Prevalence of Fabry Disease in Female Patients With Late-Onset Hypertrophic Cardiomyopathy

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Background—Fabry disease (FD) has been recognized as the cause of left ventricular hypertrophy in 6% of men with late-onset hypertrophic cardiomyopathy (HCM). Although FD is considered a recessive X-linked disorder, affected women are increasingly reported. The aim of our study was to determine the prevalence of FD in female patients with HCM.

Methods and Results—Thirty-four consecutive women (mean age, 50±13.6 years) who received an ECG and echocardiographic diagnosis of HCM were submitted to an invasive cardiac study that included a biventricular endomyocardial biopsy. Tissue samples were analyzed for histology and electron microscopy. Peripheral blood activity of α-galactosidase (α-Gal) A was assessed in all patients. None of them had a family history of FD. Histology and electron microscopy showed in 4 patients (12%; mean age, 51.5±3.9 years) the presence of cell vacuoles characterized by the accumulation of glycolipid material organized in concentric lamellar structures, diagnostic for FD. In the remaining patients, histology was consistent with HCM. In all the female carriers, the heart was the only organ clinically involved in the disease, showing concentric hypertrophy in 2 patients, asymmetric hypertrophy in 1, and apical hypertrophy in 1. The α-Gal A enzymatic activity was 44±14% of control values. Genetic analysis showed the presence of α-Gal A gene mutation in all 4 cases.

Conclusions—FD may account for up to 12% of females with late-onset HCM. Those heterozygous for FD with left ventricular hypertrophy are potential candidates for enzyme enhancement/replacement therapy. (Circulation. 2004;110:1047-1053.)

Key Words: biopsy ■ cardiomyopathy ■ hypertrophy

Fabry disease (FD) is an inborn lysosomal storage disorder characterized by a pathological intracellular glycosphingolipids deposition. The disease is caused by a deficit in the lysosomal enzyme α-galactosidase A (α-Gal A), the gene for which is located in the X chromosomal region Xq22.1 Cardiac involvement, consisting of progressive left ventricular hypertrophy (LVH), is very common and is the most frequent cause of death.1 The heart can be the only organ involved in the so-called “cardiac variant,” an entity that has been recognized in 3% of male patients with LVH and in up to 6% of men with late-onset hypertrophic cardiomyopathy (HCM).2,3

Although FD has been considered an X-linked recessive disorder,1 affected women are increasingly recognized, suggesting that the disease follows an X-linked–dominant rather than recessive transmission.4 Clinical manifestations in female carriers range from severe symptoms manifesting in childhood and adolescence, as in affected males, to a relative asymptomatic condition. In late adulthood, some carriers develop LVH and, more rarely, renal manifestations. In a recent study, cardiac involvement was detected in 56% of heterozygous female patients <38 years of age, in 86% of female patients >38 years of age, and in all patients >45 years of age.4

The prevalence of FD in female patients with HCM is still unknown. The differential diagnosis between the 2 entities is very important because effective enzyme enhancement/replacement therapy for FD has recently been made available.5,6

The aim of the present study was to investigate the prevalence of FD in a population of female patients with a clinical and instrumental diagnosis of HCM.

Methods

Study Population
From October 1996 to June 2003 in our institution, 475 consecutive unrelated female patients (age, 68±12 years) showed ECG and echocardiograph evidence of LVH (ventricular septum and/or posterior wall thickness ≥13 mm).2 Of these 475, 272 (57%) had systemic hypertension, 143 (30%) had aortic valvular stenosis, 26

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(5%) were affected by severe aortic valve regurgitation, and 34 (7%; mean age, 50±14 years) received a diagnosis of HCM on the basis of otherwise unexplained left ventricular (LV) wall thickening.7 Our institution is a tertiary referral center dedicated to the study of different types of cardiac muscle diseases, including HCM, in adults. The patients referred came mainly from the center and south of Italy, and ~30% of them were intrastitutional referrals.

Beginning in October 1996, all patients with a noninvasive diagnosis of HCM underwent systematically invasive studies, including endomyocardial biopsy, and the assessment of α-Gal A activity. The female patients studied before this period were not considered in the present study, although all women evaluated from that date until June 2003 were included, without any other selection criteria. In the same period time, 62 male patients were diagnosed as being affected by HCM, resulting in a total of 96 patients. The screening for α-Gal A activity allowed us to recognize 2 of the 62 men (3.3%) as being affected by the cardiac variant of FD, and that diagnosis was confirmed by histological and ultrastructural studies of the endomyocardial tissue and by gene mutation analysis.

Among the 34 female patients with HCM, 28 were diagnosed at ≥40 years of age (mean, 55±9 years; range, 40 to 67 years) and 6 at ≥40 years of age (mean, 27±6 years; range, 20 to 36 years). Five patients had a family history of HCM, whereas none had a family history of FD.

Clinical Studies
Clinical examination, resting ECG, Holter monitoring, 2D echocardiography with Doppler analysis, and cardiac MRI were performed in all 34 patients. Echocardiographic study was performed as previously described.8 Tissue Doppler analysis was also performed in the pulsed Doppler mode to record mitral annulus velocities at septal and lateral corners. Fifteen age-matched women with no evidence of LVH or cardiac or systemic disease were used as control subjects. The pattern of hypertrophy was defined as asymmetric septal (septal-to-posterior free-wall-thickness ratio of ≥1.3), concentric (septal-to-posterior free-wall-thickness ratio <1.3), or apical in the presence of a prevalent involvement of the LV apex.

All patients were submitted to invasive cardiac studies, including cardiac catheterization, coronary and biventricular angiography, and biventricular endomyocardial biopsy. The invasive cardiac exams were performed after informed consent was obtained and were approved by the ethics committee of our institution.

Endomyocardial biopsies (3 to 4 per ventricular chamber) were performed with a Bipal (Cordis) bioprome, approached by a 7F (501–613 and 501–613A) long sheath in the septal-apical region of both ventricles. Myocardial samples were processed for routine histological and histochemical analyses, including periodic acid-Schiff and Sudan black staining on frozen tissue, and for transmission electron microscopy as previously described.6

Biochemical Studies
A-Gal A activity was assessed in the white blood cells in all patients.6 Normal values were considered between 1012 to 2824 nmol/h per 1 mg protein. The α-Gal A enzymatic activity was also expressed as a percentage of the control mean value in each assay.

Genetic Analysis
Genomic DNA was isolated from peripheral blood (Puregene DNA Isolation Kit, Gentra System), and all α-Gal A coding regions and adjacent intronic regions were sequenced.6

Statistical Analysis
All values were expressed as mean±SD. Significance between 2 groups was determined by unpaired Student’s t test for continuous variables and by Fisher’s exact test for discrete variables. Tissue Doppler imaging variables were compared among the 3 groups by ANOVA with Bonferroni’s t test for pairwise comparisons. Values of P<0.05 were considered significant.

Results
In 4 of 34 patients (12%), histological examination of biventricular endomyocardial tissue showed the presence of regularly arranged and severely hypertrophied myocardial fibers with large perinuclear vacuoles containing material that, on frozen sections, stained positively with periodic acid-Schiff and Sudan black, suggesting the accumulation of glycolipids. At electron microscopy, the vacuoles appeared to be represented by concentric, lamellar figures in single membrane-bound vesicles (Figure 1B), consistent with the diagnosis of FD. Figure 2 shows a FD mimicking an apical HCM.

The histological findings of the 4 female patients were compared with endomyocardial biopsy samples from 5 hemizygous male patients affected by FD. In female carriers, 2 populations of cells, 1 without glycolipid accumulation and 1 with severe lipid deposits, were observed in all cases; the affected cells represented 65% to 90% of myocardicytes (Figure 3A). No right ventricular or LV specimen showed the absence of affected cells. The amount and localization of affected cells were evaluated in serial histological sections of
each endomyocardial sample, thus excluding the plane of 
sectioning as the cause of the different appearance of myo-
cytes. Conversely, in male patients, a homogeneous popula-
tion of cells with lipid storage was always detected, even in 
the presence of the same α-Gal gene mutation as in the female 
patients (Figure 3B).

In the other 30 patients, the histology was suggestive of 
HCM, showing severely hypertrophied myocytes, often in 
disarray, and short runs of myocardial fibers interrupted by 
connective tissue. These findings were observed in each 
patient in at least 1 LV endomyocardial sample and, because 
of the absence of histological markers of other specific 
diseases, were considered consistent with HCM.

At electron microscopy, the hypertrophied myocytes 
showed a disorganization of myofibrils and myofilaments, 
and no intracellular lamellar inclusions were seen.

Mean age at diagnosis and clinical manifestations did not 
differ between the FD and HCM patients (Table 1). The 
ECGs met the criteria for LVH (Sokolow-Lyon index) with 
repolarization changes in all 34 patients. At echocardiogra-
phy, there was no difference between FD and HCM in terms 
of severity of LVH and LV dimensions and contractility 
(Table 1). Tissue Doppler imaging showed a reduction in Sa, 
Ea, and Aa velocities in all patients at both corners of the 
mitral annulus compared with normal control subjects, indi-
cating an alteration in contraction and relaxation properties 
of cardiac muscle, but these parameters did not differentiate FD 
from HCM (Table 2). Moreover, the acoustic qualities of the 
LV myocardium did not differ between patients with HCM 
and FD. LV angiography showed an increase in wall thick-
ness and ventricular contractility and no abnormalities in the 
segmental wall motion. Coronary angiography was normal in 
all 34 patients. LV end-diastolic pressure was increased in all 
patients. Cardiac MRI confirmed the presence of LVH in all 
patients without specific intramyocardial signal alterations.

Characteristics of FD patients, including the pattern of 
LVH, are shown in Table 3. No significant valvular abnor-
malities, particularly mitral valve insufficiency, were 
detected.

All FD patients were ≥40 years of age at diagnosis. None 
of them was aware of being a carrier of FD or had other 
clinical manifestations of the disease. Routine laboratory 
investigations showed in patient 4 the presence of mild 
proteinuria (0.85 g/L) with normal creatinine clearance. A 
renal biopsy performed in this patient revealed the presence 
of patchy intracellular glycolipid accumulation, consistent 
with the early renal changes observed in FD.10 The same 
patient had also cornea verticillata that did not affect the 
vision, typical of FD.

No abnormalities in the laboratory tests or extracardiac 
signs of FD were detected in the other 3 cases.

Biochemical Studies
The α-Gal A enzymatic activity showed normal values in 2 
patients, nearly normal values in 1 patient, and low values in 
the remaining patient (patient 4) (Table 3). In particular, the 
percentage of mean control value was 44±14%, showing a 
high residual enzymatic activity. The α-Gal A enzymatic
activity was normal in all HCM patients (2326±453 nmol/h per 1 mg protein).

Genetic Analysis and Family Survey

The causal mutation was identified in all 4 patients affected by FD (Table 3). Although none of them were aware of being a carrier or had family members with a diagnosis of this disorder, their identification allowed the detection of FD in relatives.

Indeed, after the diagnosis of FD was made in the patients, the assessment of α-Gal A activity was extended to all available family members. In addition, a careful evaluation of the extracardiac clinical signs of FD, particularly in male subjects, and a gene mutation analysis were performed.

Affected members were detected in 3 patients’ families. Patient 1 did not have any family member affected by the disease, suggesting a de novo mutation. However, the mother, who died at 71 years of age of idiopathic heart failure, might have been affected by FD.

Mutation analysis of the mother of patient 2 revealed that she was the carrier of the disease in the family, despite normal enzymatic activity and only mild LVH misdiagnosed as caused by hypertension. The abnormal X chromosome was inherited by the patient’s sister, who was asymptomatic, and the patient’s brother, who suffered of several episodes of hypoacusia and died at 42 years of age of a cerebrovascular accident. One of the patient’s 2 sons, 24 years of age, showed at clinical evaluation the presence of angiokeratomas in the umbilical area; the other son, 21 years of age, suffered episodes of acroparesthesias. Both sons suffered from hypo-hydrosis and, at ophthalmologic examination, had whorled corneal opacity. The 17-year-old daughter of the patient’s brother was significantly clinically affected by the disease, showing acroparesthesias, hypoacusia, exercise intolerance, hypoacusia, and cornea verticillata at the initial stage, with low levels of enzymatic activity comparable to that of the affected male patients.

### TABLE 1. Clinical and Echocardiographic Data of HCM and FD Patients

<table>
<thead>
<tr>
<th></th>
<th>HCM Patients (n=30)</th>
<th>FD Patients (n=4)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>50±14.4</td>
<td>51.0±4.6</td>
<td>0.88</td>
</tr>
<tr>
<td>NYHA class, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>8 (31)</td>
<td>1 (25)</td>
<td>1</td>
</tr>
<tr>
<td>II</td>
<td>13 (50)</td>
<td>2 (50)</td>
<td>1</td>
</tr>
<tr>
<td>III</td>
<td>4 (15)</td>
<td>1 (25)</td>
<td>0.48</td>
</tr>
<tr>
<td>Ventricular arrhythmias, n (%)</td>
<td>12 (40)</td>
<td>1 (25)</td>
<td>1</td>
</tr>
<tr>
<td>Chest pain, n (%)</td>
<td>10 (33)</td>
<td>1 (25)</td>
<td>1</td>
</tr>
<tr>
<td>LVESD, mm</td>
<td>29.2±2.5</td>
<td>28±4.9</td>
<td>0.42</td>
</tr>
<tr>
<td>LVEDD, mm</td>
<td>44.2±5.8</td>
<td>44.7±4.1</td>
<td>0.86</td>
</tr>
<tr>
<td>IVS, mm</td>
<td>18.0±3.5</td>
<td>18.5±4.0</td>
<td>0.88</td>
</tr>
<tr>
<td>LVPW, mm</td>
<td>14.4±2.3</td>
<td>14.5±2.6</td>
<td>0.93</td>
</tr>
<tr>
<td>LVEF, %</td>
<td>65.6±5.1</td>
<td>66.2±8.5</td>
<td>0.83</td>
</tr>
<tr>
<td>SAM, n (%)</td>
<td>5 (16)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Outflow gradient &lt;30 mm Hg, n (%)</td>
<td>5 (16)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Left atrium, mm</td>
<td>46±5</td>
<td>47±3.6</td>
<td>0.84</td>
</tr>
</tbody>
</table>

LVESD indicates LV end-systolic diameter; LVEDD, LV end-diastolic diameter; IVS, interventricular septum; LVPW, LV posterior wall; LVEF, LF ejection fraction; and SAM, systolic anterior motion of the mitral valve. Values are mean±SD when appropriate. P<0.05 was considered significant.

### TABLE 2. Tissue Doppler Velocities in HCM Patients, FD Patients, and Normal Control Subjects

<table>
<thead>
<tr>
<th></th>
<th>HCM Patients (n=30), cm/s</th>
<th>FD Patients (n=4), cm/s</th>
<th>Control Subjects (n=15), cm/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lateral Sa</td>
<td>6.2±0.4*</td>
<td>6.1±0.5†</td>
<td>14.6±1.9</td>
</tr>
<tr>
<td>Lateral Ea</td>
<td>5.9±0.4*</td>
<td>5.8±0.2†</td>
<td>15.3±1.5</td>
</tr>
<tr>
<td>Lateral Aa</td>
<td>6.7±0.6*</td>
<td>6.6±0.2†</td>
<td>9.5±0.7</td>
</tr>
<tr>
<td>Septal Sa</td>
<td>6.0±0.6*</td>
<td>5.9±0.7†</td>
<td>14.5±1.9</td>
</tr>
<tr>
<td>Septal Ea</td>
<td>5.4±0.9*</td>
<td>5.3±0.8†</td>
<td>15.1±1.2</td>
</tr>
<tr>
<td>Septal Aa</td>
<td>6.2±0.6*</td>
<td>6.1±0.6†</td>
<td>9.7±1.0</td>
</tr>
</tbody>
</table>

*P<0.001 vs control subjects.
†P= NS vs HCM patients.
Clinical evaluation of the 2 sons (29 and 27 years old) of patient 3 revealed the presence of angiokeratomas and corneal opacity in both. The 29-year-old son referred also to suffering from exercise intolerance and episodic pain in the extremities. The patient’s brother died at 53 years of age of renal failure without being diagnosed as suffering from FD. His 25-year-old daughter was a carrier of the disease with no clinical signs and normal enzymatic activity. In this family, the carrier of the disease was presumably the patient’s grandmother, who was deceased at the time of the diagnosis of FD.

The pedigree and the clinical, biochemical, and molecular study of the family of patient 4 are shown in Figure 4.

Discussion
Although several reports exist in the literature on cardiac involvement in female carriers of FD, in terms of either systemic disease similar to hemizygous males or as the predominantly affected organ,4,11 no systematic study on the prevalence of FD in women with HCM is available.

Isolated cardiac manifestations in women without a family history of FD have included cardiomegaly with complete AV block,12 short PR interval, and cardiac sudden death.13

Our study documented, for the first time in a large cohort of female patients with a clinical and instrumental diagnosis of HCM, that up to 12% of these patients were affected by FD. This high prevalence, however, derived from a study population of ≈50 years of age and should be expected in women with late-onset (≥40 years) HCM. In a larger HCM group including a higher number of young patients, such a percentage would decrease because it is related to the typical late-age presentation of Fabry cardiomyopathy.

FD was indistinguishable from HCM in all noninvasive and invasive cardiac exams except endomyocardial biopsy. In fact, clinical features of Fabry patients were those of concentric HCM in 2 patients, asymmetric HCM in 1 patient, and apical HCM in 1 patient.

Unpredictably, histology showed in these 4 patients no cell disarray but regularly arranged myocardiocytes containing large perinuclear and cytoplasmic vacuoles that at electron microscopy consisted of concentric lamellar bodies. Histological lesions had a patchy distribution affecting 65% to 90% of myocardiocytes. This finding was absent in endomyocardial biopsies of male hemizygous patients even in the presence of the same mutation of the -Gal A gene. Indeed, the presence of 2 different populations, one with normal and one with reduced -Gal A activity, has been demonstrated in cultured skin fibroblasts from a patient heterozygous for FD.14

The higher percentage of myocardial cells involved in the disease suggested a nonrandom or skewed inactivation of the wild-type X chromosome. Skewed X inactivation, a phenomenon by which the same X chromosome is silenced in most of or all the cells of a tissue, has been shown to be common in normal female subjects, and the X-inactivation pattern can vary widely between different tissues.15–17 This variability makes inaccurate the extrapolation of the X-inactivation status from one tissue to another and explains the nearly normal values of blood -Gal A activity in most (75%) of our female patients despite severe cardiac disease and the higher prevalence of isolated cardiac involvement compared with hemizygous males. The poor diagnostic role of blood -Gal A activity (indicative of FD in only 25% of our cases) and the nonspecific contributions of noninvasive tools such as 2D echocardiography and cardiac MRI raise questions as to how Fabry cardiomyopathy can be detected in women with

<table>
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<th>TABLE 3. Characteristics of FD Patients</th>
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<tr>
<td>Age, y</td>
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<tr>
<td>Cardiac manifestations</td>
</tr>
<tr>
<td>Enzymatic activity</td>
</tr>
<tr>
<td>Percentage of normal mean values, %</td>
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<tr>
<td>LVESD, mm</td>
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<tr>
<td>LVESD, mm</td>
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<tr>
<td>LVIVS, mm</td>
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<tr>
<td>LVPW, mm</td>
</tr>
<tr>
<td>LVEF, %</td>
</tr>
<tr>
<td>Pattern of LVH</td>
</tr>
<tr>
<td>Mutation in -Gal A gene</td>
</tr>
<tr>
<td>Nucleotide change</td>
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<tr>
<td>Effect on coding sequence</td>
</tr>
<tr>
<td>Genotype</td>
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</table>

Abbreviations as in Table 1, plus VA indicates ventricular arrhythmias.
idiopathic LVH. In our series, male family members of 3 of 4 women with Fabry cardiomyopathy showed, at retrospective clinical analysis, systemic evidence of FD as angio kerascoma, cornea verticillata, or acroparesthesia associated with low levels of α-Gal A activity. Therefore, a careful family survey can in many instances provide useful indications for a correct diagnosis, which can be confirmed by appropriate genetic study. Questions remain about women with Fabry cardiomyopathy in the absence of affected male family members and with normal α-Gal A enzymatic levels, like patient 1 of our series, in whom a de novo mutation might be present. In this context, genetic screening of the α-Gal A gene, eventually implemented by an endomyocardial biopsy, particularly before the institution of a lifelong enzyme replacement treatment, should be considered. In particular, analysis of the α-Gal A gene is a reliable noninvasive approach for diagnosing FD because both we and Sachdev et al identified mutations in all patients with HCM affected by FD. The differential diagnosis of the 2 pathological conditions has important clinical implications because β-blockers, Ca²⁺ antagonists, and disopyramide, commonly adopted in HCM, may be contraindicated in Fabry cardiomyopathy patients in whom a latent contractile dysfunction is common. In addition, for FD, both enzyme enhancement therapy with galactose infusion⁶ and enzyme replacement therapy⁵ have been effective in clearing glycolipid accumulation in myocytes, with a reduction in LV hypertrophy and mass and an improvement in cardiac function, even in female patients.¹⁸

On the other hand, cardiovascular complications in the form of thromboembolic events, cardiac arrhythmias, and sudden death can occur at any time in the clinical course of the disease. Now we have a chance to prevent these complications by enzyme enhancement/replacement therapy

### Acknowledgment
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### References
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