cohort of patients classified as FD patients.

The variant p.D313Y is associated with an enzymatic pseudodeficiency,[45] as reflected by the normal plasma lyso-Gb3 values both in our female and male patients, and therefore not included in FD prevalence calculation. HCM genetic testing would be appropriate to assess a sarcomeric cause in the 36-year old male with asymmetrical non-obstructive HCM and antero-septal LGE, and in the 70-year old female with asymmetrical non-obstructive HCM and no LGE, despite the absence of a family history

of HCM in both of these p.D313Y cases. HCM genetic testing would also be appropriate to assess a sarcomeric or PRKAG2 cause in the 70-year old female with this *GLA* variant and symmetrical obstructive HCM, short PR interval, left bundle branch block, family history of HCM and no history of hypertension. It should be considered the possibility of isolated basal septal hypertrophy of the elderly in the 76-year old female with the p.D313Y variant and moderate symmetrical non-obstructive LVH, no LGE and no family history of HCM.

Finally, the variant p.D175E, predicted by Polyphen-2 as benign, was already described in a female with normal lyso-GB3 and in-vitro near-normal enzymatic activity.[33] In our patient, sarcomeric HCM and isolated basal septal hypertrophy cannot be fully excluded as causes of LVH, and proteinuria, renal failure and stroke could also be explained by the cardiovascular risk factors. Therefore, we considered p.D175E as a GVUS, probably benign.

Based on these considerations and the founder effect of FD due to the p.F113L mutation in the region of Guimarães,[29,30] FD prevalence in HCM was set at 0.9%. If we considered p.A143T and p.R118C variants to be associated with attenuated and late-onset FD, FD prevalence in HCM would be 1.4% (11/780).

Predictors of FD in patients with HCM

FD is a treatable condition and cardiologists' awareness is paramount to achieve an appropriate and timely diagnosis and treatment.

In this study, the most powerful predictors of FD among HCM patients were bifascicular block and basal inferolateral LGE. The LVH pattern is readily known as soon as the diagnosis of HCM is established and, therefore, is one of the first characteristics that will guide the etiological study of HCM. Although HCM is typically symmetrical in FD,[24] the common occurrence of fibrosis and thinning of the posterior wall with loss of the symmetrical LVH pattern at the HCM stage (≥15 mm)[24,46] make, together with the existence of this LVH pattern in other phenocopies (amyloidosis, mitochondrial disorders, Danon disease),[24] symmetrical HCM a weak predictor of FD, as was demonstrated in this study. Conversely, although basal inferolateral LGE may appear in other conditions[47,48] and LGE may involve other segments with FD progression,[49] the basal inferolateral LGE revealed to be one of the most powerful predictors of FD. Niemann et al also found that when ST segment or T wave alterations are absent, replacement fibrosis is very unlikely in FD, which might explain the finding of ST depression as a predictor of FD in HCM.[50] Cardiac conduction disorders are known cardiac manifestations of FD.[1] However, to our knowledge, this is the first study demonstrating that bifascicular block is the most powerful predictor of FD in patients with HCM.

Although extracardiac manifestations, such as acroparesthesias, angiokeratomas and cornea *verticillata*, are red-flags to FD diagnosis,[24] they are not commonly searched by cardiologists during the diagnostic study of HCM. Besides, they are frequently absent in heterozygous females and late-onset phenotypes.[1] Renal or cerebrovascular disease may also be absent in heterozygous females and in some late-onset phenotypes with a predominant organ involvement,[1] which limits their utility in the etiological study of HCM. Conversely, a score to guide the suspicion of FD based on LVH characteristics that are readily available on ECG, echocardiogram and cardiac MRI appeared to be useful in the clinical practice. However, from the analysis of the score, it became clear that its application was not needed in the clinical practice and that the algorithm to define the most appropriate diagnostic strategy in the etiological study of HCM could be simplified as illustrated in Figure 3. In simple words, in the presence of either bifascicular block or basal inferolateral LGE, targeted FD screening is the most appropriate next step in the etiological study of HCM; and in the absence of both bifascicular block and basal inferolateral LGE, FD is a less probable cause of HCM and a wide HCM gene panel is the most adequate strategy to its etiological study. The positive predictive value of the score (68.3%) is explained by the low prevalence of FD. However, given the cost difference between a targeted FD screening and a wider HCM gene panel, especially in males, it seems reasonable to proceed first with a targeted FD screening in patients with positive scores, i.e., with bifascicular block and/or basal inferolateral LGE, despite the modest positive predictive value. The score sensitivity is 63.4%, which might be explained by the fact that bifascicular block already represent an advanced stage of the disease that may be absent in the milder cases of FD at the HCM stage (≥15 mm). Although less probable, the basal inferolateral LGE may also be absent in these milder cases of Fabry HCM. Moreover, the high negative predictive value for FD (95.8%) that is achieved in patients with HCM in the absence of both bifascicular block and basal inferolateral LGE is known to be less meaningful in the field of rare diseases, as even a low number of false negatives may represent a non-negligible percentage of missed diagnoses in a universe of rare cases.

However, although the score will miss milder Fabry HCM cases and these may represent a non-negligible percentage of FD cases, it is important to emphasize that this algorithm does not intend to limit FD screening in patients with negative scores, i.e. without bifascicular block and basal inferolateral LGE, rather it intends to guide physicians on the most probable cause of HCM and therefore the most appropriate next step in the etiological study of HCM. Given the potential clinical impact of specific FD therapy, it is advisable to systematically screen all patients with unexplained LVH for FD, either by targeted screening or within a wider genetic approach. However, targeted FD screening has a lower cost than a wider gene panel of HCM. In the presence of bifascicular block or basal inferolateral LGE, this

score favors targeted FD screening. In patients with HCM in the absence of both bifascicular block and basal inferolateral LGE, it favors a wider panel of HCM genes that currently also include the *GLA* gene, despite the lower probability of FD in this case (Figure 3). Our study presents some limitations that should be acquainted. The diagnosis of FD was considered according to the current knowledge on genetics that is constantly evolving and may reveal in the future different pathophysiological roles for the *GLA* gene variants that were found in this study. The majority of the Fabry cases presented the same *GLA* gene mutation. This score only applies to adult patients with the ESC definition of HCM and therefore is not applicable to pediatric patients or patients with milder LVH (wall thickness <15 mm), in whom the early diagnosis of FD could potentially carry a higher prognostic impact. Nevertheless, it should be emphasized that the identification of Fabry cases at the HCM stage could enable the identification of several relatives[51] at earlier stages of the disease. Finally, future studies are needed to obtain an external validation of this score in other large cohorts of HCM and in patients with other *GLA* gene mutations.

In summary, this study, based on the ESC definition of HCM, using a combined enzymatic and genetic approach and taking into account the current knowledge on genetics of FD and the existence of a founder effect in a particular Portuguese region, sets FD prevalence among patients with HCM in 0.9%. This study also showed that bifascicular block and basal inferolateral LGE are the most powerful predictors of FD in patients with HCM. In the absence of both bifascicular block and basal inferolateral LGE, FD is a less probable cause of HCM, according to the ID FABRY-HCM score, being more appropriate to perform an HCM gene panel as the next step of the etiological study of HCM.

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