Cardiac and Vascular Hypertrophy in Fabry Disease
Evidence for a New Mechanism Independent of Blood Pressure and Glycosphingolipid Deposition

Frédéric Barbey, Noureddine Brakch, Aleš Linhart, Nathalie Rosenblatt-Velin, Xavier Jeanrenaud, Salah Qanadli, Beat Steinmann, Michel Burnier, Tomas Palecek, Jan Bultas, Daniel Hayoz

Objectives—Fabry disease is an X-linked disorder resulting from α-galactosidase A deficiency. The cardiovascular findings include left ventricular hypertrophy (LVH) and increased intima-media thickness of the common carotid artery (CCA IMT). The current study examined the possible correlation between these parameters. To corroborate these clinical findings in vitro, plasma from Fabry patients was tested for possible proliferative effect on rat vascular smooth muscle cells (vascular smooth muscle cell [VSMC]) and mouse neonatal cardiomyocytes.

Methods and Results—Thirty male and 38 female patients were enrolled. LVH was found in 60% of men and 39% of women. Increased CCA IMT was equally present in males and females. There was a strong positive correlation between LV mass and CCA IMT (r²=0.27; P<0.0001). VSMC and neonatal cardiomyocyte proliferative response in vitro correlated with CCA IMT (r²=0.39; P<0.0004) and LV mass index (r²=0.19; P=0.028), respectively.

Conclusions—LVH and CCA IMT occur concomitantly in Fabry suggesting common pathogenesis. The underlying cause may be a circulating growth-promoting factor whose presence has been confirmed in vitro. (Arterioscler Thromb Vasc Biol. 2006;26:839-844.)

Key Words: Fabry disease ■ growth-promoting factor ■ intima–media thickness ■ left ventricular hypertrophy

Fabry is a rare pan-ethnic X-linked lysosomal storage disease resulting from deficient activity of α-galactosidase A (α-Gal A). It is characterized by progressive accumulation of neutral glycosphingolipids (GSL), mainly globotriaosylceramide, in the plasma and within a variety of tissues throughout the body.1 In hemizygous males, Fabry disease manifests itself during childhood and adolescence by acroparesthesia, angiokeratoma, hypohidrosis, and corneal dystrophy. In adulthood, progressive ischemic damage caused by endothelial GSL accumulation in the microvasculature of the kidney, brain, and heart leads to premature death caused by renal failure, stroke, and cardiovascular disease.2 Until recently, heterozygous females were considered as asymptomatic “carriers.” Recent analysis of the Fabry Outcome Survey (FOS) database, however, has demonstrated that heterozygous females are usually affected and may even exhibit the full range of disease manifestations.3 The variability of both symptoms and circulating levels of α-Gal A in heterozygous women can most likely be explained by the Lyon hypothesis, or random X-inactivation.4,5

Recently, several reports have focused on the cardiac and vascular complications of Fabry disease. A high prevalence of left ventricular hypertrophy (LVH), which is mainly progressive, homogeneous and concentric, with well-preserved ejection fraction, and only mild to moderate degrees of diastolic filling impairment, has been observed in male and female patients with Fabry disease.1,6–9 Macrovascular involvement has also been reported but only in hemizygous patients and includes a marked and accelerated increase in intima–media thickness (IMT) of the radial artery, a medium-size muscular artery, and of the common carotid artery (CCA), an elastic artery, accompanied by hyper-distensibility of the arterial wall.10,11

The underlying mechanism leading to progressive LV hypertrophy and increased radial and CCA IMT remains unclear. It does not seem to be caused by lysosomal GSL deposits, because these represent <2% of the LV mass.12,13 The deposits are also disproportionately small compared with the increase of arterial wall mass.14 We therefore hypothesize that a circulating hypertrophy-promoting activity contributes to the cardiac and arterial remodeling observed in Fabry disease.

The first objective of the present study was to investigate LV mass and the CCA IMT in male and female patients with Fabry disease, and to examine whether there is any correlation between the severity of cardiac hypertrophy and CCA IMT. The second objective was to investigate whether plasma from patients with Fabry...
disease is able to induce a hypertrophic response in rat vascular smooth muscle cells and mouse neonatal cardiomyocytes in vitro.

**Methods**

**Clinical Protocol**

Between October 1, 2000 and December 31, 2004, 68 consecutive adults with Fabry disease (30 men, age range 24 to 60 years; 38 women, age range 18 to 80 years), from 20 Czech and Swiss kindred, were enrolled in the study. Blood pressure, echocardiography, and IMT measurements of both CCAs as well as laboratory studies were performed in all patients.

Diagnosis was made at the Charles University Hospital, Prague, and the Lausanne University Hospital. All hemizygous males had the classic form of Fabry disease, according to their clinical manifestations and a level of activity of α-Gal A of <1.5 nmol/h per mL plasma. Diagnosis of heterozygous women was confirmed by mutation analysis, because it is known that the residual activity of α-Gal A in affected females may vary between 11% and 100% of the normal value. The study population included 20 probands and/or their family members (0 to 4 others for each proband). Two males and four females were excluded because of one of the following reasons: age younger than 18 years, pregnancy, or poor cardiac echogenicity.

M-mode and 2-dimensional echocardiography were performed by 2 experienced investigators (X.J. and T.P.) using commercially available equipment (Acuson Sequoia, Siemens) and a 3.5-MHz phased-array transducer. Echocardiographic measurements were taken in triplicates according to American Society of Echocardiography (ASE) criteria. LV mass was calculated according to the ASE convention and expressed as LV mass indexed to body surface area. Interventricular-septum (IVS) thickness was measured at end-diastole and considered as increased for values >11 mm. The IMT of the CCAs was measured with high-resolution imagers (HDI-5000 and Toshiba PowerVision 6000) with a 7.5- to 9-MHz probe. Ultrason detection was performed on the far wall in both CCAs, and the largest measurement of IMT was used for analysis. Longitudinal scans of the CCAs were performed 2 cm proximal to the bifurcation (flow divider). This segment parallel to the skin surface was investigated with minimum gain adjusted to visualize the lumen–intimal and media–adventitial interfaces defining IMT in the far wall along 1 cm of length. Vessel images were stored in telediastole by electrocardiography (ECG) R-triggering and transferred to computer with a program for automatic image analysis (HDI-Laboratory). Intra- and inter-individual variations were 3.5 and 6.4%, respectively, for the CCA.

To determine the normal progression of IMT over time, 324 healthy individuals (208 males and 116 females) were studied between January 2003 and March 2004 in the Department of Vascular Medicine, University Hospital, Lausanne. Scans were analyzed by a single investigator (D.H.) blinded to the origin of the subject populations.

The study was performed according to the Declaration of Helsinki. The Institutional Review Board of the local ethic committees approved the study. All patients gave their written informed consent. The study was performed according to the Declaration of Helsinki.

**In Vitro Study**

To test the hypothesis that plasma from patients with Fabry disease has proliferative activity, plasma was collected from 27 untreated (ie, no enzyme replacement therapy), normotensive Fabry patients (15 males and 12 females) with normal renal function, as well as from 45 untreated women with newly diagnosed hypertension, with a similar CCA IMT as the Fabry patients. These hypertensive women were selected to compare the proliferative activity of their plasma with that of hemizygous male and heterozygous female patients. For proliferation assay controls, we collected plasma from 30 healthy matched subjects (15 males/15 females) from a local population which was serving as a control group for subjects with metabolic syndrome undergoing extensive cardiovascular phenotyping.

Vascular smooth muscle cells (VSMCs) were isolated by collagenase digestion of the aortic media of Wistar-Kyoto rats. Cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% fetal bovine serum and antibiotics. Rat VSMCs were not contaminated with fibroblast or endothelial cells, as evidenced by positive immunostaining of smooth muscle α-actin with fluorescein isothiocyanate (FITC)-conjugated α-actin antibody.

Neonatal cardiomyocytes were isolated from the hearts of 1- to 2-day-old C57BL/6 mice (Charles River Laboratories) as described previously. Briefly, neonatal ventricles were digested in medium containing 0.45 mg/mL collagenase ( Worthington Biochemical Corp, Lakewood, NJ) and 1 mg/mL pancreatin (Life Technologies Inc, San Diego, Calif). Cardiomyocytes and nonmyocyte cells were separated by differential plating (2× 45 minutes). The nonadherent cardiomyocytes were plated on gelatin-coated 96-well plates (15 000 cells/well) in a 3:1 mixture of DMEM and medium199 (M-199) (Invitrogen Corp) supplemented with 10% horse serum and 5% FBS.

Primary cultures of VSMCs (2000 cells/well) at early passage (≤15) and neonatal cardiomyocytes (15 000 cell/well) were seeded on 96-well plates and incubated for 24 hours. Subsequently, cells were washed three times with phosphate-buffered saline and incubated for 16 to 18 hours with 100 μL DMEM containing 12.5% plasma from; (1) patients with Fabry disease; (2) from untreated hypertensive women; and (3) from healthy controls. Cell proliferation was quantified using 2 different methods; the Cell Titer 96 Aqueous nonradioactive cell proliferation assay kit (Promega) (Method 1) and the cell proliferation enzyme-linked immunosorbent assay based on the measurement of BrdUrd incorporation during DNA synthesis (Roche) (Method 2). Cell Titer 96 Aqueous assay is based on measuring the cellular conversion of the colorimetric reagent 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium salt into formazan by dehydrogenase enzymes found only in metabolically active, proliferating cells. The absorbance was measured at 490 nm using a 96-well enzyme-linked immunosorbent assay plate reader. The proliferation assays were performed in a blinded fashion.

Nonradioactive measurement of DNA synthesis technique is based on the incorporation of the pyrimidine analogue BrdUrd instead of thymidine into the DNA synthesis of proliferating cells. After its incorporation into DNA, BrdUrd is detected by immunoassay. As indicated, cells were seeded in 96-well plate and incubated for 24 hours. Thereafter cells were washed 3 times with phosphate-buffered saline (PBS) and incubated for 16 to 18 hours with 100 μL DMEM containing plasma from patients with Fabry disease or control population. BrdUrd (10 mmol/L) was added to the culture medium for over night. After fixation of the cells, a peroxidase (POD)-labeled antibody against BrdUrd (1/100) was added for 2 hours. After washing 3 times with PBS, the POD substrate 3,3’,5,5’-tetramethylbenzidine (TMB) with PBS was added and incubated at room temperature for 30 minutes. The reaction was stopped with acid solution and the absorbance was determined at 450 nm.

**Statistical Analysis**

Results are presented as means±SEM unless otherwise indicated. Correlations of carotid IMT, IVS thickness, or LV mass index with other variables were assessed by simple regression analysis (Pearson’s correlation coefficient). The effects of relevant variables on CCA IMT, IVS thickness, and LV mass index were analyzed by multivariate stepwise regression, and adjustment was performed with the following variables: age, systolic blood pressure and body mass index (BMI). Differences between males and females, or between patients and controls, were compared using Student t test for continuous variables and Fisher exact test for qualitative variables. Statistical significance was considered for P<0.05.

**Results**

**Clinical Protocol**

The demographic and baseline clinical characteristics of the study populations (Fabry disease and controls) are shown in Table 1. Czech and Swiss male and female patients were comparable in terms of age, BMI, systolic and diastolic blood pressure, and BMI. Differences between males and females, or between patients and controls, were compared using Student t test for continuous variables and Fisher exact test for qualitative variables. Statistical significance was considered for P<0.05.
pressure, serum total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, creatinine, smoking habits, and diabetes mellitus. The 2 populations were thus considered homogeneous. All patients were white. Male and female patients differed significantly in terms of serum creatinine. smoking habits, and diabetes mellitus. The 2 populations are not significant; SBP, systolic blood pressure.

Correlations
Univariate analysis revealed a positive correlation between CCA IMT and LV mass \( r^2 = 0.27, P < 0.0001 \) in the normotensive Fabry population. We did not find any significant correlation between CCA IMT and systolic blood pressure. However, there was a significant correlation \( P < 0.001 \) between CCA IMT and age. In the hypertensive female controls, a positive correlation was observed between CCA IMT and age, and systolic blood pressure but not between CCA IMT and LV mass. LV mass was not correlated to systolic blood pressure.

Multivariate stepwise analysis revealed that only age had a significant influence on LV mass in both males \( P = 0.019 \) and females \( P = 0.036 \) with Fabry disease. The same was observed for CCA IMT in hemizygous males and heterozygous females with Fabry disease \( P < 0.001 \) for both genders.

In the hypertensive female controls, age \( P = 0.0051 \) was positively correlated with CCA IMT.

### TABLE 1. Baseline Clinical Characteristics of the Study Populations

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Fabry</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males (n=30)</td>
<td>Females (n=38)</td>
<td>P</td>
</tr>
<tr>
<td>Age, y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40.7±10.9</td>
<td>44.7±2.8</td>
<td>NS</td>
</tr>
<tr>
<td>Height, cm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>174±6.7</td>
<td>163.4±5.8</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Weight, kg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>70.4±11.0</td>
<td>64.8±10.2</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>121.5±2.3</td>
<td>126.3±2.7</td>
<td>NS</td>
</tr>
<tr>
<td>DBP, mm g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>76.4±1.5</td>
<td>76.5±1.5</td>
<td>NS</td>
</tr>
<tr>
<td>Serum total cholesterol, mmol/L</td>
<td>4.85±0.17</td>
<td>5.63±0.16</td>
</tr>
<tr>
<td>Serum HDL cholesterol, mmol/L</td>
<td>1.54±0.11</td>
<td>1.50±0.05</td>
</tr>
<tr>
<td>Serum LDL cholesterol, mmol/L</td>
<td>2.89±0.15</td>
<td>3.51±0.14</td>
</tr>
<tr>
<td>Serum triglycerides, mmol/L</td>
<td>1.28±0.14</td>
<td>1.32±0.08</td>
</tr>
<tr>
<td>Creatinine, μmol/L</td>
<td>155.1±29.6</td>
<td>83.2±2.8</td>
</tr>
<tr>
<td>Diabetes, n</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Smoking, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>8</td>
<td>21</td>
</tr>
</tbody>
</table>

Values are means±SEM.

DBP indicates diastolic blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NS, not significant; SBP, systolic blood pressure.

*P<0.05, **P<0.01; Fabry vs Controls

### TABLE 2. Cardiovascular Echocardiographic Measurements in Patients With Fabry Disease

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Males (n=30)</th>
<th>Females (n=38)</th>
<th>P (Males vs Females)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV mass index, g/m²</td>
<td>159.7±11.5 (normal&lt;134)</td>
<td>105.3±7.3 (normal&lt;110)</td>
<td>0.0004</td>
</tr>
<tr>
<td>IVS thickness, mm</td>
<td>15.1±0.8 (normal&lt;11)</td>
<td>11.4±0.7 (normal&lt;11)</td>
<td>0.003</td>
</tr>
<tr>
<td>IVS thickness&gt;11 mm</td>
<td>23 (77%)</td>
<td>19 (50%)</td>
<td>NS</td>
</tr>
<tr>
<td>CCA IMT, μm</td>
<td>693.7±34.2</td>
<td>661.1±33.1</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means±SEM.

CCA IMT indicates common carotid artery intima-media thickness; IVS, interventricular septum; LV, left ventricular; NS, not significant. Normal values are given in parentheses.
TABLE 3. Clinical Characteristics of the Hypertensive Female Control Population Recruited for the Proliferation Assay

<table>
<thead>
<tr>
<th>Hypertensive Women (n=45)</th>
<th>Mean±SE</th>
<th>95% CI</th>
<th>Mean±SE</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>62.1±6.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>26.4±4.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>160.4±12.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>94.5±9.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum total cholesterol, mmol/L</td>
<td>5.67±0.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum HDL cholesterol, mmol/L</td>
<td>1.88±0.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum LDL cholesterol, mmol/L</td>
<td>3.21±0.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum triglycerides, mmol/L</td>
<td>1.22±0.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum creatinine, µmol/L</td>
<td>88.6±11.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking, present and former</td>
<td>14%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LV mass index, g/m²</td>
<td>91.0±22.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCA IMT, µm</td>
<td>681.9±99.0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SEM. BMI indicates body mass index; LV, left ventricular.

In Vitro Study

The clinical characteristics of the untreated hypertensive women serving as controls are presented in Table 3.

The proliferation indices (method 1) and metabolic activities (method 2) of the rat VSMC and neonatal mouse cardiomyocytes obtained after cell culture with plasma from healthy controls, hypertensive subjects, and Fabry patients are presented in Table 4.

The correlations between rat VSMC and neonatal mouse cardiomyocyte proliferation and carotid IMT and LVM in- 
vivo suggest a novel mechanism that may underlie the unique and is associated with a proliferation factor present in plasma.

Discussion

Here we show that the development of vascular and cardiac hypertrophy in Fabry occurs independently of blood pressure and is associated with a proliferation factor present in plasma. Studies in rat VSMC and mouse neonatal cardiomyocytes in vitro suggest a novel mechanism that may underlie the unique pathogenesis of the cardiovascular hypertrophy.

TABLE 4. Proliferation Index and Metabolic Activity in Rat VSMC and Mouse Neonatal Cardiomyocytes

<table>
<thead>
<tr>
<th>Population</th>
<th>Method 1</th>
<th>Method 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SE</td>
<td>95% CI</td>
</tr>
<tr>
<td>VSMC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy controls M+F</td>
<td>0.148±0.003</td>
<td>0.142–0.154</td>
</tr>
<tr>
<td>M</td>
<td>0.144±0.004</td>
<td>0.136–0.152</td>
</tr>
<tr>
<td>F</td>
<td>0.151±0.004</td>
<td>0.142–0.161</td>
</tr>
<tr>
<td>Hypertensive subjects</td>
<td>0.168±0.012</td>
<td>0.143–0.194</td>
</tr>
<tr>
<td>Fabry patients M+F</td>
<td>0.215±0.017*</td>
<td>0.180–0.251</td>
</tr>
<tr>
<td>M</td>
<td>0.245±0.023</td>
<td>0.196–0.293</td>
</tr>
<tr>
<td>F</td>
<td>0.177±0.023</td>
<td>0.127–0.227</td>
</tr>
<tr>
<td>Cardiomyocytes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertensive subjects</td>
<td>0.556±0.034</td>
<td>0.487–0.625</td>
</tr>
<tr>
<td>Fabry patients M+F</td>
<td>1.107±0.038*</td>
<td>1.029–1.186</td>
</tr>
<tr>
<td>M</td>
<td>1.088±0.063</td>
<td>0.954–1.221</td>
</tr>
<tr>
<td>F</td>
<td>1.132±0.035</td>
<td>1.076–1.209</td>
</tr>
</tbody>
</table>

F indicates female; M, male; VSMC, vascular smooth muscle cells. Method 1: CellTiter 96 One solution Cell Proliferation Assay. Method 2: Cell proliferation ELISA BrdUrd DNA incorporation.

* P<0.05, Fabry patients vs Hypertensive subjects.
We observed a high prevalence of increased LV mass index both in hemizygous male and heterozygous female patients, which is consistent with previous reports. As expected, men were more severely affected than age-matched women with Fabry disease, because they carry a more severe deficit in α-Gal A activity. When a stepwise multivariate analysis was performed, LV mass index correlated with age but not with blood pressure, indicating that the pathogenesis is related to the basic enzymatic defect. Patients with Fabry disease display eccentric LV hypertrophy without systolic dysfunction and/or a severe restrictive hemodynamic filling pattern, with ECG recordings suggesting a genuine increase in muscle mass. In a subset of Fabry patients, cardiomyopathy has been reported to mimic hypertrophic cardiomyopathy, which is a genetic cardiac disease characterized by asymmetrical LV hypertrophy. However, the mechanisms and the pattern of the thickened myocardium in Fabry disease differ from those seen in hypertensive cardiomyopathy or other forms of infiltrative cardiomyopathies, such as amyloid cardiomyopathy, which is characterized by a severe restrictive filling pattern and reduced LV contraction as well as microvoltage electrocardiograms. The most commonly proposed explanation for the pathogenesis of increased LV mass in Fabry disease is progressive deposition of GSL within the myocardium. However, previous reports have demonstrated that the amount of uncleaved GSL found in the myocardium does not exceed 2% of the LV mass, suggesting that other mechanisms may be responsible for myocardial remodeling. The fact that blood pressure was not a significant determinant of LV mass index in our patients was not totally unexpected, because the patient population studied consisted mainly of normotensive or treated hypertensives. Therefore, the narrow range of blood pressure values did not allow demonstrating any significant association between blood pressure and LV mass. Other authors have reported that blood pressure is mainly low or slightly increased in patients with Fabry disease, even in those with renal insufficiency. Low blood pressure in Fabry disease could be partly explained by GSL storage in the peripheral and autonomic nervous systems, leading to sympathetic vasomotor dysfunction.

An increased IMT has been previously described in male patients at the level of the radial artery, which is a medium muscular artery. Our study is the first in which IMT was measured at the level of the CCA. The increase in mean CCA IMT and extrapolated progression rate suggest an accelerated thickening of the CCA in patients with Fabry disease as compared with older healthy controls whose IMT progression rate is well in line with previous publications. Interestingly, the arterial remodeling occurs to the same extent in males and females. This would indicate that the increase in CCA IMT is, at least in part, independent of the level of residual plasma α-Gal A activity. This is therefore the first evidence that macrovascular involvement can also occur in heterozygous females with, importantly, a penetrance comparable to that of age-matched hemizygous males. As discussed regarding cardiac hypertrophy, GSL deposits are disproportionately small compared with the increase in arterial wall mass and cannot account for the vascular hypertrophy.

A close correlation was found between CCA IMT and LV mass index, both in males and females with Fabry disease without hypertension suggesting a common mechanism. Similar observations have been made previously in hypertensive subjects. We have therefore hypothesized that the plasma of Fabry patients contains factor(s) that may trigger the development of cardiac and vascular hypertrophy. Using an in vitro assay we were able to demonstrate that plasma from both hemizygous and heterozygous patients with Fabry disease induces the proliferation of primary cultures of rat VSMC as well as neonatal mouse cardiomyocytes using 2 different proliferation assays. This proliferative effect was more pronounced in plasma from males, known to have no significant residual enzymatic activity, than in plasma from heterozygous females. The proliferative activity of plasma from the latter was higher than that from age-matched hypertensive and healthy control subjects. Moreover, the proliferative activity correlated with CCA IMT and cardiac hypertrophy features in male and female Fabry patients, but not in hypertensive and normotensive controls. The biomechanical stress stimuli induced by hypertension is a well-known risk factor for cardiac and vascular hypertrophy. Hypertensive women, with a comparable CCA IMT, were therefore used as control group to investigate the possible confounding effect of hypertension on the proliferative index. In contrast to the plasma from patients with Fabry, plasma from untreated hypertensive
women had a lower proliferative activity in our assay, again suggesting that it is not the hypertensive phenotype that is responsible for the observed cardiac and vessel wall hypertrophy, but a factor specific to Fabry. Similar mechanisms may also occur in hypertensive subjects. The weak association usually observed between the level of blood pressure and the degree of left ventricular remodeling may be related to the concomitant presence or absence of a trophic activity whose intensity is not necessarily related to the pressure level. Our in vitro results tend to favor the possibility that hyperplasia is a major factor in Fabry, but hypertrophy cannot be ruled out.

It has already been confirmed that plasma a-galactosidase A levels do not correlate with either the severity or the clinical features of the disease in females patients.35 Because of the structural and statistical relationships between CCA IMT and LV mass index in Fabry disease, CCA IMT might now be considered as a clinically relevant indicator of LV mass to be used for clinical follow-up and in intervention studies including enzyme replacement therapy.

In summary, our data demonstrate that vascular remodeling, leading to thickening of the intima-media, occurs to a similar degree in male and female patients with Fabry disease. The striking correlation between LV mass index and CCA IMT, in the absence of arterial hypertension, and the association between the VSMC and cardiomyocyte proliferative response and cardiovascular remodeling, both in males and females, as well as the results of the in vitro experiments, support the hypothesis of the existence of a growth-promoting factor.

Acknowledgments

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References

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