Screening patients with hypertrophic cardiomyopathy for Fabry disease using a filter-paper test: the FOCUS study

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ABSTRACT
Background Patients with Fabry disease (FD) show left ventricular hypertrophy (LVH) mimicking hypertrophic cardiomyopathy (HCM) of sarcomeric origin and might benefit, if detected early, from specific enzyme replacement therapy. The prevalence of FD in patients with LVH of 13 mm or greater, screened using the leucocyte alpha-galactosidase A (~gal A) activity test, a technique that is difficult to apply routinely, ranged from 0% to 6%.

Objective To screen systematically for FD in patients with a diagnosis of HCM (LVH ≥15 mm) in primary cardiology practice, a validated, physician-friendly ~gal A assay was used on dried blood spots using a filter paper test.

Design and patients A cohort of 392 adults (278 men) followed for HCM were screened for FD. A standard blood test was used for confirmation in nine men in whom the ~gal A result was 40% or less.

Results Four men (1.5%; 1.8% of men) whom the blood test was used for confirmation in nine men in whom the ~gal A result was 40% or less.

Conclusion In male patients diagnosed as having HCM, pure FD cardiac variants are not exceptional and can be specifically identified using a simple filter-paper test. The sensitivity of this test is low in female patients.

Hypertrophic cardiomyopathies (HCM) are defined by the presence of increased left ventricular thickness or mass in the absence of loading conditions (hypertension, valve disease) sufficient to cause the observed abnormality.1 Several hundred mutations involving more than 10 different genes encoding sarcomeric proteins account for approximately 60% of HCM cases.2 While echocardiographic left ventricular hypertrophy (LVH) is the hallmark of the disease, there is a great heterogeneity of symptoms and prognoses among patients with sarcomeric HCM, some having a statistically normal life expectancy and others having a high risk of sudden death, congestive heart failure, or stroke due to atrial fibrillation.2

Besides sarcomeric HCM, a complex genetic disorder inherited as a Mendelian autosomal dominant trait, defects in other genes involving general metabolic pathways can also lead to LVH as seen in HCM. Among them, mutations in the ~galactosidase A (~gal A) encoding gene on the X chromosome may cause Fabry disease (FD), a rare glycosphingolipid storage disorder3 suitable for a disease-specific treatment with enzyme replacement therapy (ERT) using recombinant ~gal A.4 LVH, most often moderate, is present in more than 80% of men,5,6 and in one of three to five women3 with FD. Its prevalence increases with age5,7 and is usually observed along with other organ failure as renal dysfunction or neurological damage.4,8 However, cardiac variants showing exclusive or predominant LVH mimicking sarcomeric HCM have been described in adult patients with FD.3,6 When prescribed early enough, ERT can stabilise renal function9 and improve LVH and diastolic function.4,10

In previous studies using plasma or leucocyte ~gal A activity and/or genotyping to screen systematically for FD cardiac variants in adult patients with apparently unexplained LVH, the prevalence of FD varies markedly between 0% and 12%.7,11–14 However, both tests are expensive and difficult to implement on a large scale and plasma ~gal A activity may lead to both false positive and false negative results.15 In addition, these studies often included selected patients from tertiary, specialised centres. Finally, they applied an unusually low echocardiographic threshold (LVH ≥15 mm) to diagnose HCM,7,11–14 which should be based in sporadic cases on the presence of unexplained LVH of 15 mm or greater.3,16 FOCUS (Fabry Or Cardiomyopathy: Use of a systematic Screening) was a multicentre study that used a simple, physician-friendly filter paper test17 for screening FD patients initially diagnosed with HCM in a general cardiology setting in France.

PATIENTS AND METHODS
Inclusion criteria From July 2006 to July 2008, patients with HCM were recruited in 29 French cardiology centres. Each cardiologist was asked to include up to five patients presenting with HCM of presumed sarcomeric origin, irrespective of the time of diagnosis. After having signed an informed consent document,
patients were included if they met the following criteria: (1) age 18–79 years; (2) HCM defined as the presence of two-dimen-
sional echocardiographic LVH of unknown cause, such as severe 
hypertension or valvular aortic stenosis, with a maximum end-
diastolic wall thickness of 15 mm or greater in any segment;16; 
and (3) no genetic testing for HCM and no known FD or 
sarcomeric mutation in the patient and their family. Only 
patients with mild to moderate hypertension were included 
in the study as most data suggest that moderate to severe hyper-
trophy (wall thickness ≥15 mm) is rare in white people with 
mild to moderate hypertension.18 Left ventricular wall thick-
nesses were measured directly at end-diastole on echographic 
two-dimensional parasternal short-axis views (either at the base, 
papillary muscle or apex levels) using fundamental imaging and 
taking care to adjust the cut-planes perpendicular to the left 
ventricular long axis; the greatest thickness as measured at any 
site within the left ventricular wall using the leading edge 
method represented the maximum wall thickness.2 16

Data collection and diagnostic tests
Patients’ demographic characteristics, HCM-related medical 
history, echocardiographic measurements and current drug 
therapy were recorded at inclusion. Blood was drawn from every 
patient by venipuncture. Five drops from each blood sample 
were directly spotted on two filter papers. The spots were dried 
at room temperature for 8–72 h and then stored at +2°C to +6°C 
for up to 2 months before being centrally processed (Laboratoire 
de génétique médicale, Garches, France); α-gal A activity was 
then measured from dried blood spots (DBS) on filter paper 
using a fluorimetric method17 with modifications as described 
previously.19

When α-gal A activity was greater than 40% compared with 
the control, a threshold determined from the results obtained 
from a different genotyped cohort (see below), the test was 
considered negative and there was no follow-up visit. The rela-
tively high activity threshold was chosen in an attempt to detect 
FD disease in women, because a high enzymatic activity in 
women cannot accurately exclude heterozygosity for FD.30

When activity was 40% or less, a second visit was scheduled to 
answer a questionnaire oriented to characterise signs or symp-
toms of FD (family history, acute or chronic peripheral neuro-
pathic pain in adolescence, decreased ability to sweat, cornea 
verticillata, angiokeratoma, gastrointestinal complaints, renal 
dysfunction, proteinuria, or stroke at age <55 years), and 
confirm FD diagnosis using a leucocyte enzyme activity measure 
test in men and genotyping in women. To do so, blood samples 
were shipped to the central laboratory within 96 h. Enzyme 
activity was assessed by a fluorimetric method on leucocytes,21 
and genotyping was performed on DNA extracted from the filter 
papers.

Accuracy of the DBS screening test
Filter paper test accuracy has been validated previously, being 
compared with the leucocyte enzyme activity measure test.19 21 23 
Furthermore, we tested the accuracy of the DBS test on an 
independent cohort of 209 subjects (128 genotyped FD patients - 
60 men, 68 women - and 81 healthy controls). The enzymatic 
activity of β-galactosidase on DBS was found to be normal for all 
subjects, suggesting the absence of any pre-analytical problem. A 
positive screening test was defined by an α-gal A activity of less 
than 1.1 (0.11±0.2) μmol/h per litre. In heterozygous women, α-
gal A activity was 2.2±1.7 μmol/h per litre, ranging from 0 to 
7.8 μmol/h per litre. Using this threshold (α-gal A <40% of 
controls), the specificity of the test was 100%, whereas the 
sensitivity was 82% (100% in men but only 66% in women).

PCR conditions
The PCR were performed as follows: final volume sample 50 μl; 
2 μm of dried blood from the filter paper as a source of DNA, 
25 μl water, 2.5 μmol of each primer, and 20 μl of Phusion Blood 
direct PCR (Finnzymes Inc, Massachusetts, USA) were used. An 
initial denaturation at 95°C for 5 min was followed by 55 cycles 
of 30 s at 95°C, 30 s at 58°C, 1 min at 72°C, and a final 10 min at 
72°C.

Genotyping
Direct sequencing was performed by Beckman Coulter Genomics (Essex, UK). PCR products were purified by Agen-
court Ampure XP (Beckman Coulter Genomics). A S750xl 
Analysrer (Applied Biosystems Inc, California, USA) was used to 
obtain DNA sequences, which were subsequently handled with 
Navigator 2.0 software.

Data analysis
At least 569 patients had to be included in the study to allow 
an accurate estimation of the prevalence of FD, with an 
expected value of 4% and a 95% CI from 2% to 6%. When 
appropriate, results are expressed as mean±standard deviation 
(SD) (range). Groups have been compared using a non-para-
metric Wilcoxon test for continuous variables and Fisher’s exact 
test for categorical variables.

RESULTS
Study population
Three-hundred and ninety-two patients (278 men) were 
included in the study (table 1).

A diagnosis of HCM was established at least 5 years earlier in 
more than 50% of patients and was new in 124 cases (51.6%). 
Fifty-one patients older than 65 years were hypertensive (61.5%) 
and all of them had been followed for HCM for more than 
5 years. A family history of HCM or sudden death was observed 
in 22% and 14% of men, respectively, and in 41% and 27% of 
women. The inheritance pattern a priori excluded the X-linked 
FD (father—son transmission) in 28 cases. Syncope or presyn-
cope, angina, heart failure and atrial arrhythmias (permanent, 
sustained, or not) were the most common symptoms. Previous 
ventricular arrhythmias were not infrequent, with 34 patients 
having experienced ventricular tachycardia, five having experi-
enced sudden death due to ventricular fibrillation (all implanted 
with an automatic internal cardiac defibrillator), and four 
patients with ventricular premature beats of Lown class 3. 
Malignant LVH (≥30 mm) was observed in 6% of men and 3% 
of women (p=0.12). During the study, two women died of 
cardiac causes (one after cardiac transplantation and one of 
sudden death), three men were resuscitated after sudden death 
(one with an appropriate shock from his de-
brillator), and six 
patients were hospitalised for worsening heart failure. No 
endomyocardial biopsy was performed in that cohort.

Screening results
Among women, all had suitable filter paper tests and enzyme 
activity greater than 40% and no FD was diagnosed. Among 
men, three had non-useful filter paper tests, as a result of
confirmed conservation problems, and nine had filter paper residual enzyme activities below the defined cut-off of 40%—markedly decreased (<17%) in four cases and slightly below the cut-off value (range 35–59%) in five cases—leading to a second enzymatic assay performed on leucocytes. Among those nine patients, the five with enzyme activities between 55% and 39% on filter papers (i.e. slightly below the initially defined cut-off value for the leucocyte assay) were found to have a leucocyte enzyme activity greater than 40%, while DNA sequencing failed to identify a pathogenic mutation in the GLA gene. In contrast, the remaining four patients with markedly reduced enzyme activity on DBS (<17%) were all confirmed as having FD, with a leucocyte enzyme activity between 5% and 17%. The prevalence of FD was thus 1.5% (95% CI 0.4 to 3.8) in our cohort of 275 men with interpretable filter paper tests and 1.8% in men above 40 years of age.

GLA gene mutations

For patients 1 and 2, sequencing was performed on PCR products obtained after amplification of DNA extracted from leucocyte pellets. For patients 3 and 4, direct sequencing was performed on purified PCR products obtained from direct amplification of DNA eluted from a 3 mm punch of DBS from filter paper in the PCR mix. Patient 1 was shown to carry a G to C transversion at position c. 486 in exon 3 of the complementary DNA sequence, leading to a missense mutation (p.Trp162Cys). Patients 2 and 3 were both shown to carry a T to C transition at position c. 537 in exon 2 the cDNA sequence of the GLA gene, also leading to a missense mutation (p.Phe113Leu). Interestingly, these two patients—who do not know each other—are both of Portuguese ancestry. Haploptype studies are ongoing to determine whether they are related or not. Patient 4 was shown to carry an A to G transition at position c. 644 in exon 5 of the cDNA sequence (missense mutation p.Asn215Ser). In all cases, sequencing of the rest of the GLA gene revealed no other abnormality.

Characteristics of FD patients

The mean age of the four men with FD was not significantly different from that of the remaining male patients of the cohort (52±8 years (42–59) vs 55±15 years (18–79), respectively, p=0.85). While all patients showed diffuse but asymmetric LVH, a septal to posterior wall ratio greater than 1.5 was not observed. Two patients presented with systolic anterior motion of the mitral valve and outflow tract obstruction, severe in one case, leading to septal ablation (table 2).

Left ventricular ejection fraction was normal in all cases. No FD patients presented with right ventricular hypertrophy or aortic root dilatation; FD patients with systolic anterior motion of the mitral valve showed mild mitral regurgitation, and one FD patient presented with minimal aortic regurgitation. Three patients (1, 2 and 4) underwent permanent pacemaker implantation due to symptomatic high-grade atriioventricular block at 43, 47 and 53 years of age, compared with only one patient in the remaining cohort. No patient reported characteristic signs or symptoms of FD. Moreover, there was no medical history of pain, angiokeratoma, cornea verticillata or proteinuria. On a systematic brain magnetic resonance imaging scan, one patient had a small lacunar infarction, whereas all the others tested normal. Another patient showed mild proteinuria, with an estimated glomerular filtration rate normal for his age. Pedigree analysis identified two half-brothers at risk of FD for patient number 4, and sequencing of the GLA gene confirmed the diagnosis of FD through the identification of a missense mutation in exon 5 of the gene (p. N215S). Interestingly, diffuse but asymmetric LVH of unknown cause existed for both of them. Furthermore, six heterozygous women were identified through pedigree analysis in three families.

DISCUSSION

In FD, α-gal A deficiency causes progressive accumulation of globotriaosylceramide and related glycosphingolipids in heart}

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**Table 1** Study group characteristics (n=392)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Study group characteristics (n=392)</th>
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</thead>
<tbody>
<tr>
<td>Demographics</td>
<td>Age, years, mean±SD (range) 53±14 (18–79)</td>
</tr>
<tr>
<td></td>
<td>≤30 (women/men), n 6/19</td>
</tr>
<tr>
<td></td>
<td>30–40 (women/men), n 9/33</td>
</tr>
<tr>
<td></td>
<td>&gt;40 (women/men), n 99/266</td>
</tr>
<tr>
<td></td>
<td>Men, n (%) 278 (71)</td>
</tr>
<tr>
<td>Medical history</td>
<td>Time since diagnosis of HCM, years, mean±SD (range) 8±9 (0–46)</td>
</tr>
<tr>
<td></td>
<td>History of hypertension 136 (34)</td>
</tr>
<tr>
<td></td>
<td>Duration of hypertension, years, mean±SD (range) 12±10 (0–45)</td>
</tr>
<tr>
<td></td>
<td>Family history of HCM 102 (26)</td>
</tr>
<tr>
<td></td>
<td>Family history of sudden death 71 (18)</td>
</tr>
<tr>
<td>Symptoms</td>
<td>NYHA class 1 118 (30)</td>
</tr>
<tr>
<td></td>
<td>2 187 (47)</td>
</tr>
<tr>
<td></td>
<td>3 82 (21)</td>
</tr>
<tr>
<td></td>
<td>4 7 (2)</td>
</tr>
<tr>
<td></td>
<td>(Pre)syncope 108 (27)</td>
</tr>
<tr>
<td></td>
<td>Angina pectoris 108 (27)</td>
</tr>
<tr>
<td></td>
<td>Atrial arrhythmias 115 (29)</td>
</tr>
<tr>
<td></td>
<td>Ventricular arrhythmias 43 (11)</td>
</tr>
<tr>
<td></td>
<td>No symptoms 64 (16)</td>
</tr>
<tr>
<td>Echocardiography</td>
<td>Maximum left ventricular wall thickness, mm, mean±SD (range) 20±5 (15–40)</td>
</tr>
<tr>
<td></td>
<td>Left ventricular wall thickness ≥30 mm 21 (5)</td>
</tr>
<tr>
<td></td>
<td>Left ventricular outflow tract gradient ≥30 mm Hg 12 (31)</td>
</tr>
</tbody>
</table>

Results are shown as number (%) unless stated otherwise. HCM, hypertrophic cardiomyopathy; NYHA New York Heart Association.

**Table 2** Clinical and genetic characteristics of men diagnosed with Fabry disease

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Age (years)</th>
<th>(Pre)syncope</th>
<th>Angina</th>
<th>NYHA class</th>
<th>FH</th>
<th>HT</th>
<th>PM</th>
<th>AF</th>
<th>VT</th>
<th>Maximum WT (mm) (IVS/PW)</th>
<th>SAM/gradient (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>59</td>
<td>+</td>
<td>0</td>
<td>I</td>
<td>0</td>
<td>0</td>
<td>Yes</td>
<td>0</td>
<td>0</td>
<td>21 (1.4)</td>
<td>no/0</td>
</tr>
<tr>
<td>2</td>
<td>48</td>
<td>0</td>
<td>+</td>
<td>III</td>
<td>0</td>
<td>+</td>
<td>No</td>
<td>+</td>
<td>+</td>
<td>19 (1.3)</td>
<td>yes/30 (140*)</td>
</tr>
<tr>
<td>3</td>
<td>59</td>
<td>+</td>
<td>0</td>
<td>II</td>
<td>0</td>
<td>0</td>
<td>Yes</td>
<td>0</td>
<td>0</td>
<td>20 (1.3)</td>
<td>no/0</td>
</tr>
<tr>
<td>4</td>
<td>41</td>
<td>+</td>
<td>0</td>
<td></td>
<td>0</td>
<td>0</td>
<td>Yes</td>
<td>0</td>
<td>0</td>
<td>21 (1.4)</td>
<td>yes/30</td>
</tr>
</tbody>
</table>

*Maximal gradient at exercise before septal embolisation. None of the patients had any other features of Fabry disease outside the heart.

AF, atrial fibrillation or arrhythmias; FH, known family history of hypertrophic cardiomyopathy or Fabry disease; gradient, peak outflow tract gradient; HT, hypertension; IVS/PW, ratio of septal thickness on posterior wall thickness; NYHA, New York Heart Association; PM, permanent pacemaker for high-degree atriioventricular block; SAM, systolic anterior motion of the mitral valve; VT, ventricular tachycardia; WT, end-diastolic left ventricular wall thickness.
tissues, such as cardiomyocytes, conduction system cells, valvular fibrocytes and both vascular endothelial and smooth muscle cells. This heart tissue accumulation potentially leads to irreversible cardiac damage. Cardiac FD manifestations include LVH, arrhythmias, conduction defects and, more rarely, coronary artery disease (mainly due to small-vessel disease). It contributes to a reduced life expectancy of affected patients due to malignant arrhythmias and heart failure. Whereas, in parallel with multivisceral involvement, nearly all FD patients will develop LVH, the extent of which both increases with age and is accompanied by a progressive reduction in left ventricular function. LVH may also be the predominant feature of the disease in some patients presenting as cardiac variants, even if the existence of pure cardiac variants remains controversial.

Previous systematic screening of FD has been performed using α-gal A enzyme activity measured in leucocytes with the fluorogenic substrate 4-methylumbelliferyl-α-D-glucopyranoside, currently the gold standard for diagnosing FD in male patients. More recently, α-gal A enzyme activity dosage on DBS using a filter paper test has been proposed as an alternative diagnostic test, and has been found to be as accurate as assays using leucocyte samples. The accuracy of this test is also demonstrated by results currently reported in a separate genotyped cohort (see Patients and methods section), with both sensitivity and specificity reaching 100% in men, whereas in women, as expected, sensitivity was lower (66%). However, the diagnosis of FD in women can occasionally be made using this method in the cases in which a markedly decreased residual enzyme activity is evidenced, and thus women were included in the current study. Moreover, those samples are easy to transport and are stable at room temperature for at least 20 days, making them suitable for obtaining a second sample may be difficult as well as for the genotyping of women for whom the enzymatic assay is not satisfactory and no efficient screening alternative is currently available.

Our results are in line with data from Montserrat et al in which a prevalence of FD of 1% has been estimated in a population of 508 men and women with HCM of presumed sarcomeric origin. Others cardiac variants have been identified in cohorts of patients with unexplained LVH of 13 mm or greater and systematically screened for FD, with an overall prevalence of approximately 3% in men (up to 6% in those aged above 40 years) and up to 12% in women (in a single study for which methodological bias cannot be excluded). Conversely, no patients with FD were found in highly selected groups with HCM, either in severely symptomatic obstructive patients who underwent surgical procedures to relieve the gradient, or in

Figure 1 Direct sequencing of polymerase chain reaction products obtained from amplification of DNA eluted directly from the 3 mm punch of dried blood spot from filter paper (patient 3). Patient 3 was shown to carry a T to C transition at position c. 337 in the cDNA sequence (arrow). This nucleotide substitution alters the codon (TTT) for phenylalanine to the codon (CTT) for leucine at position 113 of the α-galactosidase A protein (p. Phe113Leu). Despite scanning of the rest of the gene, no other sequence abnormality was found.
systematically genotyped patients. The apparent discrepancies between these different results may be related to the different methods used (type of population selected, assay methods).

While echocardiographic asymmetric LVH has been described in patients with FD, diffuse, concentric and homogeneous LVH is the more frequently reported pattern. The current study demonstrates, quite unexpectedly when compared with a previous study, that cardiac variants may show asymmetric LVH and may go unrecognized for decades. However, a septal to posterior wall ratio greater than 1.5 was not observed. Severe obstruction leading to septal ablation is infrequent, but has been reported. Syncpe associated with high-grade aortic valvular block seems to be a rather specific characteristic of the FD cardiac variant in this study, as three out of four patients diagnosed with FD presented with a syncopal high-grade aortic valvular blockage leading to permanent pacemaker implantation.

Conclusions

In men diagnosed with HCM, cardiac variants of FD are not exceptional and might benefit from specific ERT. These patients may be screened systematically for FD with a simple fluorimetric method using DBS on filter paper test, which has become a very efficient first-line diagnostic test for screening in a general cardiology setting. High-degree aortic valvular block is frequent in that population. As the outcome of specific ERT is less efficient in advanced cases of FD, before extensive myocardial fibrosis is present, such a systematic screening using a simple test appears to be clinically relevant.

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Competing interests

AAH and DPG have received research and consultancy funding from Genzyme SAS, Saint-Germain-en Laye, France.

Patient consent

Obtained.

Ethics approval

This study was conducted with the approval of the ethical committee of Pitie Salpêtrière, Paris, no 11-18, favourable advice 20 March 2006, France.

Provenance and peer review

Not commissioned; externally peer reviewed.

REFERENCES


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