Evidence for a role of sphingosine-1 phosphate in cardiovascular remodelling in Fabry disease

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Aims	A hallmark of Fabry disease is the concomitant development of left-ventricular hypertrophy and arterial intima–media thickening, the pathogenesis of which is thought to be related to the presence of a plasmatic circulating growth-promoting factor. We therefore characterized the plasma of patients with Fabry disease in order to identify this factor.
Methods and results	Using a classical biochemical strategy, we isolated and identified sphingosine-1 phosphate (S1P) as a proliferative factor present in the plasma of patients with Fabry disease. Plasma S1P levels were significantly higher in 17 patients with Fabry disease compared with 17 healthy controls (225 ± 40 vs. 164 ± 17 ng/mL; $P = 0.005$). There was a positive correlation between plasma S1P levels and both common carotid artery intima–media thickness and left-ventricular mass index ($r^2 = 0.47$; $P = 0.006$ and $r^2 = 0.53$; $P = 0.0007$, respectively). In an experimental model, mice treated with S1P developed cardiovascular remodelling similar to that observed in patients with Fabry disease.
Conclusion	Sphingosine-1 phosphate participates in cardiovascular remodelling in Fabry disease. Our findings have implications for the treatment of cardiovascular involvement in Fabry disease.
Keywords	Fabry disease • Left ventricular hypertrophy • Remodelling • Sphingosine-1 phosphate • Intima–media thickness

Introduction

Fabry disease is an X-linked lysosomal storage disorder caused by deficiency of the hydroxylase α-galactosidase A. The disease is characterized by progressive accumulation of neutral glycosphingolipids, mainly globotriaosylceramide (Gb3), in the plasma and within many cell types, including endothelial cells, vascular smooth muscle cells (VSMCs), and cardiomyocytes.[1] During childhood, early signs and symptoms include neuropathic pain, angiokeratomas, hypohidrosis, and gastrointestinal dysfunction. In adulthood, involvement of the microvasculature of the kidney, brain, and heart leads to renal failure, ischaemic stroke, and cardiovascular dysfunction.[2] Although the condition is X-linked, heterozygous females are also affected, and may even exhibit the full range of disease manifestations, but typically with a later onset than in males.[2,3]

Cardiovascular remodelling, independent of changes in blood pressure, is a hallmark of Fabry disease and occurs to a similar degree in male and female patients.[4] The main cardiac finding is concentric left-ventricular hypertrophy (LVH), which usually develops during or after the third decade of life.[5] Affected patients may ultimately develop progressive heart failure, mostly due to diastolic dysfunction.[6] Patients may also present with a marked and accelerated increase in intima–media thickness (IMT) of the radial, brachial, and common carotid arteries (CCA), as well as of the aorta.[4,7,8]

Left-ventricular hypertrophy and increased CCA IMT occur concomitantly in patients with Fabry disease suggesting a common pathogenesis.[4] As the amount of Gb3 deposited represents only a fraction of the abnormal left ventricle and arterial wall thickening (around 1–3%), a mechanism(s) other than lipid deposition must be considered in the pathogenesis of the

cardiovascular remodelling associated with this condition.[9,10] Consistent with this hypothesis, we found previously that plasma from patients with Fabry disease contains a growth-promoting factor that induces VSMC and cardiomyocyte proliferation *in vitro*.[4]

In this report, we have further characterized the plasma of male and female patients with Fabry disease and identified sphingosine-1 phosphate (S1P) as a potent growth-promoting factor that participates in both the cardiac and arterial remodelling associated with the disease.

Methods

This study was approved by the Institutional Review Board of the local ethic committees and complies with the Declaration of Helsinki. All human subjects provided written informed consent. All animal experiments were performed in accordance with the Swiss federal animal regulations and approved by the veterinary office of Vaud.

Study participants

Seventeen adults with Fabry disease (9 men: age range, 25−56 years; 8 women: age range, 24−61 years) from the cohort of patients followed at the Centre Hospitalier Universitaire Vaudois in Lausanne were enrolled in the study. No patient was receiving enzyme replacement therapy (ERT) at baseline. Both groups are part of populations that have been previously described.[4] All hemizygous males had the classic form of the disease, based on clinical manifestations and the detection of α-galactosidase A levels <1.5 nmol/h per mL plasma. Diagnosis of heterozygous females was confirmed by mutation analysis.

To determine the normal plasma level of S1P, 17 healthy individuals, matched for sex and age, were studied. Blood pressure, IMT measurements, and laboratory studies were performed in all patients and healthy controls, and echocardiography in all patients. Left-ventricular mass (LVM) index was measured in the patients and compared with normal reference values according to guidelines of the European society of cardiology.[11]

Reagents

Sphingosine-1 phosphate was purchased from Avantis Polar Lipids, anti-α-actinin antibody from Novocastra, and anti-atrial natriuretic peptide (ANP) antibody from Biomol International.

Cell culture and measurement of vascular smooth muscle cell proliferation

Vascular smooth muscle cells from Wistar rat aorta were isolated and cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum and antibiotics, as previously described.[4] Vascular smooth muscle cells were seeded on 96-well plates and incubated for 12 h. Cells were subsequently washed and incubated with various concentrations of S1P. Cell proliferation was quantified using the cell titre 96 Aqueous non-radioactive cell proliferation assay (promega) following the manufacturer's instructions.

Plasma lipid fraction extraction and fractionation

To identify the circulating growth-promoting factor(s), we isolated the lipid fraction in pooled plasma samples from the 17 patients with Fabry disease and 17 matched healthy individuals. Vascular smooth muscle cells were exposed to extracted plasma and proliferation was measured using the soluble tetrazolium salt (MTS) assay. Fresh heparinized human blood was centrifuged at 3000*g* for 5 min at 4°C. Cell-

free plasma was removed and centrifuged at 14 000*g* for 5 min at 4°C. Each plasma sample was extracted by rocking with 20 volumes of chloroform:methanol (2:1) for 15 min at 37°C and then centrifuged at 14 000*g* for 5 min at 4°C. The supernatants were transferred to fresh tubes, partitioned against one-fifth volume of distilled water at 4°C, and stored overnight prior to two-phase separation. The upper phase was removed following centrifugation and dried under nitrogen. The dry residue was resuspended in 400 µL of serum-free medium and 100 µL applied to cultured VSMCs seeded on a 96-well plate. Cells were cultured for 18 h and proliferation was quantified as described above.

A sample of 5 mL heparinized plasma from five patients was distributed in 2 mL ependorf tubes in 50 µL aliquots then extracted as described earlier. Each of 20 tubes (1 mL of plasma) of dried residues were resuspended in 1 mL water trifluoroacetic acid 1‰ and applied on C18 Sep-Pak cartridge, which was pre-equilibrated with water trifluoroacetic acid 1‰. The bound material was eluted with different concentrations of acetonitrile (0, 20, 40, 60, and 80%). An aliquot of 25 µL from each fraction was dried and resuspended in DMEM then applied to VSMCs; this step was replicated three times.

The highly active fractions from extracted Fabry plasma between 0 and 20% were mixed, dried and resuspended in 40% acetonirile/60% trifluoroacetic acid 1‰ in water. The obtained material was fractionated by gel filtration HPLC using two columns (two protein-pak 60 in tandem, Macrosphere GPC 60 A 7u Alltech) at a flow rate of 0.5 mL/min. An aliquot of 25 µL from each fraction was dried and resuspended in DMEM then applied to VSMC, this was carried out in triplicate. The tandem column was calibrated with somatostatin-28 (3000 Da) and somatostatin-14 (1500 Da).

The highly active fractions of plasma from patients with Fabry disease were mixed, dried, and resuspended in chloroform/methanol (70/30%). The obtained material was fractionated by HPLC on a Vydac C_{18} column. Elution was performed in isocratic chloroform/methanol (70/30%) for 15 min followed with a linear gradient of methanol at a flow rate of 1 mL/min from 30 to 60% in 5 min.

Mass spectrometry analysis

The lipid fraction of plasma from patients with Fabry disease were mixed, dried, resuspended in acetonitrile/acetic acid and analysed by electrospray ionization mass spectrometry (ESI-MS). Electrospray ionization mass spectrometry analysis was performed with a linear ion trap mass spectrometer (Thermo-Fisher), operating in positive ion mode, with data collected from 50 to 1000 amu.

Circulating plasma sphingosine-1 phosphate measurement

Sphingosine-1 phosphate was first measured using solid-phase extraction followed by derivatization with *o*-phthalaldehyde and fluorescence detection, as described by Butter *et al.*[12]

Blood pressure measurements, tissue analysis, and immunohistochemical staining in a Fabry mouse model

One hundred and twenty-nine strain mice were obtained from the Jackson Laboratory. Ten-week-old mice were injected intraperitonealy with S1P 0.4 mg/kg/day (*n* = 8) or with vehicle (phosphate buffered saline; *n* = 8) as a control for 4 weeks. Following 4 weeks of S1P treatment, mean arterial blood pressure was measured in control and S1P-treated mice, as described previously.[13] Subsequently, mice were killed by intravenous injection of pentobarbital 100 mg/kg. Hearts and aorta were collected. Cardiac weight index was determined. Tissue

samples were immersion-fixed in 4% paraformaldehyde, embedded in paraffin, and serial sections were cut for haematoxylin−eosin, ANP, and α-actin staining. Serial sections collected every 4 μm were analysed. Aortic IMT was evaluated by computer-assisted morphometric analysis. All measurements were performed in the same 16 mice.

Statistics

Results are reported as the mean $\pm$ SD unless otherwise indicated. GraphPad Prism Software (San Diego, USA) was used for statistical analysis. Groups were compared using the Kruskal−Wallis non-parametric one-way analysis of variance and Mann−Whitney U tests. Differences were considered significant where P-value was <0.05. Correlations between circulating levels of S1P and CCA IMT and LVM index were assessed by regression analysis (Pearson product-moment correlation coefficient).

Results

Study participants

The demographic and baseline clinical characteristics of the patients and controls are shown in *Table 1*. The male Fabry group differed significantly from the male control group in terms of CCA IMT (725.8 $\pm$ 117.0 vs. 594.0 $\pm$ 62.0 μm; $P = 0.045$). There was no significant difference between the female Fabry group and the female control group. An increase in LVM index was observed in male and female patients with Fabry disease.

Plasma of patients with Fabry disease induces vascular smooth muscle cell proliferation

Extracted plasma from patients with Fabry disease significantly increased VSMC proliferation compared with plasma from healthy subjects (*Figure 1A*, $n = 17$ $P = 0.0078$). The most abundant component present in the fractioned plasma of patients

with Fabry disease peaked at 380.6 Da, corresponding to the mass of S1P (*Figure 1B*).

Plasma sphingosine-1 phosphate levels are significantly increased in Fabry patients compared with healthy subjects

Circulating S1P levels were significantly increased in patients with Fabry disease compared with healthy controls (225 $\pm$ 40 vs. 164 $\pm$ 17 ng/mL; $P = 0.005$) (*Figure 2A*). Males with Fabry disease had significantly increased plasma S1P levels compared with healthy males (213 $\pm$ 31 vs. 161 $\pm$ 19 ng/mL; $P = 0.008$) (*Figure 2B*). Sphingosine-1 phosphate concentrations were not significantly different between heterozygous females and healthy females (198.0 $\pm$ 47.0 vs. 167.5 $\pm$ 15.0 ng/mL, $P = 0.18$); however, some heterozygous females had S1P levels as high as hemizygous males (*Figure 2C*).

Sphingosine-1 phosphate increases vascular smooth muscle cell proliferation *in vitro* in a dose-dependent manner

Sphingosine-1 phosphate significantly increased the proliferation of VSMCs in a dose-dependent manner as evaluated with optic density at 490 nm. Cell proliferation increased from 0.436 $\pm$ 0.0015 in the absence of S1P to 0.432 $\pm$ 0.06 ($P = 0.7$), 0.498 $\pm$ 0.035 ($P = 0.02$), and 0.577 $\pm$ 0.002 ($P = 0.007$) with S1P concentrations of 100 nM, 1 μM, and 10 μM, respectively (*Figure 3A*). This proliferative effect was present at S1P concentrations similar to those measured in the plasma of the patients with Fabry disease. At lower S1P concentrations (100 nM), the planar cell surface area (PCSA) of VSMCs was increased (*Figure 3B*). This suggests a hypertrophic mechanism at the origin of cardiac remodelling. In contrast, at higher S1P concentrations, while VSMC PCSA was

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Table 1 Baseline clinical characteristics of the study populations

Parameters	Males			Females		
	Fabry (*n* = 9)	Controls (*n* = 9)	*P*-value	Fabry (*n* = 8)	Controls (*n* = 8)	*P*-value
Age (years)	42.8 $\pm$ 8.8	50.0 $\pm$ 7.0	NS	40.1 $\pm$ 13.6	49.0 $\pm$ 7.5	NS
Systolic blood pressure (mmHg)	126.1 $\pm$ 12.3	118.0 $\pm$ 6.7	NS	122.7 $\pm$ 16.4	118.0 $\pm$ 9.8	NS
Diastolic blood pressure (mmHg)	75.2 $\pm$ 8.1	73.7 $\pm$ 3.2	NS	74.5 $\pm$ 5.9	69.4 $\pm$ 4.9	NS
Serum total cholesterol (mmol/L)	5.20 $\pm$ 0.99	4.81 $\pm$ 0.72	NS	5.33 $\pm$ 0.72	5.10 $\pm$ 0.49	NS
Serum HDL-cholesterol (mmol/L)	1.48 $\pm$ 0.29	1.50 $\pm$ 0.44	NS	1.51 $\pm$ 0.43	1.55 $\pm$ 0.32	NS
Total/HDL-cholesterol ratio	3.59 $\pm$ 0.25	2.90 $\pm$ 0.65	NS	3.59 $\pm$ 0.43	3.62 $\pm$ 0.10	NS
Serum LDL-cholesterol (mmol/L)	3.13 $\pm$ 0.95	3.0 $\pm$ 0.54	NS	3.20 $\pm$ 0.96	2.93 $\pm$ 0.36	NS
Serum triglycerides (mmol/L)	1.27 $\pm$ 0.56	1.10 $\pm$ 0.43	NS	1.25 $\pm$ 0.39	0.81 $\pm$ 0.31	NS
Diabetes (*n*)	0	0		0	0	
Smoking (%)	22.0	20.0		12.5	25.0	
Left-ventricular mass index (g/m^2)	149.6 $\pm$ 64.3 (normal $\leq$ 115)	−		104.9 $\pm$ 39.3 (normal $\leq$ 96)	−	
Common carotid artery IMT (μm)	725.8 $\pm$ 117.3	594.6 $\pm$ 62.4	0.045	709.4 $\pm$ 193.7	586.7 $\pm$ 81.4	NS

HDL, high-density lipoprotein; IMT, intima−media thickness; LDL, low-density lipoprotein.
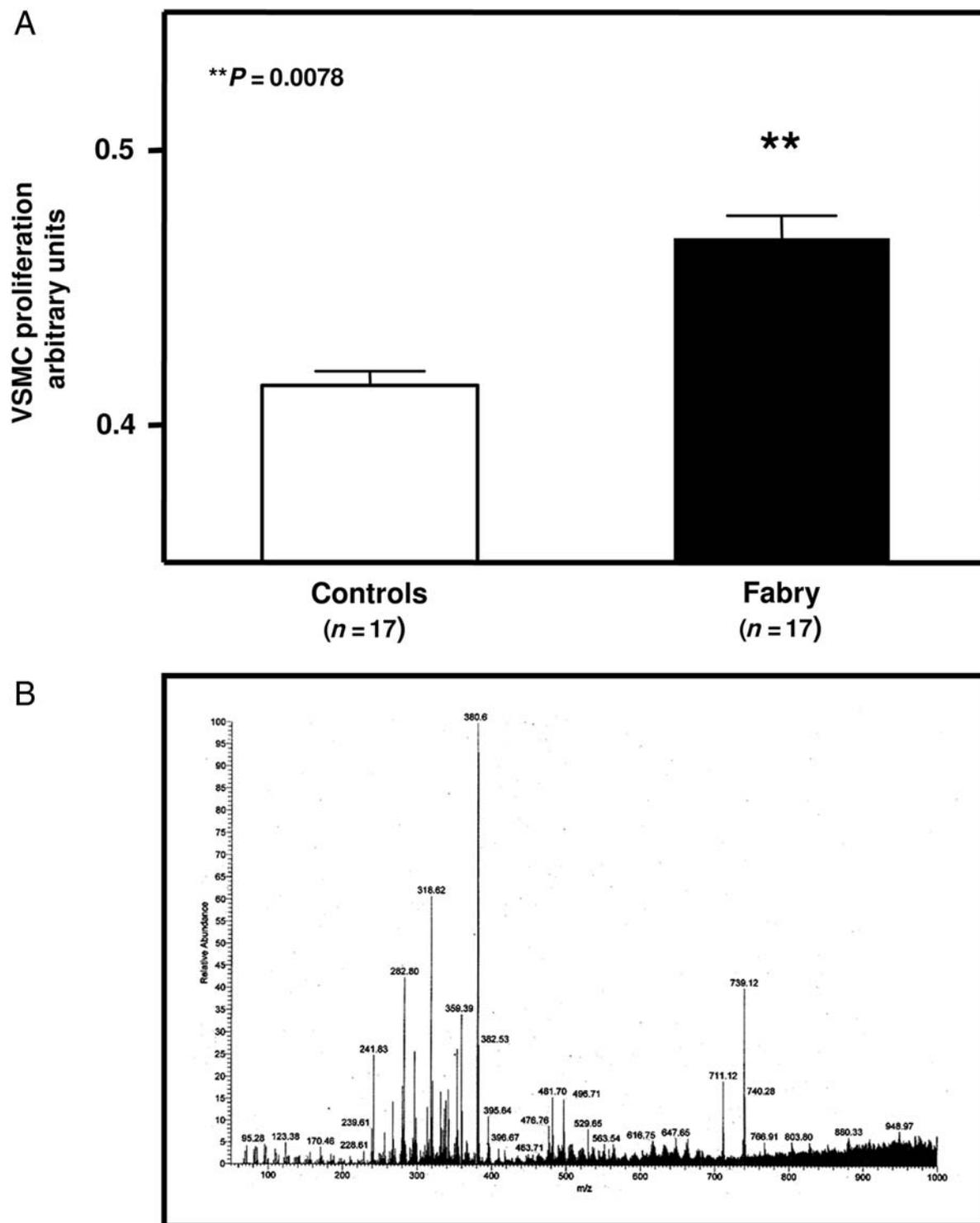
Figure 1 (*A*) Vascular smooth muscle cell proliferation is significantly increased in the presence of extracted lipid fraction from the plasma of $n = 17$ patients with Fabry disease compared with that from $n = 17$ healthy subjects. Results are expressed in arbitrary units and represent the mean of six experiments of absorbance at 490 nm, normalized with absorbance obtained from vascular smooth muscle cells incubated with Dulbecco's modified Eagle's medium alone ($P = 0.0078$). (*B*) Electrospray ionization mass spectrometry showed that the most abundant plasma lipid compound peaked at 380.6 Da, corresponding to the mass of sphingosine-1 phosphate.
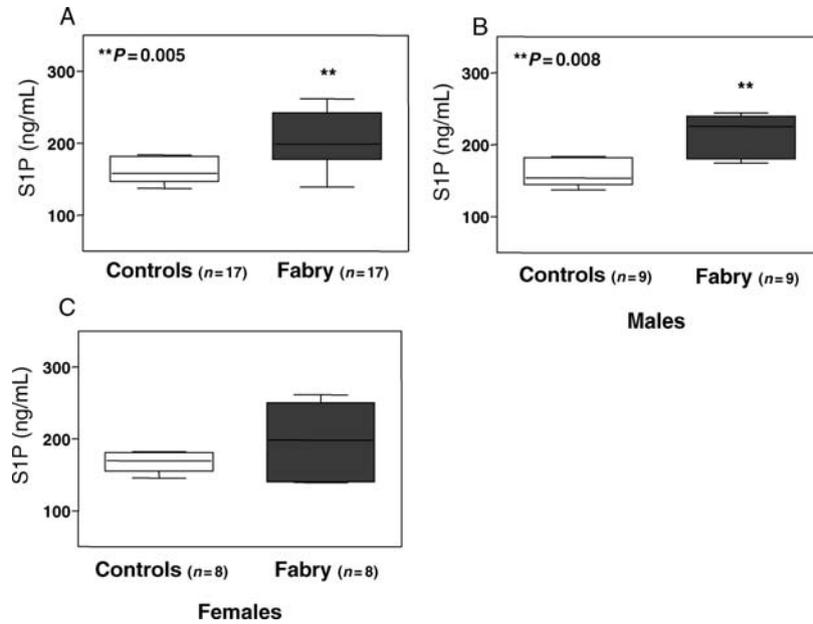
Figure 2 (A) Plasma sphingosine-1 phosphate levels of $n = 17$ healthy subjects vs. $n = 17$ patients with Fabry disease ($P = 0.005$). (B) Plasma sphingosine-1 phosphate levels of $n = 9$ healthy males vs. $n = 9$ males with Fabry disease ($P = 0.008$). (C) Plasma sphingosine-1 phosphate levels of $n = 8$ healthy females vs. $n = 8$ females with Fabry disease.
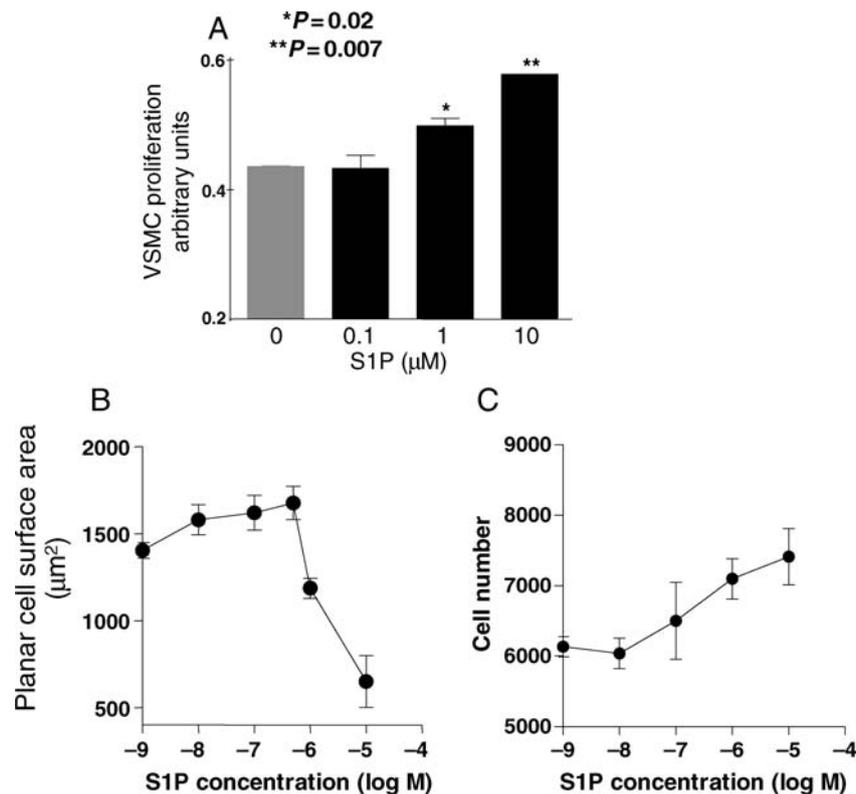

Figure 3 (A) Proliferation of vascular smooth muscle cells exposed to different concentrations of sphingosine-1 phosphate. Results are expressed in arbitrary units and represent the mean of six experiments of absorbance at 490 nm ($P = 0.02$ and $P = 0.007$ vs. untreated vascular smooth muscle cells). (B) Progressive variation of planar cell surface area (PCSA) of vascular smooth muscle cells exposed to increasing concentrations of sphingosine-1 phosphate. PCSA was quantified by computer-assisted morphometry. Values are mean ($\pm$ SD) PCSA of 150 cells. (C) Number of live vascular smooth muscle cells exposed to increasing concentrations of sphingosine-1 phosphate as assessed by fluorescence-activated cell sorting (FACS).
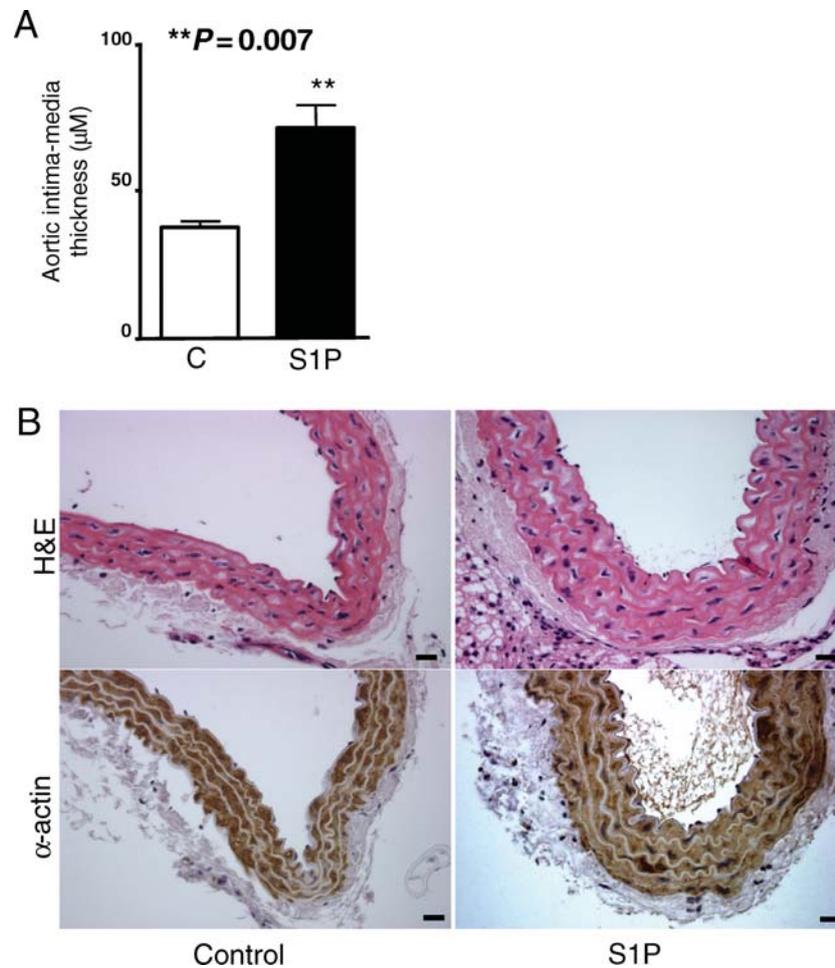
Figure 4 (*A*) Intima–media thickness (μm) in mice treated with vehicle (*n* = 8 control) or sphingosine-1 phosphate (*n* = 8 sphingosine-1 phosphate), 0.4 mg/kg/day intraperitoneally for 4 weeks (*P* = 0.0007). (*B*) Haematoxylin–eosin and α-actin staining of aorta sections from mice treated with vehicle or sphingosine-1 phosphate as under (*A*). Photomicrographs show representative sections of aorta. (Scale bar = 10 μM).

decreased, the number of cells increased as indicated by FACS analysis (*Figure 3C*), suggesting hyperplasia.

Treatment of mice with sphingosine-1 phosphate increases aortic intima–media thickness

Mean aortic IMT was significantly greater in S1P-treated mice than controls (74.1 ± 21.0 vs. 37.5 ± 5.1 μm; *P* = 0.0007) (*Figure 4A*). Haematoxylin–eosin staining of the aortic wall revealed a normal appearance and composition of the intima–media (*Figure 4B*). The predominant cellular component of the intima–media was VSMCs, as revealed by immunostaining for α-actin (*Figure 4B*).

Treatment of mice with sphingosine-1 phosphate induces cardiac hypertrophy

The cardiac weight index of S1P-treated mice was 27% greater than that of control mice (*P* = 0.03) (*Figure 5A*). The development of cardiac hypertrophy was independent of blood pressure as

S1P-treated mice had a lower mean arterial blood pressure than controls (*Figure 5B*). Furthermore, haematoxylin–eosin staining showed a hypertrophic response of cardiomyocytes to S1P. This was confirmed by the presence of diffuse ANP staining in samples from S1P-treated mice compared with patchy staining in control samples (*Figure 5B*).

Plasma sphingosine-1 phosphate levels correlate with common carotid artery intima–media thickness and left-ventricular mass index in Fabry patients

There was a positive correlation between circulating S1P levels and CCA IMT in patients with Fabry disease (r^2 = 0.47; *P* = 0.007, *Figure 6A*). Plasma S1P levels also correlated positively with LVM index in Fabry patients (r^2 = 0.53; *P* = 0.0007, *Figure 6B*). *Figure 6C* shows the correlation between S1P levels and CCA IMT (r^2 = 0.55; *P* = 0.0001) in males and females in the Fabry and control populations.
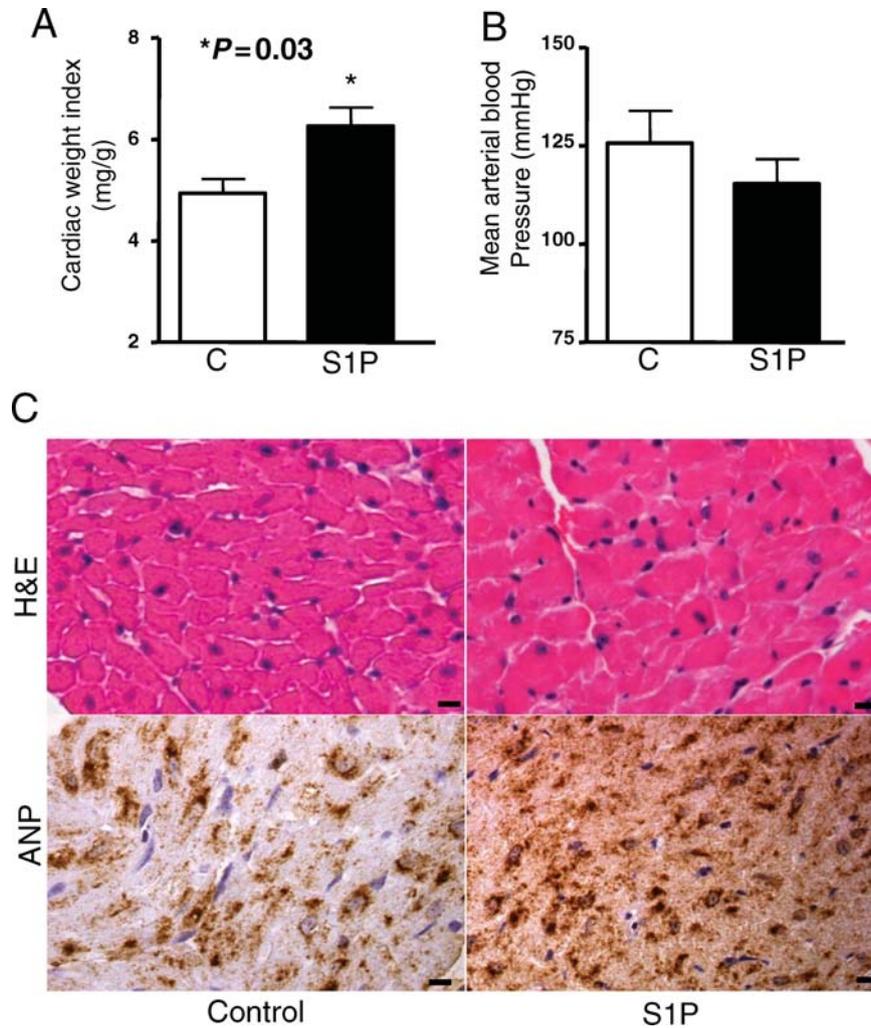
Figure 5 (A) Cardiac weight index in mice treated with vehicle (*n* = 8 control) or sphingosine-1 phosphate (*n* = 8 sphingosine-1 phosphate), 0.4 mg/kg/day intraperitoneally for 4 weeks (*P* = 0.03). (B) Arterial blood pressure of mice treated with vehicle or sphingosine-1 phosphate as under (A). Values are the means of eight animals per group. (C) Haematoxylin−eosin and atrial natriuretic peptide staining of heart sections of mice treated with vehicle or sphingosine-1 phosphate as under (A). Photomicrographs show representative sections of myocardium (Scale bar = 20 μm).

Males with Fabry disease had a greater IMT and higher S1P-values compared with male controls. The ranges of IMT and S1P-values were greater in heterozygous females than in female controls.

Discussion

In the present study, we identified S1P as a biologically active growth-promoting factor involved in cardiovascular remodelling in both males and females with Fabry disease. Male patients had significantly higher plasma S1P levels compared with healthy controls. Moreover, there was a strong correlation between plasma S1P levels and LVM index, and increased CCA IMT in patients with Fabry disease. In addition, S1P-treated mice developed comparable cardiovascular remodelling to that observed in patients with Fabry disease.

We showed previously that vascular remodelling occurs to a similar degree in male and female patients with Fabry disease.[4] Patients with Fabry disease presented with a strong correlation between LVM index and CCA IMT, in the absence of arterial hypertension, suggesting a common pathogenesis to their development. Using an *in vitro* assay, we demonstrated that plasma from both males and females with Fabry disease induced proliferation of rat VSMCs and neonatal mice cardiomyocytes.[4] These results suggested the existence of a circulating growth-promoting factor in Fabry disease. In a recent paper, Aerts *et al.*[14] reported that globotriaosylsphingosine (lyso-Gb3) was markedly elevated in the plasma of male Fabry patients but not in female patients. Interestingly, exposure of VSMCs to lyso-Gb3 promoted cell proliferation. However, the signalling pathway and cardiovascular expression of lyso-Gb3 receptors remain elusive.
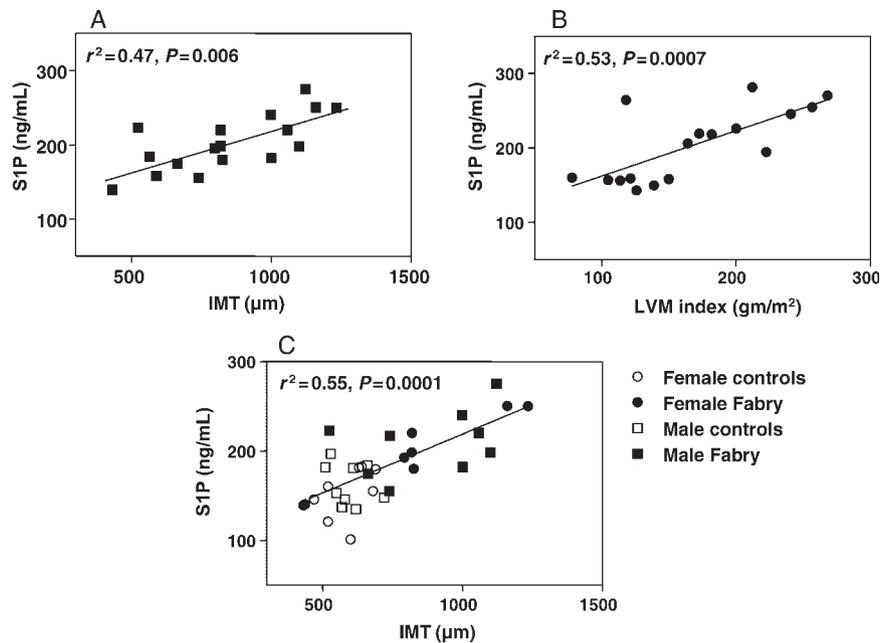
Figure 6 (*A*) Correlation between plasma sphingosine-1 phosphate levels and common carotid artery intima−media thickness (CCA IMT) in the Fabry population. (*B*) Correlation between plasma sphingosine-1 phosphate levels and left-ventricular mass in the Fabry population. (*C*) Correlation between plasma sphingosine-1 phosphate levels and CCA IMT in male and female patients with Fabry disease and controls.

In the present work, we isolated and identified S1P in the plasma of patients with Fabry disease at significantly higher concentrations compared with those seen in control plasma. Females showed a wider range of S1P concentrations than female controls, but the difference was not significant. Interestingly, we found no significant difference in plasma S1P levels between male and female Fabry patients who shared a comparable penetrance of cardiovascular remodelling, as previously shown.[6] Moreover, there was a strong correlation between S1P concentration and both CCA IMT and LVM index, in males and females with Fabry disease. This indicates that cardiovascular remodelling and plasma S1P levels are, at least in part, independent of the level of residual circulating α-galactosidase A, and that plasma S1P, unlike plasma Gb3 and lyso-Gb3, may be considered as a marker of the severity of cardiovascular remodelling in Fabry disease.[14,15]

Ceramide metabolism generates a cascade of bioactive lipids, all of which carry a specific signalling capacity.[16] This sphingolipid signalling network is expressed in the cardiovascular system.[17,18] In healthy subjects, S1P is present in the plasma at higher levels than in the tissues, and exists largely in forms bound to high-density lipoproteins.[19] S1P is generated by the phosphorylation of sphingosine by sphingosine kinases and is irreversibly degraded by a specific lyase that is exclusively located at the endoplasmic reticulum.[20]

Sphingosine-1 phosphate has been shown to induce *in vitro* VSMC proliferation, by a variety of signal transduction pathways including ERK and RhoA,[21,22] and hypertrophy of neonatal rat cardiomyocytes.[23] Our *in vitro* study showed that S1P promotes marked proliferation of VSMCs in a dose-dependent manner. These data demonstrate that S1P is a potent circulating growth-promoting factor because its effect was observed at concentrations similar to those measured in plasma from patients with Fabry disease (plasma concentrations only 1.3 times greater than in healthy controls). Interestingly, at lower S1P concentrations, the VSMC phenotype was mainly hypertrophic, whereas at higher S1P concentrations the VSMC phenotype was predominantly hyperplasic. These findings suggest that both proliferative mechanisms may be involved in the arterial remodelling of Fabry disease, with the participation of the individual mechanisms depending directly on plasma S1P levels. The mechanism responsible for increased plasma S1P levels in patients with Fabry disease has yet to be established.[16]

Overall results, i.e. the increase of plasma S1P concentrations and the strong correlation between S1P concentrations and both CCA IMT and LVM index in patients with Fabry disease, and the *in vitro* effect of S1P on VSMCs, suggest that S1P may be a key factor that triggers the development of LVH and arterial intima−media thickening in Fabry disease. We therefore injected S1P into an *in vivo* model of Fabry disease in mice. Animals developed cardiovascular remodelling that was very similar to that observed in humans with Fabry disease. Image analysis revealed a significant thickening of the aortic IMT relative to control animals. The predominant cellular component of the arterial wall was VSMCs, as revealed by immunostaining for α-actinin. Cardiac weight index was also significantly greater compared with controls. Microscopic analysis revealed a marked hypertrophy of cardiomyocytes at the origin of the increased cardiac mass as assessed by diffuse ANP expression. Significant cardiovascular remodelling developed in S1P-treated mice after only 1 month of daily injections (half-life of S1P, 3−5 min), demonstrating that S1P is also a potent hypertrophic mediator *in vivo*.[4] S1P-treated mice had lower blood pressure than controls. This is consistent with previous

animal studies which reported that systemic administration of S1P may reduce blood pressure.[24]

Sphingosine-1 phosphate interacts with high-affinity G-protein coupled receptors that can sense very small changes in S1P level, thus small increases in S1P may have functional and morphological consequences.[25] Receptors $S1P_{1-3}$ are widely expressed in endothelial cells and VSMCs of the artery tree and in cardiomyocytes,[26–28] and their biological roles have been shown by the generation of knockout mice. The importance of $S1P_1$ in angiogenesis is exemplified by the embryonic lethality of $S1P_1$ knockout.[29] There is evidence that $S1P_1$ and $S1P_3$ receptors promote, whereas $S1P_2$ receptors antagonize, VSMC proliferation *in vitro* in response to S1P.[30,31] Cardiomyocyte hypertrophy appears to be mediated by $S1P_1$ receptors as an anti-$S1P_1$ receptor antibody has been found to block S1P-mediated hypertrophy.[23]

Some reports have suggested that the biologically active high-density lipoprotein compound S1P is responsible for anti-atherogenic actions on vascular endothelial cells of the myocardium.[24,32] Recently, we showed that patients with Fabry disease are somehow protected from atherosclerotic plaques relative to controls with a similar number of cardiovascular risk factors.[33] Our data suggest that the increased plasma S1P concentration in patients with Fabry disease may be in part responsible for the cardiovascular remodelling and the anti-atherogenic effect observed in Fabry disease. These findings open new perspectives that have implications for the treatment of cardiovascular complications in Fabry disease.

In conclusion, our data provide evidence that S1P is a bioactive mediator that participates in the development of cardiovascular remodelling in patients with Fabry disease. Sphingosine-1 phosphate-treated mice developed cardiovascular anomalies similar to those observed in patients with Fabry disease, further supporting the potent cardiovascular effects of S1P. In addition, the strong correlation of plasma S1P levels with LVH and with increased CCA IMT, in the absence of arterial hypertension, provide evidence of the role of S1P as a marker of the severity of cardiovascular remodelling in Fabry disease.

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CARDIOVASCULAR FLASHLIGHT

An indirect shot to the heart

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A 49-year-old man presented with breathlessness. Chest X-ray (*Panels A–C*) showed a foreign body in line with the thoracic vertebra. Further CT imaging confirmed a metallic object embedded in the right ventricle (*Panels D–K*). He had no history of cardiac or thoracic surgery and no history of penetrating trauma. On further questioning it was discovered that he was shot in the back of the right knee with an air-rifle pellet 27 years ago (*Panel L*). This had subsequently migrated into the venous system and embolized to the right heart.

Migration and venous or arterial embolization of penetrating foreign bodies are rare, but recognized.

If they result in obstruction, then percutaneous or surgical retrieval is indicated. In this patient, this finding was considered to be incidental and unrelated to his symptoms. After joint cardiothoracic/interventional cardiology discussion, no further treatment was recommended. He is due to have CT monitoring in 2 years.