Proposed Stages of Myocardial Phenotype Development in Fabry Disease

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Brief Title: Age and sex differences in Fabry cardiomyopathy
Word Count: 4120 (excluding title page and abstract)

Source of funding: This study is funded by an investigator-led research grant from Genzyme, now Sanofi-Genzyme. Sanofi-Genzyme had no role in the study beyond the initial funding and requiring recruitment milestones as part of financial governance.

Disclosures: Sabrina Nordin is supported by investigator-led research grant by Sanofi-Genzyme and honoraria from Shire. Rebecca Kozor has received honoraria for presenting and advisory board participation, as well as support for investigator-led research from Sanofi-Genzyme. Elaine Murphy has received unrestricted educational grants from Sanofi-Genzyme and Shire and clinical trial funding from Sanofi-Genzyme, Shire, Amicus and Biomarin. Robin H Lachmann has received honoraria and travel support from Sanofi-Genzyme. Atul Mehta has received honoraria and grant support for research and educational activities from Shire, Sanofi-Genzyme and Amicus. James C Moon is the principal investigator of the Sanofi-Genzyme research grant that funded this study. No disclosures for all the other co-authors.

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Acknowledgements: This research is supported by researchers at the National Institute for Health Research University College London Hospitals Biomedical Research Centre. We are grateful for the contributions of patients, and the staff members at the Lysosomal Storage Disorder Unit, Royal Free Hospital and Charles Dent Metabolic Unit.
Abstract
Objectives: To explore the Fabry myocardium in relation to storage, age, sex, structure, function, ECG changes, blood biomarkers and inflammation/fibrosis.
Background: Fabry disease (FD) is a rare, X-linked lysosomal storage disorder. Mortality is mainly cardiovascular with males exhibiting cardiac symptoms earlier than females. By cardiovascular magnetic resonance (CMR), native T1 is low in FD because of sphingolipid accumulation.
Methods: A prospective, observational study of 182 FD (167 adults, 15 children, mean age 42±17 years, 37% male) who underwent CMR including native T1, late gadolinium enhancement (LGE) and extracellular volume fraction, 12-lead electrocardiogram and blood biomarkers (troponin and N-terminal pro-brain natriuretic peptide).
Results: In children, T1 was never below the normal range, but was lower with age (9ms/year, r=-0.78 children; r=-0.41 whole cohort; both p<0.001). Over the whole cohort, the T1 reduction with age was greater and more marked in males (males -1.9ms/year, r=-0.51 males, p<0.001; females -1.4ms/year, r=-0.47 females, p<0.001). LVH, LGE and electrocardiogram abnormalities occur earlier in males. Once LVH occurs, T1 demonstrates major sex dimorphism: with increasing LVH in females, T1 and LVH become uncorrelated (r=-0.239, p=0.196) but in males, the correlation reverses and T1 increases (towards normal) with LVH (r=+0.631, p<0.001), a U-shaped relationship of T1 to LVMi in males.
Conclusions: These data suggest that myocyte storage starts in childhood, accumulates faster in males before triggering two processes: a gender independent scar/inflammation regional response (LGE) and, in males, apparent myocyte hypertrophy diluting the T1 lowering of sphingolipid.

Keywords: Fabry disease, native T1, gender, phenotype

Condensed abstract: Fabry disease is an X-linked lysosomal storage disease. Cardiovascular involvement is the leading cause of death. Myocardial storage can now be detected as it lowers T1. By measuring multiple parameters in a large cohort (n=182), it appears that myocyte storage starts in childhood, proceeds faster in males than females and once LVH occurs, a sex-independent scar/inflammation response occurs with, separately, a sexually dimorphic myocyte hypertrophic response in males. Combined, this and other studies propose phases of cardiac involvement: accumulation phase, inflammation and myocyte hypertrophy phase and fibrosis and impairment phase suggesting additional pathways to storage that could be therapeutic targets.

Abbreviations
CMR = cardiovascular magnetic resonance
ECG = electrocardiogram
ECV = extracellular volume fraction
eGFR= estimated glomerular filtration rate
ERT = enzyme replacement therapy
FD = Fabry disease
LGE = late gadolinium enhancement
LVEF = left ventricular ejection fraction
LVH = left ventricular hypertrophy
LVMi = indexed left ventricular mass
MWT = maximum wall thickness
NT-proBNP = N-terminal pro-brain natriuretic peptide
Introduction

Fabry disease (FD) is a rare, X-linked lysosomal storage disorder caused by deficiency in the enzyme α-galactosidase A. This leads to slowly progressive sphingolipid accumulation affecting multiple organs including the heart, kidneys and brain(1). Males are affected earlier than females (average age of cardiac symptoms 32 vs 40 years)(2,3). Cardiac manifestations include left ventricular hypertrophy (LVH), arrhythmias, chronic inflammation(4), myocardial fibrosis and functional impairment(5). Cardiovascular death is the leading cause of death in both males and females(6,7), with LVH and myocardial fibrosis being amongst the suggested risk factors for ventricular arrhythmia and sudden cardiac death(8). Treatment options in FD include enzyme replacement therapy (ERT), which has been the mainstay therapy, and more recently oral chaperone therapy, which is available for amenable mutations(9). Sphingolipid storage over time appears to trigger myocardial processes including LVH and irreversible myocardial fibrosis. Although specific phases of cardiac involvement are ill defined, early initiation of treatment appears desirable to avoid irreversible and progressive phenotype alterations(10).

The cardiac phenotype in FD has been increasingly understood by cardiovascular magnetic resonance (CMR) over the past 15 years. Early data showed the presence of late gadolinium enhancement (LGE) in approximately 50% of patients, initially in the basal inferolateral wall in FD(11), sometimes occurring before LVH in females(12) or in some mutations in males(13). Recent advances in CMR allow quantitative assessment of the myocardium using fundamental magnetic tissue relaxation constants, for example T1, which are displayed as parametric color maps. Sphingolipid– or at least the specific configuration in lamellar bodies appears to be the cause of the low T1 and occurs in 85% of FD with LVH and 40-50% of FD without LVH(14-16). Low T1 has also been observed in FD with right ventricle
hypertrophy(17). The T1 signal however reflects the whole myocardium: whilst fat lowers T1, pseudonormalisation has been previously hypothesised in advanced disease – either with triggered hypertrophy diluting the storage signal or with fibrosis (high T1) normalizing it(14). There is well-documented data on differences in Fabry cardiomyopathy between male and female FD by transthoracic echocardiography and CMR with LGE(12). We sought to refine further our understanding of myocardial disease development using a multiparametric CMR approach in a large cohort of adults and children, male and female FD.

Methods

Study population

A prospective observational study in 182 FD patients. Ethical approval was obtained from the local research ethics committee. Participants were recruited from Fabry clinics at Royal Free Hospital and National Hospital for Neurology and Neurosurgery in London. Inclusion criteria included gene-positive FD; both males and females; children (<18 years old) and adults (≥18 years).

All participants underwent CMR and 12-lead electrocardiogram (ECG). Blood samples were performed on adults just before the scan and analysed for estimated glomerular filtration rate (eGFR), high-sensitivity troponin T (Roche Diagnostic; normal range 0-14ng/L) and N-terminal pro-brain natriuretic peptide (NT-proBNP) analysis (Roche Diagnostics; normal range according to age and gender)(18).

CMR imaging

CMR was performed (1.5 Tesla Avanto, Siemens Healthcare, Erlangen, Germany) using a standard clinical protocol. LGE images were acquired in the standard long axis and short axis stack using either fast long angle shot (FLASH) sequence or a
respiratory motion-corrected free-breathing single short steady state free precession (SSFP), averaged sequence (MOCO-LGE), both with phase sensitive inversion recovery. Before contrast administration (0.1 mmol/kg body weight, Gadoterate meglumine, Dotarem, Guerbet S.A., France), T1 mapping was performed using a shortened modified Look-Locker inversion recovery (ShMOLLI) sequence on basal and mid left ventricular short axis slices. The resulting pixel by-pixel T1 color maps were displayed using a customized 12-bit lookup table, where normal myocardium was green, increasing T1 was red, and decreasing T1 was blue. Post-contrast T1 mapping was performed 15 minutes after gadolinium administration for assessment of extracellular volume fraction (ECV) quantification. Contrast was not administered in participants age <18 years, eGFR <30ml/min/1.73m² or if the patient declined.

**CMR analysis**

All imaging analysis was performed using CVI42 software (Circle Cardiovascular Imaging Inc., Calgary, Canada). A region of interest for native T1 and ECV was manually drawn in the septum, taking care to avoid the blood-myocardial boundary with a 20% offset. LVH was defined as maximum wall thickness (MWT) >12mm in adults or increased indexed left ventricular mass (LVMi) on CMR according to age and gender matched normal reference ranges in adults and children(19,20). Normal T1 reference ranges (mean ± 2 standard deviations) were defined using 73 adult healthy volunteers (mean age 49±14 years): normal range total population 958±56ms, lower limit 902ms; male subgroup normal range 947±46ms, lower limit 901ms and female subgroup normal range 972±56ms, lower limit 916ms(21).
ECG analysis

The ECGs were independently analysed by 2 experienced observers (S.N, S.B). Recorded ECG variables included: heart rate, rhythm, PR interval duration (normal 120-200ms) and QRS complex duration (normal <120ms). The presence of complete left or right bundle branch block, T wave inversion in at least two contiguous leads, multifocal ventricular ectopics and Sokolow-Lyon voltage criteria for LVH (SV1 + RV5 or RV6 >35mm) were also recorded.

Statistical analysis

Statistical analyses were performed using SPSS 24 (IBM, Armonk, NY). Continuous variables were expressed as mean ± standard deviation or median ± interquartile range according to normality using Shapiro-Wilk test. Troponin and NT-proBNP values were natural log transformed for bivariate testing. Categorical variables were expressed as percentages. Group testing was independent-sample t-test, Mann-Whitney U test, Chi-squared test or Fisher exact test according to normality, categorical or continuous data. Correlations between parameters were analysed using Pearson (r) or Spearman’s rho (r_s). Difference in regression slopes was determined using analysis of covariance. The relationship between LGE and clinically relevant variable was evaluated using logistic regression model to identify the independent associations of the dependent variables. A p-value of <0.05 was considered statistically significant.

Results

There were 182 participants: 167 adults and 15 children. Baseline characteristics are shown in Table 1. Mean age of the FD cohort was 42±17 years, male gender 37% (68/182). Mean eGFR was 82±17 ml/min/1.73m². 11 patients had eGFR <60
ml/min/1.73m². 37 patients did not receive contrast at CMR. This is mainly due to patient preference, 3 patients had poor eGFR excluding contrast administration. 30% (55/182) had the cardiac variant(22). Of this group, 91% (50/55) had N215S mutation, 5% (3/55) R301Q mutation and 4% (2/55) I91T mutation. 51% (92/182) of patients were on ERT with a median ERT duration of 7±6.4 years.

In the adult cohort (n=167), mean age was 45±15 years (range 18-81 years). 28% (47/167) were of cardiac variant and 37% male (62/167), ERT in 54% (91/167). In children (n=15): median age was 11±5 years (range 6-16 years), 53% (8/15) were of cardiac variant (all N215S mutation) and 40% male (6/15). Only one child was on ERT (for uncontrolled acroparesthesia).

**Age related trends of T1**

**Children:** All children were LVH-negative with normal function (mean LVEF 68±6%, LVMi 54±12g/m², MWT 7±1mm). The children did not receive gadolinium so precluding assessment of LGE and ECV. No child had a low T1 (mean native T1 971±31ms), but T1 fell linearly with increasing age (figure 1a). Extrapolating the curve to adulthood, the curve cuts the lower limit of normal at the age of 18 (r -0.78, p<0.001; figure 1a).

**Adults:** Mean LVEF in FD adults was 73±7%. 3 adults had mildly impaired LV function with LVEF between 49-54%. Of the male FD with LVH, 98% (44/45) had low T1 (Figure 2a). The one patient with normal T1 had extensive LGE involving multiple segments including the septum. Of the female FD with LVH, 90% (28/31) had low T1 and those with normal T1 all had apical LVH.

With increasing age, T1 falls, but the trend is less strong than in children (r -0.41, p<0.001) (Figure 1b). Over the whole cohort, this was more marked in males (males -1.9ms/year, r -0.51 males, p<0.001; females -1.4ms/year, r -0.47 females, p<0.001,
When T1 is compared with LVH, however, the relationship was sex specific. Before LVH is present, with increasing LVMi, T1 falls in both sexes but more markedly in males (r -0.54, p<0.001 in male; r -0.276, p=0.01 in female; figure 2). After overt LVH is present, a major sex dimorphism is found. In males, the LVH is more extreme, and the correlation reverses (r +0.631, p<0.001), although almost all are below the lower limit of normal (pseudonormalization). In females, once LVH is present, T1 is not related to the degree of LVH (r -0.239, p>0.05).

**Age and sex related trends for all parameters**

**LVH:** LVH was more prevalent in males (66% [45/68] compared to 27% [31/114] in females, p<0.001). In both sexes, the prevalence of LVH increased with age (Figure 3a male, 3b female). There were two main differences between males and females firstly phenotype development of LVH was later in women (effectively complete penetrance by 40-49 years in males, and 60-69 years in females). Secondly, when present, the LVH was more severe in men (figure 2) - with LVMi in males ranging up to 2.6x average LVMi (based on normal reference ranges) and 1.7x average LVMi for females. The highest MWT in males was 30mm and in females was 26mm.

**LGE:** LGE prevalence was higher in males than in females (59% [32/54] vs 37% [34/91], p=0.015). Prevalence of LGE increased with age in both males and females (figure 3c and 3d). Given that LVH mainly occurs later in women, this meant a much higher frequency of LGE in LVH-negative female. The only true LVH-negative male had RV insertion points LGE, a non-specific pattern. The other two males had high normal LVMi with MWT of 13mm.

**ECV:** Male FD had lower ECV compared to female FD (ECV 0.25±0.03 vs 0.28±0.02; p<0.001), as is found in health. The correlation observed in ECV between
age and sex however is stronger in males compared to females (r 0.38 in males, p=0.04; r 0.12 in females, p>0.05).

**Blood biomarkers (Troponin and NT-proBNP):** Troponin increased with age in males and females (figure 4a and 4b). Troponin correlated with age (r_s 0.58, p<0.001) as well as LVMi (r_s 0.69), T1 (r_s -0.51) and LGE (LGE-positive 83% [34/41] vs LGE-negative 4% [2/57]); all p<0.001.

NT-proBNP increased with age (figure 4c and 4d). NT-proBNP correlated with age (r_s 0.47, p<0.001), and was associated with LVMi r_s 0.58, T1 r_s -0.34 and LGE (LGE-positive 68% [28/41] vs LGE-negative 9% [5/58]); all p<0.001. Troponin and native T1 values were independently related to LGE with troponin having the strongest association with presence of LGE (odds ratio 1.191, p=0.008). The odds ratio of NT-proBNP, LVMi, eGFR, gender and age did not reach statistical significance.

**ECG:** The prevalence of ECG abnormalities increased with age in males and females (figure 4e and 4f), occurring earlier in males (18-19 years) than females (20-29 years).

**ERT:** In general, patients on ERT had higher MWT (14±5 vs 10±3mm), LVMi (98±42 vs 69±26 g/m²) and lower septal T1 value (876±43 vs 916±60 ms), all p<0.001. ERT use was increasingly common with age in both males and females, but the age group of ERT initiation was similar between sexes (10-19 years). In males, 78% (36/46) with LVH were on ERT and 74% (23/31) in females, both p<0.005. There is no significant correlation between T1 value and ERT duration in the total cohort (r_s 0.004 p=NS). No strong correlation was found between ERT duration and between T1 values in males and females (r_s 0.290 male and -0.029 female, both p=NS). There is a downward trend between age of ERT initiation and T1 values (r_s -0.30 male and -0.44 female, both p<0.05). The trend of LVMi and T1 between males and females were similar in patients on ERT compared to patients who are ERT-naïve (Figure 5).
**Discussion**

We sought insight into myocardial phenotype development in FD by looking at a large cohort of patients (including children) and measuring multiple parameters: LVH, scar (LGE), blood biomarkers and importantly T1 – a quantitative myocardial signal that is reduced by sphingolipid storage. The data shows that no child had overt T1 lowering of storage, but that T1 falls through childhood suggesting progressive subclinical accumulation. In children and adults, the fall in T1 with age is steeper with males compared to females, suggesting storage is faster in males. Males had earlier ECG abnormalities, blood biomarker increase, LVH and slightly earlier LGE; with LGE in LVH negative subjects only occurring in females. Once LVH occurs, male hypertrophy is far more extreme than females (even when indexed), and the relationship of LVH to T1 changes: in females T1 falls until LVH is present when it is broadly flat, but in males, after LVH is present, T1 is higher (more normal) with increased LVH. What does the apparent T1 rise in males mean? Storage cannot be the cause (or T1 would be falling). T1 is a composite signal from myocardial interstitium (fibrosis, edema – high T1), capillary blood (high T1), myocyte sarcomeric protein (presumed normal T1) and sphingolipid storage (low T1). With this framework, there are multiple possible explanations: diffuse fibrosis, edema or capillary vasodilatation pseudo-normalizing T1 appears unlikely as all these increase ECV, which was normal in this study; focal fibrosis would explain the one patient with extensive LGE and normal T1, but not the others; removal of storage by ERT – a plausible scenario, but firstly, this would likely cause at least some LV mass regression and secondly, given most males and females with LVH are on ERT, enzyme would need to be more efficient in males which appears unlikely. We believe the answer is that in males, storage is triggering sarcomeric protein expression – myocyte hypertrophy(23,24),

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usual LVH rather than storage LVH, and that this is diluting the T1 lowering of sphingolipid. This would mean that there are two types of sex dimorphism in FD: that affecting all tissues (related to the second functional copy of the alpha galactosidase gene in females); and a cardiac specific sex dimorphism related to the male myocyte response to insult, here storage. Sex dimorphism in myocyte hypertrophy is familiar from other cardiac diseases: the hypertrophy in aortic stenosis is far more marked in males than females(25), and probably observed in other conditions – for example, hypertrophic cardiomyopathy, a non-sex linked disease that has a male predominance in most large studies(26). Whether FD, AS and HCM hypertrophy of sex dimorphism derive from common mechanisms is unknown at this time. Differential expression of androgen and estrogen receptors and differences in the renin-angiotensin system, nitric oxide activity, and norepinephrine release may contribute to sex differences observed in LV remodeling(25,27,28).

Based on these findings, our prior work on LVH-negative patients and on LGE including inflammation imaging(4), we hypothesize a model of myocardial phenotype evolution in Fabry disease consisting of an accumulation phase, a hypertrophy and inflammation phase, and a fibrosis and impairment (late) phase (Figure 6). This later phase is underrepresented in our study, partly as these patients have devices or have a significant mortality rate.

**Accumulation phase:**

Silent storage phase - starting in childhood, sub-clinical. Myocardial T1 is normal but falling. Minor architectural changes in cardiac morphology may be present.

Overt storage phase - T1 is now low and progressing faster in males than females, associated with LV mass within normal limits and associated with ECG changes.

**Myocyte hypertrophy and inflammation phase:**
**LGE and inflammation** mainly in the basal inferolateral wall, associated with persistent chronic troponin elevation but no thinning. This may occur before LVH in females (and Taiwan IVS4 subjects)(13).

**LVH**, demonstrating sex dimorphism: in females, consisting of likely balanced sphingolipid and myocyte hypertrophy in proportion; in males, consisting mainly of increasing myocyte contractile protein – true hypertrophy with the T1 fall becoming less prominent.

**Fibrosis and impairment phase:**
Persistent LVH and troponin elevation but now fibrosis (myocyte death) and thinning occur. LGE can be found extensively outside the basal inferolateral wall together with NT-proBNP elevation, LV impairment and clinical heart failure.

Our group and others have shown that ECV is normal in Fabry disease.(15,29) The difference in ECV between male and female is most likely explained by normal sex differences where ECV in FD(15) and in healthy controls is known to be higher in female than in male(15,29,30) with more change over time observed in males which could be related to higher prevalence of myocyte hypertrophy or myocardial fibrosis in males with increasing age (Figure 3). We have previously shown that LGE in established FD is chronic inflammation strongly correlating with troponin levels(4), supporting findings by PET/MR study(31) and endomyocardial biopsy(32). Here again, troponin was independently and strongly related to LGE, although we do not report T2 values in this paper.

Limitations of this study include no histological validation of the presence of storage with low native T1 in this study. The shape (linear/non-linear) of the relationship of sphingolipid to storage is unknown. There were no controls for the cohort of children although we note that T1 mapping in healthy controls in children might be comparative to healthy controls value in adults with no age effect in T1 values in the
pediatric group(30, 33). In addition, this is also a single center study with single timepoint data so our findings are hypothesis generating only based on our experiences and knowledge collaborated from all of our published works. We acknowledge that further longitudinal studies are needed and are currently being undertaken.

Conclusion

Sphingolipid accumulation potentially starts in childhood, proceeds more markedly in males than females before triggering a sex-independent scar or inflammation response but a sexually dimorphic myocyte hypertrophic response in males. Multi-timepoint longterm follow-up studies are needed to explore this further.

CLINICAL PERSPECTIVES

Competency in Medical Knowledge:

Fabry disease is an X-linked storage disease and the leading cause of death is cardiomyopathy. Storage can now be detected by measuring T1. This study proposes phases of cardiac involvement with storage apparently starting in childhood, accumulating faster in males before triggering new processes: regional scar and inflammation (male and female), and apparent myocyte hypertrophy (more in males) before advanced disease (extensive scar and LV impairment).

Translational Outlook:

Fabry disease is currently treated with enzyme replacement therapy. Treating cardiac disease will be key to improving outcomes. This paper suggests cardiac disease starts early, in childhood and has different stages and activated pathways. Additional
pathways to storage that could be therapeutic targets include myocardial inflammation and, particularly in men, myocyte hypertrophy.
References:


31. Nappi C, Altiero M, Imbriaco M et al. First experience of simultaneous PET/MRI for the early detection of cardiac involvement in patients with


Figure Legends

Figure 1: Relationship between age and native T1 in Fabry disease in a) children and adolescent up to 20 years old (r -0.78, p<0.001) and b) total cohort (r -0.41, p<0.001). The slope regression between male and female is statistically significant (p<0.001). Reference line on Y-axis showed lower limit of native T1 in male (dotted blue line, 901ms) and female (dotted green line, 916ms).
Figure 2: The relationship between native T1, LVMi and LGE in males and females in Fabry disease. A) Males: Dotted black line indicating the correlation between T1 and LV mass: negative when LVH is absent but positive when present. B) This reversal is not present in females. Reference line on Y-axis shows the lower limit of normal native T1 (901ms male; 916ms female), and on X-axis the lower limit of LVH (92g/m² male; 79g/m² female)

LGE=late gadolinium enhancement, LVH=left ventricular hypertrophy, LVMi=indexed left ventricular mass, NS=not significant
Figure 3: Cumulative onset of LVH and LGE by age group in male and female with Fabry disease. Red line indicates cumulative penetrance.

ECG=electrocardiogram, LGE=late gadolinium enhancement, LVH=left ventricular hypertrophy, NT-proBNP= N-terminal pro-brain natriuretic peptide
Figure 4: Cumulative onset of blood biomarkers (Troponin and NT-proBNP) and ECG abnormalities by age group in male and female with Fabry disease. Red line indicates cumulative penetrance.
**Figure 5:** The relationship between native T1, LVMi and ERT in males and females in Fabry disease.
**Figure 6:** Proposed myocardial phenotype evolution in Fabry disease. The disease developmental model consists of an accumulation phase (silent myocyte storage and over T1 lowering), a myocyte hypertrophy and inflammation phase, and a fibrosis and impairment (late) phase. Arrows refer to area of late gadolinium enhancement (LGE).
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<th>FD female (n=114)</th>
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<td>eGFR, ml/min/1.73m²</td>
<td>79±24</td>
<td>84±11</td>
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<td>Cardiac variant, n (%)</td>
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<td>Sokolow (SV1 + RV5 or RV6), mm</td>
<td>38±15</td>
<td>28±10</td>
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**CMR parameters**

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<td>LVEF, %</td>
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<td>LGE, n (%)</td>
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<td>ECV</td>
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**Drug history**

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<tr>
<td>ERT, n (%)</td>
<td>45 (67%)</td>
<td>47 (41%)</td>
<td>0.001*</td>
</tr>
<tr>
<td>ACE inhibitor/ARB, n (%)</td>
<td>22 (34%)</td>
<td>29 (26%)</td>
<td>0.245</td>
</tr>
<tr>
<td>Beta blocker, n (%)</td>
<td>9 (14%)</td>
<td>6 (5%)</td>
<td>0.048*</td>
</tr>
<tr>
<td>Statin, n (%)</td>
<td>10 (15%)</td>
<td>14 (12%)</td>
<td>0.573</td>
</tr>
<tr>
<td>Aspirin/clopidogrel, n (%)</td>
<td>13 (20%)</td>
<td>24 (21%)</td>
<td>0.845</td>
</tr>
</tbody>
</table>

**Table 1:** Demographics of the Fabry disease cohort.

ACE, angiotensin-converting enzyme; ARB, angiotensin-receptor blocker; BP, blood pressure; BPM, beats per minute; BSA, body surface area; ECV, extracellular volume fraction; eGFR, estimated glomerular filtration rate; ERT, enzyme replacement therapy.
therapy; FD, Fabry disease; LGE, late gadolinium enhancement; LVEDVi, left ventricular end diastolic volume indexed to BSA; LVEF, left ventricular ejection fraction; LVESVi, left ventricular end systolic volume indexed to BSA; LVH, left ventricular hypertrophy; LVMi, left ventricular mass indexed to BSA; MWT, maximum wall thickness; N/A, not applicable. Heart rate and blood pressure were taken for adults at the time of study visit. (*P<0.05 considered as statistically significant)

<table>
<thead>
<tr>
<th>Type of variant</th>
<th>Mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac</td>
<td>N215S, R301Q, I91T</td>
</tr>
</tbody>
</table>

**Supplemental Table:** Mutations of Fabry Disease patients.
**Supplemental Figure**: The relationship between native T1, LVMi and mutations in males and females in Fabry disease.