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Effect of enzyme replacement therapy on serum asymmetric dimethylarginine levels, coronary flow reserve and left ventricular hypertrophy in patients with Fabry disease

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Abstract

Background. Fabry disease (FD) is a rare disorder and one of the causes of progressive renal and cardiac dysfunction. FD results from an X-linked recessive lysosomal storage disorder caused by a defect in the gene encoding lysosomal α-galactosidase A. Although accumulation of globotriaosylceramide leads to renal and cardiac manifestations, the precise mechanisms remain unclear. Coronary microvascular dysfunction may be one of the causes of cardiac complications in FD. We aimed to assess coronary flow reserve (CFR) and the effect of enzyme replacement therapy (ERT) on coronary microvascular dysfunction.

Methods. Four FD patients who had never received ERT were included. The serum asymmetric dimethylarginine (ADMA) level, as a marker of endothelial dysfunction, was measured. Two-dimensional guided M-mode echocardiography was performed to measure left ventricular wall mass. Adenosine-triphosphate stress transthoracic Doppler echocardiography was used to measure CFR before starting ERT and at 3, 6 and 12 months.

Results. All the patients tolerated ERT without any side effects. At the baseline, two patients had impaired CFR, increased left ventricular mass index (LVMI) and elevated serum ADMA levels. Twelve months after starting ERT, CFR was increased in all patients, and LVMI and serum ADMA levels decreased in two patients. Furthermore, serum ADMA levels significantly correlated with CFR ($r = -0.576$, $P < 0.05$) and LVMI ($r = 0.874$, $P < 0.0001$).

Conclusions. The results suggest that ERT prevented the progression of cardiac abnormalities, possibly by improving coronary microvascular dysfunction. ADMA may be a useful surrogate marker in FD.

Keywords: asymmetric dimethylarginine; coronary flow reserve; enzyme replacement therapy; Fabry disease; left ventricular mass index

Introduction

Fabry disease (FD) is an X-linked recessive lysosomal storage disorder caused by a defect in the gene that encodes lysosomal α-galactosidase A and is known to affect various tissues including the kidney and heart. Patients with FD manifest a variety of clinical symptoms, such as acroparesthesias, angiokeratoma, hypohidrosis, corneal opacities, stroke, renal disorder and cardiac abnormalities. The manifestations are thought to be due to progressive accumulation of globotriaosylceramide (GL-3) and other glycosphingolipids within various cells such as vascular endothelium, cardiomyocytes, glomerular cells and renal tubular cells. The precise pathophysiological mechanisms of FD remain unclear, and coronary microvascular dysfunction appears to be one of the causes of cardiac complications in FD [1–4]. In several studies, GL-3 accumulation within vascular endothelial cells in the kidney and heart was cleared after enzyme replacement therapy (ERT) [5–7].

Many studies have demonstrated that nitric oxide (NO) plays an important role in the progression of endothelial dysfunction, atherosclerosis and chronic kidney disease (CKD) [8–11]. NO is synthesized by endothelial, neuronal and macrophage isoforms of the enzyme NO synthase (NOS). Asymmetric dimethylarginine (ADMA) is an endogenous competitive inhibitor of NOS, and serum ADMA levels have been suggested to be a surrogate marker of endothelial dysfunction and/or angiosclerosis.

Recently, we revealed a significant relationship between ADMA, glomerular filtration rate and endothelial dysfunction of the coronary and peripheral arteries [12, 13]. From the results of not only our studies but also various earlier studies, we thought that ADMA may be involved in the underlying mechanism connecting the two pathological conditions between the kidney and the heart and may be a useful biochemical marker even in FD.

The aim of our study was to examine the effect of ERT on cardiac abnormalities and to determine the relationship between ADMA, coronary microvascular function and left ventricular hypertrophy (LVH) in FD patients.

Materials and methods

Study population

Four FD patients who had never received ERT were included in the study. For these patients, α-galactosidase A (agalsidase β, Fabrazyme®; Genzyme Corp., Cambridge, MA, or agalsidase α, Replagal®; Shire plc, Dublin, Ireland) was administered intravenously every 2 weeks for a period of 12 months (at a dosage of 1 or 0.2 mg/kg body weight, respectively). Ten hypertensive patients without diabetes mellitus, inflammatory disease, infection, CKD or any cardiovascular complications were also evaluated as controls. In addition, 20 healthy volunteers (mean age: 29 ± 4 years) were also evaluated to determine serum ADMA levels. Patients were excluded only if they did not agree to participate in the study. For the FD patients, we prospectively performed blood examination, echocardiography, exercise stress myocardial scintigraphy and coronary flow reserve (CFR) measurements before starting ERT and at 3, 6 and 12 months. There were no changes in medication during the study period. The experimental protocols were approved by the appropriate institutional review committee and performed in accordance with the Helsinki Declaration of 1975, as revised in 2008.

Measurement of CFR

Transthoracic Doppler echocardiography, which has been described previously [14], was used to measure CFR. The ultrasound beam was transmitted toward the heart to visualize coronary blood flow in the distal portion of the left anterior descending (LAD) coronary artery by color Doppler flow mapping. First, the left ventricle was imaged in cross-section along the longitudinal axis, and then, the ultrasound beam was inclined laterally. Next, coronary blood flow in the distal LAD was examined under the guidance of color Doppler flow mapping. After positioning a sample volume on the color signal in the distal LAD, Doppler spectral tracings of flow velocity were recorded by fast Fourier transformation analysis. All of the results were recorded on 0.5-in. S-VHS videotapes for off-line analysis.

We first recorded baseline spectral Doppler signals in the distal LAD. Adenosine-5′-triphosphate was administered (140 μg/kg/min, i.v.) for 3 min to record spectral Doppler signals during hyperemic conditions. All of the patients underwent continuous heart rate and blood pressure monitoring throughout the study period.

An ultrasound system computer was used to trace the contour of the spectral Doppler signal for off-line analysis of coronary flow velocity. Peak diastolic velocity (PDV) was measured at the baseline and peak hyperemic conditions. Measurements were averaged over three cardiac cycles. CFR was defined as the ratio of hyperemic to basal PDV. We adopted a CFR of <2.0 as the cut-off value for the presence of significant coronary microvascular disease, as used previously [13].

Echocardiographic study

Two-dimensional guided M-mode echocardiography was performed to measure left ventricular wall mass. Left ventricular diastolic diameter (LVDd), left ventricular systolic diameter, diastolic thickness of the left ventricular posterior wall (LVPWT) and interventricular septum (IVST) were assessed in M-mode images in the parasternal longitudinal axis view. The M-mode analysis was performed according to the guidelines of the American Society of Echocardiography. The following formula [15] was used to calculate the left ventricular mass index (LVMI) (g/m^2):

$$\mathrm{LVMI}\,(\mathrm{g/m^2}) = \frac{[1.04 \times \{(\mathrm{IVST} + \mathrm{LVPWT} + \mathrm{LVDd})^3 - \mathrm{LVDd}^3\} - 13.6]}{\text{Body surface area}}$$

Measurement of serum ADMA levels and other laboratory determinations

On the same day as the CFR measurement, venous blood was collected from the patients after a 20-min period of supine rest in the morning following overnight fasting. In two dialysis patients, according to Japanese Society of Dialysis Therapy (JSDT) guidelines, blood samples were collected before each dialysis session at 2-day intervals. Blood was drawn into chilled citrate tubes on ice. Serum was separated by centrifugation at 2500 *g* for 10 min at 4°C and stored at −20°C until analysis. Serum ADMA levels were determined at SRL Inc. (Tokyo, Japan) with a novel high-performance liquid chromatography method. Other laboratory tests were performed by standardized clinical laboratory methods. The CKD Epidemiology Collaboration (CKD-EPI) equation was used to estimate the glomerular filtration rate for evaluation of renal function.

Statistical analysis

We used the computer software application StatView 5.0 (SAS Institute, Cary, NC) for all statistical analyses. Values are presented as means ± SDs. For continuous variables, the Mann–Whitney *U*-test was used to analyze the significance of differences between two groups. One-way ANOVA followed by the Turkey–Kramer test were used to assess data among the three groups. Pearson's correlation coefficient was used to analyze relationships between variables. Results with P-values <0.05 were considered to be statistically significant.

Results

Patient characteristics

The main characteristics of the subjects are shown in Table 1. Among the study patients, three had a history of stroke (Cases 1, 3 and 4) and two had received hemodialysis therapy (Cases 1 and 4). At the baseline, α-

Table 1. Patient characteristics

	Case 1	Case 2	Case 3	Case 4	Normal values
Age (years old)	45	19	71	51	
Sex	F	M	F	M	
HD duration (years)	12	—	—	23	
Hypertension	(+)	(–)	(+)	(–)	
Diabetes mellitus	(+)	(+)	(–)	(–)	
Hyperlipidemia	(–)	(–)	(–)	(–)	
Stroke	(+)	(–)	(+)	(+)	
Acroparesthesias	(–)	(–)	(+)	(–)	
Hypohidrosis	(–)	(–)	(–)	(–)	
Angiokeratoma	(–)	(–)	(–)	(–)	
Corneal opacities	(+)	(–)	(–)	(–)	
Chest pain	(+)	(–)	(+)	(–)	
Cr (μmol/L)	872.4	51.1	65.6	976.1	
eGFR (mL/min/1.73 m^2)	—	139	68	—	
RBC ($\times 10^{12}$/L)	4.10	5.53	3.05	4.20	
Hemoglobin (g/L)	114	161	103	121	
TP (g/L)	68	69	68	79	64–82
Alb (g/L)	39	45	39	36	35–52
T-chol (mmol/L)	3.23	6.21	4.86	4.09	3.36–6.21
TG (mmol/L)	2.01	9.04	3.92	1.26	0.34–2.03
HDL (mmol/L)	0.98	0.96	1.14	0.96	≥1.03
HbA1c (%)	6.1	12.6	5.0	4.6	4.3–5.8
Ca (mmol/L)	2.22	2.42	2.22	2.59	2.12–2.55
P (mmol/L)	1.68	1.19	0.87	2.16	0.77–1.39
BNP (ng/L)	321.5	2.4	59.6	272.2	≤18.4
hsCRP (nmol/L)	28.6	45.7	12.4	21.9	<28.6
ADMA (ng/L)	0.85	0.48	0.55	0.70	—
α-Galactosidase activity					
Dried blood spot (Agal U)	16.9	11.2	15.1	15.2	≥20
Leukocyte (nmol/mg protein/h)	61.2	52.3	75.5	32.1	49.8–116.4

HD, hemodialysis; Cr, creatinine; eGFR, estimated glomerular filtration rate; RBC, red blood cell count; TP, total protein; Alb, albumin; T-chol, total cholesterol; TG, triglyceride; HDL, high-density lipoprotein cholesterol; HbA1c, hemoglobin A1c; BNP, brain natriuretic peptide; hsCRP, high-sensitivity C-reactive protein; ADMA, asymmetric dimethylarginine.

galactosidase activity was low in all patients, as determined by the measurement of the dried blood spots in a fluorescence assay using 4-methylumbelliferyl. In contrast, α-galactosidase activity measured in leukocytes was low in only one patient (Case 4). Serum ADMA levels were significantly higher in the FD group than in the hypertensive control and healthy control groups (Figure 1A). As shown in Table 2, the LVMI increased in three patients (Cases 1, 3 and 4), and systolic function decreased in two (Cases 1 and 4). Although findings of ischemia were not observed using exercise stress myocardial scintigraphy in all the study patients, CFR decreased in three patients (Cases 1, 3 and 4) and two had angina-like chest pain (Cases 1 and 3). Needless to say, CFR was significantly lower in the FD group than in the hypertensive control group (Figure 1B).

Change of ADMA levels, CFR and LVMI during ERT

We evaluated the changes in serum ADMA levels, CFR and LVMI after starting ERT, and these parameters mostly improved 12 months after starting ERT compared with the values at baseline (Figure 2A–C). We also evaluated changes in other laboratory data (Table 3). Two study patients had angina-like chest pain before the treatment; their symptoms disappeared following ERT. Unfortunately, because of a worldwide shortage of available recombinant α-galactosidase, Cases 2 and 4 did not receive sufficient ERT for the last 2 months of treatment.

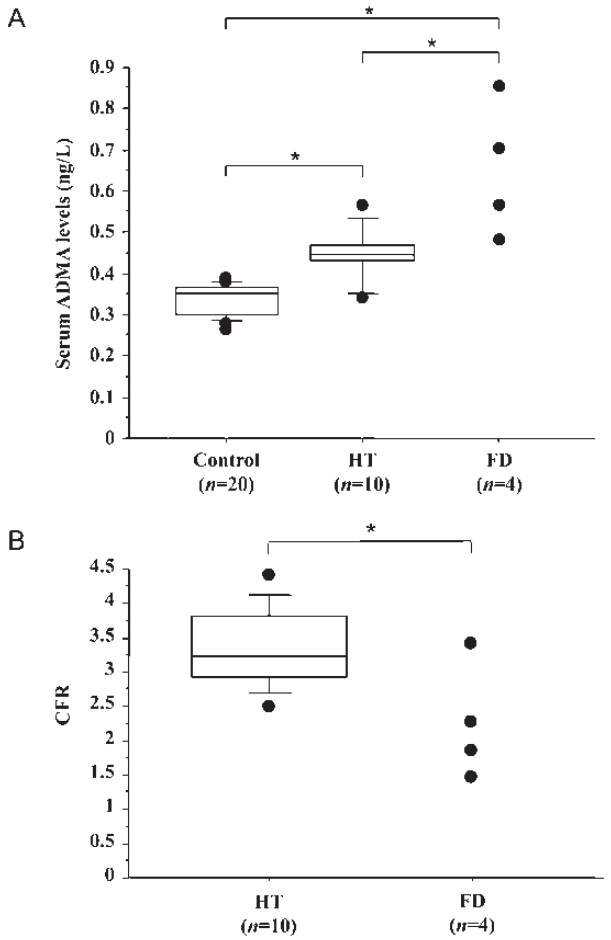

Fig. 1. Serum ADMA levels and CFR in the control patients and FD patients. (**A**) Serum ADMA levels in the healthy subjects, hypertensive patients and FD patients. (**B**) CFR in the hypertensive patients and FD patients. *$P < 0.05$

Table 2. Echocardiographic parameters at baseline

	Case 1	Case 2	Case 3	Case 4
LAD (mm)	41	39	41	44
LVDd (mm)	49	45	48	47
LVDs (mm)	37	31	33	33
IVST (mm)	16	10	12	12
PWT (mm)	13	10	11	12
FS (%)	24	30	31	29
EF (%)	58	60	66	57
LVMI (g/m^2)	203.2	93.8	158.2	151.9
E/*A* ratio	0.77	2.26	0.62	0.65
DcT (ms)	346	146	200	212
IVCD (mm)	16	8.9	5.7	13
CFR	1.77	3.41	2.31	1.45

LAD, left atrial dimension; LVDd, left ventricular end-diastolic dimension; LVDs, left ventricular end-systolic dimension; IVST, interventricular septum thickness; PWT, posterior wall thickness; FS, fractional shortening; EF, ejection fraction; LVMI, left ventricular mass index; DcT, deceleration time; IVCD, inferior vena cava diameter; CFR, coronary flow reserve.

Correlation between ADMA, CFR and LVMI

We examined the correlation of serum ADMA levels with CFR and LVMI and found that serum ADMA levels were significantly correlated with both CFR ($r = -0.576$, $P < 0.05$) and LVMI ($r = 0.874$, $P < 0.0001$) (Figure 3A and B).

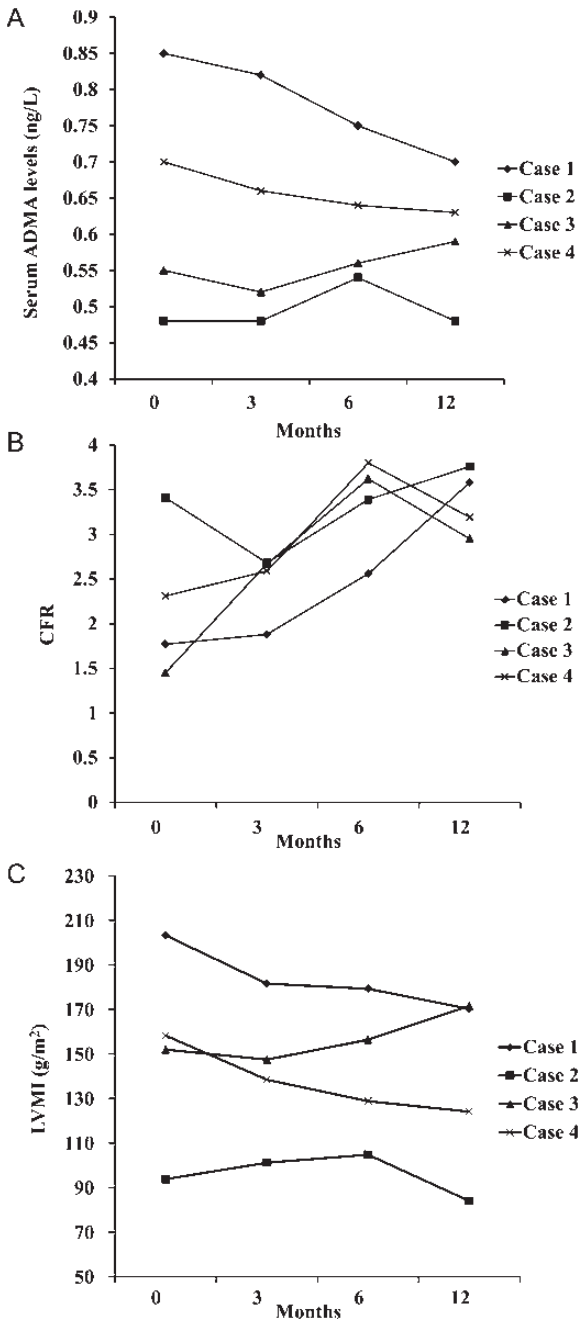

Fig. 2. Change of CFR, LVMI and ADMA during ERT. (**A**) Serum ADMA levels in individual patients. (**B**) CFR data in individual patients. (**C**) LVMI in individual patients.

There was also a significant relationship between CFR and LVMI ($r = -0.545$, $P < 0.05$) (Figure 3C). In addition, serum ADMA levels were significantly correlated with plasma brain natriuretic peptide (BNP) levels ($r = 0.770$, $P = 0.0005$).

Discussion

Our study demonstrated that (i) CFR was significantly lower and serum ADMA levels and LVMI were significantly higher in the FD group than in the control group; (ii) ERT

Table 3. Laboratory data during ERT

	Case 1				Case 2				Case 3				Case 4			
Months	0	3	6	12	0	3	6	12	0	3	6	12	0	3	6	12
Cr (μmol/L)	872.4	879.3	854.1	903.7	40.4	38.1	41.2	43.5	65.6	74.7	73.2	54.9	976.1	995.9	1060.0	986.0
eGFR (mL/min/1.73 m^2)	—	—	—	—	153	157	152	149	68	58	60	85	—	—	—	—
RBC (×10^{12}/L)	4.10	3.39	3.61	4.05	5.53	5.67	5.71	5.64	3.05	3.14	3.32	3.40	4.20	3.80	3.78	3.75
Hemoglobin (g/L)	114	92	103	116	161	163	167	167	103	102	105	110	121	108	114	114
TP (g/L)	68	69	70	71	69	69	68	70	68	63	64	69	79	72	73	73
Alb (g/L)	39	41	42	42	45	45	45	48	39	34	36	37	36	33	32	32
T-chol (mmol/L)	3.23	3.47	3.13	3.70	6.21	4.97	5.90	4.42	4.86	4.50	4.03	5.17	4.09	2.77	2.84	2.64
TG (mmol/L)	2.01	1.68	0.82	1.55	9.40	3.42	5.01	3.13	3.92	1.10	2.67	3.41	1.26	1.46	1.24	0.69
HDL (mmol/L)	0.98	1.24	1.32	1.32	0.96	0.96	0.96	0.91	1.14	1.53	1.24	1.81	0.96	0.80	0.72	0.88
HbA1c (%)	6.1	5.9	5.1	6.3	12.6	10.6	11.8	11.2	5.0	—	—	—	4.6	—	—	—
Ca (mmol/L)	2.22	2.25	2.10	2.30	2.42	2.32	2.32	2.47	2.22	2.32	2.40	2.25	2.59	2.15	2.25	2.17
P (mmol/L)	1.68	1.94	1.52	2.23	1.19	1.19	1.23	1.13	0.87	1.00	1.19	0.90	2.16	1.78	1.84	1.84
BNP (ng/L)	321.5	169.2	68.3	53.3	2.4	3.1	2.2	6.4	59.6	69.1	27.6	77.7	272.2	173.2	183.1	144.4

Cr, creatinine; eGFR, estimated glomerular filtration rate; RBC, red blood cell count; TP, total protein; Alb, albumin; T-chol, total cholesterol; TG, triglyceride; HDL, high-density lipoprotein cholesterol; HbA1c, hemoglobin A1c; BNP, brain natriuretic peptide.

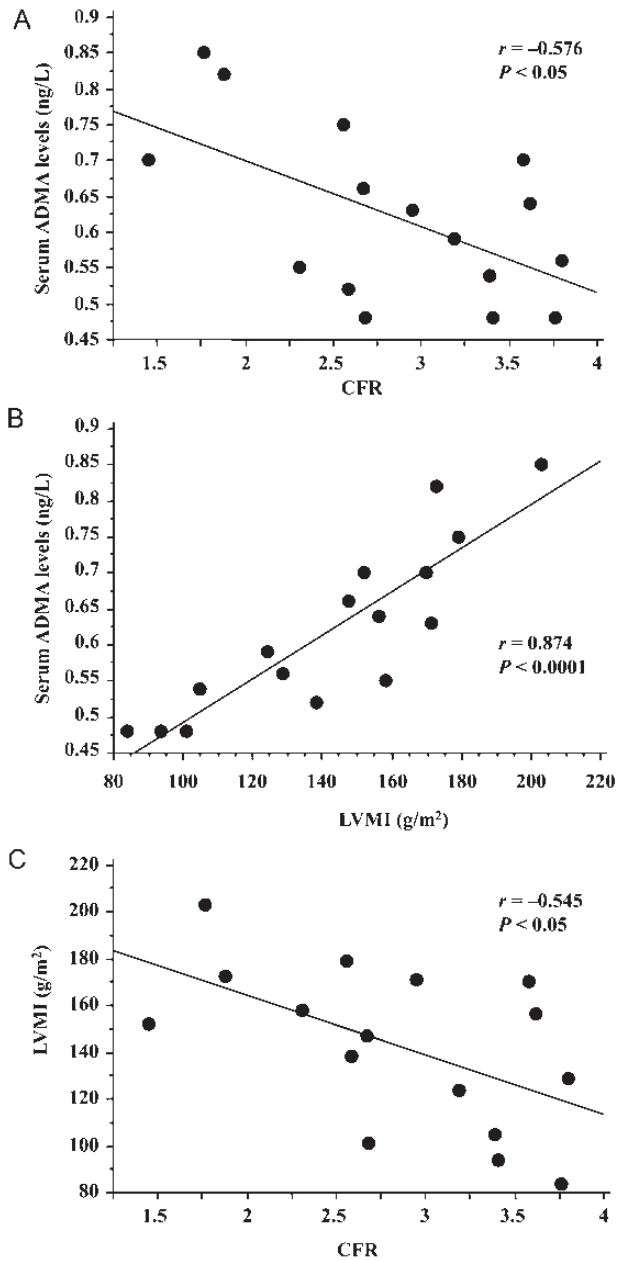

Fig. 3. Relationship between ADMA, CFR and LVMI. (**A**) Relationship between serum ADMA levels and CFR. (**B**) Relationship between serum ADMA levels and LVMI. (**C**) Relationship between CFR and LVMI.

increased the CFR, decreased the elevated serum ADMA levels and increased LVMI; and (iii) serum ADMA levels showed a significant relationship with CFR and LVMI in the FD patients.

It has been thought that FD patients show changes not only in the myocardium but also in coronary vascular function. In fact, some studies have demonstrated that CFR is markedly decreased in FD [4, 16–18]. Previous studies have also reported that ∼50% of FD patients complain of angina-like chest pain [19, 20]. In our study, two patients had angina-like chest pain and three had markedly impaired CFR, despite having no manifest coronary artery disease. Thus, coronary microvascular dysfunction may play a key role in the development of these symptoms. In many cases with cardiac symptoms, GL-3 accumulates in the endothelial, myocardial and smooth muscle cells, which causes microvascular dysfunction in coronary arteries and LVH. It has been shown that ERT reverses the accumulation of microvascular endothelial deposits of GL-3 in several organs, including the heart [1–3, 7]. Furthermore, it is thought that the increased oxygen demand of the hypertrophied myocardium is related to myocardial ischemia in FD. There are also some studies that have reported that ERT was effective in decreasing LVH and improving regional myocardial function [5, 21, 22]. Therefore, we speculated that ERT could ameliorate coronary microvascular dysfunction in FD through these two mechanisms. On the other hand, two previous studies reported that no improvements in hyperemic myocardial blood flow, CFR and LVMI were observed by ERT [23, 24]. However, the result of one study showed that the study patients felt subjectively better and suffered less pain after starting ERT. In addition, plasma GL-3 levels decreased significantly after ERT in both studies. From the results of these studies, ERT could prevent the progression of impaired CFR and LVH but could not improve CFR and LVH in their study patients. Severe LVH is thought to be associated with marked myocardial fibrosis in FD [25]. Therefore, it also has been speculated that ERT is less effective in patients with severe LVH. In the present study, ERT improved not only CFR but also LVMI in most patients. We believe that the reason is that our study patients did not have such severe LVH.

Several studies have demonstrated that serum ADMA levels increased in CKD patients and correlated with the severity of atherosclerotic lesions [9–11]. Thus, ADMA is thought to be an available biochemical marker of endothelial dysfunction and/or vascular lesions in patients with CKD. Our previous studies demonstrated that ADMA was significantly associated with CFR, endothelium-mediated vasodilation of the brachial artery and renal function [12, 13]. ADMA is also known to be related to LVH in patients with CKD [26, 27]. In the present study, to ascertain a possible role of ADMA in FD and determine whether ADMA is a useful marker of cardiac manifestation in FD, we assessed the relationship between serum ADMA levels, CFR and LVMI. We found that serum ADMA levels were significantly correlated with CFR and LVMI in patients with FD as well as in non-FD patients. Furthermore, the levels tended to decrease with improving CFR and LVMI after starting ERT. In the present study, serum BNP levels were also significantly associated with CFR and LVMI. However, because many papers demonstrated that ADMA induces cardiovascular dysfunction, it is reasonable to suppose that ADMA plays a key role in kidney and heart disease. Moreover, it has been reported that ADMA is more sensitive than N-terminal pro-BNP to diagnose heart failure in patients with congenital heart disease [28]. Considering these findings, there is a possibility that serum ADMA levels may reflect the degree of cardiac damage even in FD.

Acroparesthesia is one of the major symptoms in FD and it is known that ERT could reduce this symptom. A recent study reported that ADMA might affect nociception in CKD [29]. As mentioned above, our results show that serum ADMA levels increased in FD. Taken together, we speculated that ADMA might be involved in the acroparesthesia of FD though we do not have objective data on polyneuropathy.

The main limitation of our study was the small number of study patients and heterogeneous patient group. However, our patients were carefully and appropriately treated. Moreover, we performed a close observation for our study patients prospectively and longitudinally. We believe this is important because there are a few prospective and longitudinal studies that evaluated cardiac parameters on this topic. Therefore, we consider the results of the present study to be valuable for the management of patients with FD. Another limitation of the present study was that two study patients did not get adequate therapy for the last 2 months of the study period because of a worldwide shortage of recombinant α-galactosidase. There is a possibility that the insufficient therapy might have affected these patients' conditions and clinical data. Clearly, if we were to evaluate a larger number of appropriately treated patients, the efficacy of ERT and the usefulness of serum ADMA measurement would be more evident.

Conclusions

Our data suggest that ERT prevents progression of cardiac abnormalities, possibly by improving coronary microvascular dysfunction. In addition, we believe that ADMA may be a useful surrogate marker for cardiac lesions in FD. Further study is required to elucidate the precise mechanisms underlying ERT and the change in ADMA among patients with FD and to establish a useful strategy for the prevention of organ damage.

Transparency declaration

This study was presented in part at the annual meeting of the American Society of Nephrology, 2011.

Conflict of interest statement. None declared.

References

1. Linhart A, Elliott PM. The heart in Anderson-Fabry disease and other lysosomal storage disorders. *Heart* 2007; 93: 528–535
2. Kampmann C, Baehner F, Ries M *et al*. Cardiac involvement in Anderson-Fabry disease. *J Am Soc Nephrol* 2002; 13: S147–S149
3. Kampmann C, Wiethoff CM, Perrot A *et al*. The heart in Anderson Fabry disease. *Z Kardiol* 2002; 91: 786–795
4. Kalliokoski RJ, Kalliokoski KK, Sundell J *et al*. Impaired myocardial perfusion reserve but preserved peripheral endothelial function in patients with Fabry disease. *J Inherit Metab Dis* 2005; 28: 563–573
5. Eng CM, Guffon N, Wilcox WR *et al*. International Collaborative Fabry Disease Study Group. Safety and efficacy of recombinant human alpha-galactosidase A—replacement therapy in Fabry's disease. *N Engl J Med* 2001; 345: 9–16
6. Germain DP, Waldek S, Banikazemi M *et al*. Sustained, long-term renal stabilization after 54 months of agalsidase beta therapy in patients with Fabry disease. *J Am Soc Nephrol* 2007; 18: 1547–1557
7. Thurberg BL, Fallon JT, Mitchell R *et al*. Cardiac microvascular pathology in Fabry disease: evaluation of endomyocardial biopsies before and after enzyme replacement therapy. *Circulation* 2009; 119: 2561–2567
8. Cooke JP, Tsao PS. Is NO an endogenous antiatherogenic molecule? *Arterioscler Thromb* 1994; 14: 653–655
9. Miyazaki H, Matsuoka H, Cooke JP *et al*. Endogenous nitric oxide synthase inhibitor. A novel marker of atherosclerosis. *Circulation* 1999; 99: 1141–1146
10. Kielstein JT, Boger RH, Bode-Boger SM *et al*. Marked increase of asymmetric dimethylarginine in patients with incipient primary chronic renal disease. *J Am Soc Nephrol* 2002; 13: 170–176
11. Kielstein JT, Boger RH, Bode-Boger SM *et al*. Asymmetric dimethylarginine plasma concentrations differ in patients with end-stage renal disease: relationship to treatment method and atherosclerotic disease. *J Am Soc Nephrol* 1999; 10: 594–600
12. Takiuchi S, Fujii H, Kamide K *et al*. Plasma asymmetric dimethylarginine and coronary and peripheral endothelial dysfunction in hypertensive patients. *Am J Hypertens* 2004; 9: 802–808
13. Fujii H, Takiuchi S, Kawano Y *et al*. Putative role of asymmetric dimethylarginine in microvascular disease of kidney and heart in hypertensive patients. *Am J Hypertens* 2008; 21: 650–656
14. Hozumi T, Yoshida K, Ogata Y *et al*. Noninvasive assessment of significant left anterior descending coronary artery stenosis by coronary flow velocity reserve with transthoracic color Doppler echocardiography. *Circulation* 1998; 97: 1557–1562
15. Devereux RB, Reichek N. Echocardiographic determination of left ventricular mass in man. Anatomic validation of the method. *Circulation* 1977; 55: 613–618
16. Elliott PM, Kindler H, Shah JS *et al*. Coronary microvascular dysfunction in male patients with Anderson-Fabry disease and the effect of treatment with alpha galactosidase A. *Heart* 2006; 92: 357–360
17. Fujii H, Kono K, Goto S *et al*. Prevalence and cardiovascular features of Japanese hemodialysis patients with Fabry disease. *Am J Nephrol* 2009; 30: 527–535
18. Dimitrow PP, Krzanowski M, Undas A. Reduced coronary flow reserve in Anderson-Fabry disease measured by transthoracic Doppler echocardiography. *Cardiovasc Ultrasound* 2005; 3: 11
19. MacDermot KD, Holmes A, Miners AH. Anderson-Fabry disease: clinical manifestations and impact of disease in a cohort of 60 obligate carrier females. *J Med Genet* 2001; 38: 769–807
20. MacDermot KD, Holmes A, Miners AH. Anderson-Fabry disease: clinical manifestations and impact of disease in a cohort of 98 hemizygote males. *J Med Genet* 2001; 38: 750–760
21. Hughes DA, Elliott PM, Shah J *et al*. Effects of enzyme replacement therapy on the cardiomyopathy of Anderson-Fabry disease: a randomised, double-blind, placebo-controlled clinical trial of agalsidase alfa. *Heart* 2008; 94: 153–158
22. Weidemann F, Niemann M, Breunig F *et al*. Long-term effects of enzyme replacement therapy on Fabry cardiomyopathy: evidence for a better outcome with early treatment. *Circulation* 2009; 119: 524–529
23. Kalliokoski RJ, Kantola I, Kalliokoski KK *et al*. The effect of 12-month enzyme replacement therapy on myocardial perfusion in patients with Fabry disease. *J Inherit Metab Dis* 2006; 29: 112–118
24. Koskenvuo JW, Hartiala JJ, Nuutila P *et al*. Twenty-four-month alpha-galactosidase A replacement therapy in Fabry disease has only minimal effects on symptoms and cardiovascular parameters. *J Inherit Metab Dis* 2008; 31: 432–441
25. Weidemann F, Breunig F, Beer M *et al*. The variation of morphological and functional cardiac manifestation in Fabry disease: potential implications for the time course of the disease. *Eur Heart J* 2005; 26: 1221–1227
26. Kielstein JT, Zoccali C. Asymmetric dimethylarginine: a cardiovascular risk factor and a uremic toxin coming of age? *Am J Kidney Dis* 2005; 46: 186–202
27. Zoccali C, Mallamaci F, Maas R *et al*. CREED Investigators. Left ventricular hypertrophy, cardiac remodeling and asym-

metric dimethylarginine (ADMA) in hemodialysis patients. *Kidney Int* 2002; 62: 339–345
28. Tutarel O, Denecke A, Bode-Böger SM *et al*. Asymmetrical dimethylarginine–more sensitive than NT-proBNP to diagnose heart failure in adults with congenital heart disease. *PLoS One* 2012; 7: e33795
29. Kielstein JT, Suntharalingam M, Perthel R *et al*. Asymmetric dimethylarginine may mediate increased heat pain threshold in experimental chronic kidney disease. *Nephrol Dial Transplant* 2012; 27: 899–902

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