Journal of the American College of Cardiology
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Published by Elsevier Inc.

Vol. 50, No. 25, 2007
ISSN 0735-1097/07/$32.00
doi:10.1016/j.jacc.2007.06.062

Prevalence of Fabry Disease in a Cohort of 508 Unrelated Patients With Hypertrophic Cardiomyopathy

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Objectives We aimed to study the prevalence of Fabry disease (FD) in patients with hypertrophic cardiomyopathy (HCM).

Background There are limited and controversial data about the prevalence of FD in patients with HCM.

Methods We screened the plasma α-galactosidase A activity from 508 unrelated patients with HCM (328 men, 180 women, ages 58 $\pm$ 16 years). Patients with low activity (0% to 30% of the normal control in men, and 0% to 50% in women) underwent genetic study of the *GLA* gene.

Results We found low plasma activity in 15 patients (3%). Three men had *GLA* mutations (0.9%): S238N (novel) in 2 and E358del (described) in 1. Two women had described mutations (1.1%): L89P and A143T. Three unrelated men had the D313Y variant previously associated with enzyme pseudo-deficiency. Two women had polymorphisms that did not segregate with the disease in their families. Five women (activitiy 39% to 47%) had no sequence variants. The familial studies allowed the diagnosis of 14 carriers: 6 women without Fabry manifestations, 3 women with cardiomyopathy, 2 men with renal and cardiac disease, 1 man with microhematuria, 1 woman with first-degree atrioventricular block, and a 32-year-old woman with only renal disease.

Conclusions By means of a screening based on genotyping of patients with low plasma enzymatic activity, the prevalence of FD in our population of HCM is 1% (0.9% in men and 1.1% in women). This diagnosis is relevant, because it allows the identification of disease carriers that might benefit from enzyme replacement therapy. (J Am Coll Cardiol 2007;50:2399–403) © 2007 by the American College of Cardiology Foundation

More than 400 different mutations in genes encoding sarcomeric proteins have been associated with hypertrophic cardiomyopathy (HCM) (1,2); however, even after a systematic screening, mutations in these genes are not found in 30% to 40% of the patients (1). Recently, several studies have identified Fabry disease (FD) as a relatively frequent cause of idiopathic left ventricular hypertrophy (3–5). Fabry disease is an X-linked lysosomal α-galactosidase A deficiency that causes multisystemic problems, including renal, neurological, ocular, skin, and cardiac manifestations. There is a classical form of the disease characterized by early manifestation of the multisystemic disorder. But there are also atypical forms with late-onset isolated renal or cardiac

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manifestations, usually in men with some residual plasma enzymatic activity or in female carriers (3–6). Cardiac involvement in FD is related to the accumulation of glycosphingolipids in the myocardium, valvular, and conduction tissues, leading to increase in wall thickness, mitral valve thickening, and conduction system disease (7). Although patients with apparently isolated cardiac involvement might be diagnosed with HCM, there are very few studies of the prevalence of FD

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Manuscript received February 12, 2007; revised manuscript received May 25, 2007, accepted June 5, 2007.

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December 18/25, 2007:2399–403

Abbreviations and Acronyms

FD = Fabry disease

HCM = hypertrophic cardiomyopathy

in cohorts of patients with HCM, with controversial results (3–6).

The objective of this study is to evaluate the prevalence of FD in a wide nonselected population of patients previously diagnosed with HCM.

Methods

We studied 508 consecutive unrelated patients from 3 regional centers (Coruña, in northern Spain; Murcia and Alicante, in southern Spain) with HCM diagnosed according to the criteria of the World Health Organization and of the European Society of Cardiology Working Group on Myocardial and Pericardial Diseases (1,8,9). For each patient, a complete pedigree was drawn, and all of the first-degree relatives were invited to be screened. All patients gave written informed consent for the clinical, molecular biology, and genetic studies, and the institutional ethics committees approved the protocol.

Plasma and genomic deoxyribonucleic acid (DNA) extracted from lymphocytes were stored at −80°C for subsequent analysis. The α-galactosidase A was assayed fluorometrically as described by Beutler and Kuhl (10) with the modifications of Mayes et al. (11). The specific activity was expressed as percentage of the mean values of normal control subjects matched by age and gender. Patients with low activity (0% to 30% of the normal control subjects in men and 0% to 50% in women) were submitted to genetic study of the *GLA* gene (direct sequencing of exons 1 to 7). The DNA mutations are described according to *GLA* complementary DNA sequence (12), with the A of the ATG initiation codon being +1. The diagnosis of FD was done in those patients with low enzymatic activity who also potentially had disease-causing mutations in the *GLA* gene (6).

Table 1 Clinical Characteristics and Sudden Death Risk Factors of the Index Patients

	Coruña	Murcia	Alicante	Total
Maximum left ventricular wall thickness (mm)	20 ± 6	20 ± 4	21 ± 5	20 ± 5
Subaortic obstruction (%)	25	32	56	36
Nonsustained ventricular tachycardia (%)	25	30	26	26
Previous syncope (%)	13	37	21	17
Abnormal exercise blood pressure response (%)	34	23	46	37
Maximum left ventricular wall thickness ≥30 mm (%)	7	3	8	6
Family history of premature sudden death (%)	10	18	31	19

Table 2 Summary of the Enzymatic and Genetic Screening of the Index Patients

	Coruña	Murcia	Alicante	Total
Patients (n)	200	148	160	508
Male gender (%)	64	76	55	65
Age (yrs)	58 ± 15	55 ± 16	59 ± 18	58 ± 16
Age ≥40 yrs (%)	87	83	82	84
Plasma enzymatic activity (% of control)	83 ± 26	112 ± 47	104 ± 29	99 ± 37
Men with enzymatic activity ≤30, n (%)	1 (0.7)	3 (2.4)	2 (2.3)	6 (1.8)
Women with enzymatic activity ≤50, n (%)	9 (12.5)	0	0	9 (5)
Patients with low enzymatic activity, n (%)	10 (5)	3 (2)	2 (1.3)	15 (3)
Patients with *GLA* polymorphisms, n (%)	3 (1.5)	2 (1.4)	0	5 (1)
Patients with *GLA* mutations, n (%)	2 (1)	1 (0.7)	2 (1.3)	5 (1)

Table 3 Genotype and Phenotype in the Index Patients With Low Enzymatic Activity

	Patient #														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Center	C	C	M	A	A	M	M	C	C	C	C	C	C	C	C
Age (yrs)	70	74	50	48	46	81	54	55	24	60	76	59	50	74	58
Gender	F	F	M	M	M	M	M	M	F	F	F	F	F	F	F
Enzymatic activity (% of control)	19	25	12	20	9	22	22	23	42	44	39	43	44	46	47
GLA mutation	L89P	A143T	E358del	S238N	S238N	–	–	–	–	–	–	–	–	–	–
GLA polymorphism	–	–	–	–	–	+	+	+	+	+	+	–	–	–	–
Maximum left ventricular wall thickness (mm)	14	21	20	17	20	20	25	15	42	21	21	15	17	25	33
Renal disease	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–
Conduction defect	+	–	–	+	+	+	–	–	–	+	+	–	–	–	–
Cardiac disease in the family	+	+	+	+	+	–	+	–	+	–	–	+	+	–	–
Renal disease in the family	+	+	–	+	+	–	–	–	–	–	–	–	–	–	–

n = 15.
A = Alicante; C = Coruña; M = Murcia.

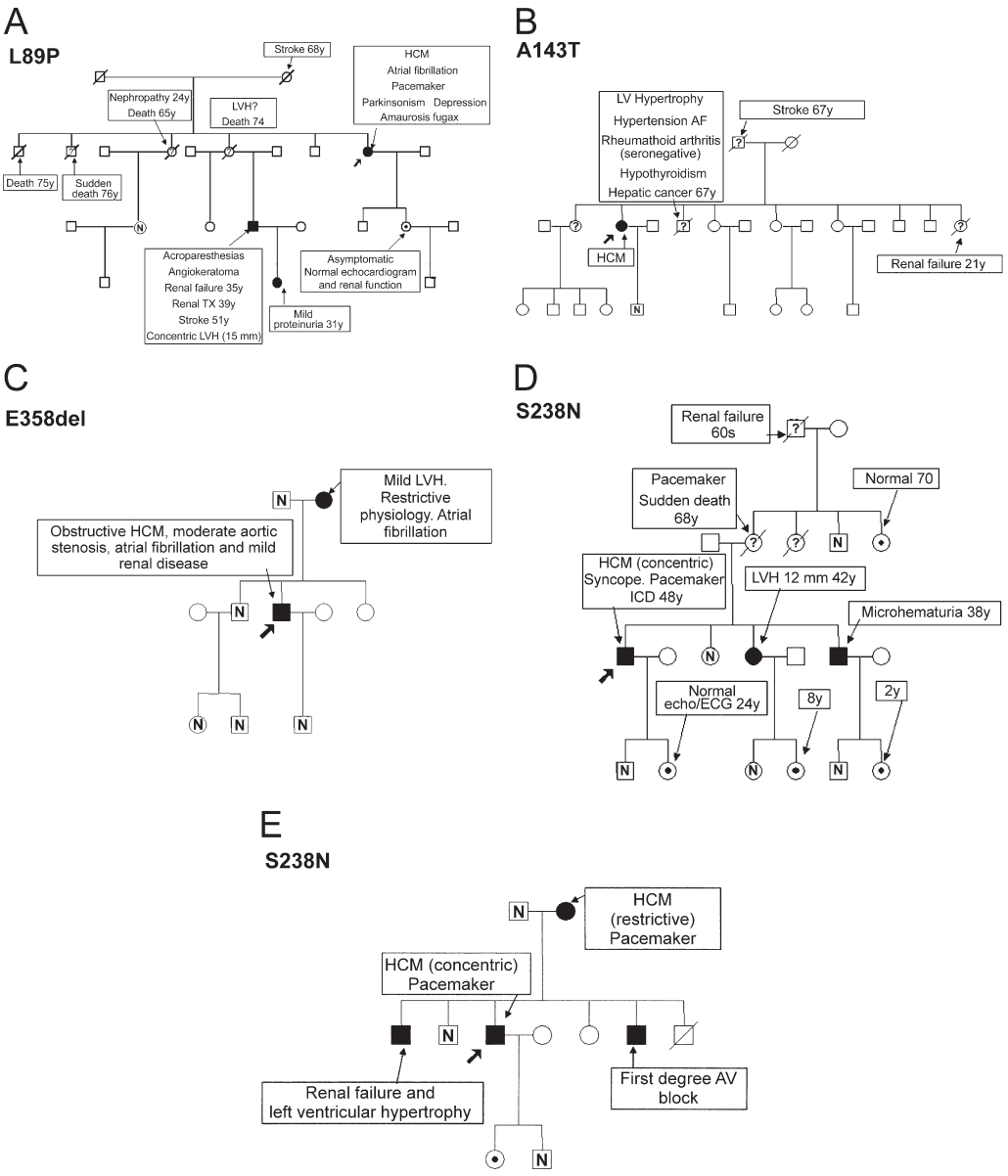

Figure 1 **Pedigrees of the Families With Fabry Disease**

Circles are women and **squares** are men. **Filled symbols** are affected patients (all with mutation). Symbols with an "**N**" indicate unaffected relatives without mutation. **Dotted symbols** are patients with mutation without apparent clinical manifestations. **Dashed symbols** are deceased patients. **Question marks** indicate unknown or not confirmed phenotype but possibly affected. **Numbers** are ages. Index patients are indicated by the **thick arrows**. AF = atrial fibrillation; AV = atrioventricular; ECG = electrocardiogram; echo = echocardiogram; HCM = hypertrophic cardiomyopathy; ICD = implantable cardioverter-defibrillator; LV = left ventricular; LVH = left ventricular hypertrophy; TX = transplant.

Results

We studied 328 men and 180 women. Tables 1 and 2 summarize their clinical characteristics and the results of the enzymatic screening. We found low plasma activity in 15 patients (3%): 6 men (1.8%) and 9 women (5%). The clinical characteristics and the results of the genetic study of these 15 patients are shown in Table 3. We found 3 previously described mutations (L89P, A143T, and E358del) and 1 novel mutation: S238N (in 2 patients). Three men had the D313Y variant that has been previously associated with enzymatic pseudo-deficiency. Two women had polymorphisms that did not segregate with the disease in their families. In 5 women with low plasma enzymatic activity, no sequence variant was found in the *GLA* gene. The prevalence of FD was 1% in the whole population

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(0.9% in men and 1.1% in women): 1.0% in Coruña, 0.7% in Murcia, and 1.3% in Alicante. Figure 1 summarizes the results of the familial studies.

Discussion

Our study shows a 1% prevalence of FD in unselected patients with HCM. Previous studies with lower number of patients had found higher prevalences (3–5). Nakao et al. (3) identified 7 patients with FD among a cohort of 230 men (3%) with left ventricular hypertrophy. Sachdev et al. (4) found 6 patients with FD among 153 men with HCM (4%), with a prevalence of 6% in men diagnosed at older than 40 years of age. Of note, 3 of the 6 patients carried the same mutation, suggesting a potential founder effect. In contrast, in 100 HCM patients that underwent septal ablation, Ommen et al. (13) could not find any FD. The reason for this negative finding could be that the hypertrophy secondary to FD is usually concentric and not associated with the generation of dynamic subaortic obstruction (3–5,7,13). Chimenti et al. (5) identified 4 patients with FD in a group of 34 women (12%) with HCM who underwent endomyocardial biopsy. We can speculate about a potential selection bias in this study, because endomyocardial biopsy is not a routine test in patients with HCM, but it is usually done in patients with restrictive physiology or with a clinical suspicion of a myocardial infiltrative disease. We think that the prevalence of FD found in our cohort is probably more representative of the true prevalence of the disease in HCM. The prevalence of HCM in the adult general population is 1 in 500 (1). So, with a prevalence of FD of 1 in 100 in patients with HCM, the prevalence of FD with left ventricular hypertrophy would be 1 in 50,000 in the general population (1:500 × 1:100), which is near to the current estimation of FD prevalence (6,14). However, a recent study suggests that milder forms of FD with late or incomplete penetrance and probably without overt cardiac manifestations might be more frequent (15).

We have found a similar prevalence of FD in men and women with a screening based in the measurement of the α-galactosidase A enzymatic activity in plasma. This method is useful for the diagnosis of male patients, who usually present with very low enzymatic activity (6); but in female carriers, plasma enzymatic activity might be within the normal range, and the screening could have a significant number of false negative results (5,6). To minimize this possibility, we have sequenced the *GLA* gene in 7 women with a borderline activity (30% to 50% of the control) that represents 4% of our female cohort, and we found several polymorphisms but no pathogenic mutation. With these results, we consider that a screening strategy based on the plasma enzymatic activity measurement is cost-effective not only in men but also in women with HCM. Sequencing of the *GLA* gene would be limited to the men and women with low activity and to women that even with a normal or borderline activity had other clinical or familial characteristics that could suggest the diagnosis of FD (renal disease, early cerebrovascular disease, angiokeratoma, corneal opacities, heat intolerance, hypoacusia, and so forth).

Genotype–phenotype correlations and clinical implications of the diagnosis. We have found 3 previously described mutations that had been associated with classical (L89P, E358del) or late-onset (A143T) forms of the disease (15–19) and show a quite variable clinical presentation in different members of our families. This clinical heterogeneity could be explained in part in women by the effect of the random inactivation of the wild-type or the mutant X chromosomes in different organs (lyonization) (20,21). However, the variability of the clinical presentation is also remarkable in men. We have also found 1 novel mutation (S238N). The S238N is a missense mutation in exon 5 of the *GLA* gene that shows incomplete penetrance in young carriers (even in men), suggesting that it might be related to a late-onset form of the disease. The S238 amino acid is close to the active D231, and the change from Ser to Asn at 238 can adversely affect the folded state of the molecule. Mutations in the neighbor W236 and S235 have also been associated with FD (22–24).

One of the most relevant results of our study is that the diagnosis of FD in 5 index patients has allowed the identification of 14 additional carriers. Fabry disease had not been previously diagnosed in any of the families. Some carriers are now receiving enzyme replacement therapy that might improve the natural course of their disease. A careful follow-up is indicated in all the mutant carriers.

Conclusions

With a screening based on genotyping of patients with low plasma enzymatic activity, the prevalence of FD in our population with HCM was 1% (0.9% in men and 1.1% in women). This diagnosis is relevant because it allows the identification of disease carriers that might need enzyme replacement therapy.

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