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Hybrid positron emission tomography-magnetic resonance imaging for assessing different stages of cardiac impairment in patients with Anderson–Fabry disease: AFFINITY study group

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Aims Anderson–Fabry disease (AFD) is an X-linked lysosomal storage disorder associated with multi-organ dysfunction. While native myocardial T1 mapping by magnetic resonance (MR) allow non-invasive measurement of myocyte sphingolipid accumulation, ^{18}F-fluorodeoxyglucose (^{18}F-FDG) positron emission tomography (PET) and MR are able to identify different pathological patterns of disease progression. We investigated the relationship between T1 mapping and ^{18}F-FDG uptake by hybrid PET-MR cardiac imaging in AFD female patients.

Methods and results Twenty AFD females without cardiac symptoms underwent cardiac PET-MR using ^{18}F-FDG for glucose uptake. In all patients and in seven age- and sex-matched control subjects, T1 mapping was performed using native T1 Modified Look-Locker Inversion-recovery prototype sequences. ^{18}F-FDG myocardial uptake was quantified by measuring the coefficient of variation (COV) of the standardized uptake value using a 17-segment model. T1 values of AFD patients were lower compared with control subjects (1236 ± 49 ms vs. 1334 ± 27 ms, $P < 0.0001$). Focal ^{18}F-FDG uptake with COV >0.17 was detected in seven patients. COV was 0.32 ± 0.1 in patients with focal ^{18}F-FDG uptake and 0.12 ± 0.04 in those without ($P < 0.001$). Patients with COV >0.17 had higher T1 values of lateral segments of the mid ventricular wall, compared with those with COV ≤0.17 (1216 ± 22 ms vs. 1160 ± 59 ms, $P < 0.05$).

Conclusion In females with AFD, focal ^{18}F-FDG uptake with a trend towards a pseudo-normalization of abnormal T1 mapping values, may represent an intermediate stage before the development of myocardial fibrosis. These findings suggest a potential relationship between progressive myocyte sphingolipid accumulation and inflammation.

Keywords hybrid PET-MR • T1 mapping • Anderson–Fabry disease • Cardiac

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Introduction

Anderson–Fabry disease (AFD) is an X-linked lysosomal storage disorder associated with severe multi-organ dysfunction and characterized by mutations in the gene encoding the enzyme α-galactosidase A.[1] This enzymatic deficiency leads to progressive accumulation of globotriaosylceramide (Gb3) in lysosomes of a variety of cell types and tissues. The biology of myocardial storage is not completely clear but there are strong suggestions to an earlier enzyme replacement therapy (ERT) initiation, preferably before the development of fibrosis. As an early therapy would have societal cost implications, there is a pressing need to identify non-invasive biomarkers of myocardial storage which can detect initial stage of the disease, and potentially determine treatment response. Recently published data indicated that late gadolinium enhancement (LGE) as assessed by cardiac magnetic resonance (MR) occurs in up to 64% of patients,[2,3] with the majority of patients displaying enhancement localized to the basal infero-lateral wall. The presence of LGE was seen most commonly, but not uniquely, in those with increased left ventricular (LV) mass index. Previous reports have also suggested that LGE in the context of normal mass is a phenomenon seen predominantly in women. While native myocardial T1 mapping by cardiac MR may allow non-invasive measurement of myocyte sphingolipid accumulation,[4–6] both ^{18}F-fluorodeoxyglucose (^{18}F-FDG) positron emission tomography (PET) and cardiac MR are able to identify different pathological patterns of disease progression. In particular, the hybridization of PET and MR is a major advance in non-invasive imaging with numerous promising applications in the cardiovascular field.[7–9] Intrinsic co-registration of metabolic/molecular probe imaging offered by ^{18}F-FDG-PET combined with morphological, functional and tissue imaging of MR, opens new opportunities for disease characterization and early detection of tissue damage, such as inflammation and fibrosis in the heart. There is general agreement that early ERT particularly in pre-hypertrophic stage, prevents progression of the disease; thus, there is an increased need to identify AFD patients that can benefit from early ERT before multi-organ involvement occurs.

This study was undertaken to investigate the potential relationship between T1 mapping and ^{18}F-FDG uptake by hybrid PET-MR cardiac imaging in AFD female patients, without evidence of irreversible myocardial damage or LV hypertrophy.

Methods

Study population

Between June 2015 and April 2018, we prospectively enrolled 20 females (mean age 38 ± 12 years) with genetically proven AFD from 11 unrelated families, who did not present cardiac symptoms and with normal LV function. No patient had received ERT before study entry. Exclusion criteria were pregnancy, breast-feeding, and standard contraindication for MR imaging. As part of the baseline examination, the clinical team collected information from each patient on traditional cardiovascular risk factors and history of AFD-associated pain in hands and feet, decreased sweating, gastrointestinal problems, and neurological symptoms (including stroke and headache). Coronary artery disease was ruled out based on the clinical history plus a negative maximal exercise electrocardiography stress test or pharmacological stress echocardiography. In each patient, high-sensitivity troponin I (HS-troponin I) and N-terminal β-natriuretic peptide 1 were assayed and renal function was investigated by the measurement of glomerular filtration rate and 24-h urine protein excretion. Each patient underwent hybrid PET-MR study within a week. A cohort of seven healthy women undergoing cardiac MR with balanced steady state free precession sequences and native T1 mapping only, served as control group. This group consisted of healthy volunteers or outpatient subjects referred for non-specific symptoms and in whom a detailed diagnostic workup and clinical follow-up was unremarkable. All patients provided informed and written consent. The study conformed to the principles outlined in the Declaration of Helsinki and was approved by the local Ethics Advisory Committee.

PET-MR imaging

All patients underwent cardiac PET-MR hybrid imaging (Biograph mMR; Siemens Healthcare, Erlangen, Germany) according to the Society of Nuclear Medicine and Molecular Imaging and the American Society of Nuclear Cardiology guidelines for cardiac PET-computed tomography.[10] To ensure optimal suppression of ^{18}F-FDG uptake, patients were instructed to consume two high fat, low carbohydrate meals the day before the study and then fasted for at least 6 h before the test.[11] As illustrated in *Figure 1*, all patients were intravenously injected with 370 MBq of ^{18}F-FDG and imaging was performed 60–90 min later.[12] Before starting the examination, MR-compatible leads were placed on patients' chest and attached to PET-MR unit in order to synchronize images with the onset of systole and offset cardiac motion for electrocardiographic gating. Standard cardiac MR protocol sequences were performed for morphological and functional studies, including T1-weighted sequences, balanced steady state free precession, T2-weighted-short tau inversion recovery (STIR) sequences, phase-sensitive inversion recovery. Early and late enhancement inversion recovery sequences were obtained from 5 to 15 min after administration of a 0.15-mmol/kg-body weight gadolinium-DTPA (Magnevist, Schering, Berlin, Germany) bolus. An inversion time scout scan was acquired, and the optimal inversion time value was found. A single bed position PET emission scan was acquired over 20 min simultaneously with a whole heart cardiac MR sequence. All patients and control subjects underwent cardiac MR T1 mapping by using pre-contrast T1 Modified Look-Locker Inversion-recovery (MOLLI) prototype sequences [sampling scheme 5 s (3 s) 3 s with motion correction] performed at the basal, mid, and apical slices of the LV short-axis view. The T1 mapping acquisition parameters were: echo time, 1.08 ms; flip angle, 35°; slice thickness, 8 mm; field of view, 400 mm; matrix, 256 × 168; PAT factor, 2.

PET-MR analysis

A radiologist and a nuclear medicine physician in consensus analysed cardiac PET-MR images on a dedicated workstation, as previously described.[11] Morphological and functional MR data were analysed to assess LV anatomy, kinetic, wall thickness, and early and LGE patterns. PET images were classified according to ^{18}F-FDG uptake pattern: homogeneous, heterogeneous, and focal. Images with a homogeneous or a heterogeneous pattern show the outline of the LV wall with uniform or inconsistent tracer distribution, respectively, whereas images with a focal pattern show a segmental increase in tracer uptake. Only focally increased cardiac uptake was considered a positive finding for the presence of active cardiac inflammation.[9,11] The standardized uptake value (SUV) was also determined. The intensity of ^{18}F-FDG uptake was quantified by measuring the SUV in 17 myocardial segments and the average SUV and standard deviation (SD) of the SUV were calculated for each patient. The coefficient of variation (COV) of the SUV in each patient was calculated as the SUV SD divided by the average SUV as an index of heterogeneity of ^{18}F-FDG uptake.[9,11] Two nuclear medicine physicians blindly measured SUV, and the measurements were averaged. The intra-

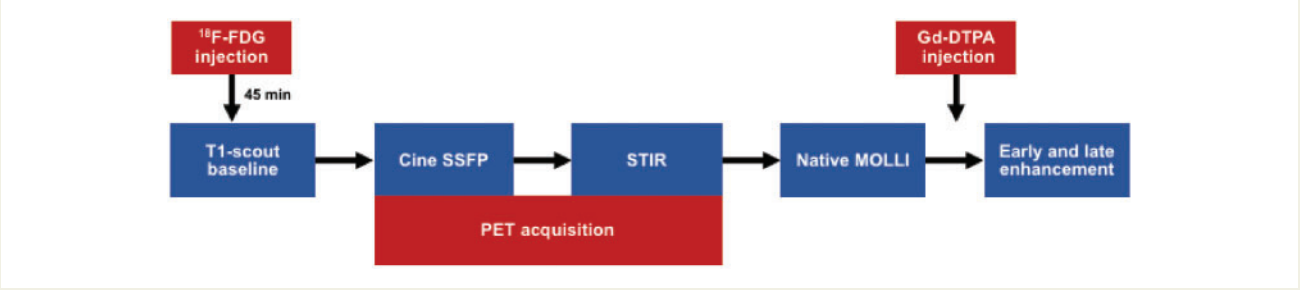

Figure 1 Cardiac PET-MR imaging acquisition protocol.

Table 1 Genetic characteristics of 14 females with Anderson–Fabry disease

Patients	α-GLA mutation	α-GLA protein effect
1	c.950T>C	p.Ile317Thr
2	c.1021dupG	Frameshift and premature stop codon
3	c.1021dupG	Frameshift and premature stop codon
4	c.1066C>T	p.R356W
5	c.863C>A	p.A288D
6	c.508G>A	p.D170N
7	c.352C>T	p.R118C
8	c.352C>T	p.R118C
9	c.901C>G	p.R301G
10	c.1066C>T	p.R356W
11	c.680G>C	p.R227P
12	c.901C>G	p.R301G
13	IVS4 + 5G>T	Splicing alteration
14	c.424T>C	p.C142R

α-GLA, α-galactosidase A.

observer and inter-observer variability of SUV measurements were <5%. As previously shown, a COV value >0.17 was considered as an index of abnormal tracer uptake.[13] For the T1 mapping quantification, LV apical, mid-ventricular, and basal short-axis slices were considered. Native mean T1 values were measured by drawing a 6-pixel size region of interest in the anterior, septal, inferior, and lateral segments of each slice. Any segment with artefacts affecting the measurements was eliminated.

Statistical analysis

Results are expressed as mean ± SD, or medians and interquartile range. The Kolmogorov–Smirnov test was used to evaluate if the continuous variables fit a normal distribution. For comparison of groups, unpaired *t*-test or Wilcoxon rank-sum test were performed depending on whether the distribute on was normal or not. A *P*-value <0.05 was considered statistically significant. Statistical analyses of all data were performed using SPSS software (SPSS 21.0 for Windows, IBM, Chicago, IL, USA).

Results

The individual genetic data of AFD patients are reported in *Table 1*. As far it was possible to extend the pedigree analysis, no link between families sharing the same mutation was found. According to selection criteria, all patients carrying α-galactosidase A mutation had normal LV mass index, some reported mild neuropathic pain and isolated episodes of gastrointestinal complaints and two patients had cornea verticillata with normal visual acuity. No patient was affected by diabetes. Clinical data from control subjects and AFD patients are shown in *Table 2*.

PET-MR imaging

PET-MR procedure was successfully performed in all individuals without sequence or contrast agent-related side effects. All patients exhibited negative T2-STIR images. Six patients had focal LGE indicating intra-myocardial fibrosis and were excluded from the final analysis. Focal ^{18}F-FDG uptake with COV >0.17 was detected in seven patients out of the remaining 14 patients with focal FDG uptake in the infero-lateral wall, suggesting inflammation pattern. COV was 0.32 ± 0.1 in patients with focal ^{18}F-FDG uptake and 0.12 ± 0.04 in those without ($P < 0.001$). When AFD population was categorized according to ^{18}F-FDG PET results, no statistically significant differences were observed between patients with normal and abnormal COV, as regard to clinical characteristics (*Table 3*). Similarly, no significant differences were

Table 2 Clinical data from control subjects and Anderson–Fabry disease patients

	Control subjects ($n = 7$)	AFD patients ($n = 14$)	*P*-value
Age (years)	35 ± 3	34 ± 12	0.61
Body mass index (kg/m^2)	23 ± 3	24 ± 4	0.67
GFR (mL/min/m^2 × 1.73)	120 ± 10	109 ± 14	0.17
Heart rate (bpm)	70 ± 9	71.4 ± 13	0.68
Systolic blood pressure (mmHg)	119 ± 11	120 ± 22	0.77
Diastolic blood pressure (mmHg)	72 ± 9	73 ± 11	0.76

Values are expressed as mean ± standard deviation or median (interquartile range).
AFD, Anderson–Fabry disease; GFR, glomerular filtration rate.

Table 3 Clinical data from Anderson–Fabry disease patients according to ^{18}F-FDG PET results

	AFD patients ($n = 14$)	AFD with COV ≤ 0.17 ($n = 7$)	AFD with COV > 0.17 ($n = 7$)	*P*-value
Age (years)	34 ± 12	33 ± 11	35 ± 13	0.77
Body mass index (kg/m^2)	24 ± 4	25 ± 4	23 ± 3	0.36
GFR (mL/min/m^2 × 1.73)	109 ± 14	108 ± 20	111 ± 9	0.76
Urine protein (mg/24 h)	131 (100–180)	152 (0.20–283)	128 (0.40–210)	0.33
HS-troponin I (pg/mL)	0.02 ± 0.02	0.03 ± 0.03	0.01 ± 0.01	0.15
NT-proBNP (pg/mL)	63.2 ± 30	69 ± 32	59 ± 29	0.58
Heart rate (bpm)	71.4 ± 13	71 ± 12	72 ± 16	0.87
Systolic blood pressure (mmHg)	120 ± 22	127 ± 17	115 ± 26	0.37
Diastolic blood pressure (mmHg)	73 ± 11	77 ± 15	69 ± 6	0.26

Values are expressed as mean ± standard deviation or median (interquartile range).
AFD, Anderson–Fabry disease; COV, coefficient of variation; GFR, glomerular filtration rate; HS, high sensitivity; NT-proBNP, N-terminal pro-brain natriuretic peptide.

observed in the LV mass index, LV end-diastolic volume index, LV end-systolic volume index, and LV ejection fraction between patients and control subjects (*Table 4*). Of note, no wall motion abnormalities were observed in all LV myocardial segment. Measurement of T1 mapping of the 14 patients with T2-STIR negative and no evidence of LGE, demonstrated that the mean and the segmental native T1 value were significantly lower in AFD patients compared with control subjects (1236 ± 49 ms vs. 1334 ± 27 ms, $P < 0.001$) (*Table 4*).

T1 mapping and ^{18}F-FDG PET findings

Bull's eyes of the mean native T1 values of LV myocardial segments, in patients with normal and abnormal COV are illustrated in *Figure 2*. Interestingly, only in the lateral segments of the mid-LV wall, patients with focal FDG uptake and COV >0.17 showed higher mean native T1 values (1216 ± 22 ms vs. 1160 ± 59 ms, $P < 0.05$) (*Figure 3*), suggesting a potential relationship between progressive myocyte sphingolipid accumulation and inflammation. No significant differences were observed in the remaining myocardial regions. An example of AFD patient, exhibiting high T1 values in the lateral segments of the mid-LV wall and abnormal COV is shown in *Figure 4*.

Discussion

In patients with AFD, knowledge of the presence or absence of myocardial fibrosis is crucial with respect to the treatment expectations and several studies have shown that the earlier the treatment is started, the better the long-term outcome is.[14] Thus, there is an increasing need to identify biomarkers which can detect early cardiac involvement, before the development of irreversible myocardial fibrosis, and potentially influence treatment response. Several studies focused on individual biomarkers. In particular, previous data demonstrated that LGE occurs in up to 64% of patients, with the majority of patients displaying enhancement localized to the basal infero-lateral wall.[2,3] The presence of LGE was seen most commonly, but not uniquely, in those with increased LV mass. Accordingly, in the present study focal FDG uptake involved the infero-lateral wall, suggesting a possible later development of fibrosis in this specific myocardial region. Previous reports also suggested that LGE in the context of normal LV mass is a phenomenon seen predominantly in women. However, LGE cannot be considered a very early biomarker to be used for identification of myocardial involvement in AFD.

T1 mapping is an additional and powerful diagnostic tool in AFD, which has been recently proposed to discriminate AFD from other

Table 4 Cardiac MR characteristics of study population

	Control subjects (*n* = 7)	AFD patients (*n* = 14)	*P*-value
LV mass index (mg/m$^{2)}$	58 ± 5	55 ± 10	0.467
LVEDV index (mL/m^2)	74 ± 4	73 ± 15	0.856
LVESV index (mL/m^2)	27 ± 4	23 ± 8	0.14
LV ejection fraction (%)	66 ± 3	69 ± 7	0.302
Mean native T1 (ms)	1334 ± 27	1236 ± 49	<0.001
Apical lateral native T1 (ms)	1439 ± 133	1247 ± 118	0.003
Apical septal native T1 (ms)	1492 ± 139	1315 ± 140	0.02
Mid-lateral native T1 (ms)	1295 ± 56	1188 ± 52	<0.001
Mid-septal native T1 (ms)	1294 ± 28	1230 ± 47	0.004
Basal lateral native T1 (ms)	1300 ± 65	1236 ± 51	0.02
Basal septal native T1 (ms)	1305 ± 67	1219 ± 83	0.03

Values are expressed as mean ± standard deviation.
AFD, Anderson–Fabry disease; LV, left ventricular; EDV, end-diastolic volume; ESV, end-systolic volume.

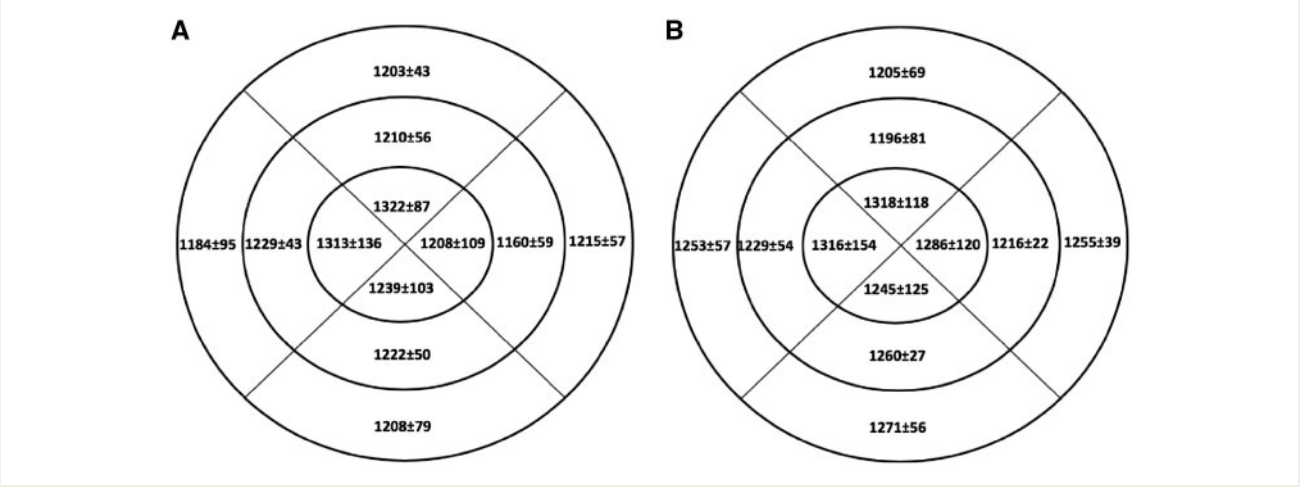

Figure 2 Bull's eyes of mean native T1 values of left ventricular myocardial segments, in patients with normal (*A*) and abnormal (*B*) coefficient of variation.

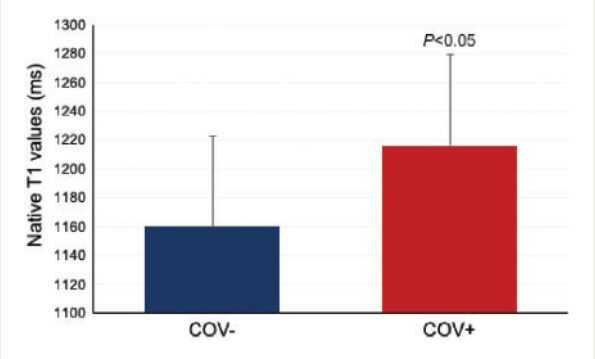

Figure 3 Mean native T1 values of mid-lateral segments, in patients with normal (blue bar) and abnormal (red bar) coefficient of variation.

causes of LV hypertrophy. Previous studies have shown that decreased native myocardial T1 is highly prevalent in AFD patients with LV hypertrophy.[4,6] In particular, Pica *et al.*[4] demonstrated that in subjects without LV hypertrophy, reduced myocardial T1 has a 50% prevalence and is associated with echocardiographic parameters of cardiac dysfunction, suggesting that a low T1 is detecting early cardiac disease. As a candidate biomarker, native myocardial T1 in AFD is useful both in established and early disease. Cardiac MR derived myocardial T1 mapping has previously been shown to have very high sensitivity and specificity to discriminate AFD patients with LV hypertrophy. Low T1 in AFD is likely a consequence of the progressive sphingolipid storage in the myocardium. Pica *et al.*[4] also suggested four different phases of myocardial involvement in AFD: phase 1 normal; phase 2 low T1, indicating early myocardial dysfunction; phase 3 LV hypertrophy with low T1; and phase 4 'pseudo-normalization' of T1, indicating fibrosis and heart failure. However, even in

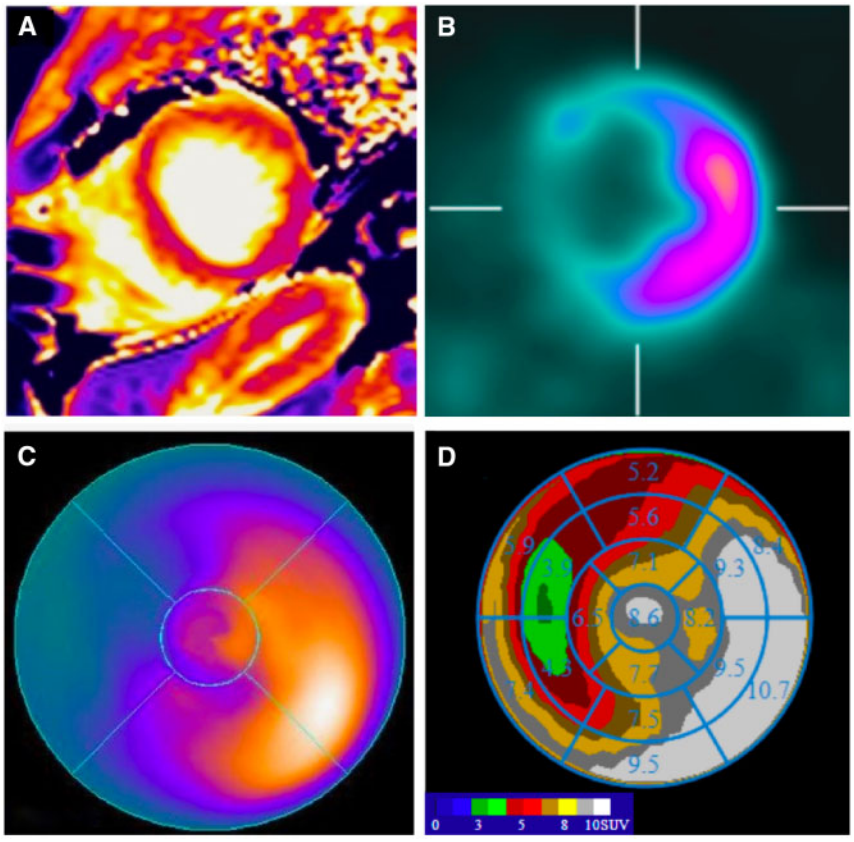

Figure 4 (*A*) T1 mapping and (*B*) ^{18}F-fluorodeoxyglucose (FDG) PET images on short-axis view of a patient with abnormal coefficient of variation (0.3) and high T1 values in the lateral segments of the mid-left ventricular wall. (*C*) ^{18}F-FDG polar map and (*D*) corresponding standard uptake values (SUV) in individual myocardial segments. Increased SUV is observed in the same territory of high T1 values.

those with normal wall thickness, T1 values in the infero-lateral wall are significantly higher than in 'remote' myocardium (i.e. myocardium not including the infero-lateral wall). These data suggest that tissue composition in the infero-lateral wall is already altered prior to the development of LV hypertrophy and/or LGE, a finding further supported by global and regional T2 value abnormalities, irrespective of cardiac morphology. The use of T1 values in isolation, either for disease evaluation or disease monitoring is problematic, because (i) Gb3 storage and myocardial fibrosis have opposing effects on native T1 and (ii) change in T1 values may reflect either disease progression or treatment effect. As suggested by Sado *et al.*[6] in patients with severe disease, septal T1 values can pseudo-normalize. Importantly, as LV hypertrophy increases, the 'spread' of T1 values increases; i.e. as progressive sphingolipid deposition occurs, low T1 values get lower, and as fibrosis develops, higher T1 values get higher. More recently, Nordin *et al.*[15] in a prospective multicentre observational study of 100 LV hypertrophy-negative AFD patients, showed that low native myocardial T1 is more common than previously reported,[4,6] with a prevalence of 59%. The authors conclude that there is a detectable pre-hypertrophic phenotype in AFD consisting of storage (low native T1), structural, functional, and electrocardiogram changes. Nappi *et al.*[9] studied 13 AFD patients, without cardiac symptoms and with normal LV function using a hybrid cardiac PET-MR imaging approach. They found in six patients focal LGE indicating intra-myocardial fibrosis, and in four of them positive STIR sequences. All patients with LGE and positive STIR MR images had focal ^{18}F-FDG uptake in the corresponding myocardial segments indicating myocardial inflammation. These results suggest the feasibility of PET-MR imaging for the early detection of cardiac involvement and in particular of focal inflammation, even in non-hypertrophic stage.[9] Spinelli *et al.*[13] recently demonstrated the relation between impaired LV longitudinal function and myocardial metabolic abnormalities, detected by hybrid cardiac PET-MR imaging in early phase of AFD related cardiomyopathy. The authors compared the results of PET-MR cardiac imaging with those of speckle tracking echocardiography in 24 heterozygous females carrying α-galactosidase A mutation and without LV hypertrophy, showing worse global longitudinal systolic strain, in patients with COV >0.17 compared with those with COV ≤0.17 (-18.5 ± 2.7% vs. -22.2 ± 1.8%). The authors conclude that in females carrying α-galactosidase A mutation, focal ^{18}F-FDG uptake represents an early sign of disease-related myocardial damage and is associated with impaired LV longitudinal function, further supporting the hypothesis that inflammation plays an important role in glycosphingolipids storage disorders. Furthermore, in this study, in order to distinguish physiological from pathological FDG uptake, the authors demonstrated normal ^{18}F-FDG uptake in a cohort of 10 age-matched

control women, who underwent PET imaging for other reasons and served as control group. These subjects had no evidence of active inflammatory, coronary or valvular diseases, of diabetes mellitus or severe hepatic, renal, malignant, and haematologic diseases and were not receiving corticosteroids.[13]

Our study is the first to correlate T1 mapping parameters and COV values, showing that COV as assessed by ^{18}F-FDG-PET, is significantly impaired in the infero-lateral basal regions, in patients with AFD. This abnormality is probably associated with a trend towards pseudo-normalization of T1 values, as assessed by cardiac MR and may represent an intermediate stage, before the development of myocardial fibrosis and possibly myocardial inflammation. These data are further supported by a previous paper, demonstrating the coexistence of two different pathological phenomena, by means myocardial inflammation and fibrosis.[9] In addition, Frustaci *et al.*[16] more recently reported for the first time, a high histological incidence (56%) of myocarditis in a large series of patients with AFD, undergoing endo-myocardial biopsy. These authors suggest that myocarditis may occur with interstitial widening through inflammatory cell infiltration, oedema, and cell necrosis and may also be a source of myocardial fibrosis through the activation of transforming growth factor b1. The concept of myocardial inflammation has been largely investigated in the past, by using FDG-PET as compared to MR imaging. Nensa *et al.*[17] prospectively compared ^{18}F-FDG-PET with LGE and T2-weighted MR sequences, using integrated PET-MR in patients with suspected myocarditis, showing that pathological myocardial ^{18}F-FDG uptake is in overall good agreement with LGE and T2 hyperintensity. Increased glucose uptake is a hallmark of inflammation; as neutrophils, cells of the monocyte/macrophage family and lymphocytes are able to express high levels of glucose transporters (in particular GLUT1 and GLUT3) and hexokinase activity. Thus, compared with cardiac MR, ^{18}F-FDG-PET allows a different and more direct visualization of inflammation by quantifying the metabolic activity of inflammatory cell infiltrates. Several studies have focused on inflammation markers and leucocyte activity in AFD patients. In particular, leukocytes and endothelium from patients with AFD show signs of inflammatory activation,[18–20] characterized by increased expression of adhesion molecules, such as CD31 in CD3+ lymphocytes, monocytes, and granulocytes, when compared with healthy controls.[20] Another study by Hayashi *et al.*[21] demonstrated increased levels of the macrophage-related markers CD68, CD163, and CD45 in endo-myocardial biopsy samples from patients with AFD. Moreover, serum levels of IL-6, IL-1β, TNF-α, and soluble vascular adhesion molecule were significantly higher in AFD patients[22] indicating that pro-inflammatory cytokines might play a role in the progression of AFD-related cardiomyopathy. These data suggest that inflammation might play a key role in the early stages of AFD, before the development of structural changes and myocardial fibrosis; however, more evidence is required before the involvement of classical inflammatory pathways in Fabry disease can be confirmed. In a previous work, Nordin *et al.*[23] assessed T2 mapping and troponin in a cohort of AFD patients, the majority of which with LV hypertrophy, and concluded that inflammation related to cardiomyocyte Gb3 storage contributed to LGE. The finding that in our study, the presence of myocardial focal FDG uptake was not associated to increased levels of HS-troponin I, may suggest that HS-troponin I is not a marker of very early cardiac involvement in AFD.

Finally, the results of our study further support the evidence that an integrated PET-MR technique, is particularly well suited for comparative studies, and represents a valuable non-invasive diagnostic tool that can provide unique and simultaneous information regarding the presence of myocardial inflammation that likely represents a clue of response to cardiomyocyte Gb3 storage.

Limitations and strengths

T1 values are affected by confounding variables such as field strength, body composition, and scanning parameters and it should be taken into account correction for these variables. Extracellular volume fraction could contribute to normalize myocardial T1 according to blood T1. Therefore, the lack of data regarding extracellular volume should be considered as limitation of this study, as well as the lack of myocardial T2 mapping. The relatively small sample size could represent another limitation, but it reflects the rarity of the clinical condition under evaluation and the single-centre nature of the study. The absence of validation with endo-myocardial biopsy should also be considered as a possible limitation. However, the value of biopsy as gold standard is questionable unless a targeted approach is used, for example using LGE-cardiac MR imaging to guide biopsy.[24] Furthermore, while biopsy is often accepted as a standard of reference, it has limited sensitivity due to sampling errors.[25] The use of imaging techniques able to assess cardiac metabolism, structure and function represents a major strength of the study. Yet, criteria applied for analysis of both ^{18}F-FDG-PET and cardiac MR are well established and validated in clinical studies.

Conclusions

This study highlights the role of hybrid PET-MR imaging in the early detection of cardiac involvement in AFD patients allowing to identify different stages of disease progression. The evidence of a trend towards pseudo-normalization of abnormal T1 values, associated with abnormal COV values, may represent an intermediate stage (possibly myocardial inflammation) allowing an early and more effective therapeutic approach, thus preventing the development of irreversible myocardial damage and fibrosis. It is also conceivable to hypothesize that a focal ^{18}F-FDG uptake in female patients carrying AFD related mutation is a predictor of the development of LV hypertrophy, although further research is needed to establish this issue.

Conflict of interest: none declared.

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