Circulation: Cardiovascular Imaging

ORIGINAL ARTICLE

Myocardial Edema, Myocyte Injury, and Disease Severity in Fabry Disease

BACKGROUND: Cardiovascular magnetic resonance can demonstrate myocardial processes in Fabry disease (FD), such as low native T1 (sphingolipid storage) and late gadolinium enhancement (LGE, scar). Recently, high T2 (edema) has been observed in the basal inferolateral wall along with troponin elevation. We hypothesized that edema and myocyte injury would be chronically associated and have electrical, mechanical, and disease associations in FD.

METHODS: A prospective international multicenter study was conducted on 186 consecutive FD patients (45.2±1.1 years, 58% females). Additionally, 28 patients with hypertrophic cardiomyopathy, 30 with chronic myocardial infarction and 59 healthy volunteers were included. All study participants underwent comprehensive cardiovascular magnetic resonance with T1 and T2 mapping, cines, and LGE imaging.

RESULTS: LGE in the basal inferolateral wall in FD had T2 elevation (FD 58.2±5.0 ms versus hypertrophic cardiomyopathy 55.6±4.3 ms, chronic myocardial infarction 53.7±3.4 ms and healthy volunteers 48.9±2.5 ms, $P<0.001$), but when LGE was present there was also global T2 elevation (53.1±2.9 versus 50.6±2.2 ms, $P<0.001$). Thirty-eight percent of FD patients had high troponin. The strongest predictor of increased troponin was high basal inferolateral wall T2 (odds ratio, 18.2 [95% CI, 3.7–90.9], $P<0.0001$). Both T2 and troponin elevations were chronic over 1 year. High basal inferolateral wall T2 was associated with baseline global longitudinal strain impairment ($P=0.005$) and electrocardiographic abnormalities (long PR, complete bundle branch block, left ventricular hypertrophy voltage criteria, long QTc, and T-wave inversion, all $P<0.05$) and predicted clinical worsening after 1 year (Fabry stabilization index >20%, $P=0.034$).

CONCLUSIONS: LGE in Fabry has chronic local T2 elevation that is strongly associated with chronic troponin elevation. In addition, there is slight global T2 elevation. Both are associated with ECG and mechanical changes and clinical worsening over 1 year.

João B. Augusto, MD*
Sabrina Nordin, MBBS*
Ravi Vijapurapu, MBBS
Shanat Baig, MBBS
Heerajnarain Bulluck, MBBS, PhD
Silvia Castelletti, MD
Mashael Alfarih, MSc
Kristopher Knott, MBBS
Gabriella Captur, PhD
Tushar Kotecha, MBChB
Uma Ramaswami, MD
Michel Tchan, PhD
Tarekegn Geberhiwot, PhD
Marianna Fontana, PhD
Richard P. Steeds, MD
Derralynn Hughes, PhD
Rebecca Kozor, PhD
James C. Moon, MD

*Drs Augusto and Nordin are joint first authors.

Key Words: edema ■ Fabry disease ■ healthy volunteers ■ myocardial infarction ■ troponin

https://www.ahajournals.org/journal/circimaging

CLINICAL PERSPECTIVE

The main cause of death in Fabry disease is cardiac, through heart failure or arrhythmia. Treatment for Fabry disease is available (enzyme replacement or oral chaperone therapies), but the effect is incomplete likely due to sub-optimal tailoring of these therapies. Left ventricular hypertrophy typically qualifies a patient to receive treatment, but this alone does not reflect myocardial biology. Multiparametric cardiovascular magnetic resonance has been able to measure sphingolipid infiltration (low native T1 mapping) and late gadolinium enhancement (LGE) typically in the basal inferolateral wall. More recently, myocardial edema by means of high T2 mapping has also been shown in the basal inferolateral wall LGE areas. In this prospective international multicenter study, we have explored multiparametric markers of cardiac involvement in Fabry disease, including markers of myocyte injury (troponin) and LV pressure/volume overload (NT-proBNP [N-Terminal Pro-B-Type Natriuretic Peptide]). These data show that when LGE is present, Fabry patients have increased T2 values in the LGE areas but also globally. Interestingly, LGE T2 elevation was higher in Fabry disease than in other diseases with LGE, such as chronic myocardial infarction or hypertrophic cardiomyopathy. This regional T2 elevation was strongly associated with elevated troponin and increased NT-proBNP, with weaker associations with global T2 elevation. A high basal inferolateral wall T2 was also associated with both electrocardiographic changes and global longitudinal strain impairment. Over a year, T2, troponin and NT-proBNP elevations were chronic (chronic myocardial edema and injury), and basal inferolateral wall T2 elevation was associated with clinical worsening, suggesting a potential new treatment/disease monitoring target that should be further investigated.

Fabry disease (OMIM 301500; FD) is a rare X-linked lysosomal storage disorder caused by mutations in the gene (*GLA*) encoding for α-galactosidase A. Progressive sphingolipid accumulation affects multiple organs, including the heart,[1] where it leads to left ventricular hypertrophy (LVH), myocardial fibrosis, and cardiomyopathy.[2] The heart is the leading cause of death in patients with FD,[3] but treatment with enzyme replacement therapy or oral chaperone therapy is available. The challenge, however, is how to tailor these therapies to better prevent disease progression. For this reason, modalities for early cardiac disease detection and monitoring are important.

Cardiovascular magnetic resonance (CMR) parametric mapping approaches[4] detect changes preceding LVH, such as low native (noncontrast) T1 mapping reflecting sphingolipid accumulation.[5–7] Late gadolinium enhancement (LGE), typically occurring in the basal inferolateral (BIFL) wall[8] is associated with a poor response to therapy and adverse outcomes[9–11] with histological correlation in advanced disease suggesting focal fibrosis.[12] Recent research, however, has suggested that LGE composition in FD might be more complex than once thought. Hybrid imaging with positron emission tomography/MR[13] has shown focal fludeoxyglucose uptake and short-TI inversion recovery sequence signal suggesting local inflammation with one preliminary report showing T2 elevation suggesting edema.[14] Furthermore, these high-LGE T2 values were strongly associated with troponin release, a marker of cardiac injury. However, these studies were single-center, single time point, small in sample size, and focused on local changes only. Recently, a high histological incidence of myocarditis (over half of the cases, mostly CD3+ T lymphocytes) has been observed in septal endomyocardial biopsies suggesting distributed inflammation.[15]

Using multicenter, multinational prospective data, we hypothesized that in FD,[1] T2 elevation would be global,[2] would be associated with cardiac injury (elevated troponin), LV pressure/volume overload (elevated NT-proBNP [N-Terminal Pro-B-Type Natriuretic Peptide]), and cardiac electrocardiographic and mechanical changes and[3] that these changes would be chronic and associated with disease progression.

METHODS

The data that support the findings of this study are available from the corresponding author on reasonable request.

Study Population and Data Collection

Participants were recruited from 4 Fabry clinics as part of the prospective, multicenter international observational Fabry400 study (URL: https://www.clinicaltrials.gov. Unique identifier: NCT03199001)—United Kingdom: recruited from the National Hospital for Neurology and Neurosurgery London and scanned at The Heart Hospital London; recruited and scanned at the Royal Free Hospital London and Queen Elizabeth Hospital Birmingham; Australia: recruited from Westmead Hospital and scanned at North Shore Radiology, Sydney. Ethical approval was obtained in all 4 cohorts and the study conformed to the principles of the Helsinki Declaration. Written informed consent was obtained from all participants. We included all consecutive FD patients who underwent CMR (both T1 and T2 mapping performed) and same day high-sensitivity troponin. All FD patients had a confirmed *GLA* mutation (Table I in the Data Supplement). We excluded patients[1]: aged under 18,[2] with standard contraindications to CMR and[3] known pregnancy.

In addition to the FD cohort (n=186), we included 3 other groups to assess native T2 mapping LGE changes across

different diseases: (1) 28 patients with gene-positive hypertrophic cardiomyopathy (HCM), asymmetrical LV hypertrophy and LGE; (2) 30 patients with chronic myocardial infarction (cMI) 6 months post-reperfused ST-elevation MI; and (3) 59 healthy volunteers with no history of cardiovascular disease (normal health questionnaire, normal ECG, no cardioactive medication unless for primary prevention) were also prospectively recruited as controls. A subset of patients with FD was assessed at both baseline and at 1-year follow-up to assess for chronicity (using T2 mapping, high-sensitivity troponin T, NT-proBNP, and Fabry stabilization index [FASTEX] score).

Clinical Data and Blood Biomarkers

Clinical data collected included enzyme replacement therapy status, cardiovascular medication, and *GLA* variant. All patients with FD had blood collected just before the scan and analyzed for high-sensitivity troponin T (United Kingdom) and high-sensitivity troponin I (Australia; both measured by ELISA, Roche Diagnostics). Normal ranges were high-sensitivity troponin T <15 ng/L and high-sensitivity troponin I <16 ng/L (below the 99th percentile upper reference limit for the test). NT-proBNP normal ranges were United Kingdom <47 pmol/L and Australia <14 pmol/L. Missing values (n=8) for NT-proBNP were due to hemolysis.

FASTEX Analysis

FASTEX analysis was performed to evaluate overall clinical stability or progression of FD at follow-up using an online application (https://www.fastex.online) as previously described.[16,17] A FASTEX score of ≥20% is an indication of overall clinical worsening or clinical instability at follow-up.[17]

ECG Analysis

Twelve-lead ECG was performed on the same day and independently analyzed by 2 experienced observers (Drs Nordin and Augusto). Recorded ECG variables were heart rate, rhythm, PR interval duration (normal 120–200 ms), QRS complex duration (normal <120 ms), and QTc interval duration (normal >440 ms for males and >460 ms for females). The presence of complete left or right bundle branch block, T-wave inversion in at least 3 contiguous leads, Sokolow-Lyon voltage criteria (SV1+RV5 or RV6 >35 mm) and Cornell voltage criteria for LVH (R wave in aVL + SV3 >20 mm in females or >28 mm in males) were also recorded.

CMR Acquisition

All participants underwent CMR at 1.5 Tesla (Avanto—The Heart Hospital, Queen Elizabeth Hospital and Sydney—and Aera—Royal Free Hospital—Siemens Healthcare, Erlangen, Germany). Standard cine imaging for volume analysis was performed. LGE images followed a bolus of 0.1 mmol/kg gadolinium contrast agent (Gadoterate meglumine, Dotarem, Guerbet S.A., France) using a phase-sensitive inversion recovery sequence. Gadolinium was not administered if there was known allergy, if estimated glomerular filtration rate was <30 mL/min per 1.73 m^2 or in the case of patient preference. Native (precontrast) T1 mapping was performed on 4-chamber, 3-chamber views and 3 (basal, mid, and apical)

Table 1. Baseline Characteristics of the FD Cohort

	Male FD (n=78)	Female FD (n=108)	*P* Value
Age, y	46.6±15.4	44.1±14.1	0.250
BSA, m^2	1.9±0.2	1.8±0.2	<0.001*
Cardiac variant mutation, n (%)	24/78 (30.8)	37/108 (34.3)	0.638
Drug history			
ERT, n (%)	47/78 (60.3)	40/108 (37.0)	0.002*
ACE inhibitors/ARB, n (%)	18/70 (25.7)	24/100 (24.0)	0.857
β-blocker, n (%)	10/70 (14.3)	6/100 (6.0)	0.107
Statin, n (%)	14/70 (20.0)	10/100 (10.0)	0.076
Aspirin/clopidogrel, n (%)	15/70 (21.4)	11/100 (11.0)	0.083
ECG parameters			
ECG abnormality, n (%)	33/43 (76.7)	31/83 (37.3)	<0.001*
Short PR, n (%)	4/42 (9.5)	6/83 (7.2)	0.731
Long PR, n (%)	2/42 (4.8)	5/83 (6.0)	1.000
Complete BBB, n (%)	13/42 (31.0)	7/83 (8.4)	0.002*
Long QTc, n (%)	6/42 (14.3)	7/83 (8.4)	0.358
T-wave inversion, n (%)	13/43 (30.2)	14/83 (16.9)	0.109
Sokolow-Lyon criteria, n (%)	22/43 (51.2)	10/83 (12.0)	<0.001*
Cornell criteria, n (%)	3/42 (7.1)	5/83 (6.0)	1.000
CMR parameters			
LVEDVi, mL/m^2	77.1±19.0	68.7±13.2	0.001*
LVESVi, mL/m^2	23.3±11.4	19.5±7.5	0.012*
LVEF, %	70.5±9.8	72.2±7.8	0.200
LVMi, g/m^2	106.0 (80.9–143.9)	61.8 (54.1–73.6)	<0.001*
MWT, mm	14.0 (12.0–18.0)	9.0 (8.0–11.2)	<0.001*
LVH, n (%)	57/78 (73.1)	24/108 (22.2)	<0.001*
Low septal T1, n (%)	58/78 (74.4)	48/108 (44.4)	<0.001*
High BIFL wall T2, n (%)	33/78 (42.3)	26/108 (24.1)	0.011*
High septal T2, n (%)	10/78 (12.8)	17/108 (15.7)	0.675
LGE, n (%)	35/66 (53.0)	27/99 (27.3)	0.001*
LV LGE, %	1.2 (0–5.4)	0 (0–1.7)	0.001*
Global longitudinal strain, %	−14.5±5.5	−18.0±3.6	<0.001*
Blood biomarkers			
eGFR, mL/min per 1.73 m^2	82.0 (54.0–90.0)	89.5 (76.0–90.0)	0.011*
High troponin, n (%)	42/78 (53.8)	28/108 (25.9)	<0.001*
High NT-proBNP, n (%)	37/76 (48.7)	38/102 (37.3)	0.167

ACE indicates angiotensin-converting enzyme; ARB, angiotensin II receptor blocker; BBB, bundle branch block; BIFL, basal inferolateral; BNP, brain natriuretic peptide; BSA, body surface area; CMR, cardiovascular magnetic resonance; eGFR, estimated glomerular filtration rate; ERT, enzyme replacement therapy; FD, Fabry disease; LGE, late gadolinium enhancement; LVEDVi, left ventricular end-diastolic volume indexed to body surface area; LVEF, left ventricular ejection fraction; LVESVi, left ventricular end-systolic volume indexed to body surface area; LVH, left ventricular hypertrophy; LVMi, left ventricular mass indexed to body surface area; MWT, maximum wall thickness; and troponin, high-sensitivity troponin.

*Significant *P* value (<0.05).

LV short-axis slices using a modified Look-Locker inversion recovery sequence (5b[3s]3b). Native T2 mapping was performed on the same slices using a steady-state free-precession sequence. The resulting pixel-by-pixel color maps were displayed using a custom 12-bit lookup table, where normal myocardium was purple in native T1 and red in native T2. The T1 mapping and ECV Standardization Program (T1MES) phantom were used to control quality across all sites, measuring magnet stability for native T1 and T2 sequences (Methods in the Data Supplement).

CMR Analysis

All images were analyzed using CVI42 software (Circle Cardiovascular Imaging Inc v.5.9.4, Calgary, Canada). Measurements were performed by 2 readers (Drs Augusto and Alfarih). LV volumes, LV ejection fraction, and mass (papillary muscles included as part of the LV mass) were measured using a semiautomated threshold-based technique and indexed. LV hypertrophy was defined as a maximum wall thickness >12 mm and/or an increased LV mass index indexed to the body surface area with reference to age- and gender-adjusted CMR nomograms.[18]

Global longitudinal 2-dimensional strain (GLS) values were obtained using feature tracking analysis. Endocardial and epicardial borders were drawn in end-diastole and end-systole in 4-, 2-, and 3-chamber and short-axis cine stack. Feature tracking was used to assess the extent of myocardial deformation determined by the motion of an imaginary line placed between endocardial and epicardial boundaries throughout the cardiac cycle.

LV LGE quantification was performed in the short-axis slices using manually drawn endocardial and epicardial borders and a semiautomated 5 SDs above the mean signal in remote myocardium approach, with minimal manual adjustment and expressed as a percentage of total LV mass.

For global native (precontrast) T1 and T2 mapping analysis, endocardial and epicardial borders were manually drawn in the short-axis slices (20% offset to avoid the blood-myocardial interface) and mean values were obtained. Regions of interest with 20% offset were drawn for T1 and T2 mapping in the areas of LGE in the BIFL wall (or in the BIFL wall alone if without LGE) and in the basal septum (or remote areas without LGE if LGE was present in the septum). An additional mid septal regions of interest was also drawn for T1 mapping as previously described, and the lowest septal T1 value was considered for low T1 recognition. Normal native septal T1 and T2 mapping ranges (mean±SD and limits of normal) for each center (total and according to gender) are described in detail in the Methods in the Data Supplement section. T2 mapping was measured per segment in patients with FD according to the 16-segment American Heart Association model. Of the

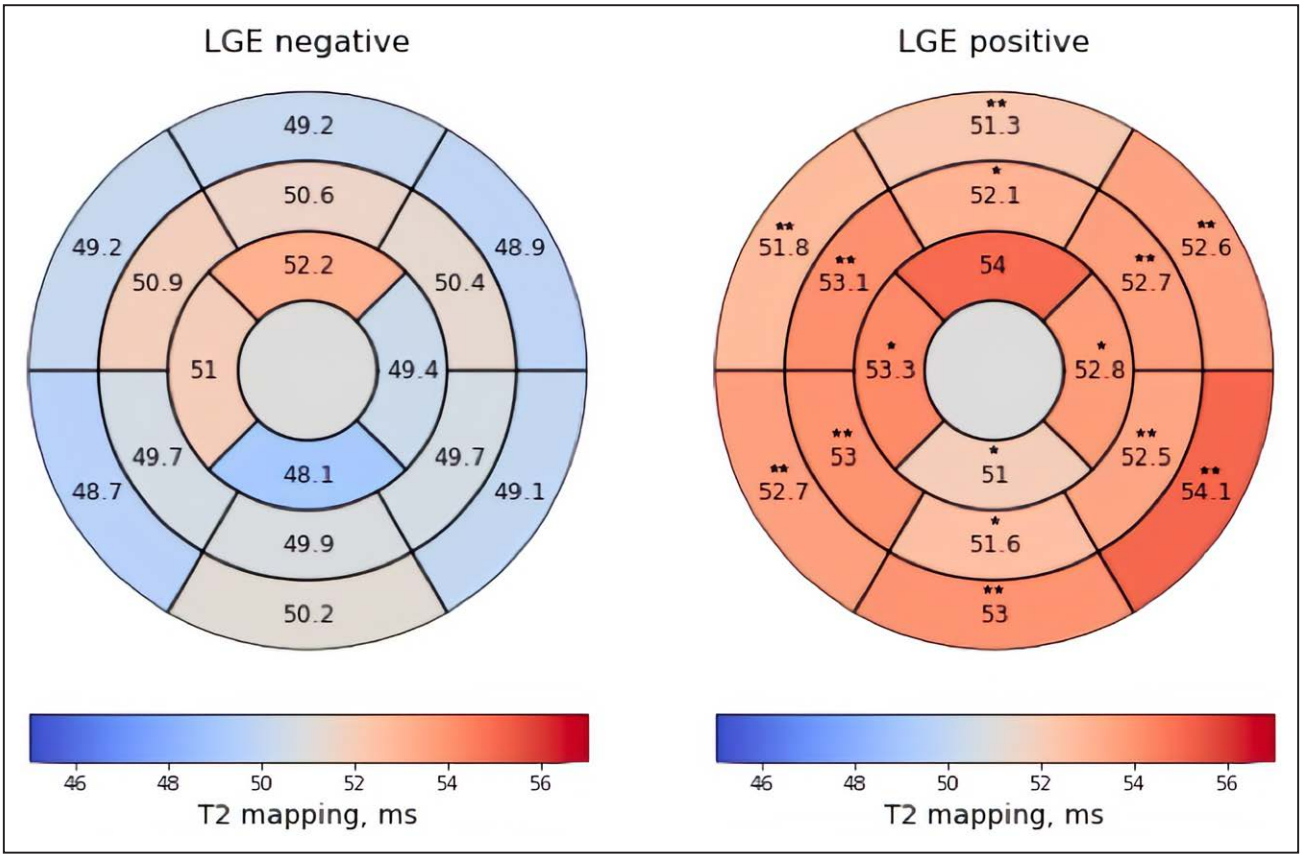


Figure 1. Distribution of native T2 mapping per segment (American Heart Association 17-segment model) according to late gadolinium enhancement (LGE) status.

**P*<0.05–0.001 and ***P*<0.001 vs the corresponding segment in patients without LGE.

2976 segments in 186 patients, T2 was available in 2290 segments (77%); reasons for segment exclusion included mainly incomplete acquisitions (eg, apical SAX slice missing) and some (limited) artifact.

Statistical Analysis

Statistical analysis was performed using SPSS (version 24.0, IBM Corp, Armonk, NY, Methods in the Data Supplement). Comparisons between groups were performed using the Student *t* test or Mann-Whitney *U* test; categorical variables were compared using the Fisher's exact test. Correlations were performed using Pearson's correlation (*r*). Paired-samples *t* test was used to compare T2 mapping values between different segments and between baseline and follow-up; related-samples Wilcoxon signed-rank test was used to compare troponin values between baseline and follow-up. Differences between cohorts (FD, HCM, cMI, and healthy volunteers) were assessed using 1-way ANOVA for parametric data or Kruskal-Wallis test for nonparametric data; categorical variables were compared using the χ^2 test. Significant effects were further evaluated with post hoc pairwise comparisons using Bonferroni adjustment. Regression analysis was performed and is further described in the Methods in the Data Supplement. Reproducibility analysis is detailed in Table II in the Data Supplement. Two-sided $P<0.05$ were considered significant.

RESULTS

One hundred eighty-six patients with FD were included: 93 from The Heart Hospital London, ditto 11 in the Royal Free Hospital London, 44 in the Queen Elizabeth Hospital Birmingham and 38 in Sydney. Mean age of the FD cohort was 45.2±1.1 years with 58% female. Mean estimated glomerular filtration rate was 80.3±1.6 mL/min per 1.73 m^2: 5 patients (3%) with estimated glomerular filtration rate <30 mL/min per 1.73 m^2 and 155 patients (83%) with estimated glomerular filtration rate >60 mL/min per 1.73 m^2. Sixty-one patients with FD (33%) had a cardiac variant mutation (Table I in the Data Supplement). Eighty-seven patients (47%) were on enzyme replacement therapy, none on chaperone therapy, and 51% (64/126) patients had at least one ECG abnormality. Baseline characteristics by sex are shown in Table 1.

CMR in Fabry Patients

Twenty-one patients did not receive gadolinium contrast (5 due to contraindications; 16 patient preference). Mean LV ejection fraction was 71.5±6.4%, 6 patients (3%) had mildly impaired LV ejection fraction, 4 males and 2 females. Mean LV end-diastolic volume index was 72.2±1.2 mL/m^2, 12 patients (7%) with dilated LV cavity and 46 (25%) with small LV cavity. Of the FD cohort, 44% had LVH, 57% had low T1, and 38% (62 of 165) had LGE. Male patients with FD had more LVH than females (73 versus 22%, $P<0.001$), higher prevalence of low T1 (74% versus 44%, $P<0.001$), and LGE (53% versus 27%, $P=0.001$). Of the female patients with FD with low T1, 27% had LVH (versus 79% in males with low T1, $P<0.001$).

T2 Mapping in FD

FD had regionally high (more abnormal) T2 values when LGE was present ($P<0.05$ versus corresponding seg-

Table 2. Comparison of CMR Characteristics Across Different Cohorts With LGE

	FD (n=56)	HCM (n=28)	Chronic MI (n=30)	Controls (n=59)	*P* Value
Age, y	55.8±10.2*	49.8±15.0	57.9±12.6*	48.2±13.8	0.001
Male, n (%)	32 (57.1)†	18 (64.3)	26 (86.7)	35 (59.3)	0.038
CMR parameters					
LVEDV, mL	130.7±37.2†	136.9±33.5†	164.8±33.1*	132.3±28.5	<0.001
LVESV, mL	33.0±14.9†	33.0±12.3†	84.3±28.4*	39.2±11.7	<0.001
LVEF, %	75.0±8.1*†	75.9±7.4*†	49.9±8.7*	70.5±5.7	<0.001
LVM, g	198.5 (149–265)*†	201.5 (146–230)*†	111 (86–123)	105 (83–125)	<0.001
LVH, n (%)	49 (87.5)*†	28 (100)*†	0	0	<0.001
Septal/remote T1, ms	907.6±61.1*†‡	1020.2±39.3	1003.2±41.5	1014.6±36.5	<0.001
LGE/BIFL wall T1, ms	1034.6±104.0*†‡	1180.4±66.1*	1137.8±51.6*	996-3±32.6	<0.001
Septal/remote T2, ms	51.4±3.3*†	50.9±2.6*†	47.8±2.1	49.2±2.2	<0.001
LGE/BIFL wall T2, ms	58.2±5.0*†‡	55.6±4.3*	53.7±3.4*	48.9±2.5	<0.001

BIFL indicates basal inferolateral; CMR, cardiovascular magnetic resonance; FD, Fabry disease; HCM, hypertrophic cardiomyopathy; LGE, late gadolinium enhancement; LVEDV, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; LVESV, left ventricular end-systolic volume; LVH, left ventricular hypertrophy; LVM, left ventricular mass; and MI, myocardial infarct.

P value for trend between groups.

Pairwise comparisons using Bonferroni correction:

*$P<0.05$ vs controls.

†$P<0.05$ vs chronic MI.

‡$P<0.05$ vs HCM.

ments in patients without LGE, except for the apical anterior wall, *P*=0.154, Figure 1) with T2 being highest in the BIFL wall (54.1±5.2 ms, Figure 1) especially if a regions of interest was drawn exactly in-line with the distribution of LGE (58.2±5.0 ms). Global T2 was also high when LGE was present (53.1±2.9 versus 50.6±2.2 ms without LGE, *P*<0.001).

Patients with LVH had higher BIFL and septal T2 values (BIFL 57.0±6.1 versus 48.8±3.7 ms and septal 50.6±3.7 versus 48.0±3.4 ms, both *P*<0.001), but low T1 did not significantly affect T2 in the BIFL (53.1±6.9 versus 51.4±5.6 ms, *P*=0.070) or in the septum (49.1±3.9 versus 49.3±3.6 ms, *P*=0.731).

T2 Mapping Across Different Diseases

As expected, septal T1 values measured away from LGE were lower in FD than cMI, HCM, and health (*P*<0.001, Table 2). T2 in LGE was higher in FD than in other diseases (FD 58.2±5.0 ms, HCM 55.6±4.3 ms, cMI 53.7±3.4 ms, controls 48.9±2.5 ms, *P*<0.001, Figure 2A and 2B, Table 2)—although we note that there were some areas of LGE in HCM that did have T2 elevation (Figure 2A). Interestingly, the increased T2 values in FD were not restricted to the LGE area but also observed in the septum/remote to LGE: septal T2 in FD 51.4±3.3 ms versus HCM 50.9±2.6 ms, cMI 47.8±2.1, controls, 49.2±2.2, *P*<0.05 for FD versus cMI and versus controls, Figure 2C.

T1-T2 Mapping Relationship

There is a positive correlation between T1 and T2 across diseases in the same LGE regions of interest (T2 increasing with higher T1 values) but for a given T1 value, T2 is higher in FD than in other cohorts (Figure I in the Data Supplement). The increase in LGE T1 over remote T1 was similar across diseases: average in FD +127 ms (+13%), in HCM +160 ms (+16%), and in cMI +135 ms (+13%), pairwise comparison not significant. However, the increase in LGE T2 over remote T2 was significantly higher in FD than HCM (*P*=0.023): +6.8 ms (+13%) in FD, +4.7 ms (+9%) in HCM, and +5.9 ms (+12%) in cMI.

T2 Mapping and Blood Biomarkers in FD

T2 and Troponin

Troponin was measured in all patients. High troponin was observed in 70 patients (38%). Median troponin T was 7 (interquartile range, 1–25) ng/L (maximum 223 ng/L) and median troponin I was 5 (interquartile range, 1–55) ng/L (maximum 1450 ng/L). Troponin elevation was related both to LVH (75% versus 9% in LVH negative, *P*<0.001) and to LGE (79% versus 7% in LGE negative, *P*<0.001).

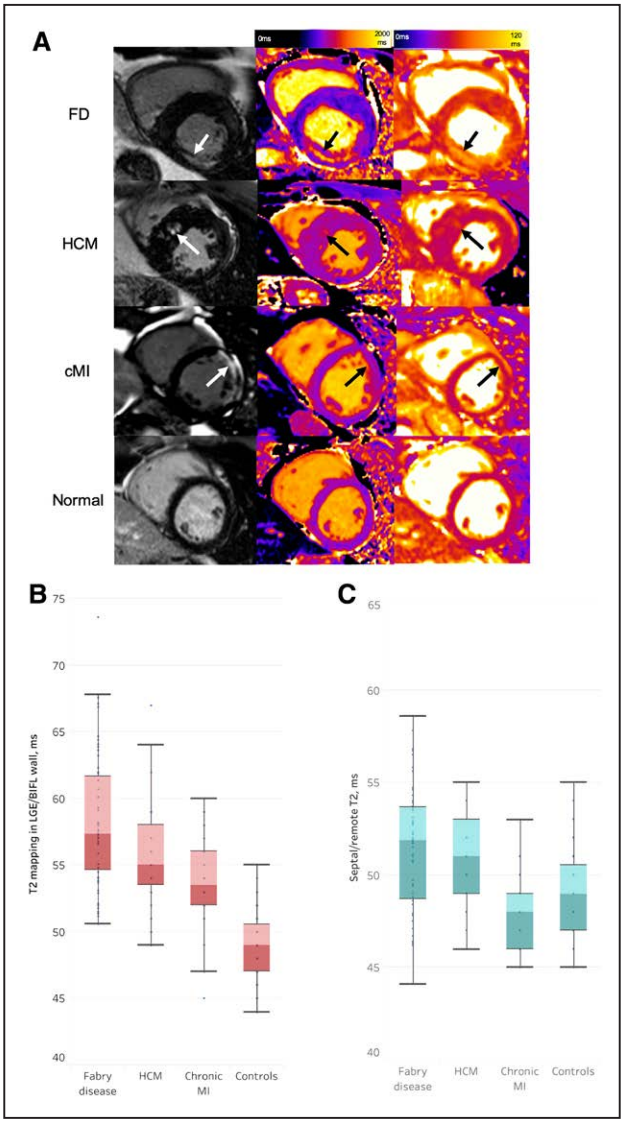


Figure 2. T2 mapping across different cohorts.
A, Corresponding short-axis slices in (from **top** to **bottom**) Fabry disease (FD), hypertrophic cardiomyopathy (HCM), chronic myocardial infarction (cMI), and normal controls. **Left** column shows late gadolinium enhancement (LGE) images, **middle** column shows modified Look-Locker inversion (MOLLI) T1 maps, and **right** column T2 maps. Box and whisker plot comparing T2 values in LGE/basal inferolateral (BIFL) wall (**B**) and in the septum/remote areas (**C**) for each cohort. T2 values were higher for Fabry disease. White arrows point to the LGE; black arrows mark matching areas of abnormal T1 and T2 signal.

High troponin values were strongly associated with T2 in the BIFL wall (*r*=0.85, *P*<1.0×10^{-21}, Figure 3A) but less so with septal/remote T2 (*r*=0.53, *P*<0.001) or global T2 (*r*=0.42, *P*<0.001). Thus, BIFL T2 was included in the regression model. Following univariable analysis (9 variables), multivariable analysis to predict increased troponin identified one strong predictor: high BIFL T2 (odds ratio [OR], 17.3 [95% CI, 3.6–83.4], *P*<0.001) and 3 weaker predictors: age (OR, 1.1, *P*=0.039), LVH (OR, 10.9, *P*=0.017), and LGE (OR, 5.2, *P*=0.034), Table 3.

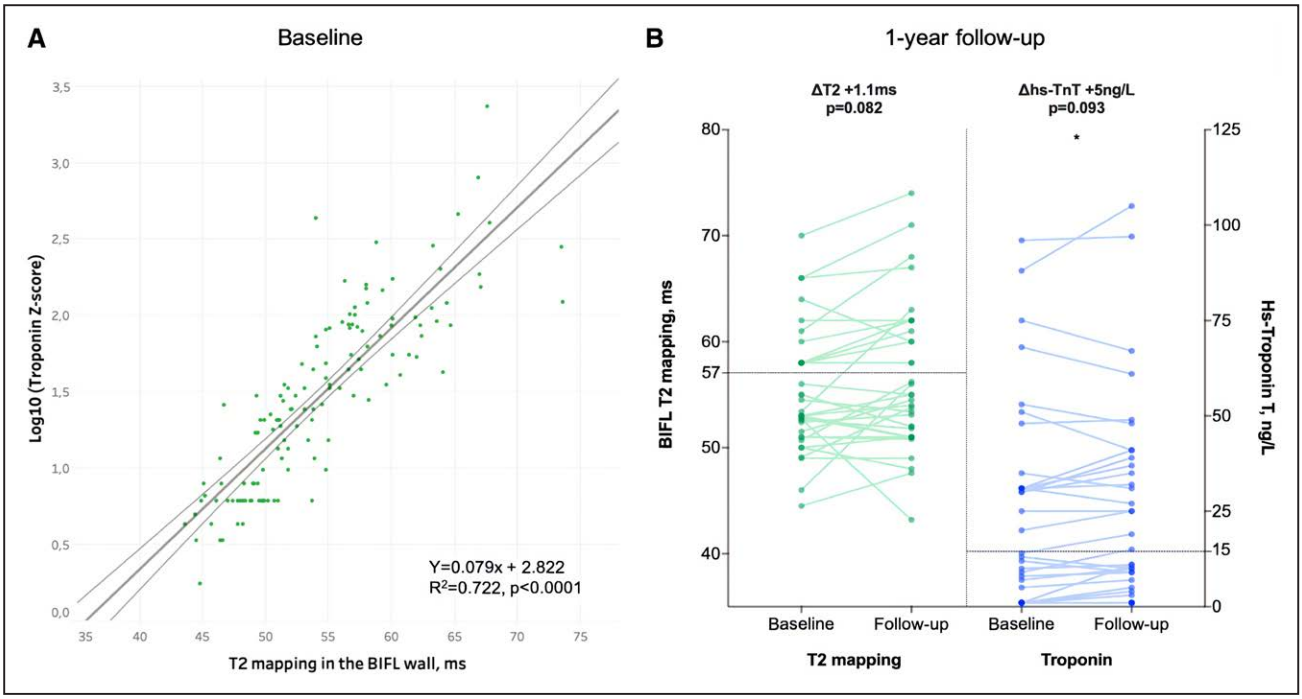


Figure 3. Troponin and T2 elevation are correlated and chronically elevated.
A, Troponin elevation was highly correlated with basal inferolateral (BIFL) wall T2 elevation. Troponin values below the level of detection have been excluded; to combine troponin I (TnI) and troponin T (TnT) scores, Z-scores are plotted with log transformation after adding a constant. **B**, Both troponin and T2 elevation are broadly stable over 1 y (the horizontal line represents the upper limit of normal) with no patients normalizing for either variable. Linear trendline and 95% CI are shown in (**A**). *One outlier not shown (TnT changing from 223 to 254 ng/L).

T2 and NT-proBNP

BIFL T2 was the sole independent predictor of increased NT-proBNP (OR, 3.9 [1.2–11.8], *P*=0.019, Table 3)—results are detailed in the Data Supplement.

T2 Mapping and Electrical Abnormalities

BIFL T2 was selected for the following analyses given the strong aforementioned associations.

Contemporaneous ECG (same day as CMR scan) was available in 126 patients. Overall, patients with ECG abnormalities had higher BIFL T2 (53.8±6.8 versus 48.7±3.4 ms, *P*<0.001), specifically: long PR (54.4±4.1 versus 51.1±6.1 ms, *P*=0.041), complete bundle branch block (57.0±6.2 versus 50.2±5.3 ms, *P*<0.001), LVH voltage criteria (using either Sokolow-Lyon or Cornell criteria, 54.4±7.8 versus 49.9±4.4 ms, *P*=0.003), long QTc (56.5±7.2 versus 50.7±5.6 ms, *P*=0.003), and T-wave inversion (59.2±6.0 versus 49.1±3.8 ms, *P*<0.001). Short PR was not associated with higher BIFL T2 (*P*=0.999). These results are summarized in Figure 4. High BIFL T2 remained a predictor of LVH voltage criteria in multivariable analysis (Tables III through IX in the Data Supplement).

T2 Mapping and Myocardial Mechanics

GLS was more impaired in patients with high BIFL T2 (−14.7±6.8% versus −17.4±3.2%, *P*=0.006, Figure 5). High BIFL T2 predicted GLS worsening (*P*=0.005, Figure 5) in a linear regression model that included maximum wall thickness, LV mass indexed, low septal T1, and percentage of LV LGE (Figure 5), with % LV LGE being the strongest predictor of impaired GLS.

Interval Change in Biomarkers and FASTEX

Thirty-four patients were re-investigated at 1 year (mean 1.1±0.1 years). After 1 year, there was a slight increase in BIFL T2 (from 55.2±5.8 to 56.3±6.9 ms, delta mean T2 +1.1 ms, *P*=0.081), with a similar trend found in troponin T (from 17 [1–35] to 22 [7–41] ng/L, delta median +5 ng/L, *P*=0.094) and NT-proBNP (from 18 [11–82] to 30 [8–108] pmol/L, delta median +12 pmol/L, *P*=0.079). Per patient interval change data for T2 and troponin is presented in Figure 3B.

Ten patients (29%) had an increase in FASTEX >20% (clinical worsening) after 1 year. BIFL T2 and troponin at baseline predicted clinical worsening (*P*=0.004 and *P*=0.016, respectively), unlike NT-proBNP (*P*=0.128) or %LV LGE (*P*=0.279). In multivariable analysis (baseline BIFL T2 and troponin), BIFL T2 was the sole predictor of clinical worsening (OR, 1.4 [95% CI, 1.0–1.9], *P*=0.034).

DISCUSSION

In this prospective international multicenter study, we have explored multiparametric markers of myocardial

Table 3. Univariable and Multivariable Analysis in FD of the Determinants of Increased High-Sensitivity Troponin (Top) and NT-proBNP (Bottom) Elevation

Dependent Variables	Variables in Model	Univariable Exp(B) (95% CI)	*P* Value	Multivariable Exp(B) (95% CI)	*P* Value
High troponin	High BIFL wall T2	36.2 (14.9–88.3)	<0.001*	17.3 (3.6–83.4)	<0.001*
	LVH	32.5 (13.9–76.1)	<0.001*	10.9 (1.5 –78.2)	0.017*
	LGE	51.7 (19.4–137.9)	<0.001*	5.2 (1.1–23.5)	0.034*
	Age (per year)	1.1 (1.1–1.2)	<0.001*	1.1 (1.0–1.2)	0.039*
	eGFR (per 1 mL/min per 1.73 m^2)	1.1 (1.0–1.1)	<0.001*	1.0 (0.98–1.1)	0.227
	GLS (per 1%)	1.1 (1.1–1.4)	<0.001*	1.1 (0.9–1.3)	0.456
	LVEF	175.1 (3.4–8988.1)	0.010*	13.1 (0–359792)	0.622
	Male gender	3.3 (1.8–6.2)	<0.001*	0.6 (0.1–4.3)	0.631
	ERT status	3.9 (2.1–7.3)	<0.001*	1.1 (0.2–5.2)	0.902
	LVEDVi	1.02 (1.00–1.04)	0.038*	1.0 (0.96–1.05)	0.917
	Low septal T1	2.4 (1.3–4.5)	0.006*	1.1 (0.2–4.9)	0.980
High NT-proBNP	High BIFL wall T2	12.5 (5.8–27.0)	<0.001*	3.9 (1.2–11.8)	0.019*
	LVEDVi	1.03 (1.01–1.05)	0.004*	1.03 (0.998–1.06)	0.070
	LGE	8.4 (4.0–17.5)	<0.001*	2.1 (0.6–7.1)	0.231
	Age (per year)	1.1 (1.1–1.1)	<0.001*	1.02 (0.98–1.07)	0.307
	eGFR (per 1 mL/min per 1.73 m^2)	1.1 (1.0–1.1)	<0.001*	1.02 (0.99–1.05)	0.315
	GLS (per 1%)	1.3 (1.1–1.3)	<0.001*	1.0 (0.9–1.2)	0.468
	LVH	7.6 (3.9–14.9)	<0.001*	1.3 (0.4–4.3)	0.664
	ERT status	3.2 (1.7–5.9)	<0.001*	1.2 (0.5–3.0)	0.727
	Low septal T1	1.8 (0.9–3.3)	0.066	-	-
	LVEF	23.7 (0.6–977.9)	0.095	-	-
	Male gender	1.6 (0.9–2.9)	0.128	-	-

BIFL indicates basal inferolateral; BNP, brain natriuretic peptide; eGFR, estimated glomerular filtration rate; ERT, enzyme replacement therapy; FD, Fabry disease; GLS, global longitudinal strain; LGE, late gadolinium enhancement; LVEDVi, left ventricular end-diastolic volume index; LVEF, left ventricular ejection fraction; and LVH, left ventricular hypertrophy.

*Significant *P* value (<0.05).

disease and pathological processes, including markers of storage (T1), edema (T2), myocyte injury (troponin), and LV pressure/volume overload (NT-proBNP) as well as their regionality, chronicity, and impact on electrical and mechanical myocardial function, and disease progression. These data show that when LGE is present, Fabry patients have increased T2 values in the BIFL but also globally. This was different to cMI or healthy volunteers, although interestingly, some HCM patients had some degree of T2 elevation. The regional T2 elevation was strongly associated with cardiac injury (elevated troponin) and increased LV pressure/volume overload (elevated NT-proBNP), with weaker associations with global T2 elevation. A high BIFL T2 was also associated with both electrocardiographic changes and mechanical (GLS) impairment. Over a year, T2, troponin, and NT-proBNP elevations were chronic, and T2 BIFL elevation was associated with clinical worsening.

Myocardial Edema and Myocyte Injury

Whether T2 just detects superimposed edema in LGE or if it can also detect global myocardial edema had not been investigated before until now. Here, we have shown that when LGE is present in FD, T2 mapping is increased in most myocardium segments, and both septal and LGE T2 are significantly higher in FD than in cMI or healthy volunteers. However, the BIFL LGE T2 is likely a better reflection of the underlying pathological process than other segments because[1]: its T2 value is higher than other segments,[2] the signal difference versus other diseases with LGE is more pronounced than septal T2,[3] it shows chronicity and a much tighter association with troponin and NT-proBNP than septal or global T2.

As alluded by Rozenfeld et al,[19] the high levels of sphingolipids in the plasma and cells alone are insufficient to explain the pathophysiology of FD. We think the answer could be in inflammation. A high T2 signal reflects an increased water content (ie, edema) which can be inflammatory or noninflammatory. A previous study combining T2 short-TI inversion recovery with positron emission tomography-fludeoxyglucose uptake[13] showed locally increased signal in LGE, suggesting this could be inflammation. However, a formal diagnosis of inflammation cannot be made in this study.

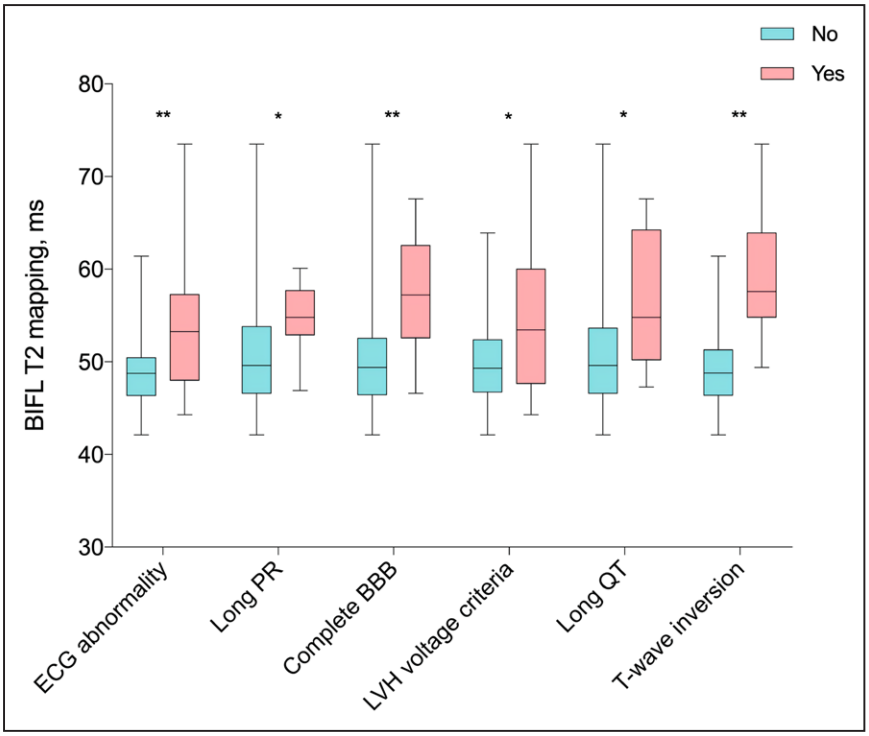


Figure 4. Box-plots of basal inferolateral T2 mapping according to ECG abnormalities in Fabry patients.
BBB indicates bundle branch block; BIFL, basal inferolateral; and LVH, left ventricular hypertrophy. *P<0.05 and **P<0.001.

There are 2 steps first that are needed: first, to confirm by diverse means that there is inflammation (this requires histology); second, to show that inflammation is causal. Regarding the latter, a primary autoimmune process by anti-Gb3 antibodies could be initiating inflammation possibly through complement fixation. This could be tested by looking for antibodies tissue histologically, ex vivo modeling, or possibly by looking at elements of the complement cascade. The alternative could be a secondary event due to direct toxicity of GB3 resulting in clearance of effete cells. However, this is a complex and dynamic process to understand and exploration of the specific mechanisms of inflammation in FD was not within the scope of our article.

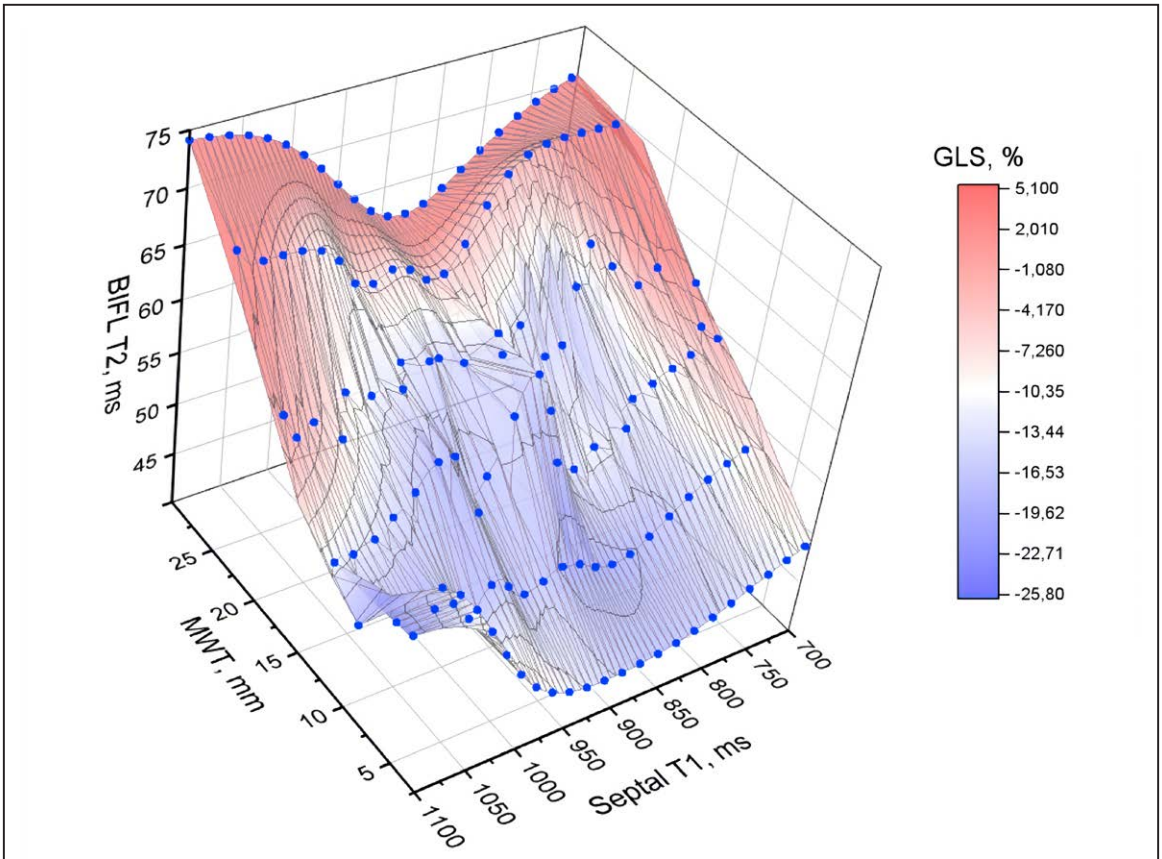
T2 Mapping in Clinical Practice

Unlike T2, T1 mapping does not follow a linear progression with disease; it rather has a biphasic response: normal pre-LVH, then lowers with storage, and finally increases in advanced disease (pseudo-normalizing or becoming high). Low T1 has value in identifying early FD but fails to predict high troponin as non-low T1 includes advanced FD. In contrast, T2 increases with disease progression. This is the first time that T2 mapping was shown to have a prognostic role in FD. Indeed, high BIFL T2 was associated with clinical worsening (FASTEX >20%) after 1 year. Not only that, but it was also associated with LV mechanical (GLS) impairment and electrocardiographic changes.

We have previously shown that GLS impairment in FD precedes any measurable reduction in LV ejection fraction.[20] Here, we go on to show that GLS impairment is worse with increased BIFL T2 values even after adjusting for LGE, LVH, and septal T1. BIFL T2 was also higher in Fabry patients with ECG abnormalities like long PR, complete bundle branch block, LVH voltage criteria, long QTc, and T-wave inversion. However, high BIFL T2 only remained a predictor of LVH voltage criteria in multivariable analysis. Further more detailed work is required in relation to ECG abnormalities and CMR characteristics.

Our results offer new mechanistic insights and exciting new roads to drive development and management of Fabry patients. It has been ≈16 years[8] and 6 years[6] since LGE and then T1 mapping were first described in FD. The former is now essential for care and the latter becoming increasingly used. The same may occur for T2, particularly if therapy starts targeting this marker. However, because T2 is so tightly concordant with troponin, we suspect that troponin could be the next key cardiac biomarker in FD.

Limitations of our study include no histological validation and no other additional inflammatory markers, such as high-sensitivity C-reactive protein or interleukin-6. Although T2 values were significantly different between cohorts, serum troponin was not measured in either HCM or chronic MI cohorts. Higher T1 and T2 values in the apical segments might have been due to blood pool signal contamination, as apical segments are usually thinner. T1 and T2 could be interacting in some unknown way. The degree of error in T2 is higher than in T1 (a higher relative SD is commonly found in T2 than in T1). Therefore, small increases in T2 like the ones we see in global myocardium



Dependent variable	Variables in Model	Univariate B coefficient and 95% CI	P-value	Multivariable B coefficient and 95% CI	P-value
GLS	LV LGE (%)	0.6 (0.4–0.7)	**<0.001**	0.4 (0.2 – 0.6)	**<0.001**
	High BIFL T2	2.7 (1.2 – 4.1)	**<0.001**	2.3 (0.7 – 3.9)	**0.005**
	LVMI (g/m^2)	0.07 (0.06 – 0.09)	**<0.001**	0.03 (-0.001 – 0.07)	0.055
	MWT (mm)	0.6 (0.5 – 0.8)	**<0.001**	0.2 (-0.07 – 0.6)	0.126
	Low septal T1	1.3 (-0.1 – 2.7)	0.072	-	-

Figure 5. Four-dimensional plot illustrating the impairment of global longitudinal strain (GLS) with higher values of basal inferolateral (BIFL) T2 (*r*=0.463, *P*<0.001), maximum wall thickness (*r*=0.582, *P*<0.001), and lower values of septal T1 (*r*=−0.286, *P*<0.001).
A grid and surface colormap for GLS was built using the thin plate spline method for data interpolation and smoothing. A linear regression model is shown below (GLS as a dependent variable) considering the aforementioned factors, the percentage of late gadolinium enhancement (LGE) and left ventricular (LV) mass index. Both higher percentage of LV LGE and high BIFL T2 predicted GLS impairment. LVMI indicates left ventricular mass index; and MWT, maximum wall thickness.

should be interpreted with caution. Nevertheless, the local T2 signal is encouraging and supported by other findings (eg, blood biomarkers). However, the presence of T2 elevation with correlating troponin elevation is not the same as inflammation—no correlations with histology or direct measures of the immune system have been made. An histology study would greatly improve our understanding of the relevance of cardiac imaging in FD.

Conclusions

Using a combination of blood and CMR multiparametric imaging biomarkers, we have shown that FD (when LGE is present) has chronic myocardial edema that is strongly associated with chronic troponin elevation. This edema has a prognostic role in FD and is associated with baseline cardiac electromechanical changes and clinical worsening after 1 year, suggesting a potential new treatment/disease monitoring target that should be further investigated.

ARTICLE INFORMATION

Received August 16, 2019; accepted February 7, 2020.

The Data Supplement is available at https://www.ahajournals.org/doi/suppl/10.1161/CIRCIMAGING.119.010171.

Correspondence

James C. Moon, MD, Barts Heart Centre, St Bartholomew's Hospital, W Smithfield, London EC1A 7BE, United Kingdom. Email j.moon@ucl.ac.uk

Affiliations

Institute of Cardiovascular Science, University College London, United Kingdom (J.B.A., S.N., M.A., K.K., G.C., T.K., J.C.M.). Cardiac Imaging Department, Barts Heart Centre, St Bartholomew's Hospital, London, United Kingdom (J.B.A., S.N., H.B., M.A., K.K., J.C.M.). Cardiology Department (R.V., S.B., R.P.S.) and Inherited Metabolic Disorders Unit (T.G.), University Hospitals Birmingham, United Kingdom. Hatter Cardiovascular Institute, London, United Kingdom (H.B.). Istituto Auxologico Italiano IRCCS Center for the Cardiac Arrhythmias of Genetic Origin, Milan, Italy (S.C.). Royal Free London NHS Foundation Trust and University College London, United Kingdom (T.K., U.R., M.F., D.H.). Department of Genetic Medicine, Westmead Hospital, Sydney, Australia (M.T.). Sydney Medical School, University of Sydney, Australia (R.K.).

Sources of Funding

This study was funded by an investigator-led research grant from Sanofi-Genzyme. Sanofi-Genzyme had no role in the study beyond the initial funding. Dr Kozor was sponsored by Heart Research Australia. Dr Castelletti was funded by the European Society of Cardiology Research Grant.

Disclosures

Dr Augusto is supported by investigator-led research grant by Sanofi-Genzyme. Dr Ramaswami has received travel grants and honoraria for lectures and advisory boards from Takeda, Sanofi-Genzyme, Amicus, Idorsia, and Chiesi. The other authors report no conflicts.

REFERENCES

1. Zarate YA, Hopkin RJ. Fabry's disease. *Lancet*. 2008;372:1427–1435. doi: 10.1016/S0140-6736(08)61589-5
2. Desnick RJ, Brady R, Barranger J, Collins AJ, Germain DP, Goldman M, Grabowski G, Packman S, Wilcox WR. Fabry disease, an under-recognized multisystemic disorder: expert recommendations for diagnosis, management, and enzyme replacement therapy. *Ann Intern Med*. 2003;138:338–346. doi: 10.7326/0003-4819-138-4-200302180-00014
3. Baig S, Edward NC, Kotecha D, Liu B, Nordin S, Kozor R, Moon JC, Geberhiwot T, Steeds RP. Ventricular arrhythmia and sudden cardiac death in Fabry disease: a systematic review of risk factors in clinical practice. *Europace*. 2018;20(FI2):f153–f161. doi: 10.1093/europace/eux261
4. Messroghli DR, Moon JC, Ferreira VM, Grosse-Wortmann L, He T, Kellman P, Mascherbauer J, Nezafat R, Salerno M, Schelbert EB, et al. Clinical recommendations for cardiovascular magnetic resonance mapping of T1, T2, T2* and extracellular volume: a consensus statement by the Society for Cardiovascular Magnetic Resonance (SCMR) endorsed by the European Association for Cardiovascular Imaging (EACVI). *J Cardiovasc Magn Reson*. 2017;19:75. doi: 10.1186/s12968-017-0389-8
5. Pica S, Sado DM, Maestrini V, Fontana M, White SK, Treibel T, Captur G, Anderson S, Piechnik SK, Robson MD, et al. Reproducibility of native myocardial T1 mapping in the assessment of Fabry disease and its role in early detection of cardiac involvement by cardiovascular magnetic resonance. *J Cardiovasc Magn Reson*. 2014;16:99. doi: 10.1186/s12968-014-0099-4
6. Sado DM, White SK, Piechnik SK, Banypersad SM, Treibel T, Captur G, Fontana M, Maestrini V, Flett AS, Robson MD, et al. Identification and assessment of anderson-fabry disease by cardiovascular magnetic resonance noncontrast myocardial T1 mapping. *Circ Cardiovasc Imaging*. 2013;6:392–398. doi: 10.1161/CIRCIMAGING.112.000070
7. Nordin S, Kozor R, Baig S, Abdel-Gadir A, Medina-Menacho K, Rosmini S, Captur G, Tchan M, Geberhiwot T, Murphy E, et al. Cardiac phenotype of prehypertrophic fabry disease. *Circ Cardiovasc Imaging*. 2018;11:e007168. doi: 10.1161/CIRCIMAGING.117.007168
8. Moon JC, Sachdev B, Elkington AG, McKenna WJ, Mehta A, Pennell DJ, Leed PJ, Elliott PM. Gadolinium enhanced cardiovascular magnetic resonance in anderson-fabry disease. Evidence for a disease specific abnormality of the myocardial interstitium. *Eur Heart J*. 2003;24:2151–2155. doi: 10.1016/j.ehj.2003.09.017
9. Krämer J, Niemann M, Störk S, Frantz S, Beer M, Ertl G, Wanner C, Weidemann F. Relation of burden of myocardial fibrosis to malignant ventricular arrhythmias and outcomes in fabry disease. *Am J Cardiol*. 2014;114:895–900. doi: 10.1016/j.amjcard.2014.06.019
10. Kozor R, Grieve SM, Tchan MC, Callaghan F, Hamilton-Craig C, Denaro C, Moon JC, Figtree GA. Cardiac involvement in genotype-positive fabry disease patients assessed by cardiovascular MR. *Heart*. 2016;102:298–302. doi: 10.1136/heartjnl-2015-308494
11. Niemann M, Herrmann S, Hu K, Breunig F, Strotmann J, Beer M, Machann W, Voelker W, Ertl G, Wanner C, et al. Differences in fabry cardiomyopathy between female and male patients: consequences for diagnostic assessment. *JACC Cardiovasc Imaging*. 2011;4:592–601. doi: 10.1016/j.jcmg.2011.01.020
12. Moon JC, Sheppard M, Reed E, Lee P, Elliott PM, Pennell DJ. The histological basis of late gadolinium enhancement cardiovascular magnetic resonance in a patient with anderson-fabry disease. *J Cardiovasc Magn Reson*. 2006;8:479–482. doi: 10.1080/10976640600605002
13. Nappi C, Altiero M, Imbriaco M, Nicolai E, Giudice CA, Aiello M, Diomiaiuti CT, Pisani A, Spinelli L, Cuocolo A. First experience of simultaneous PET/MRI for the early detection of cardiac involvement in patients with anderson-fabry disease. *Eur J Nucl Med Mol Imaging*. 2015;42:1025–1031. doi: 10.1007/s00259-015-3036-3
14. Nordin S, Kozor R, Bulluck H, Castelletti S, Rosmini S, Abdel-Gadir A, Baig S, Mehta A, Hughes D, Moon JC. Cardiac fabry disease with late gadolinium enhancement is a chronic inflammatory cardiomyopathy. *J Am Coll Cardiol*. 2016;68:1707–1708. doi: 10.1016/j.jacc.2016.07.741
15. Frustaci A, Verardo R, Grande C, Galea N, Piselli P, Carbone I, Alfarano M, Russo MA, Chimenti C. Immune-mediated myocarditis in fabry disease cardiomyopathy. *J Am Heart Assoc*. 2018;7:e009052. doi: 10.1161/JAHA.118.009052
16. Camporeale A, Pieroni M, Pieruzzi F, Lusardi P, Pica S, Spada M, Mignani R, Burlina A, Bandera F, Guazzi M, et al. Predictors of clinical evolution in prehypertrophic fabry disease. *Circ Cardiovasc Imaging*. 2019;12:e008424. doi: 10.1161/CIRCIMAGING.118.008424
17. Mignani R, Pieruzzi F, Berri F, Burlina A, Chinea B, Gallieni M, Pieroni M, Salviati A, Spada M. FAbry STabilization indEX (FASTEX): an innovative tool for the assessment of clinical stabilization in fabry disease. *Clin Kidney J*. 2016;9:739–747. doi: 10.1093/ckj/sfw082
18. Maceira AM, Prasad SK, Khan M, Pennell DJ. Normalized left ventricular systolic and diastolic function by steady state free precession cardiovascular magnetic resonance. *J Cardiovasc Magn Reson*. 2006;8:417–426. doi: 10.1080/10976640600572889
19. Rozenfeld P, Feriozzi S. Contribution of inflammatory pathways to fabry disease pathogenesis. *Mol Genet Metab*. 2017;122:19–27. doi: 10.1016/j.ymgme.2017.09.004
20. Vijapurapu R, Nordin S, Baig S, Liu B, Rosmini S, Augusto J, Tchan M, Hughes DA, Geberhiwot T, Moon JC, et al. Global longitudinal strain, myocardial storage and hypertrophy in fabry disease. *Heart*. 2019;105:470–476. doi: 10.1136/heartjnl-2018-313699