Vascular Aspects of Fabry Disease in Relation to Clinical Manifestations and Elevations in Plasma Globotriaosylsphingosine

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Abstract—Fabry disease is an X-linked hereditary lysosomal storage disorder attributed to a deficiency of α-galactosidase A leading to increased plasma levels of globotriaosylsphingosine (lysoGb3). The disease presents as a vascular disease, with cerebral, cardiac, and renal complications. Carotid intima-media thickness (IMT), brachial flow-mediated dilation (FMD), pulse wave velocity, and advanced glycation end products were measured in 57 classically affected patients (22 men and 35 women), 55 healthy matched controls (20 men and 35 women), and 10 atypical Fabry disease patients (5 men and 5 women). Most patients received enzyme replacement therapy. In classically affected male patients, brachial FMD was decreased (2.9% [95% CI, 0.8% to 7.9%] versus 5.9% [2.1% to 8.5%] in controls; \( P = 0.01 \)), and carotid IMT was increased (0.67 mm [95% CI, 0.50–0.96 mm] versus 0.59 mm [95% CI, 0.40–0.76 mm] in controls; \( P = 0.01 \)). In women and atypical patients these vascular parameters were comparable with controls. Pulse wave velocity was not different; advanced glycation end products were only slightly increased in atypical patients. In classically affected women, a small increase in lysoGb3 was associated with an increase in IMT independent of age. In the classically affected men, all with increased IMT and high levels of plasma lysoGb3, lysoGb3 levels did not add to a higher IMT, suggestive of a ceiling effect. For FMD, elevated lysoGb3 levels (>7 nmol/L) contributed to a 2.9% lower FMD independent of age and sex (\( P = 0.02 \)). Increased carotid IMT and decreased brachial FMD occur in classic Fabry disease, which is associated with plasma lysoGb3 level independent of age and sex. These observations still exist despite enzyme replacement therapy. (Hypertension. 2012;60:00-00.)

Key Words: Fabry disease ■ globotriaosylsphingosine ■ intima-media thickness ■ flow-mediated dilation ■ pulse wave velocity ■ advanced glycation end products

Fabry disease is an X-linked hereditary lysosomal storage disorder attributed to a deficiency of α-galactosidase A. Its distribution is panethnic, with an estimated birth prevalence of 1:40000 to 117000, although recent screening studies suggest higher prevalence rates. The primary cause of disease is a deficient activity of the lysosomal α-galactosidase A (EC 3.2.1.22). Because of this, globotriaosylceramide (Gb3; also named GL-3 or CTH) is stored in various cell types, especially endothelial cells. Clinically, the disease presents as a vascular disease, with development of cerebral white matter lesions (WMLs), cardiac hypertrophy, and kidney disease. Premature coronary artery and cerebral artery disease were found in one cohort but was not confirmed in other studies. A proportion of female carriers with α-galactosidase A deficiency also develop disease, albeit with a more protracted course. The chronic presence of a large amount of endogenous circulating α-galactosidase A enzyme is apparently unable to correct the lack of enzyme in those cells that only express the mutated allele. Atypical variants of Fabry disease, consisting of individuals with more subtle α-galactosidase A abnormalities, present with an atypical course with limited manifestations, including isolated cardiomyopathy or renal insufficiency. The latter group of individuals constitutes a major problem in diagnosis, which is solely based on the demonstration of a predicted amino acid substitution as revealed by the gene analysis and/or detection of reduced enzyme activity in assays using artificial substrates. In these individuals, there may be an absence of elevated levels of Gb3 in urine and Gb3 and globotriaosylsphingosine (lysoGb3) in plasma, which questions the relationship between storage and clinical symptoms in these patients.

Since 2001, enzyme replacement therapy (ERT) has been available. Two commercial products, agalsidase-α and agalsidase-β, reduce cardiac mass, reduce pain, improve quality of life, and stabilize kidney function in some patients.
with preserved renal function. However, despite this endothelial Gb3 reduction, disease progression does occur, suggesting another mechanism causing vasculopathy. The pathogenesis of the vasculopathy in Fabry disease is, however, poorly understood. Abnormal functional control of the vessel and the presence of endothelial dysfunction, as well as an increased prothrombotic state with the formation of reactive oxygen species, have all been suggested to underlie the vasculopathy.

Fabry disease patients show an increased intima-media thickness (IMT) of the common carotid, brachial, and radial artery compared with controls. Flow-mediated dilation (FMD), a measure of endothelial dysfunction, is impaired in Fabry disease. Furthermore, Fabry disease patients before ERT treatment have an increased pulse wave velocity (PWV), a measure of aortic stiffness. Recently, accumulation of advanced glycation end products (AGES) has been reported as a marker of cardiovascular disease in individuals with an increased IMT and patients at risk for cardiovascular complications but not yet in Fabry disease. In addition, none of these vascular measures have been studied in Fabry disease.24

A potential pathogenic factor in Fabry disease may be lysoGb3, a water-soluble lipid that is highly elevated in the plasma of classic Fabry disease patients. LysoGb3 induces smooth muscle cell proliferation in vitro and might, therefore, contribute to increased IMT in Fabry disease patients.

The primary aim of the study was to compare carotid IMT, FMD, PWV, and AGES in patients with Fabry disease with healthy matched controls. We evaluated how these parameters of vascular damage relate to disease severity and clinical parameters. The second aim of the study was to investigate the hypothesis that an increase in plasma lysoGb3 levels is associated with an increase in IMT.

Methods

Participants

All adult patients (age >18 years) with a confirmed diagnosis of Fabry disease by enzyme analysis and genotyping, were invited to participate from March 2009 through July 2010. Patients with the R112H and P60L substitutions are considered atypical Fabry disease patients based on their atypical course of disease and apparent lack of lipid abnormalities. Exclusion criteria were diabetes mellitus, primary dyslipidemia, or other relevant comorbidity. The participating patients were matched with healthy controls for age, sex, and smoking status. Healthy controls were patient relatives with an excluded diagnosis of Fabry disease, friends, or were recruited by advertisement. Participants were instructed to fast overnight and refrain from smoking. A full medical and family history was obtained. Blood pressure (BP) was measured using a validated automatic oscillometric device (Omron 705it). Hypertension was defined as a systolic BP ≥140 mm Hg and/or a diastolic BP ≥90 mm Hg in 3 consecutive measurements or use of medication to lower the BP. The estimated glomerular filtration rate was calculated by the abbreviated Modification of Diet in Renal Disease equation. In the Fabry disease patients, data on left ventricular mass, cerebral WMLs, and stroke were available as part of ongoing data collection. Left ventricular hypertrophy was defined as left ventricular mass >51 g/m² in men and >48 g/m² in women. A WML was diagnosed by a neuroradiologist on T2-weighted MRI images. The study was approved by an institutional review committee, and all of the subjects gave informed consent.

Laboratory Assessments

Creatinine, glucose, and total cholesterol profile were assessed. In Fabry disease patients, lysoGb3 levels in plasma were measured with a newly developed method based on tandem mass spectrometry with isotope-labeled lysoGb3 (5,6,7,8,9,13C6-lysoGb3) as an internal standard.

Imaging

All of the measurements were performed at the Academic Medical Center Vascular Imaging Laboratory. The performing analyst was blinded for the clinical status of the subject.

Carotid IMT

For carotid IMT measurements, bilateral B-mode ultrasound DICOM still images of 6 predefined carotid arterial far wall segments of the right and left common carotid artery carotid bulb and internal carotid artery were acquired. The per-subject carotid IMT was defined as the average of the 6 IMT measurements.

Brachial FMD

A BP cuff was placed around the right forearm and the arm stabilized using a custom-built probe holder/arm rest. Brachial artery lumen diameter at the level of the antecubital crease was measured for a period of 1 minute ("baseline"), followed by 5 minutes of arterial cuff occlusion and forearm ischemia. On cuff release, lumen was measured for 3 minutes. FMD was expressed as follows: (maximum lumen diameter after cuff release − diameter at baseline)/diameter at baseline × 100%.

Pulse Wave Velocity

Pressure waveforms were recorded at the carotid and femoral arteries sequentially. Wave transit time was calculated by the SphygmoCor (version 8, AtCor) system software, using the R wave of a simultaneously recorded ECG as a reference frame. Surface distance between the 2 recording sites was measured to determine the PWV. The measurements were done on the right side of the subject twice and averaged.

Advanced Glycation End Products

Skin autofluorescence was measured with the AGE Reader (DiagnOptics Technologies BV, Groningen, the Netherlands) at the volar site of the forearm in a semidark environment.

Statistical Analysis

Values are expressed as median (range) or mean ± SD. Comparison between groups was made by using the Mann-Whitney test or the Kruskal-Wallis test where appropriate. Univariate, multiple linear regression, and logistic regression were applied to determine the association among the vascular parameter, lysoGb3, and disease manifestations. These analyses were adjusted for cardiovascular risk factors, including age, sex, body mass index, smoking status, a history of hypertension, systolic and diastolic BPs, total cholesterol/high-density lipoprotein ratio, and low-density lipoprotein cholesterol. The predictors and their interactions were kept in the model if significant at P = 0.05. Lifetime exposure to plasma lysoGb3 was assessed as follows: age at baseline × lysoGb3 at baseline + years of ERT treatment × lysoGb3 at the time of evaluation. As part of a sensitivity analysis, multiple imputation for missing values for FMD and PWV data were performed, using age, sex, and patient/control group, as well as BP for PWV as predictor variables, which led to the same conclusions (data not shown). A P value <0.05 was considered statistically significant. Statistical analyses were performed with SPSS 17.0.

Measurements

In total, 57 classically affected patients (22 men and 35 women), 10 atypical patients (5 men and 5 women), and 55 controls (20 men and 35 women) were investigated. Forty-four patients received treatment with ERT (Table 1). PWV data were available for 76% of the participants (40 classic patients, 8 atypical patients, and 45 controls). The main reasons for unsuccessful PWV measurements were cardiac complications but not yet in Fabry disease.

2 Hypertension October 2012
rhythm disturbances. FMD data were available for 78% of the participants (46 classic patients, 8 atypical patients, and 41 controls). Twenty-three FMD measurements were rejected because of inferiority of the images. AGES were measured in 96% of the participants.

Results

Classically Affected Men and Women
Comparing the total cohort of classic patients with matched controls, FMD was significantly lower (4.7% versus 5.8%; P=0.02; Table 2). Carotid IMT was increased (0.65 versus 0.60 mm in controls; P=0.02; Table 2). PWV and AGES were not significantly different between classic Fabry disease patients and controls. Analysis by sex revealed that, in men (age, 38.4±14.3 years), IMT was increased and FMD decreased compared with male controls. In Fabry disease women (age, 45.7±13.3 years), IMT, FMD, PWV, and AGES were comparable to female controls (Table 2; Figure 1).

Atypical Patients
Comparing the atypical patients (age, 52.0±13.3 years) with controls revealed that none of the vascular parameters were significantly different with the exception of AGES in men (Table 2).

Enzyme Replacement Therapy
Forty-two classic patients received ERT with a median ERT duration of 5.7 years (95% CI, 0.5–9.9 years). Almost all classically affected male patients (21 of 22) received ERT; the one not treated received treatment in the past. In the 21 ERT-treated classically affected women, IMT, FMD, PWV, and AGES were comparable to the 21 female matched controls. These parameters were also comparable for the 14 untreated classically affected female patients compared with the 14 matched controls.

Table 1. Baseline Clinical Characteristics of the Study Population

<table>
<thead>
<tr>
<th>Variable</th>
<th>Classic Mutation</th>
<th>Atypical</th>
<th>Control</th>
<th>P Value Classic vs Controls</th>
<th>P Value Atypical vs Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>57</td>
<td>10</td>
<td>55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men, n (%)</td>
<td>22 (39)</td>
<td>5 (50)</td>
<td>20 (36)</td>
<td>0.81</td>
<td>0.42</td>
</tr>
<tr>
<td>Women, n (%)</td>
<td>35 (61)</td>
<td>5 (50)</td>
<td>35 (64)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, mean±SD</td>
<td>42.9±18.8</td>
<td>52.0±13.3</td>
<td>44.2±14.5</td>
<td>0.63</td>
<td>0.14</td>
</tr>
<tr>
<td>Median (range)</td>
<td>44.2 (19–76)</td>
<td>50.9 (27–74)</td>
<td>47.2 (19–83)</td>
<td>0.63</td>
<td>0.14</td>
</tr>
<tr>
<td>Ethnicity (W; M; A)</td>
<td>55;1;1</td>
<td>6;4;0</td>
<td>52;2;1</td>
<td>0.83</td>
<td>0.001</td>
</tr>
<tr>
<td>Agal α</td>
<td>17</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Agal β, 0.2 mg/kg/2 wk</td>
<td>3</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Agal β, 0.25–0.50 mg/kg/2 wk</td>
<td>12</td>
<td>1</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Agal β, 1.0 mg/kg/2 wk</td>
<td>10</td>
<td>1</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>No ERT (n)</td>
<td>15</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>History of hypertension, n (%)</td>
<td>11 (19)</td>
<td>2 (20)</td>
<td>2 (3.6)</td>
<td>0.01</td>
<td>0.05</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>121±19</td>
<td>122±12</td>
<td>126±14</td>
<td>0.03</td>
<td>0.38</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>73±9</td>
<td>78±10</td>
<td>77±11</td>
<td>0.11</td>
<td>0.54</td>
</tr>
<tr>
<td>BP-lowering medication, n (%)</td>
<td>30 (53)</td>
<td>3 (30)</td>
<td>4 (7)</td>
<td>&lt;0.001</td>
<td>0.03</td>
</tr>
<tr>
<td>ACE-ARB, n (%)</td>
<td>26 (49)</td>
<td>3 (30)</td>
<td>2 (4)</td>
<td>&lt;0.001</td>
<td>0.02</td>
</tr>
<tr>
<td>Glucose</td>
<td>4.9±0.6</td>
<td>4.9±0.5</td>
<td>5.0±0.5</td>
<td>0.27</td>
<td>0.68</td>
</tr>
<tr>
<td>TC/HDL ratio</td>
<td>3.2±0.8</td>
<td>4.2±0.9</td>
<td>3.5±1.1</td>
<td>0.18</td>
<td>0.05</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>2.9±0.9</td>
<td>3.3±0.7</td>
<td>3.2±0.9</td>
<td>0.08</td>
<td>0.54</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>0.87±0.40</td>
<td>1.2±0.78</td>
<td>1.0±0.69</td>
<td>0.80</td>
<td>0.30</td>
</tr>
<tr>
<td>Statin use, n (%)</td>
<td>3 (5.3)</td>
<td>1 (10)</td>
<td>0 (0)</td>
<td>0.09</td>
<td>0.02</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.4±4.1</td>
<td>27.7±4.9</td>
<td>24.6±3.7</td>
<td>0.52</td>
<td>0.06</td>
</tr>
<tr>
<td>Current smoking, n (%)</td>
<td>11 (19)</td>
<td>2 (20)</td>
<td>10 (18)</td>
<td>0.88</td>
<td>0.89</td>
</tr>
<tr>
<td>eGFR, median (range)</td>
<td>88 (23–148)</td>
<td>85 (8–111)</td>
<td>86 (45–129)</td>
<td>0.84</td>
<td>0.79</td>
</tr>
<tr>
<td>LVH, n (%)</td>
<td>35 (61)</td>
<td>2 (20%)</td>
<td>ND</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>WML, n (%)</td>
<td>39 (68)</td>
<td>6 (60)</td>
<td>ND</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>LysoGb3 pretreatment</td>
<td>10.7 (2–124)</td>
<td>0.7 (0.3–3)</td>
<td>ND*</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>LysoGb3</td>
<td>8.4 (2–87)</td>
<td>0.7 (0.4–1.6)</td>
<td>ND*</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

BP-lowering medication includes ACE inhibitors and ARBs prescribed for microalbuminuria/proteinuria. A indicates Asian; W, white; M, Mediterranean; NA, not applicable; ND, not determined; BMI, body mass index; BP, blood pressure; LVH, left ventricular hypertrophy; WML, white matter lesion; eGFR, estimated glomerular filtration rate; TC, total cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; ERT, enzyme replacement therapy; LysoGb3, globotriaosylsphingosine.

*Reference interval for lysoGb3 in healthy controls is 0.3 to 0.5 nmol/L. Some patients received an adjusted ERT dose because of participation in a previous clinical trial or the global shortage of Fabrazyme.
Vascular Parameters and Clinical Parameters

Classically Affected Patients

IMT was associated with renal function, left ventricular mass, and WMLs, including previous strokes (Table 3). There was no correlation between IMT and microalbuminuria. However, if adjusted for age and sex, the relationship between IMT and the clinical manifestations lost significance.

FMD and PWV were related to left ventricular hypertrophy and WML, respectively, and the AGES were related to LVH and WML, but none of these were significant when adjusted for age and sex. The AGES, however, were inversely related to renal function. If adjusted for age and sex, an increase of the AGES by 1 U reflected a decrease of renal function by 20.3 mL/min per 1.73 m² ($P<0.02$).

Table 2. PWV, FMD, IMT, and AGES for the Total Cohort and for Men and Women Separately

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Classic</th>
<th>Atypical</th>
<th>Control</th>
<th>$P$ Value Classic vs Control</th>
<th>$P$ Value Atypical vs Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>PWV, m/s</td>
<td>6.4 (4.5–17.8)</td>
<td>7.2 (5.6–9.7)</td>
<td>6.6 (4.8–9.7)</td>
<td>0.59</td>
<td>0.24</td>
</tr>
<tr>
<td>FMD, %</td>
<td>4.7 (0.8–17.4)</td>
<td>5.4 (0.6–7.5)</td>
<td>5.8 (1.9–13.6)</td>
<td>0.02</td>
<td>0.16</td>
</tr>
<tr>
<td>IMT, mm</td>
<td>0.65 (0.43–0.96)</td>
<td>0.64 (0.48–0.98)</td>
<td>0.60 (0.39–1.09)</td>
<td>0.02</td>
<td>0.22</td>
</tr>
<tr>
<td>AGES</td>
<td>1.9 (1.3–3.9)</td>
<td>2.5 (1.5–3.4)</td>
<td>1.3 (1.9–2.9)</td>
<td>0.58</td>
<td>0.002</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Classic</th>
<th>Atypical</th>
<th>Control</th>
<th>$P$ Value Classic vs Control</th>
<th>$P$ Value Atypical vs Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>PWV, m/s</td>
<td>6.3 (4.5–7.9)</td>
<td>6.8 (6.1–9.2)</td>
<td>6.1 (5.3–9.7)</td>
<td>0.53</td>
<td>0.26</td>
</tr>
<tr>
<td>FMD, %</td>
<td>2.9 (0.8–7.9)</td>
<td>5.4 (0.6–7.5)</td>
<td>5.9 (2.1–8.5)</td>
<td>0.01</td>
<td>0.36</td>
</tr>
<tr>
<td>IMT, mm</td>
<td>0.67 (0.50–0.96)</td>
<td>0.62 (0.56–0.98)</td>
<td>0.59 (0.40–0.76)</td>
<td>0.01</td>
<td>0.22</td>
</tr>
<tr>
<td>AGES</td>
<td>1.9 (1.4–3.9)</td>
<td>2.0 (2.0–2.7)</td>
<td>1.8 (1.4–2.6)</td>
<td>0.10</td>
<td>0.008</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Classic</th>
<th>Atypical</th>
<th>Control</th>
<th>$P$ Value Classic vs Control</th>
<th>$P$ Value Atypical vs Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>PWV, m/s</td>
<td>6.8 (5.1–17.8)</td>
<td>7.5 (5.6–9.7)</td>
<td>6.8 (4.4–8.8)</td>
<td>0.78</td>
<td>0.45</td>
</tr>
<tr>
<td>FMD, %</td>
<td>5.4 (1.8–17.4)</td>
<td>5.5 (4.2–5.5)</td>
<td>5.7 (1.9–13.6)</td>
<td>0.23</td>
<td>0.47</td>
</tr>
<tr>
<td>IMT, mm</td>
<td>0.65 (0.43–0.88)</td>
<td>0.66 (0.48–0.89)</td>
<td>0.60 (0.39–1.09)</td>
<td>0.39</td>
<td>0.58</td>
</tr>
<tr>
<td>AGES</td>
<td>1.9 (1.3–2.8)</td>
<td>2.5 (1.5–3.4)</td>
<td>2.0 (1.3–2.9)</td>
<td>0.51</td>
<td>0.06</td>
</tr>
</tbody>
</table>

$P$ values are calculated for the differences among the 3 groups (classic, atypical, and control group). PWV indicates pulse wave velocity; FMD, flow-mediated dilation; IMT, intima-media thickness; AGES, advanced glycation end products.

Figure 1. Intima-media thickness (IMT) in men (A) and women (B) with classic phenotypes compared with controls, according to age. In men, the slope of the regression line is comparable, whereas the intercept is 0.12 mm higher in classic men compared with controls ($P<0.001$). In women, the intercept and slope did not differ. C, In classic men, flow-mediated dilation (FMD) corrected for age is 1.8% lower compared with controls ($P=0.02$), whereas the slopes are comparable. D, In classically affected women, FMD is comparable to controls.
Atypical Patients
In the atypical patients, only IMT and left ventricular mass were associated (Table 3).

Classically affected men all showed markedly elevated lysoGb3, as well as an increase in IMT compared with controls. Classically affected women showed more modest abnormalities in plasma lysoGb3 and IMT. The atypical cases with no increased lysoGb3 showed no clear differences compared with controls. In the total cohort of classically affected male and female patients, there were no correlations between IMT and plasma lysoGb3 or pretreatment lysoGb3 (lysoGb3 levels before initiation of treatment; Figure 2). A more detailed analysis of female patients with classic Fabry disease alone revealed that their lysoGb3 levels were associated with IMT thickness. When adjusted for age, lysoGb3 (median, 6 nmol/L [range, 2–11 nmol/L]) at the time of evaluation was associated with a trend toward increase in IMT ($p=0.08$), and a significant correlation between pretreatment lysoGb3 (median, 8 nmol/L [range, 2–24 nmol/L]) and IMT was observed (0.006-mm increase in IMT per 1-nmol/L pretreatment lysoGb3 increase; $p=0.03$). In men, neither lysoGb3 (median, 24 nmol/L [range, 7–87 nmol/L]) nor pretreatment lysoGb3 added to the prediction of the IMT thickness.

For FMD a slightly different pattern was observed. In classically affected patients, there was a trend toward a lower FMD with increasing lysoGb3 level ($p=0.07$). There was a correlation with pretreatment lysoGb3: a 0.35% decrease in FMD per 10-nmol/L increase of pretreatment lysoGb3 ($p=0.01$) and a trend when adjusted for age, sex, and cardiovascular risk factors ($p=0.07$). Analysis for men and women with classic Fabry disease separately again only showed a significant correlation in women ($p=0.01$). There were no correlations between lysoGb3 and PWV or lysoGb3 and AGES.

Lifetime Exposure to LysoGb3 and Aging
There were modest correlations between IMT and lifetime exposure to lysoGb3 ($R^2=0.21$; $p<0.001$) and FMD and lifetime exposure to lysoGb3 ($R^2=0.12$; $p=0.01$) in the

### Table 3. IMT and Clinical Disease Manifestations: Univariate Analysis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Classic Phenotypes Only (n=57)</th>
<th>Atypical Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>eGFR, mL/min/1.73 m²</td>
<td>$-7.6$ (2.8) 0.009</td>
<td>$-5.6$ (7.7) 0.47</td>
</tr>
<tr>
<td>Microalbuminuria, mg/24 h</td>
<td>24.9 (35.2) 0.48</td>
<td>7.9 (30.5) 0.81</td>
</tr>
<tr>
<td>LVmass, g/m².7</td>
<td>33.1 (7.7) &lt;0.001</td>
<td>15.4 (5.1) 0.02</td>
</tr>
<tr>
<td>eGFR, mL/min/1.73 m²</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microalbuminuria, mg/24 h</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVmass, g/m².7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVH</td>
<td>$6.7$ (2.4–18.5) &lt;0.001</td>
<td>2.2 (0.7–7.1) 0.19</td>
</tr>
<tr>
<td>WML</td>
<td>$4.0$ (1.8–9.1) 0.001</td>
<td>2.7 (0.6–11.1) 0.18</td>
</tr>
</tbody>
</table>

Changes (B-value) in disease parameters and odds ratios (OR) are presented per 0.1-mm increase in IMT. LVmass indicates left ventricular mass; LVH, left ventricular hypertrophy; WML, white matter lesions; eGFR, estimated glomerular filtration rate.

**Atypical Patients**

In the atypical patients, only IMT and left ventricular mass were associated (Table 3).

**IMT, FMD, PWV, AGES, and Plasma LysoGb3**

Classically affected men all showed markedly elevated lysoGb3, as well as an increase in IMT compared with controls. Classically affected women showed more modest abnormalities in plasma lysoGb3 and IMT. The atypical cases with no increased lysoGb3 showed no clear differences compared with controls. In the total cohort of classically affected male and female patients, there were no correlations between IMT and plasma lysoGb3 or pretreatment lysoGb3 (lysoGb3 levels before initiation of treatment; Figure 2). A more detailed analysis of female patients with classic Fabry disease alone revealed that their lysoGb3 levels were associated with IMT thickness. When adjusted for age, lysoGb3 (median, 6 nmol/L [range, 2–11 nmol/L]) at the time of evaluation was associated with a trend toward increase in IMT ($p=0.08$), and a significant correlation between pretreatment lysoGb3 (median, 8 nmol/L [range, 2–24 nmol/L]) and IMT was observed (0.006-mm increase in IMT per 1-nmol/L pretreatment lysoGb3 increase; $p=0.03$). In men, neither lysoGb3 (median, 24 nmol/L [range, 7–87 nmol/L]) nor pretreatment lysoGb3 added to the prediction of the IMT thickness.

For FMD a slightly different pattern was observed. In classically affected patients, there was a trend toward a lower FMD with increasing lysoGb3 level ($p=0.07$). There was a correlation with pretreatment lysoGb3: a 0.35% decrease in FMD per 10-nmol/L increase of pretreatment lysoGb3 ($p=0.01$) and a trend when adjusted for age, sex, and cardiovascular risk factors ($p=0.07$). Analysis for men and women with classic Fabry disease separately again only showed a significant correlation in women ($p=0.01$). There were no correlations between lysoGb3 and PWV or lysoGb3 and AGES.

**Lifetime Exposure to LysoGb3 and Aging**

There were modest correlations between IMT and lifetime exposure to lysoGb3 ($R^2=0.21$; $p<0.001$) and FMD and lifetime exposure to lysoGb3 ($R^2=0.12$; $p=0.01$) in the

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**Figure 2.** A, Intima-media thickness (IMT) and globotriaosylsphingosine (lysoGb3) in Fabry disease men and women with classic phenotypes (n=57) and (B) in atypical patients (n=10). Below (C) flow-mediated dilation (FMD) and lysoGb3 in Fabry disease men and women with classic phenotypes (n=46) and (D) atypical patients (n=8).
classic Fabry disease cohort. Similar observations were found in men and women separately.

**Discussion**

Present results show that increased carotid IMT and decreased brachial FMD are associated with lysoGb3 level and occur despite treatment. In the atypical patients, all with very low lysoGb3 levels, IMT, FMD, and PWV were comparable to controls. The atypical patients from 3 different families were only very mildly affected, with the exception of the index cases who presented with renal insufficiency (n=2) and multiple transischemic attacks (n=1). The latter patient also had the highest IMT, despite very low lysoGb3 levels but having another cardiovascular risk factor (smoking). The AGES in atypical men were increased, possibly because of other cardiovascular risk factors, such as (a trend toward) a higher total cholesterol/high-density lipoprotein profile and body mass index compared with controls (Table 1).

In classically affected men, IMT was increased compared with controls. This is consistent with previous cross-sectional studies and one longitudinal study in untreated and treated Fabry disease patients.6,21–23 However, in classically affected women, IMT was comparable to healthy controls, which is in contrast with one other report.6 However, in that study there was a mean age difference of 6 years between patients and controls, which might be an explanation.6 FMD was decreased in classically affected men and comparable to healthy controls in female patients. There are few studies on FMD in Fabry disease reporting that FMD is impaired, but these studies did not differentiate the results for sex.21,22,24–26 PWV was not different between patients and controls, despite an increased IMT illustrating that an increase in IMT does not necessarily lead to increased arterial stiffness.27 A large proportion of patients received antihypertensive medication (including angiotensin-converting enzyme/angiotensin receptor blocker medication), which might have contributed to a lower PWV. Although the AGES were not different between classic patients and controls, an increase in AGES was associated with a decrease of renal function, which is in line with a previous study.28

Carotid IMT, brachial artery FMD, and PWV are indicators for atherosclerosis.38–40 No atherosclerosis of significance was observed, confirming observations by others.6,7,41–43 Increased IMT was related to some disease manifestations but lost significance when adjusted for age and sex. In general, an increase in IMT is associated with an increased risk of stroke.44 The persistent increase in IMT and occurrence of new WMLs despite ERT are both indicative of ongoing vascular disease manifestations.

The second aim of this study was to evaluate the hypothesis that increased levels of lysoGb3 are associated with increased IMT. Although no difference in IMT thickness was determined between female patients and controls, IMT thickness differed among female patients not explained by age alone (Figure 1B). Apart from age, lysoGb3 was associated with IMT thickness in female patients. However, in classically affected men showing higher IMT and lysoGb3 levels than women, no strict association was discovered. This would suggest that already very low levels of lysoGb3 can add to an increase in IMT, and when a certain ceiling is exceeded, higher lysoGb3 values do not add to a higher IMT. Indeed, if the entire cohort, including classic and atypical patients, is separated into 2 groups, based on the median lysoGb3 value, patients with a lysoGb3 >7 nmol/L showed, on top of age and sex, a 0.044-mm higher IMT compared with those with a lysoGb3 <7 nmol/L (P=0.034). In classically affected patients, there was an association with the pretreatment lysoGb3 only: the IMT was 0.056 mm higher in patients with pretreatment lysoGb3 levels above the median (>10 nmol/L) compared with patients with levels <10 nmol/L independent of age and sex (P=0.02).

Similarly, in the total cohort of classic and atypical patients, a lysoGb3 >7 nmol/L was associated with a 2.2% lower FMD as compared with patients with a lysoGb3 <7 nmol/L after adjustment of age and sex (P=0.02). In classic patients, this was −2.9% in FMD in the case of lysoGb3 >7 nmol/L (P=0.02).

In previous experiments, lysoGb3 induced smooth muscle cell proliferation, which could suggest that an increase in IMT develops first or an increase in IMT and an impaired FMD develop concomitantly. However, the exact pathophysiology in Fabry disease is unclear. Previously we hypothesized that excess of lysoGb3 induces smooth muscle cell proliferation and remodeling, leading to shear stress.19 This may increase expression of both angiotensin 1 receptor and angiotensin 2 receptor, activating integrin-mediated signaling, thereby inducing alterations of extracellular matrix and cytoskeletal composition.45 Increased angiotensin 2 activity before enzyme infusion has been reported recently, which strengthens this hypothesis.46 An alternative explanation for increased IMT in Fabry disease patients could be elevation of sphingosine-1-phosphate levels, although this is not a disease-specific marker, and the exact mechanism for increased sphingosine-1-phosphate levels in Fabry disease could not be established.47–49

A limitation of our study was that PWV and FMD could not be measured in a subset of patients. However the sensitivity analyses did not alter the overall conclusions. Another limitation of our investigation is its cross-sectional design and the fact that a large proportion of patients received ERT, including different products and dosages, as well as antihypertensive treatment. Because the patients receiving angiotensin-converting enzyme/angiotensin receptor blocker medication were in general more severely affected (because of the presence of microalbuminuria and proteinuria), further stratification by angiotensin-converting enzyme/angiotensin receptor blocker was not performed.

**Perspectives**

The present study showed that IMT is increased and FMD decreased in classic Fabry disease, in particular men, all with elevated lysoGb3 levels. The persistence of vascular abnormalities despite ERT and comedication suggests only a modest effect of the current treatment modalities. Longitudinal investigations are warranted, as well as new interventional, aiming at early as well as more effective, lowering of lysoGb3.
Acknowledgments
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Disclosures
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References


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**Novelty and Significance**

_What Is New?_

- IMT is increased and FMD is decreased in men with classic Fabry disease, all with elevated lysoGb3 levels.
- In female patients, lysoGb3 level correlates with IMT and FMD, independent of age, indicating that already low levels of lysoGb3 are associated with vascular abnormalities.
- Abnormalities remain present despite treatment with ERT.

_What Is Relevant?_

- The present results suggest that treatment interventions should ideally result in preventing the increase of IMT and decrease of FMD by early intervention, as well as a more effective lowering of plasma lysoGb3.
- New treatment strategies should be developed.

**Summary**

Already low levels of lysoGb3 are related to vascular changes in Fabry disease that eventually may lead to cerebrovascular complications.

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Vascular Aspects of Fabry Disease in Relation to Clinical Manifestations and Elevations in Plasma Globotriaosylsphingosine

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