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# Caractérisation de l'atteinte cardiaque de la maladie de Fabry selon le strain longitudinal, les rehaussements tardifs et le T1 mapping : relation avec le statut fonctionnel, et prédiction des évènements cardiovasculaires

Émilie Testet

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**CARACTERISATION DE L'ATTEINTE CARDIAQUE DE LA  
MALADIE DE FABRY SELON LE STRAIN LONGITUDINAL,  
LES REHAUSSEMENTS TARDIFS ET LE T1 MAPPING :  
RELATION AVEC LE STATUT FONCTIONNEL,  
ET PREDICTION DES EVENEMENTS CARDIOVASCULAIRES**

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Directrice

## **RESUME**

**CONTEXTE :** La Maladie de Fabry est une maladie de surcharge lipidique liée à l'X dans laquelle l'atteinte cardiaque est une cause majeure de morbidité. La relation entre les paramètres échographiques et d'imagerie par résonance magnétique (IRM) innovants et les paramètres d'épreuve d'effort, ainsi que leur valeur pronostique, n'ont à ce jour pas été évalués.

**OBJECTIF :** Nous avons évalué la relation entre les paramètres d'imagerie cardiaque (masse ventriculaire gauche, strain longitudinal (SL), T1 natif et réhaussements tardifs) et d'une part les paramètres de VO<sub>2</sub> à l'effort ; d'autre part la survenue d'évènements cardiovasculaires durant le suivi.

**METHODES :** Dans cette étude prospective, monocentrique, tous les patients atteints de Maladie de Fabry référés auprès de notre centre tertiaire ont bénéficié d'un électrocardiogramme (ECG), d'un Holter ECG, d'une échocardiographie, d'une IRM, et d'une épreuve d'effort avec VO<sub>2</sub> et ont été comparés à des témoins sains.

**RESULTATS :** De juin 2016 à novembre 2017, nous avons inclus 31 patients atteints de maladie de Fabry (âge moyen 42 ± 16 ans, 39% d'hommes) ayant un degré moyen de sévérité de la maladie (selon le MMSI), que nous avons comparé à 14 témoins appariés. Treize patients (42%) avaient une hypertrophie ventriculaire gauche (>11.0 mm). Le T1 moyen était significativement plus faible chez les patients par rapport aux témoins (934 ± 60 vs. 999 ± 45 ms,  $p < 0.05$ ). Une bonne corrélation était observée entre le pic de VO<sub>2</sub> et le SL transmural ( $r = -0.55$ ) et endocardique ( $r = -0.53$ ). La pente VE/VCO<sub>2</sub> était corrélée à l'épaisseur ventriculaire gauche ( $r = 0.48$ ), et au T1 moyen ( $r = -0.57$ ). Durant un suivi moyen de 23 mois, 6 patients ont présenté un événement cardiovasculaire. En analyse univariée, le SL transmural global et plus spécifiquement le SL transmural septal basal ; le SL épicaudique global et le SL épicaudique antérolatéral basal et moyen ; et le T1 moyen étaient significativement prédicteurs d'événement cardiovasculaire ( $p < 0.05$ ).

**CONCLUSION :** Notre étude suggère que le strain longitudinal et le T1 mapping sont utiles dans la prédiction du statut fonctionnel et des évènements cardiovasculaires chez les patients atteints de Maladie de Fabry.

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**DISCIPLINE :** CARDIOLOGIE

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**MOTS CLES :** Maladie de Fabry · Echocardiographie · Strain longitudinal · Strain multi-couches · Imagerie par résonance magnétique · T1 mapping · Réhaussements tardifs · Prognostic

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**INTITULE et ADRESSE de l'UFR :** UFR des sciences médicales – 146 rue Léo Saignat, 33076 Bordeaux

## Serment d'Hippocrate

Au moment d'être admise à exercer la médecine, je promets et je jure d'être fidèle aux lois de l'honneur et de la probité.

Mon premier souci sera de rétablir, de préserver ou de promouvoir la santé dans tous ses éléments, physiques et mentaux, individuels et sociaux.

Je respecterai toutes les personnes, leur autonomie et leur volonté, sans aucune discrimination selon leur état ou leurs convictions. J'interviendrai pour les protéger si elles sont affaiblies, vulnérables ou menacées dans leur intégrité ou leur dignité. Même sous la contrainte, je ne ferai pas usage de mes connaissances contre les lois de l'humanité.

J'informerai les patients des décisions envisagées, de leurs raisons et de leurs conséquences. Je ne tromperai jamais leur confiance et n'exploiterai pas le pouvoir hérité des circonstances pour forcer les consciences.

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# **Characterization of Fabry Disease cardiac involvement according to longitudinal strain, late gadolinium enhancement, and T1 mapping: relation to functional status, and prediction of cardiovascular outcomes**

## **ABSTRACT**

**BACKGROUND:** Anderson-Fabry disease (FD) is a rare X-linked glycolipid storage disease in which cardiac involvement is a leading cause of morbimortality. Neither relation between echocardiographic and cardiovascular magnetic resonance (CMR) innovative parameters, and exercise testing parameters, nor their prognosis value, have been evaluated yet.

**AIMS:** We sought to evaluate the relation between cardiac imaging parameters (i.e. left ventricular (LV) mass, multi-layer longitudinal strain (LS), native T1 and late gadolinium enhancements) and on one hand cardiopulmonary exercise capacity in FD patients; and on the other hand, cardiovascular (CV) outcomes during follow-up.

**METHODS:** In this prospective, monocentric study, we evaluated all consecutive FD patients referred in our tertiary center, performing electrocardiogram (ECG), Holter ECG monitoring, echocardiography, CMR, and cardiopulmonary exercise testing, that we compared with healthy controls.

**RESULTS:** From June 2016 to November 2017, we included 31 FD patients (mean age  $42 \pm 16$  years, 39% male) with an overall mild degree of disease severity, that were compared with 14 matched healthy controls. Thirteen FD patients (42%) had left ventricular hypertrophy (LVH) (wall thickness  $>11.0$  mm). Mean T1 in FD patients was significantly lower than in controls ( $934 \pm 60$  vs.  $999 \pm 45$  ms,  $p < 0.05$ ). Regarding functional capacity, a good correlation was observed between  $VO_2$  peak and global ( $r = -0.55$ ) and endocardial ( $-0.53$ ) LS. VE/ $VC_{O_2}$  slope was correlated with maximal LV wall thickness ( $r = 0.48$ ), and mean T1 value ( $r = -0.57$ ). During a median follow-up of 23 months, 6 patients presented CV events. In the Cox univariate analyses, global transmural LS and more specifically basal septal LS; epicardial global LS, and particularly basal and mid anterolateral LS; and anterior T1 value were significantly predictive of CV event ( $p < 0.05$ ).

**CONCLUSIONS:** Our study suggests that longitudinal strain and T1 mapping are relevant for prediction of functional status and cardiovascular events in FD patients.

**Key words:** Fabry disease · Echocardiography · Longitudinal strain · Multi-layer longitudinal strain · Cardiac magnetic resonance · T1 mapping · Late gadolinium enhancement · Prognosis

**Abbreviations list:**

CMR: Cardiac magnetic resonance

CV: Cardiovascular

ECG: Electrocardiography

ECV: Extracellular volume

ERT: Enzyme replacement therapy

FD: Fabry disease

HR: Hazard ratio

LGE: Late gadolinium enhancement

LS: Longitudinal strain

LV: Left ventricle

LVH: Left ventricular hypertrophy

MMSI: Mainz Severity Score Index

NYHA: New York Heart Association

RVH: Right ventricular hypertrophy

## Introduction

Anderson-Fabry disease (FD) is a X-linked glycolipid storage disease caused by a deficiency of  $\alpha$ -galactosidase A enzyme due to a mutation in the galactosidase alpha gene<sup>1,2</sup>. The prevalence of FD is historically estimated between 1 in 17,000 to 1 in 117,000 individuals<sup>3,4</sup>, but it is probably significantly underestimated. The deficiency in the enzymatic activity leads to progressive lysosomal accumulation of complex sphingolipids in vascular endothelial and smooth-muscle cells throughout the body, particularly in the skin, kidney, nervous system, eyes, and heart<sup>5,6</sup>.

Cardiac involvement in FD represents a leading and increasing cause of morbidity and mortality<sup>7,8</sup> and a major determinant for reduction of life-expectancy in this disease<sup>9</sup>. Accumulation of sphingolipids in cardiac tissues can progressively induce left ventricular hypertrophy (LVH) and myocardial fibrosis, causing a true infiltrative cardiomyopathy. Its complications are various, and can include heart failure with preserved ejection fraction, valvular regurgitation, conduction system disease, atrial and ventricular arrhythmias, and microvascular angina. A specific treatment exists, enzyme replacement therapy (ERT), consisting in administrating the lacking enzyme to restore its role in complex lipids metabolism<sup>10-12</sup>. It permits a reduction of glycosphingolipids accumulation in cardiac tissue<sup>13</sup>, demonstrated by significant decrease of left ventricular mass<sup>14</sup>. Novel chaperone therapy also appeared more recently<sup>15</sup>.

Echocardiography is a substantial non-invasive technique for evaluation and follow-up of patients with FD. Doppler tissue imaging (DTI) and assessment of myocardial deformation by strain in speckle tracking have been shown to allow the early recognition of intrinsic impairment of respectively myocardial relaxation and systolic function, before LVH even develops<sup>17-19</sup>. However, multi-layer analysis of speckle-tracking strain is a more recently-developed method enabling evaluation of endocardial and epicardial strain<sup>20-22</sup>, that has not been evaluated yet in FD. Furthermore, neither the prognosis value of these parameters, nor their relation with cardiopulmonary exercise testing parameters, have been assessed yet.

Currently, cardiovascular magnetic resonance (CMR) imaging represents the leading imaging modality for the detection of cardiac involvement in Fabry disease<sup>23</sup>, because of its valuable capabilities of tissue characterization. Indeed, late gadolinium enhancement (LGE) sign the presence of focal fibrosis in Fabry disease<sup>24-26</sup>, and its expansion is a major prognostic determinant for ventricular arrhythmias and cardiac outcomes in this disease<sup>27</sup>. More recently, it has been shown that native T1 assessment permits early detection of cardiac involvement in

FD as it reflects sphingolipids accumulation<sup>28</sup>. However, their ability to predict cardiovascular (CV) outcomes and functional status have not been studied.

We hypothesized that these recently-developed cardiac imaging parameters used to detect cardiac involvement in FD can also predict functional status and CV outcomes of FD patients.

The aims of this prospective monocentric study were to evaluate the relation between cardiac imaging parameters (i.e. LV mass, longitudinal strain, native T1 and LGE) and cardiopulmonary exercise capacity; and to evaluate the relation between cardiac imaging parameters and CV outcomes during follow-up.

## Methods

All patients with genetically confirmed FD, followed at Bordeaux University Hospital, who were referred to the expert center in hereditary cardiomyopathies at the Cardiologic Hospital Haut-Lévêque (Pessac, France) between June 2016 and November 2017, were included and were prospectively evaluated. They were compared with 14 healthy age-matched and gender-matched controls (healthy volunteers). Information concerning the study and data collection was provided to all patients, and the study protocol was approved by the institutional review board. Patients and healthy subjects were included after providing informed consent. For all patients, previous medical histories and clinical characteristics were obtained from the hospital database. The Mainz Severity Score Index (MSSI) was used to reflect the clinical spectrum of FD<sup>29</sup>; scores were divided into severity bands of mild (<20), moderate (20–40), and severe (>40) affliction. Participants underwent 12-lead electrocardiography (ECG), 24-hour Holter ECG monitoring, blood biomarker (brain natriuretic peptide) measurement, transthoracic echocardiography, cardiopulmonary exercise testing, and, if possible, CMR.

LVH on ECG was assessed using Sokolow-Lyon and Cornell criteria and was considered positive when the sum of voltage in (SV1 + RV5) or (SV3 + RaVL) exceeded 35 and 28 mm, respectively, in men, or 35 and 20 mm, respectively, in women. A 24-hours Holter ECG monitoring was performed to detect conduction abnormalities and atrial or ventricular arrhythmias. Atrial fibrillation was considered significant when lasting more than 30 seconds. Non-sustained ventricular tachycardia was defined as 3 or more consecutive beats at a rate of at least 100 beats/min and lasting less than 30 s.

### Standard Echocardiography

Echocardiography was performed using M3S probe, Vivid 9 or E95 (GE® Vingmed Ultrasound AS, Horten, Norway). All images were digitally stored for offline analysis using EchoPAC® version 113.1 (GE Vingmed Ultrasound®). Complete 2D and Doppler images were acquired according to standard techniques<sup>30,31</sup>, 2D measurements were obtained in conventional views over 3 separate cardiac cycles and were averaged. Maximal left atrial volumes were calculated using the biplane area-length formula and indexed to body surface area<sup>32</sup>. Maximal LV wall thickness was measured on two-dimensional imaging. LVH in echocardiography was defined by a maximal wall thickness > 11.0 mm<sup>33</sup>. LV end-systolic and end-diastolic volumes were calculated using Simpson's biplane method of disks, and indexed to body surface area. LV ejection fraction was

subsequently derived. Evaluation of diastolic function included conventional Doppler-based measurements. First, pulsed-wave Doppler velocities were measured from the apical four-chamber view placing a 2-mm sample volume at the tips of the mitral leaflets. Transmitral E and A diastolic velocities and E-wave deceleration time were measured. Tissue Doppler was applied in the pulsed Doppler mode to record E' mitral annular velocities at the septal and lateral corners, and the measurements were averaged to calculate mean E/E' ratio to estimate LV filling pressures<sup>34</sup>. Severity of mitral regurgitation was determined using an integrative approach including semiquantitative and quantitative color Doppler-based parameters as recommended by current guidelines<sup>35</sup>. Systolic pulmonary artery pressure was calculated as recommended<sup>36</sup>.

### **Speckle-Tracking Imaging**

To study longitudinal LV systolic function, two-dimensional recordings of apical four-chamber, two-chamber, and three-chamber views were obtained using 2D-speckle-tracking imaging at a frame rate > 50 Hz. The endocardial border was manually traced, and the region of interest width was adjusted to include the entire myocardium. The software package (EchoPAC<sup>®</sup> version 113.1) automatically tracks and accepts segments of good tracking quality, and modifications of the region of interest can be manually modified if necessary to ensure optimal tracking of LV hypertrophic endocardium. Individual global strain curves were obtained automatically from each view. We analyzed transmural, endocardial and epicardial strain separately in the 17 segments.

### **Cardiac Magnetic Resonance Imaging**

CMR imaging was performed on a 1.5-T scanner (Magnetom Avanto<sup>®</sup>, Syngo MR B15 version; Siemens Medical Solutions<sup>®</sup>, Erlangen, Germany). LGE sequences were performed, with images acquired 10–20 min following intravenous administration of 0.2 mmol/kg gadolinium-DTPA with breath-hold 2D segmented inversion-recovery sequences or phase-sensitive inversion-recovery sequences in identical planes as in cine images. Regional LGE extent was assessed qualitatively. The presence and regional extent of LGE was assessed by two independent observers. Control subjects, and a patient refusing the injection, did not receive gadolinium.

CMR images were viewed using Osirix<sup>®</sup> software (Pixmeo, Switzerland). Analysis was performed using the MRHeart<sup>®</sup> analysis tool plug-in for Osirix. LV end-diastolic volume and LV end-systolic volume, LV mass and LV ejection fraction were obtained from the short-axis stack by manually contouring end-diastolic and end-systolic endocardial borders and end-diastolic epicardial

borders from the base to the apex. Total LV mass was defined including papillary muscles and trabeculations in the myocardial mass. Papillary muscle mass was calculated as previously described<sup>37</sup>. LVH was defined as an increased LV mass index according to published data of normal limits for age and gender<sup>38</sup> or as maximum wall thickness > 11.0 mm in adults. Asymmetric wall thickness was defined as a regional wall thickness of  $\geq 13.0$  mm in  $\geq 1$  myocardial segment, in accordance with European guidelines on the diagnosis of hypertrophic cardiomyopathy in patients with genetic disorders<sup>39</sup>, and segmental end-diastolic wall thickness > 1.3-fold the opposing LV segment. Right ventricular hypertrophy (RVH) was defined by maximal wall thickness > 7 mm.

Before contrast administration, T1 mapping was performed using the modified Look-Locker inversion recovery (MOLLI) sequence in the basal and mid-short-axis planes. Sequences were optimized for a heart rate of 60-80/min using a single-breathhold T1 mapping pulse sequence (8 cm slice thickness) before and after administration of an intravenous bolus of gadolinium-based contrast agent (0.2 mmol/kg of gadoterate meglumine). Structures have been acquired orthogonal to the imaging plane to minimize obliquity. To minimize off resonance, the shim has been properly adjusted. Native and post contrast T1 maps have been acquired using the same slice prescription parameters and the same cardiac phase. The resulting pixel-by-pixel color T1 maps were displayed using a customized 12-bit look-up table, where normal myocardium was green, increasing T1 was red, and decreasing T1 was blue. The color map is visible immediately after data acquisition. For the septal T1, a region of interest in the basal and midseptum was drawn with adequate margins of separation from tissue interfaces prone to partial volume averaging (such as between myocardium and blood pool), and the average T1 was obtained, avoiding areas of LGE. Lateral, anterior and inferior T1 were obtained using the same method. Post-contrast T1 mapping was performed 15 min after gadolinium administration for assessment of extra-cellular volume (ECV) fraction quantification, calculated using hematocrit value measured contemporaneously with the CMR study.

### **Metabolic exercise stress test**

The metabolic exercise stress test was carried out using bicycle ergometry with computerized respiratory gas exchange measurements, (Ergo Card, Medisoft®, Soriennes, Belgium), under the supervision of a cardiologist, in patients whose general health status allowed testing. Patients peddled for 2 min with zero resistance, after which the bicycle ergometer gradually increased the resistance at a ramp rate of 20, 25 or 30 W/min according to sex and previous performance. With each breath, oxygen uptake ( $\text{VO}_2$ ), carbon dioxide production ( $\text{VCO}_2$ ) and ventilation rate were

recorded, whereas heart rate, ECG and workload were continuously recorded. Exercise was stopped when patients reached maximal workload (exhaustion), at which point, the  $VO_2$  max reading was obtained. The anaerobic threshold was reached when there was a rapid increase in  $CO_2$  production. The respiratory exchange ratio ( $VCO_2 /VO_2$ ) was measured as an indication of the volume of  $CO_2$  produced in relation to the volume of  $O_2$  consumed. Increased ventilatory drive was reflected by the increase of the slope of ventilation relative to carbon dioxide production ( $VE/VCO_2$  slope).  $O_2$  pulse was obtained by dividing the  $VO_2$  value by the heart rate obtained every 10 seconds of exercise. Predicted maximum watts,  $VO_2$  max,  $O_2$  pulse and oxygen uptake at anaerobic threshold adjusted for age and sex were calculated according to Wasserman's equations<sup>40</sup>.

### **Follow-up and end-points**

Long-term follow-up was achieved using regular consultation every 1 or 2 years in our hospital and via patients recalls. The follow-up period for each patient was determined using the time from initial consultation to the last data recorded from the patient. Cardiovascular outcomes included CV death, worsening of NYHA functional class, hospitalization for heart failure, onset of atrial fibrillation or ventricular arrhythmia, and stroke.

### **Statistical Analysis**

Continuous variables were presented as mean  $\pm$  SD. Categorical data were summarized as frequencies and percentages. To compare continuous variables, we used a Mann-Whitney  $U$  test. To compare categorical variables, we used a Pearson Chi-square test. Linear correlations were analyzed using Pearson test. Survival without CV event, defined as the end-point, was determined from inclusion until the first end-point or last follow-up and estimated using Cox proportional univariate hazards regression analyses. Survival without event was shown in Kaplan-Meier curves.  $p$  values  $< 0.05$  was considered significant. Software package SPSS® for Windows version 17.0 (SPSS Inc, Chicago, IL, USA) was used for all statistical calculations.

## Results

### Baseline participant characteristics (Table 1)

A total of 31 FD subjects were included in the study (mean age  $42 \pm 16$  years, 39% male), together with 14 matched participants (mean age  $42 \pm 17$  years,  $p=0.62$ , 38% male). FD patients had a mean MSSSI of  $17 \pm 16$  (range 1-48), reflecting an overall relative mild degree of disease severity. All patients who had a severe degree of disease activity (4 patients) were male. Fifty-five percent of our population was treated by ERT at the time of the inclusion (83% of male patients, 37% of female patients). Six patients had arterial hypertension, well controlled by medical therapy. Three patients had treated coronary artery disease, 3 had known atrial fibrillation. Two patients (1 male, 1 female) had previously had non-sustained ventricular tachycardia, and they both carried an implantable cardioverter defibrillator. Four patients had a pacemaker implanted for conduction disorders. One patient had had an alcohol septal ablation as treatment of cardiac obstruction. Eleven patients (35%) had renal involvement due to the disease, including 5 male patients who underwent a kidney transplantation. Nine patients had a medical history of stroke or transient ischemic attack.

### Exercise, electrical and biological status (Table 2)

Nine patients (32%) had electrical LVH, among which 6 were male. Patients achieving metabolic exercise stress test had a mean performance of  $99 \pm 27$  W (range 50-160), representing  $62 \pm 24$  % of theoretical total wattage. Male performances were significantly poorer than female ones, representing respectively  $41 \pm 15\%$  vs.  $75 \pm 20\%$  of theoretical wattage according to sex ( $p<0.05$ ). Mean  $VO_2$  peak was  $19.5 \pm 4.6$  ml/kg/min, which represents respectively  $54 \pm 10\%$  and  $78 \pm 28\%$  of theoretical  $VO_2$  peak according to sex ( $p<0.05$ ). Mean oxygen uptake at anaerobic threshold was  $13.1 \pm 2.7$  ml/kg/min, which represents  $41 \pm 14\%$  of theoretical peak  $VO_2$  with a significant difference between male and female patients ( $32 \pm 4$  % vs.  $47 \pm 15$  %,  $p<0.05$ ). Mean  $CO_2$  ventilatory efficiency ( $VE/VCO_2$  slope) was  $34 \pm 8$ .

### Echocardiography (Table 3 and Figure 1)

Mean maximal LV wall thickness at end-diastole was  $11.9 \pm 4.3$  mm in FD patients, and was significantly higher in FD patients than healthy controls ( $8.0 \pm 1.7$  mm,  $p<0.001$ ). Both septal and posterior walls were significantly thicker in male compared to female patients (respectively  $13.9 \pm 5.0$  vs.  $9.7 \pm 1.9$  mm,  $p<0.05$ , and  $13.1 \pm 4.6$  vs.  $9.3 \pm 2.1$  mm,  $p<0.05$ ) and in FD patients compared to controls ( $p<0.001$ ). Thirteen patients (42%) had echocardiographic LVH. Mean

indexed left atrial volume, LV ejection fraction, global longitudinal strain (LS), right ventricle systolic tissue Doppler imaging, E/E' ratio and systolic pulmonary artery pressure were significantly higher in FD patients than controls ( $p<0.05$ ). None of our patients encountered impaired LV ejection fraction, mitral regurgitation or elevation of systolic pulmonary artery pressure. Global and regional transmural, endocardial and epicardial LS were represented **Figure 1**.

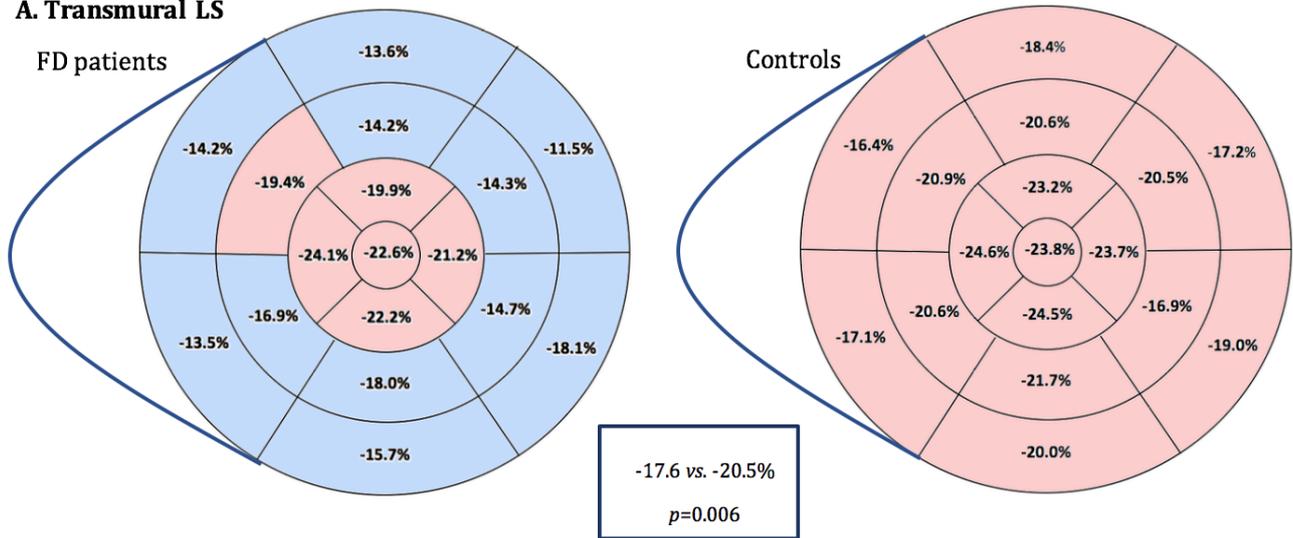
Intra- and interobserver mean percentage variability of multi-layer LS analysis had already been assessed: variability was between 3.1 and 6.1 % for global LS, and between 7.8 and 13.0% for regional LS<sup>41</sup>.

<b>Table 1 Baseline characteristics of FD patients</b>				
	<b>FD Patients (n= 31)</b>	<b>Male FD patients (n= 12)</b>	<b>Female FD patients (n= 19)</b>	<b>p value male vs. female FD patients</b>
<b>Clinical characteristics</b>				
Age (years)	42 ± 16	41 ± 14	43 ± 18	0.77
Body mass Index (kg/m <sup>2</sup> )	24 ± 5	22 ± 3	25 ± 6	0.14
MSSI <sup>1</sup>	17 ± 16	26 ± 19	11 ± 10	0.048*
mild, n (%)	21 (68%)	6 (50%)	4 (21%)	0.09
moderate, n (%)	6 (19%)	2 (16%)	4 (21%)	0.76
severe, n (%)	4 (13%)	4 (21%)	0 (0%)	0.007*
Enzyme replacement therapy, n (%)	17 (55%)	10 (83%)	7 (37%)	0.011*
Heart rate (bpm)	75 ± 11	76 ± 10	74 ± 12	0.91
Systemic blood pressure (mmHg)	117 ± 12	115 ± 12	118 ± 11	0.50
Diastolic blood pressure (mmHg)	68 ± 12	70 ± 15	66 ± 10	0.79
NYHA class				
I, n (%)	20 (65%)	6 (50%)	14 (74%)	0.33
II, n (%)	10 (32%)	5 (42%)	5 (26%)	0.37
III, n (%)	1 (3%)	1 (8%)	0 (0%)	0.20
IV, n (%)	0 (0%)	0 (0%)	0 (0%)	
History of syncope, n (%)	4 (13%)	2 (16%)	2 (11%)	0.57
<b>Cardiovascular history</b>				
Arterial hypertension, n (%)	6 (19%)	4 (33%)	2 (11%)	0.12
Dyslipidemia, n (%)	10 (32%)	4 (33%)	6 (32%)	0.80
Diabetes mellitus, n (%)	0 (0%)	0 (0%)	0 (0%)	
Current smoking, n (%)	9 (29%)	4 (33%)	5 (26%)	0.67
Coronary artery disease, n (%)	3 (10%)	1 (8%)	2 (11%)	0.84
Atrial fibrillation, n (%)	3 (10%)	2 (17%)	1 (5%)	0.30
Nonsustained ventricular tachycardia, n (%)	2 (6%)	1 (8%)	1 (5%)	0.74
History of sudden cardiac death in relatives <sup>2</sup> , n (%)	3 (10%)	1 (8%)	2 (11%)	0.84
Pacemaker for conduction disorders, n (%)	4 (13%)	2 (17%)	2 (11%)	0.62
Implantable Cardioverter Defibrillator, n (%)	2 (6%)	1 (8%)	1 (5%)	0.74
Interventional treatment of cardiac obstruction, n (%)	1 (3%)	1 (8%)	0 (0%)	0.20
<b>Medications</b>				
Beta blockers, n (%)	6 (19%)	3 (25%)	3 (16%)	0.53
Calcium channel blockers, n (%)	2 (6%)	1 (8%)	1 (5%)	0.74
Angiotensin-converting enzyme inhibitors, n (%)	3 (10%)	0 (0%)	3 (16%)	0.15
Angiotensin receptor blockers, n (%)	4 (13%)	3 (25%)	1 (5%)	0.11
Statins, n (%)	10 (32%)	4 (33%)	6 (32%)	0.92
Aldosterone-blocking agents, n (%)	1 (3%)	0 (0%)	1 (5%)	0.42
Antiplatelet agents, n (%)	5 (16%)	2 (17%)	3 (16%)	0.95
Anticoagulant therapy, n (%)	4 (13%)	3 (25%)	1 (5%)	0.11
<b>Renal involvement, n (%)</b>				
Low creatinine clearance (< 60 ml/min), n (%)	2 (7%)	1 (14%)	1 (5%)	0.74
Significant proteinuria (> 0,3 g/24h), n (%)	4 (15%)	0 (0%)	4 (21%)	0.09
Dialysis, n (%)	0 (0%)	0 (0%)	0 (0%)	
Kidney transplantation, n (%)	5 (16%)	5 (42%)	0 (0%)	0.002*
<b>Other clinical manifestations</b>				
Stroke/ transient ischemic attack, n (%)	9 (29%)	5 (42%)	4 (21%)	0.11
Cutaneous manifestations, n (%)	10 (32%)	5 (42%)	5 (26%)	0.37
Acroparesthesia, n (%)	17 (55%)	9 (75%)	8 (42%)	0.44
Values are mean ± SD or n (%). *p < 0.05 considered statistically significant.				
<sup>1</sup> scores were divided into severity bands of mild (<20), moderate (20–40), and severe (>40) affliction				
<sup>2</sup> in ≥ 1 first degree relatives < 40 years or sudden cardiac death in a first degree relative with confirmed hypertrophic cardiomyopathy at any age				
FD : Fabry disease ; ERT : enzyme replacement therapy ; MMSI : Mainz Severity Score Index ; NYHA : New York Heart Association				

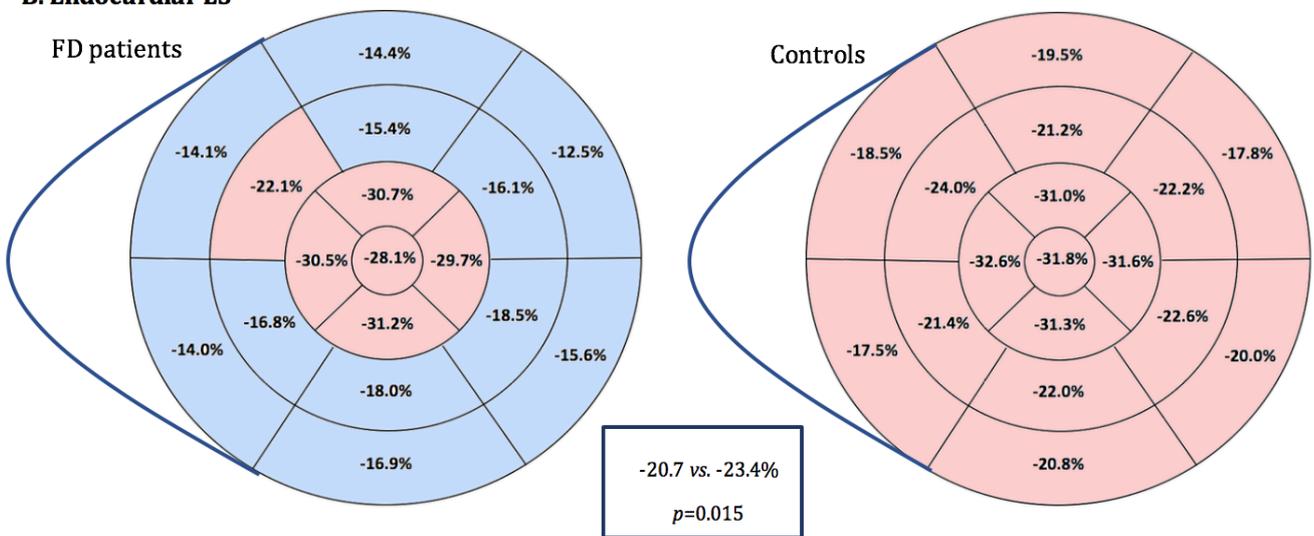
	<b>FD Patients (n= 31)</b>	<b>Male patients (n= 12)</b>	<b>Female patients (n= 19)</b>	<b>p value : Males vs. Female patients</b>
<b>Electrocardiography</b>				
First degree atrioventricular block	2 (7%)	1 (10%)	1 (6%)	0.53
Complete Bundle-branch block	2 (7%)	1 (10%)	1 (6%)	0.35
Left ventricular hypertrophy	9 (32%)	6 (60%)	3 (17%)	0.040*
<b>24-Hours holter monitoring</b>				
Atrial fibrillation	4 (15%)	3 (30%)	1 (6%)	0.09
Nonsustained ventricular tachycardia	3 (11%)	2 (20%)	1 (6%)	0.26
<b>Brain natriuretic peptide (pg/ml)</b>	121 ± 218	209 ± 316	75 ± 135	0.25
<b>Metabolic exercise stress test</b>				
Total wattage (Watts)	99 ± 27	94 ± 34	102 ± 23	0.44
Total wattage (Mets)	5.5 ± 1.4	5.2 ± 1.6	5.8 ± 1.2	0.22
% Theoretical total wattage	62 ± 24	41 ± 15	75 ± 20	0.002*
Systolic blood pressure peak (mmHg)	166 ± 25	167 ± 33	165 ± 19	0.83
Heart rate peak (beats per minute)	146 ± 24	139 ± 21	149 ± 25	0.26
Respiratory exchange ratio (VCO <sub>2</sub> /VO <sub>2</sub> )	1.2 ± 0.2	1.2 ± 0.2	1.3 ± 0.1	0.15
VO <sub>2</sub> peak (ml/kg/min)	19.5 ± 4.6	19.9 ± 5.5	19.2 ± 4.2	0.70
% Theoretical VO <sub>2</sub> peak	68 ± 25	54 ± 10	78 ± 28	0.045*
CO <sub>2</sub> ventilatory efficiency (VE/VCO <sub>2</sub> slope)	34 ± 8	36 ± 10	34 ± 6	0.85
Peak oxygen pulse (VO <sub>2</sub> /heart rate) (ml/beat/kg)	8.5 ± 1.5	8.9 ± 1.8	8.2 ± 1.2	0.49
% Theoretical peak oxygen pulse	66 ± 27	60 ± 24	70 ± 30	0.57
Oxygen uptake at anaerobic threshold (ml/kg/min)	13.1 ± 2.7	12.6 ± 1.5	13.4 ± 3.3	0.91
% Theoretical oxygen uptake at anaerobic threshold	41 ± 14	32 ± 4	47 ± 15	0.029*
Values are mean ± SD or n (%). *p < 0.05 considered statistically significant.				
FD : Fabry disease				

	<b>Patients (n= 31)</b>	<b>Male patients (n= 12)</b>	<b>Female patients (n= 19)</b>	<b>p value male vs. female FD patients</b>	<b>Controls (n= 14)</b>	<b>p value FD patients vs. controls</b>
<b>Morphology</b>						
Interventricular septum thickness (mm)	11.3 ± 4.0	13.9 ± 5.0	9.7 ± 1.9	0.033*	8.0 ± 1.7	< 0.001*
Posterior wall thickness (mm)	10.8 ± 3.7	13.1 ± 4.6	9.3 ± 2.1	0.043*	7.0 ± 2.0	< 0.001*
Maximal LV wall thickness (mm)	11.9 ± 4.3	14.6 ± 5.4	10.3 ± 2.4	0.07	8.2 ± 1.6	< 0.001*
Indexed LV diastolic volume (ml/m <sup>2</sup> )	48 ± 18	60 ± 20	41 ± 11	0.002*	43.2 ± 10.1	0.17
Indexed LV systolic volume (ml/m <sup>2</sup> )	17 ± 7	21 ± 8	14 ± 4	< 0.001*	15.6 ± 4.7	0.70
Indexed left atrial volume (ml/m <sup>2</sup> )	38 ± 23	48 ± 30	33 ± 16	0.24	22.4 ± 7.8	0.002*
<b>Systolic function</b>						
LV : Ejection fraction (%)	66 ± 5	65 ± 5	67 ± 5	0.35	63.8 ± 6.0	0.029*
LV : Global longitudinal strain (%)	-17.7 ± 4.5	-15.2 ± 3.2	-18.8 ± 4.7	0.07	-20.5 ± 7.9	0.006*
RV : Systolic velocity Doppler TDI (m/s)	12 ± 2	12 ± 2	12 ± 2	0.72	13.8 ± 1.5	0.006*
<b>Diastolic function</b>						
Mitral E/A inflow ratio	1.4 ± 0.6	1.2 ± 0.3	1.5 ± 0.7	0.22	1.3 ± 0.4	0.80
Deceleration time (ms)	220 ± 89	242 ± 130	207 ± 52	0.81	205 ± 62	0.53
Mean E/E' ratio	10.7 ± 4.9	11.8 ± 5.2	10.0 ± 4.8	0.35	5.8 ± 1.4	< 0.001*
<b>Mitral regurgitation</b>						
None or mild, n(%)	31 (100%)	12 (100%)	19 (100%)			
Moderate or severe, n(%)	0 (0%)	0 (0%)	0 (0%)			
<b>Systolic pulmonary artery pressure (mmHg)</b>	26 ± 6	25 ± 6	26 ± 6	0.28	22 ± 4	0.019*
Values are mean ± SD or n (%). *p < 0.05 considered statistically significant.						
FD : Fabry disease ; LV : Left ventricle ; RV : Right ventricle ; TDI : Tissue Doppler Imaging						

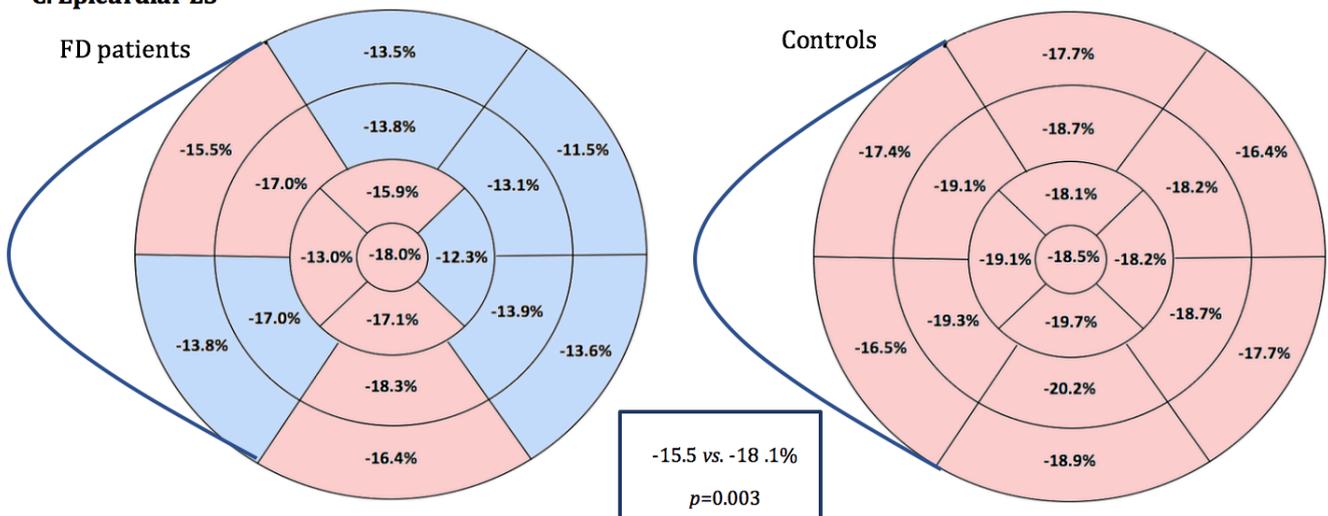
### A. Transmural LS



### B. Endocardial LS



### C. Epicardial LS



**Figure 1 Comparison of transmural, endocardial and epicardial LS between FD patients and controls**

Blue segments: significant difference between FD patients and controls LS; Red segments: absence of significant difference between FD patients and controls LS.  $p$  value  $<0.05$  was considered significant.

FD: Fabry Disease; LS: Longitudinal strain

## Cardiovascular magnetic resonance imaging (Table 4 and Figure 2)

Four patients did not perform CMR, 3 because they carried non CMR compatible pacemaker or defibrillator, and 1 because of claustrophobia; they all had cardiac impairment due to FD. One patient refused gadolinium injection.

LV mass index was  $94 \pm 56$  g/m<sup>2</sup> and was significantly higher in male than female FD patients ( $132 \pm 73$  vs.  $71 \pm 26$  g/m<sup>2</sup>,  $p < 0.05$ ). Among 9 patients (33%) with LVH, 6 had concentric hypertrophy, 2 had asymmetric septal hypertrophy, 1 had asymmetric inferolateral hypertrophy. Mean septal to lateral ratio in these patients was 1.15. Three patients (11%) had RVH, all were male ( $p < 0.05$ ).

For all patients who had LGE (9 patients, 35%, no significant difference between male and female patients), its localization was mid-wall, and affected basal inferolateral region. Most of the time (6 patients, 67%), it was confined in one segment, and 2 patients had 4 segments with LGE. One male patient had very extensive LGE with 12 affected segments in the anterior, lateral and inferior walls. One female patient had LGE without increased LV mass. Mean native T1 in patients

	<b>FD Patients (n= 27)</b>	<b>Male patients (n= 10)</b>	<b>Female patients (n= 17)</b>	<b>p value male vs. female FD patients</b>	<b>Controls (n=11)</b>	<b>p value FD patients vs. controls</b>
<b>Volumes and function</b>						
LV end diastolic volume index (ml/m <sup>2</sup> )	81 ± 5	95 ± 18	72 ± 16	0.005*		
LV end systolic volume index (ml/m <sup>2</sup> )	28 ± 10	34 ± 11	24 ± 7	0.004*		
LV ejection fraction (%)	67 ± 7	68 ± 7	66 ± 7	0.84		
<b>Maximal LV diastolic wall diameter</b>	11.8 ± 4.7	15.1 ± 5.9	9.9 ± 2.3	0.024*	8.0 ± 1.1	< 0.001*
septal (mm)	11.3 ± 4.7	14.5 ± 6.0	9.4 ± 2.3	0.015*	7.8 ± 0.9	< 0.001*
lateral (mm)	10.3 ± 4.0	12.7 ± 5.1	9.0 ± 2.5	0.034*	6.7 ± 1.1	< 0.001*
anterior (mm)	9.6 ± 3.8	12.1 ± 4.6	8.1 ± 2.2	0.17	6.9 ± 1.0	< 0.001*
posterior (mm)	10.3 ± 4.0	13.2 ± 4.8	8.6 ± 2.0	0.042*	6.4 ± 1.2	< 0.001*
<b>LV mass index (g/m<sup>2</sup>)</b>	94 ± 56	132 ± 73	71 ± 26	0.006*	56 ± 8	< 0.001*
<b>LV hypertrophy, n (%)</b>	9 (33%)	6 (60%)	3 (18%)	0.024*	0 (0%)	0.032*
Maximal LVH localization, n (% of LVH patients)						
concentric	6 (67%)	5 (83%)	1 (33%)	0.12		
asymmetric septal	2 (22%)	1 (18%)	1 (33%)	0.74		
asymmetric inferolateral	1 (11%)	0 (0%)	1 (33%)	0.20		
<b>RV hypertrophy, n (%)</b>	3 (11%)	3 (30%)	0 (0%)	0.017*	0 (0%)	0.48
<b>Late gadolinium enhancement, n (%)</b>	9 (35%)	4 (40%)	5 (31%)	0.65	0 (0%)	0.05
<b>Native T1 value (ms)</b>	934 ± 60	899 ± 53	955 ± 53	0.016*	999 ± 45	< 0.001*
septal (ms)	931 ± 55	898 ± 53	951 ± 46	0.025*	994 ± 42	< 0.001*
lateral (ms)	933 ± 52	903 ± 50	952 ± 45	0.046*	985 ± 40	< 0.001*
anterior (ms)	941 ± 67	893 ± 63	971 ± 51	0.016*	1015 ± 43	< 0.001*
posterior (ms)	930 ± 68	904 ± 53	946 ± 72	0.025*	1002 ± 53	< 0.001*
late gadolinium enhancement areas (ms)	966 ± 57	957 ± 79	972 ± 42			
<b>Extracellular volume (%)</b>	24.7 ± 3.0	23.9 ± 3.1	25.2 ± 2.9	0.22		
septal (%)	24.9 ± 3.0	24.1 ± 2.6	25.5 ± 3.2	0.23		
lateral (%)	28.8 ± 2.8	24.6 ± 3.9	25.0 ± 2.0	0.36		
anterior (%)	24.5 ± 3.5	23.3 ± 3.2	25.3 ± 3.6	0.24		
posterior (%)	24.5 ± 2.9	23.7 ± 2.9	25.0 ± 3.0	0.37		
Values are mean ± SD or n (%). *p < 0.05 considered statistically significant.						
CMR: Cardiovascular magnetic resonance ; FD: Fabry Disease ; LV: Left ventricle ; LVH: Left ventricular hypertrophy ; RV: Right ventricle						

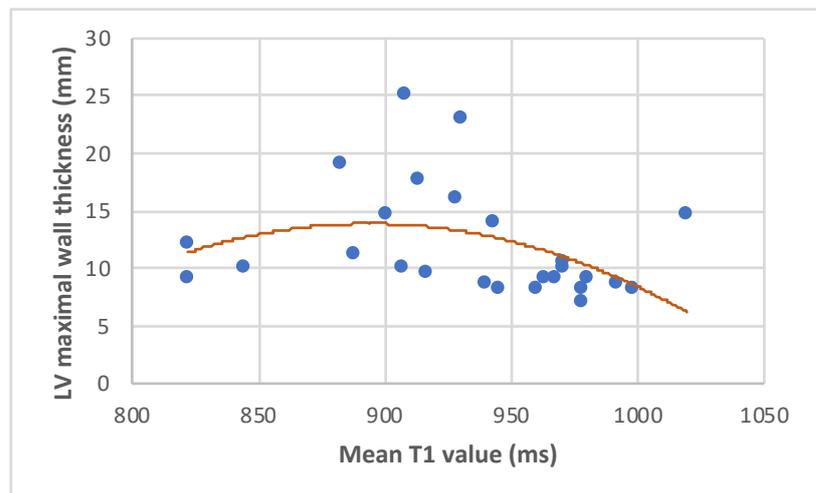
with FD was significantly lower than in healthy volunteers ( $934 \pm 60$  vs.  $999 \pm 45$  ms,  $p < 0.05$ ). When considering the whole FD group, male mean T1 was significantly lower than female mean T1 ( $899 \pm 53$  vs.  $955 \pm 53$  ms,  $p < 0.05$ ) and this significant difference was found separately in the septal, anterior, lateral and inferior walls. Ninety-percent (9/10) of male patients had lower global native T1 than healthy subjects. The only patient with normal T1 was aged seventeen and was the only adolescent of our cohort. Mean T1 was higher in LGE areas than in other zones. LV maximal wall thickness according to mean T1 value is represented **Figure 2**.

Intra- and interobserver mean percentage variability of native T1 analysis were respectively 3.4% and 2.9% (Appendix 1 and 2).

Mean ECV was  $24.7 \pm 3.0$  % in our FD patients, with no significant difference between male and female FD patients ( $23.9 \pm 3.1$  % vs.  $25.2 \pm 2.9$  %,  $p = 0.22$ ).

**Figure 2: LV maximal thickness according to T1 value.**

LV: Left ventricle



### Correlations (Table 5)

A good inverse correlation was observed between VO<sub>2</sub> peak and global ( $r = -0.55$ ) and endocardial ( $r = -0.53$ ) LS ( $p < 0.05$ ). A weaker inverse correlation was observed between VO<sub>2</sub> peak and epicardial LS ( $r = -0.40$ ) and mean E/E' ratio ( $r = -0.42$ ) as  $p$  value was not significant.

VE/VC<sub>02</sub> slope was correlated with maximal LV wall thickness at end-diastole ( $r = 0.48$ ), LV ejection fraction ( $r = 0.49$ ) and mean T1 value ( $r = -0.57$ ) ( $p < 0.05$ ).

### Prognosis analysis (Table 6 and Figure 3)

During a median follow-up of 23 months, six patients (19%) had CV events (2 strokes, 1 non-sustained ventricular tachycardia, 1 atrial fibrillation, 1 appropriate implantable cardiac defibrillator shock, 1 degradation of functional status).

<b>Table 5 Correlations between VO2 peak and VE/VC02 slope and different echocardiographic and CMR parameters</b>		
	<b>r value</b>	<b>p value</b>
<b>VO2 peak</b>		
Maximal LV wall thickness at end-diastole	-0.38	0.092
LV ejection fraction	0.10	0.650
Indexed left atrial volume	-0.13	0.620
Mean E/E' ratio	-0.42	0.067
Global transmural LS	-0.55	0.012*
Global endocardial LS	-0.53	0.025*
Global epicardial LS	-0.40	0.122
Indexed LV mass	-0.14	0.560
Number of segments with LGE	-0.28	0.250
Mean T1 value	-0.11	0.654
Mean extracellular Volume	-0.23	0.420
<b>VE/VC02 slope</b>		
Maximal LV wall thickness at end-diastole	0.48	0.026*
LV ejection fraction	0.49	0.021*
Indexed left atrial volume	0.08	0.782
Mitral E/E' septal-lateral ratio	0.42	0.066
Global transmural LS	0.22	0.392
Global endocardial LS	0.16	0.587
Global epicardial LS	0.09	0.730
Indexed LV mass	0.32	0.161
Number of segments with LGE	0.10	0.684
Mean T1 value	-0.57	0.011*
Mean extracellular Volume	0.11	0.820
CMR : Cardiovascular magnetic resonance, LGE : late gadolinium enhancement ; LV : Left ventricle ; LS : Longitudinal strain		

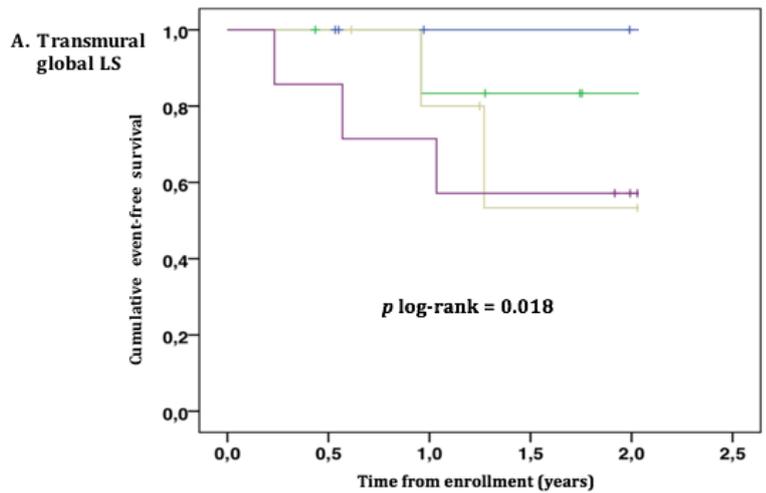
In the Cox univariate analyses, male sex and history of syncope were strongly associated with CV events (HR 5.29, 95%CI 1.02-27.57,  $p < 0.05$ , and HR 6.72, 95%CI 1.39-32.53,  $p < 0.05$ ). Increased echocardiographic mean E/E' ratio was significantly predictive of CV outcomes ( $p < 0.05$ ). Altered transmural global LS, and more specifically basal septal and mid anterolateral LS; and epicardial global LS, and particularly basal and mid anterolateral LS, were significantly predictive of CV outcomes ( $p < 0.05$ ). Among LS, the most significantly predictive of CV outcomes was basal antero-septal transmural LS (HR 1.27, CI95% 1.09-1.47,  $p < 0.05$ ). In CMR, maximal wall thickness (HR 1.34, CI95% 1.12-1.61,  $p < 0.05$ ) and LV mass index (HR 1.02, CI95% 1.01-1.04,  $p < 0.05$ ) predicted the upcoming of a CV outcome whereas a high anterior native T1 value was a protective factor (HR 0.98, 95%CI 0.97-1.00,  $p < 0.05$ ).

Kaplan-Meier curves (Figure 3) show significantly different event-free survival according to global transmural LS, basal antero-septal transmural LS and anterior T1 value ( $p$  log rank  $< 0.05$ ).

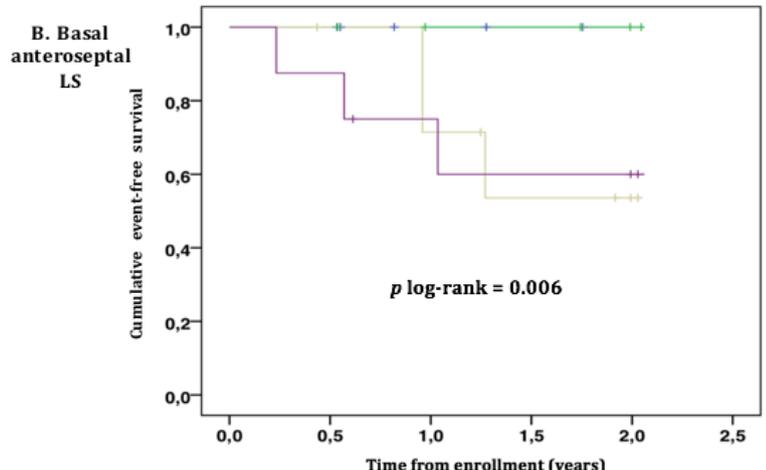
<b>Table 6 Univariate Cox analyses for predicting cardiovascular outcomes in FD patients</b>			
	<b>HR</b>	<b>CI 95%</b>	<b>p</b>
Age	1.049	1.00-1.10	0.065
Male sex	5.290	1.02-27.57	0.048*
History of syncope	6.720	1.39-32.53	0.018*
Ventricular non sustained tachycardia	3.738	0.65-21.38	0.138
Theoretical VO2 peak	0.995	0.97-1.03	0.744
VE/VCO2 slope	1.156	1.03-1.30	0.011*
Echocardiographic maximal LV wall thickness	1.341	1.12-1.61	0.002*
LV ejection fraction	1.000	0.88-1.13	0.998
Indexed left atrial volume	1.008	0.98-1.03	0.596
Mean E/E' ratio	1.180	1.03-1.35	0.015*
CMR maximal LV wall thickness	1.253	1.06-1.48	0.008*
<b>Transmural global LS</b>	1.338	1.05-1.71	0.019*
Basal inferoseptal transmural LS	1.236	1.06-1.45	0.008*
Basal anteroseptal transmural LS	1.267	1.09-1.47	0.002*
Basal inferolateral transmural LS	1.083	0.94-1.25	0.265
Mid inferolateral transmural LS	1.027	0.96-1.10	0.462
Basal anterolateral transmural LS	1.066	0.98-1.16	0.156
Mid anterolateral transmural LS	1.127	1.03-1.23	0.010*
<b>Endocardial global LS</b>	1.201	0.96-1.50	0.106
Basal inferolateral endocardial LS	1.122	0.97-1.30	0.120
Mid inferolateral endocardial LS	1.075	0.95-1.22	0.250
Basal anterolateral endocardial LS	1.107	1.01-1.21	0.030*
Mid anterolateral endocardial LS	1.092	0.99-1.21	0.093
<b>Epicardial global LS</b>	1.344	1.01-1.79	0.045*
Basal inferolateral epicardial LS	1.082	0.93-1.27	0.321
Mid epicardial inferolateral epicardial LS	1.100	0.95-1.28	0.219
Basal anterolateral epicardial LS	1.160	1.04-1.30	0.110
Mid anterolateral epicardial LS	1.146	1.02-1.29	0.027*
<b>LV mass index</b>	1.020	1.01-1.04	0.010*
<b>Late gadolinium enhancement</b>	3.713	0.61-22.43	0.153
Number of segments with LGE	1.255	1.99-1.59	0.063
<b>Global native T1 value</b>	0.986	0.97-1.00	0.074
Septal native T1 value	0.987	0.97-1.00	0.114
Lateral native T1 value	0.985	0.97-1.00	0.104
Anterior native T1 value	0.984	0.97-1.00	0.048*
Inferior native T1 value	0.997	0.99-1.01	0.673
<b>Global extracellular volume</b>	1.092	0.76-1.58	0.638
Septal extracellular volume	1.150	0.79-1.66	0.471
Lateral extracellular volume	1.055	0.75-1.49	0.761
Anterior extracellular volume	1.052	0.79-1.40	0.732
Inferior extracellular volume	0.994	0.70-1.41	0.973

\* $p < 0.05$  considered statistically significant.

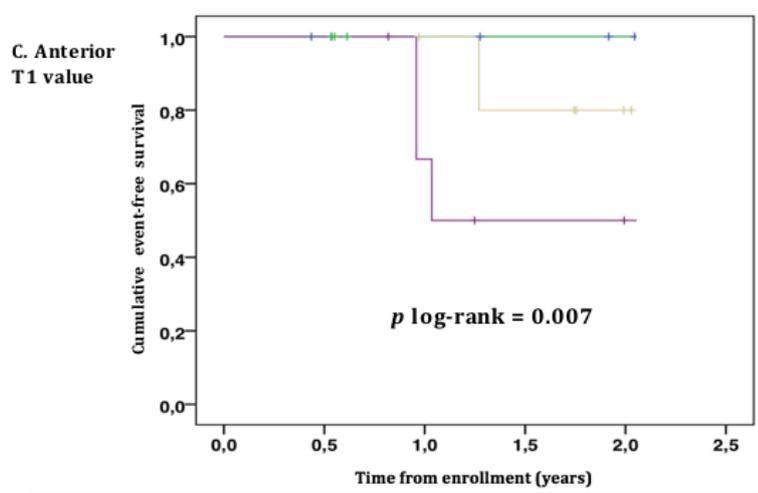
CMR : Cardiovascular magnetic resonance ; CI : Confidence interval ; HR : Hazard ratio ; LS : Longitudinal strain ; LV : Left ventricle



LS	No at risk				
$\leq -20.9\%$	7	6	3	3	2
$-20.8 ; -18.1\%$	7	6	5	4	2
$-18.0 ; -13.9\%$	6	6	4	2	2
$\geq -13.8\%$	7	6	5	4	2



LS	No at risk				
$\leq -18.1\%$	8	8	5	4	3
$-18.0 ; -15.1\%$	7	7	5	5	3
$-15.0 ; -12.0\%$	8	7	5	3	1
$\geq -11.9\%$	8	7	5	4	3



T1	No at risk				
$\geq 987 \text{ ms}$	7	6	5	4	3
$941 ; 986 \text{ ms}$	6	6	3	3	3
$905 ; 940 \text{ ms}$	6	6	5	4	1
$\leq 904 \text{ ms}$	7	7	4	2	1

**Figure 3: Kaplan-Meier event-free survival curves for transmural global LS (A), basal anteroseptal LS (B), and anterior T1 value (C) for prediction of CV events in FD.**

$p \text{ log rank} < 0.05$  was considered statistically significant.

CV: Cardiovascular; FD: Fabry disease; LS: Longitudinal strain

## **Discussion**

The aim of this study was to investigate the prognosis value of recently-developed echocardiographic and CMR parameters and their relation with functional status in FD. Firstly, we showed a global alteration of basal segments in LS in FD patients compared to healthy subjects; and a lower mean native T1, notably without overlap between adult male patients and healthy controls values. Secondly, we highlighted a significant correlation between VO<sub>2</sub> peak and endocardial and transmural LS; as well as between VE/VCO<sub>2</sub> slope and mainly T1 value; highlighting the clinical value of these parameters. Thirdly, we demonstrated transmural and epicardial global LS, and regionally septal and lateral LS values, were predictive of CV outcomes.

### **Echocardiographic and CMR characterization of FD patients**

Our study confirms the frequent cardiac involvement in FD patients, as described in other studies<sup>16</sup>. Our population, though, had an overall mild degree of disease severity according to MSS1. As expected, male patients were more affected than females, because of the X-linked mutation that causes the disease. Repartition of LVH in our population was consistent with published literature<sup>42</sup>, although no patient had apical hypertrophy in our study. LGE had always a midwall distribution when present, as previously described<sup>43</sup>.

LV ejection fraction is not impaired in patients with FD; however, LS is significantly altered. In global and endocardial strain, the loss of global deformation is mainly due to basal segments, resulting in an 'apical sparing' pattern. This pattern can also be observed in cardiac amyloidosis<sup>44</sup>. A slightly different pattern is observed in epicardial strain, with a predominant alteration of anterior, lateral and inferoseptal segments, but with also an 'apical sparing' pattern. Kramer et al.<sup>45</sup> had compared FD patients with and without LGE, and identified a predominant alteration of LS in basal posterior and lateral segments which was correlated with the amount of LGE. Our results tend to show a more global basal alteration of LS compared to healthy controls. This may indicate that there is more lipid deposition in basal segments than apical ones, inducing a more important resistance to deformation. This hypothesis is concordant with the predominant hypertrophy in basal segments in our population. With regard to diastolic function, classical mitral E/A inflow ratio was not different between FD patients and healthy controls. However mean E/E' ratio and indexed atrial volume were significantly increased. Systolic pulmonary artery pressures are also slightly but significantly increased. These parameters confirm the coexistence of diastolic and systolic LV dysfunction in FD.

The myocardial native T1 in patients with FD was significantly lower than in healthy volunteers. All adult male patients had lower mean T1 than healthy subjects. When considering the whole FD group, the male mean T1 was significantly lower than the female mean T1, as observed in previous studies<sup>28,46</sup>. One male patient had a remarkably a low mean T1, measured at 822 ms, although he didn't have LVH. It is also interesting to note that this patient had low global LS (-16.4 %), without any other echocardiographic abnormalities. To our knowledge, the effect of specific therapies in patients with cardiac involvement revealed by low LS or low native T1 has not being studied and should be analyzed in future studies.

Mean ECV was  $24.7 \pm 3.0$  % in our FD patients, and did not differ from healthy controls. This is concordant with the pathophysiology of the disease, which is due to an intracellular (lysosomal) storage disorder. Moreover, we only measured ECV in segments without LGE, to avoid fibrosis. This is consistent with Sado et al. study<sup>47</sup> in which ECV measured in septum was not different from healthy subjects.

### **Relation between echocardiographic and CMR parameters and functional status in metabolic exercise test**

Global and endocardial LS were significantly correlated to VO<sub>2</sub> peak. Epicardial LS and mean E/E' ratio also seemed correlated to VO<sub>2</sub> peak, but without any significance. Peak VO<sub>2</sub>, reflecting the maximal aerobic metabolism capacity, provides objective assessment of functional capacity in patients with heart failure, and has been shown to be of great importance in predicting survival from heart failure<sup>48-50</sup>. LS provides a good evaluation of functional status and seem superior to classical systolic function parameters; however multi-layer analysis doesn't seem to be superior to transmural LS.

Furthermore, T1 value had a strong correlation with the VE/VCO<sub>2</sub> slope. LV wall thickness and LV ejection fraction were also significantly correlated to VE/VCO<sub>2</sub> slope. On the contrary, mean E/E', LV mass, LGE, and ECV failed to predict functional status. VE/VCO<sub>2</sub> slope is a recently developed parameter that reflects respiratory efficiency; it has been shown to be significantly better than peak VO<sub>2</sub> in predicting cardiac-related mortality<sup>51</sup>. Increase in VE/VCO<sub>2</sub> slope, at least partly explained by a decrease in pulmonary perfusion, reflects a reduction of cardiac output at peak exercise.

Only Lobo et al.<sup>52</sup> had already studied exercise parameters in FD, and have found no significant correlation between echocardiographic features and any exercise performance parameter. This

is the first study that demonstrates the usefulness of echocardiographic systolic function parameters, and CMR T1 value, in predicting functional status in FD. However, multi-layer LS failed to show a superiority compared to transmural LS in predicting exercise capacity in FD.

Also, we found a major alteration of exercise capacity in our patients compared to normative population data, reflected by low theoretical VO<sub>2</sub> peak, particularly in males. This is partly explained by deconditioning, as % theoretical mean oxygen uptake at anaerobic threshold is < 50% in our patients.

### **Prognosis factors**

In our population, 19.3% of FD patients presented a CV event during a median follow-up of 23 months. All of them were treated by ERT. The reduction of glycosphingolipid accumulation under ERT has been demonstrated in various tissues<sup>11-13,53,54</sup>; particularly in the myocardium<sup>55</sup>. However, despite the relatively low degree of disease severity (reflected by MSS1) in our population and the relatively good results of the therapy, CV outcomes are frequent.

As expected, male sex is a strong prognosis factor in our study (HR=5.29). History of syncope is also a strong predictive factor of CV event in univariate analysis. In cardiopulmonary exercise testing, only VE/VCO<sub>2</sub> slope was significantly predictive of CV event, suggesting its prognosis value in heart failure can probably be extended in FD cardiomyopathy.

We tried to find interesting LS pattern, studying global, multi-layer and regional strain. On the one hand, global transmural and epicardial LS were significantly predictive of CV outcomes in univariate analysis. On the other hand, basal inferoseptal and anteroseptal transmural LS, and basal and mid anterolateral epicardial LS were significantly predictive of CV outcomes in univariate analysis. The absence of clear interesting multi-layer pattern in FD is consistent with predominant involvement of mesomyocardial areas in CMR. Anyhow, in addition to the ability of speckle-tracking imaging to early detect myocardial dysfunction<sup>18</sup>, to predict functional status<sup>56</sup>, to reflect myocardial fibrosis<sup>44</sup>, and to assess treatment efficiency<sup>57</sup> in FD, global and regional strain have a good prognosis value. Global transmural LS and basal anteroseptal transmural LS seem to have the strongest prognosis value and should be used to identify the most severe patients.

Kaplan Meier's analysis of event-free survival show a significant impact on prognosis when global LS value is higher than -18.0%; and when basal anteroseptal LS value is higher than -15.0%.

All patients who encountered a cardiac event during follow-up had LGE areas, signaling local myocardial fibrosis. Presence of LGE failed to predict CV outcomes, it can probably be explained by the small sample size of our study; but the number of LGE segments tend towards significance in predicting CV outcomes in univariate analysis. A relationship between LGE and malignant ventricular arrhythmias has been shown by Kramer et al.<sup>27</sup> in a 73-patients' study, in which 18% of patients with LGE vs. 0% of patients without LGE experienced ventricular arrhythmias. ERT did not seem to reduce the risk of progression of fibrosis in this study, and the only independent predictive factor of ventricular arrhythmias was the annual increase in fibrosis during follow-up. Furthermore, Weideman et al.<sup>58</sup> showed that under ERT, reduction of LVH was significant in patients without fibrosis and accompanied with improvement of myocardial function, whereas patients with mild or severe fibrosis showed a minor reduction in LVH and no improvement in myocardial function. Moreover, Pieroni et al.<sup>59</sup> presented the case of a 55-year old man that developed a quick and significant progression of cardiac involvement of his disease despite ERT, while cutaneous and renal involvement remained stable. These studies suggest that the effect of ERT depends on the stage of the disease at baseline. Patients with evidence of fibrosis in CMR may have limited or even no benefits at all from ERT, and fibrotic remodeling seems to be even worse at a late stage of the disease. Therefore, substitutive treatment should probably be introduced early in disease progression, before the development of myocardial fibrosis, to achieve at least a stabilization of cardiac involvement. Reduction of progression of myocardial fibrosis would probably prevent global CV events as well as ventricular arrhythmias.

### **Considerations about localization of LVH and LGE**

In our study, LVH predominate in septal segments in FD patients, whereas LGE, predominant in inferolateral basal area, never affects the septal wall. Frustaci et al.<sup>60</sup> showed in a FD family that patients with most LVH had larger and more vacuolated cells, with marked necrosis and apoptosis. Glycolipid accumulation was shown to induce oxidative damage of proteins in cardiomyocytes, leading to degradation of contractile elements, reduced ATP synthesis, and cell death<sup>61,62</sup>. Therefore, fibrosis seems to be caused by apoptosis of enlarged, lipid-engorged cardiomyocytes, and should be found in the most hypertrophic areas. However, in our study, LVH does not match with LGE localization.

We can speculate LVH initially predominates in the inferolateral wall, and the expansion of fibrosis leads to a thinning of this area. Hasegawa et al.<sup>63</sup> published the case of a patient whose LVH slowly evolved from concentric to asymmetric, because of progressive fibrosis of apical and lateral walls. They hypothesized that a reduced microcirculatory myocardial perfusion led to

cellular apoptosis. Moreover, Kawano et al.<sup>64</sup> showed that LV basal posterior thinning, defined as septum/posterior wall thickness ratio > 1.3, was a prognosis factor in FD. Development of fibrosis would occur in most hypertrophic areas as a progression of myocardial involvement.

However, in our study, 2 females had myocardial fibrosis without LVH, and all females who had LVH had fibrosis. In contrast, all males who had fibrosis had invariably LVH. These observations have already been made on a larger scale in Niemann et al. study<sup>65</sup>. In females, progression toward severe LVH is delayed, probably because of residual activity of alpha-galactosidase, and development of fibrosis does not necessarily require LVH. Furthermore, Moon et al.<sup>25</sup> studied myocardial histological sections of a deceased patient who had massive concentric LVH: he had a thinned inferolateral region, concordant with location of LGE and collagen, but without increased lipid accumulation. Evolution towards fibrosis in the inferolateral wall doesn't seem to depend on early LVH of this zone, and the fact that maximal fibrosis has a peculiar predilection for the inferolateral wall is thereby unexplained. Moon et al.<sup>24</sup> hypothesized that cardiac involvement in FD could impair resistance to physical stress within the myocardium, that might be most prominent in the inferolateral region, where significant shear forces combine with a watershed vascular territory. Indeed, several other cardiomyopathies have predilection for lateral wall involvement<sup>66,67</sup>.

In conclusion, the physiopathology of development of cardiac fibrosis still remains unclear in FD.

### **Considerations about normalized or elevated T1 value in most hypertrophied areas or with fibrosis in FD**

We noticed a higher native T1 value in LGE areas compared to other segments. Sado et al.<sup>46</sup> had highlighted normal or even elevated T1 in the inferolateral wall in FD patients, where is found myocardial fibrosis. As a reminder, T1 mapping was at first studied for assessment of diffuse interstitial fibrosis, revealed by elevated T1 value<sup>68</sup>. In FD, however, the lipid storage yields low T1, making it a useful biomarker for early detection of cardiac involvement. Sado et al.<sup>46</sup> hypothesized that the inferolateral wall myocardium in FD underwent a 4-phase transition from normal (pre-detectable storage) to low (storage) to pseudonormalization (storage and fibrosis) to elevation (fibrosis). As fibrosis distorts T1 value, it has to be measured in areas without LGE.

In our study, patients with the highest LV maximal wall thickness did not have the lowest mean T1 value (Figure 2). Nordin et al.<sup>69</sup> even recently showed that from a certain degree of LVH, in men, the correlation between T1 and LVH reverses and T1 increases (toward normal) with LVH, whereas ECV is not impacted. They hypothesized that, in males, storage is triggering sarcomeric

protein expression via myocyte hypertrophy<sup>70,71</sup> and usual LVH rather than storage LVH, which are diluting the T1 lowering of sphingolipid. This hypertrophic and inflammation phase precede the diffuse fibrosis phase.

### **Study limitations**

Limitations of this study include no histological validation of the presence of storage with low native T1 and the presence of fibrosis with LGE. The relatively small number of patients, leading to a low power study, could explain the lack of statistical difference. This is inherent in a monocentric study, carried out on a rare disease. Furthermore, there is a large heterogeneity in our population, as we know that male and female patients do not have the same phenotype, and two phenotypes exist in male patients: classical and late-onset phenotypes<sup>72</sup>. Cardiovascular outcomes covered a broad spectrum of CV events in our study. Number of events was insufficient to permit multivariate analysis.

Among our patients, some of them could not perform MRI, unfortunately reducing the percentage of patients with cardiac involvement performing MRI.

### **Conclusions**

Our study suggests that longitudinal strain and T1 mapping are reproducible, enables characterization of cardiac involvement, and predicts functional status and cardiovascular outcomes in FD patients. These data should be confirmed in larger studies.

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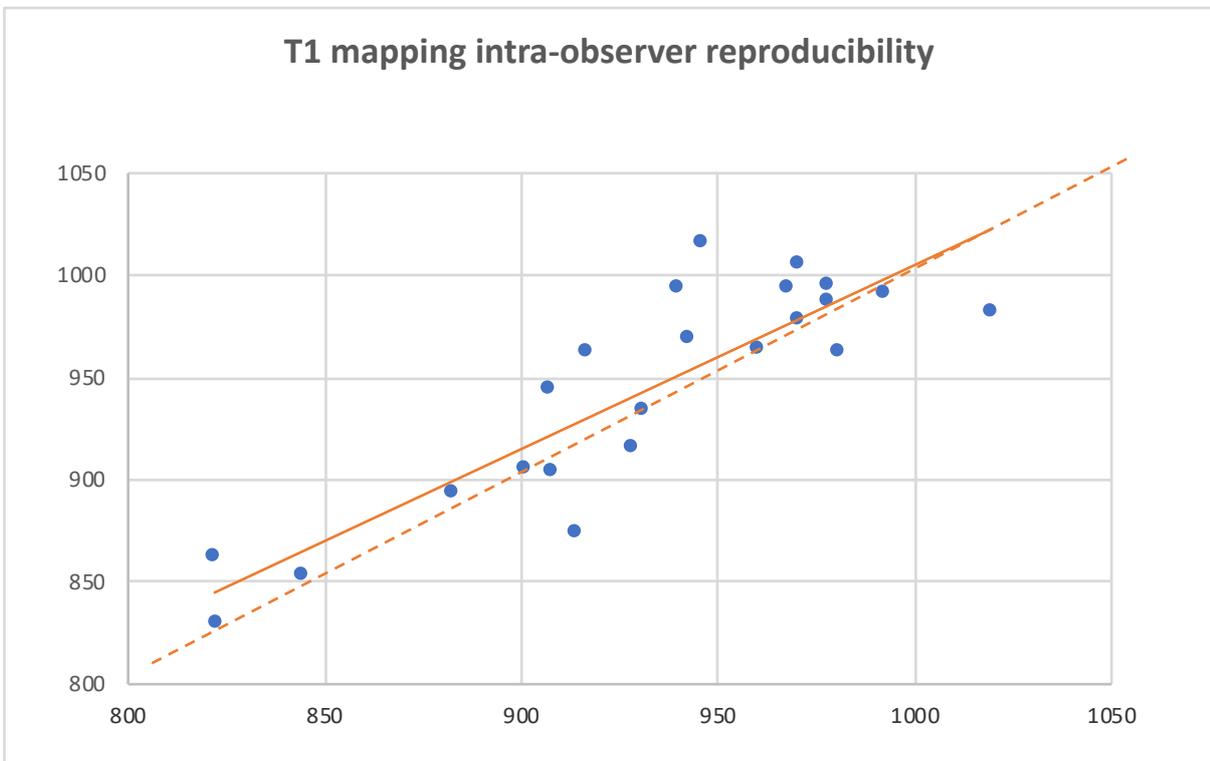
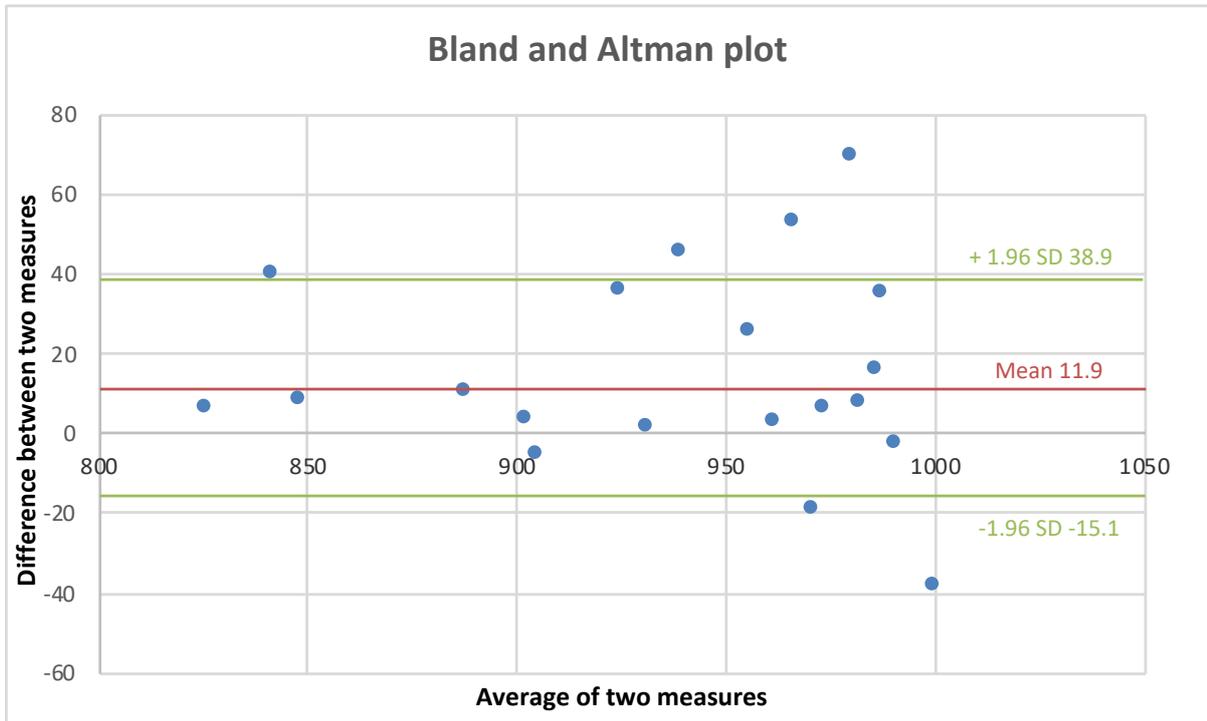
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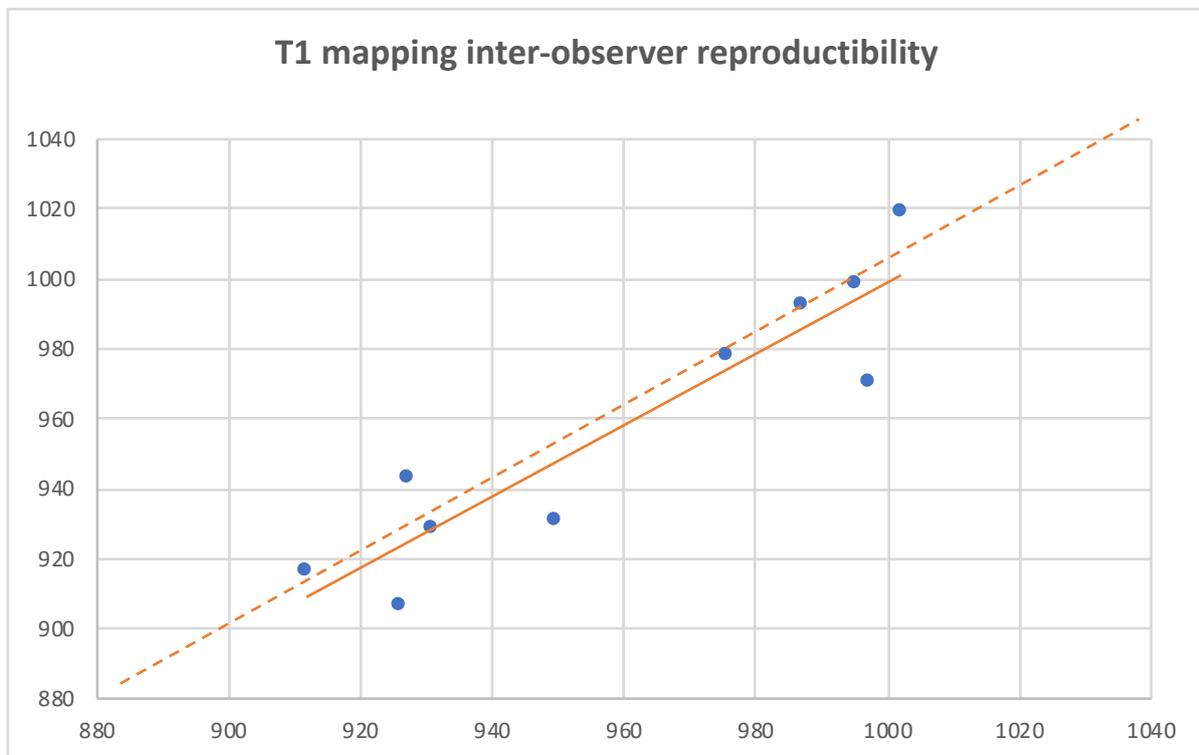
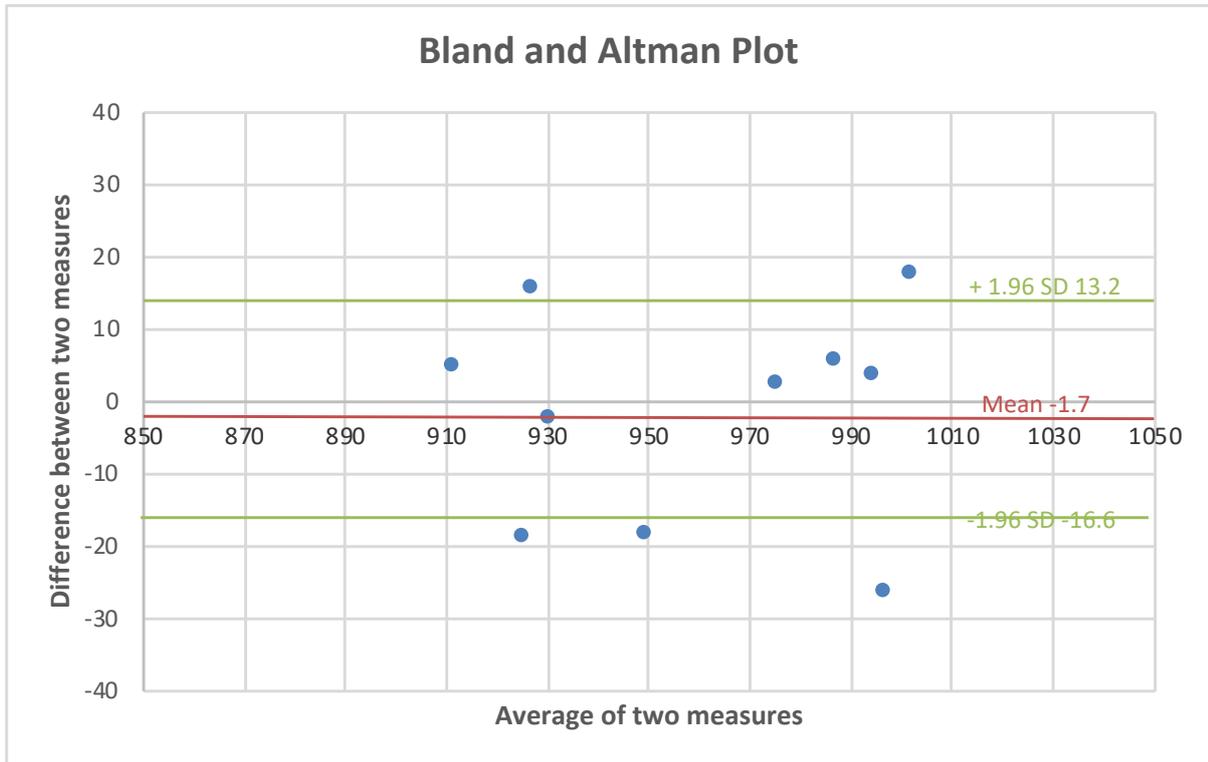
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### Appendix 1: T1 mapping intra-observer reproducibility



## Appendix 2: T1 mapping inter-observer reproducibility



# **Characterization of Fabry Disease cardiac involvement according to longitudinal strain, late gadolinium enhancement, and T1 mapping: relation to functional status, and prediction of cardiovascular outcomes**

## **ABSTRACT**

**BACKGROUND:** Anderson-Fabry disease (FD) is a rare X-linked glycolipid storage disease in which cardiac involvement is a leading cause of morbimortality. Neither relation between echocardiographic and cardiovascular magnetic resonance (CMR) innovative parameters, and exercise testing parameters, nor their prognosis value, have been evaluated yet.

**AIMS:** We sought to evaluate the relation between cardiac imaging parameters (i.e. left ventricular (LV) mass, multi-layer longitudinal strain (LS), native T1 and late gadolinium enhancements) and on one hand cardiopulmonary exercise capacity in FD patients; and on the other hand, cardiovascular (CV) outcomes during follow-up.

**METHODS:** In this prospective, monocentric study, we evaluated all consecutive FD patients referred in our tertiary center, performing electrocardiogram (ECG), Holter ECG monitoring, echocardiography, CMR, and cardiopulmonary exercise testing, that we compared with healthy controls.

**RESULTS:** From June 2016 to November 2017, we included 31 FD patients (mean age  $42 \pm 16$  years, 39% male) with an overall mild degree of disease severity, that were compared with 14 matched healthy controls. Thirteen FD patients (42%) had left ventricular hypertrophy (LVH) (wall thickness  $>11.0$  mm). Mean T1 in FD patients was significantly lower than in controls ( $934 \pm 60$  vs.  $999 \pm 45$  ms,  $p < 0.05$ ). Regarding functional capacity, a good correlation was observed between  $VO_2$  peak and global ( $r = -0.55$ ) and endocardial ( $-0.53$ ) LS.  $VE/VCO_2$  slope was correlated with maximal LV wall thickness ( $r = 0.48$ ), and mean T1 value ( $r = -0.57$ ). During a median follow-up of 23 months, 6 patients presented CV events. In the Cox univariate analyses, global transmural LS and more specifically basal septal LS; epicardial global LS, and particularly basal and mid anterolateral LS; and anterior T1 value were significantly predictive of CV event ( $p < 0.05$ ).

**CONCLUSIONS:** Our study suggests that longitudinal strain and T1 mapping are relevant for prediction of functional status and cardiovascular events in FD patients.