European Journal of Heart Failure Advance Access published June 30, 2013

European Journal of Heart Failure
doi:10.1093/eurjhf/hft104

Coronary microvascular dysfunction is an early feature of cardiac involvement in patients with Anderson–Fabry disease

Benedetta Tomberli[1,2]*, Franco Cecchi[1], Roberto Sciagrà[3], Valentina Berti[3], Francesca Lisi[4], Francesca Torricelli[5], Amelia Morrone[6], Gabriele Castelli[2], Magdi H. Yacoub[7], and Iacopo Olivotto[1,2]

[1]Department of Clinical and Experimental Medicine, University of Florence, Italy; [2]Referral Center for Myocardial Diseases, Department of Cardiology, Careggi University Hospital, Florence, Italy; [3]Nuclear Medicine Unit, Department of Clinical Physiopathology, Careggi University Hospital, Florence, Italy; [4]Department of Radiology, Careggi University Hospital, Florence, Italy; [5]Unit for Genetic Diagnosis, Careggi University Hospital, Florence, Italy; [6]Metabolic and Muscular Unit, Clinic of Pediatric Neurology, Meyer University Hospital, Florence, Italy; and [7]Heart Science Centre, Imperial College London, Harefield, UK

Received 27 February 2013; revised 31 May 2013; accepted 7 June 2013

Aims Male patients with Anderson–Fabry disease (AFD) often exhibit cardiac involvement, characterized by LV hypertrophy (LVH), associated with severe coronary microvascular dysfunction (CMD). Whether CMD is present in patients without LVH, particularly when female, remains unresolved. The aim of the study was to investigate the presence of CMD by positron emission tomography (PET) in AFD patients of both genders, with and without evidence of LVH.

Methods and results We assessed myocardial blood flow following dipyridamole infusion (Dip-MBF) with ^{13}N-labelled ammonia by PET in 30 AFD patients (age 51 $\pm$ 13 years; 18 females) and in 24 healthy controls. LVH was defined as echocardiographic maximal LV wall thickness $\geq$13 mm. LVH was present in 67% of patients ($n = 20$; 10 males and 10 females). Dip-MBF was reduced in all patients compared with controls (1.8 $\pm$ 0.5 and 3.2 $\pm$ 0.5 mL/min/g, respectively, $P < 0.001$). For both genders, flow impairment was most severe in patients with LVH (1.4 $\pm$ 0.5 mL/min/g in males and 1.9 $\pm$ 0.5 mL/min/g in females), but was also evident in those without LVH (1.8 $\pm$ 0.3 mL/min/g in males and 2.1 $\pm$ 0.4 mL/min/g in females; overall $P = 0.064$ vs. patients with LVH). Analysis of variance (ANOVA) for the 17 LV segments showed marked regional heterogeneity of MBF in AFD ($F = 4.46$, $P < 0.01$), with prevalent hypoperfusion of the apical region. Conversely, controls showed homogeneous LV perfusion ($F = 1.25$, $P = 0.23$).

Conclusions Coronary microvascular function is markedly impaired in AFD patients irrespective of LVH and gender. CMD may represent the only sign of cardiac involvement in AFD patients, with potentially important implications for clinical management.

Keywords Fabry disease • Coronary microvascular dysfunction • PET • Early phenotype

Introduction

Anderson–Fabry disease (AFD) is an X-linked disorder of glycosphingolipid catabolism caused by deficient or absent activity of the lysosomal enzyme α-galactosidase A (α-gal A). AFD is considered a rare disease, with a reported prevalence in the general population of ~ 1:117 000.[1,2] Recently, however, newborn screening initiatives have found an unexpectedly high prevalence of AFD, as high as 1 in ~3900 newborns,[3,4] suggesting that previous figures may represent a substantial underestimate.

As a consequence of α-gal A deficiency, progressive accumulation of globotriaosylceramide (Gb3) occurs in various tissues throughout the body, including the endothelium, and is associated with protean clinical manifestations, including renal failure, juvenile stroke, dyspnoea, angina, and sudden cardiac death.[5,6] Cardiac involvement

* Corresponding author. Dipartimento Cuore e vasi, San Luca Vecchio, Centro di Riferimento Cardiomiopatie, Azienda Ospedaliero Universitaria Careggi, Largo Brambilla 3, 50134, Firenze, Italy. Tel +39 055 7945138, Fax +39 055 7949335, Email: benedetta.tomberli@gmail.com

has been described in both genders and is mainly characterized by variable degrees of LV hypertrophy (LVH) and interstitial fibrosis.[7] Although commonly classified as a storage disease of the heart, AFD cardiomyopathy is characterized by true LVH, as Gb3 accumulation only accounts for a very limited proportion of cardiac mass increase.[8]

Coronary microvascular dysfunction (CMD) is an important feature of AFD cardiomyopathy, accounting for the considerable prevalence of angina, in the absence of epicardial coronary disease.[8,9] CMD has mostly been described in male AFD patients with LVH.[9–11] However, in patients with sarcomeric hypertrophic cardiomyopathy, a different model of genetically determined LVH, CMD is a diffuse phenomenon within the LV, may involve non-hypertrophied LV walls, and may possibly precede LVH development.[12–15] Whether CMD may be present in AFD patients without LVH remains unresolved. Furthermore, the prevalence and severity of CMD in female AFD patients, generally exhibiting more subtle cardiac manifestation than men, are undefined. Both issues are potentially relevant to early diagnosis of AFD cardiomyopathy and long-term risk stratification.[12–15]

Thus, the present study was undertaken to investigate coronary microvascular function in a cohort of male and female AFD patients at different stages of disease, with and without evidence of LVH, in order to assess whether CMD may occur independently of LVH and in the absence of any other sign of cardiomyopathy. We therefore assessed maximal global and regional myocardial blood flow after dipyridamole (Dip-MBF), as an expression of coronary microvascular function, using positron emission tomography (PET).

Methods

Study population

Thirty patients from 13 families (12 males, 18 females; mean age 51 $\pm$ 13 years) with AFD were included in a cross-sectional study. Diagnosis was based on measurement of α-gal A enzyme activity in leucocytes and confirmed by direct sequencing of the α-gal A gene (*GLA* gene) on genomic DNA isolated from whole blood. Enzyme activity and genetic analysis were assessed as previously described.[16] In two patients (individual II-1 from family 3 and individual III-7 from family 7), the final diagnosis of AFD was achieved by means of endomyocardial biopsy from the right interventricular septum. Between 2006 and 2010, all patients underwent thorough cardiac evaluation by clinical examination, resting ECG, Holter monitoring, and two-dimensional (2D) echocardiography including tissue Doppler analysis.

Coronary microvascular function was assessed by PET as described below. CAD was excluded in AFD patients at the time of enrolment by maximal, symptom-limited treadmill or cycloergometer exercise test, in asymptomatic patients with low pre-test probability (i.e. young and without cardiovascular risk factors), followed by coronary angiography or computed tomography (CT)-angiography in the presence of a positive or dubious test result; patients with a high risk profile and/or angina were directly referred for coronary angiography or CT-angiography. Furthermore, patients with diabetes were excluded. The same study protocol was employed in 24 healthy controls, who were investigated for exclusion of heart disease, and were comparable with the patients in terms of gender and age (13 males and 11 females, $P = 0.55$ vs. patients; age 46 $\pm$ 16 years, $P = 0.23$ vs. patients). Healthy controls had no evidence of cardiomyopathy, with normal ECG, echocardiographic examination, and without common cardiovascular risk factors. The study protocol was approved by the local research ethics committee, and written informed consent in lay Italian language was obtained from each subject included in the study

Echocardiography

Standard echocardiographic studies were performed with commercially available instruments. Clinical diagnosis of LVH was based on the demonstration by 2D echocardiogram of a hypertrophied and non-dilated LV (wall thickness $\geq$ 13 mm) in the absence of another cardiac or systemic disease capable of producing a similar degree of hypertrophy. The LV end-diastolic and end-systolic diameter, left atrial diameter, and magnitude and distribution of LVH were assessed as previously described.[17] In all patients, maximum LV wall thickness values were measured at the time of PET. The LVEF was measured in the standard four-chamber view by the area–length method. Obstruction of the LV outflow was considered present when a peak outflow gradient of $\geq$ 30 mmHg was present under basal conditions. Diastolic function was assessed accordingly to current guidelines.[18]

Positron emission tomography

All cardiac PET scans were performed in the Nuclear Medicine Laboratory (Nuclear Medicine Unit, Department of Clinical Physiopathology, Careggi University Hospital) in Florence between 2006 and 2011, after an appropriate period of pharmacological wash-out for patients receiving pharmacological treatment. Patients were positioned on the couch of the PET scanner (General Electrics Advance PET, Milwaukee, WI, USA) and a 5 min transmission scan was recorded for subsequent attenuation correction of emission data, according to a previously described procedure.[19] Then, near maximal hyperaemia was induced by i.v. administration of dipyridamole (0.56 mg/kg of body weight over 4 min). Three minutes following the end of dipyridamole infusion, a bolus of 370 MBq of nitrogen-13 ammonia (^{13}N-labelled ammonia) diluted in 10 mL of saline solution was injected i.v. over a period of 15–20 s and followed by a 10 mL saline solution flush at a rate of 2 mL/s. A dynamic scan with 15 frames of increasing duration was acquired for 4 min, followed by a prolonged static acquisition of 15 min. Particular care was taken to avoid any patient motion in order to minimize possible misalignment problems between transmission and emission scans. Data were analysed with an operator interactive computer program [PMOD Cardiac Modelling (PCARD), version 3.3, PMOD Technologies, Zurich, Switzerland]. Briefly, anatomic images were reconstructed using the static acquisition, and reoriented according to the heart axis. After reconstruction, the dynamic images were reoriented as well. On the short axis slices, regions of interest were manually drawn including: (i) the right ventricular cavity; (ii) the LV cavity; and (iii) the LV wall from the apex through the base (identified by the appearance of the membranous septum). The regions of interest were edited by the standard PMOD volume of interest (VOI) tool to derive the related VOIs. The three VOIs were then copied on all ^{13}N-labelled ammonia dynamic images to extract the corresponding time–activity curves. The arterial input function was obtained from the LV cavity time–activity curve. The myocardial uptake was derived from the LV wall VOI. Myocardial perfusion was calculated from model fitting of the arterial input function and tissue time–activity curves.[20] The LV wall was divided into 17 segments: septal (apical septal, midinferoseptal, midanteroseptal, basal inferoseptal, and basal anteroseptal), anterior (apical anterior, midanterior, and basal anterior), lateral (apical lateral, midlateral, and basal lateral), inferior (apical inferior, midinferolateral, midinferior, basal inferolateral, and basal inferior), and apical.[21] Mean hyperaemic MBF for the entire left ventricle was obtained by volume-weighted averaging of the 17 LV segment territories (apex, septum, anterior, lateral, and inferior).

All images studies were analysed by one expert observer (R.S.), blinded to patients' genetic, clinical, and echocardiographic data.

Statistical analysis

Data are expressed as mean ± standard deviation. Two-tailed unpaired Student's *t*-test was employed for the comparison of normally distributed data. χ^2 or Fisher's exact test, as appropriate, were utilized to compare non-continuous variables expressed as proportions. Independent determinants of CMD were evaluated by logistic regression analysis. *P*-values are two-sided and considered significant when <0.05. Calculations were performed using the SPSS 12.0 software (Chicago, IL, USA).

Results

Demographic features and mutational status

The mean age of the 30 AFD patients, at the time of PET, was 51 ± 13 years (range 23–75); 12 patients (40%) were male (*Table 1*). Genetic analysis identified 11 distinct mutations in the *GLA* gene (*Table 2*, *Figure 1*); three index patients and seven family members shared the c.644A > G *GLA* gene mutation leading to the p.Asn215Ser amino acid substitution. As expected, mean α-gal A activity in leucocytes was lower in males than in females (2.1 ± 1.4 vs. 21 ± 12 nmol/mg/h, respectively; $P < 0.001$); conversely, mean levels were comparable in patients with and without echocardiographic evidence of LVH (13 ± 13 and 14 ± 14 nmol/mg/h, respectively; $P = 0.75$). The two patients with endomyocardial biopsy, both with echocardiographic evidence of LVH, had typical AFD histological findings.

Overall, 8 had a history of smoking, 5 were current smokers, and 11 had hypercholesterolaemia (*Table 1*). Three patients were obese [body mass index (BMI) >30; 10%], while hypertension, well controlled by medical therapy, was present in 12 patients (40%) without differences between the two subgroups. Evidence of extracardiac involvement was present in 19 patients (63%) (detailed in *Table 2*): 12 patients (40%, 9 males) had proteinuria, 5 males had mild to moderate renal impairment (17%), and 2 patients (6%, 1 male) underwent renal transplantation. Five patients had prior stroke (17%, 2 males), while neuropathic pain was present in 11 patients (37%, 5 males). Nine patients with angina and dubious stress test underwent coronary angiogram or coronary CT-angiogram (as reported in *Table 2*), in order to exclude epicardial CAD. In the remaining patients, coronary disease was excluded by symptom-limited exercise test.

Eleven patients (37%) were receiving enzyme replacement therapy (ERT) at the time of the study, eight patients with LVH and three without LVH. Most patients were on ACE inhibitors/ARBs (57%) and antiplatelet agents (43%), whereas only a minority were on beta-blockers or calcium channel antagonists (17% for each class).

Evidence of Anderson–Fabry disease cardiomyopathy

Twenty of the 30 AFD patients (67%, 10 males, 10 females) had echocardiographic evidence of LVH, with a maximum LV wall thickness value of 20 ± 4 mm (range 13–27 mm). The remaining 10 patients (33%, 2 males, 8 females) had normal LV wall thickness values. Most patients with LVH (76%) had ECG abnormalities compatible with LV hypertrophy/strain. Conversely, only one of the patients without LVH (10%) had an abnormal ECG morphology (*Table 1*).

Compared with patients without LVH, those with LVH were more often males (10/20 or 50% vs. 2/10 or 20% respectively, $P = 0.23$) and more often symptomatic for chest pain or dyspnoea ($P < 0.05$ for both; *Table 1*). Patients with LVH had larger atrial volumes and more frequent evidence of diastolic dysfunction. None of the patients had LV outflow tract obstruction at rest. Of note, patients with LVH were older (56 ± 9 years vs. 42 ± 16 in those without LVH, $P = 0.005$), with a strong direct relationship between age and degree of hypertrophy, particularly in males, suggesting age-related development of AFD cardiomyopathy (*Figure 2A*). Finally, 8 of the 10 patients without LVH (i.e. 7 females and 1 males) showed normal tissue Doppler imaging mitral septal annulus velocities (adjusted per age) and absence of late gadolinium enhancement (LGE) at cardiac magnetic resonance imaging (MRI).

Evidence of coronary microvascular dysfunction by positron emission tomography

Coronary microvascular response to dipyridamole was blunted in all AFD patients, as compared with control subjects (1.8 ± 0.5 and 3.2 ± 0.5 mL/min/g, respectively; $P < 0.001$) (*Figure 3*). Dip-MBF in AFD patients ranged from 0.8 to 2.6 mL/min/g, and was <1.25 mL/min/g, reflecting severe impairment of coronary microvascular function, in 7 patients (23%). Patients with LVH invariably showed marked degrees of CMD (*Figure 2B*). However, patients without LVH also showed blunted Dip-MBF values, ranging from 2.4 to as low as 1.2 mL/min/g. As a result, CMD in patients without LVH was on average milder, but not statistically different, from that of those with LVH (mean 2.0 ± 0.4 vs. 1.6 ± 0.5 mL/min/g, respectively; $P = 0.064$) (*Figure 3*).

Analysis of variance (ANOVA) for the 17 LV segments showed marked regional heterogeneity of MBF in AFD ($P < 0.01$), with prevalent hypoperfusion of the apical region (*Figure 4*). Conversely, controls showed homogeneous perfusion of the LV (patients, $F = 4.46$, $P < 0.01$; controls, $F = 1.25$, $P = 0.23$).

To exclude the possible influence of common CMD risk factors, other than AFD, we compared MBF values between patients with no history of smoking, hypertension, and dyslipidaemia with the rest of the cohort. Patients without risk factors ($n = 12$) showed a similar degree of MBF impairment, as compared with other patients ($n = 18$; MBF = 1.6 ± 0.5 mL/min/g vs. MBF = 1.9 ± 0.4 mL/min/g, respectively; $P = 0.12$). Furthermore, there was no difference in MBF between patients on ERT vs. naïve patients (1.7 ± 0.5 mL/min/g vs. 1.7 ± 05 mL/min/g, respectively; $P = 0.9$).

Impact of gender on microvascular function

Coronary microvascular dysfunction was more severe in males, compared with female AFD patients (1.5 ± 0.4 and 1.9 ± 0.5 mL/min/g, respectively, $P = 0.01$) (*Figure 3*). Unexpectedly, however, a blunted Dip-MBF was also present in the 18 female patients, irrespective of the presence of LVH (1.9 ± 0.5 mL/min/g in females with LVH and 2.1 ± 0.4 mL/min/g in females without LVH; $P = 0.38$). Of note, a 61-year-old female patient without LVH had

Table 1 Baseline and cardiac characteristics of 30 patients with Anderson–Fabry disease

	All patients ($n = 30$)	LVH – ($n = 10$)	LVH + ($n = 20$)	*P*-value
Age, years	51 ± 13	42 ± 16	56 ± 9	0.005
Age <40 years, *n* (%)	6 (10)	5 (50)	1 (5)	0.009
Gender				0.23
Male, *n* (%)	12 (40)	2 (20)	10 (50)	
Female, *n* (%)	18 (60)	8 (80)	10 (50)	
Height, cm	167 ± 8	165 ± 9	168 ± 7	0.42
Weight, kg	69 ± 12	65 ± 10	72 ± 13	0.15
BMI	26 ± 7	27 ± 11	26 ± 4	0.63
Genotype				0.98
p.Asn215Ser (c.644A > G), *n* (%)	10 (33)	3 (30)	7 (35)	
Other mutations, *n* (%)	20 (67)	7 (70)	13 (65)	
Cardiovascular risk factors				
Smoking				
Smokers, *n* (%)	5 (17)	1 (10)	4 (20)	0.64
Ex-smokers, *n* (%)	8 (27)	3 (30)	5 (25)	0.93
Hypercholesterolaemia, *n.* (%)	11 (37)	2 (20)	9 (45)	0.25
Hypertension, *n* (%)	12 (40)	2 (20)	10 (50)	0.23
BMI >30, *n* (%)	3 (10)	1 (10)	2 (10)	0.96
Therapy				
Beta-blockers, *n* (%)	5 (17)	0	5 (25)	0.14
Calcium channel antagonists, *n* (%)	5 (17)	1 (10)	4 (20)	0.64
Amiodarone, *n* (%)	2 (7)	0	2 (10)	0.54
ACE inhibitors, *n* (%)	8 (27)	1 (10)	7 (35)	0.21
ARBs, *n* (%)	9 (30)	1 (10)	8 (40)	0.20
Antiplatelets, *n* (%)	13 (43)	1 (10)	12 (60)	0.017
ERT, *n* (%)	11 (37)	3 (30)	8 (40)	0.70
NYHA class				0.02
I, *n* (%)	17 (57)	9 (90)	8 (40)	
II, *n* (%)	10 (33)	1 (10)	9 (45)	
III/IV, *n* (%)	3 (10)	0	3 (15)	
Dyspnoea, *n* (%)	13 (43)	1 (10)	12 (60)	0.017
Chest pain, *n* (%)				
At rest	2 (7)	1 (10)	1 (5)	0.95
On effort	11 (37)	1 (10)	10 (50)	0.049
Palpitations, *n* (%)	16 (53)	5 (50)	11 (55)	0.99
Syncope, *n* (%)	5 (17)	2 (20)	3 (15)	0.97
NSVT, *n* (%)	3 (10)	0	3 (15)	0.53
PAF, *n* (%)	4 (13)	1 (10)	3 (15)	0.97
Pacemaker, *n* (%)	3 (10)	0	3 (15)	0.53
ECG				
LVH, *n* (%)	14 (52)	1 (10)	13 (76)	0.001
Bradycardia, *n* (%)	11 (37)	4 (40)	7 (35)	0.94
Heart rate, b.p.m.	61 ± 8	61 ± 12	62 ± 7	0.79
Short PR, *n* (%)	3 (10)	1 (10)	2 (10)	0.97
Mean flow <1.25 mL/min/g, *n* (%)	7 (23)	1 (10)	6 (30)	0.37
Echocardiography				
LV max WT, mm	16 ± 6	9 ± 1	20 ± 4	<0.01
IV septum, mm	15 ± 6	9 ± 1	18 ± 5	<0.01
LV posterior wall, mm	12 ± 3	9 ± 1	13 ± 2	<0.01
Left atrium, mm	37 ± 7	31 ± 5	40 ± 6	<0.01
Left atrium volume, mm/m^2	39 ± 13	29 ± 9	45 ± 11	<0.01

Continued

Table 1 *Continued*

	All patients ($n = 30$)	LVH − ($n = 10$)	LVH + ($n = 20$)	*P*-value
LV telediastolic diameter, mm	48 ± 6	48 ± 5	49 ± 6	0.75
LV telesystolic diameter, mm	27 ± 8	27 ± 5	27 ± 7	0.86
LV telediastolic volume, mL	99 ± 32	91 ± 19	102 ± 37	0.40
LV telesystolic volume, mL	37 ± 20	33 ± 13	39 ± 23	0.42
EF, %	63 ± 10	65 ± 9	63 ± 10	0.62
Diastolic pattern				0.03
Normal, *n* (%)	6 (20)	6 (60)	0	
Delayed relaxation, *n* (%)	12 (40)	4 (40)	8 (40)	
Pseudonormal, *n* (%)	11 (37)	0	11 (50)	
Restrictive, *n* (%)	1 (3)	0	1 (5)	
LVOT obstruction, *n* (%)	0	0	0	N/A
Mitral regurgitation (mild or moderate)	16 (53)	4 (40)	12 (60)	0.44
Aortic regurgitation (mild or moderate)	5 (17)	2 (20)	3 (15)	0.92

Data are shown as mean ± SD or *n* (%).
BMI, body mass index; ERT, enzyme replacement therapy; IV, interventricular; LVH −, patients without LV hypertrophy; LVH +, patients with LV hypertrophy; LV max WT, LV maximal wall thickness; LVOT, LV outflow tract obstruction; N/A, not applicable; NSVT, non-sustained ventricular tachycardia; PAF, paroxysmal atrial fibrillation.

extreme degrees of coronary flow impairment (Dip-MBF = 1.2 mL/min/g) (family 6, subject II-1 in *Table 2*). An inverse relationship between mean Dip-MBF and degree of LVH, suggesting more severe CMD in the presence of increasing LVH, was present only in males (*Figure 2B*). The 10 patients with the p.Ans215Ser *GLA* gene mutation showed significant blunting of MBF as compared with patients with other mutations (1.5 ± 0.5 vs. 1.9 ± 0.5 mL/min/g, respectively; $P = 0.019$), despite a similar degree of hypertrophy (maximum LV wall thickness 18 ± 8 mm vs. 15 ± 5 mm, respectively, $P = 0.28$).

Discussion

Coronary microvascular dysfunction is independent of left ventricular hypertrophy in Anderson–Fabry disease

The present study demonstrates that microvascular function is markedly impaired in AFD patients, irrespective of any other evidence of cardiac involvement. In our cohort, all AFD patients with normal echocardiographic findings exhibited blunting of the vasodilator response to dipyridamole, reflecting impaired microvascular function, with a mean Dip-MBF of only 2.0 ± 0.4 mL/min/g, compared with 3.2 ± 0.5 mL/min/g in normal controls, i.e. a 60% reduction in coronary flow. Such a degree of CMD was only slightly milder than that observed in patients with clear evidence of AFD cardiomyopathy and LVH. Indeed the most severe impairment in microvascular function was observed in a 61-year-old female patient, without evidence of LVH (Dip-MBF 1.2 mL/min/g).

Of note, while MBF was globally impaired in AFD hearts, there was marked heterogeneity in flow, with prevalent hypoperfusion of the apical region of the left ventricle, suggesting a regional nature of cardiac involvement. While counterintuitive in a systemic storage disease, the novel idea of a non-homogeneous cardiac involvement in AFD is in line with prior observations in a number of cardiomyopathies, and particularly those related to storage or infiltrative disorders, such as, for example, amyloidosis.[22] The mechanisms accounting for such regionally heterogeneous manifestations remain unresolved.

Altogether, these findings point to CMD as a constant manifestation of AFD, which may be present independent of other instrumental evidence of cardiac involvement. In males, the correlation between LV wall thickness and maximal MBF, and the direct relationship of LV wall thickness to age, both suggest that a reduced coronary maximal blood flow may precede development of LVH (*Figure 2*). This concept is novel and strongly supported by the young age of patients with CMD in the absence of LVH. Furthermore, AFD patients without LVH and CMD generally had none of the other features that are considered very early disease manifestations, such as reduced diastolic mitral annulus velocities or presence of LGE.[23,24] Nevertheless, longitudinal studies are required to understand whether flow abnormalities can indeed be considered to represent initial cardiac involvement followed by overt cardiomyopathy in AFD patients.

The consistent and severe blunting of MBF in patients with the p.Ans215Ser *GLA* gene mutation may suggest a role for genetic status in determining the severity of MBF. Furthermore, the concept of the possible influence of genetic status on microvascular function has been proved in another more common genetic cardiomyopathy (i.e. hypertrophic cardiomyopathy).[25] However, in the present study, the relatively small number of patients did not allow a meaningful comparison between individual mutations.

Coronary microvascular dysfunction in female Anderson–Fabry disease patients

In our cohort, females with LVH showed blunted Dip-MBF, although to a less severe degree than males (1.9 ± 0.5 and 1.5 ± 0.4 mL/min/g,

Table 2 Clinical and genetic features of the study population at the time of positron emission tomography

Family	Genotype (effect on protein)	Subject	Sex	Age			α-Gal	ERT	Symptoms (NYHA class)	LVH	Max LVWT	EF	Dip-MBF	CAD exclusion	Major organ involvement
				At clinical onset	At diagnosis	At PET									
1	p.Met1Val (c.1 A > G)	III-1	M	4	28	29	0.9	Yes	Palpitations (1)	Yes	16	67	2.4	Stress test	Proteinuria, acroparaesthesia, abdominal pain
		II-1	F	16	47	48	46	Yes	Dyspneoa, palpitations, syncope, angina (2)	Yes	16	62	2.3	Coronary angiogram	Acroparaesthesia
2	p.Trp44X (c.131 G > A)	III-1	M	12	52	54	0.01	Yes	Dyspnoea, palpitations (2)	Yes	23	70	1.1	Coronary CT-angiogram	Acroparaesthesia, RI, proteinuria
		III-2	F	-	39	41	11.8	No	Palpitations (1)	No	9	60	1.8	Stress test	None
3	p.Arg112Cys (c.334 C > T)	II-1	M	11	34	52	1.9	Yes	Angina (1)	Yes	23	66	0.8	Coronary angiogram	Acroparaesthesia stroke, renal transplant
4	p.Ala143Thr (c.427 G > A)	III-2	F	-	40	42	5.8	No	Palpitations (1)	No	8	64	2.4	Stress test	None
5	p.Asn215Ser (c.644 A > G)	II-1	M	44	56	60	1.82	No	None (1)	Yes	25	59	1.7	Coronary CT-angiogram	None
6	p.Asn215Ser (c.644 A > G)	II-1	F	-	56	60	13	No	Dyspnoea, palpitations (2)	No	11	67	1.2	Stress test	None
		II-2	M	48	55	55	3.4	No	Dyspnoea, angina (2)	Yes	28	58	1.2	Coronary angiogram	RI, proteinuria
		II-3	F	N/A	53	56	16	No	Dyspnoea (2)	Yes	13	70	0.9	Stress test	Acroparaesthesia
		II-4	M	N/A	46	53	4.2	No	Dyspnoea, palpitations (2)	Yes	27	62	1.0	Stress test	RI, proteinuria
		II-5	M	–	51	48	3.6	No	Dyspnoea, angina (2)	Yes	22	70	1.3	Stress test	None
		III-3	F	-	24	27	44	No	None (1)	No	7	76	2.2	Stress test	None
		III-5	F	20	25	31	5.3	No	Syncope, palpitations (1)	No	9	67	2.3	Stress test	Acroparaesthesia
7	p.Asn215Ser (c.644 A > G)	III-7	M	59	69	69	3.2	No	Palpitations, dyspnea, angina (3)	Yes	23	33	1.2	Coronary angiogram	Proteinuria
		III-12	M	50	68	69	2.8	No	Dyspnea, angina (2)	Yes	23	71	1.5	Stress test	Proteinuria, RI, stroke
8	p.Arg220X (c.658 C > T)	II-2	F	22	37	47	26.6	Yes	None (1)	Yes	21	82	1.6	Coronary angiogram	Renal transplant
9	p.Leu243Ser (c.728 T > C)	III-1	F	N/A	63	62	26	No	Palpitations, dyspnoea (2)	Yes	16	62	2.3	Stress test	None
		III-2	F	36	56	57	22	No	Dyspnoea, palpitations, angina (3)	Yes	23	47	2.2	Stress test	Proteinuria
10	p Asp299Gly (c.896 A > C)	II-1	F	N/A	66	68	21	No	None (1)	Yes	17	58	1.9	Stress test	None
		III-1	F	-	49	49	31	No	Palpitations (1)	No	11	59	1.9	Stress test	None
		III-2	M	18	46	49	0.01	Yes	None (1)	Yes	15	61	2.0	Stress test	Proteinuria, RI, acroparaesthesia, abdominal pain
11	p.Arg301Pro (c.902 G > C)	III-2	F	16	49	63	13.5	Yes	Palpitations, dyspnoea, syncope, angina (2)	Yes	21	58	1.6	Stress test	Proteinuria, acroparaesthesia, strokes
		III-4	F	N/A	46	56	31.6	No	None (1)	Yes	14	63	2.6	Stress test	None
		III-5	F	N/A	55	62	18.2	No	Dyspnoea (2)	Yes	15	63	1.4	Stress test	Stroke
		IV-1	M	25	30	40	0.9	Yes	None (1)	No	10	59	2.0	Stress test	Proteinuria
12	p.Arg301X (c.901 C > T)	I-1	F	–	70	75		No	Palpitations (1)	No	9	77	2.2	Coronary angiogram	None

Continued

13	c.1235_1236 delCT	II-1	F	15	40	54	6.6	Yes	Dyspnoea, palpitations (2)	Yes	13	76	1.8	Coronary CT-angiogram	Acroparaesthesia, strokes, abdominal pain
		III-1	M	4	15	28	2.4	Yes	None (1)	No	10	53	1.5	Stress test	Acroparaesthesia, abdominal pain, proteinuria
		III-3	F	14	10	23	13.8	Yes	None (1)	No	8	63	2.3	Stress test	Acroparaesthesia, abdominal pain

α-Gal = α-galactosidase A activity assayed on leucocytes (normal values 20–60 nmol/mg/h); CT-angiogram, computed tomography angiogram; Dip-MBF, mean myocardial blood flow following dipyridamole infusion; ERT, enzyme replacement therapy; LVH, left ventricular hypertrophy; Major organ involvement, only kidney (RI, renal impairment diagnosed as glomerular filtration rate < 60 mL/min), peripheral and central nervous system involvement are reported in this table; Max LVWT, maximal left ventricular wall thickness; N/A, age at onset unknown (diagnosis made by family screening).

respectively; $P = 0.01$). In addition, and rather unexpectedly, women without LVH also had considerable degrees of CMD, representing the only evidence of AFD in some of these individuals. As a result, there should be heightened awareness of the consideration of cardiac involvement in female patients with AFD, even in the absence of an echocardiographic phenotype. Although AFD is an X-linked disease, female patients are well known to develop the clinical manifestations of the disease, which can be severe and determine outcome.[26] Cardiomyopathy is not an exception to this rule, and has been described in women; however, cardiac manifestations generally have a later onset and are less marked than those occurring in men (*Figure 4*).[27,28]

Potential mechanisms of coronary microvascular dysfunction and implication for management

There are several potential pathophysiological mechanisms underlying coronary blood flow impairment in AFD, and these include those mediated by LVH (reduced capillary density, extravascular compression forces) as well as those directly affecting the microvasculature (endothelial dysfunction due to Gb3 storage, nitric oxide pathway dysregulation, or microvascular remodelling).[13] However, in patients without LVH, only the latter are present, suggesting that LVH *per se* is indeed a likely contributor to CMD, rather than a cause.

Despite considerable advances in our understanding of AFD, the mechanisms leading to LVH are not well understood. Storage of Gb3 within cardiac myocytes accounts for a small amount of the whole LV mass, which is mainly represented by true myocardial hypertrophy.[8] One hypothesis is that intracellular accumulation of Gb3 may disturb cardiac energetic metabolism, representing an early trigger for the activation of the intracellular signalling pathways leading to hypertrophy, fibrosis, apoptosis, and necrosis.[29] Furthermore, microvascular dysfunction and chronic hypoperfusion, due to Gb3 accumulation in endothelial cells, may also play a crucial role in the activation and perpetuation of these pathophysiological processes, even at early stages.[13]

The issue of identification of early organ damage has become critical following the introduction in clinical practice of ERT and novel therapeutic molecules, including pharmacological chaperone therapy.[30–33] To date, evidence of benefit of ERT in AFD patients with overt signs of cardiomyopathy and LVH is disappointing, and earlier timing of treatment initiation has been advocated in order to improve efficacy.[11,30]

Thus, early detection of subclinical cardiac involvement, as allowed by PET studies of microvascular function, may become a critical element in clinical decision-making especially in young AFD patients. In particular, CMD may represent a viable treatment target, potentially relevant to prevention of disease progression and outcome.[30] In the future, the advent of cardiac magnetic resonance (CMR), by virtue of its wider availability and more favourable safety profile compared with PET, may allow large-scale investigation of CMD in AFD patients. To date, however, quantitative assessment of microvascular flow by CMR is time-consuming and largely limited to research purposes.[15]

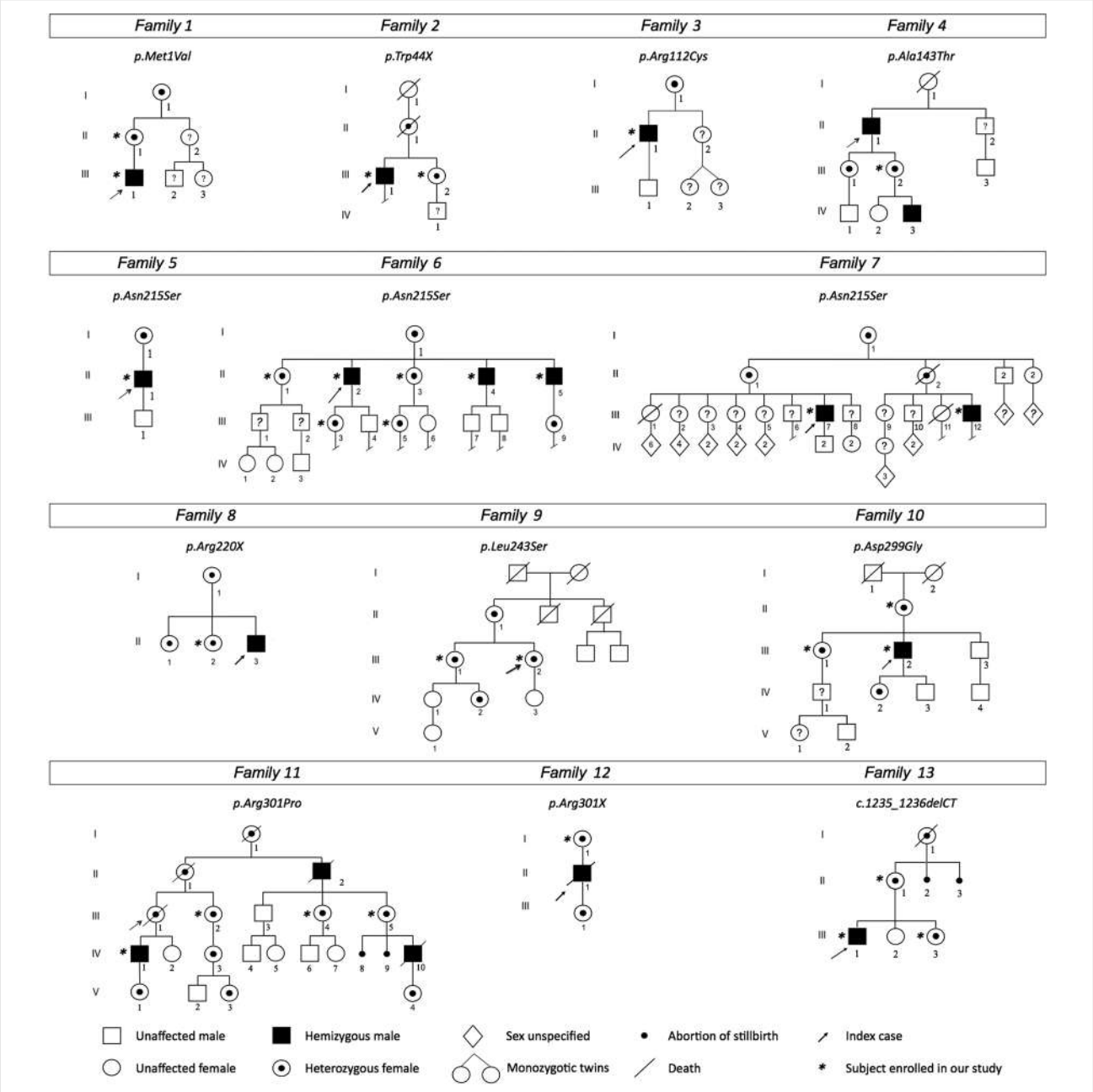

Figure 1 Pedigree of the 13 index patients.

Furthermore, is it possible to hypothesize that the severity of CMD may be an important predictor of adverse outcome, as for other genetically determined cardiomyopathies.[12,34]

Limitations of the study

An unavoidable limitation of the present study lies in the small sample size and heterogeneity of our study population. Selecting a more homogeneous study cohort, by excluding patients with co-morbidities or cardiovascular risk factors, would not have been feasible in a rare and multisystemic disorder such as AFD. Thus, myocardial blood flow may have been affected in our cohort by the interplay of co-morbidities such as hypertension, smoking, or dyslipidaemia, as well as ERT itself. This may have potentially led to overestimation of CMD in our patients with regard to the healthy controls, in whom none of the conventional cardiovascular risk factors was present. The presence of epicardial coronary disease was not systematically excluded by coronary angiogram or coronary CT-angiogram in all patients, based on radioprotection and safety considerations. Nevertheless, none of our enrolled AFD patients, at >3 years average from the execution of PET studies, has been found to have significant CAD during follow-up.

While we acknowledge this unavoidable bias, however, MBF values in AFD with no history of smoking, hypertension, and dyslipidaemia,

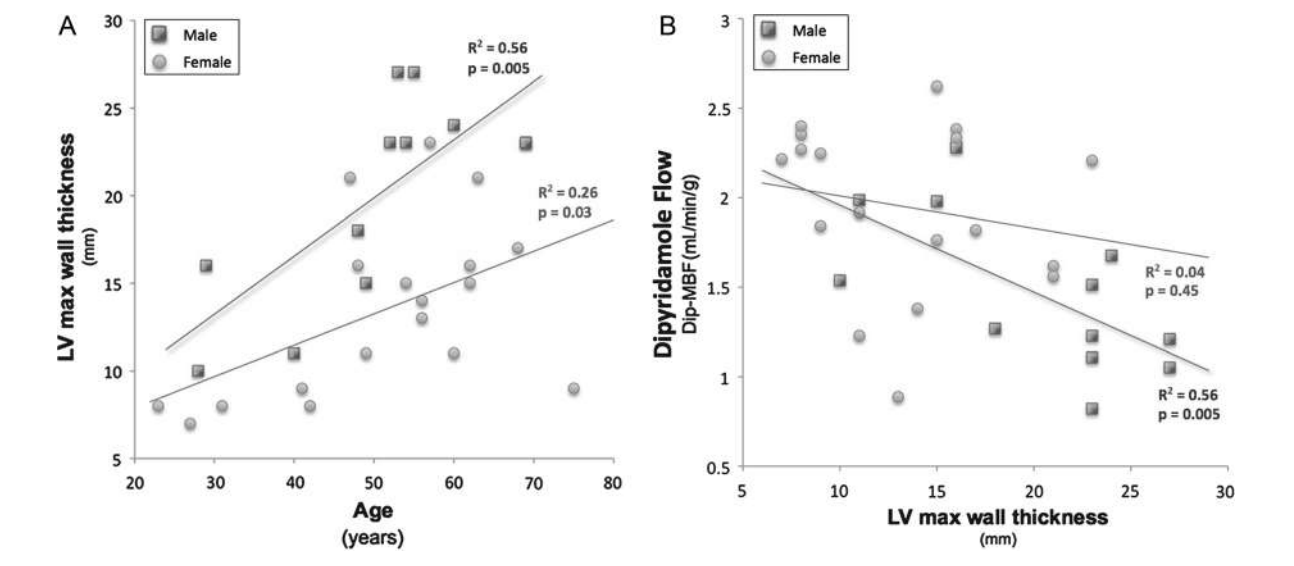

Figure 2 Relationship between LV wall thickness and myocardial flow. Linear regression analysis showed a direct relationship between age and degree of LV hypertrophy (LVH) (*A*) and between degree of LVH and myocardial blood flow following dipyridamole infusion. (*B*). Both relationships were stronger in males, suggesting that the development of Anderson–Fabry cardiomyopathy is age related and that coronary microvasculature dysfunction may precede LVH.

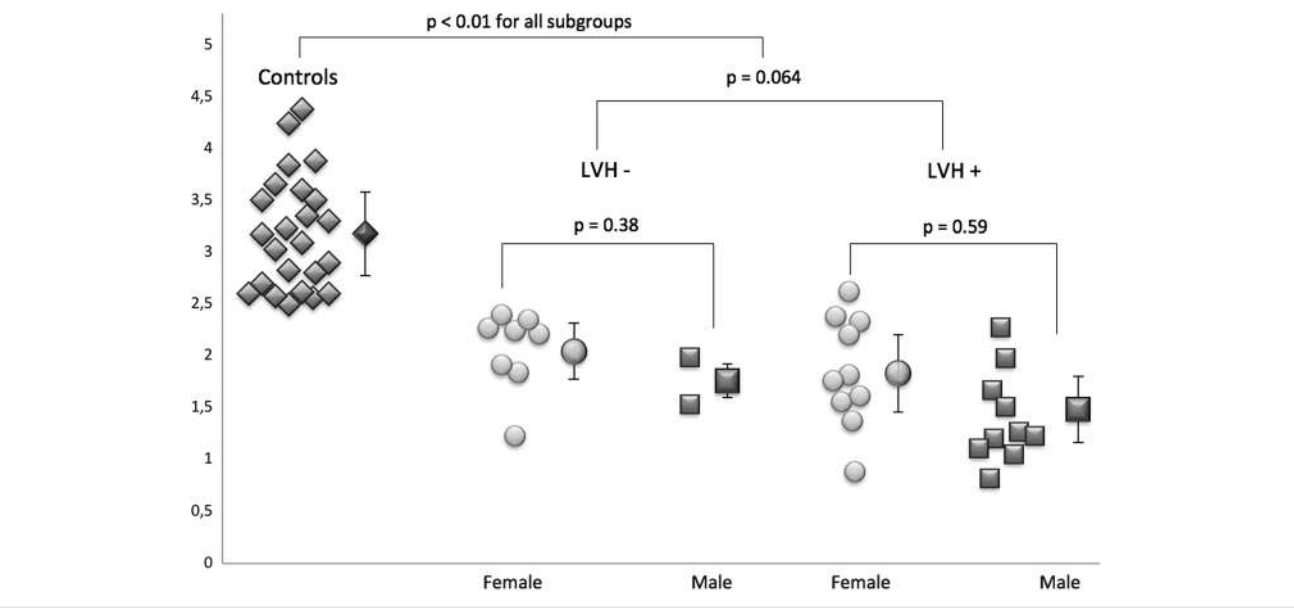

Figure 3 Comparison of microvascular dysfunction in patients with Anderson–Fabry disease (AFD), based on the presence of LV hypertrophy (LVH) and gender. Myocardial blood flow following dipyridamole infusion is markedly impaired in AFD patients compared with control subjects. Values are shown as mean ± SD.

ideally representing 'pure' AFD patients, were comparable with the rest of the cohort. Likewise, MBF in patients who were on ERT at the time of the study was comparable with that of those who were untreated at that time, consistent with previous studies which confirmed the questionable efficacy of ERT in patients with clear-cut cardiomyopathy.[9,11,30] Thus, our data are consistent with a disproportionate microvascular involvement in AFD, overruling the effect of these potential environmental confounders.

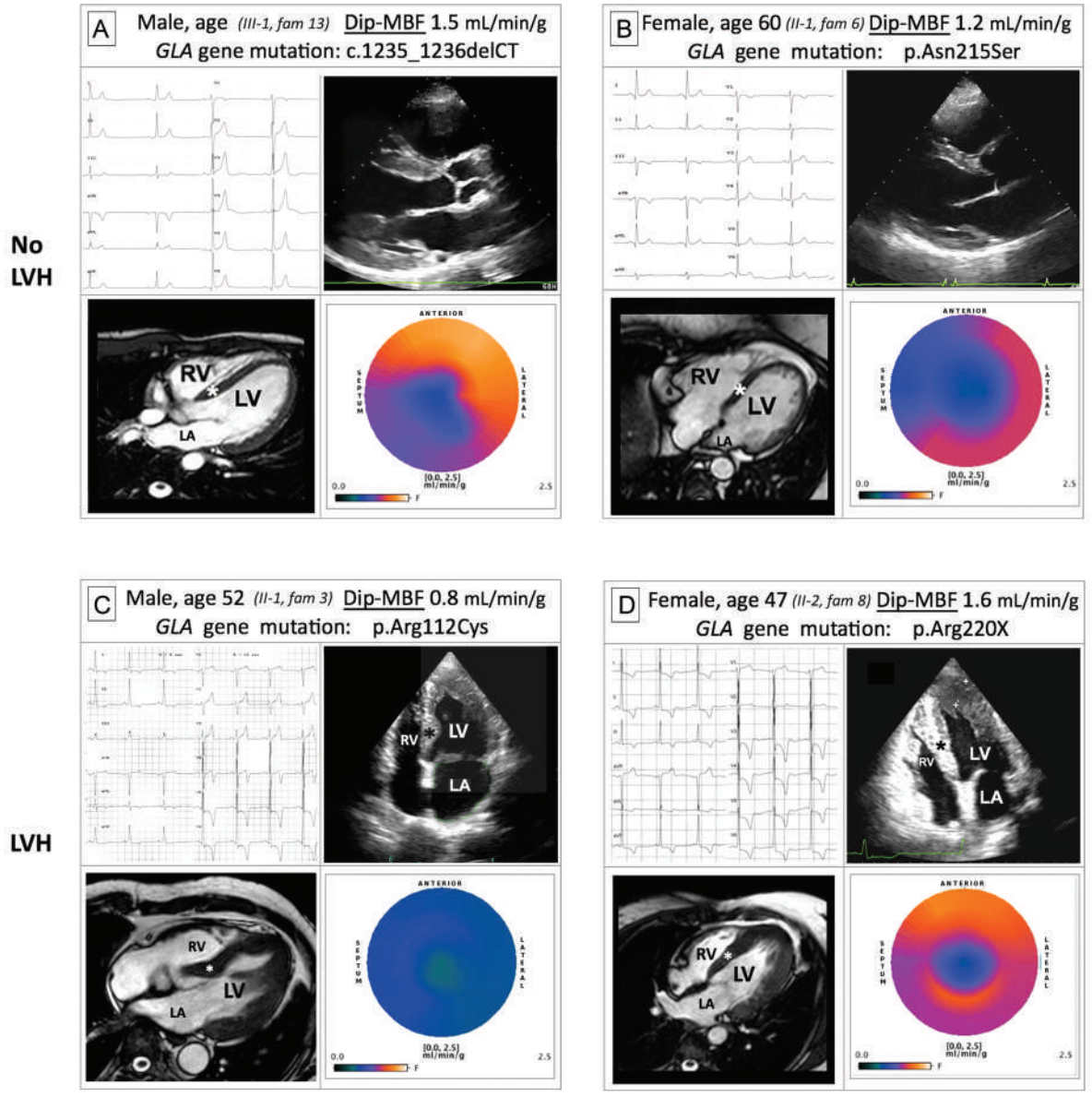

Figure 4 Relationship of coronary microvasculature dysfunction (CMD) to phenotype in individual patients with Anderson–Fabry disease (AFD). (*A*) A 28-year-old male with extreme sinus bradycardia (36 b.p.m.) and normal interventricular septal thickness values (*echocardiographic parasternal long axis and cardiac magnetic resonance four-chamber views). Colour-coded polar map obtained by positron emission tomography (PET) after dipyridamole infusion showing regional CMD, with relatively preserved flow in the antero-lateral region (family 13, subject III-1; *Table 2*). (*B*) A 60-year-old female with normal ECG, without normal LV wall thickness values. The polar map shows severe CMD, despite normal LV wall thickness values (family 6, subject II-1; *Table 2*). (*C*) A 52-year-old male with marked ECG abnormalities, severe LV hypertrophy (LVH) (*echocardiographic apical four-chamber view and cardiac magnetic resonance four-chamber view). The polar map shows severe CMD (family 3, subject II-1; *Table 2*). (*D*) A 47-year-old female with markedly abnormal ECG, and severe LVH. The polar map shows relatively preserved microvascular function, despite the LVH (family 8, subject II-2; *Table 2*). Dip-MBF, myocardial blood flow following dipyridamole infusion,

Comparably, due to the small and heterogeneous patient cohort and the cross-sectional design of the study, it is not possible to extrapolate whether CMD represents an early phase of cardiac involvement in AFD, invariably followed by onset of a clear-cut cardiomyopathy over time. Likewise, it is not possible at this stage to envisage whether these abnormalities are susceptible to regression with specific therapy; both such hypotheses need to be specifically addressed in longitudinal studies. However, our findings imply that a normal echocardiogram cannot exclude deterioration of microvascular function related to the disease, raising important issues regarding risk stratification and management.

Conclusions

Coronary microvascular function is markedly impaired in AFD patients irrespective of LVH and gender. CMD may represent the first sign of cardiac involvement in patients who would be otherwise considered unaffected. These findings suggest that coronary blood flow impairment may precede the development of cardiac hypertrophy in AFD, representing an important element for patient clinical management and decision-making in this challenging disease.

Acknowledgements

The authors would like to thank Katia Baldini, professional nurse (Referral Center for Myocardial Diseases, Department of Cardiology, Careggi University Hospital, Florence, Italy).

Conflict of interest: F.C., B.T., and A.M. received speaker's fees and research support from Shire HGT, Genzyme Corporation. All other authors report no potential conflict of interest.

References

1. Meikle PJ, Hopwood JJ, Clague AE, Carrey WF. Prevalence of lysosomal storage disorders. *JAMA* 1999;**281**:249–254.
2. Poorthuis BJ, Wevers RA, Kleijer WJ, Groener JE, de Jong JG, van Weely S, Niezen-Koning KE, van Diggelen OP. The frequency of lysosomal storage diseases in The Netherlands. *Hum Genet* 1999;**105**:151–156.
3. Spada M, Pagliardini S, Yasuda M, Tukel T, Thiagarajan G, Sakuraba H, Ponzone A, Desnick RJ. High incidence of later-onset fabry disease revealed by newborn screening. *Am J Hum Genet* 2006;**79**:31–40.
4. Mechtler TP, Stary S, Metz TF, De Jesús VR, Greber-Platzer S, Pollak A, Herkner KR, Streubel B, Kasper DC. Neonatal screening for lysosomal storage disorders: feasibility and incidence from a nationwide study in Austria. *Lancet* 2012;**379**:335–341.
5. Germain DP. Fabry disease. *Orphanet J Rare Dis* 2010;**5**:30
6. Mehta A, Ricci R, Widmer U, Dehout F, Garcia de Lorenzo A, Kampmann C, Linhart A, Sunder-Plassmann G, Ries M, Beck M. Fabry disease defined: baseline clinical manifestations of 366 patients in the Fabry Outcome Survey. *Eur J Clin Invest* 2004; **34**:236–242.
7. Eng CM, Fletcher J, Wilcox WR, Waldek S, Scott CR, Sillence DO, Breunig F, Charrow J, Germain DP, Nicholls K, Banikazemi M. Fabry disease: baseline medical characteristics of a cohort of 1765 males and females in the Fabry Registry. *J Inherit Metab Dis* 2007;**30**:184–192.
8. O'Mahony C, Elliott P. Anderson–Fabry disease and the heart. *Prog Cardiovasc Dis* 2010;**52**:326–335.
9. Elliott PM, Kindler H, Shah JS, Sachdev B, Rimoldi OE, Thaman R, Tome MT, McKenna WJ, Lee P, Camici PG. Coronary microvascular dysfunction in male patients with Anderson–Fabry disease and the effect of treatment with alpha galactosidase A. *Heart* 2006;**92**:357–360.
10. Kalliokoski RJ, Kalliokoski KK, Sundell J, Engblom E, Penttinen M, Kantola I, Raitakari OT, Knuuti J, Nuutila P. Impaired myocardial perfusion reserve but preserved peripheral endothelial function in patients with Fabry disease. *J Inherit Metab Dis* 2005;**28**:563–573.
11. Kalliokoski RJ, Kantola I, Kalliokoski KK, Engblom E, Sundell J, Hannukainen JC, Janatuinen T, Raitakari OT, Knuuti J, Penttinen M, Viikari J, Nuutila P. The effect of 12-month enzyme replacement therapy on myocardial perfusion in patients with Fabry disease. *J Inherit Metab Dis* 2006;**29**:112–118.
12. Cecchi F, Olivotto I, Gistri R, Lorenzoni R, Chiriatti G, Camici PG. Coronary microvascular dysfunction and prognosis in hypertrophic cardiomyopathy. *N Engl J Med* 2003;**349**:1027–1035.
13. Camici PG, Olivotto I, Rimoldi OE. The coronary circulation and blood flow in left ventricular hypertrophy. *J Mol Cell Cardiol* 2012;**52**:857–864.
14. Maron MS, Olivotto I, Maron BJ, Prasad SK, Cecchi F, Udelson JE, Camici PG. The case for myocardial ischemia in hypertrophic cardiomyopathy. *J Am Coll Cardiol* 2009;**54**: 866–875.
15. Petersen SE, Jerosch-Herold M, Hudsmith LE, Robson MD, Francis JM, Doll HA, Selvanayagam JB, Neubauer S, Watkins H. Evidence for microvascular dysfunction in hypertrophic cardiomyopathy: new insights from multiparametric magnetic resonance imaging. *Circulation* 2007;**115**:2418–2425.
16. Ferri L, Guido C, la Marca G, Malvagia S, Cavicchi C, Fiumara A, Barone R, Parini R, Antuzzi D, Feliciani C, Zampetti A, Manna R, Giglio S, Della Valle CM, Wu X, Valenzano KJ, Benjamin R, Donati MA, Guerrini R, Genuardi M, Morrone A. Fabry disease: polymorphic haplotypes and a novel missense mutation in the GLA gene. *Clin Genet* 2012;**81**:224–233.
17. Maron MS, Olivotto I, Betocchi S, Casey SA, Lesser JR, Losi MA, Cecchi F, Maron BJ. Effect of left ventricular outflow tract obstruction on clinical outcome in hypertrophic cardiomyopathy. *N Engl J Med* 2003;**348**:295–303.
18. Nagueh SF, Appleton CP, Gillebert TC, Marino PN, Oh JK, Smiseth OA, Waggoner AD, Flachskampf FA, Pellikka PA, Evangelisa A. Recommendations for the evaluation of left ventricular diastolic function by echocardiography. *J Am Soc Echocardiogr* 2009;**22**:107–133.
19. Sotgia B, Sciagrà R, Olivotto I, Casolo G, Rega L, Betti I, Pupi A, Camici PG, Cecchi F. Spatial relationship between coronary microvascular dysfunction and delayed contrast enhancement in patients with hypertrophic cardiomyopathy. *J Nucl Med* 2008; **49**:1090–1096.
20. DeGrado TR, Hanson MW, Turkington TG, Delong DM, Brezinski DA, Vallée JP, Hedlund LW, Zhang J, Cobb F, Sullivan MJ, Coleman RE. Estimation of myocardial blood flow for longitudinal studies with ^{13}N-labeled ammonia and positron emission tomography. *J Nucl Cardiol* 1996;**3**:494–507.
21. Cerqueira MD, Weissman NJ, Dilsizian V, Jacobs AK, Kaul S, Laskey WK, Pennell DJ, Rumberger JA, Ryan T, Verani MS; American Heart Association Writing Group on Myocardial Segmentation and Registration for Cardiac Imaging. Standardized myocardial segmentation and nomenclature for tomographic imaging of the heart. *Circulation* 2002;**105**:539–542.
22. Perugini E, Rapezzi C, Piva T, Leone O, Bacchi-Reggiani L, Riva L, Salvi F, Lovato L, Branzi A, Fattori R. Non-invasive evaluation of the myocardial substrate of cardiac amyloidosis by gadolinium cardiac magnetic resonance. *Heart* 2006;**92**:343–349.
23. Pieroni M, Chimenti C, Ricci R, Sale P, Russo MA, Frustaci A. Early detection of Fabry cardiomyopathy by tissue Doppler imaging. *Circulation* 2003;**107**:1978–1984.
24. Niemann M, Herrmann S, Hu K, Breunig F, Strotmann J, Beer M, Machann W, Voelker W, Ert F, Wanner C, Weidemann F. Differences in Fabry cardiomyopathy between female and male patients consequences for diagnostic assessment. *JACC Cardiovasc Imaging* 2011;**4**:592–601.
25. Olivotto I, Girolami F, Sciagrà R, Ackerman MJ, Sotgia B, Bos JM, Nistri S, Sgalambro A, Grifoni C, Torricelli F, Camici PG, Cecchi F. Microvascular function is selectively impaired in hypertrophic cardiomyopathy patients with sarcomere myofilament gene mutation. *J Am Coll Cardiol* 2011;**58**:839–848.
26. Wilcox WR, Oliveira JP, Hopkin RJ, Ortiz A, Banikazemi M, Feldt-Rasmussen U, Sims K, Waldek S, Pastores GM, Lee P, Eng CM, Marodi L, Stanford KE, Breunig F, Wanner C, Warnock DG, Lemay RM, Germain DP, Fabry Registry. Females with Fabry disease frequently have major organ involvement: lessons from the Fabry Registry. *Mol Genet Metab* 2008;**93**:112–128.
27. Pinto LL, Vieira TA, Giugliani R, Schwartz IV. Expression of the disease on female carriers of X-linked lysosomal disorders: a brief review. *Orphanet J Rare Dis* 2010;**5**:14.
28. Kampmann C, Baehner F, Whybra C, Martin C, Wiethoff CM, Ries M, Gal A, Beck M. Cardiac manifestations of Anderson–Fabry disease in heterozygous females. *J Am Coll Cardiol* 2002;**40**:1668–1674.
29. Machann W, Breunig F, Weidemann F, Sandstede J, Hahn D, Köstler H, Neubauer S, Wanner C, Beer M. Cardiac energy metabolism is disturbed in Fabry disease and improves with enzyme replacement therapy using recombinant human galactosidase A. *Eur J Heart Fail* 2011;**13**:278–283.
30. Weidemann F, Niemann M, Breunig F, Herrmann S, Beer M, Störk S, Voelker W, Ertl G, Wanner C, Strotmann J. Long-term effects of enzyme replacement therapy on Fabry cardiomyopathy: evidence for a better outcome with early treatment. *Circulation* 2009;**119**:524–529.
31. Ishii S. Pharmacological chaperone therapy for Fabry disease. *Proc Jpn Acad Ser B Phys Biol Sci* 2012;**88**:18–30.
32. Porto C, Pisani A, Rosa M, Acampora E, Avolio V, Tuzzi MR, Visciano B, Gagliardo C, Materazzi S, la Marca G, Andria G, Parenti G. Synergy between the pharmacological chaperone 1-deoxygalactonojirimycin and the human recombinant alpha-galactosidase A in cultured fibroblasts from patients with Fabry disease. *J Inherit Metab Dis* 2012;**35**:513–520.
33. Motabar O, Sidransky E, Goldin E, Zheng W. Fabry disease—current treatment and new drug development. *Curr Chem Genomics* 2010;**4**:50–56.
34. Olivotto I, Cecchi F, Gistri R, Lorenzoni R, Chiriatti G, Girolami F, Torricelli F, Camici PG. Relevance of coronary microvascular flow impairment to long-term remodeling and systolic dysfunction in hypertrophic cardiomyopathy. *J Am Coll Cardiol* 2006;**47**:1043–1048.