UCL INSTITUTE OF CARDIOVASCULAR SCIENCE

The biology of myocardial Fabry Disease-insights from Cardiovascular Magnetic Resonance

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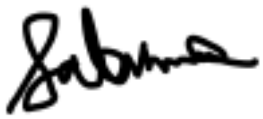
MD(Res) Thesis

UCL

2019

Declaration

I, **Sabrina Binti Nordin**, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Signature: 

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Date: 30th April 2019

Acknowledgements

I would like to thank both my supervisors, Professor James Moon and Professor Derralynn Hughes, for their mentorship and expert guidance throughout my research. I would also like to thank all the patients and staff members in all the participating Lysosomal Storage Disorder units for their participation and support. Without them this research would not have been possible. I am grateful to all the research fellows, radiographers and administrative staff who have helped me throughout my research. They have made this experience an unforgettable one. Finally, I would like to thank my family and friends, particularly my husband, Radzi, for his support and understanding.

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Abstract

Fabry disease (FD) is a rare, X-linked lysosomal storage disease leading to sphingolipid accumulation in multiple organs including the heart. Cardiovascular involvement is the leading cause of death with males affected earlier than females. Treatment is expensive and is less effective once overt cardiac involvement occurs. Ten years after the initial observation of a specific pattern of late gadolinium enhancement (LGE) in FD by our group, we observed that FD had reduced native myocardial T1 in the majority of patients with LVH and a smaller proportion in pre-hypertrophic FD.

In this thesis, I have shown that the prevalence of low native T1 in pre-hypertrophic FD could be underestimated due to 'patchy' appearance on visual colour map. Early native T1 lowering forms part of a detectable pre-hypertrophic phenotype in FD consisting of storage (low native T1), structural, functional and ECG changes. Continuing the development of new techniques for FD, I show that LGE in FD has elevated T2, typically a marker of oedema, and that this relates to blood troponin levels leading to the new hypothesis that LGE, when there is no thinning, may be inflammation, and that FD with late gadolinium enhancement (LGE) should be considered a chronic inflammatory cardiomyopathy. By assembling the largest ever FD CMR cohort, Fabry 400 with single timepoint imaging (during my thesis), I present data that is most consistent with myocyte storage starting in childhood, accumulating faster in males with two, partially independent processes: a gender independent scar/inflammation regional response (LGE) and, in

males, apparent triggered myocyte hypertrophy – a process that appears to "dilute" the T1 lowering of sphingolipid in more advanced disease. Future multi-timepoint imaging by others on Fabry 400 should confirm or refute these hypotheses.

Combining these insights, I proposed different phases of cardiac involvement by CMR: an accumulation phase; an inflammation and myocyte hypertrophy phase; and later a fibrosis and impairment phase – creating a more complex and nuanced description of myocardial FD and highlighting additional potentially therapeutic pathways beyond simply storage.

Finally, I have shown that the cardiac phenotype changes with time depending on the stage of the disease. After one year of ERT, there was a small improvement in left ventricle mass and myocardial storage measured by T1.

Impact Statement

Building on the work of others, using an exemplar, "clean" rare monogenic disease, FD, at scale and by applying new techniques I have created a richer, more complex disease model of myocardial phenotype development with new insights and new potential therapeutic targets for FD, but also with potential insights into other diseases.

Funding

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

Abbreviations

ACEi	Angiotensin converting enzyme inhibitor
AF	Atrial fibrillation
AMVL	Anterior mitral valve leaflet
ARB	Angiotensin receptor blocker
AV	Atrioventricular
BIFL	Basal inferolateral
BP	Blood pressure
BSA	Body surface area
CCS	Canadian Cardiovascular Society
cMI	Chronic myocardial infarction
CMR	Cardiovascular magnetic resonance
DCM	Dilated Cardiomyopathy
ECG	Electrocardiogram
ECV	Extracellular volume
eGFR	Estimated glomerular filtration
ERT	Enzyme replacement therapy
FD	Fabry Disease
FLASH	Fast low angle shot
Gb3	Globotriaosylceramide
GCS	Glucosylceramide synthase
GLA	α-galactosidase A
GLS	Global longitudinal strain
HCM	Hypertrophic Cardiomyopathy
HV	Healthy volunteer

ICC	Intra-class correlation coefficient
ICD	Implantable cardioverter defibrillator
IQR	Interquartile range
LGE	Late gadolinium enhancement
LV	Left ventricular
LVEDV	Left ventricular end-diastolic volume
LVEF	Left ventricular ejection fraction
LVESV	Left ventricular end systolic volume
LVH	Left ventricular hypertrophy
LVMi	Indexed left ventricular mass
LVOT	Left ventricular outflow tract
LVPM	Left ventricular papillary muscle
Lyso-Gb3	Globotriaosylspingosine
MOLLI	Modified Look-Locker Inversion recovery
MS	Milliseconds
MWT	Maximum wall thickness
NT-proBNP	N-terminal pro-brain natriuretic peptide
NSAIDS	Non-steroidal anti-inflammatory drugs
NYHA	New York Heart Association
PSIR	Phase sensitive inversion recovery
ROI	Region of interest
PET/MR	Positron emission tomography/magnetic resonance
SAPPHIRE	Saturation Pulse Prepared Heart rate independent Inversion-Recovery
SASHA	Saturation recovery Single Shot Acquisition
SCD	Sudden cardiac death

SCMR	Society (for) Cardiovascular Magnetic Resonance
SD	Standard deviation
SA	Short axis
ShMOLLI	Shortened Modified Look-Locker Inversion recovery
S1P	Sphingosine-1-phosphatase
STIR	Short tau inversion recovery
TI	Inversion time
TTE	Transthoracic echocardiography
TnT	Troponin T
XCI	X-chromosome inactivation

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Chapter 1: Introduction

This chapter is partly based on the following publications:

- **Nordin S**, Dancy L, Moon JC, Sado DM. Clinical applications of multiparametric CMR in left ventricular hypertrophy. *International Journal of Cardiovascular Imaging.* 2018 Apr;34(4):577-585.
- Baig S, Edwards NC, Kotecha D, Liu B, **Nordin S**, Kozor R, Moon JC, Geberhiwot T, Steeds RP. Ventricular arrhythmia and sudden cardiac death in Anderson-Fabry disease: A systematic review and identification of risk factors in clinical practice. *Europace*. 2018. 1;20(FI2):f153-f161.
- Baig S, Vijapurapu R, Alharbi F, **Nordin S**, Kozor R, Moon J, Bembi B, Geberhiwot T, Steeds RP. Diagnosis and Treatment of the Cardiovascular Consequences of Fabry Disease. QJM. 2018.

1.1 What is Fabry Disease

Fabry Disease (FD) is a rare, X-linked lysosomal storage disorder caused by mutations in the GLA (α-galactosidase A) gene leading to deficiency of α-galactosidase A level.(1) This results in progressive intracellular accumulation of its substrate sphingolipids, particularly globotriaosylceramide (Gb3) in lysosomes of various tissues and multiple organs including the heart, kidneys and brain.(2) More than 800 mutations have been described.(3) Both males and females have reduced life expectancy with males more limited than females (58.2 years vs. 75.5 years), particularly beyond 40 years in males and 60 in females.(4,5) Cardiovascular-related death was common in FD (6), and we show through meta-analysis that it, particularly sudden cardiac death is the leading reported cause of death in FD patients.(5,6)

1.1.1 Epidemiology

The reported incidence of overt FD has been estimated around 1:40,000 in males and 1:117,000 in the general population.(2,7) However, the incidence is likely under-reported due to presenting symptoms being non-specific. In addition, residual enzyme activity leads to slowly progressive disease resulting in the later-onset presentation, often with single organ involvement (cardiac or renal).(2) It has been reported that there is an average delay of 14 years in males and 19 years in females between onset of symptoms and diagnosis.(8) FD can be found at higher prevalences in patient groups with unexplained cardiac, cerebrovascular or renal disease: for example undiagnosed FD is found in up to 3-4% of males with hypertrophic cardiomyopathy, 1% in patient with unexplained left ventricular hypertrophy (LVH, n=508) (9); between 0.25% and 1% of males who are on haemodialysis and in approximately 5% males with acute cryptogenic stroke.(10)

In some jurisdictions, newborn screening has detected a higher incidence of GLA mutations recently whereby 1:3100 was found in Italian males and 1:1600 in Taiwan males.(11,12) However, the pathogenicity found in all mutations found in newborns is unknown. Screening in Taiwan has uncovered a large population of patients with a single GLA intronic mutation, IVS4+919>A. This results in a later onset (>40 years of age) cardiac-predominant phenotype of FD with pressing impact on older, particularly male relatives (for example parents and grandparents) rather than the newborn index case.(12,13)

1.1.2 Pathophysiology

FD is cause by the deficiency of α-galactosidase A level leading to loss of function mutation in the GLA gene located on the Xq22.1 chromosome locus. This leads to sphingolipid accumulation, particularly Gb3 in all body cells containing lysosomes including vascular endothelium and smooth muscle cells.(14) Intracellular accumulation starts in utero and is probably the pathogenic trigger event of the disease.(15,16) The mechanism linking sphingolipid accumulation and clinical manifestations is not sufficiently clear.(17) It has been shown that Gb3 accumulation increases oxidative stress by inducing excessive production of reactive oxygen species in cultured vascular endothelial cells.(18) Gb3 may also lead to increase pro-inflammatory cytokines, especially dendritic cells and monocytes.(17) Thus, it has been hypothesised that progressive storage of these molecules triggers a series of pathological process including inflammation or fibrosis, or both processes.(2) This leads to cellular and tissue dysfunction, and eventually organ failure (Figure 1).(14)

One of the additional factors in the pathogenesis of FD includes the deacylated metabolite of Gb3- globotriaosylsphingosine (lyso-Gb3), which is an α-galactosidase A inhibitor. This promotes the accumulation of Gb3 and proliferation of vascular smooth muscles.(19,20) Sphingosine-1-phosphatase (S1P), an active growth-promoting factor has also been identified in the plasma of Fabry patients and has been thought to contribute in cardiovascular

remodelling due to its proliferative mechanism.(21) In-vitro, S1P has been shown to induce cardiomyocyte hypertrophy. In vivo, left ventricular mass correlated strongly with plasma level of S1P.(14,21)

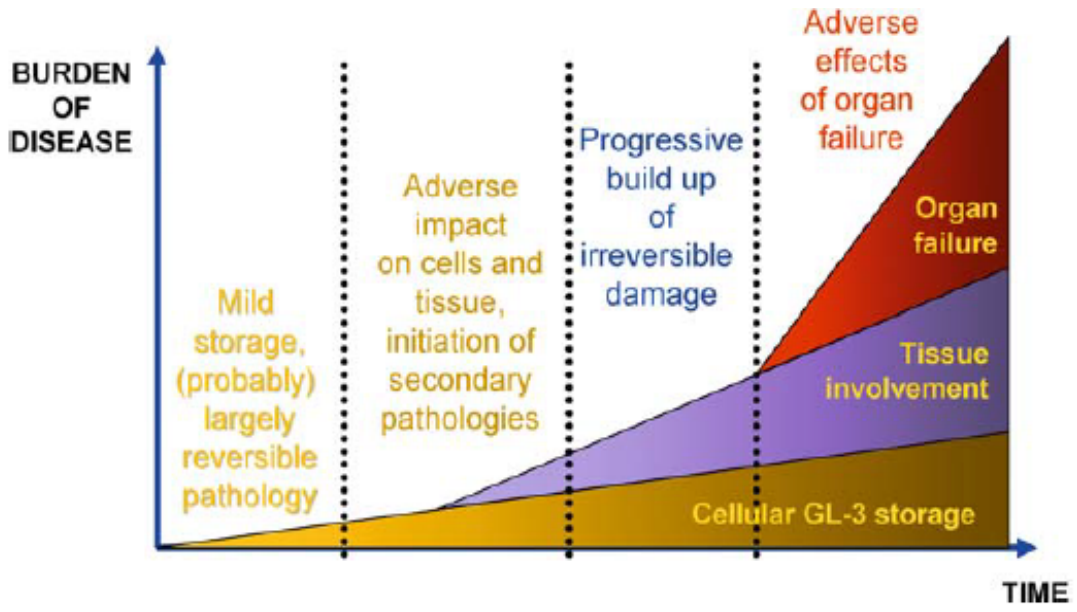

Figure 1: Schematic model of progression of FD (adapted from Eng CM et al).(8)

1.1.3 Clinical presentation

In general, two phenotypes have been described: an early onset classical form presenting in childhood and a later onset form which often affects a single organ without peripheral manifestations.(12) Although the disease process starts early with evidence of storage in the prenatal period, symptoms do not develop until early childhood typically presenting between the age of 5-10 years.(2,15) In the classical (early onset) patients, male with absent or low enzyme activity presents mainly with acroparasthesia, lack of sweating (hypohidrosis) and gastrointestinal symptoms such as irritable bowel

syndrome and diarrhoea.(4) Painful episodes may be triggered by heat intolerance and exertion. Other childhood manifestations include angiokeratoma and cornea verticillata. Angiokeratoma in characteristic regions mainly around the umbilicus, mouth and hands may occur.(22) Whorl-like bilateral corneal opacities not affecting visual acuity (cornea verticillata) can also be observed by slit-lamp examination.(22)

In a proportion of young patients, microalbuminuria may develop and is a precursor to progressive Fabry nephropathy. By the age of 20–30 years, proteinuria becomes evident and progressive, predictable decline in the glomerular filtration rate may occur.(2,7,23) By the fourth decade of life, affected men normally develop renal failure.(7) Neurological manifestations include stroke, TIA, hearing loss and white matter lesions of unknown pathogenesis.(24) Early stroke (ischaemic or haemorrhagic) has been reported in 6.9% of untreated males and 4.3% of females, occurring before the age of 30 years in one-fifth of the patients.(25) Cardiac manifestations will be discussed in detail in a later section.

One aspect to highlight – fatigue and exercise intolerance is highly prevalent and ill understood. Impaired cardiopulmonary exercise capacity in FD has been demonstrated in various studies, even without significant cardiac involvement.(26,27) There have also been variable results reported with initiation of therapy.(28,29) Possible mechanisms hypothesised were lipid deposition in the conduction system and autonomic dysfunction causing

blunted heart rate response to peak exercise in FD.(27,28) Figure 1 illustrates the common clinical manifestations of FD.

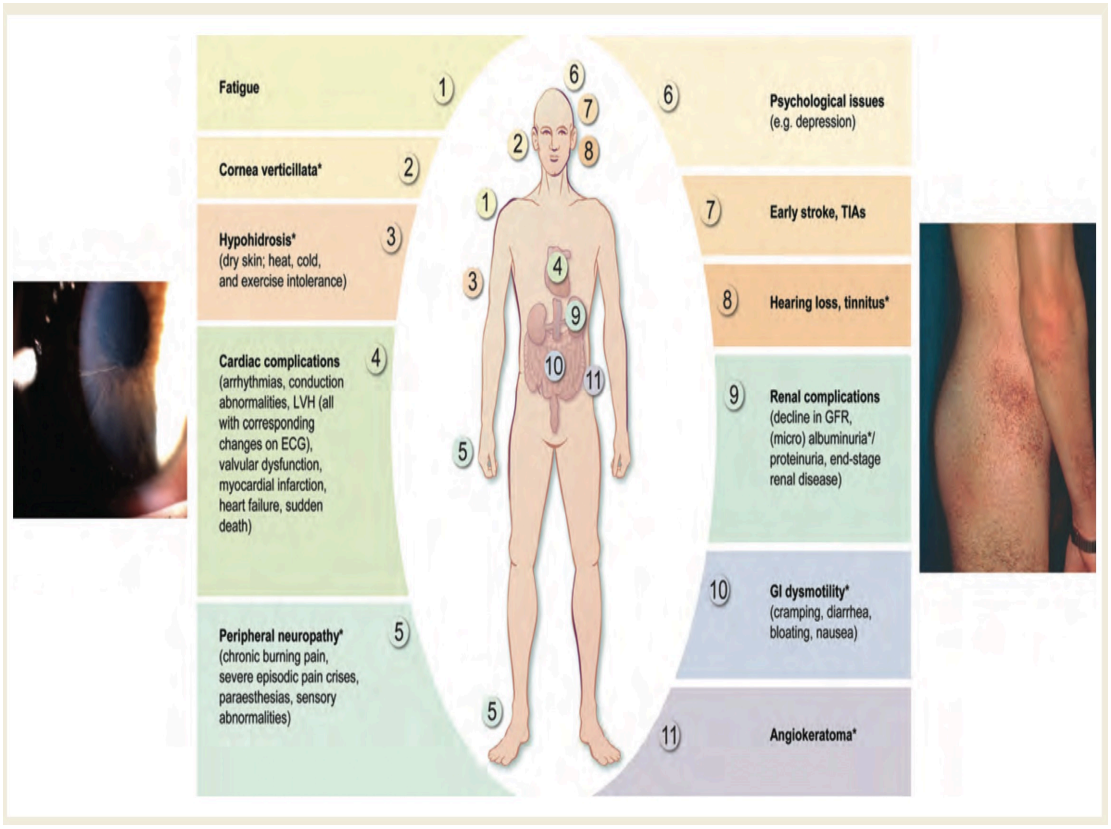

Figure 2: Common signs and symptoms of Fabry disease. Left panel image showing typical corneal verticillata and right panel image showing angiokeratoma. (*indicating onset often in childhood/adolescence). Adapted from Yousef Z et al.(7)

A group of mutations known as the 'cardiac variants' (Table 1) presents with later onset and often prominent or exclusively cardiac manifestations although cardiac involvement is also prevalent in the classical variant.(30) Individuals with cardiac variant may be more prone to develop clinically significant conduction disease.(30) However adverse cardiac outcomes are associated

with age, global disease severity and advance cardiac disease rather than presence of cardiac genetic variants.(30)

α-galactosidase mutations associated with cardiac variant		
c.644A>G, p.N215S	c.888G>A, p.M296I	c.334G>A, p.R112H
c.886A>G, p.M296V	c.58G>C, p.A20P	c.982G>A, p.G328R
c.835C>G, p.Q279E	c.5171T>C, p.I91T	IVS4+919G>A
c.902G>A, p.R301Q	c.5236T>C, p.F113L	

Table 1: Mutations associated with 'cardiac variants' in FD. Adapted from Patel V et al.(30)

Despite possessing a normal copy of the GLA gene, clinical manifestations of FD with variable severity can also occur in women. Presentation may be in part variable due to X-chromosome inactivation (XCI), which may be random or skewed.(2,31) A recent study has demonstrated relationship between clinical manifestations, enzyme activity and XCI in peripheral blood. (12,31) In females FD with random XCI, clinical features worsen with age whilst disease progression in females with skewed XCI profile is predominantly dependent on the expressed allele where early onset disease, rapid progressions with age and poorer prognosis is associated with predominant expression of the mutant GLA allele.(31) Despite most presentations in women being milder in severity with later onset usually delayed by 10 years, women may develop the

full phenotype of disease manifestations including progressive cardiomyopathy, arrhythmia and renal failure.(7) Females are now mostly considered to be equally affected but just later (with the possible exception of IVS4) - thus, women should be included in screening programmes.

1.2 Cardiac features in Fabry Disease

Cardiac involvement is frequent in Fabry patients (approximately 60% in cross-sectional surveys) and is the leading cause of death in FD.(32,33) The average age of overt cardiac disease has been reported to be 32 years in men and 40 years in women.(32,34) Cardiac manifestations in FD include LVH, arrhythmia, small vessel disease, progressive myocardial fibrosis, heart failure and sudden cardiac death (SCD).(4,14,35)

1.2.1 LVH in FD

LVH is the conventional clinical marker of cardiac involvement in FD. At a histopathological level, there are a number of processes other than myocyte size which may increase the wall thickness eg myocardial oedema. LVH can be measured and identified with transthoracic echocardiography (TTE) and cardiovascular magnetic resonance (CMR). Using either technique, LV wall thickness measuring >12mm in the Caucasian population is taken to make a diagnosis of LVH.(36) LVH in FD is by no means specific – LVH is found in many cardiac scenarios ranging from physiological hypertrophy in athletes, in response to afterload (aortic stenosis and hypertension) where it may be

initially adaptive, later maladaptive and as part of overt genetic cardiomyopathy (HCM or DCM), as part of infiltration (amyloid) storage (including FD, but also other lysosomal diseases, glycogen, mucopolysaccharides) and other range of conditions eg myocardial oedema and systemic diseases. Tables 2 and 3 show a detailed list of causes of LVH. Distinguishing the aetiology of LVH is important and although usually straightforward, there may be overlap. Non-invasive imaging plays an increasingly important role as part of investigation of LVH and specifically in FD, which will be discussed later in this thesis.

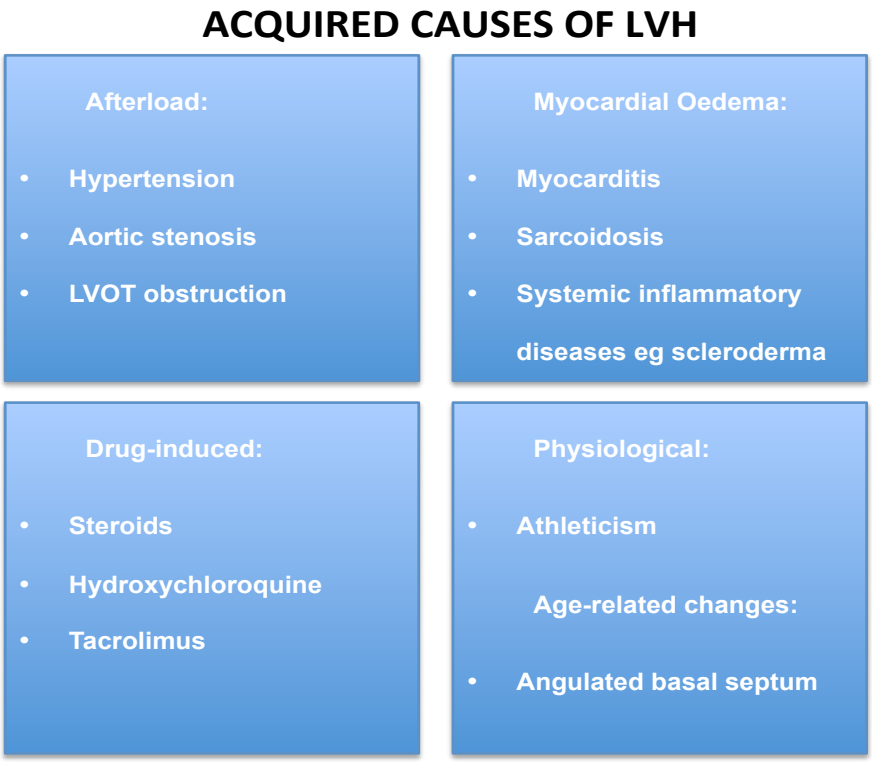

Table 2: Acquired causes of LVH. Adapted from Elliott PM et al.(36)

Table 3: Inherited causes of LVH. Adapted from Elliott PM et al.(36)

AL=amyloid light chain, ATTR=amyloidosis, transthyretin type, CFC=cardiofaciocutaneous, FHL-1=Four and a half LIM domains protein I; GSD=glycogen storage disease; LEOPARD=lentigines, ECG abnormalities, ocular hypertelorism, pulmonary stenosis, abnormal genitalia,retardation of growth, and sensorineural deafness; MELAS=mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes; MERFF=myoclonic epilepsy with ragged red fibres. Adapted from Elliott PM et al.(36)

LVH occurs in up to 50% of FD males and one third of females at a single timepoint, the prevalence and extent of LVH increase with age.(37) FD LVH is typically concentric, but in a small number of cases asymmetrical and (particularly females) apical hypertrophy can occur.(38) Sphingolipid accumulation can occur within myocytes, valves and vascular endothelium of the heart.(14,39,40) Myocyte hypertrophy and vacuolation can be observed histologically in FD as demonstrated in Figures 3 and 4.

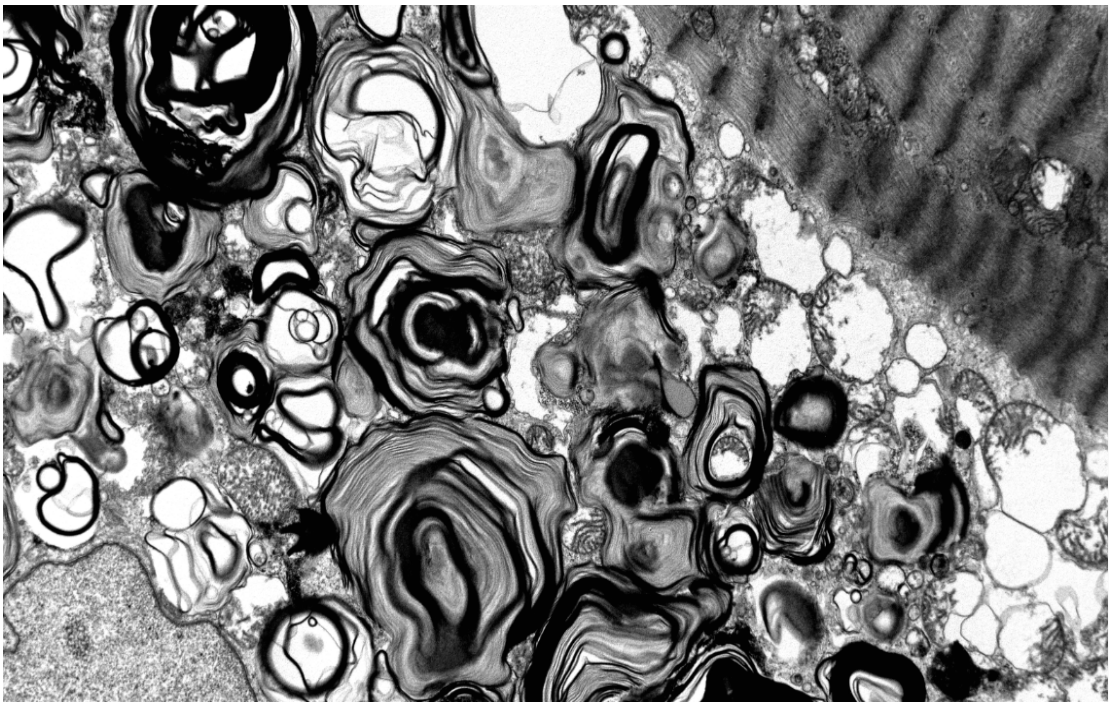

Figure 3: Electron microscopy of an endomyocardial biopsy specimen from one of our Fabry patients showing typical lysosomal inclusions “zebra bodies” within the myocyte consisting of concentric, lamellar configuration.

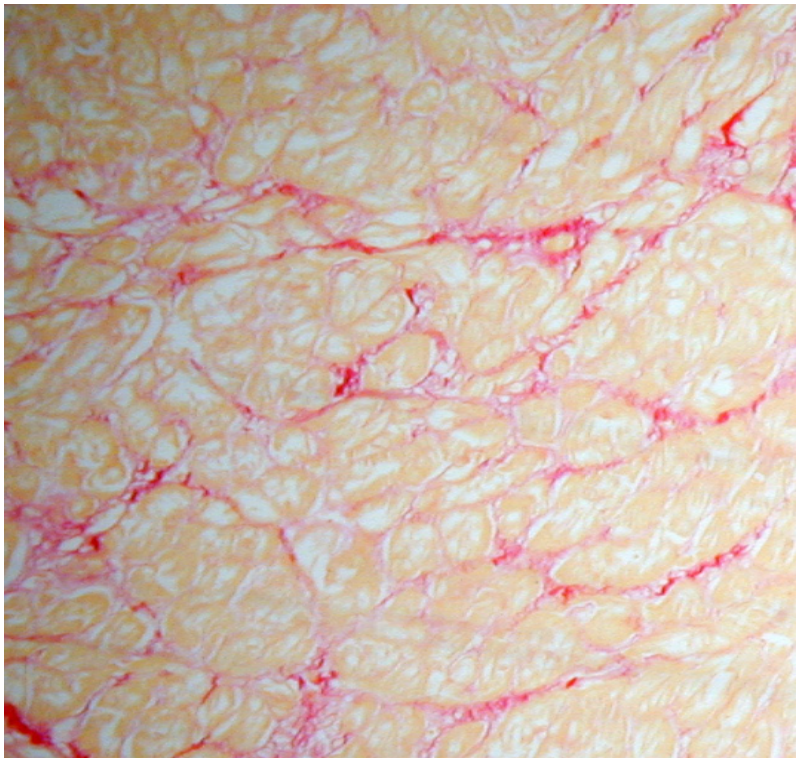

Figure 4: Histology of a myocardial biopsy specimen with Sirius red staining from a Fabry patient showing highly vacuolated cardiomyocytes. (Courtesy of James Moon)

Chemical extraction experiments have suggested that Gb3 deposition represents only a small proportion of the abnormal left ventricle and arterial wall thickening suggesting storage alone may not explain the full extent of hypertrophy found in FD.(34,41,42) Apart from Gb3, trophic and growth promoting factors such as lyso-Gb3 and S1P have been shown to participate in cardiac remodelling and triggering hypertrophic activation in FD.(19,21) Others have investigated whether Gb3 can cause oxidative stress and upregulates cell adhesion that might trigger small vessel coronary ischaemia(18) or disrupts mitochondrial energy metabolism(43,44), as

mechanisms that might induce hypertrophy. Inflammation and neuro-hormal dysregulation are also proposed stimuli to LVH manifestation in FD.(41,45) Therefore, the pathophysiology of LVH in FD remains partly obscure currently.

1.2.2 Arrhythmia and conduction abnormalities in FD

Arrhythmia has been reported to occur in 27–42% of men and 27% of women with FD.(23,32) Typical electrocardiogram (ECG) findings include P–R interval shortening without pre-excitation.(12,46) This is likely due to Gb3 accumulation around the atrioventricular node causing PR-interval shortening, therefore accelerating atrioventricular conduction rather than accessory pathways.(47-51) Over time there is evidence of P-R prolongation, increasing QRS duration, repolarization abnormalities, and atrioventricular block.(50) This is likely due to progressive deposition of Gb3 in cardiac conduction tissue causing cellular dysfunction. LVH voltage criteria ECG changes can also be observed (Figure 5).

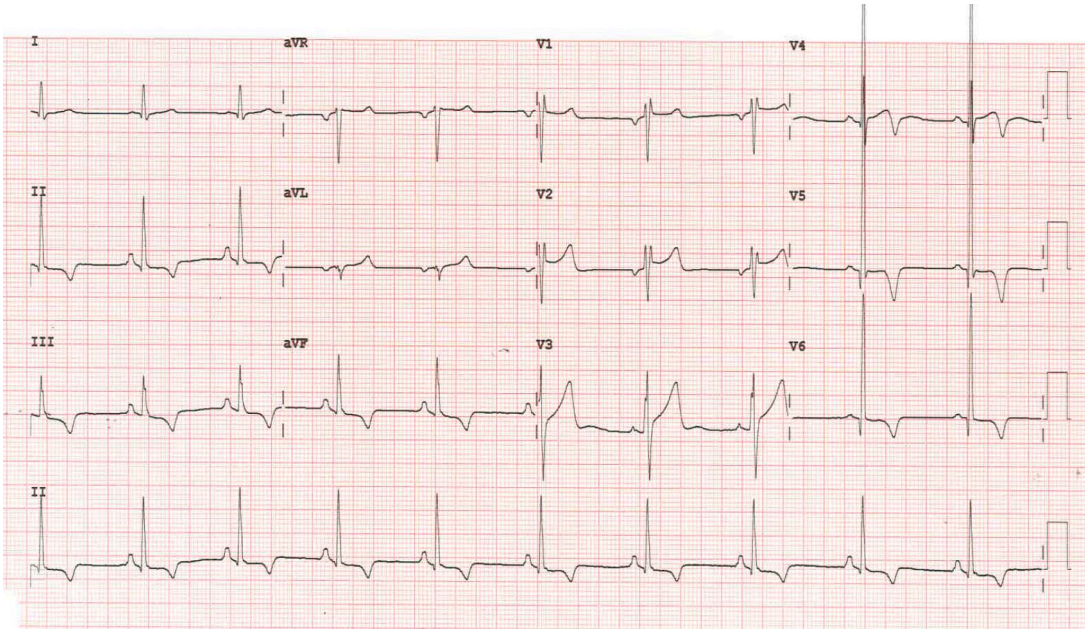

Figure 5: Typical ECG findings in a 53-year old FD male with LVH showing LVH voltage criteria and repolarization abnormalities.

Malignant ventricular arrhythmias can also occur in FD and varies widely between 5 to 30%.(52-54) A recent systematic review we conducted suggests that male gender, older age (>40 years in men), LVH, late gadolinium enhancement (LGE) on CMR and previous non-sustained ventricular tachycardia are risk factors for ventricular arrhythmias and sudden cardiac death.(55) Conventional HCM criteria for implantable cardioverter defibrillator (ICD) implantation do not apply to FD patients, and despite the lack of evidence, best practice suggests patients, particularly when LVH and/or LGE is present should be screened regularly for cardiac arrhythmias using 24-hour Holter monitoring and, potentially the implantation of loop recorders in patients with pre-syncope or syncope episodes. Given the progressive nature of the FD cardiac disease and uncertainty about therapy halting progression, a

relatively low threshold for pacemaker or ICD implantation may be warranted.

1.2.3 Other cardiac manifestations in FD

Myocardial infarction is not common in FD (2%) however involvement of coronary microcirculation can lead to angina (13-23%).(7,23,32) Chest pain with or without significant LVH has been associated with coronary microvascular dysfunction.(56) Several underlying mechanisms may contribute to the microvascular dysfunction in FD, including endothelial deposition of sphingolipids such as Gb3 as well as hypertrophy and hyperplasia of smooth muscles.(57) Other cardiac changes may also contribute to symptoms such as myocyte hypertrophy and fibrosis, which can cause raised coronary vascular resistance and increased myocardial oxygen demand.(32)

FD patients with LVH may present with left ventricular outflow tract (LVOT) obstruction. Left ventricular (LV) systolic function in these patients is usually normal or supranormal. Diastolic dysfunction may occur in FD. A small proportion developing a progressive decline in ejection fraction, eventually leading to a “burnt out” dilated cardiomyopathy-like phenotype.

Other findings include prominent papillary muscles (58-60) and valve disease.(61,62) Valves are thickened and distorted, with mainly left-sided

valve regurgitation (aortic and mitral insufficiency) observed.(2) Haemodynamic significant valve abnormalities are uncommon in FD with little clinical significance.(62) Hypertension is generally uncommon in FD however may occur and is associated with cardiovascular events in both males and females.(63) It could also be indirectly attributed to renal insufficiency and becomes more prevalent with progression of the disease.(7,64)

1.3 Diagnostic Testing for Fabry Disease

Once FD is suspected, diagnosis in males can be made by reduced or absent plasma or leucocyte α-galactosidase A enzyme activity followed by confirmatory gene testing.(65) Normal α-galactosidase A enzyme activity does not exclude FD in female heterozygotes therefore diagnosis in females require GLA mutation analysis. As Gb3 deposition starts in utero, prenatal diagnosis by demonstrating low α-galactosidase A enzyme activity and gene sequencing can also be performed from chorionic villi or culture of amniotic cells.(2) More recently, dried blood spot enzyme assay on filter paper samples has been used as a screening test for FD with the advantage that batch testing can be performed.(2,34) Samples can be stored for some weeks before being transported and analysed in a central laboratory.

It is also important to highlight that patients with suspected FD will require genetic counselling and information about possible prognosis and therapy before diagnostic testing. Confirmation of FD diagnosis may have

consequences towards family planning, insurance and employment.(12) Proactive and early identification of individuals with FD can be done using pedigree analysis (X-linked inheritance) in families of index patients with a confirmed diagnosis.(9,34)

1.4 Imaging techniques in cardiac Fabry Disease

1.4.1 Transthoracic echocardiogram

TTE has been the primary imaging investigation for detection of cardiac involvement in FD. This imaging method is widely available, cost-effective and can be generally performed on any patient. It can show the cardiac structure including the LV size, wall thickness and cardiac function. Assessment of valves and left ventricular outflow tract or intracavitary obstruction can be performed. Conventionally, clinical identification of cardiac involvement in FD primarily uses echocardiographic assessment of the maximal wall thickness (MWT) of the LV. However, this is not an early marker of cardiac involvement and there are certain limitations with TTE. This technique is operator-dependent and image quality can be challenging in certain patients. The apex of the LV and the anterolateral wall can be difficult to assess with TTE.(36) Furthermore, it is very limited in myocardial tissue characterisation.

TTE is the tool for assessing diastolic dysfunction with reduction in early myocardial relaxation velocity (e') and late diastolic relaxation velocity but

elevated early mitral filling velocity. This leads to increased E/e' ratio, a marker of high LV end-diastolic filling pressure.(66) In addition, TTE is an excellent tool in measuring flow in valve disease and assessing gradients in LV outflow tract or intracavitary obstruction. Using strain rate imaging on echocardiography tissue Doppler imaging, impaired longitudinal contraction can be observed starting in the basal inferolateral wall segments with progressive reduced regional longitudinal and radial function in more advance disease (Figure 6).(14,35,67,68)

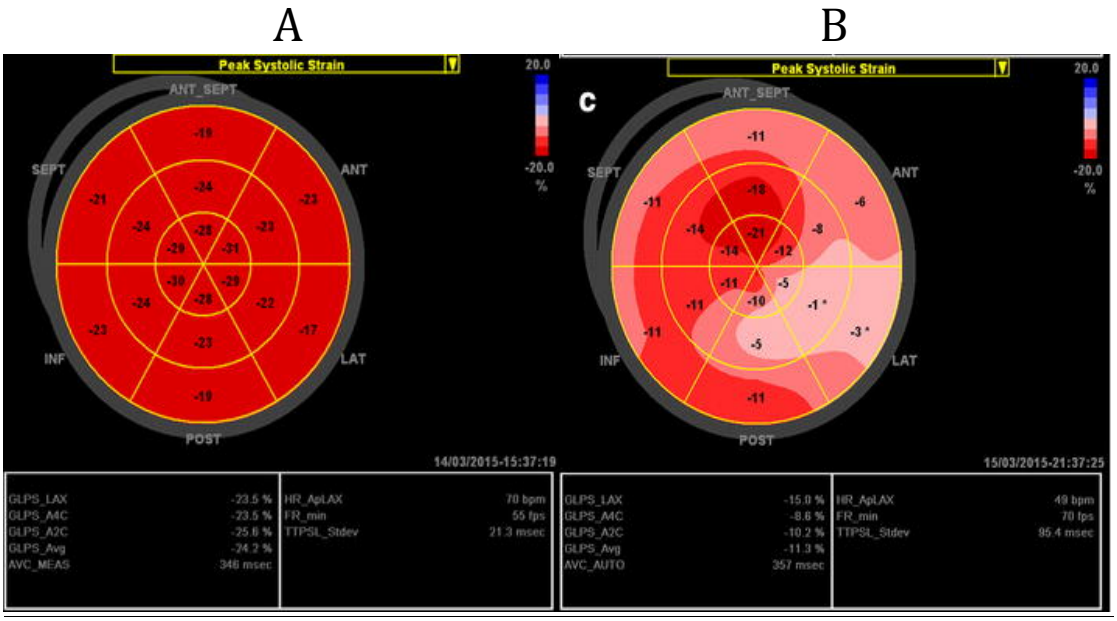

Figure 6: Examples of the longitudinal strain bull's eye plot derived from two-dimensional speckle tracking imaging in A) healthy subject B) Fabry disease. Adapted from Liu D et al.(69)

1.4.2 Cardiovascular Magnetic Resonance

CMR has emerged as a useful non-invasive technique offering precise and highly reproducible assessment of LV size, function and mass using steady state free precession cine imaging.(70) In patients with suspected hypertrophic cardiomyopathy (including FD), CMR is recommended in patients with inadequate echocardiographic windows and should be considered in suspected apical hypertrophy and aneurysm (Table 4). (36)

Recommendations	Class
It is recommended that CMR studies be performed and interpreted by teams experienced in cardiac imaging and in the evaluation of heart muscle disease.	I
In the absence of contraindications, CMR with late gadolinium enhancement (LGE) is recommended in patients with suspected HCM who have inadequate echocardiographic windows, in order to confirm the diagnosis.	I
In the absence of contraindications, CMR with LGE should be considered in patients fulfilling diagnostic criteria for HCM, to assess cardiac anatomy, ventricular function, and the presence and extent of myocardial fibrosis.	IIa
CMR with LGE imaging should be considered in patients with suspected apical hypertrophy or aneurysm.	IIa
CMR with LGE imaging should be considered in patients with suspected cardiac amyloidosis.	IIa

Table 4: European CMR guideline evaluation recommendations in hypertrophic cardiomyopathy. Adapted from Elliott PM et al.(36)

1.4.3 Late gadolinium enhancement

The LGE technique remains to be the cornerstone of tissue characterisation in CMR. Gadolinium decreases the T1 magnetic resonance time constant of any tissue in which it is present. Chelated gadolinium intravenously is able to

leave capillaries and enter the extracellular space in the body, however in healthy myocardium it cannot pass through intact cell membranes.(71) In areas where there is extracellular space expansion, there is a change in gadolinium volume of distribution and slower kinetics such that it takes longer for the agent to accumulate and lingers in this area hence shortening the T1.(71) CMR sequences which are T1 weighted can be used following gadolinium administration where an inversion recovery sequence is performed. The inversion time (TI) is set manually by the operator to produce an image in which the null “normal” myocardium appears black and the areas of extracellular space expansion are white.(71,72) If the myocardium is therefore imaged at a suitable time-point following gadolinium administration (approximately 5 minutes or more), there will be more gadolinium found in areas of extracellular space expansion (for example in areas of fibrosis, oedema or infiltration) than in surrounding “normal” myocardium.(71)

There are known limitations with the LGE technique. Firstly, it is semi-quantitative technique where either LGE is present or absent and the amount can be quantified as a percentage of the overall LV mass if LGE is deemed present.(71) However, actual extracellular volume measurement cannot be obtained using this technique. It also depends on comparing “normal” to “abnormal” areas. It is excellent in showing focal fibrosis but cannot be used to accurately assess diffuse fibrosis.(71) Furthermore, LGE demonstrates increased regional extracellular water – but does not discriminate between fibrosis, amyloid or oedema as the underlying process – and oversimplistic assumptions mistakes can and have been made (see later – LGE is chronic

inflammation in FD LGE). In addition, gadolinium is relatively contraindicated in patients with an estimated glomerular filtration (eGFR) of <30ml/min/1.73m^2 due to the risk of nephrogenic systemic fibrosis hence, excluding FD patients with severe renal involvement from receiving it. This risk was related to "linear" chelators, which bind gadolinium less strongly than cyclic agents, and has now effectively disappeared with the market withdrawal of linear agents.

1.4.4 Multiparametric mapping

The CMR mapping techniques discussed here were designed with a view to complementing the LGE technique. To date, four methods have emerged – T1, T2, T2* and extracellular volume fraction (ECV) mapping. T1, T2 and T2* are basic magnetic signal of tissue (measured in milliseconds) which may alter in certain disease states.(71)

1.4.5 T1 mapping techniques

The native T1 is the longitudinal or spin-lattice relaxation time (T1) of a given tissue without contrast administration.(73) The T1 relaxation time is determined by how quickly protons re-equilibrate their spins following excitation by a radiofrequency pulse. The initial methods of measurement of T1, as first described by Look and Locker, which was subsequently called the Look-Locker sequence required multiple breath-holds in order to acquire the recovery curve from multiple different time points after an initial excitation

pulse.(74) This method was based on regions of interest (ROI) schemes and defined manually for every frame. (74) This was then subsequently adapted as the Modified Look-Locker Inversion recovery (MOLLI) sequence enabling generation of pixel-wise “T1 mapping” where each pixel carries the measured value of the T1 of a given tissue. The original sampling method 3(3)3(3)5 enables T1 times measurement over a single breath hold over 17 successive heart beats.(75,76) This includes acquisition of 11 images in total with 3 inversions (3, 3, and 5 images acquired respectively in the beats following inversion with 3 heart beat recovery periods between inversion).(Figure 7) Further modifications of this sequence has been proposed to shorten the acquisition durations and improve accuracy and precision eg by reducing heart rate sensitivity and mitigate respiratory motion by applying motion correction.(77)

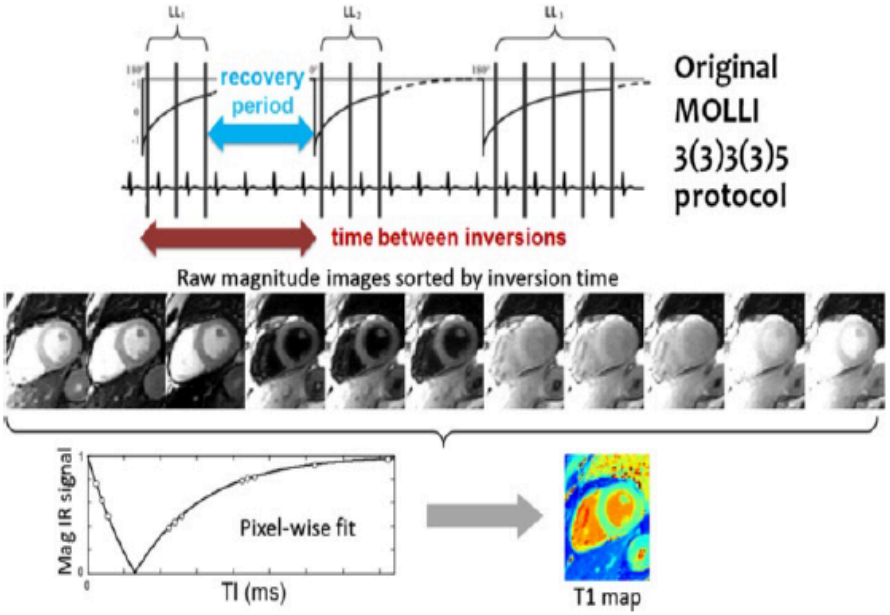

Figure 7: Modified Look-Locker Inversion Recovery (MOLLI) sequence for T1 mapping. Adapted from Kellman P et al (77).

The Shortened MOLLI (ShMOLLI) method uses sequential inversion-recovery measurements with a single breath hold of nine successive heartbeats and a conditional fitting algorithm to account for short recovery period between inversion pulses.(76) There are other sequences which use saturation recovery method such as Saturation recovery Single Shot Acquisition (SASHA) and hybrid approach eg Saturation Pulse Prepared Heart rate independent Inversion-Recovery (SAPPHIRE), which is heart rate independent and uses both saturation and inversion recovery.(78,79)

1.4.6 Other mapping techniques

T2 or spin-spin relaxation property of a tissue measures its transverse relaxation time and is dependent on water content. T2* relaxation is the combination of T2 relaxation effect with additional dephasing caused by local inhomogeneities in the applied magnetic field.(80) The ECV measures the extracellular interstitial compartment of the myocardium. It can be calculated using native and post contrast T1 myocardial and blood pool assessment together with measurement of blood haematocrit using the following equation(81):

$$ECV = (1\text{-haematocrit}) \times \frac{(1/\text{myocardial T1 post contrast} - 1/\text{myocardial T1 pre contrast})}{(1/\text{blood T1 post contrast} - 1/\text{blood T1 pre contrast})}$$

Recently “synthetic ECV” technique has emerged whereby haematocrit can be estimated from native values of blood pool T1 (blood T1 increases with anaemia) hence simplifying the assessment of ECV by precluding the need for haematocrit.(82)

All parametric mapping techniques result in an image being presented in which each pixel carries a measured value and is given a colour based upon its underlying T1, T2, T2* or ECV value respectively. This is based upon a colour look up table. Regions of interest (ROI) can be drawn on the image which will allow calculation of mean values as demonstrated in Figure 8.

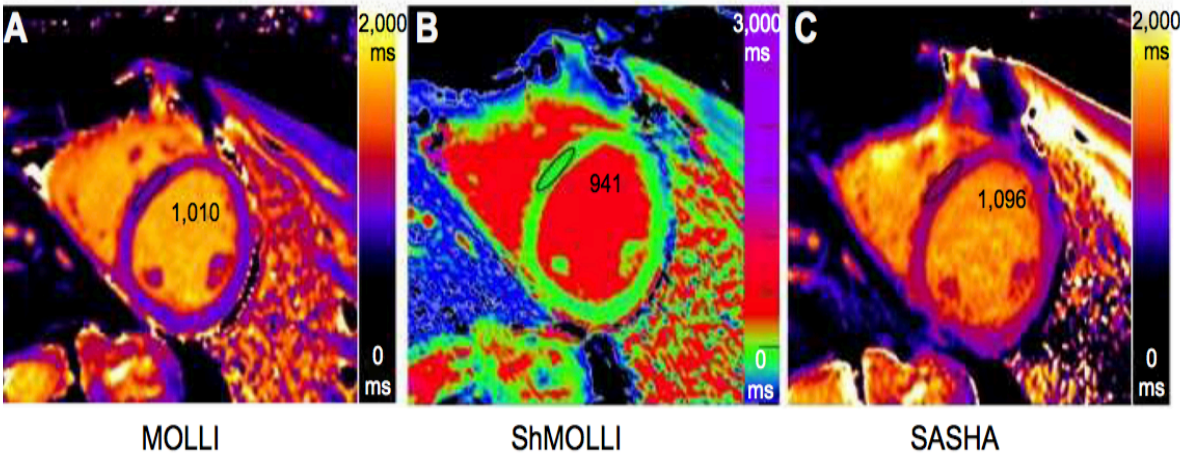

Figure 8: Native T1 maps of a healthy volunteer using three different sequences. Note the difference in the myocardial T1 values of the same healthy volunteer with each sequence and the difference in look up tables displayed on the right panel of each colour maps. A) MOLLI, modified Look-Locker inversion recovery; B) ShMOLLI, shortened modified Look-Locker

inversion recovery; C) SASHA, saturation recovery single-shot acquisition. Adapted from Abdel-Gadir A et al.(83)

1.4.7 Clinical utility of multiparametic mapping

There are a number of advantages of parametric mapping techniques. Firstly, by being fully quantitative, they permit between group comparison and within an individual over time.(71) It also allows assessment of diffuse diseases for diagnosis, early disease recognition and monitoring (with time or with therapy) provided a pathological process generates sufficient signal to noise.(71) Each of these 4 parameters (including ECV) may have different pathological sensitivities, with the potential for combination for greater discrimination. In addition, no contrast administration is required for native T1, T2 and T2* mapping.

Currently, T2* is used clinically for assessment of cardiac and liver iron. T2 is increased in areas of oedema such as in acute myocarditis or in connective tissue diseases such as systemic sclerosis, systemic lupus and rheumatoid arthritis. This oedema is typically assumed to represent inflammation, particularly if there is myocyte death and blood troponin elevation.(84) Native T1 is typically increased slightly by myocardial fibrosis, and more by myocardial oedema and infiltrative disease such as cardiac amyloidosis. Native T1 is low in areas where fat or iron are present. Apart from amyloid and oedema, ECV can be increased due to excessive collagen deposition.

ECV has been shown to be a robust marker of diffuse fibrosis and has been shown to be prognostic in certain cohorts eg cardiac amyloidosis.(85,86)

Practically, normal myocardial T1 values vary particularly between different sequences and different field strengths (T1 value is higher with higher field strengths) so it is essential that any centre performing these techniques has its own normal healthy volunteer ranges.(73,87) In addition, to ensure no drift over time, phantom quality control is recommended in the interval between setting reference ranges and patient scanning.

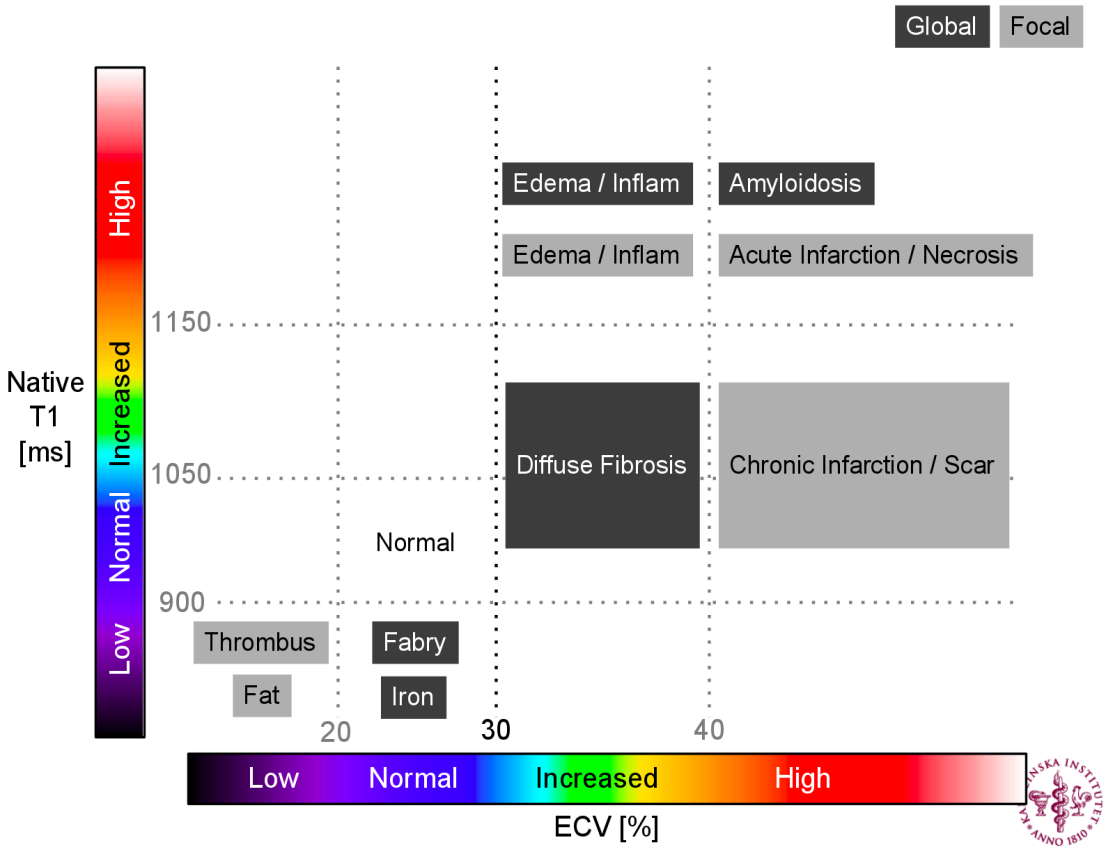

Figure 9: Tissue characterisation of different diseases using native T1 and ECV as presented by Martin Ugander at SCMR conference 2014.

1.5 CMR in Fabry Disease

In FD, CMR adds real value by its ability to interrogate tissue characterisation. Recently, CMR has been shown to be an important tool in aiding diagnosis in FD with promising potential in early detection of cardiac involvement.(88,89)

1.5.1 LGE in FD

LGE, first described in 2003 in FD, has been shown by histological correlation at autopsy, initially in one case, to represent focal fibrosis in FD and is detectable in approximately 50% of patients.(39,90) The LGE is characteristic and typically observed in the basal inferolateral midwall as demonstrated in Figure 10. Other diseases also typically impact this area (eg dystrophies, myocarditis). Later in the disease, the LGE pattern can also be extensive. There is also a reported positive correlation in males between left ventricular mass index and the extent of myocardial enhancement suggesting that LVH my in some way lead to fibrosis, initially in the basal inferolateral wall.(39) However this linear order may not occur in females – 17% of females without LVH have been reported to have LGE in FD.(91) More recently, LGE was also found in a small proportion of LVH-negative males with IVS4+919>A, a late onset cardiac variant mutation highly prevalent in Taiwan.(91)

The presence of progressive fibrosis is an important prognostic determinant for malignant ventricular arrhythmias and cardiac outcome in FD.(55,92) Previous study have shown less impact on the heart when ERT is started

after fibrosis is present, and this has been taken as evidence that ERT is best started before myocardial fibrosis has developed to achieve long-term improvement in myocardial morphology and function and exercise capacity.(68)

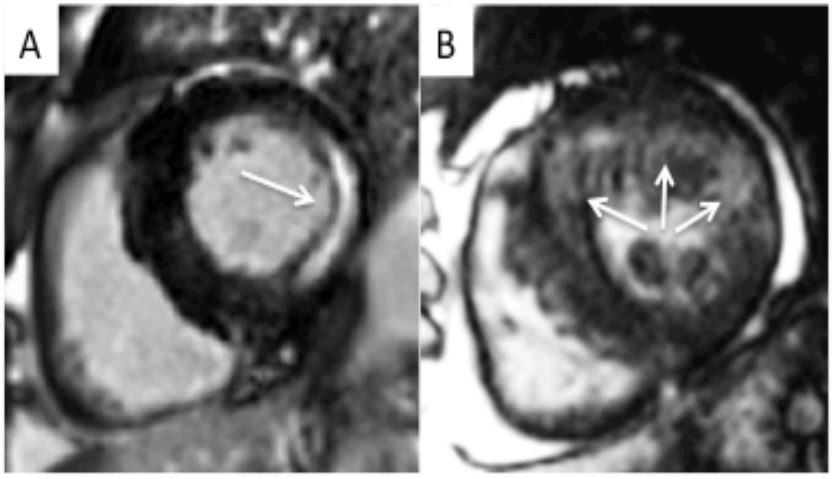

Figure 10: Late gadolinium enhancement in Fabry disease. Typical basal inferolateral LGE as shown in (A) however this can also be extensive in advanced disease as shown by arrows in (B)

1.5.2 Native T1 and ECV in FD

The development of T1 mapping promises improved tissue characterisation without the need for contrast agents, complementing the LGE technique. The CMR LGE technique can only demonstrate areas of fibrosis in FD. A method to directly assess the amount of intracellular glycosphingolipid would provide a number of benefits in this disease, for example earlier diagnosis and the opportunity to monitor and tailor treatment. Since fat is known to decrease

native T1, two separate groups investigated the use of T1 mapping in FD.(89,93) Patients with LVH were found to have a low T1 (Figure 11). However, in areas where the LGE is present the native T1 is either “pseudo” normal or elevated.(89) In patients with LVH, T1 in FD appears to be discriminatory of other diseases causing LVH such as aortic stenosis, hypertension, hypertrophic cardiomyopathy and amyloidosis, with no apparent overlap. (89) Technically, the lowering of native T1 may not be solely the fat content of myocardium, but may be related to the ultrastructure – with the trapping/alteration of water behaviour when trapped between the layers of the lamellar bodies providing the T1 lowering signal, in a manner analogous to the T1 signal differences between “grey” and “white” matter of the brain (where there is glial cell insulation of axons that evoke lamellar bodies.

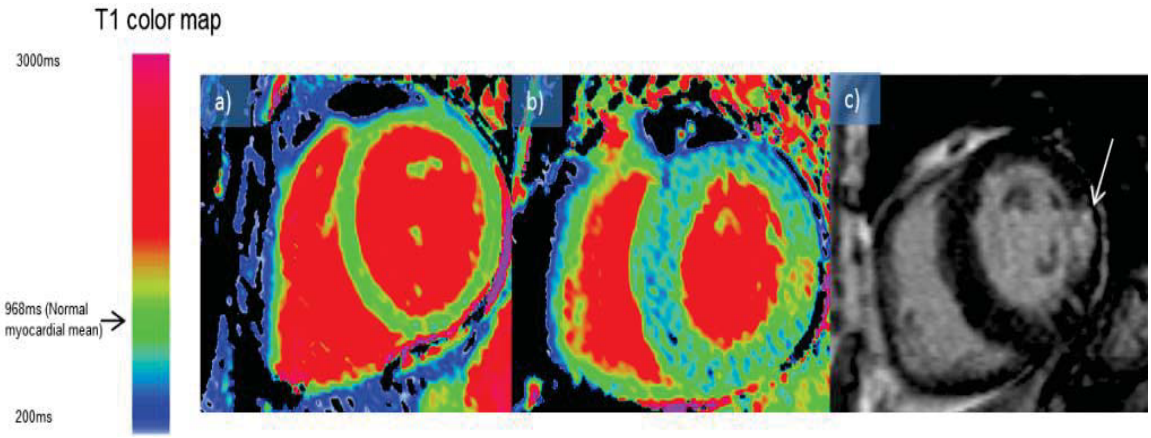

Figure 11: Native T1 map from A) a healthy volunteer and B) a Fabry disease patient. Blue areas (low T1) are seen in the FD septum and red (high T1) in the inferolateral wall corresponding with the arrow in LGE area in the same patient as demonstrated in (C). Adapted from Sado DM et al.(89)

Approximately 40-50% of patients without LVH were also found to have low T1 values, suggesting that native T1 is an early disease marker in FD – or at least a marker of storage that occurs early (although reasonable, it is an assumption that all storage represents “disease”).(88,89) LVH-negative FD with low T1 was associated with subtle diastolic dysfunction.(88) One small study performed ^{1}H NMR spectroscopy and native T1 on some FD patients showing correlation between the presence of fat signal with low native myocardial T1.(93) These data together suggests the potential of native myocardial T1 being an early marker of cardiac involvement in FD than any of the current clinical markers in this disease with further work needed to address the question as to whether it should guide the initiation of therapy. The reproducibility of native myocardial T1 with inter-study, intra-observer and inter-observer variability has been studied using the intra-class correlation coefficient (ICC). This found high level of reproducibility: ICCs of 0.99, 0.98, 0.97 for ShMOLLI T1 mapping and 0.98, 0.98, 0.98 for MOLLI T1 mapping.(88). A few studies have demonstrated that ECV in FD to be similar to ECV in healthy controls and is higher in females compared to males, findings which are also found in healthy controls.(93,94) Currently most FD studies are single centre and magnet standardization for mapping biomarkers is currently being explored for example the T1MES program has created a T1 mapping phantom for scanning in centres worldwide.(95) In this thesis, we will explore the utilisation of T1 mapping in FD on early detection of cardiac involvement in FD by CMR.

1.5.3 T2 in FD

The use of T2 mapping in FD has been previously unexplored. T2 map is used to detect inflammation or oedema. A previous study has found a prolonged T2 relaxation time in FD compared to other diseases with LVH and controls.(96) Another study of 13 FD patients using hybrid positron emission tomography/magnetic resonance (PET/MR) imaging has shown that patients with LGE and positive short tau inversion recovery (STIR) MR images showed focal FDG uptake in the corresponding myocardial segments hinting possible inflammation in FD.(97) These studies included small samples of FD patients. In this thesis, we will explore the role and importance of T2 map in FD.

1.6 Blood biomarkers in FD

Gb3 and lyso-Gb3 accumulate due to the enzymatic defects and may be used as diagnostic tools or as screening parameter for high-risk patients.(14,98-100) Gb3 concentration can be measured in plasma and urine and are increased in FD males with classic variant. However these biomarkers are not completely reliable to monitor therapeutic effects and does not detect patients with cardiac variant.(14,99,101) Plasma and urinary Gb3 is also not helpful in identifying disease severity and clinical manifestations in FD.(102) Lyso-Gb3, a degradation product of Gb3 accumulation is an important biomarker. The plasma concentration is severely elevated in all classical variant patients and slightly elevated in late onset mutations.(14) The level of plasma lyso-Gb3 can also potentially be an additional useful tool for the confirmation of FD in

individuals with *GLA* mutation of unknown significance and is closely related to disease severity.(102,103)

Cardiac troponin is a well-known parameter reflecting cardiac muscular damage.(104) Cardiac troponin I has been observed to be persistently elevated in approximately 20% of FD patients and is associated with LGE.(105) More recently, a prospective study of 75 FD patients has shown a strong positive correlation between high sensitive cardiac troponin T, LVH and the amount of LGE.(106) In the same study, retrospective longitudinal data of 58 patients showed decreased LV wall thickness and LVEF (although the latter is not statistically significant) over time in patients with elevated troponin levels indicating potential cardiomyopathy progression.(106) Brain natriuretic peptide and the N-terminal fragment of its pro-hormone (NT-proBNP) are established biomarkers in the diagnosis and prognosis of heart failure. NT-proBNP has been found to correlate with symptom class, echocardiographic markers of diastolic dysfunction (107) and with severity of FD cardiomyopathy.(108) Elevated, age-corrected NT-proBNP has also been found in patients without echocardiographic evidence of LVH, which is thought to be potentially useful in detecting early cardiac involvement.(107)

1.7 Management of FD

1.7.1 Fabry-specific treatment

In the last 15 years, enzyme replacement therapy (agalsidase alfa and

agalsidase beta) has been the mainstay treatment of FD. This is administered intravenously every two weeks and is very costly. Early work in determining efficacy of ERT in FD has been based on Gb3 clearance demonstrated on biopsy, decreased severity of Fabry-related pain and improvement in quality of life.(40,109) Initial results in a prospective, observational study of 16 FD patients reported a significant reduction in myocardial mass after 12 months of treatment with ERT (agalsidase beta), $p<0.05$ with improved regional mycocardial function using strain rate imaging.(110) There is currently no sufficient evidence that ERT is able to reverse fibrosis. ERT is currently indicated in all classically affected patients with symptoms as well as in patients with earliest signs of organ involvement. Table 5 shows the current European consensus recommendations criteria for ERT initiation for cardiac involvement.(111)

	Cardiac involvement*
All FD males and females	: cardiac hypertrophy (MWT>12mm) without (or only minimal signs of) fibrosis (Class 1) : signs of cardiac rhythm disturbances^{+} (Class 1)

Table 5: European consensus criteria for initiation of ERT in cardiac involvement.*consistent with FD, not fully explained by other pathology; $^{+}$sinus bradycardia, atrial fibrillation, repolarization changes. Adapted from Biegstraaten M et al.(111)

There has been increasing evidence that early treatment is crucial in management of FD. Presence of overt LVH and myocardial fibrosis have been shown to affect ERT outcome suggesting early treatment is important to achieve long-term improvement in myocardial morphology and function as well as exercise capacity.(68,112) Longer-term clinical endpoints in FD patients have now been reported and have shown benefits of early initiation of treatment in FD. A recent study of 10-year outcome of 52 classic FD patients from the original phase 3 clinical trials that received agalsidase beta has been reported.(113) 81% of patients did not report any severe clinical events (defined as chronic dialysis, kidney transplant, myocardial infarction, congestive cardiac failure, major cardiac procedures, stroke and death) and 94% were alive at the end of the study at 10 years. Patients who were classified as low renal involvement (n=32) started therapy 13 years younger than patients with high renal involvement (n=20). The mean interventricular septum and left posterior wall thickness were unchanged suggesting stabilisation over time. Patients who started treatment at age ≥40 years mainly have significant increase in LV wall thickness indicating the most favourable treatment responses were in younger patients with less organ damage and in patients who commenced treatment at a younger age.(12,113)

A 10-year single centre, retrospective analysis of 45 adult patients receiving agalsidase alfa showed an improvement in New York Heart Association (NYHA) heart failure classification in at least 1 class in 52% and stable or

improvement in Canadian Cardiovascular Society (CCS) angina scores in 98%.(12,114) Indexed left ventricular mass (LVMi) in the normal range were maintained in both males and females. Where LVH was present prior to ERT, a reduction in LVMi were apparent after 1 year, with benefits maintained for 10 years in males however this is not maintained in females.(114)

1.7.2 Novel and emerging therapies

The newly available oral pharmacologic chaperone stabilizes certain mutant α-galactosidase enzymes by binding reversibly to it hence facilitating better enzyme trafficking into the lysosomes.(115) This treatment is currently indicated for patients over 16 years of age (by European Medicines Agency; over 18 by FDA) with amenable mutations and is administered orally every other day. It is contraindicated in patients with renal impairment with eGFR <30ml/min, during pregnancy and breast-feeding. The availability of this drug may address certain unmet needs for FD patients receiving treatment for example antibody formation to enzyme replacement therapy leading to infusion-associated reaction and interfering with efficacy of treatment as well as providing convenience to patients as regular intravenous infusions is not required. However monitoring of treatment compliance may be an issue. Results from the initial phase 3 study has shown that treatment with oral chaperone for up to 24 months has shown a significant reduction in LVMi in particular in patients with LVH at baseline.(115) When compared to ERT at 18 months, LVMi decreased significantly with oral chaperone (-6.6 g/m^2 (-11.0 to

-2.2)) with no significant change with ERT.(116) These are promising results however they need to be confirmed by larger cohort studies.

Another oral therapy that is currently in early clinical development is substrate reduction therapy. The method of action is by inhibition of glucosylceramide synthase (GCS) and aims to complement enzyme replacement therapy. Fabry mice treated with GCS inihibitor (Genz-682452) resulted in reduced tissue substrate and a delayed loss of thermosensory responses.(12,117) The effects were most notable in younger mice before development of overt pathology however this product currently remains in the investigational phases.(117)

Gene therapy is another promising therapeutic strategy. This first human clinical trial, FACT study (NCT02800070) is currently being conducted and uses autologous stem cell transplantation with CD34+ cells that are transduced with the lentivirus vector containing human α-galactosidase A gene.

1.7.3 Concomitant cardiac therapy

Apart from Fabry-specific treatments such as ERT or oral chaperone therapy, additional treatment may be required in patients with advanced cardiomyopathy to manage cardiac symptoms such as heart failure and

brady- or tachyarrhythmias. Management of heart failure and arrhythmias (including device therapies) should follow standard guidelines.(36,118) Anticoagulation should be commenced in FD patients with history of atrial fibrillation (AF) regardless of the CHA_2DS_2VASc score, because the score underestimates thromboembolic risk in FD.(36,118) Lifelong anticoagulation is recommended in those with AF even when sinus rhythm has been achieved due to the high incidence and risk of stroke.(66) Fabry cardiomyopathy is excluded from the HCM risk-SCD model(119) and there is currently no clear risk stratification model of SCD in FD to guide ICD implantation.(55) Consequently cardiac devices implantation usually occurs on a secondary prevention basis. Cardiac transplantation is potentially an option for patients who remain symptomatic (NYHA IV) despite optimal medical therapy (including implantation of cardiac resynchronization therapy, if appropriate). However this is usually limited by the presence of concomitant renal impairment. FD does not appear to develop in the allograft presumably due to presence of residual enzyme activity in the donor organ.(66)

Other drugs used in the disease may have cardiac impact, for example renin-angiotensin system blockade for the kidneys and hypertension, aspirin or clopidogrel for cerebrovascular disease, statins, potentially vitamin D replacement (many FD patients are head intolerant so do not expose themselves to sunlight), and, adversely, potentially non-steroidal anti-inflammatory drugs (NSAIDs) for acroparasthesia

Chapter 2: Research Aims

Fabry Disease remains a diagnostic challenge and there is still a paucity of knowledge about Fabry cardiomyopathy. There remains great scope to refine our understanding of FD cardiac pathophysiology. This includes early detection of storage in the myocardium, but also the downstream adaptive and maladaptive consequences of this storage. Together such markers could help time and monitor current therapies, but also highlight new areas for therapeutic manipulation. For storage, as described in the introduction chapter, initial results from my predecessor Dr Daniel Sado in the use of T1 mapping in FD have been promising(89), leading to the conception of the Fabry400 study.

Fabry400 (NCT03199001) is an international multicenter study aiming to understand the biology of Fabry Disease and its relationship to non-invasive T1 mapping by CMR. Four centres (United Kingdom: 2 centres in London-Chief Investigator Professor James Moon and Birmingham-Principal Investigator (PI) Dr Richard Steeds; Australia: Sydney-PI Dr Rebecca Kozor) formed the nucleus of this project. The target recruitment for the project is to scan a total of 400 FD patients. I was the lead research fellow for this project and the majority of my research time was dedicated to this work. The data originating from this is the basis for my thesis and multiple submitted papers.

The main aim of this thesis is to explore and develop the use of novel multiparametric CMR to provide insights into the disease biology of FD by assembling the world's largest CMR cohort of FD patients within the study Fabry400.

2.1 Early detection of cardiac involvement in FD

Background: 40-50% LVH-negative patients has been shown to have low T1 indicating possibility of visualizing storage earlier prior to LVH (88,89). ECG changes and blood biomarkers eg NT-proBNP has also been shown to precede LVH (47,107). In this thesis, the potential role of T1 mapping with technique refinement in early detection of cardiac involvement is further explored.

Hypothesis: Storage (low native T1) in LVH-negative FD can be associated with structural and functional abnormalities, ECG changes and blood biomarkers

2.2 Inflammation in FD

Background: Troponin has been found to be elevated in FD especially in patients with LGE and LVH (105,106). A small study using PET/MR has shown that FD is possibly inflammation (97). The link between the intramyocardial storage of sphingolipids in FD and extracellular scarring is poorly understood. T2 mapping in FD has not been explored before. Multiparametric mapping using native T1 (to assess storage), T2 (to assess

inflammation) and blood biomarkers are used in this thesis to establish whether LGE in FD is inflammation.

Hypothesis: Inflammation is present in FD with LGE, measurable using T2 mapping and troponin.

2.3 Age and sex differences in FD

Background: Males develop cardiac involvement earlier than females and are generally more severely affected. There is currently a paucity of knowledge of the age and sex differences of the Fabry myocardium in relation to storage and inflammation or fibrosis.

Hypothesis: There is an age and sex differential response in the Fabry myocardium in relation to storage (assessed by native T1), hypertrophy and inflammation (assessed by T2, LGE and blood biomarkers) response by multiparametric mapping.

2.4 Longitudinal assessment of FD

Background: The effect of ERT on myocardial storage, inflammation and the cardiac phenotype in FD has not been previously assessed longitudinally using CMR (a non-invasive modality).

Hypothesis: After one year of ERT, there is improvement in LVMi and reduction in myocardial storage (assessed by native T1). In the ERT-naïve group, there is increase in LVMi with reduction in myocardial storage. In the ERT-established group, there is no change in LVMi and myocardial storage.

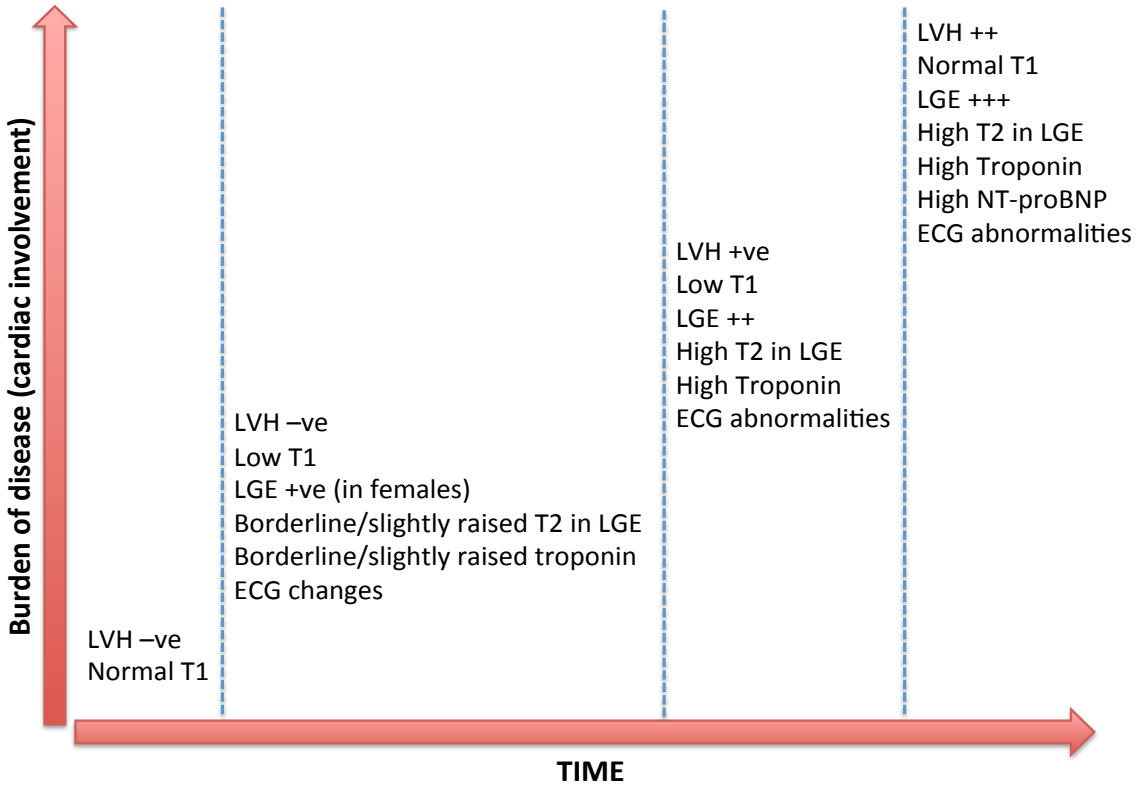

Figure 12: Hypothesised Fabry Cardiomyopathy model using multiparametric mapping and blood biomarkers.

Chapter 3: General Methodology

3.1 Ethical approval

Ethical approval was obtained from the United Kingdom (UK) National Research Service (14/LO/1948) for the UK (London and Birmingham) Fabry cohort and Northern Sydney Local Health District Ethics Committee for Australia Fabry cohort as part of the multi-centre Fabry400 study. Dr Rebecca Kozor (Australia Principal Investigator), obtained ethical approval for the Fabry cohort in Australia. All patients were prospectively recruited and provided informed written consent.

Healthy volunteers and patients with other cardiac diseases included in this thesis were recruited from on-going studies (05/Q0502/102 for chronic myocardial infarction cohort and 07/H0715/101 for healthy volunteers and hypertrophic cardiomyopathy cohorts).

3.2 Study population

3.2.1 Fabry disease

FD participants were recruited from Fabry clinics (UK): Royal Free Hospital London, National Hospital for Neurology and Neurosurgery London, Queen Elizabeth Hospital Birmingham; Australia: Westmead Hospital Sydney). Inclusion criteria were gene-positive FD males and females. Study exclusion criteria were pregnancy and standard contraindications to CMR (e.g claustrophobia and unsafe implanted ferromagnetic materials).

3.2.2 Healthy volunteers

HV were recruited from an ongoing study of 94 healthy volunteers (age range 20-76 years) with no history of cardiovascular disease (normal health questionnaire and on no cardioactive medication unless for primary prevention). All volunteers had normal CMR scans as reported by Level 3 CMR physicians. ECGs were performed and blood samples were collected for kidney function and haematocrit for ECV quantification. Healthy volunteers were used in Chapter 4 to compare the CMR and ECG parameters between the LVH-negative FD and healthy controls and Chapter 5 as a comparator cohort to establish normal native T1 and T2 reference ranges.

3.2.3 Other disease cohorts

HCM and chronic myocardial infarction (cMI) participants were used in Chapter 5 as a comparator cohort for inflammation in FD. This will be described in more detail in Chapter 5.

3.3: CMR imaging

Only the routine CMR sequences performed in this thesis will be described in this section. All participants were scanned on a 1.5 Tesla scanner: Avanto, Siemens medical solutions for London (The Heart Hospital, University College London Hospitals Trust) and Birmingham (Queen Elizabeth Hospital) and Aera, Siemens medical solutions for Sydney, Australia (Royal North Shore Hospital) using a 32-channel phased-array cardiac coil. Breath-hold images

were always acquired at end-expiration. The CMR protocol used in this thesis is demonstrated in Figure 13.

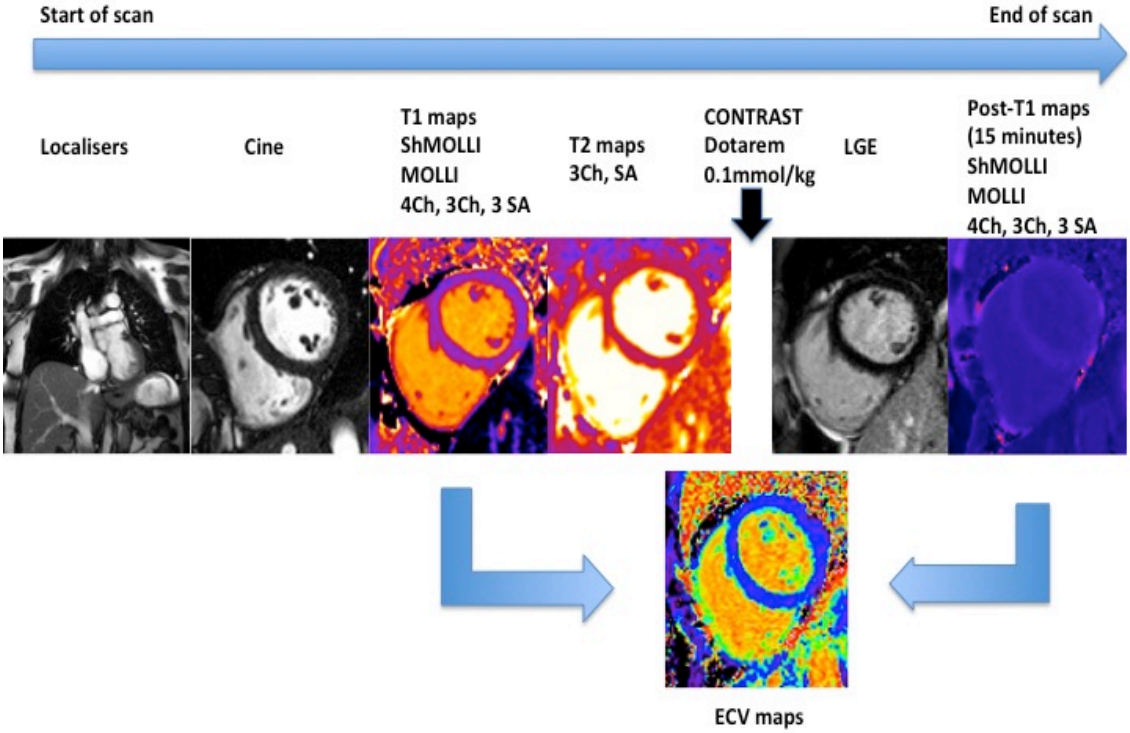

Figure 13: CMR protocol used in this thesis

3.3.1 Cine images

Steady state free precession cine imaging was performed in the long axis planes then short axis (SA) for structural and function assessment. For the short axis stack, a slice thickness of 7mm with a 3mm gap was performed. Retrospective ECG gating was used with 25 phases however prospective ECG gating was used if the patient had an arrhythmia if best cine imaging is not obtained despite optimising parameters on retrospective gating.

3.3.2 Late gadolinium enhancement

Gadolinium (Dotarem, Guerbet S.A., France) was administered intravenously (0.1mmol/kg) as a bolus for LGE assessment. In this thesis, gadolinium is not administered when the eGFR<30ml/min/1.73m^2, in children or if patient declines gadolinium. LGE images was acquired in the standard long axis and short axis stack using either fast low angle shot (FLASH) sequence with phase sensitive inversion recovery (PSIR) (120) or a respiratory motion-corrected, free-breathing single shot SSFP averaged PSIR sequence (121,122) at 5-15 minutes after gadolinium injection.

3.3.3.Multiparametric mapping

3.3.3.1 Native T1 mapping

T1 maps were acquired pre-contrast using either ShMOLLI, or MOLLI with a 5s(3s)3s sampling protocol.(77,123) Motion correction and a non-linear least square curve fitting were performed with the set of images acquired at different inversion times to generate a pixel-wise coloured T1 map, which was displayed using a colour map.

MOLLI with 5s(3s)3s sampling protocol uses time in seconds rather than heartbeats for sampling (2 inversions with images acquired for at least 5 seconds with subsequent recovery of at least 3 seconds and followed by a second inversion with acquisition of images for at least 3 seconds).(77) A fixed minimum time period for acquisition and recovery helps gain

independence of heart rate and improves precision at higher heart rates as the recovery curve remains well sampled across its range.(77) Each slice required one breath hold.

The acquisition parameters were: pixel bandwidth 977 Hz/pixel; echo time = 1.1 ms; flip angle = 35°; matrix= 256x144; slice thickness 6mm. Motion correction and a non-linear least-square curve fitting were performed with the set of images acquired at different inversion times to generate a pixel-wise color T1 map displayed using a customized lookup table.

3.3.3.2 T2 mapping

T2 maps were acquired pre-contrast in one breath-hold per slice (Work In Progress package 448B, Siemens Healthcare). This consisted of 3 single-shot images at different T2 preparation times (0ms, 24ms and 55ms) with the following parameters: pixel bandwidth 930 Hz/pixel; echo time = 1.1 ms; repetition time = 4 x R-R interval (3 recovery heart beats); flip angle =70°; acquisition matrix = 116x192; slice thickness = 6mm. Following motion correction and fitting, a coloured T2 map consisting of pixel-wise T2 values was generated.(124)

3.3.3.3 Post-contrast T1 map and ECV

Post-contrast T1 mapping was acquired 15 minutes after gadolinium administration using either ShMOLLI or MOLLI with 4s(1s)3s(1s)2s sampling protocol to improve accuracy of T1s in the 200-600ms range.(77) Similar

acquisition parameters as for native T1 maps were used. Using an off-line software (ECV Mapping Tool Version 1.1), pixel-wise ECV maps were generated from co-registration of the native and post-contrast T1 pixel maps together with the patient's haematocrit taken at time of the scan.(125)

3.4 Imaging analysis

3.4.1 Cines

All volume analysis was performed using CVI42 software (Circle Cardiovascular Imaging Inc., Calgary, Canada). Semi-automated contours were drawn on the short-axis cine images using the threshold segmentation option for the endocardial border and manual tracing for the epicardial border. The left ventricular end-diastolic volume (LVEDV), left ventricular end systolic volume (LVESV), LV mass (LVM) and LVEF were quantified using rounded endocardial contours and included papillary muscles as part of the LVM and not as LV volume. Papillary muscle mass was also manually contoured as part of the LVM assessment and for comparing papillary muscles in FD with HV (Figure 14).

This method was chosen as it has been reported that papillary muscle mass in FD is significant compared to other diseases with LVH and may occur early.(58,59) The basal cine slice was included if at least 50% of the cavity circumference was surrounded by ventricular myocardium and this principle was used for both end-systole and end-diastole.

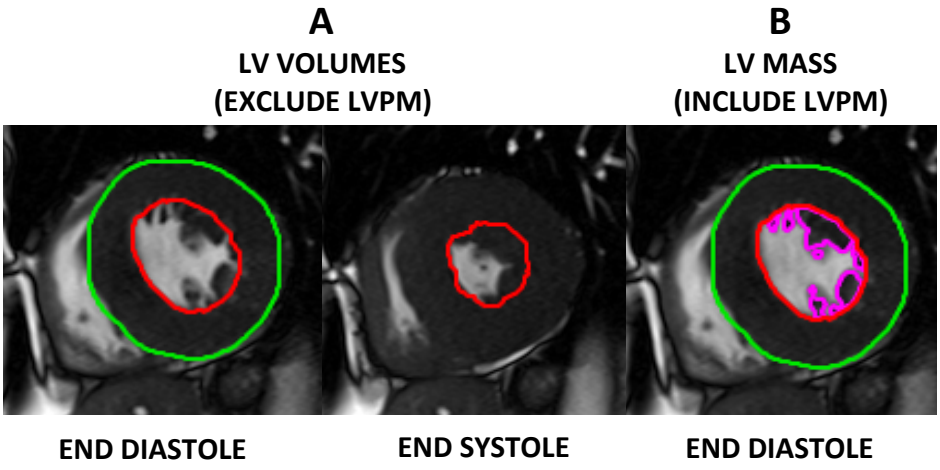

Figure 14: A) A single short axis example of LV volume assessment showing epicardial and endocardial contours excluding the left ventricular papillary muscle (LVPM). B) Epicardial and endocardial contours including manual contours of the LVPM for LV mass.

3.4.2 T1,T2 and ECV maps

All the parametric maps were analysed using CVI42. The short axis slices of the maps were segmented into AHA segments with a 20% offset at the endocardial and epicardial borders to reduce any effects of partial voluming from blood pool. A region of interest (ROI) in the septum was then manually drawn within these borders as shown in Figure 15. Other additional ROIs for mapping used in this thesis will be detailed in the relevant chapters.

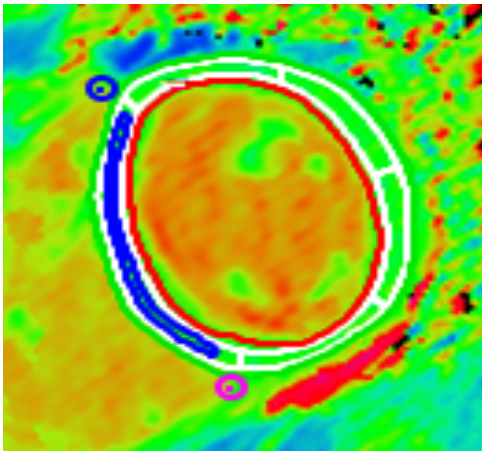

Figure 15: Example of ROI drawn in the septum in a ShMOLLI map with a 20% offset.

3.5 ECG analysis

12-lead ECGs were performed and were independently analysed by 2 experienced observers. Recorded ECG variables included: heart rate, rhythm, PR interval duration (normal 120-200ms), QRS complex duration (normal <120ms) and QTc interval duration (normal ≤440ms for males and ≤460ms for females). The presence of complete left or right bundle branch block (BBB), T wave inversion in at least two contiguous leads, multifocal ventricular ectopics (VE) and Sokolow-Lyon voltage criteria for LVH (SV1 + RV5 or RV6 >35mm) were also recorded.

3.6 Statistical analysis

Statistical analysis was performed using either SPSS 23 or SPSS 24 (IBM, Armonk, NY). Normality was determined by performing Shapiro-Wilk test

where a $p>0.05$ was considered as normal or parametric distribution. Continuous variables were expressed as mean ± standard deviation (SD) for parametric data or median (interquartile range (IQR)) for non-parametric data. Categorical variables were expressed as percentages.

Group testing was compared using independent-sample t-test, Mann-Whitney U, Chi-squared test or Fisher exact test as appropriate (normality, categorical or continuous). Comparisons between multiple groups were performed by 1-way ANOVA with post hoc Bonferroni correction. Data between baseline and follow up visits were compared using either paired *t*-test or Wilcoxon Signed Rank test according to normality.

Correlation was assessed using either Pearson's correlation coefficient for parametric data or Spearman's rho for non-parametric data. Troponin and NT-proBNP values were natural log transformed for bivariate testing.

For regression analysis, univariate analysis was performed initially to identify which factors are associated with the dependent variables. This is followed by backwards step-wise linear regression analysis for continuous data or logistic regression analysis for binomial data to identify the most important independent associations of the dependent variables. The unstandardized coefficient B or odds ratio and its 95% confidence interval were noted.

Bland-Altman analysis was performed to assess agreement between different analysis techniques. To assess intra- and inter-observer reproducibility, intra-class correlation coefficient (ICC) was performed. A p-value of <0.05 was considered significant.

Chapter 4 Results: Early Changes in Fabry Cardiomyopathy

This chapter is based on the following publication: **Nordin S,** Kozor R, Baig S, Abdel-Gadir A, Medina-Menacho K, Rosmini S, Captur G, Tchan M, Geberhiwot T, Murphy E, Lachmann R, Ramaswami U, Edwards NC, Hughes DA, Steeds RP, Moon JC. The cardiac phenotype of pre-hypertrophic Fabry disease. *Circulation Cardiovascular Imaging.* 2018 Jun;11(6):e007168.

My contribution was recruiting and performing CMR scans on 70% of the FD patients. I analysed all the data as first operator, did the statistical analysis and wrote the paper.

4.1 Introduction

Slowly progressive sphingolipid accumulation affects multiple organs, including the heart.(2) Cardiac involvement drives outcome in FD with cardiovascular death being the leading cause of death in FD.(6,55,63) Amongst the suggested risk factors for ventricular arrhythmias and sudden cardiac death were LVH and myocardial fibrosis.(55) Although oral chaperone has now become available for amenable mutations,(115) the mainstay of therapy has been ERT, which is expensive, less effective after the onset of fibrosis, and does not entirely reverse established LVH suggesting that best outcomes may occur with early initiation of ERT.(68) Early phenotypic markers of FD cardiac involvement are therefore needed.

Native T1 is low in FD, representing sphingolipid accumulation,(88,93) and is found in approximately 40-50% of LVH-negative patients, suggesting storage

occurs early prior to development of LVH.(88,89) ECG changes (47,126) and raised serum cardiac biomarkers (eg troponin and NT-proBNP) (107,127,128) have been previously reported to precede LVH. A preclinical phenotype has also been previously described in sarcomeric hypertrophic cardiomyopathy (HCM). The features described were multiple myocardial crypts, elongated anterior mitral valve leaflet (AMVL), abnormal trabeculation, and a higher or supranormal ejection fraction.(129)

4.2 Hypothesis

Storage (low native T1) in LVH-negative FD can be associated with structural and functional abnormalities, ECG changes and blood biomarkers.

4.3 Methods

4.3.1 Study population

This is a prospective, multicentre international observational study in 100 LVH-negative FD patients. Recruitment was from London (n=80), Birmingham (n=13) and Sydney (n=7).

Inclusion criteria were:

1. FD (n=100): all gene-positive LVH-negative males and females with LV maximum wall thickness (MWT) <13mm and LVMi within the normal reference ranges by CMR according to body surface area (BSA), age and gender.(130)
2. Healthy volunteers (HV, n=35): as described in the Methods chapter.

High-sensitivity Troponin T (London and Birmingham) and high-sensitivity Troponin I (Sydney) (Roche Diagnostic; normal range 0-14ng/L for Troponin T and 0-15ng/L for Troponin I), and NT-proBNP were obtained at the time of CMR scanning (Roche Diagnostics; normal range according to age and gender).(107)

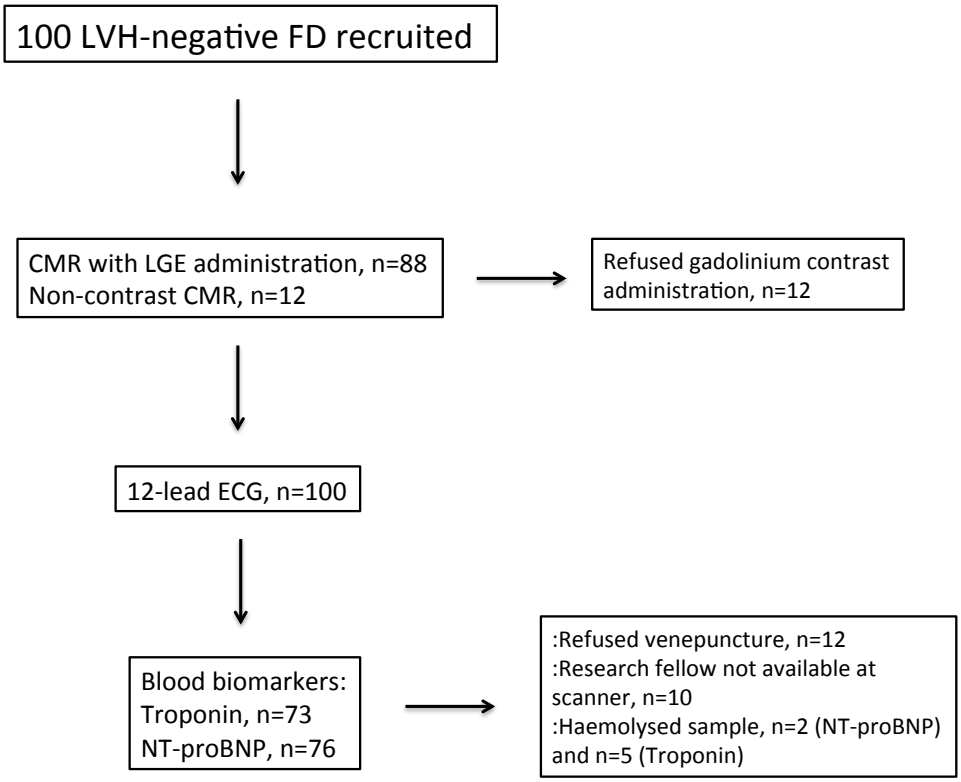

Figure 16: Consort diagram showing data collection in the FD participants (CMR, ECG and blood biomarkers).

4.3.2 CMR acquisition

All participants underwent CMR at 1.5 Tesla (Avanto (UK) and Aera (Australia); Siemens Healthcare, Erlangen, Germany) using a standard clinical protocol with LGE imaging using phase sensitive inversion recovery.

T1 mapping was performed pre-contrast bolus administration (0.1 mmol/kg body weight, Gadoterate meglumine, Dotarem, Guerbet S.A., France) on basal and mid left ventricular SA slices using ShMOLLI sequence. Post-T1 mapping was performed 15 minutes after contrast administration for ECV quantification.

4.3.3 CMR analysis

All images were centralised and analysed using CVI42 software. The native T1 analysis plan was initially for a ROI avoiding the blood-myocardial boundary (20% offset) manually drawn in the basal septum,(73) but visual inspection of the colour maps showed that native T1 lowering (blue areas) could be patchy so six segments in basal and mid SA slices were used in further analyses. Normal native T1 reference ranges (mean ± 2 SD) were defined using age and gender-matched HV from each individual centre, taking into account the native T1 regional variations in HV from each centre.

The normal native septal T1 reference ranges (mean, SD, then lower limit of normal – defined as mean - 2SD) for each centre were as follows: London centre total population mean 968±32ms, lower limit 904ms; male subgroup mean 956±27ms, lower limit 902ms and female subgroup mean 978±34ms, lower limit 910ms. Birmingham centre total population mean 959±31ms, lower limit 891ms; male subgroup mean 947±28ms, lower limit 890ms and female subgroup mean 958±30ms, lower limit 898ms. Sydney centre total population mean 958±31ms, lower limit 896ms; male subgroups mean 947±24ms, lower

limit 893ms and female subgroups mean 965ms±31ms, lower limit 903ms in females.

The T1MES phantom was scanned as part of the T1MES multicentre study.(95) The London site coefficient of variation (CoV) for reads varies from 0.017% to 2.267% at mean temperature of 20°C. Birmingham site CoV for reads varies from 0.512% to 1.460% at mean temperature of 21°C. Sydney site CoV for reads varies from 0.033% to 1.169% at mean temperature 21°C. Inter-centre reads across the 9 tubes were consistent (all $p > 0.05$): the mean differences in measured T1 across all tubes were 10.30 ± 5.71 (SD) ms between centres. Considering only the tubes emulating normal native myocardial T1 and low native myocardial T1 (that are relevant to FD) the mean differences in measured T1 between sites was 9.52 ± 3.64 (SD) ms.

The methods for quantification of LV volumes and LV mass by manual contouring of LVPM(58,130,131) were used as described in methodologies chapter. The LVPM were reported as absolute mass. AMVL measurements and myocardial crypt counts (considering only ≥2) followed previous published methodologies.(129,132) Abnormal myocardial trabeculation can be measured using fractal analysis to define myocardial trabecular complexity in individuals. This derives the following fractal dimensions (a non-integer value between 1 (least complex trabeculae) and 2 (most complex trabeculae)). LV fractal analysis was performed on the LV short-axis cine stack using the cvi42 fractal plugin for trabeculae analysis (133). This derived the maximal fractal

dimension of the apical segment of the LV (Fractal $Dimension_{MaxApical}$), which has been previously shown to best represent abnormal myocardial trabeculation in various pathologies eg left ventricular non-compaction and sarcomeric HCM including preclinical HCM. (129, 133)

4.3.4 Statistical analysis

Statistical analyses were as described. In addition, the CoV between repeated phantom scans was calculated as a measure of reproducibility of the phantoms between the centres.

4.4 Results

Baseline demographic characteristics are shown in Table 6. Mean age of the FD cohort was 39±15 years. 81% of the participants were female. Mean eGFR was 86±8 ml/min/1.73m^2. 34% of the FD participants were of cardiac (later onset) variant.(30) Of the cardiac variant group, 85% (29/34) with N215S mutation, 9% (3/34) I91T mutation and 6% (2/34) R301Q mutation. 38% of FD patients were on ERT.

The median ERT duration was 7 (IQR 4.3-9) years. The main indications for ERT initiation were due to non-cardiac manifestations including uncontrolled acroparesthesia, neurological involvement such as stroke and transient ischaemic attack and renal involvement such as proteinuria. One female patient had started ERT mainly due to conduction abnormalities on ECG.

There was no correlation between ERT with native T1 or ECG changes**:** 41% (24/59) vs 34% (14/41), of low vs normal native T1 patients were on ERT (p=0.51), and 42% (17/41) vs 36% (21/59), of abnormal ECG vs normal ECG patients were on ERT (p=0.55). ERT duration did not correlate with native T1 or ECG abnormalities.

	Healthy Volunteer (n=35)	Fabry Disease (n=100)	P value
Age, years	40±14	39±15	0.724
Male, n (%)	9 (25%)	19 (19%)	0.235
BSA, m^2	1.73±0.20	1.79±0.21	0.104
Heart rate, bpm	66±10	62±12	0.084
Systolic BP, mmHg	121±13	116±14	0.038*
Diastolic BP, mmHg	76±10	72±9	0.012*
Hypertension, n (%)	0	8 (8%)	0.112
CAD, n (%)	0	2 (2%)	1
eGFR (ml/min/1.73m^2)	86±6	86± 8	0.782
ERT, n (%)	N/A	38 (38%)	
Cardiac variant, n (%)	N/A	34 (34%)	
LVEF (%)	67±4	71±7	0.002*
MWT, mm	9±1.3	9±1.5	0.368
LVMi, g/m^2	63±14	61±10	0.243
LVEDVi, ml/m^2	73±12	72±11	0.728
LVESVi, ml/m^2	24±6	21±7	0.012*

Table 6: Demographic characteristics of the healthy volunteers and FD cohort.

4.4.1 Myocardial abnormalities:

Function and Mass: All FD patients had normal MWT, LV cavity size and mass and mass-to-volume ratios less than 1. MWT in the FD and HV cohort were similar (9±1.5mm vs 9±1.3mm, p=0.4). Indexed LV mass (LVMi) and end diastolic volume (LVEDVi) were similar to HV (LVMi 61±10 g/m^2 vs 63±14 g/m^2, p=0.20; LVEDVi 72±11 ml/m^2 vs 73±12 ml/m^2, p=0.7), but end systolic volume (LVESVi) was smaller in FD (21±7 ml/m^2 vs 24±6 ml/m^2, p=0.012). All patients had normal or supra-normal LV systolic function, and overall, the LVEF in FD was higher than HV (71±7% vs 67±4%, p=0.002). Papillary muscle mass was found to be higher in FD (8±4g vs 6±2g, p<0.05).

Myocardial Native T1: Using a basal septal ROI, 41 of 100 FD participants (41%) had a low native T1. However, native T1 lowering was observed visually on the colour maps to be 'patchy' in many. Therefore, we analysed a second slice with further ROIs, Figure 17. This increased the low native T1 prevalence to 59% (an additional of 18 patients) – but preserved the 0% prevalence in HVs. It was these 59% FD patients that were considered as having low native T1 for subsequent analyses in this chapter.

Native T1 reproducibility: Native T1 intra- and inter-observer variability (n=30) were good – the low native T1 areas are obvious when displayed with an appropriate look-up table, Figure 18. ICCs (intra-observer then inter-observer) in the septum were 0.995 and 0.986 respectively and ROIs

elsewhere: 0.983 and 0.986. Bland Altman graphs did not show evidence of systemic bias (Figure 19).

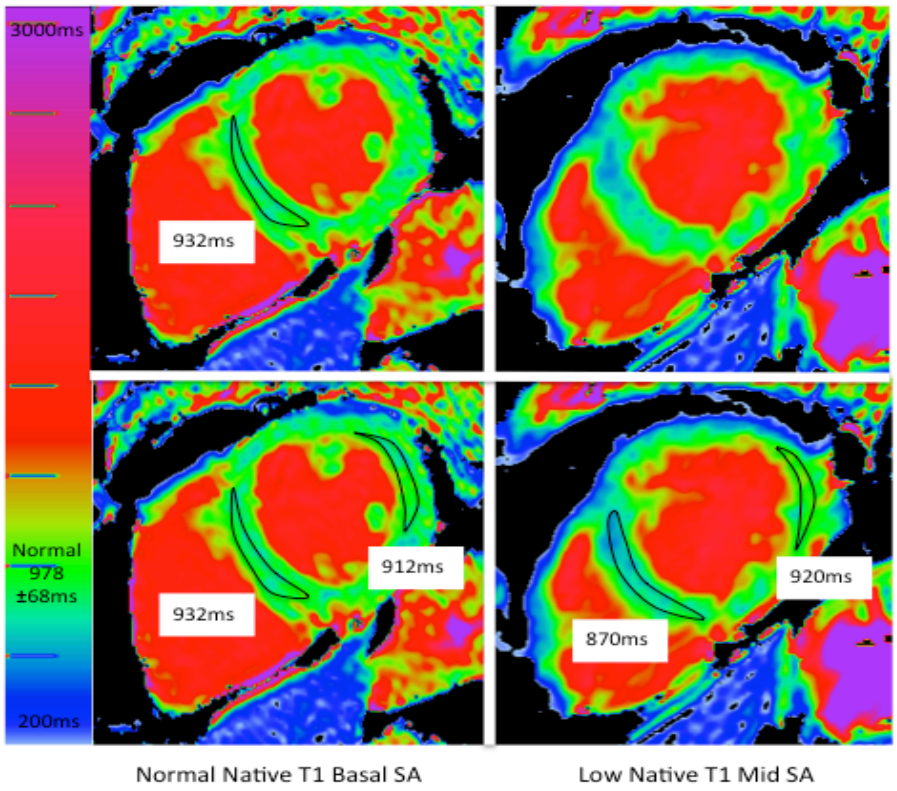

Figure 17: An example of an LVH-negative patient with obvious but missed low native T1 using the conventional approach of drawing one ROI in a single short axis slice in the top row. This generated a normal native T1 (932ms). Note however there is "blue" myocardium in the second, unmeasured slice. The low native T1 is captured by drawing other ROIs on both slices (bottom row).

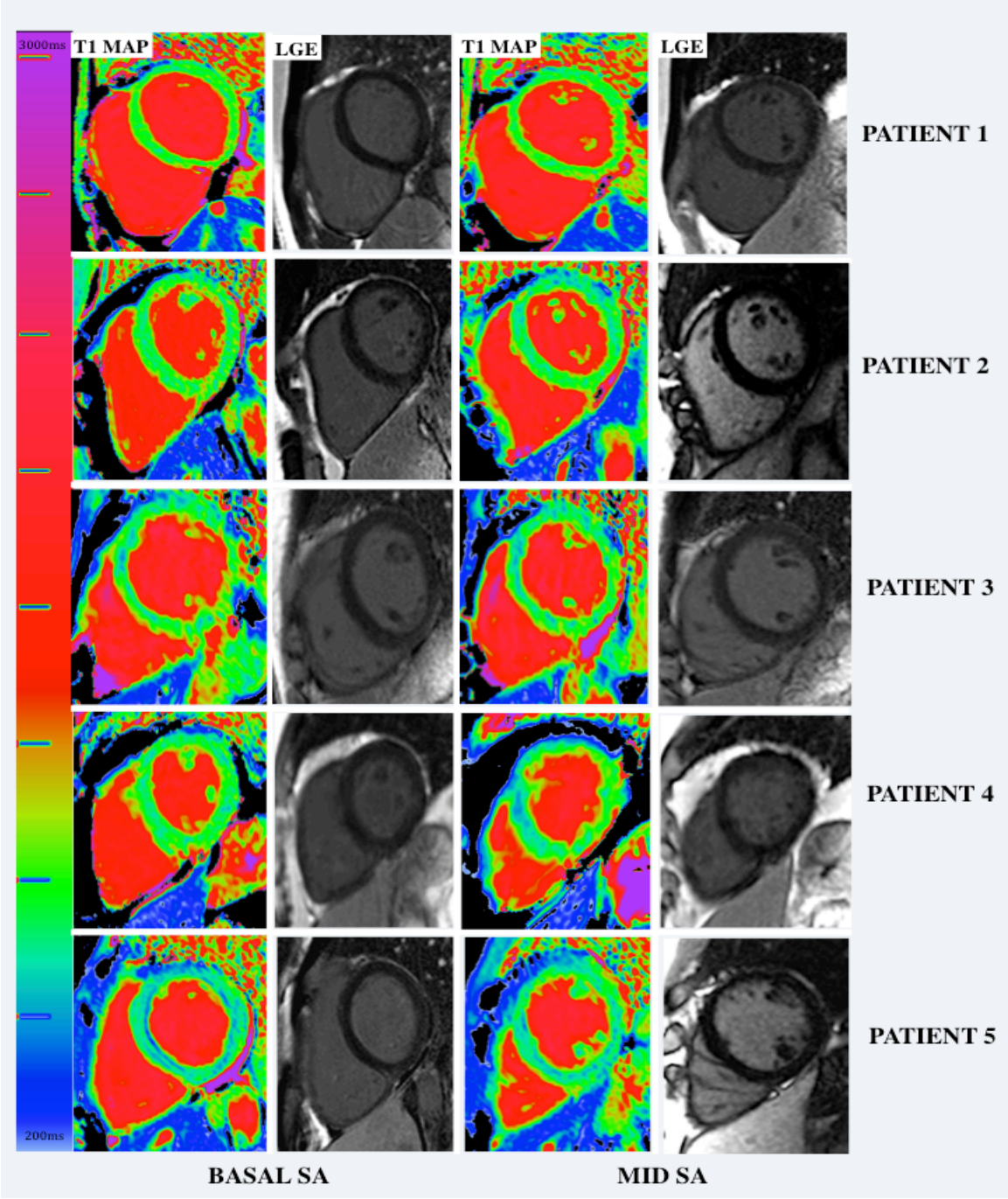

Figure 18: Spectrum of native T1 in LVH-negative Fabry Disease. Patient 1 - Normal native T1 in both SA slices. Patient 2 - Patchy low native T1 (blue myocardium) in the lateral walls. Patient 3- Low native T1 mainly at the septal regions in both SA slices. Patient 4 – Low native T1 in the mid SA septum only. Patient 5 - Low native T1 all across both SA slices.

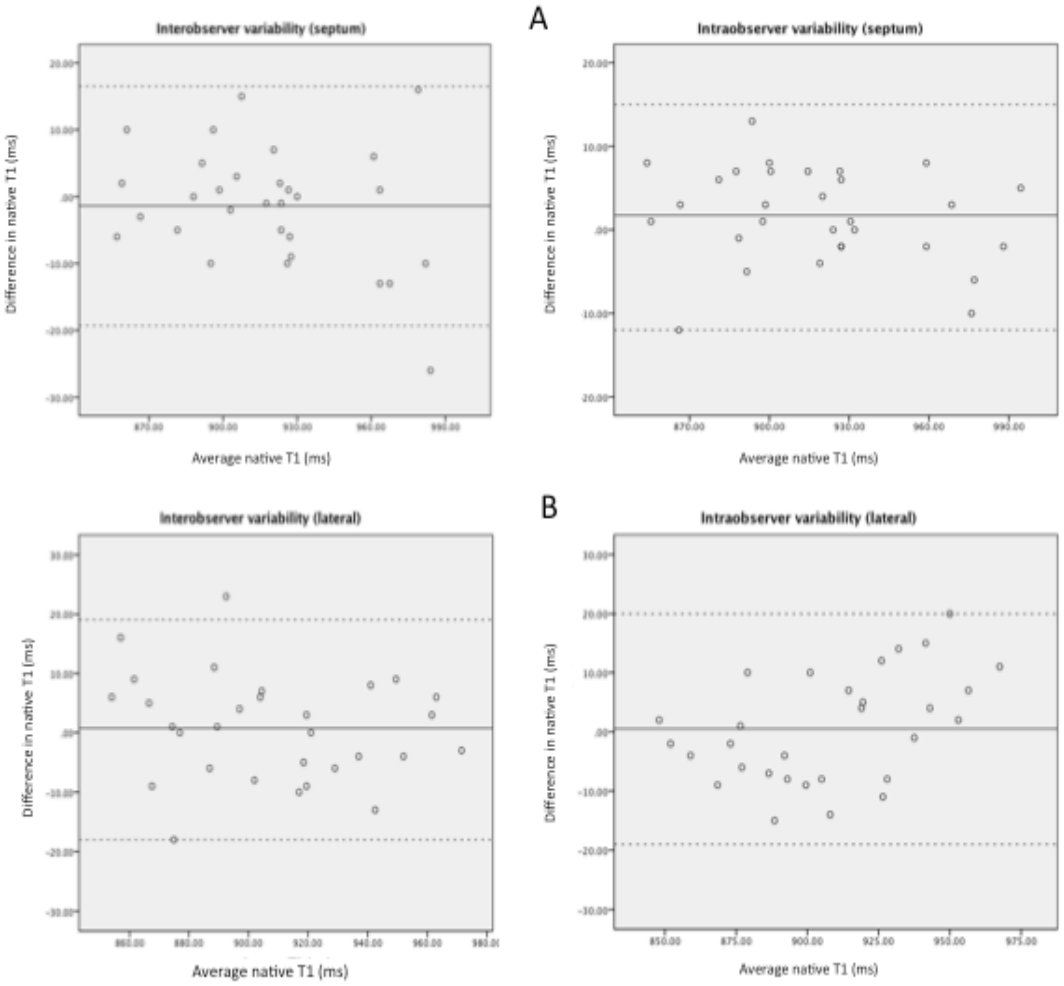

Figure 19: Bland Altman graphs of native T1 analyses of (A) inter-observer and intra-observer variability at the septum, and (B) inter-observer and intra-observer variability of the other segments apart from septum. The mean difference of each plot is shown as a solid line and dotted lines showing 95% limits of agreement.

Myocardial LGE: 16 FD had LGE (13 basal inferolateral wall; 3 right ventricular insertion points). LGE was not detected in any of the healthy volunteers.

Myocardial ECV: The ECV measured from a septal ROI was normal in all participants (FD 0.27±0.03 vs HV 0.28±0.03), p=0.46.

Myocardial architecture: In FD compared to HV, the anterior mitral valve leaflet was longer (23±2mm vs 21±3mm, p=0.002). 9% (9/100) of FD and 3% (1/35) of HV had crypts (p>0.05). Compared to HV, FD had abnormal trabeculae at the apex resulting in higher Fractal $Dimension_{MaxApical}$: 1.27±0.06 vs 1.24±0.04, p<0.005. Fractal $Dimension_{Mean}$ were similar to HV (1.22±0.03 vs 1.23±0.03, p=0.10).

ECG: In FD, 41% (41/100) had abnormal ECGs. 4 patients had other potential causes of ECG abnormalities (n=1 CAD; n=3 hypertension). All HV ECGs were normal.

Blood Biomarkers: Of the FD patients, 10% (7/73) had raised hsTroponin levels and 16% (12/76) had raised NT-proBNP.

4.4.2 Comparison with native T1:

Native T1 and ECG: Of the patients with abnormal ECGs, 76% (31/41) had low native T1. ECG abnormalities were more than twice as common when the T1 was low (53%, 31/59 vs 24%, 10/41, p=0.005). The ECG abnormalities observed were: (low native T1 vs normal native T1) Sokolow (19% vs 7%), T wave inversion (13% vs 7%), short PR (12% vs 5%) and long PR intervals (9% vs 2%), Figure 20. QRS interval, QTc interval and Sokolow-Lyon voltages were higher in the low native T1 subgroup compared to the normal native T1 FD subgroup and HV, Table 7.

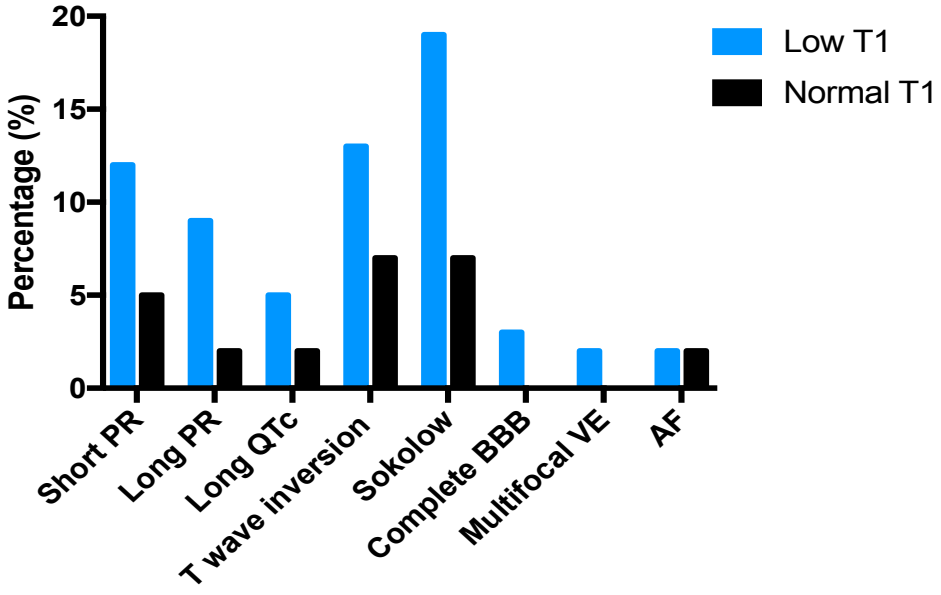

Figure 20: Comparison of ECG changes between low native T1 and normal native T1 Fabry Disease subgroups.

	Healthy Volunteer	Low Native T1 FD	Normal Native T1 FD	P value
PR interval (ms)	158±17	147±29	147±26	0.08
QRS duration (ms)	85±9	93±14	88±10	0.005
QTc interval (ms)	394±26	411±27	407±21	<0.01
Sokolow (mm)	21±7	27±8	23±10	<0.005

Table 7: ECG parameters of healthy volunteers, low native T1 and normal native T1 Fabry Disease subgroups.

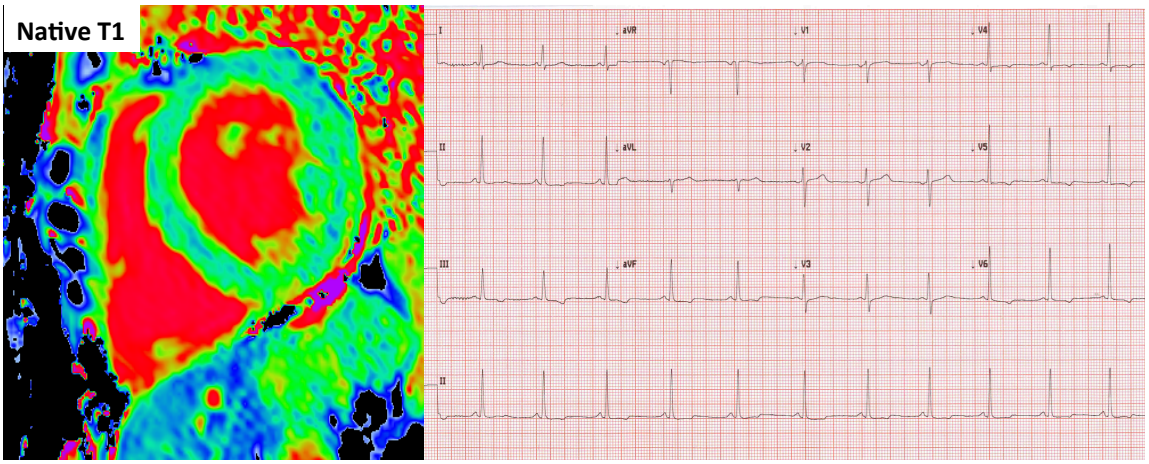

Figure 21: Example of an FD patient with low native T1 with abnormal ECG (T wave inversion in leads III, aVF, V4-6).

Native T1 and LV function/mass: MWT, LVMi and EF were higher in low native T1 compared to normal native T1 FD (MWT 9±1.5mm vs 8±1.4mm, $p<0.005$, LVMi 63±10g/m^2 vs 58±9g/m^2, $p<0.05$; EF 73±8% vs 69±7%, $p<0.01$). LVEDVi, LVESVi and mass to volume ratio were the same. Normal native T1 patients were the same as HV for all parameters.

Native T1 and LGE: LGE was approximately five times as common in the low native T1 FD compared to the normal native T1 FD (27%, 14/52 vs 6%, 2/34, $p=0.01$). 88% (14/16) with LGE had low native T1. One patient with normal native T1 and LGE had both a history of hypertension and coronary artery disease; however, the LGE pattern in this patient was typical of Fabry (midwall basal inferolateral).

Native T1 and ECV: ECV was normal and similar in all the subgroups (low native T1 0.27±0.03 vs normal native T1 0.28±0.03 vs HV 0.28±0.03, all $p=0.56$).

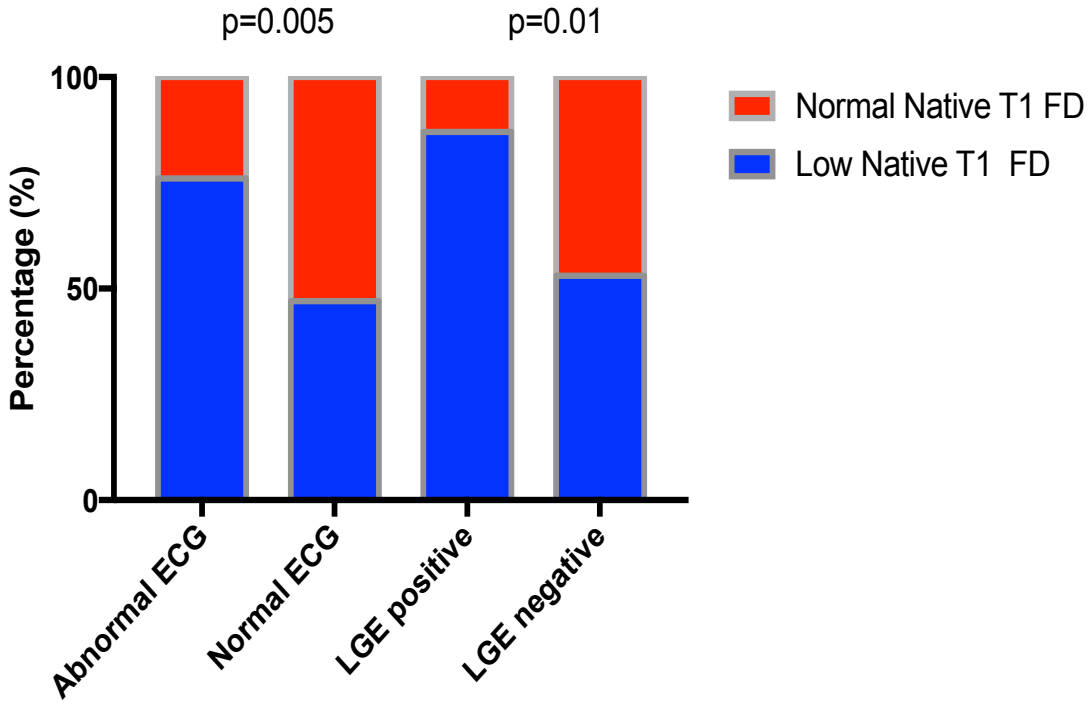

Figure 22: Normal vs Abnormal ECG and LGE-positive vs LGE-negative in the low native T1 and normal native T1 Fabry Disease subgroups

Native T1 and papillary muscle mass: With low native T1, papillary muscle mass was higher (p=0.003). Normal native T1 patients had similar papillary muscle mass as HV (low native T1 FD 8±4g vs normal native T1 FD 6±3g vs HV 6±2g). Papillary muscle mass increase was not out of proportion to compacted myocardial hypertrophy however.

Native T1 and AMVL/fractal dimension: AMVL elongation and the increased apical fractal dimension were storage independent and not confined to the low native T1 group - AMVL elongation and increased apical fractal dimension were the only variables different between *normal* native T1 FD and

HV. (AMVL normal native T1 vs HV: 24±3mm vs 21±3mm, p=0.004; Fractal $Dimension_{MaxApical}$ normal native T1 vs HV 1.27±0.05 vs 1.24±0.04, p=0.01).

Native T1 and blood biomarkers: Although most patient with elevated biomarkers had low T1 (71%, 5/7 for hsTroponin; 58%; 7/12 for NT-proBNP), differences were not significant.

4.4.3 Other Comparisons:

LGE and blood biomarkers: Half of the patients with LGE had raised troponin. No other patients had raised troponin (45%, 5/11 vs 0%, 0/57; p<0.001). There was no significant difference in the prevalence of raised NT-proBNP in the LGE-positive and LGE-negative subgroups (17%, 2/10 vs 14%, 8/59, p=0.29).

ERT: There is no significant difference between the MWT, LVMi, LVPM mass and LVEF between the ERT and ERT-naïve subgroup.There was also similar proportion of low T1, abnormal ECG, LGE and raised blood biomarkers (troponin and NT-proBNP) between these subgroups.

Gender: There are higher proportions of males with low T1 and ECG changes compared to females (low T1 male 79%, 15/19 vs female 54%, 44/81; p<0.05 and abnormal ECG male 63%, 12/19 vs female 36%, 29/81; p<0.05). Males have higher MWT (10±1.3mm vs 9±1.5mm, p<0.001) and LVMi (71±10g/m^2

vs 59±9g/m^2, p<0.001) and females have higher LVEF (73±7% vs 66±8%, p=0.001). 94% (15/16) with LGE were female; the one male had LGE at RV insertion point.

4.5 Discussion

This study shows that a pre-hypertrophic FD phenotype is present. Storage (low native T1) is patchy and more common (prevalence 59%) than previously reported in pre-hypertrophic FD.(88,89) Other abnormalities are found and most of these co-segregate with low native T1 – ECG changes, higher mass (within the normal range), LGE, larger papillary muscle mass and higher EF (also observed in FD with hypertrophy). There are however two exceptions: firstly, troponin is associated with LGE rather than directly with storage (as in overt disease(127)); secondly elongated anterior mitral valve leaflet and abnormal apical trabeculae are also present in pre-hypertrophic FD, and appear to be storage independent. Diffuse fibrosis (ECV), and NT-proBNP were not useful. Overall, these data suggest firstly that storage is a key part of FD phenotype development, and secondly that there is a storage independent FD phenotype with some of its features may be similar to preclinical sarcomeric HCM.(129)

Earlier phenotypic markers of FD cardiomyopathy are needed as cardiac involvement drives prognosis and ERT may be more successful if initiated before the cardiomyopathic cascade of secondary changes (LVH, inflammation and fibrosis) is established.(54,68) Treatment is expensive so is

not feasible without phenotypic justification, although new oral chaperone treatment may aid therapy in patients with amenable mutations.

My findings support the utility of a low native T1 to identify pre-hypertrophic or early cardiac involvement in FD, and may be a candidate starting criteria for early treatment in either practice or in clinical trials. More than one ROI and inspection of colour maps with appropriate look-up tables is needed to maximize sensitivity for early storage. ECG changes have been known to precede LVH in TTE studies (47,126) but may be non-specific.(134) A low native T1 if at the detection limit could also generate false positives. How could ECG and native T1 be used in combination clinically? Cardiac involvement by ECG abnormalities only was 41%; 59% by low native T1 only. However, cardiac involvement by either ECG abnormalities or low native T1 approach is 69% and 31% by both approach (Table 8). In pre-hypertrophic FD, it is the low native T1 and abnormal ECG group that I hypothesised will most likely progress and may benefit most from early treatment.

	Low Native T1	Normal Native T1	P value
ECG (n=100)			
Abnormal	**31**	**10**	**0.005**
Normal	**28**	**31**	
LGE (n=88)			
Positive	**14**	**2**	**0.01**
Negative	**38**	**34**	
Troponin (n=73)			
Raised	**5**	**2**	**0.45**
Normal	**35**	**31**	
NT-proBNP (n=76)			
Raised	**7**	**5**	**0.89**
Normal	**36**	**28**	
Structure and Function (n=100)			
MWT (mm)	**9±1.5**	**8±1.4**	**<0.005**
LVMi (g/m^2)	**63±10**	**58±9**	**<0.05**
LVEF (%)	**73±8**	**69±7**	**<0.01**

Table 8: Summary of comparison between low native T1 and normal native T1 Fabry Disease with other CMR parameters, ECG and blood biomarkers.

The mechanism linking low native T1 with ECG abnormalities has not been previously explored but others have shown sphingolipid deposition around the atrioventricular (AV) node might cause PR-interval shortening, therefore accelerating AV conduction rather than accessory pathways.(47-51) PR prolongation and increasing QRS duration occur with time.(50) This is likely

due to progressive accumulation of sphingolipid in cardiac conduction tissue causing cellular dysfunction.(50,135-137) No studies have shown an association between native T1 with T-wave inversion and LVH ECG voltage criteria such as Sokolow criteria in LVH-negative FD cohort; however these ECG changes have been previously observed in pre-hypertrophic FD mainly in TTE studies.(107) ECG abnormalities were at least twice as common when the native T1 was low in FD supporting a role for storage in the pathogenesis. However, a small minority of patients (n=10) had ECG abnormalities before detectable storage (normal native T1). This could be due to other effects such as circulating metabolites(21), inflammation(138) or T1 mapping test insensitivity. It is also possible that low T1 can still be missed in the pre-hypertrophic cohort with the current proposed technique.

In future, this technique can potentially be refined further by adding more SA slices to improve diagnostic sensitivity (eg by using >2 slices or by using 3D whole heart T1 mapping) if low T1 is not detected using the current proposed technique. Future longitudinal work is required to evaluate the progression of hypertrophy in these patients, it is the low native T1 group that I hypothesise will develop LVH.

Limitations of this study include no direct histological validation of low T1 with sphingolipid in this paper. Other T1 lowering influences (iron and other forms of fat) or confounding by T1 elevators (eg fibrosis, amyloid, vasodilatation and oedema) in native T1 are possible. In addition, there is no outcome data,

although a 5-year follow-up to determine disease progression is currently planned. It is unclear how and if these patients would benefit from early institution of ERT. The significance of the type of ECG changes is also unknown due to lack of prospective studies in this population. The healthy volunteers were mainly Caucasian, which may impact comparison of trabeculation in the normal cohort. The population was dominated by female heterozygous patients, (partly as females are 2x as common, partly as males remain LVH negative for a shorter time. This potentially skews the results partly.

4.6 Conclusion

Storage (low native T1) in pre-hypertrophic FD is more common than previously described because early native T1 lowering may be patchy. There is a measurable early phenotype in pre-hypertrophic FD consisting of low native T1, ECG changes, LGE and elevated LV mass, wall thickness and ejection fraction.

Chapter 5 Results: Chronic Myocardial Inflammation in Fabry Disease

This chapter is based on the following publication: **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(15):1707-1708.

My contribution was recruiting all the FD patients, Dr Rebecca Kozor and I then equally performed the CMR scans, analysed the data and wrote the manuscript.

5.1 Introduction

The link of sphingolipid storage with cardiac disease is not well understood in FD. An advance was the identification of myocardial fibrosis by CMR using LGE technique, characteristically occurring initially in the basal inferolateral wall,(39) and later affecting other segments(139), with histological correlation (in advanced disease) indicating it is focal fibrosis.(90) It is associated with a poor response to therapy and adverse outcomes(140), but is mainly a late feature(141), although it can occur before hypertrophy in females(142). Recently, hybrid imaging with PET/MR has shown that some FD LGE may be inflammation.(97) Native T1 is low in FD, most likely due to sphingolipid accumulation(88). However, the links between intracellular accumulation and extracellular scar remains obscure. T2 mapping may be useful as it is a sensitive detector of inflammation and oedema, eg discriminating acute from chronic myocardial infarction (cMI)(143) or myocarditis.(144) Although T2 has been measured in FD previously(96), mapping has not been explored in FD.

The traditional measures of cardiac inflammation and myocyte stress include the use of blood biomarkers eg troponin and NT-pro brain natriuretic peptide (NT-proBNP). Multiple studies have shown increased troponin values in FD patients, correlating with LGE and LVH.(105,106)

5.2 Hypothesis

Inflammation is present in FD with LGE, measurable using T2 mapping and troponin.

5.3 Method

CMR (T1 and T2 mapping with LGE) and blood biomarkers (high sensitivity troponin T (TnT) and NT-proBNP) were collected in FD with CMR performed in comparator diseases: hypertrophic cardiomyopathy (HCM), chronic infarction and healthy controls.

5.3.1 Subject population

The following subgroups were included in this study:

1. FD (n=47): all gene-positive; both males and females.
2. HCM (n=28): randomly selected gene-positive patients with asymmetrical LVH and LGE.
3. Chronic myocardial infarction (cMI, n=30): 6 months post-reperfused ST-elevation myocardial infarction.
4. Healthy controls (n=60): as described in Methods chapter.

All patients underwent CMR (1.5 Tesla, Avanto, Siemens) at a single UK centre. FD patients had bloods collected just before the scan and analysed for high-sensitivity TnT (Roche Diagnostics; normal range 0-14ng/L), NT-pro BNP (Roche Diagnostics; normal <47pmol/L), and eGFR calculation.

5.3.2 CMR acquisition

Structure and function was performed with long axis and short axis cines acquisition using methods described in Chapter 3. T1 mapping was acquired pre-contrast on basal and mid left ventricular SA slices in diastole using a MOLLI 5s(3s)3s sequence (77). T2 mapping was performed pre-contrast on co-registered SA slices. LGE imaging was acquired using a FLASH sequence with PSIR (120), 5-15 minutes after contrast administration.

5.3.3 CMR analysis

All imaging analysis was performed using CVI42 software. LVH was defined as increased LVMi on CMR according to age and gender matched normal reference ranges.(130) The presence of LGE was assessed by 2 independent observers. The amount of LGE was quantified using the threshold method of 5 standard deviations above the mean remote myocardium(145) presented in grams and also expressed as a percentage of the LV myocardium. For remote area native T1 and T2, a ROI was manually drawn in the basal septum (or a segment without LGE if the basal septum was LGE-positive),

with a 20% offset. For the native T1 and T2 at the LGE area, the ROI was manually drawn over areas corresponding to LGE. An additional ROI was drawn in the basal inferolateral segment in cases without LGE and in controls. Normal reference ranges (mean ± 2 SD) were defined using the healthy volunteers (n=60), with the group considered as a whole for T2 and split by gender for T1 as T1 is known to vary with gender.(146)

5.3.4 Statistical analysis

Statistical analyses were carried out using SPSS 23 (IBM, Armonk, NY) as described in the methodologies chapter. A p-value of <0.05 was considered significant.

5.4 Results

5.4.1 CMR results

There were 165 participants in total: 47 FD, 28 HCM, 30 cMI, and 60 healthy controls, Table 9. All FD patients had either good or hyperdynamic LV systolic function with normal LV cavity size. The mean eGFR of the FD cohort was 78±16 ml/min/1.73m^2, with all cases >30 ml/min/1.73m^2 but with 15% (7/47) having renal impairment (eGFR<60 ml/min/1.73m^2).

Seven FD cases did not receive gadolinium due to patient preferences. Of the remaining 40 cases, 18 had LGE (14/21 males, all LVH-positive; 4/19 females, 3 were LVH-positive. One female patient had LGE without

hypertrophy). All LGE occurs in the basal inferolateral wall, of which 8 also had extensive LGE in other myocardial segments. 22 FD cases had no LGE (4/22 were LVH-positive). The median LGE extent was 18(12-24) g.

	Controls (n=60)	HCM (n=28)	cMI (n=30)	FD (n=47)	Male FD (n=22)	Female FD (n=25)
Age (yrs)	48±14	49±15	58±13	45±12	47±12	43±12
Male sex	34/60	18/28	26/30	22/47	22	0
ERT	-	-	-	26/47	17/22	9/25
LVEDV (ml)	132±28	137±34	172±38	132±26	146±22	119±24
LVEF (%)	71±6	76±7	49±8	75±8	73±8	76±7
LVM (g)	106±27	205±79	107±30	163±91	231±92	103±25
LVH+ve	0	28/28	3/30	23/47	18/22	5/25
LGE+ve	0	28/28	30/30	18/47	14/22	4/19

Table 9: Baseline characteristics of the different cohorts (healthy controls, HCM, chronic MI and FD).

The T1 in the remote myocardium in FD was lower than in HCM, cMI and controls (934±61ms vs 1021±40, 1003±41, 1015±36 ms; $p<0.001$; Figure 20A). T1 was elevated in the LGE areas in all diseases studied but T1 in LGE was not as high in FD compared to HCM and cMI (FD: 1096±93ms, HCM: 1180±66ms, cMI: 1138±52ms; $p<0.05$; Figure 23A). However, the increase over remote T1 was higher in FD (FD: 204±90ms, HCM: 158±62ms, cMI: 135±57ms, $p<0.05$; Figure 23B).

T2 values were normal in the remote areas in all groups (FD: 51±3ms, HCM: 51±3ms, cMI: 48±2ms, HV: 49±2ms; Figure 24). However, T2 values in the LGE areas were very high in FD compared to HCM and cMI (FD: 64±7ms,

HCM: 55±4ms, cMI: 54±3ms; p<0.001; Figure 21). Mean T2 values in LGE in HCM and cMI were similar (p=0.1), and just above the upper limit of normal (normal T2 range 45-53ms from healthy volunteers).

All 18 FD cases with LGE had a high T2 (range 55-81ms) in the areas of LGE. When there was no LGE, the T2 in the BIFL wall was normal in all 22 cases, although slightly higher than controls, 51±3ms vs 49±2ms, p=0.006).

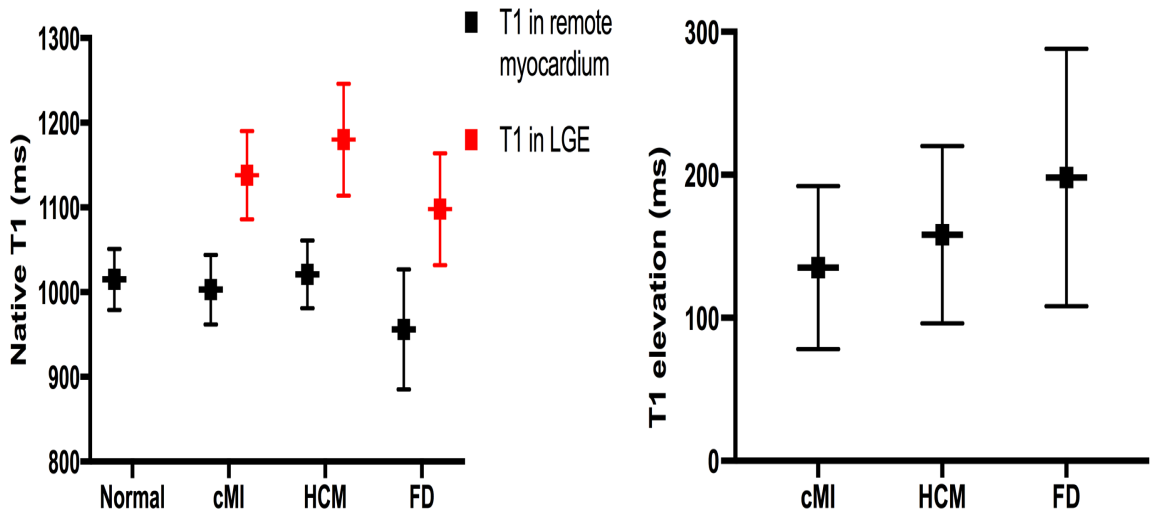

Figure 23: (A) Mean T1 values in remote myocardium in FD vs controls, HCM and cMI (p<0.001) and in the LGE area in FD compared to HCM and cMI (p<0.05). (B) Mean difference in T1 between the LGE and remote areas in FD compared to HCM and cMI (p<0.05).

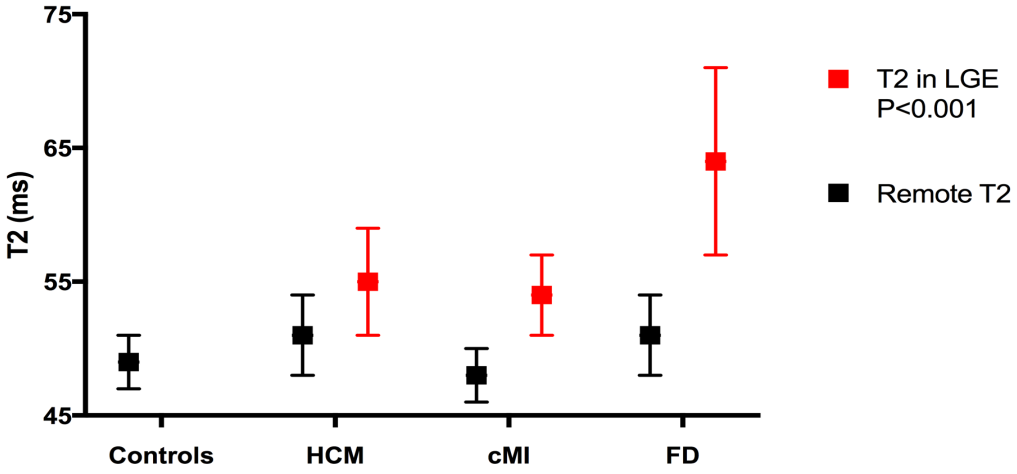

Figure 24: Mean T2 values in the remote myocardium in normal controls, cMI, HCM and FD and LGE area in cMI, HCM and FD (p<0.001).

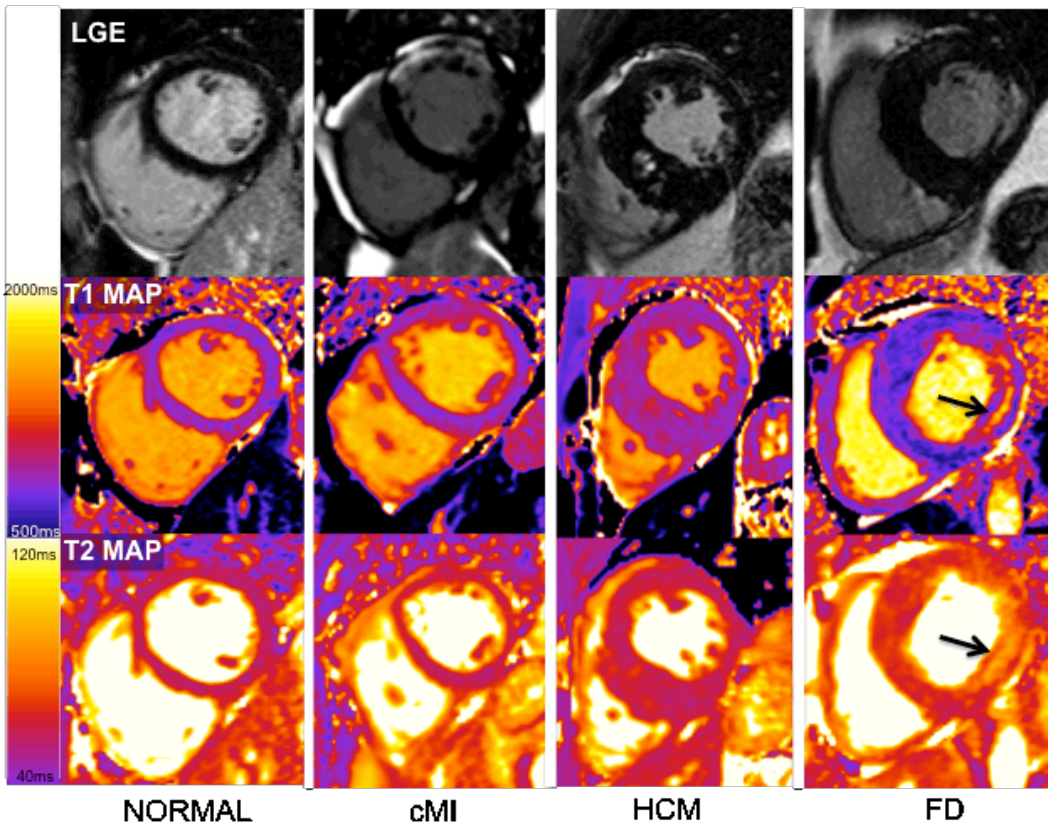

Figure 25: Corresponding short axis slices in normal controls, chronic MI, HCM and FD. Top row are late gadolinium images, middle row are T1 maps (MOLLI) and bottom row are T2 maps.

5.4.2 Blood biomarkers in FD

Of the FD patients, 89% (42/47) had troponin measured, of which 40% (17/42) were elevated. Median troponin was 10(1-32)ng/L (range 1-93ng/L). Troponin elevation only occurred when there was LGE (LGE-positive vs LGE-negative: 83% (15/18) vs 0% (0/20), p<0.001) and was strongly related to LVH (LVH-positive vs LVH-negative: 94% (16/17) vs 4% (1/25), p<0.001). On univariate analysis, T2 in the BIFL wall/LGE area correlated with natural log troponin (r_s=0.82, p<0.001) and LVM (r_s=0.69, p<0.001) but not LGE extent (r_s =0.42, p=0.08).

Median NT-proBNP (n=41) was 41(28-344)pmol/L (range 5-1116pmol/L) with 27% of the total FD cohort having an elevated NT-proBNP. T2 in the BIFL wall/LGE area also correlated with the rise in NT-proBNP (r_s =0.5, p<0.001).

To further investigate the predictors of troponin and NT-proBNP in FD, multivariate analyses were performed. In univariate analysis, troponin was associated with age, gender, LVM, eGFR, remote T1, and T2 in the LGE/BIFL segment. On multivariate analysis, the only associations were eGFR and T2, with T2 in LGE/BIFL segment being the strongest association with troponin (B=2.4, p<0.001; Table 10). A similar analysis for NT-proBNP demonstrated only eGFR as a multivariate predictor (B=6.0, p<0.01; Table 11).

Dependent Variables	Variables in Model	Univariate B (95% CI)	P value	Multivariate B (95% CI)	P value
Troponin	Age	1.3 (0.7, 2.0)	<0.001	-	
	Male	-20.4 (-37.2,-3.6)	0.02	-	
	ERT status	9.2 (-9.5, 27.5)	0.3	-	
	LVM	0.2 (0.1, 0.3)	<0.001	-	
	eGFR	-0.9 (-1.4, -0.5)	<0.001	-0.4 (-0.7, 0.004)	0.05
	Remote T1	-0.2 (-0.3, -0.1)	0.005	-	
	T1 in LGE/BIFL wall	-0.001 (-0.004, 0.01)	0.6	-	
	T2 in LGE/BIFL wall	2.7 (2.1, 3.4)	<0.001	2.4 (1.7, 3.1)	<0.001

Table 10: Univariate and multivariate analyses in FD of troponin.

Dependent Variables	Variables in Model	Univariate B (95% CI)	P value	Multivariate B (95% CI)	P value
NT-pro BNP	Age	4.6 (-0.3, 9.5)	0.07	-	
	Male	-122.0 (-239.8, -4.2)	0.04	-	
	ERT status	-42.3 (-168.6, 83.9)	0.5	-	
	LVM	0.8 (0.2, 1.4)	0.01	-	
	eGFR	-7.0 (-10.1, -3.8)	<0.001	-6.0 (-9.2, -2.7)	0.01
	Remote T1	-1.0 (-2.0, -0.1)	0.03	-	
	T1 in LGE/BIFL wall	-0.003 (-0.04, 0.03)	0.9	-	
	T2 in LGE/BIFL wall	9.3 (2.6, 16.0)	0.008	-	

Table 11: Univariate and multivariate analyses in FD of NT-pro BNP.

5.5 Discussion

The link between the intramyocardial storage of sphingolipids in FD and extracellular scarring is unknown. In this study, the main findings are:

1) In FD, remote myocardial native T1 was low (this is known), but remote T2 was normal (new finding).
2) LGE in FD was different to LGE in HCM or cMI: with lower absolute elevation but a higher rise over background, and a much higher T2 in almost every case.
3) Troponin is elevated in nearly every FD case with LVH or LGE. However, the single key factor associated with troponin was the T2 at the LGE segments.

We believe that the best explanation of these results – which combine blood and imaging biomarkers – is that LGE in the BIFL wall is not just 'scar', but also inflammation. Furthermore, the inflammation would appear to be chronic as the troponin sampling and CMR was at a single (random) time-point however further serial evaluation is required. This leads to the conclusion that once LGE has developed; FD cardiomyopathy is not only a storage disease but also a chronic inflammatory cardiomyopathy – at least until thinning is present. This result has real mechanistic insight and suggests potential novel therapeutic avenues, but appears surprising. Reviewing the literature more widely, however, provides pointers with troponin elevation observed in FD related to LGE and LVH(105,106) in addition to the finding from the PET/MR study.(97)

This result is striking. It contradicts the near universal assumption that FD LGE is scar. This impression arose from the work of my supervisor based on

the histological analysis of one sudden death FD patient where the LGE was associated with thinning. Retrospective review of the images did not show inflammation in this case – just fibrosis. With almost 15 years of an LGE=scar mindset, much work will be needed to change this impression. Further scientific work will be required. In particular, whilst the oedema and cell death explanations of the data are secure, this is not quite the same as "inflammation". Similarly, the type of inflammation is not apparent – is this inflammation "mopping up" myocyte death, or is inflammation causing the mycyte death? We also do not know why the inflammation is sustained and chronic.

Following the publication of my work, preliminary work with endomyocardial biopsies have shown that immune-mediated myocardial inflammation, defined as $CD3^+$ T lymphocytes >7/mm^2 associated with necrosis of glycolipid-laden cardiomyocytes, can be found in FD and correlated with disease severity. (149) However, troponin was not evaluated in this study and T2 weighted-STIR sequence was used instead of T2 mapping, which excluded quantification of T2. Therefore, further histological work is required to prove the correlation between LGE and T2 with inflammation in FD however obtaining endomyocardial biopsies or autopsy from post-mortem may be difficult due to the rarity of the disease.

Limitations of this study include no histological validation, no other additional inflammatory markers such as high sensitivity C-reactive protein, no analysis

of lymphocyte populations, a relatively small sample size, and single center acquisitions. T1 and T2 could be interacting in some unknown way – but even so, the concordance of the results with blood biomarkers is convincing. Thus, this study has potentially important implications for new avenues of therapy given its inflammatory nature. Further larger and more in-depth studies are required to explore these findings.

5.6 Conclusion

Using a combination of blood biomarkers and multiparametric CMR, cardiac FD (when LGE is present) is potentially a chronic inflammatory cardiomyopathy. These data suggest inflammation links storage to extracellular LGE in FD.

Chapter 6 Results: Age and Sex Differences in Fabry Cardiomyopathy

This chapter is based on the following publication: **Nordin S**, Kozor R, Medina-Menacho K, Abdel-Gadir A, Baig S, Sado DM, Lobascio I, Murphy E, Lachmann RH, Mehta A, Edwards NC, Ramaswami U, Steeds RP, Hughes DA, Moon JC. Proposed stages of Myocardial Phenotype Development in Fabry Disease. *JACC Cardiovascular Imaging.* 2018.

My contribution was recruiting and performing CMR scans on 75% of the FD patients. I analysed all the data as first operator, did the statistical analysis and wrote the paper.

6.1 Introduction

Cardiac manifestations in males FD occur earlier than females (average age of cardiac symptoms 32 vs 40 years)(32,34). This include LVH, arrhythmias, chronic inflammation(127), myocardial fibrosis and functional impairment(65). Cardiovascular death is the leading cause of death in both males and females(6,63), with LVH and myocardial fibrosis being amongst the suggested risk factors for ventricular arrhythmia and sudden cardiac death(55). 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(54).

Early data showed the presence of LGE in approximately 50% of patients, initially in the basal inferolateral wall in FD(39), sometimes occurring before

LVH in females(142) or in some mutations in males.(91) There is well-documented literature on differences in Fabry cardiomyopathy between male and female FD by transthoracic echocardiography and CMR with LGE but not in relation to mapping.(142)

6.2 Hypothesis

There is an age and sex differential response in the Fabry myocardium in relation to storage, hypertrophy and inflammation response.

6.3 Methods

6.3.1 Study population

This is a prospective observational study in 182 FD patients in a single UK centre. 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. 12-lead ECG and blood samples were performed on adults just before the scan and analysed for 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).(107)

6.3.2 CMR acquisition

CMR was performed (1.5 Tesla Avanto, Siemens Healthcare, Erlangen, Germany) using a standard clinical protocol as described. Contrast was not administered in participants age <18 years, eGFR <30ml/min/1.73m^2 or if the patient declined.

6.3.4 CMR analysis

All imaging analysis was performed using CVI42 software. A region of interest for native T1 and ECV was manually drawn in the septum with a 20% offset. LVH was defined as MWT >12mm in adults or increased LVMi on CMR according to age and gender matched normal reference ranges in adults and children.(130,147) Normal T1 reference ranges (mean ± 2SD) 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.(59)

6.3.5 Statistical analysis

Statistical analyses were performed using SPSS 24 (IBM, Armonk, NY) as described in the methodologies chapter. In addition, difference in regression slopes was determined using analysis of covariance. A p-value of <0.05 was considered statistically significant.

6.4 Results

There were 167 adults and 15 children included in this study. Baseline characteristics are shown in Table 12. Mean age of the FD cohort was 42±17 years, male gender 37% (68/182). Mean eGFR was 82±17 ml/min/1.73m^2. 11 patients had eGFR <60 ml/min/1.73m^2. 37 patients did not receive contrast at CMR. This is mainly due to patient preference, 3 patients had poor eGFR excluding contrast administration. 51% (92/182) of patients were on ERT with a median ERT duration of 7(IQR 6.4) years. 30% (55/182) had the cardiac variant(30) where 91% (50/55) had N215S mutation, 5% (3/55) R301Q mutation and 4% (2/55) I91T mutation.

	FD male (n=68)	FD female (n=114)	P value
Age, years	41±17	43±17	0.458
BSA, m^2	1.87±0.3	1.75±0.2	0.001*
Heart rate, bpm	60±14	62±11	0.338
Systolic BP, mmHg	122±16	119±18	0.349
Diastolic BP, mmHg	74±12	72±9	0.179
eGFR, ml/min/1.73m^2	79±24	84±11	0.064
Cardiac variant, n (%)	25 (37%)	30 (26%)	0.159
Sokolow (SV1 + RV5 or RV6), mm	38±15	28±10	<0.001*
CMR parameters			
LVEF, %	71±8	73±7	0.010*
LVEDVi, ml/m^2	78±15	69±11	<0.001*
LVESVi, ml/m^2	23±8	19±6	<0.001*
MWT, mm	15±6	10±3	<0.001*
LVMi, g/m^2	111±46	67±19	<0.001*
LVH, n (%)	45 (66%)	31 (27%)	<0.001*
Native T1, ms	861±62	917±49	<0.001*
Low T1, n (%)	53 (78%)	67 (59%)	0.010*
LGE, n (%)	32 (59%)	34 (37%)	0.015*
ECV	0.25±0.03	0.28±0.02	<0.001*
Drug history			
ERT, n (%)	45 (67%)	47 (41%)	0.001*
ACE inhibitor/ARB, n (%)	22 (34%)	29 (26%)	0.245
Beta blocker, n (%)	9 (14%)	6 (5%)	0.048*
Statin, n (%)	10 (15%)	14 (12%)	0.573
Aspirin/clopidogrel, n (%)	13 (20%)	24 (21%)	0.845

Table 12: Demographics of the Fabry disease cohort.

6.4.1 Age related trends of T1 in children and adults in FD

Children: All children were LVH-negative with normal function (mean LVEF 68±6%, LVMi 54±12g/m^2, MWT 7±1mm). LGE and ECV were no assessed because the children did not receive gadolinium. No child had a low T1 (mean native T1 971±31ms), however T1 fell linearly with increasing age (figure 26a). 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 26a).

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. 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 26b). 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, figure 26b). The slope regression between male and female is statistically significant (p<0.001).

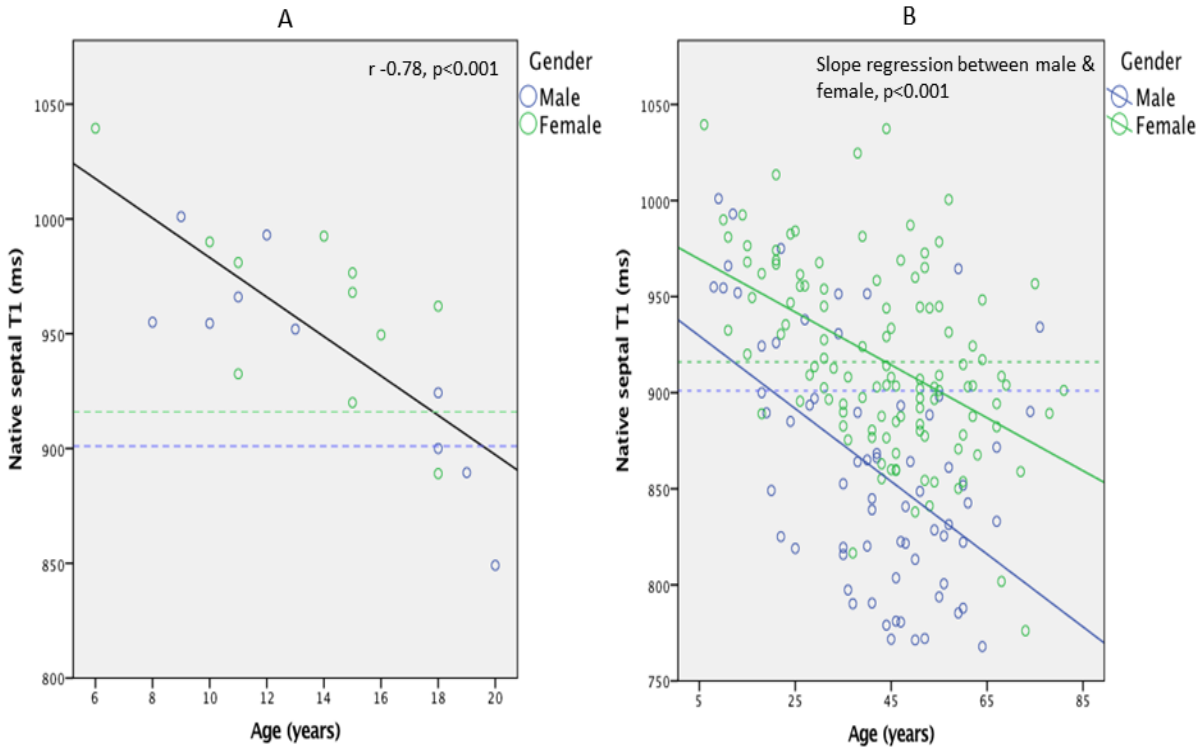

Figure 26: Relationship between age and native T1 in Fabry disease in a) children and adolescent up to 20 years old (r -0.78, p<0.001) and b) total cohort (r -0.41, p<0.001). Reference line on Y-axis showed lower limit of native T1 in male (dotted blue line, 901ms) and female (dotted green line, 916ms).

When T1 is compared with LVH, however, the relationship was sex specific. Before LVH is present, with increasing LVMi, T1 falls in both males and females but more markedly in males (r -0.54, p<0.001 in male; r -0.276, p=0.01 in female; figure 27). After overt LVH is present, a major sex dimorphism is found. In males, the LVH is more marked, 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 uncorrelated to the degree of LVH (r -0.239, p>0.05).

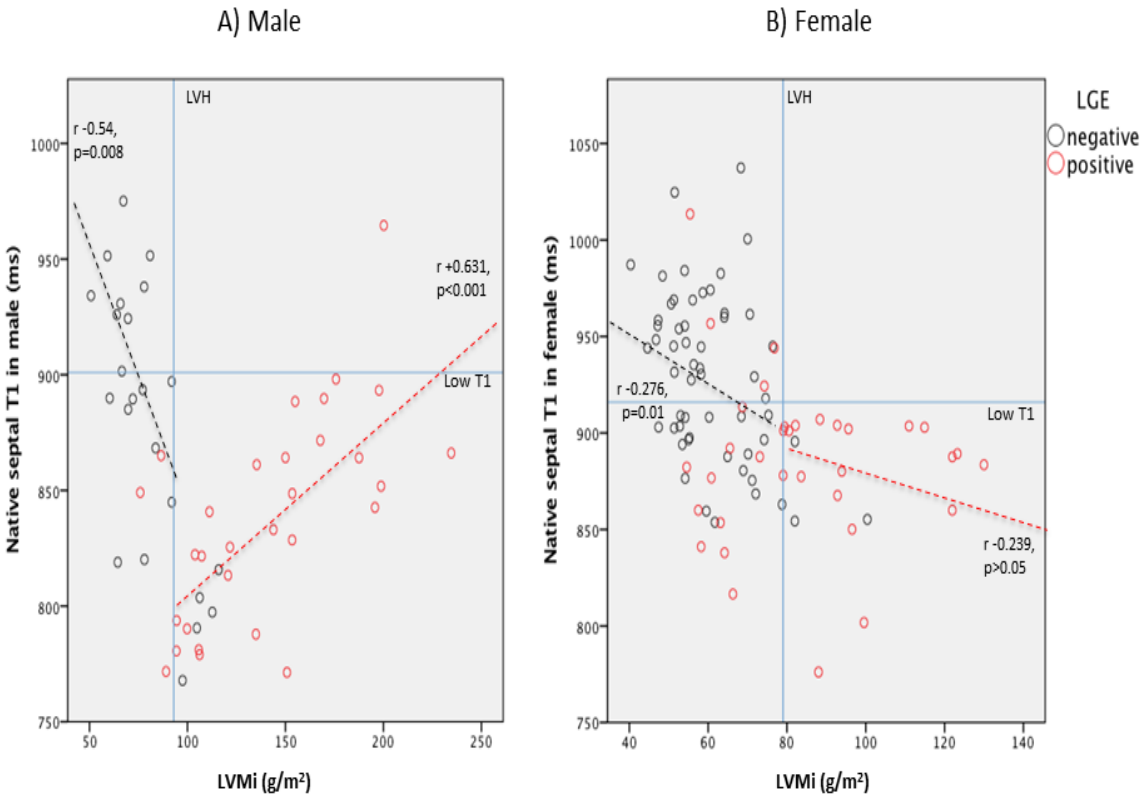

Figure 27: The relationship between native T1, LVMi and LGE in males and females in Fabry disease. A) Males: Dotted black line indicating the correlation between T1 and LV mass: negative when LVH-negative but positive when LVH present. B) This reversal is not present in females. Reference line on Y-axis shows the lower limit of normal native T1 (901ms male; 916ms female), and on X-axis the lower limit of LVH (92g/m^2 male; 79g/m^2 female)

6.4.2 Comparison of CMR parameters, blood biomarkers and ECG changes between age and sex in FD

LVH: LVH prevalence was higher in males than in females (66% [45/68]) vs 27% [31/114]), p<0.001). In both sexes, the prevalence of LVH increased with age (Figure 28a male, 28b female). There were two main differences observed 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 27) - with LVMi in males ranging up to 2.6x average LVMi (based on normal reference ranges) and 1.7x average LVMi for females. MWT was also higher in males (maximum 30mm) and in females (maximum 26mm).

LGE: LGE was more prevalent in males compared to females (59% [32/54] vs 37% [34/91], p=0.015). Prevalence of LGE increased with age in both males and females (figure 28c and 28d). Given that LVH mainly occurs later in women, this meant a much higher frequency of LGE in LVH-negative female.

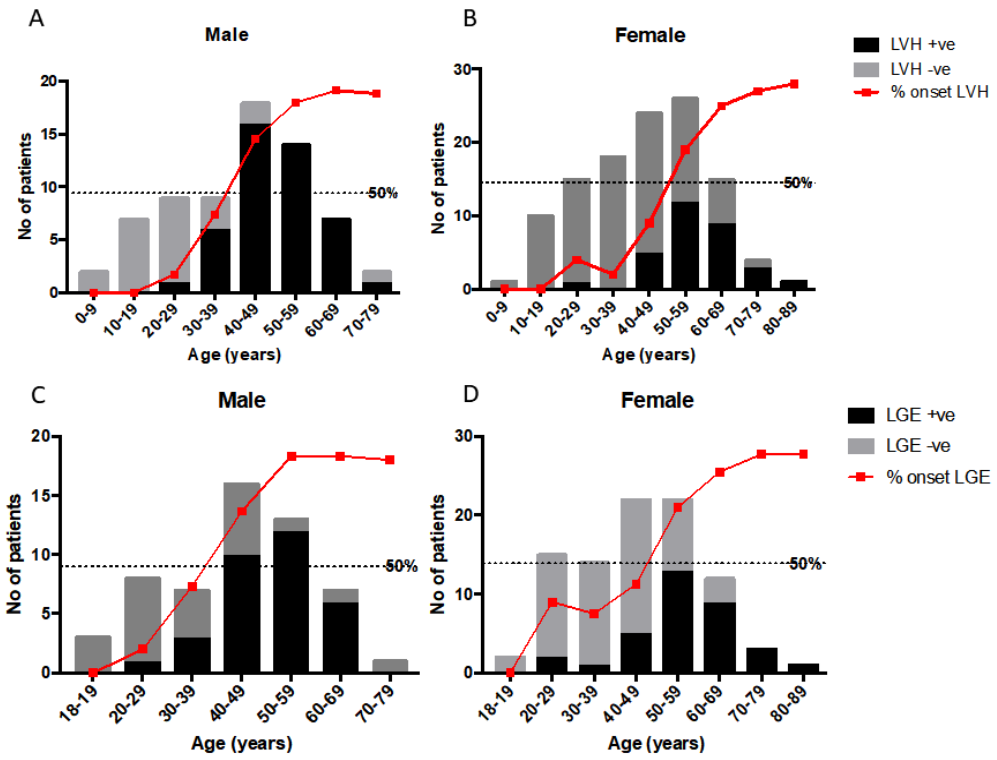

Figure 28: Cumulative onset of LVH and LGE by age group in males and females with Fabry disease. Red line indicates cumulative penetrance.

ECV: Male FD had lower ECV compared to female FD (ECV 0.25±0.03 vs 0.28±0.02; p<0.001). The correlation between ECV and age 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 29a and 29b). 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 29c and 29d). 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 associated with LGE where troponin having the strongest association with presence of LGE (odds ratio 1.191, p=0.008) on multivariate logistic regression analysis. (Table 13)

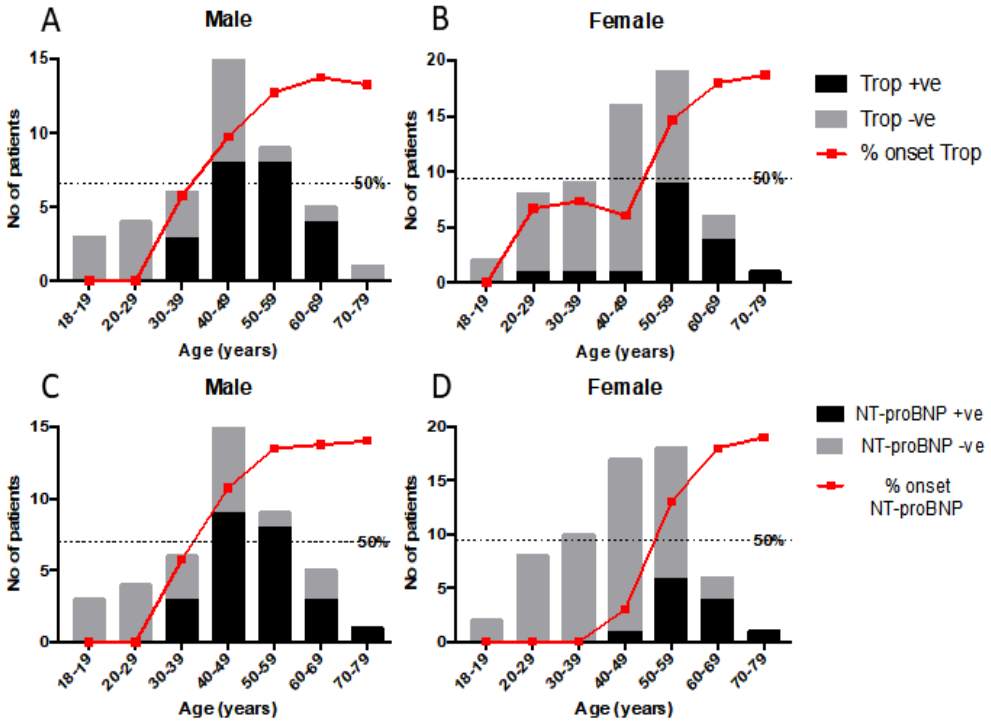

Figure 29: Cumulative onset of blood biomarkers (Troponin and NT-proBNP) by age group in males and females with Fabry disease. Red line indicates cumulative penetrance.

	Odds Ratio	95% CI	P-value
Troponin	1.191	1.047-1.354	0.008*
NT-proBNP	1.017	0.994-1.041	0.153
LVMi	0.996	0.952-1.042	0.873
Native T1	0.978	0.957-0.999	0.041*
Age	1.029	0.958-1.106	0.429
Gender	1.831	0.188-17.833	0.603
eGFR	1.039	0.974-1.108	0.25

Table 13: Multivariate logistic regression analysis of LGE in Fabry Disease (*P<0.05 considered as statistically significant).

ECG: The prevalence of ECG changes increased with age in males and females (figure 30a and 30b), observed to be earlier in males (18-19 years) than females (20-29 years). Table 14 shows the types of ECG changes found in this cohort of patients.

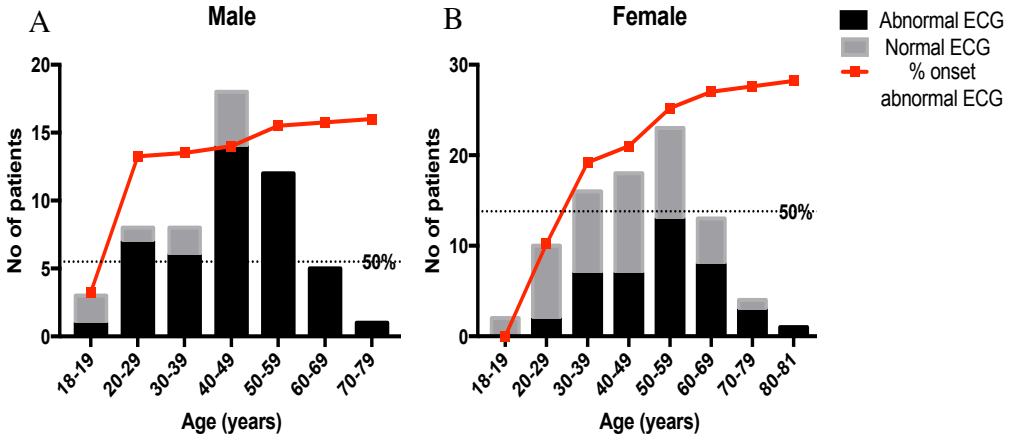

Figure 30: Cumulative onset of ECG changes by age group in males and females with Fabry disease. Red line indicates cumulative penetrance.

ECG changes	Percentage (%)
1st degree AV block (PR >200ms)	7
Short PR interval (PR <120ms)	17
Sokolow criteria (SV1+RV5 or RV6)	57
Atrial fibrillation	5
T wave inversions/repolarisation abnormalities	63
Complete LBBB/RBBB	15

Table 14: ECG changes in males and females with Fabry disease.

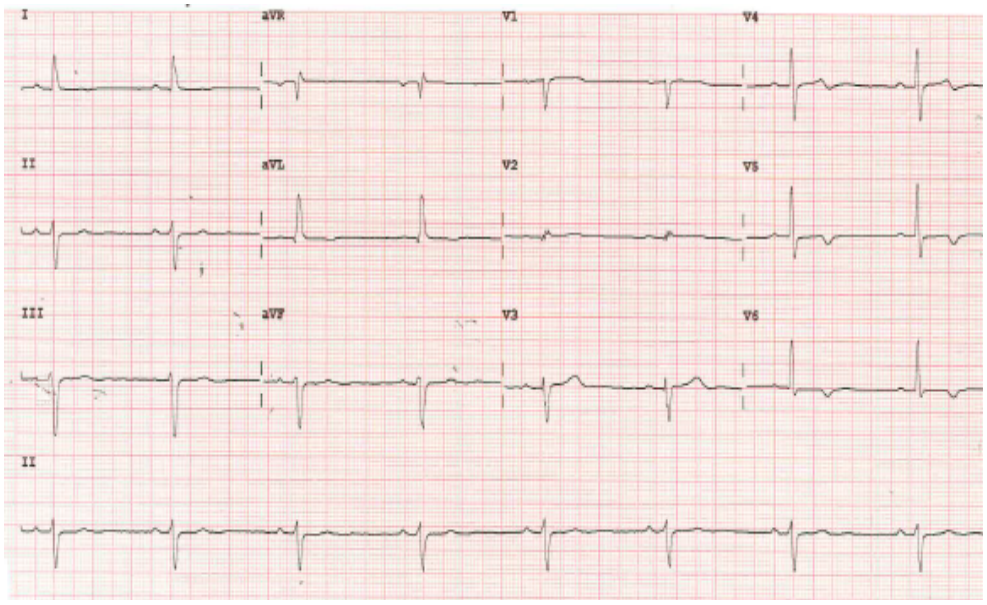

Figure 31: Example of a female LVH-negative FD patient with low T1 and LGE at the basal inferolateral wall with ECG changes (sinus bradycardia [heart rate 47 beats per minute] and T wave inversion V4-V6).

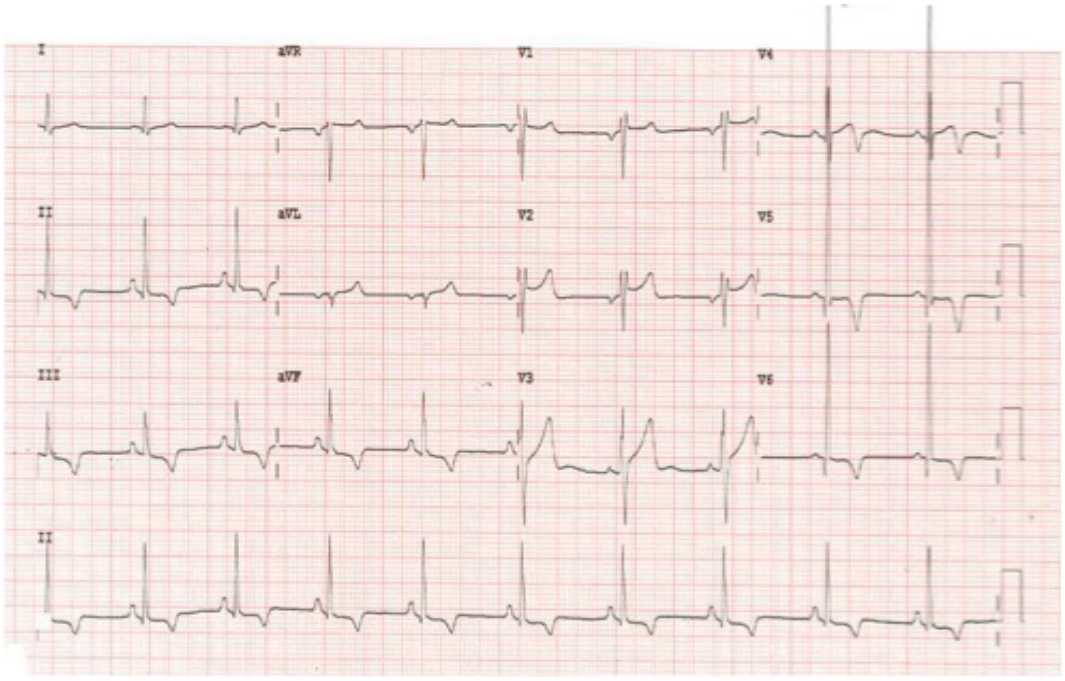

Figure 32: Example of a male LVH-positive FD patient with low T1 and LGE with ECG changes (LVH voltage criteria and repolarization abnormalities).

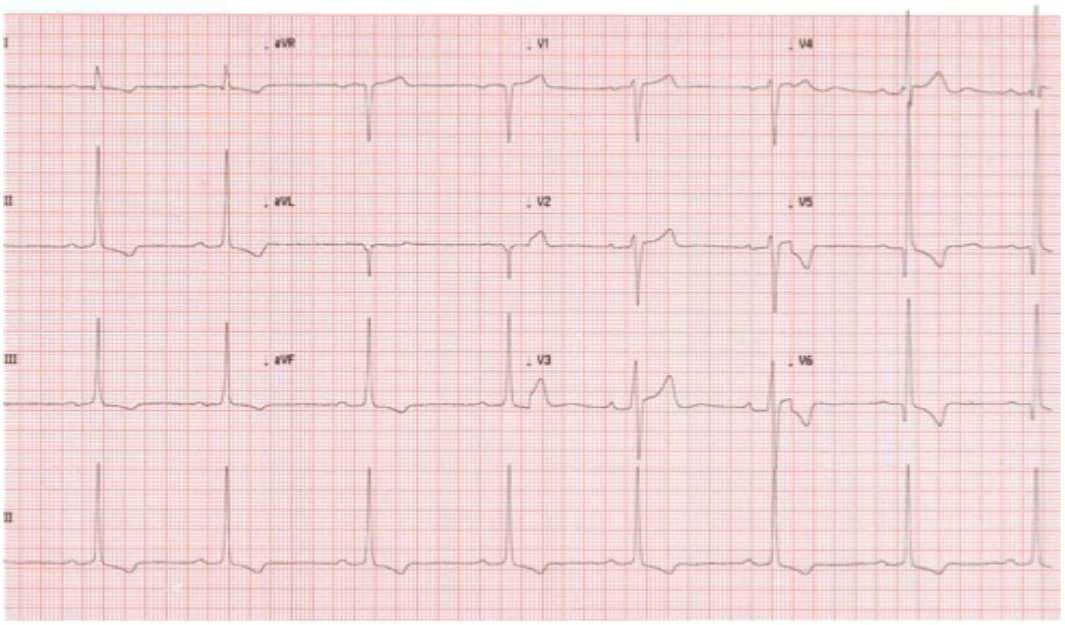

Figure 33: Example of a male LVH-positive FD patient with low T1 and LGE with ECG changes (sinus bradycardia [heart rate 46 beats per minute], 1st AV block [PR interval 235ms] with LVH voltage criteria and repolarization abnormalities.

ERT:

Patients on ERT had higher MWT (14±5 vs 10±3mm), LVMi (98±42 vs 69±26 g/m^2) and lower septal T1 value (876±43 vs 916±60 ms), all p<0.001. The use of ERT was increasingly prevalent with age in both sexes, however the age group of ERT initiation was similar (10-19 years). In males, 78% (36/46) with LVH were on ERT and 74% (23/31) in females, both p<0.005. T1 values were not correlated with ERT duration in the total cohort (r_s 0.004 p=NS). ERT duration between T1 values in males and females were uncorrelated (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 ERT patients compared to ERT-naïve patients (Figure 34).

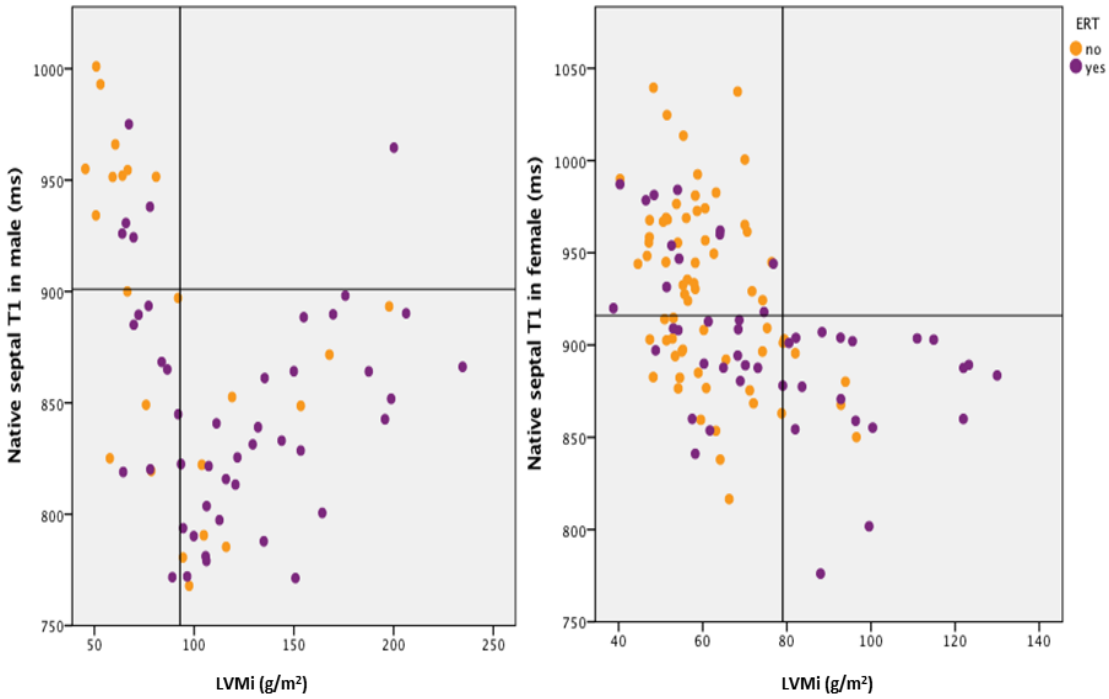

Figure 34: The relationship between native T1, LVMi and ERT in males and females in Fabry disease.

6.5 Discussion

This study aims to seek insight into myocardial phenotype development in FD by looking at a large cohort of patients (including children for the first time) at a single timepoint 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 more marked with males compared to females, suggesting storage is faster in males.

Males had earlier ECG changes, blood biomarker increase and LVH; 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 starts to plateau, but in males, after LVH is present, T1 is higher (normalizing T1) with increased LVH. What does the apparent T1 rise in males mean? Storage cannot be the cause of this (otherwise T1 would be falling instead). T1 is a composite signal from myocardial interstitium (fibrosis, oedema – 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, oedema 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. It is likely the answer is that in males, storage is triggering sarcomeric protein expression – causing myocyte hypertrophy(19,21) 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.

Multiple groups have shown that ECV is normal in Fabry disease.(93,94) The difference in ECV between male and female is most likely explained by normal sex differences where ECV in FD(93) and in healthy controls is known to be higher in female than in male(93,94,148) 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. In Chapter 5, I have shown that LGE in established FD is chronic inflammation strongly correlating with troponin levels, supporting findings by PET/MR study(97) and endomyocardial biopsy.(149) Here again, troponin was independently and strongly related to LGE, although we did not report T2 values in this study.

Based on these findings and combined with my prior work on LVH-negative patients in Chapter 4 and on LGE including inflammation imaging in Chapter 5, 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 is hypothesized. This later phase is likely to be underrepresented in our study, partly as these patients have devices or have a significant mortality rate. These phases will be discussed in more detail in the discussion chapter (Chapter 8).

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 significant age effect in T1 values in the paediatric group(148,150). In addition, this is also a single centre study with single time-point data therefore the findings in this study are hypothesis generating only based on experiences and knowledge collaborated from my published works. Further longitudinal studies are needed (and planned by my successor) to explore this further.

6.6 Conclusion

Sphingolipid accumulation potentially starts in childhood, proceeds more markedly in males compared to females before triggering an apparently sex-independent scar or inflammation response but a sexually dimorphic myocyte hypertrophic response in males.

Chapter 7 Results: Serial multiparametric assessment in Fabry Cardiomyopathy with enzyme replacement therapy

This chapter has been submitted for publication: **Nordin S,** Kozor R, Vijapurapu R, Augusto J, Knott K, Captur G, Treibel TA, Ramaswami U, Tchan M, Geberhiwot T, Steeds RP, Hughes DA, Moon JC. Myocardial storage, inflammation and cardiac phenotype in Fabry Disease after one year of enzyme replacement therapy.

My contribution was recruiting and performing CMR scans on 70% of the FD patients. I analysed all the data as first operator, did the statistical analysis and wrote the paper.

7.1 Introduction

ERT has been the mainstay of treatment with oral chaperone newly available for patients with amenable mutations. Timing of initiation of treatment seems important. The presence of overt LVH and myocardial fibrosis have been shown to negatively affect ERT outcome suggesting the importance of early initiation of treatment (68,112). Cardiac response to ERT is typically assessed by measuring the LVMi using either TTE or CMR. However, this method does not quantify myocardial biology. The effect of treatment on myocardial storage is not widely reported, as invasive myocardial biopsy was required to measure this previously (40,151).

Multiparametric mapping by CMR is now a non-invasive tool to assess myocardial storage and other disease processes such as inflammation. Myocardial strain measured by global longitudinal strain (GLS) using feature-tracking CMR has been recently shown to be impaired with hypertrophy, storage (measured by low native T1) and LGE in FD (152). Understanding the effect of ERT on these now measurable pathways is difficult as it is not acceptable to randomize patients fulfilling treatment criteria. We therefore designed an observational study to map the one-year evolution of these processes in stable untreated and stable treated disease, and to compare this to the changes of a group of patients starting ERT for the first time.

7.2 Hypothesis

After one year of ERT, there is improvement in LVMi and reduction in myocardial storage. In the ERT-naïve group, there is increase in LVMi with reduction in myocardial storage. In the ERT-established group, there is no change in LVMi and myocardial storage.

7.3 Methods

7.3.1 Study population

This was a prospective, multicenter, international observational study of 56 FD patients. Participants were recruited from three Fabry clinics (United Kingdom (UK): Royal Free Hospital London, Queen Elizabeth Hospital Birmingham; Australia: Westmead Hospital Sydney).

Inclusion criteria included gene-positive FD males and females age 18 years who underwent assessment at baseline and 12 months. These were in 3 groups: FD participants starting ERT, and two further groups: FD patients stable on ERT (‘established on ERT’), and FD ERT naïve participants. FD is a disease that evolves slowly. Across the different pathological processes measured in this study, and reflecting the X-linked nature of the disease and treatment guidelines, change over one year in the ERT naive group will reflect early untreated disease (likely more female, more LVH negative) and changes over one year in the ERT treated group will reflect more advanced treated disease trajectory (likely more male, more LVH positive). The group starting ERT will likely represent an intermediate stage group – but with the potentially powerful impact of 1 year of new ERT detectable when compared to the other two groups. Patients on oral chaperone were excluded.

Baseline and one year assessment included CMR, estimated glomerular filtration rate (eGFR) and blood biomarkers (Troponin and NT-proBNP).(107)

7.3.2 CMR

All participants underwent CMR at 1.5 Tesla at 4 sites (London – Avanto and Aera, Birmingham – Avanto, Sydney – Avanto); Siemens Healthcare, Erlangen, Germany) using a standard clinical protocol with LGE imaging using phase sensitive inversion recovery. T1 mapping using a MOLLI sequence and T2 mapping were performed pre-contrast bolus administration (0.1 mmol/kg

body weight, Gadoterate meglumine, Dotarem, Guerbet S.A., France) on basal left ventricular SA slices. Post-T1 mapping was performed 15 minutes after contrast administration for ECV quantification. Contrast was not administered if eGFR<30ml/min/1.73m^2 or if the patient declined. Paired scans used identical sequences and magnets without upgrades, and phantom controls measured magnet stability for native T1 and T2 sequences.

7.3.3 CMR analysis

All analysis was blinded to clinical status. The presence of LGE was assessed by 2 independent observers. The amount of LGE was quantified using the threshold method of 5 standard deviations above the mean remote myocardium presented in grams. 2-dimension GLS were obtained by manually drawing epicardial and endocardial contours on the end diastolic frame of long axis images (4-chamber, 2-chamber and 3-chamber views) with strain obtained using applied automatic feature-tracking algorithm (152).

A ROI for native T1 and ECV was manually drawn in the septum with a 20% offset, taking care to avoid the blood-myocardial boundary. T2 at the LGE area assessment was performed by manually drawing a ROI over areas corresponding to LGE (127). For cases without LGE, a ROI was drawn in the basal inferolateral segment. Normal native T1 and T2 reference ranges (mean ± 2 SD) were defined using age and gender-matched healthy controls from each individual center, with the group considered as a whole for T2 and split by gender for native T1 as native T1 is known to vary with gender (146,153).

The T1MES phantom was scanned as part of the T1MES multicenter study according to the user manual instructions distributed to centers and as previously described.(95) Scanner room temperatures were stable throughout the test period at 20±0 °C for the 2 London sites, and 21±1 °C for Birmingham and Sydney sites. Unadjusted for temperature, serial MOLLI T1 times across the 9 tubes were highly stable with site-specific coefficient of variation (CoV) of 0.805% and 0.751% for the London sites (Avanto and Aera scanners respectively), 0.625% Birmingham, and 0.846% Sydney. Serial T2 times across the 9 tubes were also stable with site-specific CoV of 1.10% and 1.07% for the London sites, 1.06% Birmingham, and 1.06% Sydney.

7.3.4 Statistical analysis

Statistical analyses were performed using SPSS 24 (IBM, Armonk, NY) as described in the methodologies chapter The CoV between serial repeated phantom scans was calculated as a compound measure of all causes of change in the estimated native T1 and T2 of all 9 tubes. A p-value of <0.05 was considered significant.

7.4 Results

56 participants were scanned at baseline and follow up (mean 1.1±0.2 years). Baseline characteristics are shown in Table 15. Five additional recruited

patients were excluded: one patient underwent permanent pacemaker insertion and four patients who started ERT did not attend follow up.

	FD initiated on ERT (n=20)	FD established ERT (n=18)	FD treatment naïve (n=18)
Age, years	49±10	53±13	41±12
Male, n (%)	7 (35%)	10 (56%)	3 (17%)
BSA, m^2	1.86±0.20	1.78±0.19	1.84±0.27
Heart rate, bpm	62±12	58±10	61±13
Systolic BP, mmHg	127±15	129±20	118±18
Diastolic BP, mmHg	74±9	76±12	71±10
Hypertension, n (%)	2 (11%)	4 (22%)	2 (11%)
Atrial fibrillation, n (%)	1 (5%)	3 (17%)	1 (6%)
eGFR (ml/min/1.73m^2)	85±18	63±27	83±12
Cardiac variant, n (%)	7 (35%)	2(11%)	9 (50%)
Drug history			
Aspirin/clopidogrel, n (%)	1 (5%)	5 (28%)	2 (11%)
ACEi/ARB, n (%)	3 (15%)	8 (44%)	3 (17%)
Beta-blocker, n (%)	3 (15%)	2 (11%)	0 (0%)
Statin, n (%)	3 (15%)	6 (33%)	0 (0%)
CMR parameters			
MWT, mm	14.8±5.9	17.5±4.7	9.8±2.8
LVMi, g/m^2	93±42	124±45	65±15
LVH, n (%)	12 (60%)	15 (83%)	3 (17%)
LVEF, %	74±7	71±5	70±7
LVEDVi, ml/m^2	74±14	64±12	67±9
LVESVi, ml/m^2	19±6	18±5	20±6
GLS, %	-16.6±4.0	-13.2±3.4	-19.2±2.6
Low native T1, n (%)	16 (80%)	13 (72%)	8 (44%)

Table 15: Baseline demographic characteristics of the FD cohort according to treatment status.

7.4.1 Group initiated on ERT:

20 participants were scanned at baseline (pre-ERT) and at 1 year, mean age 49±10 years. 10 were on agalsidase alfa (Replagal) and 10 on agalsidase beta (Fabrazyme). 60%(12/20) had baseline LVH, 80%(16/20) had low T1 and 74%(14/19) had LGE. One patient did not received contrast at baseline due to previous allergic reaction. 35%(7/20) were males; 35%(7/20) had a known cardiac variant (6 N215S mutation and 1 R301Q mutation).

Of these 20 patients, 12 (60%) were LVH positive. The ERT indication was mainly LVH. Of those with LVH, 58%(7/12) were males, mean age 53±12 years. All had LGE at baseline. 83% (10/12) had low T1 at baseline. The 2 LVH-positive patients with normal native T1 included a female with apical hypertrophy and a male with extensive LGE including the septal area. 83%(10/12) had an increase in T1 value after 1 year of ERT. 8(40%) were LVH negative. The ERT indication in these 8 patients was still mainly cardiac eg LGE on CMR, but also other organ involvement eg transient ischaemic attack, renal decline, or gastrointestinal symptoms. All LVH-negative patients started on ERT were female, mean age 43±9 years. Of the LVH-negative patients, 71%(5/7) had LGE (one patient did not received contrast at baseline due to previous allergic reaction) and 75%(6/8) had low native T1 at baseline. 50% (4/8) had an increase in T1 value after 1 year of ERT.

Over 1 year of ERT initiation, there was a small reduction in MWT (14.8±5.9mm vs 14.4±5.7mm, $p<0.05$) but no change in LVMi (93±42g/m^2 vs

92±40g/m^2, p>0.05). There was a small reduction in T1 lowering (partial normalization) (917±49ms vs 931±54ms, p<0.05). T2 in the LGE area, LGE presence, absence or extent, septal ECV and GLS were unchanged (T2 55±6ms vs 56±6ms, LGE 5.6(0-16.9)g/m^2 vs 4.3(1.8-18.5)g/m^2, ECV 0.26±0.04 vs 0.26±0.04, GLS -16.6±4.0 vs -16.2±3.9; all p>0.05), as were troponin and NT-proBNP levels (Troponin 20(7-34)ng/L vs 23(9-40)ng/L and NT-proBNP 18(12-83)pmol/L vs 27(13-114)pmol/L; all p>0.05).

These changes were driven by the LVH-positive group, who had a small reduction in LVMi (117±38 g/m^2 vs 114±36 g/m^2, p<0.05) and reduction in T1 lowering (partial normalization) (902±47ms vs 920±48, p<0.01), Figure 35. There was no significant change in other parameters (MWT 17.9±5.6mm vs 17.6±5.1mm, T2 57±5ms vs 58±6ms, ECV 0.27±0.036 vs 0.27±0.043, LGE quantification 9(5-19)g/m^2 vs 15(4-22)g/m^2, GLS -15.3±3.6 vs -14.6±4.2, troponin 31(28-50)ng/L vs 37(29-45)ng/L and NT-proBNP 78(20-218)pmol/L vs 97(33-154)pmol/L (Table 16). All markers were unchanged in the LVH-negative group (LVMi 58±10g/m^2 vs 58±10g/m^2, MWT 10.1±1.9mm vs 9.6±2.3mm, native T1 940±46ms vs 948±60ms, T2 52±3ms vs 52±4ms, ECV 0.25±0.04 vs 0.25±0.02, LGE quantification 0(0-2) g/m^2 vs 1(0-3) g/m^2, GLS -18.6±4.0 vs -18.6±1.8, Troponin 7(1-13)ng/L vs 10(1-15)ng/L and NT-proBNP 13(6-18)pmol/L vs 17(1-24)pmol/L; all p>0.05), Table 17.

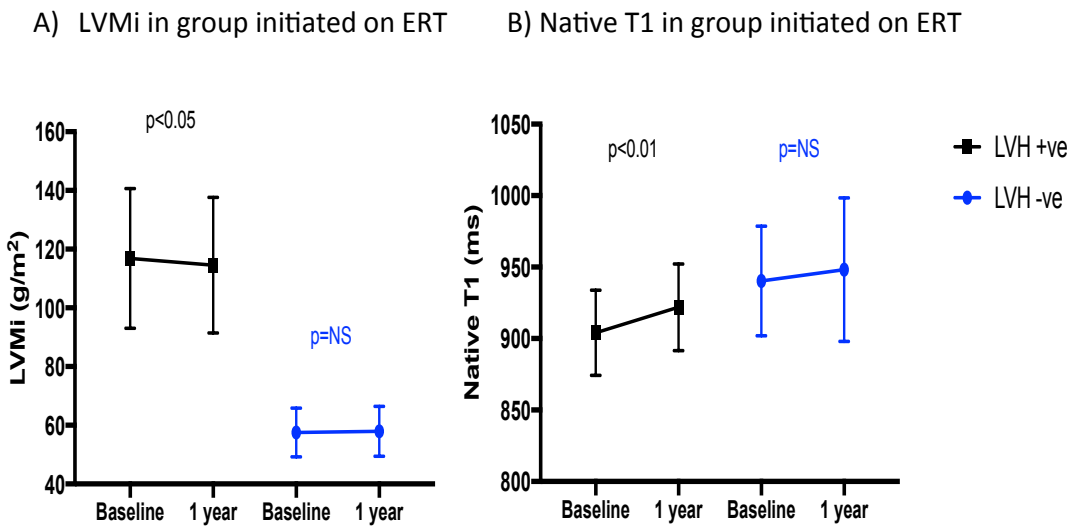

Figure 35: LVMi and native T1 before and after initiation of ERT at 12 months in LVH-positive and LVH-negative FD. Error bars represent 95% confidence interval.

LVH-positive	Initiated on ERT (n=12)			Established on ERT (n=15)		
	0 month	12 months	P value	0 month	12 months	P value
MWT (mm)	17.9±5.6	17.6±5.1	NS	19.1±3.4	19.4±3.5	NS
LVMi (g/m^2)	117±38	114±36	<0.05	138±35	139±35	NS
Native T1 (ms)	902±47	920±48	<0.01	912±54	905±49	NS
T2 (ms)	57±5	58±6	NS	59±5	62±6	<0.05
ECV	0.27±0.04	0.27±0.04	NS	0.28±0.03	0.28±0.04	NS
GLS (%)	-15.3±3.6	-14.6±4.2	NS	-12.7±3.1	-11.2±4.8	<0.05
Troponin (ng/L)	31(28-50)	37(29-45)	NS	53(30-92)	61(34-101)	<0.05
NT proBNP (pmol/L)	78(20-218)	97(33-154)	NS	74(38-270)	71(29-223)	NS

Table 16: CMR and blood biomarkers parameters LVH-positive FD prior to initiation of ERT and 12 months after ERT and LVH-positive FD established on ERT.

LVH-negative	Initiated on ERT (n=8)			Treatment naïve (n=15)		
	0 month	12 months	P value	0 month	12 months	P value
MWT (mm)	10.1±1.9	9.6±2.3	NS	8.9±1.6	9.3±1.7	<0.01
LVMi (g/m^2)	58±10	58±10	NS	60±10	62±11	0.01
Native T1 (ms)	940±46	948±60	NS	990±50	975±51	<0.01
T2 (ms)	52±3	52±4	NS	50±4	49±4	NS
ECV	0.25±0.04	0.25±0.02	NS	0.29±0.04	0.27±0.03	NS
GLS (%)	-18.6±4.0	-18.6±1.8	NS	-19.9±2.0	-19.6±2.0	NS
Troponin (ng/L)	7(1-13)	10(1-15)	NS	1(1-4)	4.5(1-10)	<0.05
NT proBNP (pmol/L)	13(6-18)	17(1-24)	NS	1(1-12)	1(1-7)	NS

Table 17: CMR and blood biomarkers parameters of LVH-negative FD prior to initiation of ERT and 12 months after ERT and LVH-negative FD who are treatment naive.

7.4.2 Advanced stable disease group (established on ERT):

The 18 patients established on ERT were 53±13years with median ERT duration 4.2(1.4-12.2)years. 10 were on Replagal and 8 on Fabrazyme. 83% (15/18) had LVH and 56% (10/18) were males. 72%(13/18) had low T1 and 73%(11/15) had LGE. 3 did not receive contrast at baseline due to poor renal function. 11% (2/18) had a known cardiac variant (N215S and I901T mutations).15/18 (83%) patients were LVH-positive of which 60%(9/15) were males. 4 LVH-positive patients with normal T1 had septal LGE to explain the 'pseudonormal' T1.

Over 1 year, there was no significant difference in MWT and LVMi (17.5±4.7mm vs 17.8±4.9mm and 124±45g/m^2 vs 125±45g/m^2 respectively; $p>0.05$), Figure 29. Native T1, ECV and LGE quantification were unchanged (916±52ms vs 912±50ms, 0.28±0.03 vs 0.28±0.04 and 13(4-28)g/m^2 vs 15(6-29)g/m^2, respectively, $p>0.05$). However, there was a significant increase in T2 in the LGE area and troponin at 1 year (T2 57±6ms vs 60±7ms; troponin 43(29-90)ng/L vs 48(30-99)ng/L; both $p<0.05$). GLS also became more impaired after 1 year (-13.2±3.4 vs -12.1±4.8, $p<0.05$) but NT-proBNP was unchanged after 1 year (60(16-233) pmol/L vs 70(21-220) pmol/L, $p>0.05$). Within this group, comparing LVH negative and positive (baseline, follow-up) subgroups was not statistically possible as there were only 3/18 that were LVH negative.

7.4.3 Group not on ERT (treatment-naïve):

The 18 patients not on ERT were 41±12 years. 17% (3/18) were males. 17% (3/18) had LVH. 44%(8/18) had low T1 and 21%(3/14) had LGE. 4 patients declined contrast administration. 50% (9/18) had a known cardiac variant (N215S mutation). 15/18 (83%) patients were LVH-negative of which 93%(14/15) were females.

Over 1 year, there was a small increase in MWT and LVMi (9.8±2.7mm vs 10.2±2.6mm; p=0.01 and 65±15g/m^2 vs 67±16g/m^2; p=0.005), Figure 36. 1 participant who was LVH-negative with low native T1 at baseline scan was found to progressed to LVH. No patients developed new LGE. There was a reduction in native T1 981±58ms vs 959±61ms; p=0.002, no patient became low T1. T2, ECV and GLS were all normal at baseline and unchanged over 1 year (T2 50±3ms vs 50±4ms, ECV 0.29±0.04 vs 0.27±0.03, GLS -19.2±2.6 vs -19.1±2.3, p>0.05. There was an increase in Troponin 1(1-4) ng/L vs 4.5(1-10) ng/L; p<0.05. NT-proBNP was unchanged 1(1-12) pmol/L vs 1(1-7) pmol/L; p>0.05. Within this group, comparing LVH positive to LVH negative (baseline, follow-up) subgroups is not statistically possible as there were only 3/18 that were LVH positive.

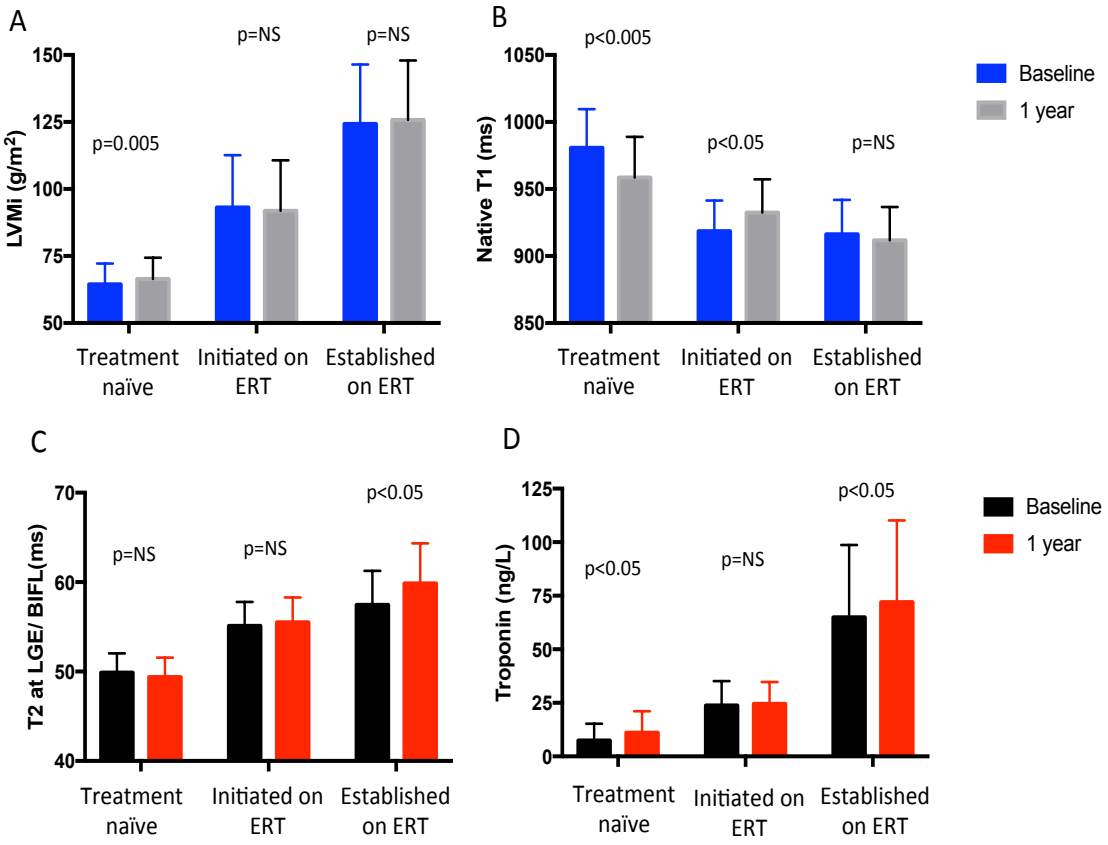

Figure 36: Left ventricular mass indexed, native T1, T2 at LGE area or basal inferolateral wall if no LGE and troponin levels at baseline and 12 months in all 3 groups. Error bars represent 95% confidence interval.

7.5 Discussion

In this study, insight into the biology of the FD myocardium is non-invasively gained by serially evaluating the effect of ERT on the FD myocardium after one year of initiation using multiparametric CMR and blood biomarkers. These patients were compared with two stable control groups - one early untreated group and one advanced treated group. The study found that, over 1 year, early, stable (more female, mainly LVH negative) treatment naïve FD have an

increase in LV mass, and a fall in T1 and a small increase in mean troponin. More advanced, stable on treatment (more male, more LVH positive) FD has no change in mass, T1 or LGE area – but T2 and troponin increase with increased impairment in myocardial strain (GLS). What did the new ERT treated group do? If ERT had no cardiac effect, then the expectation would that they would be intermediate between these two groups: ie no or a small increase in mass; no or a small decrease in T1 with no change or a small increase in T2 and troponin. Instead, I found that they did not change at all if they were LVH negative; but if LVH positive, they had a small improvement in LV mass and a small improvement in T1 (partial normalization).

The changes I observed in myocardial native T1 and T2 times in the study participants was unlikely due to technical instability in the CMR systems across sites or to environmental shifts, since the phantom data suggested a high level of data consistency with no drift over time. Changes were however small, each group was also small and we caution against over-fitting and over-interpreting the data.

The LV mass results with ERT initiation is consistent with previous findings where a decrease in LVMi was observed in FD with LVH and no change observed in those without LVH during the first year,(154) although here the changes were small.(151,155-157). Reductions in LVMi and myocardial Gb3 content have been previously demonstrated by myocardial biopsy (40,151). Data on untreated FD is limited however previous studies have demonstrated

an increase in LVM in untreated FD (37,158). Future studies – larger, longer or with new therapeutic approaches (chaperone/gene therapy) will provide more insights.

Study limitations include no histology, the lack of a full understanding of the quantitative link between storage and T1 lowering, the relatively small sample size reflecting the rarity of the disease, and the study design given the non-availability of randomization. The link between pathophysiological process and outcome is unknown.

7.6 Conclusion:

Over 1 year using multiparametric CMR and biomarkers, the FD myocardial phenotype changes. These changes are different in early (untreated mainly female, mainly LVH negative) disease compared to more advanced (treated, mainly male, mainly LVH positive): with early disease small increases in LV mass and more storage, and with late disease small increases in inflammation and small reductions in strain – both have small increases in troponin. Intermediate patients starting ERT are different with a small improvement in LV mass and a small improvement in myocardial storage, compatible with a small ERT but clear treatment effect on myocardium. Whether or how these effects translate to patient morbidity or mortality reduction with cumulative lifetime use, and whether the effects represent solely endothelial or endothelial and myocardial effects remain unclear.

Chapter 8: DISCUSSION AND CONCLUSION

Cardiovascular death is the leading cause of the death in FD in both males and females.(5,6) Detection of early cardiac involvement is important. Treatment appears less effective once LGE (considered to date fibrosis) and overt left ventricular hypertrophy occur (110) however, treatment is onerous and expensive thus phenotypic justification is required to commence patients on treatment.

CMR has shown to be a valuable non-invasive tool in the diagnosis and management of FD. The development of multiparametric mapping in combination with use of other modalities eg blood biomarkers and ECG has provided a platform to aid early diagnosis of FD and further refine our understanding of this complex disease.

As a results of the work for this thesis:

1) I assembled the largest CMR cohort ever performed at 4 sites.
2) I have explored the early changes in pre-hypertrophic or LVH-negative FD patients in relation to storage measured by native T1 in chapter 4.
3) I have also refined the technique of low native T1 detection in the LVH-negative cohort in chapter 4.
4) I have shown that late gadolinium enhancement in FD, when there is no thinning is possibly more inflammation than fibrosis in chapter 5.

5) In chapter 6, I then explored the relation of the Fabry myocardium with multiple parameters (multiparametric mapping, ECG and blood biomarkers) in a large cohort of FD patients (children and adults). With the findings in this chapter and from insights I gained from previous chapters, I then developed a proposed myocardial phenotype evolution in FD by CMR.
6) By using multiparametric CMR and blood biomarkers in chapter 7, I have shown that FD myocardial phenotype changes after one year depending on the stage of the disease. After one year of ERT initiation, there is a small improvement in LV mass and myocardial storage measured by T1.

8.1 Key findings and implications (clinical insights and potentials)

8.1.1 Assessment in LVH-negative FD

Prior to my work, low native T1 has been observed in approximately 40-50% of LVH-negative FD and has promising potential in early detection of cardiac involvement in FD.(88,89) ECG changes(47,126) and elevated blood biomarkers (eg troponin and NT-proBNP) have also been previously described.(107,127,128) In this thesis, native T1 lowering when observed visually on the colour maps was found to be ‘patchy’. By increasing the number of SA slices and segments for ROI assessment, the prevalence of low native T1 increased from 41% to 59%.

Certain similarities to pre-clinical HCM were observed in pre-hypertrophic FD eg higher LVEF, longer AMVL and increased trabeculations, which were native T1-independent in the latter two features.(129) Differences include the native T1 lowering which is unique to FD, the absence of crypts (crypts occur in preclinical HCM),(159) early papillary muscle mass increase, and LGE as a pre-LVH FD feature observed in females, linking to troponin elevation.

Multiple abnormalities were found to be associated with low native T1. ECG abnormalities were present in 41% of the LVH-negative cohort and were twice more likely with low native T1. LV maximum wall thickness, indexed mass and ejection fraction were higher with low native T1. LGE was five times more common with low native T1. 31% of patients were found to have both low native T1 and ECG abnormalities therefore, this subgroup – with evidence of both storage and an electrical response to storage is hypothesized most likely to progress and early treatment in this subgroup may have the most clinical impact in this subgroup. By contrast, those without storage are hypothesised to be stable and potentially less in need of ERT.

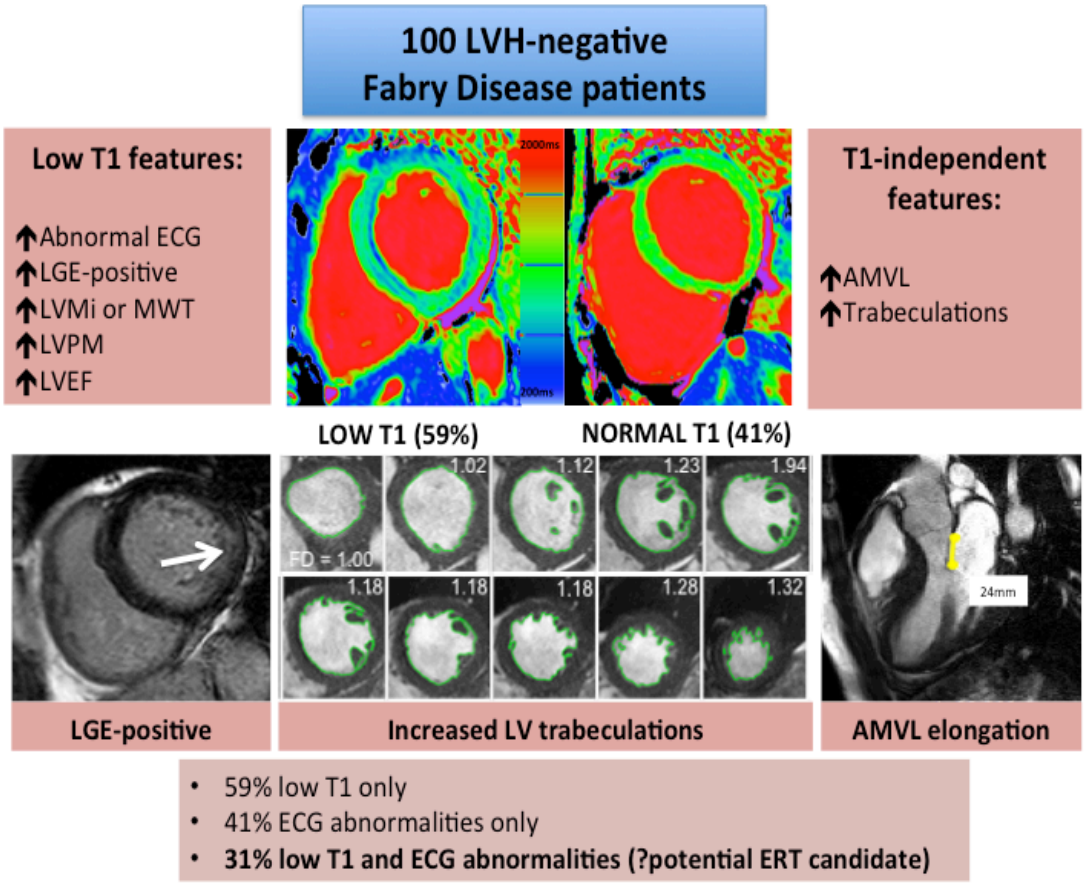

Figure 37: Summary of findings of early changes in Fabry cardiomyopathy

8.1.2 Inflammation in FD

Prior to my work, there has been hints in various publications about possibility of inflammation in FD. Troponin levels were observed to be high in FD and was thought to be related to LGE and LVH.(105,106) A small study of 13 FD patients using PET/MR has demonstrated possibility of inflammation in FD.(97)

In this thesis, I have shown that FD with LGE is likely chronic inflammation by using a combination of multiparametric mapping and blood biomarkers. T2 in

LGE area is found to be significantly elevated in FD patients compared to other comparator disease cohorts. T2 in LGE area were significantly correlated with troponin. The single best multivariate predictor of troponin in FD was T2 in LGE area. This data suggest inflammation may be the link between storage and extracellular LGE in FD, thus creating potential for new therapeutic targets. Following the publication of this work, several publications have emerged recently on inflammation on FD with histological validation(149) and using other inflammatory biomarkers.(160)

Similar concept has been demonstrated in other cardiomyopathies. Recently, it has been shown that T2 is elevated in AL cardiac amyloidosis and was associated with poor outcome. The underlying pathogenesis in these patients was thought to be myocardial oedema.(161) The association of T2 and troponin have also been recently explored in HCM.(162,163)

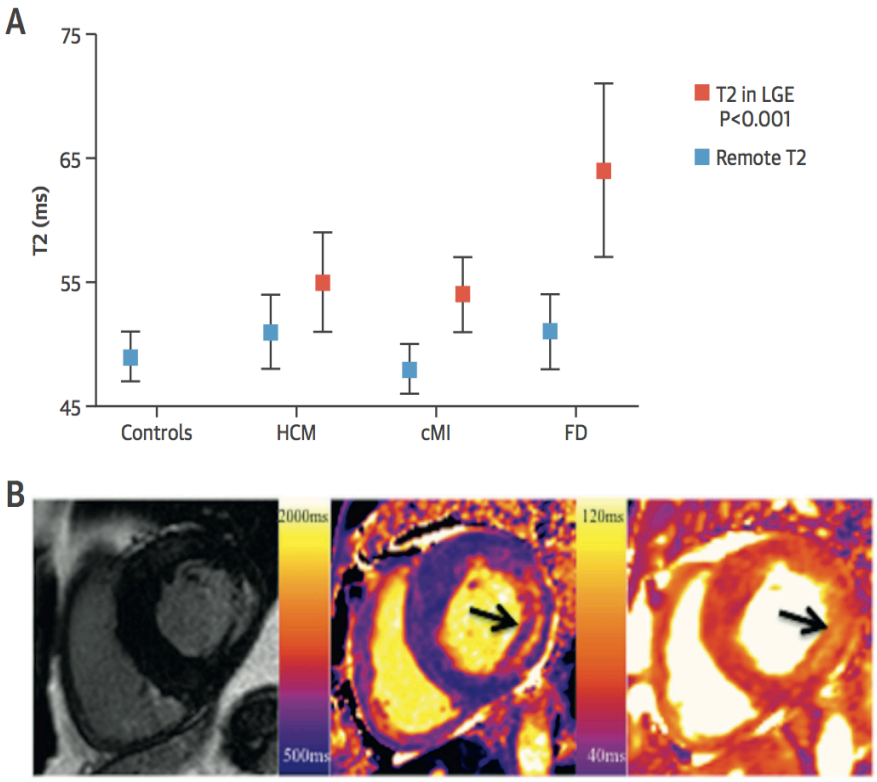

Figure 38: Myocardial T2 is elevated in FD with LGE. Adapted from Nordin et al.(127)

8.1.3 Stages of FD by CMR

The leading cause of death in FD is cardiac involvement with males affected earlier and generally present with more severe cardiac manifestations than females in FD. Sex dimorphism in myocyte hypertrophy is observed in other cardiac diseases: the hypertrophy in aortic stenosis is far more marked in males than females(164), and probably observed in other conditions – for example, hypertrophic cardiomyopathy, a non-sex linked disease that has a male predominance in most large studies.(165) Whether sex dimorphism of

the myocyte hypertrophy in these diseases derive from common mechanisms is unknown at this time. Differences in the renin-angiotensin system, nitric oxide activity, and norepinephrine release and differential expression of androgen and oestrogen receptors could potentially contribute to sex differences observed in LV remodelling.(164,166,167)

Little is known of the age and sex difference in storage, inflammation and myocyte responses in FD. There has also been no published work on T1 mapping in FD children prior to my work. In this chapter, an FD developmental model based on 3 phases was developed: 1) Accumulation phase. 2) Myocyte hypertrophy and inflammation phase and 3) Fibrosis and impairment phase. These phases represent potential different stages and activated pathways that could potentially be therapeutic targets.

Accumulation phase:

Silent storage phase - starting in childhood, pre-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:

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

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).(91)

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 heart failure clinically.

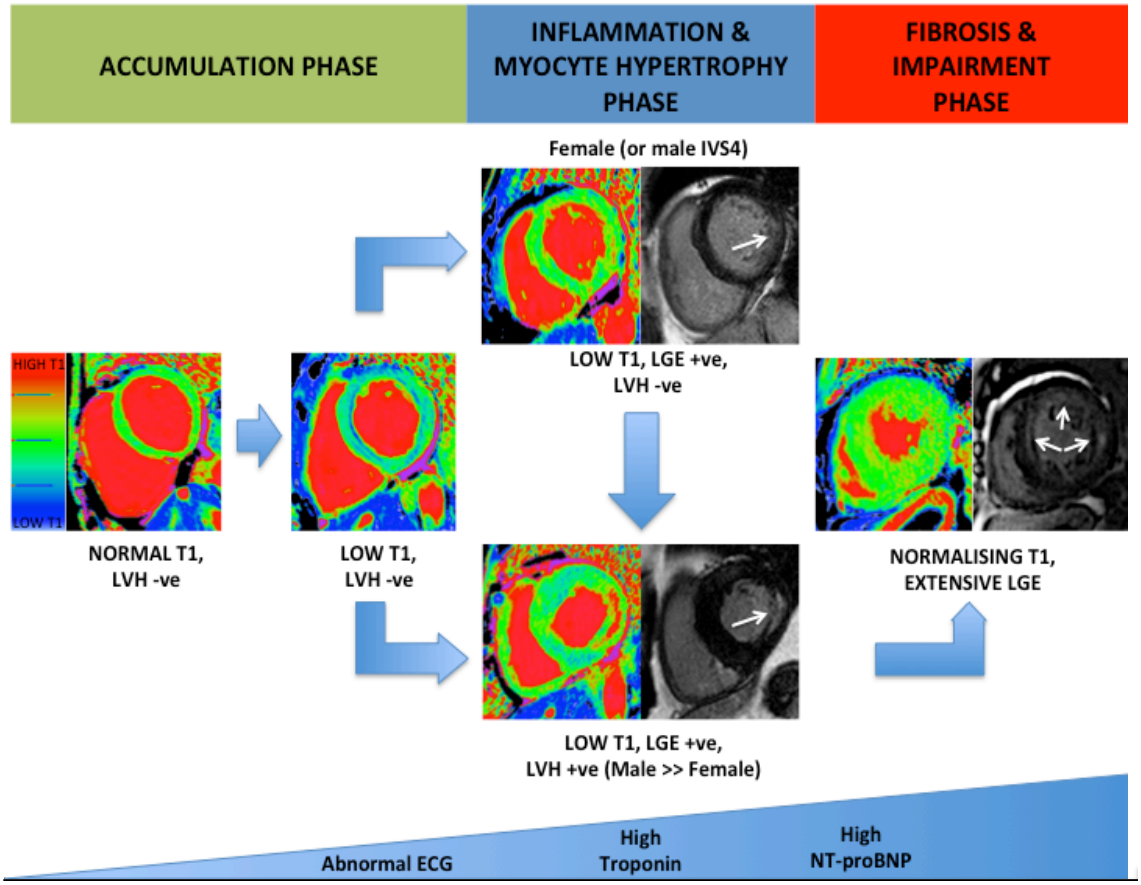

Figure 39: Proposed myocardial phenotype development in Fabry disease by CMR. The disease developmental model consists of an initial accumulation phase (silent myocyte storage and overt T1 lowering) leading to a myocyte hypertrophy and inflammation phase, and eventually a fibrosis and impairment (late) phase.

8.1.4 Serial multiparametric assessment with ERT

Prior to my work, there has been no published work on serial assessment of myocardial phenotype using multiparametric mapping. This is the first prospective study exploring longitudinal change in native T1 and T2 in FD, here also exploring treatment impact. This requires quality control to ensure

measurement system stability. The serial measurement supports proposed models of phenotype development developed out of single timepoint data discussed in the previous section. (168)

I believe the best interpretation of these data is this: Firstly, from the advanced stable disease data, it is clear that chronic ERT use does not completely normalize LVH, T1, T2 or troponin, supporting single time point observational data and evidence that ERT in more advanced disease may be less effective (54,68). Secondly, that the disease is slowly progressing in both early untreated and more advanced treated disease – but in different ways (early disease, more storage, LVH developing; advanced disease more inflammation, increasingly impaired strain; both: slowly increasing troponin). Thirdly, from the newly ERT treated group there is a clear signal of an ERT effect once LVH is present with a small reduction in LV mass and normalization of T1. However the effect size is small, but is in the opposite direction to the treatment naïve group. How a small effect accumulates to alter outcome over years of therapy is unknown.

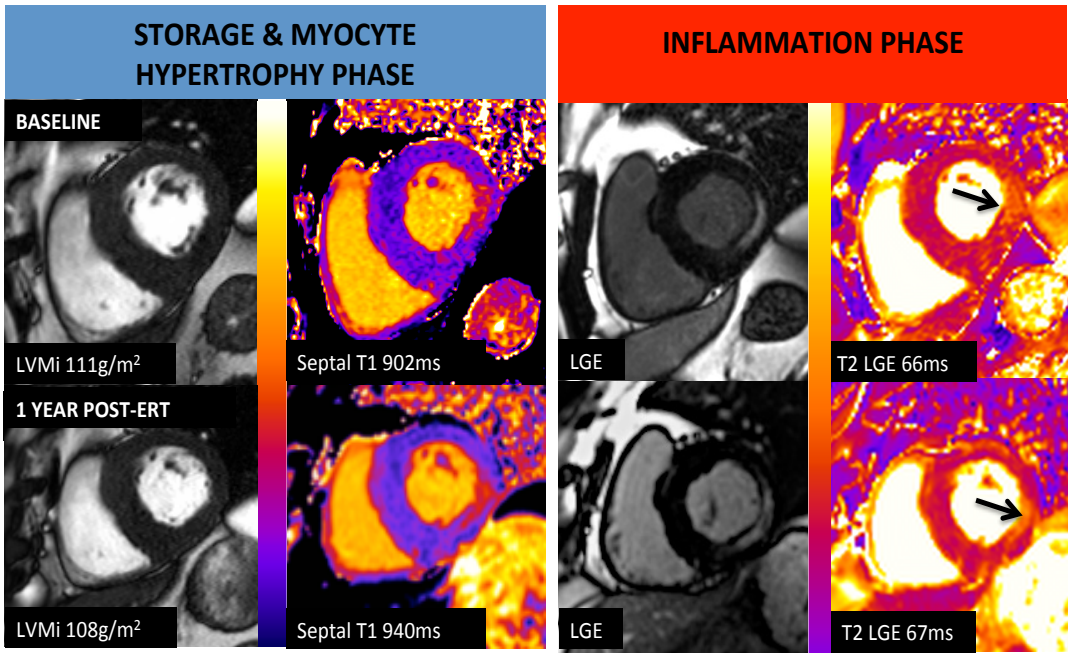

Figure 40: Example case. A 60-year-old FD male with LVH, low T1 (902ms) and basal inferolateral late gadolinium enhancement (LGE) before initiation of ERT. T2 corresponding to the LGE area is high at 66ms with high troponin level. After 1 year of initiation of ERT, T1 partially normalized to 940ms and limited regression of LVMi was observed (-4g/m^2). T2 at the LGE area remained high at 67ms with no troponin level change.

8.2 On-going and Future Work

This thesis has provided pioneering work in the field of multiparametric mapping in FD. Further work is required to explore its potential. Another centre in Melbourne, Australia has recently joined the project with a particular interest in exploring exercise physiology in FD (PI Dr Andre Le Gerche). Two new research fellows Dr Joao Augusto (London) and Dr Ravi Vijapurapu (Birmingham) will continue to explore some of the following:

1. In LVH-negative FD, further longitudinal study is required to monitor progression or outcome specifically in patients with low native T1 and ECG abnormalities. This should also include children. Longitudinal follow up for this should be at least 3-5 years as the assumption is that FD is a slowly progressive disease especially when in the LVH-negative phase.

2. Larger cohort study of inflammation in FD using T2 mapping and other inflammatory biomarkers with outcome data is desirable. Ideally there should be an assessment of histology in human or animal models – targeting particularly the LGE area of the heart. There should be additional peripheral blood biomarkers of inflammation, and an assessment of lymphocyte populations.

3. Arrhythmia. Given the new biomarkers and disease models, a study of predictors of arrhythmia seeking a FD specific disease model and algorithms would be important. Such study could include a large registry with this new

baseline phenotyping, but also substudies using loop recorder devices and CMR. This is currently funded and in the contracting and permissions phase.

3. Phenotype/genotype correlation study with native T1 and lyso-Gb3. Little is known about this currently although some of the native T1 data has been explored in this thesis.

4. Histological validation of native T1 in FD. However this may be more difficult to perform due to FD being a rare disease.

5. CMR perfusion mapping for assessment of microvascular dysfunction in FD. Previous literature has shown some evidence of microvascular dysfunction in FD in small population sample. Perfusion mapping by CMR can give further insight of this in relation to hypertrophy, storage and presence of inflammation.

6. Use of T1 mapping as a surrogate marker in clinical trials eg with oral chaperone or ERT.

Related projects

Two additional research projects have been developed to investigate some of the work suggested in the previous section.

Project 1: The Blood Determinants of Myocardial Storage, Inflammation and Scar in Fabry Disease

Chief Investigator: Professor James Moon

Co-Investigator and collaborators: Professor Derralynn Hughes, Dr Tarek Hiwot and Dr Kevin Mills

The three key workstreams in this project are as follows:

Workstream 1: LIPIDOMICS

Aim: To comprehensively and quantitatively characterize the lipidome of blood and urine in FD, to elucidate mechanistic links with myocardial storage and inflammation.

Workstream 2 - INFLAMMASOME

Aim: To conduct a targeted, high-throughput, digest-based, multiple reaction monitoring, quantitative proteomics analysis of the plasma “inflammasome” in FD.

Workstream 3 - HYPERTROPHY PATHWAYS

Aim: To compare the lipidome and inflammasone of FD to that of other key cohorts with and without LVH: HCM, aortic stenosis and healthy volunteers.

This research grant of £156,093.44 has been agreed by Sanofi-Genzyme and is currently undergoing contracting. I have been involved in the grant application and the urine and blood biomarkers I have collected from the FD participants will be used for this study.

Project 2: Arrhythmia burden, risk of sudden death and stroke in patients with Fabry Disease: Role of implantable loop recorders (RaILRoAD)

Chief Investigator: Dr Richard Steeds

There is currently no clear guidance on device therapy in FD. This multi-centre prospective randomised control trial evaluates the use of implantable loop recorder in Fabry Disease. The aims of this project are to identify arrhythmic burden in FD, compare rates of arrhythmia between different surveillance modalities (Holter testing and implantable loop recorder) and define arrhythmic risk factors in FD. This study is co-funded by Sanofi-Genzyme and Shire and is currently undergoing contracting.

Primary endpoints:

- AF requiring anticoagulation
- Bradyarrhythmia requiring pacing
- Supraventricular tachycardia requiring drug treatment or ablation
- Non-sustained ventricular tachycardia requiring drug treatment, ICD implant, ablation

Secondary endpoints:

- Assessment of arrhythmia burden
    - ERT vs. no ERT
    - Myocardial fibrosis (presence, location)
    - Predictive value of resting ECG, TTE & CMR

8.3 Conclusion

This thesis has provided further valuable insight into the field of Fabry disease. CMR with multiparametric mapping and LGE have the unique ability to non-invasively demonstrate and quantify storage and inflammation in FD. My thesis has demonstrated the enormous potential of these techniques not only in aiding early detection of cardiac involvement but also to gain further understanding into the cardiac pathophysiology in FD creating new avenues to therapy in this complex but treatable disease.

Chapter 9: Publications

9.1 1st author publications on related topics during this thesis:

- **Nordin S**, Kozor R, Medina-Menacho K, Abdel-Gadir A, Baig S, Sado DM, Lobascio I, Murphy E, Lachmann RH, Mehta A, Edwards NC, Ramaswami U, Steeds RP, Hughes DA, Moon JC. Proposed stages of Myocardial Phenotype Development in Fabry Disease. *JACC Cardiovascular Imaging.* 2019 Aug; 12(8 Pt 2): 1673-1683.

- **Nordin S,** Kozor R, Baig S, Abdel-Gadir A, Medina-Menacho K, Rosmini S, Captur G, Tchan M, Geberhiwot T, Murphy E, Lachmann R, Ramaswami U, Edwards NC, Hughes DA, Steeds RP, Moon JC. The cardiac phenotype of pre-hypertrophic Fabry disease. *Circulation Cardiovascular Imaging.* 2018 Jun;11(6):e007168.

- **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(15):1707-1708. *Joint first author.

- **Nordin S**, Dancy L, Moon JC, Sado DM. Clinical applications of multiparametric CMR in left ventricular hypertrophy. *International Journal of Cardiovascular Imaging.* 2018 Apr;34(4):577-585.

9.2 Co Author publications on related topics during this thesis:

- Knott K, Augusto J, **Nordin S,** Kozor R, Camaioni C, Xue H, Hughes R, Manisty C, Brown Louise, Kellman P, Ramaswami U, Hughes DA, Plein S, Moon JC. Quantitative myocardial perfusion in Fabry Disease. *Circulation Cardiovascular Imaging*. 2019. 12(7):e008872.

- Vijapurapu R, Geberhiwot, T, Jovanovic A, Baig S, **Nordin S**, Kozor R, Levya F, Kotecha D, Wheeldon N, Deegan P, Rusk R, Moon JC, Hughes DA, Woolfson P, Steeds RP. A multicentre study of indications for cardiac device implantation and utilization in Fabry cardiomyopathy. Heart. 2019.

- Kotecha T, Martinez-Naharro A, Treibel TA, Francis R, **Nordin S**, Abdel-Gadir A, Knight DS, Zumbo G, Rosmini S, Maestrini V, Bulluck H, Rakhit RD, Wechalekar AD, Gilbertson G, Sheppard MN, Kellman P, Gillmore JD, Moon JC, Hawkins PN, Fontana M. Myocardial edema in amyloidosis: new insight into pathogenesis. *J Am Coll Cardiol.* 2018 Jun 26;71(25):2919-2931.

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- Vijapurapu R, **Nordin S,** Baig S, Liu B, Rosmini S, Augusto J, Tchan M, Hughes DA, Hiwot T, Moon JC, Steeds RP, Kozor R. Global longitudinal strain, myocardial storage and hypertrophy in Fabry disease. *Heart* 2018.

- Baig S, Vijapurapu R, Alharbi F, **Nordin S**, Kozor R, Moon J, Bembi B, Geberhiwot T, Steeds RP. Diagnosis and Treatment of the Cardiovascular Consequences of Fabry Disease. *QJM*. 2018.

- Bulluck H, Rosmini S, Abdel-Gadir A, Bhuva A, Treibel T, Fontana M, Knight D, **Nordin S,** Sirker A, Herrey A, Manisty C, Moon JC, Hausenloy DJ. Redefining viability by cardiovascular magnetic

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- Abdel-Gadir A, Vorasettakarnkij Y, Ngamkasem H, **Nordin S**, Ako EA, Tumkosit M, Sucharitchan P, Uaprasert N, Kellman P, Piechnik SK, Fontana M, Fernandes JL, Manisty C, Westwood M, Porter JB, Walker JM, Moon JC. Ultrafast Magnetic Resonance Imaging for Iron Quantification in Thalassemia Patients In The Developing World: The TIC-TOC Study. *Circulation.* 2016; 134:432-434.

9.3 Manuscripts submitted for publication related to this thesis:

- **Nordin S,** Kozor R, Vijapurapu R, Augusto J, Knott K, Captur G, Treibel TA, Ramaswami U, Tchan M, Geberhiwot T, Steeds RP, Hughes DA, Moon JC. Myocardial storage, inflammation and cardiac phenotype in Fabry Disease after one year of enzyme replacement therapy.

- Augusto J*, **Nordin S***, Vijapurapu R, Baig S, Bulluck H, Castelletti S, Alfarih M, Knott K, Captur G, Kotecha T, Ramsawami U, Tchan M, Geberhiwot T, Fontana M, Steeds R, Hughes D, Kozor R, Moon JC. Chronic myocardial edema and troponin release in Fabry Disease. *Joint first author

- Lundin M, Heiberg E, Nordlund D, Gyllenhammar T, Steding-Ehrenborg, K, Engblom H, Carlsson, M, Atar D, Van der Pals J, Erlinge D, Borquist R, Khoshnood A, Ekelund U, Nickander J, Themudo, R, **Nordin S,** Kozor R, Bhuva A, Moon JC, Maret E, Caidahl K, Sigfridsson A, Sorensson P, Szhelbert E, Arheden H, Ugander M. Left ventricular mass and global wall thickness-prognostic utility and characterization of left ventricular hypertrophy.

- Vijapurapu R, Baig S, **Nordin S,** Augusto J, Price A, Kozor R, Kotecha D, Hodson J, Hughes DA, Moon JC, Geberhiwot T, Steeds RP. Longitudinal assessment of cardiac involvement in Fabry disease using cardiovascular magnetic resonance imaging.

9.4 Abstract Presentations related to this thesis

I have only included abstracts where I have presented. I have also contributed to multiple other related abstracts as a co-author.

- Oral abstract presentation, CMR 2018 in Barcelona. *Phenotype development in cardiac Fabry disease proceeds through four stages: a prospective 182-patient study.*

- Oral and moderated poster presentation in CMR 2018, Barcelona. *Subclinical Fabry Cardiomyopathy.*

- Oral and moderated poster presentation in SCMR conference 2017, Washington DC. *Fabry disease is a chronic inflammatory cardiomyopathy – insights from multiparametric mapping and blood biomarkers.*

- Oral abstract presentation, EuroCMR conference 2017 in Prague. *ECG abnormalities in LVH-negative Fabry Disease: presence of low native T1 makes a difference but may be missed.*

- Oral case presentation on Acute florid HIV-related myocarditis with rapid response of clinical and CMR parameters to anti-retroviral therapy, SCMR conference 2016 in Los Angeles.

- Moderated poster presentation in ESC Myocardial and Pericardial meeting 2017 in Nyborg, Denmark. *Proposed stages of phenotype*

development in Fabry cardiomyopathy: a prospective 182-patient study by cardiovascular magnetic resonance.

- Moderated posters in WORLD symposium 2018 in San Diego. *Proposed stages of phenotype development in cardiac Fabry disease: a prospective 182-patient study by CMR.*

- Moderated posters in WORLD symposium 2018 in San Diego. *The Subclinical Phenotype of Cardiac Fabry Disease.*

- Moderated poster presentation, EuroCMR conference 2016 in Florence. *Papillary muscles offer further insight into hypertrophied hearts: a cardiovascular magnetic resonance study*.

- Moderated poster presentation on ECG, LVH and T1 changes in Fabry disease – implications for screening and understanding of the disease model, SCMR conference 2016 in Los Angeles.

- Poster presentation in ESC Myocardial and Pericardial meeting 2017 in Nyborg, Denmark. *Preclinical Fabry Cardiomyopathy.*

- Poster presentation, UCL Postgraduate Research Symposium 2017 in London. *Fabry Disease Is A Chronic Inflammatory Cardiomyopathy: Insights from multiparametric mapping and blood biomarkers.*

- Poster presentation in EuroCMR 2019 in Venice. *Clinical utility of multiparametric CMR in the assessment of cardiac involvement in Becker muscular dystrophy with raised troponin levels.*

- Poster presentation in International Myotonic Dystrophy Consortium meeting (IDMC-12) 2019 in Sweden. *Cardiac evaluation in Myotonic Dystophy Type 1.*

9.5 Prizes and grants involvement:

- Scottish Cardiac Society Emily Taylor Travel Grant 2019.

- Society for Cardiovascular Magnetic Resonance 2017 Regional Travel Award, Washington DC.

- Finalist in “Best Moderated ePoster” in CMR 2018, Barcelona. *Subclinical Fabry Cardiomyopathy.*

- Finalist in “Best of Walking Poster” in SCMR conference 2017, Washington DC. *Fabry disease is a chronic inflammatory cardiomyopathy – insights from multiparametric mapping and blood biomarkers.*

- The Blood Determinants of Myocardial Storage, Inflammation and Scar in Fabry Disease grant-funded by Sanofi-Genzyme

- Arrhythmia burden, risk of sudden death and stroke in patients with Fabry Disease: Role of implantable loop recorders (RaILRoAD) grant – co-funded by Sanofi-Genzyme and Shire

Chapter 10: References

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