FABRY CARDIOMYOPATHY

Towards Early Diagnosis And Rational Follow-Up

Mohamed El Sayed

Fabry cardiomyopathy
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ACADEMISCH PROEFSCHRIFT

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geboren te Port Said

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The heart has its own language. The heart knows
a hundred thousand ways to speak

-Rumi-

Chapter 1

General introduction and thesis outline

General introduction

Background
Fabry disease (FD) is a rare, inherited, slowly progressive X-linked lysosomal storage disorder (OMIM 301500). The worldwide prevalence is estimated at 1:40,000 to 1:117,000 in males [1]. The substantial disparity in the reported disease prevalence may be explained by variations in the definition of FD, which in some studies individuals with non-pathogenic variants or variants of unknown significance in the GLA gene are classified as having FD [2].

Mutations in the galactosidase alpha gene (GLA) are the primary cause of FD, leading to a decreased activity of the lysosomal enzyme alpha-galactosidase A (AGAL) (enzyme commission no.3.2.1.22) [3, 4]. This results in intracellular accumulation of the enzymes' main substrate, globotriaosylceramide (Gb3) in various organs, including the vascular endothelium, kidneys, brain, peripheral nerves and heart [5-8]. Several processes may be set into motion by the increasing cellular accumulation of Gb3 and its derivative Globotriaosylsphingosine (lysoGb3), that ultimate lead to organ damage.

Cardiac involvement is common in FD and can manifest as left ventricular hypertrophy (LVH) and myocardial interstitial fibrosis formation. During adulthood, symptomatic cardiac disease in the form of conduction abnormalities, arrhythmias, ischemic heart disease and heart failure may arise, ultimately leading to cardiac death in many patients [9]. Alterations in electrophysiological markers and cardiac morphology and function precede the development of clinical heart disease. Currently, the knowledge on the course of these changes and in which patients do they arise is lacking. Also, data on the age of occurrence, the progression rate and how these clinical markers are linked to clinical outcomes is limited. The main goal of this thesis is to study the cardiac manifestations of FD, the course of cardiac disease as reflected in electrophysiological and echocardiographic features in men and women with FD throughout adult life, and how they differ from the healthy population.

Pathophysiology of cardiac involvement in FD
Gb3 accumulates in all cardiac cell types and tissues, including myocytes, endothelial and smooth muscle cells of intramyocardial vessels and conduction tissue [10, 11]. Although Gb3 is expected to have direct toxic effects, secondary alterations of cellular processes in response to accumulation of storage material are likely to have significant pathological consequences. This theory was supported by the finding that only 1-2% of the left ventricle volume consisted Gb3 in post-autopsy cardiac material from FD patients [12]. Moreover, despite Gb3 clearance from the vascular endothelium, many patients still develop organ-

related complications whilst being treated with enzyme replacement therapy (ERT) [13, 14]. This may be partly explained by the fact that in cardiac biopsies of FD patients, treatment with ERT did not result in significant Gb3 clearance from cardiomyocytes within five months [15]. However, the pathophysiology is probably more complex, several specific hypothetical pathways, that link the Gb3 storage to the cardiac impairment in FD have been described. First, as a consequence of Gb3 accumulation, cardiomyocytes, endothelial and smooth muscle cells proliferate. This proliferation leads to an increased oxygen demand of the cardiac muscle, which cannot be met because of microvascular dysfunction caused by low nitric oxide synthase (eNOS) expression in the affected endothelial cells. The microvascular dysfunction limits capillary elasticity and resulting oxygen deficit leads to ischemia and fibrosis [16]. Second, lysosomal glycosphingolipid accumulation suppresses the autophagic processes in the cell [17]. A disturbed autophagic flux also affects mitophagy, which in turn interferes with the mitochondrial energy production, resulting in reduced activity of the respiratory chain complexes I, IV and V and further drop in cellular levels of energy-rich phosphates (e.g. ATP). This cardiac energy metabolism dysfunction and the increased oxygen demand due to LVH may result in decreased ischemic tolerance [18-20]. Third, a deficiency of alpha-galactosidase A limits the degradation of lipidic antigens. In FD, Gb3 and lysoGb3 (a water- soluble deacylated form of Gb3) act as antigens, activating NK T-cells. This leads to the secretion of pro-inflammatory factors and oxidative species, which may enhance the processes of cell damage and death [21, 22]. Lastly, Birkel et al. (2019) also found an impaired sodium and calcium channel function in FD cardiomyocytes, derived from pluripotent stem cells, with a higher and shorter action potential [23]. These findings support the hypothesis that conduction abnormalities in FD are not only explained by tissue damage and fibrosis, but that an altered ion channel expression on cell membranes may contribute to the increased electrical depolarization velocities observed in FD [24].

The phenotype and genotype of FD
Because of the X-linked chromosomal inheritance of FD, men typically have more severe symptoms and are affected earlier in life than women with FD. Additionally, classical and non-classical disease phenotypes are distinguished, with substantial variations in symptomatology, organ involvement and prognosis.

Classical FD is characterized by a significantly reduced or, in men (almost), absent AGAL activity. These male patients often present in childhood with typical classical symptoms, including angiokeratomas, cornea verticillata and neuropathic pain. The presence of this triad is a strong predictor for the diagnosis of classical FD, in which the majority of classical FD patients develop multi-organ involvement of the kidneys, brain and heart during late adolescence or

young adulthood, which is progressive throughout adulthood [25, 26]. In women angiokeratoma are not observed, cornea verticillata and neuropathic pain are present in a subset of patients and cardiac and cerebral disease develop in average 10 years later and not all patients are affected [27, 28].

Non-classical FD patients have higher residual activity of the AGAL enzyme due to the less disruptive GLA mutations. In the non-classical patient group, involvement of only the heart is more common. The diagnosis of non-classical FD is usually made at a later age than classical FD, as it often presents with more atypical symptoms and lacks the 'classical triad' of FD symptoms [29-31]. The abovementioned categorization by phenotype and sex is essential for prognostication, guiding treatment and follow-up.

Diagnosing FD
Diagnosing FD in individuals with non-classical disease and women with classical FD, which often lack Fabry specific- symptoms, can be challenging since not all GLA- gene variants cause disease [26]. Genetic testing panels may help diagnose Fabry cardiomyopathy, which can mimic hypertrophic cardiomyopathy (HCM) [32]. If there is any uncertainty regarding the pathogenicity of a GLA variant this can be assessed by biochemical and enzymatic test, segregation studies in the family and if uncertainty persists by organ imaging and biopsies [33]. A GLA variant is deemed to be pathogenic if it leads to: 1) an increased level of lysoGb3 in the range of FD patients (male and female patients) [34, 35], 2) a significantly reduced enzyme activity level (in all male patients and some female patients) and if 3) a biopsy of an affected organ showing typical zebra body inclusions in a relevant cell type or the appearance of a low native T1-value on cardiac MRI, most likely representing myocardial sphingolipid accumulation [36].

Cardiac involvement of FD
Common cardiac manifestations of FD include LVH [37-40], valvular heart disease [41], myocardial fibrosis [20, 42], microvascular disease [43, 44] and conduction abnormalities [45, 46]. Clinically, Fabry cardiomyopathy is manifested by chronic heart failure with a preserved ejection fraction (HFpEF) [47], angina pectoris (often without underlying coronary artery disease) [44] and/or (supra)ventricular arrhythmias (e.g., sinus bradycardia, atrial fibrillation (AF), and (non)sustained ventricular tachycardia). Fabry patients often suffer from brady-tachycardia syndromes warranting the implantation of a pacemaker and/or implantation of a ICD to prevent acute cardiac death due to malignant arrhythmias [48].

Cardiovascular death is the most common cause of death in patients with FD [7]. Given the cardiac morbidity and mortality in FD patients, research should

be aimed at identifying cardiac involvement at an earlier stage of the disease, so that timely preventive therapy can be started. Currently, the most commonly used treatment is enzyme replacement therapy (ERT), which was introduced in 2001 [49]. ERT is an expensive and burdensome treatment. As such, being able to identify which patient needs treatment and at what time point in the development of FD manifestations is essential. These therapy initiation decisions are especially relevant in patients with non-classical FD and women with classical FD given the high disease heterogeneity in these patient groups, as only a subset of patients develops cardiovascular events with a highly variable age of onset [26, 28].

An increasing number of studies showed that initiation of Fabry specific treatment when there is myocardial fibrosis, impaired cardiac function or when clinical complications have already occurred, is less effective in preventing the progression of heart disease compared to earlier treatment initiation [50-52]. These findings emphasize the need for identification of cardiac disease markers indicative of early cardiac involvement, at a stage in which structural changes of the heart have not yet occurred. In addition, knowledge of the cardiac disease course may be helpful in monitoring whether or not the progression of FD cardiomyopathy is halted by new FD-specific therapies [53]. The following description is a hypothesized three-stage model for the development of Fabry cardiomyopathy, based on the available literature, emphasizing potential biochemical, electrophysiological and echocardiographic biomarkers. However, this model is mainly based on historical cross-sectional data, where the distinction between classical and non-classical FD is not always made.

1. **The accumulation phase:** sphingolipids (particularly Gb3) accumulate in the cardiac tissue but clinical signs of cardiac disease are not yet present [11, 54, 55]. At which age this cardiac accumulation starts is unknown, as the youngest patient who underwent an endomyocardial biopsy published in literature was 17 years old [15]. Although the primary substrate of AGAL is Gb3, and it seems obvious that Gb3 could serve as a potential biomarker, Gb3 is often not elevated in men with non-classical FD and women with classical FD, even though these patients may develop significant cardiac pathology [56]. In contrast to Gb3, plasma concentrations of lysoGb3, are increased in all patients with FD and are stable throughout life in an untreated patient (**chapter 5**) and have a clear correlation with (cardiac) disease severity [25, 57, 58].
Endomyocardial biopsies are too invasive in clinical settings for patients to be used to detect early cardiac involvement. A low native T1 value on cardiac MRI (CMR) is likely a manifestation of myocardial sphingolipid overload [59, 60]. Studies correlating the low T1 values on CMR with the degree of Gb3 accumulation in histopathological examinations are lacking. Low T1 values are found in FD patients, in presence but also in absence of LVH, suggesting

the detection of glycosphingolipid accumulation even prior to the hypertrophy response [59, 61, 62]. In addition to biochemical and imaging markers, early electrocardiogram (ECG) alterations, present in the accumulation phase, have been described in the literature, of which the most important (listed from most to least common) are [24, 45, 63, 64]:

- A short PR- interval and P- wave duration;
- QRS- and QTc prolongation;
- T- wave inversion and meeting the Sokolov-Lyon or Cornell index criteria prior to the onset of LVH on cardiovascular imaging;
- Complete bundle branch block, multifocal extra systoles and atrial fibrillation.

Symptomatic Fabry cardiomyopathy is characterized by diastolic dysfunction of the left ventricle with normal left ventricular ejection fraction (known as heart failure with preserved ejection fraction, HFpEF) [47, 65]. Pica et al. (2014) described in FD patients with a low T1 and a normal left ventricular mass (suggestive of the accumulation phase), several early LV diastolic function alterations, including an aberrant global longitudinal strain (GLS), higher ratio of early diastolic mitral inflow velocity/ early diastolic septal tissue mitral annulus velocity (E/e') and a larger left atrial volume index (LAVI) [61]. Abnormal GLS and E/e' values were associated with the development of adverse cardiac events later on in the disease development [65, 66].

2. **The hypertrophy and fibrosis phase:** as Gb3 accumulation progresses, the myocardium will exhibit LVH with interstitial fibrosis formation, detectable by late gadolinium enhancement (LGE) on CMR. The median age at which FD patients develop these features is 40 years for men and 50 years for women (no distinction was made between classical and non-classical patients) [67]. Interestingly, 25% of women with FD without LVH still develop myocardial fibrosis, while fibrosis in men always occurs in the presence of LVH [68]. In other conditions that can lead to cardiac hypertrophy (e.g., aortic stenosis and other genetic hypertrophic cardiomyopathies), sex differences in ventricular hypertrophy are observed, with males again having a greater tendency to develop LVH, thus this sex effect appears to be independent of the underlying pathology [69, 70]. For the FD population, this would mean that 1) the current reference values are too insensitive for the detection of mildly elevated left ventricular mass in women [71] or 2) that we should look for morphological markers of cardiac involvement other than LVH in females with FD. At this disease stage, plasma troponin and N-terminal pro b- type natriuretic peptide (NT-proBNP) also rise and levels are associated with increases in left ventricular mass and the presence of fibrosis. In addition ECG abnormalities, most likely related to LVH and fibrosis, increase (left axis deviation,

T-wave inversion and pronounced LVH voltage criteria) [67]. Besides the aforementioned echocardiographic features, HFpEF patients without FD often have elevated Tricuspid regurgitant jet velocity (TR velocity) [72]. However, this marker was found to be normal in a smaller study, including FD patients [73].

3. **The heart failure phase:** as the disease progresses, myocardial fibrosis will expand throughout the heart, and the hypertrophic heart tissue will be partly replaced by atrophic tissue with further rising of the plasma troponin and NT-proBNP [67]. Secondary to the extensive cardiac fibrosis, ST-segment alterations [74] and ultimately ventricular arrhythmias [46] can occur. End-stage diastolic heart failure as well as systolic left ventricular dysfunction occur during this stage [65], with heart failure being the most common cause of death [75].

Aims and outline

The above mentioned sequence of events is hypothetical and, as said, based primarily on cross- sectional studies describing the cardiac manifestations of FD. Hence the precise longitudinal course of Fabry cardiomyopathy in patient groups stratified by sex and disease phenotype has not been documented. Studying the progression of Fabry cardiomyopathy is crucial for early diagnosis and establishing rational group-specific follow-up protocols to detect complications, but also prevent over- medicalizing patients. The Amsterdam University Medical Centre (AUMC), the national referral centre for patients with FD in the Netherlands has a longitudinal clinical dataset and biobank, which are unique in both their size and the length of systematic follow-up of patients, providing detailed clinical data. To this end, we performed several longitudinal studies at the AUMC, aiming to:

1) Map the course of cardiac manifestations of FD, from pre-symptomatic phase through to complication development, in different FD patient groups (men and women, patients with classical and non-classical FD).
2) Detect electrophysiological, imaging and biochemical characteristics of FD that precede deterioration of cardiac function to be able to detect early cardiac involvement.

In **Chapter 2**, we describe the effect of disease phenotype and sex on the occurrence of cardiac events in FD. In **Chapter 3**, the evolution of ECG parameters in a large population of adults with classical FD are assessed and compared to those of apparently healthy control subjects. **Chapter 4** reports on the development of morphological and functional echocardiographic features

of FD cardiomyopathy during adult life in classical FD patients and compares them to those of healthy control subjects. In **Chapter 5**, we investigated the predictive value of lysoGb3 in untreated FD patients for the (cardiac) disease course. **Chapter 6** holds a summary and general discussion of this thesis.

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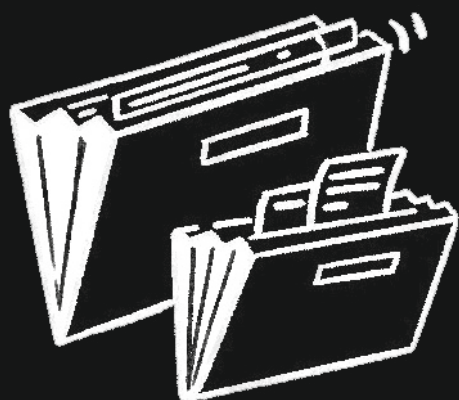
Chapter 2

Influence of sex and phenotype on cardiac
outcomes in patients with Fabry disease

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Mareen Datema; Carla E.M. Hollak; Mirjam Langeveld

Abstract

Objective
This study describes the influence of sex and disease phenotype on the occurrence of cardiac events in Fabry disease (FD).

Methods
Cardiac events from birth to last visit (median age 50) were recorded for 213 FD patients. Patients were categorized as follows: men with classical FD (n=57), men with non-classical FD (n=26), women with classical FD (n=98) and women with non-classical FD (n=32), based on the presence of classical FD symptoms, family history (men and women), biomarkers and residual enzyme activity (men). Event rates per 1000 patient years after the age of 15 years and median event- free survival (EVS) age were presented. Influence of disease phenotype, sex and their interaction was studied using Firth's penalized Cox regression.

Results
The event rates of major cardiovascular events (MACE) (combined endpoint cardiovascular death (CVD), heart failure (HF) hospitalization, sustained ventricular arrhythmias (SVA) and myocardial infarction) were: 11.0 (95% CI: 6.6-17.3) in men with classical FD (EVS 55 years), 4.4 (2.5-7.1) in women with classical FD (EVS 70 years) and 5.9 (2.6-11.6) in men with non-classical FD (EVS 67 years). None of these events occurred in women with non-classical FD. Sex and phenotype significantly influenced the risk of MACE. CVD was the leading cause of death (75%) to which HF contributed most (42%). The overall rate of SVA was low (14 events in 9 patients (4%)).

Conclusions
Sex and phenotype greatly influence the risk and age of onset of cardiac events in FD. This indicates the need for patient group-specific follow-up and treatment.

Key questions

What is already known on this subject?
Early cardiac manifestations of FD are left ventricular hypertrophy, fibrosis formation and conduction abnormalities. As the disease progresses, cardiac complications, such as arrhythmias and heart failure, occur in a significant proportion of FD patients, yet others remain asymptomatic throughout long-term follow-up. The evolution of cardiac events and the influence of sex and disease phenotype on this trajectory has not been described previously.

What might this study add?
This is the first large longitudinal cohort study describing the effect of disease phenotype and sex on occurrence of cardiac events in FD. The event rates of major adverse cardiovascular events (combined endpoint cardiovascular death (CVD), heart failure (HF) hospitalization, sustained ventricular arrhythmias (SVA) and myocardial infarction) were: 11.0 in men with classical FD (95% CI: 6.6-17.3) (event free survival (EVS) 55 years), 4.4 (2.5-7.1) in women with classical FD (EVS 70 years) and 5.9 (2.6-11.6) in men with non-classical FD (EVS 67 years). None of these events occurred in women with non-classical FD. Cardiovascular death was the leading cause of death (75%) to which heart failure contributed most (42%). The overall rate of sustained ventricular arrhythmias was low (14 events in 9 patients (4%)).

How might this impact on clinical practice?
Cardiac care in FD should be tailored to the sex and disease phenotype of the patient and focus on early detection and treatment of heart failure.

2

Introduction

Fabry disease (FD) is a rare X-linked lysosomal storage disease that is caused by mutations in the galactosidase alpha (GLA) gene, resulting in reduced alpha-galactosidase A enzyme activity [1, 2]. Accumulation of the enzyme's substrate globotriaosylceramide (Gb3) and its derivatives is the primary trigger for damage and dysfunction of various tissues and organs, including vascular endothelium and the heart [3-5].

Due to the X-linked mode of inheritance, men are generally more severely affected and disease manifestations occur earlier compared to women. In addition, a distinction is made between classical and non-classical disease phenotype, with significant differences in onset and progression of symptoms, organ damage, and outcome between these two groups. The classical form of FD in men is characterized by greatly reduced or absent alpha-galactosidase A activity, resulting in manifestations in multiple organs, starting from late adolescence [6, 7]. Non-classical disease manifests itself later in adulthood and often affects only the heart [8-10].

Early cardiac manifestations of FD are bradycardia, shortened PR interval, low native T1 value on cardiac MRI and, in male and a subset of female patients, cardiac hypertrophy [11, 12]. As the disease progresses, conduction abnormalities (CA), supraventricular arrhythmias, ischemic heart disease, diastolic and systolic dysfunction and ultimately overt heart failure (HF), leading to cardiac death, may occur [3, 13-22]. On the other hand, a significant number of patients will remain asymptomatic, even at an advanced age [7]. Limited evidence is available on the risk and timing of cardiac manifestations and events in different patient groups (i.e. men versus women, classical versus non-classical FD phenotype) [3, 23]. It is important to generate these data, as it will guide patient-specific follow-up and timing of treatment initiation, risk assessment (e.g. for sudden cardiac death) and evaluation of new FD therapies that are currently in various stages of development. To answer the open questions, we performed a retrospective study in a FD cohort under follow-up at the Amsterdam UMC, which is unique in both its size, as well as the length of systematic follow-up of patients, providing detailed clinical data on cardiac outcome.

Methods

Patient and public involvement

This is an observational longitudinal retrospective study, using data from all adult patients with a definite diagnosis of FD (figure 1) that have been under follow-up at any time at the Amsterdam University Medical Centers (AUMC), the national referral center for FD patients in the Netherlands.
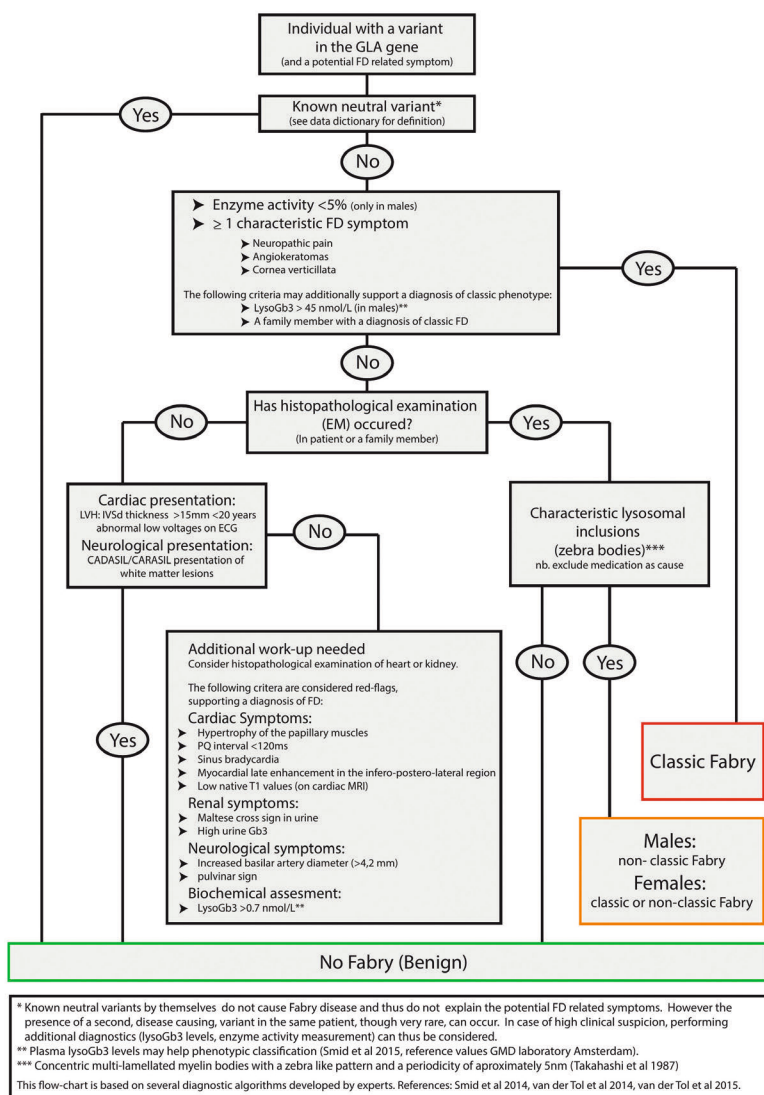
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Figure 1: Flowchart for the diagnosis and phenotype allocation in Fabry disease.

Ethics
The Medical Ethics Review Committee of the AUMC confirmed that the Medical Research Involving Human Subjects Act (WMO) does not apply to this study (W19-438 # 19.505), because of the retrospective character of this study, using historical data obtained in context of regular patient care. The study was conducted in accordance with the Declaration of Helsinki.

Data collection
Patients were divided into 4 groups: men with classical FD, men with non-classical FD, women with classical FD and women with non-classical FD, based on the presence of classical FD symptoms (cornea verticillata, acroparesthesia or angiokeratoma), family history or known mutation-phenotype associations (men and women) and biomarkers and residual alpha-galactosidase A activity (men) [24] (see **figure 1** for details). Patients attended the clinic for standardized follow-up visits.

Between September 2018 and January 2019, all available patients charts, clinical letters, cardiac imaging reports from birth until the last outpatient clinic visit were investigated to record the following events: cardiovascular death (CVD), HF hospitalization, sustained ventricular arrhythmias (SVA), myocardial infarction (MI), CA, pacemaker or implantable cardiac defibrillator (ICD) implantation, atrial fibrillation (AF), coronary artery disease (CAD), percutaneous coronary intervention (PCI), coronary artery bypass graft (CABG) surgery, systolic dysfunction on cardiac MRI or if unavailable on echocardiography, left ventricular outflow tract (LVOT) obstruction, moderate or severe valve disease and other heart surgery/intervention. The combined endpoint major adverse cardiovascular event (MACE) was defined as the occurrence of at least one of the following events: CVD, HF hospitalization, SVA or MI. SVA included sudden cardiac death (SCD), sudden cardiac arrest, sustained ventricular tachycardia lasting >30 sec, appropriate ICD shock, and ventricular fibrillation. CA were defined as a composite of second-degree atrioventricular block type II, third- degree atrioventricular block, sinusarrest and device implantation for CA. Endpoint definitions are provided in supplemental table 1. Events were adjudicated by a panel of experts consisting of 2 cardiologists (AH and SMB) and 1 metabolic specialist (ML).

Statistical analysis
For all cardiac events, the event rate per 1000 patient-years was calculated in order to correct for unequal follow-up duration between the different patient groups. The corresponding 95% confidence intervals (CI) were reported for both individual and composite cardiac events, using the Mid-P exact test (**table 2, supplemental table 2**). For the event rates calculation, follow-up duration from

the age of 15 years onwards was used, because this study shows that cardiac events do not occur before the age of 15 (**figure 2, table 2**, **supplemental table 2**) and the event-free follow-up duration of especially the younger men with classical FD would impact unevenly on the event rate without this correction.

Event-free survival was analyzed using the Kaplan-Meier (KM) method in which patients where stratified according to phenotype and sex. If less than 50% of the patients developed a specific event, the median age of onset for those patients that experienced the event was reported instead. Pair-wise comparison between FD patient groups was performed using a log-rank test, with Bonferroni correction. Next, these analysis were performed correcting for competing risks (CR) (e.g. non- cardiovascular death for the outcome of cardiovascular death, **supplemental figures 2-6**). Cox regression analyses were performed to assess the effect of phenotype and sex and the interaction between these variables on the occurrence of events. Since no events occurred in the subgroup of women with non-classical FD, we used Firth's penalized Cox regression to obtain stable models. *P* values <0.008 (after Bonferroni correction) were considered statistically significant. SPSS (v.25) and R (version 3.6.1) were used for statistical analysis.

Results

213 patients were included: 57 (27%) men and 98 (46%) women with classical FD, 26 (12%) men and 32 (15%) women with non-classical FD. Median age at last outpatient visit or death was 50 years (range 19-83). Patients characteristics are described in **table 1**. **Figure 2** shows the occurrence of cardiac events for all patients in the 4 different groups.

Table 1: Patient characteristics

	All	Men		Women	
		Classical	Non-classical	Classical	Non-classical
General					
Number of patients, n (%)	213 (100%)	57 (27%)	26 (12%)	98 (46%)	32 (15%)
Age at last outpatient visit or death (years), median (range)	50 (19-83)	45 (19-66)	64 (26-78)	51 (19-83)	47 (23-79)
Cumulative follow up from age of 15 (years)	7090	1546	1190	3213	1140
Age at first outpatient clinic visit (years) median (range)	42 (3-77)	30 (3-58)	60 (22-70)	41 (7-71)	44 (19-77)
Comorbidities					
Number of patients with CVA at any time, n (%)	25 (12%)	9 (16%)	5 (19%)	11 (11%)	0 (0%)
Obesity†, n (%)	30/198 (15%)	1/51 (2%)	4/24 (17%)	16/93 (17%)	9/30 (30%)
Smoking†, n (%)	75/177 (42%)	17/44 (39%)	14/23 (61%)	35/87 (40%)	9/23 (39%)
Hypertension†, n (%)	48/199 (24%)	8/51 (16%)	11/26 (42%)	18/95 (19%)	11/27 (41%)
Dyslipidemia†, n (%)	14/187 (8%)	0/43 (0%)	7/23 (30%)	5/91 (6%)	2/30 (7%)
Diabetes mellitus†, n (%)	4/203 (2%)	0/52 (0%)	2/25 (8%)	1/96 (1%)	1/30 (3%)
Cardiac imaging					
Echocardiography					
Available echocardiography at any time, n (%)	181 (85%)	49 (86%)	21 (81%)	87 (89%)	24 (75%)
Left ventricular hypertrophy on echocardiography at any time, n (%)*	90/181 (50%)	24/49 (49%)	16/21 (76%)	43/87 (49%)	7/24 (29%)
*Cardiac MR**					
Available cardiac MR at last follow-up, n (%)	141 (66%)	41 (72%)	11 (42%)	71 (73%)	18 (56%)
Left ventricular ejection fraction (%), median (range)	61 (24-84)	57 (24-70)	57 (48-67)	63 (51-84)	61 (57-70)
Available cardiac MR, where myocardial fibrosis was assessed by late gadolinium enhancement, n (%)	139/141 (99%)	39/41 (95%)	11/11 (100%)	71/71 (100%)	18/18 (100%)
Myocardial fibrosis on cardiac MR, n (%)	54/139 (39%)	13/39 (33%)	6/11 (55%)	29/71 (41%)	6/18 (33%)

Table 1: Patient characteristics (continued)

Cardiac outcomes in Fabry disease

Table 1: Patient characteristics (continued)

	All	Men		Women	
		Classical	Non-classical	Classical	Non-classical
ERT					
Patients on ERT, n (%)	128 (60%)	47 (83%)	12 (46%)	62 (63%)	7 (22%)
Age start ERT (years), median (range)	42 (10-77)	27 (10-58)	59 (20-68)	46 (16-71)	60 (30-77)
Untreated patients, n (%)	85 (40%)	10 (17%)	14 (54%)	36 (37%)	25 (78%)
Reasons for not starting ERT					
Diagnosis through family screening, absent or minimal organ involvement, n (%)	41 (19%)	1 (2%)	3 (12%)	17 (17%)	20 (63%)
Diagnosis not through family screening, absent or minimal organ involvement, n (%)	2 (1%)	0 (0%)	0 (0%)	1 (1%)	1 (3%)
Advanced disease stage, n (%)	18 (9%)	2 (4%)	9 (35%)	5 (5%)	2 (6%)
Follow-up ended before ERT was available, n (%)	8 (4%)	5 (9%)	0 (0%)	3 (3%)	0 (0%)
Other, n(%)	16 (8%)	2 (4%)	2 (8%)	10 (10%)	2 (6%)

CVA=cerebrovascular accident; ERT=enzyme replacement therapy, MR= magnetic resonance

* Definition of cardiac hypertrophy on echocardiography: (Males >51 g/m2.7 and females >48 g/m2.7), calculated with the Devereux formula: 0.8{1.04[([LVEDD + IVSd +PWd]3 - LVEDD3])]} + 0.6. LVEDD: LV end diastolic dimension (mm), IVSd: Interventricular septal thickness at end- diastole (mm), PWd: Posterior wall thickness at end- diastole (mm).

** The included cardiac MRI's were obtained at the time of the last follow-up (with a maximum range of 2 year between the MRI and the last follow-up date). Severely affected FD patients with a non-MRI compatible cardiac device were not included in the routine imaging follow-up.

† cardiovascular risk factors assessed at first outpatient clinic visit:

- Obesity: Body Mass Index ≥ 30 kg/m2

- Smoking: patients who have ever smoked

- Hypertension: antihypertensive medication use or systolic blood pressure of >140 mmHg and/ or diastolic blood pressure of >90 mmHg, measured at least twice

- Dyslipidemia: elevated levels of total cholesterol (>6.5 mmol/l) or low density lipoprotein (LDL) cholesterol (>2.5 mmol/l) or triglycerides (>3.0 mmol/l), or low levels of high-density lipoprotein (HDL) cholesterol (men: <1.0 mmol/l, women <1.2 mmol/l), or medication prescribed for the indication dyslipidemia

- Diabetes mellitus: type I or type II if reported by a medical doctor in the medical chart or when the patient is using anti-diabetic medication.

Table 2: Prevalence, event rates, and age at onset of death and cardiovascular events

	All (213)	Men (83)		Women (130)	
		Classical (57)	Non-classical (26)	Classical (98)	Non-classical (32)
Death					
Number of patients, n (%)	24 (11%)	15 (26%)	3 (12%)	6 (6 %)	0 (0%)
Deaths per 1000 person years (from the age of 15), with 95%-CI	3.4 (2.2-5.0)	9.7 (5.7-15.6)	2.5 (0.6-6.9)	1.9 (0.8-3.9)	0 (-)
Age at death (years), median (range)	58 (26-77)	56 (26-66)	65 (64-68)	72 (57-77)	(-)
Cause of death: heart failure, n (%)	10 (42%)	4 (27%)	2 (67%)	4 (67%)	0 (0%)
Cause of death: myocardial infarction, n (%)	2 (8%)	2 (13%)	0 (0%)	0 (0%)	0 (0%)
Cause of death: ischemic or hemorrhagic cerebrovascular accident, n (%)	4 (17%)	2 (13%)	0 (0%)	2 (33%)	0 (0%)
Cause death: sudden cardiac death during hemodialysis	2 (8%)	2 (13%)	0 (0%)	0 (0%)	0 (0%)
Cause death: other, n (%)	6 (25%)	5 (33%)	1 (33%)	0 (0%)	0 (0%)
Major adverse cardiovascular events¥					
Number of patients	38 (18%)	17 (30%)	7 (27%)	14 (14%)	0 (0%)
Event rate, with 95%-CI	5.4 (3.9-7.3)	11.0 (6.6-17.3)	5.9 (2.6-11.6)	4.4 (2.5-7.1)	0 (-)
Age at first event	54 (33-75)	52 (33-66)	64 (34-67)	54 (34-75)	(-)
Cardiovascular death					
Number of patients	18 (9%)	10 (18%)	2 (8%)	6 (6 %)	0 (0%)
Event rate, with 95%-CI	2.5 (1.6-3.9)	6.5 (3.3-11.5)	1.7 (0.3-5.6)	1.9 (0.8-3.9)	0 (-)
Age at CVD	58 (47-77)	55 (47-66)	66 (65-68)	72 (57-77)	(-)
Heart failure hospitalization					
Number of patients	18 (9%)	8 (14%)	4 (15%)	6 (6%)	0 (0%)
Event rate, with 95%-CI	2.5 (1.6-3.9)	5.2 (2.4-9.8)	3.4 (1.1-8.1)	1.9 (0.8-3.9)	0 (-)
Age at first event	63 (43-77)	54 (43-66)	68 (64-69)	69 (52-77)	(-)

Table 2: Prevalence, event rates, and age at onset of death and cardiovascular events (continued)

	All (213)	Men (83)		Women (130)	
		Classical (57)	Non-classical (26)	Classical (98)	Non-classical (32)
Sustained ventricular arrhythmias ‡					
Number of patients	9 (4%)	4 (7%)	3 (12%)	2 (2%)	0 (0%)
Event rate, with 95%-CI	1.3 (0.6-2.3)	2.6 (0.8-6.2)	2.5 (0.6-6.9)	0.6 (0.1-2.6)	0 (-)
Age at first event	56 (46-73)	48 (46-56)	67 (64-67)	62 (51-73)	(-)
Myocardial infarction					
Number of patients	22 (10%)	8 (14%)	4 (15%)	10 (10%)	0 (0%)
Event rate, with 95%-CI	3.1 (2.0-4.6)	5.2 (2.4-9.8)	3.4 (1.1-8.1)	3.1 (1.6-5.6)	0 (-)
Age at first event	51 (33-75)	51 (33-58)	57 (34-67)	51 (34-75)	(-)
Conduction abnormalities §					
Number of patients	29 (14%)	7 (12%)	8 (50%)	12 (12%)	2 (6%)
Event rate, with 95%-CI	4.1 (2.8-5.8)	4.5 (2.0-9.0)	6.7 (3.1-12.8)	3.7 (2.0-6.4)	1.8 (0.3-5.8)
Age at first documentation	60 (48-74)	56 (48-60)	62 (50-65)	63 (49-74)	72 (71-72)
Atrial fibrillation					
Number of patients	44 (21%)	20 (35%)	7 (27%)	16 (16%)	1 (3%)
Event rate, with 95%-CI	6.2 (4.6-8.3)	12.9 (8.1-19.6)	5.9 (2.6-11.6)	5.0 (3.0-7.9)	0.9 (0.04-4.3)
Age at first event	55 (18-69)	49 (18-61)	57 (34-69)	58 (47-67)	68 (-)

Data in table 2 is presented as number (percentage) or median (range). All event rates are per 1000 patient years from the age of 15 onwards.
¥ Major adverse cardiac events: composite of cardiovascular death, heart failure hospitalization, sustained ventricular arrhythmias and myocardial infarction.
‡ Sustained ventricular arrhythmias: composite of sudden cardiac death, sudden cardiac arrest, sustained ventricular tachycardia including appropriate ICD shock, and ventricular fibrillation.
§ Conduction abnormalities: composite of second-degree AV block Mobitz II, third-degree AV block, sinusarrest, and pacemaker or implantable cardiac defibrillator device implantation for conduction abnormalities.

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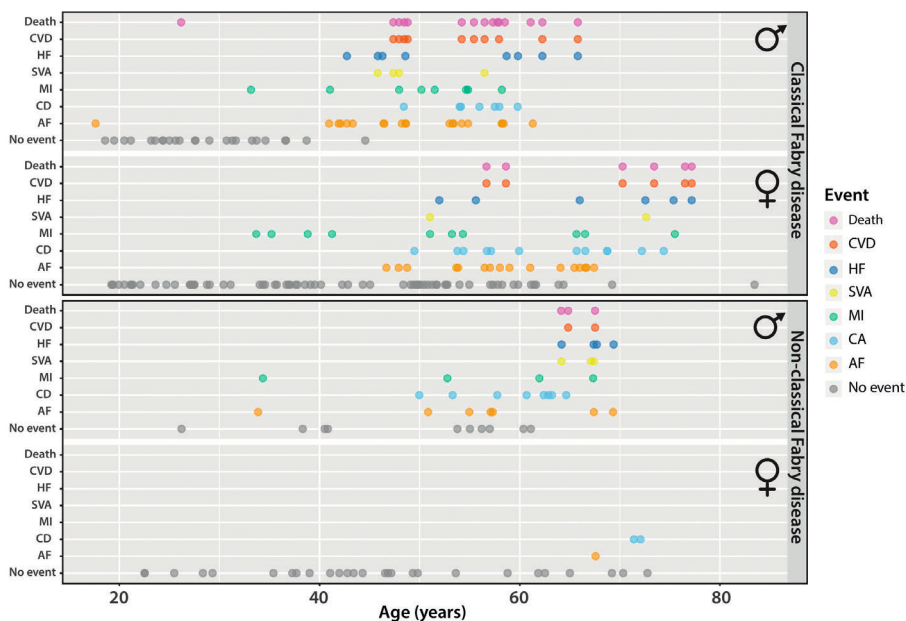
Figure 2: The occurrence of cardiac events for all 213 FD patients, stratified by sex (♂=men, ♀=women) and phenotype. Included events are: death, cardiovascular death (CVD), heart failure (HF) hospitalization (first event), sustained ventricular arrhythmias (SVA) (first event), myocardial infarction (MI) (first event), conduction abnormalities (CA) (first recorded), and atrial fibrillation (AF) (first recorded). No event was scored if none of the predefined events was recorded at the time of the last outpatient visit.

Enzyme replacement therapy

Sixty percent (128/213) of the included patients were treated with enzyme replacement therapy (ERT). Decisions to initiate treatment were based on the presence of symptoms as described earlier [25] or based upon the recommendations of the European Fabry Working Group once they became available [26]. The majority of the untreated patients were either diagnosed through family screening, without signs of organ involvement at last follow-up (48% of untreated patients) or diagnosed at an advanced disease stage, at which point no benefit of ERT was to be expected (21%) (**table 1**).

Major adverse cardiovascular events

The event rate (after age 15 years) for MACE was 11.0 per 1000 patient-years (95% CI: 6.6-17.3) for men with classical FD, versus 4.4 (2.5-7.1) in women with classical FD, and 5.9 (2.6-11.6) in men with non-classical FD. None of the women with non-classical FD developed MACE. KM analysis showed a significant difference between the four subgroups (**figure 3**, see **supplemental figure 2**

for CR analysis). Having a classical phenotype and being male significantly increased the risk of MACE (**table 3**). Hazard ratio (HR) for MACE in men with a classical versus non-classical phenotype was 6.1 and in men versus women with a classical disease phenotype 5.0 (**table 4**). Of the patients who developed MACE, MI was the first recorded event in 47% of men with classical FD, 64% of women with classical FD, and 57% of men with non-classical FD. Excluding MI from the MACE analysis, did not change the observed differences between the patient groups (see supplemental results).

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Figure 3: Kaplan-Meier curves (with 95%-confidence intervals) including data of 213 FD patients, stratified by sex and phenotype, for major adverse cardiovascular events (clustered endpoint of cardiovascular death, heart failure hospitalization, sustained ventricular arrhythmias, and myocardial infarction). Patients are censored if a MACE did not occur at time of last follow up contact. Median event-free survival is given for each group. Pairwise comparisons between patient groups are given.

Table 3: Firth's penalized Cox regression

Outcome	Covariate	Coef	Exp (coef)	95% CI
MACE	Phenotype, classical	2.99	19.89**	2.61-2554.03
	Sex, male	2.79	16.35**	1.94-2138.95
	Phenotype:Sex	-1.19	0.31	0.002-2.96
CVD	Phenotype, classical	2.29	9.84*	1.13-1292.61
	Sex, male	1.88	6.56	0.51-916.78
	Phenotype:Sex	0.81	2.25	0.01-38.56
HF hospitalization	Phenotype, classical	2.36	10.56*	1.23-1383.10
	Sex, male	2.35	10.51*	1.08-1407.18
	Phenotype:Sex	0.38	1.47	0.01-22.14
SVA	Phenotype, classical	1.07	2.91	0.23-404.25
	Sex, male	1.81	6.14	0.58-831.73
	Phenotype:Sex	0.55	1.74	0.01-39.94
MI	Phenotype, classical	2.42	11.19*	1.41-1446.63
	Sex, male	2.21	9.12	0.94-1221.07
	Phenotype:Sex	-1.28	0.28	0.00-3.26

*$P<0.05$, **$P<0.008$*

Table 4: Hazard ratio's for the comparison of different patient groups. These HR where obtained from Firth's penalized Cox regression models in which sex and phenotype where combined in 1 variable with 4 patient groups

Comparison	MACE	CVD	HF	SVA	MI
Men classical FD vs Men non-classical FD	6.1 (2.4-17.0)**	22.2 (5.0-19.3)**	15.5 (3.7-82.7)**	5.1 (0.9-41.1)	3.1 (1.0-11.7)
Men classical FD vs Women classical FD	5.0 (2.3-11.0)**	14.8 (4.3-67.0)**	15.4 (4.1-73.8)**	10.7 (1.9-92.0)**	2.5 (1.0-6.7)
Men non-classical FD vs Women classical FD	0.8 (0.3-2.0)	0.7 (0.1-2.7)	1 (0.3-3.4)	2.1 (0.4-13.0)	0.8 (0.2-2.4)

Cardiovascular death

In men with classical FD, 10 out of 15 deaths (67%) were cardiovascular deaths (event rate 6.5 per 1000 patient years). CVD was also the major cause of death in women with classical FD (6/6 deaths (100%), event rate 1.9) and men with non-classical FD (2/3 deaths (67%), event rate 1.7). KM analysis showed a significant difference between the four subgroups (**figure 4A**, see **supplemental figure 3** for CR analysis). Having a classical phenotype increased the risk of
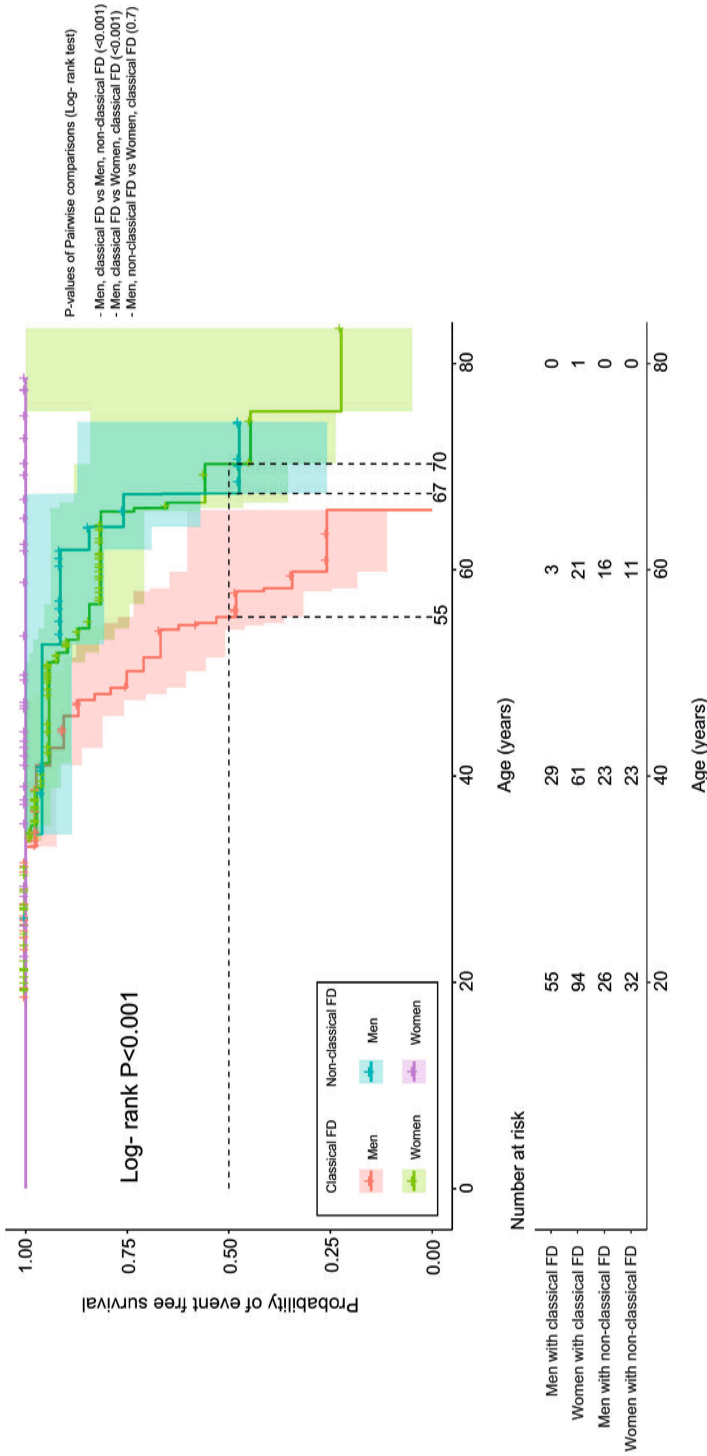
Figure 4: Kaplan-Meier curves (with 95%-confidence intervals) including data of 213 FD patients, stratified by sex and phenotype for A. Cardiovascular death, B. Heart failure hospitalization, C. Sustained ventricular arrhythmias, D. Myocardial infarction. Patients are censored if event did not occur at time of last follow up contact. Median event-free survival is given for each group in which events occurred. Pairwise comparisons between patient groups are given for each cardiac outcome.

CVD, but this effect did not hold after correcting for multiple testing (**table 3**). Group comparisons showed significant higher risk in men with classical vs non-classical disease (HR: 22.2) and men versus women with classical disease (HR: 14.8) (**table 4**). HF was an important cause of CVD (40% of CVD in men with classical FD, 67% in women with classical FD, and 100% in men with non-classical FD) (**table 2, supplemental table 2**).

Heart failure hospitalization
The event rate for HF hospitalization was 5.2 in men with classical FD, versus 1.9 in women with classical FD. In men with non-classical FD the event rate was 3.4. KM analysis showed a significant difference between the four subgroups (**figure 4B**, see **supplemental figure 4** for CR analysis).
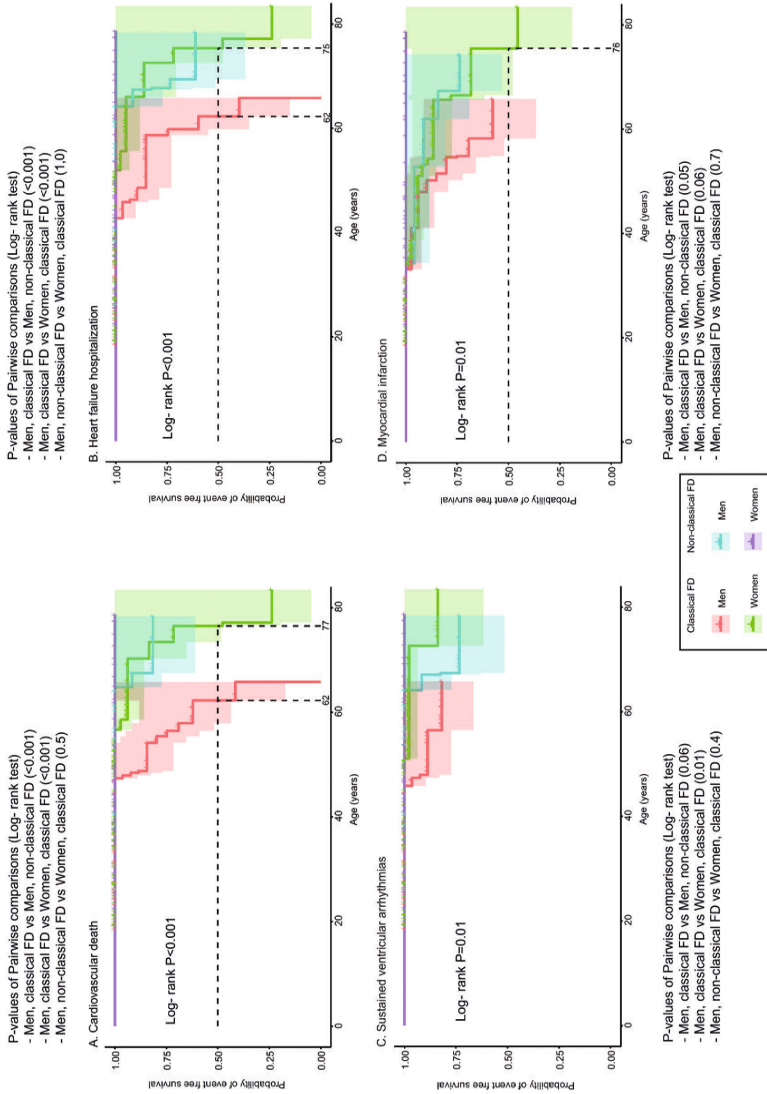
Classical phenotype and male sex increased the risk of HF hospitalization, but this effect did not hold after correcting for multiple testing (**table 3**). Group comparisons showed significant higher risk in men with classical vs non-classical disease (HR: 15.5) and men versus women with classical disease (HR: 15.4) (**table 4**). The left ventricle ejection fraction prior or at the time of the event was known in 17 out of 18 patients, who were hospitalized because of HF. In 12/17 patients (71%), the ejection fraction was <50% (5/7 in men with classical FD, 3/6 in women with classical FD and 4/4 in men with non-classical FD).

Sustained ventricular arrhythmias and implantable cardiac defibrillator device implantation
The event rate of SVA was low (14 events in 9 patients in the total cohort) (**figure 4C**, see **supplemental figure 5** for CR analysis, for between group comparison see **table 3 and 4**). The first SVA episode occurred at the time of an MI in 2 patients and in one patient at the time of HF hospitalization. Of the remaining 6 patients who experienced an SVA, 2 patients had an ejection fraction <35%, in 2 patients the first event occurred during hemodialysis, and in one patient an allergic reaction to clopidogrel was described as a possible trigger. In the last patient, no trigger could be identified. Of all patients who suffered SVA, 3 died as a result of the first episode (1 due to MI and 2 during hemodialysis), 1 patient died 3 years after the first SVA episode due to MI. The other 5 patients had an ICD implanted after the first event. In 3 patients, the ICD successfully aborted an episode of ventricular arrhythmia during follow-up. Of these 3 patients, 2 died of HF respectively 22 months and 6 years after ICD implantation. One patient was alive at last follow-up (ICD was 4.8 years in situ). In 2 patients, no shocks were administered by the ICD. Of these 2, 1 patient died 9 months after ICD implantation as a result of HF (no documented SVA), whereas in the other patient no shocks were documented at the time of a sudden cardiac arrest with pulseless electrical activity. He was resuscitated and survived the episode. In the

entire cohort of 213, 19 patients (including the 5 patients described above) had an ICD implanted. The total time the 19 ICDs were in situ was 71 years (range 0.1-9.3 years per patient).

Myocardial infarction

MI occurred in 22 patients. MI event rate was 5.2 in men with classical FD, 3.1 in women with classical FD and 3.4 in men with non-classical FD. The median event-free survival was not significantly different between the 3 affected patient groups, although the KM curves do show a similar pattern as seen for the other cardiac events (**figure 4D, supplemental figure 6**). A classical phenotype increased the risk of MI, but this effect did not hold after correcting for multiple testing (**table 3**). Group comparisons showed no significant differences in the HRs between the patient groups (**table 4**). For further MI classification see supplemental **table 3**. Cardiovascular risk factors (obesity, smoking, hypertension, dyslipidemia and diabetes mellitus) in patients suffering an MI were present in 4 out of 8 men with classical FD (data not available for 2 patients), 8 out of 10 women with classical FD and 4 out of 4 men with non-classical FD (data not shown).

Data on other non-major cardiac events *can be found in the supplemental results section.*

Discussion

This is the first longitudinal study that describes the prevalence and timing of cardiac events in a large cohort of male and female FD patients, categorized into classical and non-classical patient groups. The results show that these sex- and phenotype-defined patient groups differ substantially in terms of their risk of major cardiac events. The risk for all events showed the same trend: highest in men with a classical FD, intermediate in women with classical and men with non-classical FD and low in women with non-classical FD. For the individual events differences between the groups were not always significant, due to the relative low event rate. In men with classical FD, events occur mainly from the fifth decade of life onwards, resulting in a reduced life expectancy (no men with classical FD in our cohort survived beyond 66 years of age). In women with classical FD and men with non-classical FD events are observed from the sixth decade onwards, with more variability regarding the age of onset. MI is the first major adverse cardiac event observed in 21/38 (55%) of patients with a MACE. Most likely, both macrovascular and microvascular coronary artery disease contribute to the development of ischemia, due to endothelial dysfunction and cardiac hypertrophy in FD [17, 27, 28]. The relative contribution of FD related endothelial pathology and hypertrophy versus that of general risk factors (e.g.

smoking) cannot easily be determined, given that the majority of patients had cardiovascular risk factors at the time of MI.

Importantly, none of the women with non-classical FD developed a MACE. This variation means that if "cardiac events" is a clinical endpoint in a FD treatment study, men with classical FD should be treated as a separate group, from women with classical FD and men with non-classical FD, and women with non-classical FD should not be included at all. This also means that results from previous clinical trials, in which phenotypic distinction was not made, should be interpreted with caution.

Fabry cardiomyopathy is associated with a risk of sudden cardiac death (SCD) and in advanced disease ICD implantation should be considered [15, 29] . The most frequently observed cause of death in the current study was heart failure (42% of all deaths), whereas SCD comprised 17% of all deaths. This finding is in contrast with a recent meta-analysis of Baig et al., that identified SCD as the most common cause of death (62% of all deaths). However, the context of SCD and the Fabry disease phenotype of the included patients were not clear for most studies included in this meta-analysis. In addition, most included studies were performed in small cohorts, with relatively short follow-up duration. In our FD patient cohort SVA rate was low and more than half of the patients developed their first event in the context of either an MI or HF (hospitalization or ejection fraction <35%). An earlier study of Vijapurapu et al. (2019) reported a higher event rate in FD patients with an ICD, however sex and phenotype distribution in this group was not reported. Large multicenter cohort studies are needed to develop a FD SCD risk calculator to guide ICD implantation decision making.

Our findings, if confirmed in other longitudinal cohort studies, may change decision-making regarding ICD implantation policy in FD. With the knowledge that HF is the main contributor to death in FD, future studies should aim to detect and treat HF in a much earlier phase of the disease. This highlights the urgent need for biomarkers that predict future development of HF, separating low and high-risk patients, as clearly not all women with classical FD and men with non-classical FD develop HF.

A limitation of the current study is its retrospective design, which may lead to under-recording and subsequent underestimation of the event risk, even though we accessed historical files for the majority of patients. Nevertheless, we cannot rule out that for some deceased patients, especially those who died over a decade ago, some cardiac events may have been missed. In addition, the reported age of onset of cardiac manifestations such as systolic dysfunction, LVOT obstruction and heart valve disease depends on the time at which imaging was performed

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and may thus not be fully accurate. The contribution of enrollment bias on the observed outcomes between sexes remains unknown. In theory, more women could have been identified through family screening and more men because of clinical manifestations, which could have contributed to the observed differences in the occurrence of cardiac events. The influence of chronic kidney disease on MACE occurrence, which may have increased the risk, was not analyzed as the sample size and differences in follow-up duration between patients did not allow for this analysis. Although, a GFR below 60 ml/min was only present in 20% of classically affected male patients and only in a single patient from other groups (**supplemental table 4**). In addition, the effect of ERT on the occurrence of cardiac events was not analyzed, because of the same reasons, as well as variability in the age of therapy initiation and indication bias for start of treatment.

An important strength of the study is the fact that it was a long-term longitudinal study on a large FD cohort, and the detailed data on predefined clinical cardiac events, in contrast to earlier studies which often used composites for cardiac involvement in Fabry disease including symptoms of angina pectoris, palpitations, and microscopic Gb3 storage [22, 23].

Conclusions

This large longitudinal study confirms that men with classical FD develop severe cardiac events, mainly from the fifth decade of life onwards. For women with classical FD and men with non-classical FD, cardiac events occur approximately a decade later and in a smaller proportion of patients. None of the non-classically affected women in this cohort developed a major cardiac event. More than half of the first observed sustained ventricular arrhythmias occurred in the context of either an MI or HF, and HF was the most common cause of death. These findings shed new light on the clinical course and cardiac outcomes in FD cardiomyopathy and emphasize the need for new treatments, primarily to prevent heart failure in FD.

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SUPPLEMENTAL MATERIAL

Supplemental results

Conduction abnormalities
Occurrence of CA showed the same distribution pattern across the four patient groups (p=0.03 for comparison of men with classical FD versus women with classical FD; p=0.18 for men with classical FD versus men with non-classical FD; and p=0.005 for men with classical FD versus women withnon-classical FD) (Supplemental figure 1A). The median event-free survival was: 60, 69, and 65 years for men and women with classical FD and men with non-classical FD, respectively. CA were present in two women with non-classical disease .

Atrial fibrillation
The event rate for AF was 12.9 per 1000 patient-years in men with classical FD, 5.0 in women with classical FD and 5.9 in men with non-classical FD. Median event-free survival to first recorded AF event in men with classical FD was 53 years, compared to 66 years in women with classical FD. The median age of AF onset in men with non-classical FD was 57 years. Results of the log-rank test were p<0.001 for men with classical FD versus both other patient groups, (Supplemental figure 1B, table 2).

Systolic dysfunction, left ventricular outflow tract obstruction, and heart valve disease
The event rate for systolic dysfunction was the highest in men with classical and non-classical FD (7.1 and 6.7 respectively) and was substantially lower in women with classical and non-classical FD (2.5 and 0.9, respectively). First detection of systolic dysfunction occurred approximately a decade earlier in men with classical FD (median age 52, range 29-64) compared to women with classical FD (median age 65 years, range 54-77) and men with non-classical FD (64 years, range 53-69). For moderate/severe heart valve disease comparable results were found: men with classical FD had the highest event rate (9.1) and the lowest median age of onset (51 years, range 36-6l) in comparison with the other 3 patient groups (supplemental table 2). The event rate of LVOT obstruction was low (1.1) in the total cohort (supplemental table 2).

Heart surgery/ intervention
Rates of cardiothoracic surgery/ interventions (other than PCI and CABG surgery) were low in all patient groups (17 interventions in 10 patients), with the highest event rate (2.5) in men with non- classical FD (supplemental table 2). Types of surgery/interventions were heart valve repair and/ or replacement (n=9), ablation after ventricular or supraventricular arrhythmias (n=5), myectomy because of

severe LVOT obstruction (n=2), and correction of an anomalous pulmonary vein (n=1). Of the total cohort, 7 patients underwent PCI and 4 CABG surgery. There were no patients who underwent a heart transplantation.

Cardiac events in female patients with non-classical Fabry disease
No MACEs were detected in women with non-classical FD. A total of 5 patients developed 8 non- major cardiac events (2 CA (1 tachycardia- bradycardia syndrome and 1 left/right bundle branch block for which a pacemaker was implanted, 1 AF, 3 moderate/ severe heart valve disease, 1 systolic dysfunction and 1 LVOT obstruction). All events in this group occurred from age 65 years onwards.

MACE (excluding MI from the analysis)
13 men with classical FD, 4 men with non-classical FD and 8 women with classical FD experienced a MACE. None of the women with non-classical FD developed MACE. The event rate (after age 15 years) for MACE was 8.4 per 1000 patient-years for men with classical FD, versus 2.5 in women with classical FD, and 3.4 in men with non-classical FD.

Median event- free survival was 60 years in men with classical FD, 75 years for women with classical FD. The median age of onset for MACE in men with non-classical FD was 67 years (p<0.001 for comparison men with classical FD versus the other three patient groups; p=0.11 for comparison men and women with classical FD).

Supplemental table 1: Definitions of cardiac events

Events	Definition
Major adverse cardiovascular events (MACE): composite of cardiovascular death, heart failure hospitalization, sustained ventricular arrhythmias (SVA) and myocardial infarction	
1. Cardiovascular death*	Death as a result of one of the following diseases/ syndromes: - Acute coronary syndrome - Sudden cardiac death (SCD) - Hypertensive crise - Ischemic or hemorrhagic stroke - Cardiomyopathy - Other cardiovascular cause such as: pulmonary embolism, peripheral vascular disease
2. Heart failure hospitalization*	Hospital admission (at least one night) with the following clinical manifestations of heart failure: dyspnea, reduced exercise tolerance, fluid retention in peripheral and/ or splanchnic vessels, seen as peripheral edema

Supplemental table 1: Definitions of cardiac events (continued)

Events	Definition
3. Sustained ventricular arrhythmia (SVA)	composite of sudden cardiac death (SCD), sudden cardiac arrest (SCA), sustained ventricular tachycardia (VT) including appropriate ICD shock, and ventricular fibrillation (VF)
4. Myocardial infarction*	Acute myocardial injury with clinical evidence of acute myocardial ischemia. Definition according to the Fourth Universal Definition of Myocardial Infarction

Sustained ventricular arrhythmias (SVA): composite of sudden cardiac death (SCD), sudden cardiac arrest (SCA), sustained ventricular tachycardia (VT) including appropriate ICD shock, and ventricular fibrillation (VF)

5. Sudden cardiac death*	Sudden cessation of cardiac activity, resulting in hemodynamic collapse and death. Often typically due to sustained ventricular arrhythmias (VT, VF). This occurs either : - ≤ 1 hour after observed cardiac symptoms/ abnormalities *or* - If the patient is found dead, he or she was seen alive in the previous 24 hours without cardiac symptoms. Trauma, overdose, drowning and suicide exclude SCD
6. Sudden cardiac arrest*	Sudden cessation of cardiac activity so that the person becomes unresponsive, with no normal breathing and no signs of circulation
7. Sustained ventricular tachycardia*	Ventricular tachycardia lasting for >30 seconds or ended <30 seconds by a defibrillator
8. Ventricular fibrillation*	Inconsistent depolarization of the ventricles with AV- dissociation resulting in mechanical cardiac arrest (resuscitationand/or defibrillation often necessary)

Conduction abnormalities (CA): composite of second-degree atrioventricular (AV) block Mobitz II, third-degree AV block, sinusarrest (SA) and pacemaker (PM) or implantable cardiac defibrillator device (ICD) implantation for conduction abnormalities

9. Second-degree AV block, Mobitz II	Intermittent nonconducted P waves not preceded by PR prolongation and not followed by PR shortening.
10. Third-degree AV block	Interruption of impulse transmission from the atria to the ventricles

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Supplemental table 1: Definitions of cardiac events (continued)

Events	Definition
11. Sinusarrest	An alteration in discharge by the sinusnode pacemaker, resulting in no P- waves and associated QRS-T during sinus pause. This pause is sometimes followed by junctional rhythm or idioventricular rhythm. Absence of escape rhythm results in asystole
12. Implantable cardiac defibrillator implantation*	First ICD or Cardiac resynchronization therapy device (CRT-D) implantation, indication of primary or secondary prevention was registered
13. Pacemaker implantation*	First pacemaker implantation, indication for implantation was registered
Other events or interventions	
14. Atrial fibrillation	Irregular heart rhythm without identifiable p-waves recorded on ECG
15. Coronary artery disease*	At least 50% stenosis of luminal diameter of left main coronary artery or at least 70% stenosis of luminal diameter of at least one of the major epicardial coronary arteries on coronary angiogram. Patients were also classified as having coronary atherosclerosis if there was a clear indication of myocardial ischemia on non-invasive imaging (i.e. stress CMR, SPECT)
16. Percutaneous coronary intervention (PCI)	Non-surgical intervention in which coronary stenosis is resolved with coronary angioplasty with or without the placement of a coronary stent
17. Coronary artery bypass graft (CABG) surgery	Open heart surgery where a bypass is placed around one or more (stenotic) coronary arteries
18. Systolic dysfunction on MRI or echocardiography	Left ventricular ejection fraction <50% on MRI. If no MRI is available: left ventricular ejection fraction <55% on echocardiography
19. Left ventricular outflow tract (LVOT) obstruction	Dynamic gradient of ≥30 mmHg on echocardiogram in the left ventricular outflow tract measured at rest, during Valsalva procedure or during exercise
20. Moderate or severe valve disease	First ultrasound report mentioning moderate to severe stenosis of insufficiency of the mitral, tricuspid or aortic valve. Or heart valve dysfunction that required surgery where no previous ultrasound reports were available

Supplemental table 1: Definitions of cardiac events (continued)

Events	Definition
21. Heart surgery	Heart surgery, with exception of isolated CABG or PCI. This includes: myectomy due to LVOT obstruction with dynamic gradient, heart valve surgery, pulmonary vein isolation (PVI), or other interventions

* Events discussed with the expert panel

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Supplemental table 2: Prevalence, event rates and age at onset of individual outcomes †

	All (213)	Men (83)		Women (130)	
		Classical (57)	Non-classical (26)	Classical (98)	Non-classical (32)
1. Sudden cardiac death					
Number of patients	4 (2%)	4 (7%)	0 (0%)	0 (0%)	0 (0%)
Event rate, with 95%-CI	0.6 (0.2-1.4)	2.6 (0.8-6.2)	0 (-)	0 (-)	0 (-)
Age at death	48 (47-56)	48 (47-56)	(-)	(-)	(-)
2. Sudden cardiac arrest					
Number of patients	6 (3%)	1 (2%)	3 (12%)	2 (2%)	0 (0%)
Event rate, with 95%-CI	0.8 (0.3-1.8)	0.6 (0.03-3.2)	2.5 (0.6-6.9)	0.6 (0.1-2.1)	0 (-)
Age at first event	66 (46-73)	46 (-)	67 (64-67)	65 (57-73)	(-)
3. Sustained ventricular tachycardia					
Number of patients	4 (2%)	0 (0%)	2 (8%)	2 (2%)	0 (0%)
Event rate, with 95%-CI	0.6 (0.2-1.4)	0 (-)	1.7 (0.3-5.6)	0.6 (0.1-2.1)	0 (-)
Age at first event	66 (51-73)	(-)	66 (64-67)	62 (51-73)	(-)
4. Ventricular fibrillation					
Number of patients	2 (1%)	1 (2%)	0 (0%)	1 (1%)	0 (0%)
Event rate, with 95%-CI	0.3 (0.1-0.9)	0.6 (0.03-3.2)	0 (-)	0.3 (0.02-1.5)	0 (-)
Age at first event	59 (46-73)	46 (-)	(-)	73 (-)	(-)
5. Second- degree AV block, Mobitz II					
Number of patients	1 (1%)	0 (0%)	1 (4%)	0 (0%)	0 (0%)
Event rate, with 95%-CI	0.1 (0.01-0.7)	0 (-)	0.8 (0.04-4.1)	0 (-)	0 (-)
Age at first documentation	65 (-)	(-)	65 (-)	(-)	(-)
6. Third- degree AV block					
Number of patients	9 (4%)	1 (2%)	4 (15%)	4 (4%)	0 (0%)
Event rate, with 95%-CI	1.3 (0.6-2.3)	0.6 (0.03-3.2)	3.4 (1.1-8.1)	1.2 (0.4-3.0)	0 (-)
Age at first documentation	60 (50-72)	60 (-)	58 (50-63)	59 (54-72)	(-)

Supplemental table 2: Prevalence, event rates and age at onset of individual outcomes † (continued)

	All (213)	Men (83)		Women (130)	
		Classical (57)	Non-classical (26)	Classical (98)	Non-classical (32)
7. Sinusarrest					
Number of patients	7 (3%)	3 (5%)	2 (8%)	2 (2%)	0 (0%)
Event rate, with 95%-CI	1.0 (0.4-2.0)	1.9 (0.5-5.3)	1.7 (0.3-5.6)	0.6 (0.1-2.1)	0 (-)
Age at first documentation	58 (48-69)	58 (48-60)	60 (58-62)	59 (49-69)	(-)
8. PM, ICD and CRT-D implantation					
Number of patients	36 (17%)	9 (16%)	11 (42%)	14 (14%)	2 (6%)
PM	14 (7%)	4 (7%)	3 (12%)	5 (5%)	2 (6%)
PM + ICD upgrade at later stage	3 (1%)	0 (0%)	2 (8%)	1 (1%)	0 (0%)
ICD	7 (3%)	0 (0%)	3 (12%)	4 (4%)	0 (0%)
DDD-ICD	9 (4%)	3 (5%)	2 (8%)	4 (4%)	0 (0%)
CRT-D	2 (1%)	2 (4%)	0 (0%)	0 (0%)	0 (0%)
PM + CRT-D upgrade at later stage	1 (0.5%)	0 (0%)	1 (4%)	0 (0%)	0 (0%)
9. Coronary artery disease					
Number of patients	18 (9%)	9 (16%)	3 (12%)	6 (6%)	0 (0%)
Event rate, with 95%-CI	2.5 (1.6-3.9)	5.8 (2.8-10.7)	2.5 (0.6-6.9)	1.9 (0.8-3.9)	0 (-)
Age at first documentation	55 (39-77)	51 (46-64)	55 (53-67)	57 (39-77)	(-)
10. Percutaneous coronary intervention					
Number of patients	7 (3%)	3 (5 %)	2 (8%)	2 (2%)	0 (0%)
Event rate, with 95%-CI	1.0 (0.4-2.0)	1.9 (0.5-5.3)	1.7 (0.3-5.6)	0.6 (0.1-2.1)	0 (-)
Age at first documentation	53 (39-67)	50 (48-64)	61 (55-67)	46 (39-53)	(-)
11. Coronary artery bypass graft surgery					
Number of patients	4 (2%)	3 (5%)	1 (4%)	0 (0%)	0 (0%)
Event rate, with 95%-CI	0.6 (0.2-1.4)	1.9 (0.5-5.3)	0.8 (0.04-4.1)	0 (-)	0 (-)
Age at first surgery	58 (54-69)	58 (54-58)	69	(-)	(-)

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Supplemental table 2: Prevalence, event rates and age at onset of individual outcomes † (continued)

	All (213)	Men (83)		Women (130)	
		Classical (57)	Non-classical (26)	Classical (98)	Non-classical (32)
12. Systolic dysfunction on MRI or echocardiography					
Number of patients	28 (13%)	11 (19%)	8 (31%)	8 (8%)	1 (3%)
Event rate, with 95%-CI	3.9 (2.7-5.6)	7.1 (3.7-12.4)	6.7 (3.1-12.8)	2.5 (1.2-4.7)	0.9 (0.04-4.3)
Age at first documentation	60 (29-77)	52 (29-64)	64 (53-69)	65 (54-77)	72 (-)
13. Left ventricular outflow tract obstruction					
Number of patients	8 (4%)	2 (4%)	1 (4%)	4 (4%)	1 (3%)
Event rate, with 95%-CI	1.1 (0.5-2.1)	1.3 (0.2-4.3)	0.8 (0.04-4.1)	1.2 (0.4-3.0)	0.9 (0.04-4.3)
Age at first documentation	56 (31-65)	43 (31-55)	58 (-)	57 (46-61)	65 (-)
14. Moderate or severe heart valve disease					
Number of patients	39 (18%)	14 (25%)	6 (23%)	16 (16%)	3 (9%)
Event rate, with 95%-CI	5.5 (4.0-7.4)	9.1 (5.2-14.8)	5.0 (2.0-10.5)	5.0 (3.0-7.9)	2.6 (0.7-7.2)
Age at first documentation	58 (36-77)	51 (36-61)	65 (51-70)	61 (47-77)	74 (65-76)
15. Heart surgery/ intervention*					
Number of patients	10 (5%)	2 (4%)	3 (12%)	5 (5 %)	0 (0%)
Event rate, with 95%-CI	1.4 (0.7-2.5)	1.3 (0.2-4.3)	2.5 (0.6-6.9)	1.6 (0.6-3.5)	0 (-)
Age at first surgery/ intervention	63 (49-72)	53 (49-57)	64 (64-68)	61 (52-72)	(-)

Data are presented as number (percentage) or median (range). All event rates are per 1000 patient years from age 15 onwards.
† Events per 1000 person years from age of 15 = (Number of events/ Cumulative follow up from age 15)x1000
* heart surgery or intervention other than percutaneous coronary intervention or coronary artery bypass graft surgery

AV = atrioventricular; CRT = cardiac resynchronization therapy; ICD = implantable cardiac defibrillator; PM = pacemaker

Supplemental table 3: Classification of myocardial infarctions

|	All (213)	Men (83)		Women (130)	
		Classical (57)	Non-classical (26)	Classical (98)	Non-classical (32)
Type I, n	7	3	1	3	0
STEMI, n	3	1	1	1	-
NSTEMI, n	3	1	0	2	-
Unknown, n	1	1	0	0	-
Type II, n	5	2	0	3	0
NSTEMI, n	5	2	-	3	-
Unknown type of infarction, n	10	3	3	4	0
NSTEMI, n	4	1	1	2	-
Unknown, n	6	2	2	2	-

n = number of patients; STEMI = ST-segment elevation myocardial infarction; NSTEMI = Non-ST-segment elevation myocardial infarction.

Supplemental table 4: Patient kidney characteristics

	All (213)	Men (83)		Women (130)	
		Classical (57)	Non-classical (26)	Classical (98)	Non-classical (32)
KDIGO CKD stages**					
Patients with a known CKD stage, n (%)	152 (71%)	41 (72%)	13 (50%)	82 (84%)	16 (50%)
G1 (eGFR >90), n (%)	75 (49%)	23 (56%)	3 (23%)	37 (45%)	12 (75%)
G2 (eGFR 60-89), n (%)	48 (32%)	9 (22%)	4 (31%)	33 (40%)	2 (13%)
G3a (eGFR 45-59), n (%)	13 (9%)	1 (2%)	2 (15%)	8 (10%)	2 (13%)
G3b (eGFR 30-44), n (%)	12 (8%)	7 (17%)	2 (15%)	3 (4%)	0 (0%)
G4 (eGFR 15-29), n (%)	3 (2%)	1 (2%)	1 (8%)	1 (1%)	0 (0%)
G5 (eGFR <15), n (%)	1 (1%)	0 (0%)	1 (8%)	0 (0%)	0 (0%)
Proteinuria (microalbuminuria > 300 milligrams/ 24 hours)**					
Patients with a known proteinuria status, n (%)	71 (33%)	16 (28%)	7 (27%)	41 (42%)	7 (22%)
Present proteinuria, n (%)	12 (17%)	4 (25%)	3 (43%)	5 (12%)	0 (0%)
Absent proteinuria, n (%)	59 (83%)	12 (75%)	4 (57%)	36 (88%)	7 (100%)

KDIGO= Kidney Disease Improving Global Outcomes; CKD= Chronic Kidney Disease; eGFR= estimated Glomerular Filtration Rate, according to the Chronic Kidney Disease Epidemiology Collaboration equation and measured in milliliters/ minute per 1.73 meter^2
** The KDIGO CKD and proteinuria status are measurements, which are known at the time of the last follow-up (with a minimum and maximum range of 1 year between the measurement and the last follow-up date).

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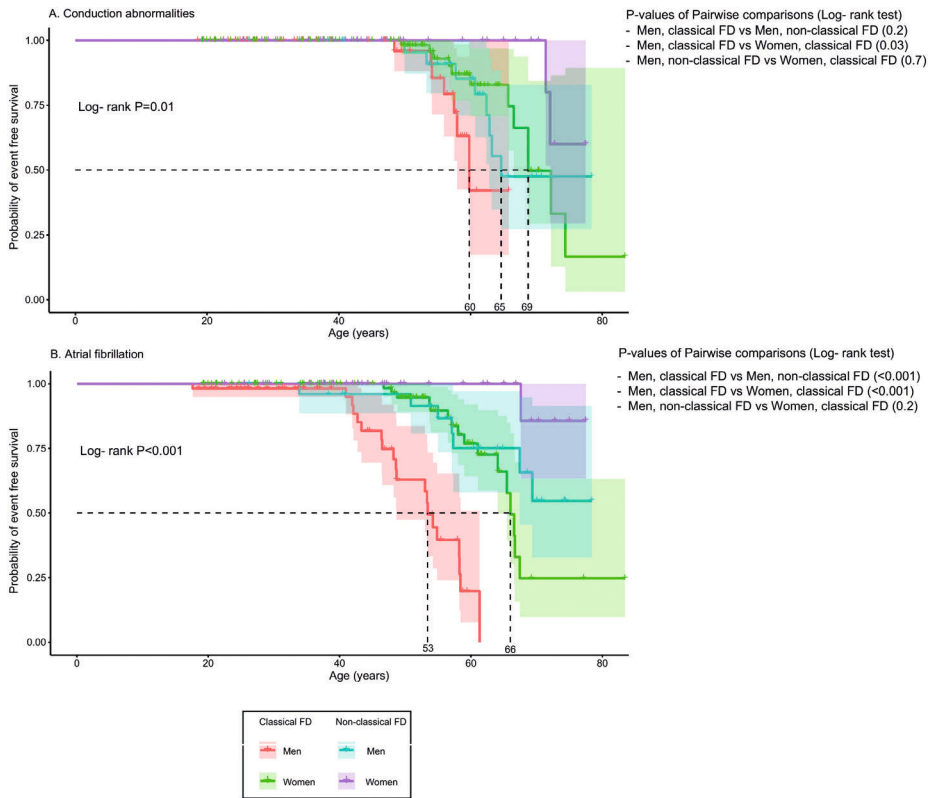
Supplemental figure 1: Kaplan-Meier curves (with 95%-confidence intervals) including data of 213 FD patients, stratified by sex and phenotype for A. Conduction abnormalities, B. Atrial fibrillation. Patients are censored if event did not occur before last follow up. Median event-free survival is given for each group in which events occurred. Pairwise comparisons between patient groups are given.

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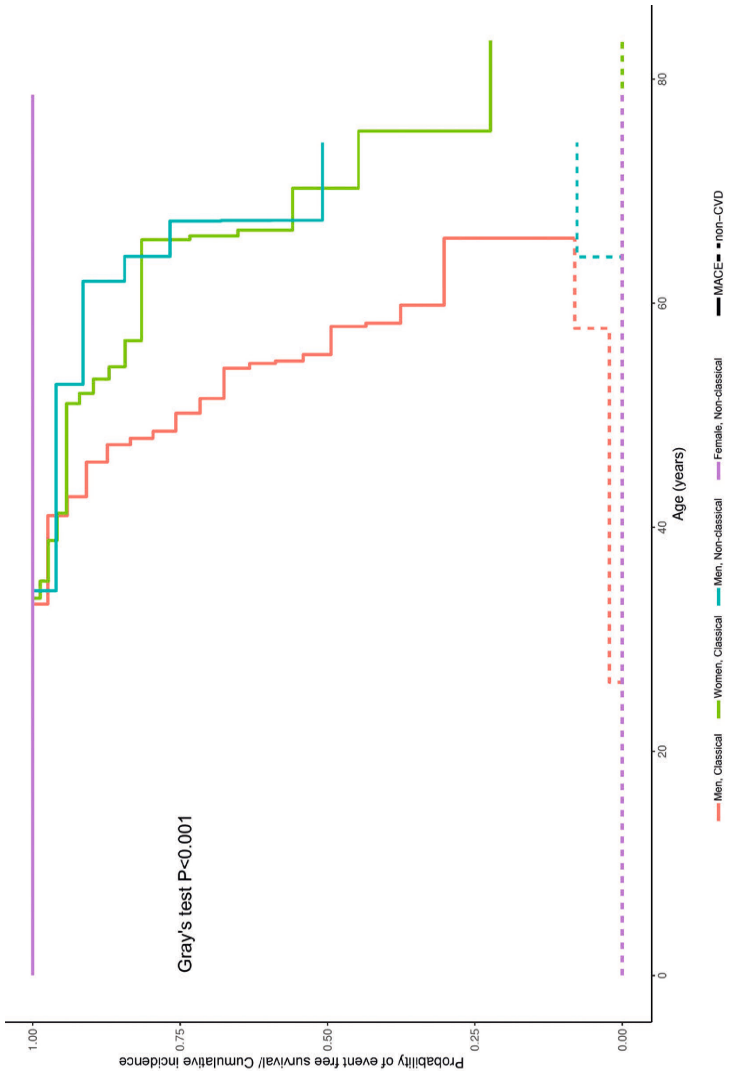
Supplemental figure 2: The smooth lines represent the Kaplan-Meier curves including data of 213 FD patients, stratified by sex and phenotype, for Major adverse cardiovascular events (clustered endpoint of cardiovascular death, heart failure hospitalization, sustained ventricular arrhythmias, and myocardial infarction). The broken lines represent the cumulative incidence curves from the competing risk (CR) analyses, where non- cardiovascular death is accounted as competing risk. The Event-free survival in men with classical FD is 55 years and in women with classical FD 70 years. The P- value of the Gray's test is given.
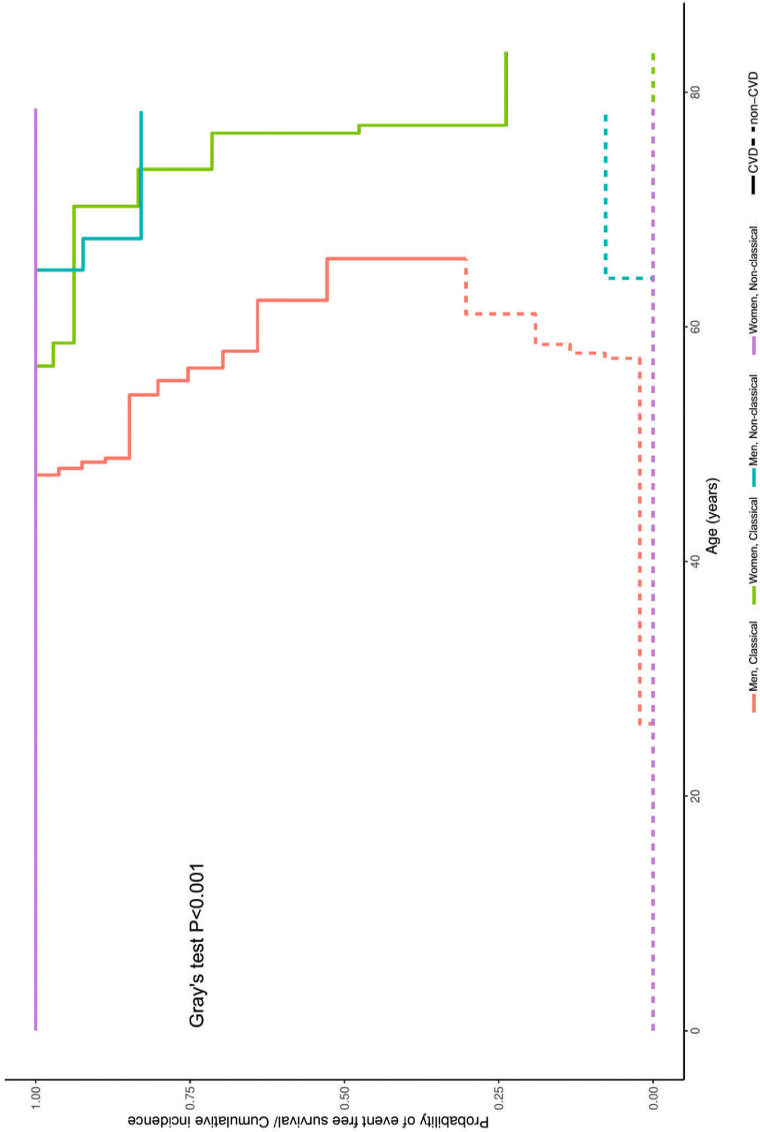
Supplemental figure 3: The smooth lines represent the Kaplan-Meier curves including data of 213 FD patients, stratified by sex and phenotype, for Cardiovascular death. The broken lines represent the cumulative incidence curves from the competing risk (CR) analyses, where non- cardiovascular death is accounted as competing risk. The Event-free survival in men with classical FD is 66 years and in women with classical FD 77 years. The P-value of the Gray's test is given.
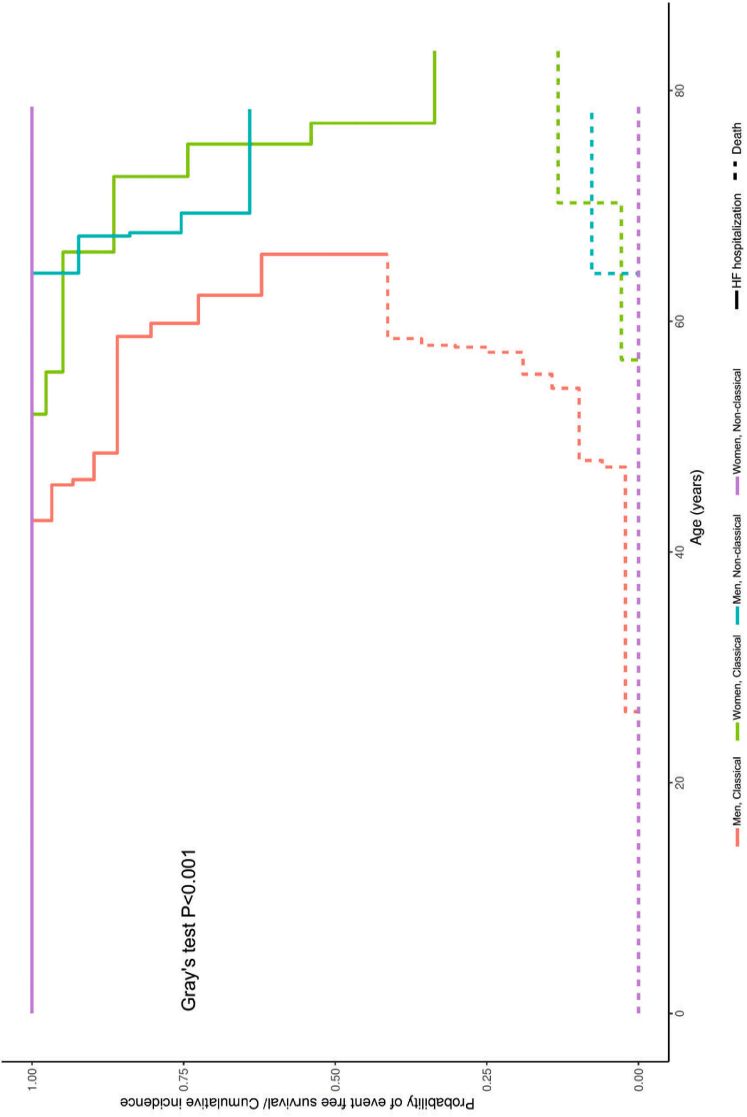
Supplemental figure 4: The smooth lines represent the Kaplan-Meier curves including data of 213 FD patients, stratified by sex and phenotype, for Heart failure hospitalization. The broken lines represent the cumulative incidence curves from the competing risk (CR) analyses, where non- cardiovascular death is accounted as competing risk. The Event-free survival in men with classical FD is 66 years and in women with classical FD 77 years. The P-value of the Gray's test is given.

Supplemental figure 5: The smooth lines represent the Kaplan-Meier curves including data of 213 FD patients, stratified by sex and phenotype, for Sustained ventricular arrhythmias. The broken lines represent the cumulative incidence curves from the competing risk (CR) analyses, where death is accounted as competing risk. The P- value of the Gray's test is given.
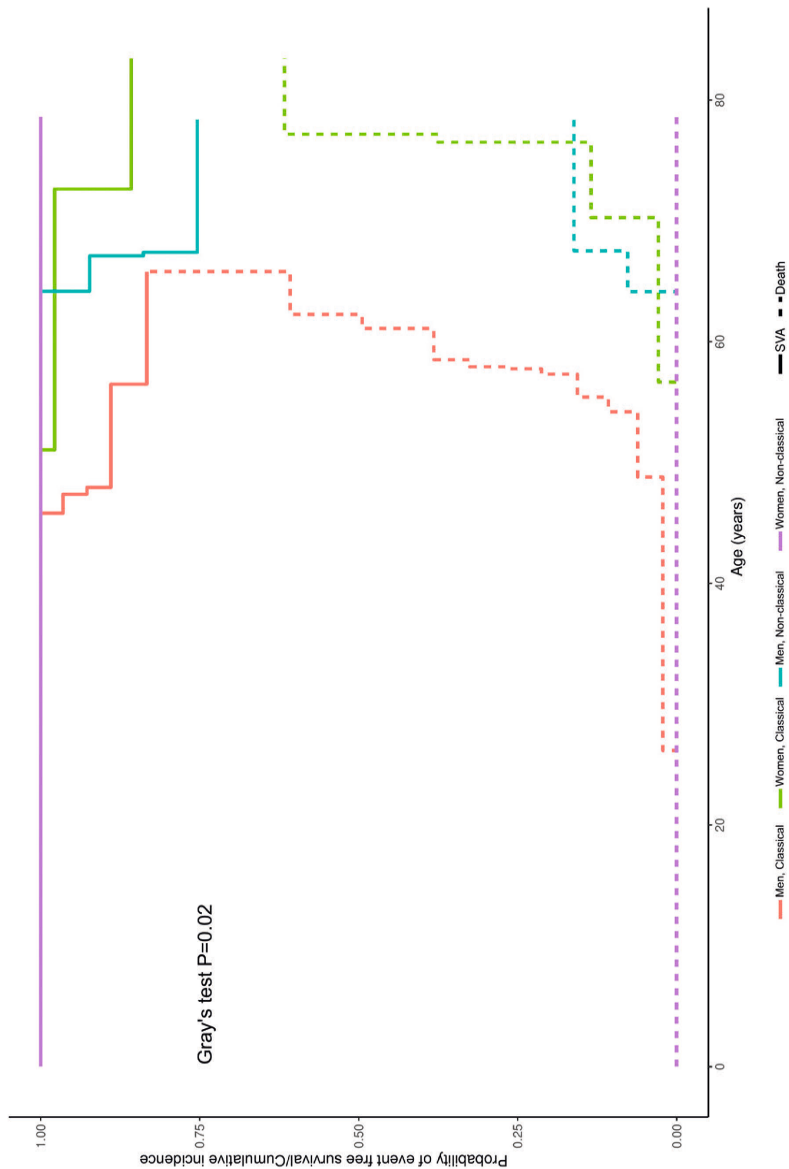
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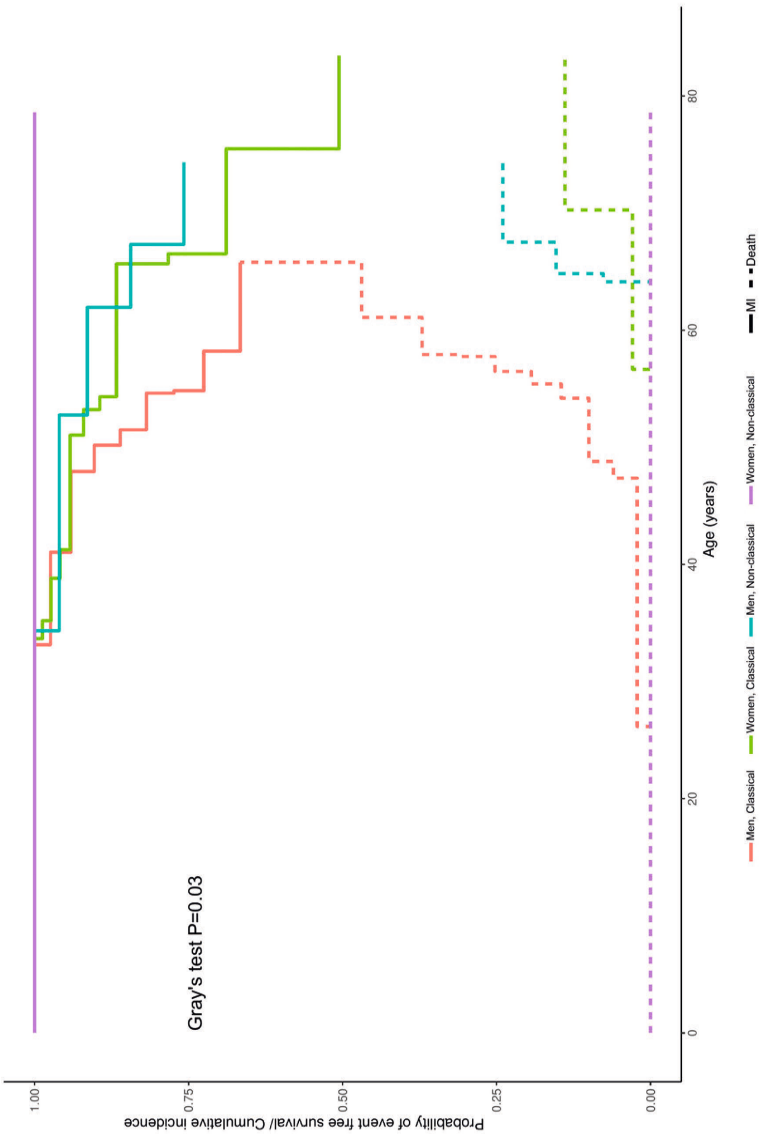
Supplemental figure 6: The smooth lines represent the Kaplan-Meier curves including data of 213 FD patients, stratified by sex and phenotype, for Myocardial infarction. The broken lines represent the cumulative incidence curves from the competing risk (CR) analyses, where death is accounted as competing risk. The P- value of the Gray's test is given.
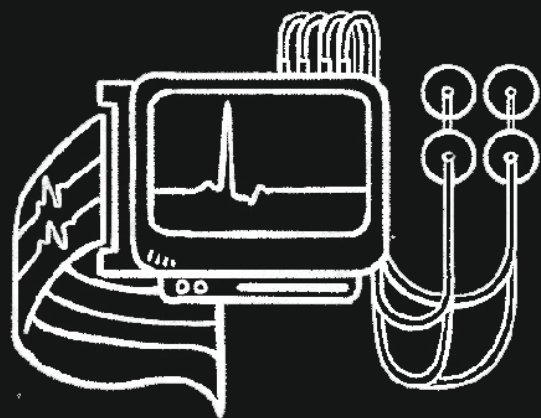
Chapter 3

ECG Changes During Adult Life in Fabry Disease:
Results from a Large Longitudinal Cohort Study

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Abstract

Background
Fabry disease (FD) is an X-linked, lysosomal storage disorder leading to severe cardiomyopathy in a significant proportion of patients. To identify ECG markers that reflect early cardiac involvement and disease progression we conducted a long term retrospective study in a large cohort of FD patients.

Methods
A total of 1,995 ECGs from 133 patients with classical FD (64% females, 80% treated with enzyme replacement therapy), spanning 20 years of follow-up, were compared to ECGs from 3,893 apparently healthy individuals. Generalized linear mixed models were used to evaluate the effect of age, FD and sex on: P-wave duration, PR-interval, QRS-duration, QTc, Cornell index, spatial QRS-T angle and frontal QRS-axis. Regression slopes and absolute values for each parameter were compared between FD patients and control subjects.

Results
At a younger age (< 40 years), Cornell index was higher and frontal QRS-axis more negative in FD patients compared to controls ($p<0.05$). For the other ECG parameters, the rate of change, more than the absolute value, was greater in FD patients compared to controls ($p<0.05$). From the fifth decade (men) or sixth (women) onwards, absolute values for P-wave duration, QRS-duration, QTc and spatial QRS-T angle were longer and higher in FD patients compared to control subjects.

Conclusions
ECG abnormalities indicative of FD are age and sex dependent. Tracking the rate of change in ECG parameters could be a good way to detect disease progression, guiding treatment initiation. Moreover, monitoring ECG changes in FD can be used to evaluate effectiveness of treatment.

Introduction

Fabry disease (FD) is an X-linked lysosomal storage disease with slowly progressive and highly variable clinical expression. The disorder is caused by mutations in the galactosidase alpha (GLA) gene, leading to reduced activity of the lysosomal enzyme alpha-galactosidase A. The enzymes' substrate globotriaosylceramide (Gb3) and its derivates accumulate in various tissues and organs, including the heart [1, 2]. Over decades, lysosomal dysfunction, disturbed autophagy, inflammatory and fibrotic changes eventually lead to permanent cardiac damage [3-6]. Initially, signs of cardiac involvement may be subtle, such as a low native T1 value on cardiac magnetic resonance imaging (CMR) and a short PR-interval on ECG. At that early stage there are most often no clinical symptoms of cardiac disease and overall cardiac function on echocardiography and CMR is normal [7, 8]. At later disease stages, conduction abnormalities, overt left ventricular hypertrophy (LVH), myocardial fibrosis and eventually symptomatic cardiac disease (heart failure, arrhythmias and sudden cardiac death) occurs [9-12].

Since FD is an X-linked disorder the disease is generally more severe in men. The disease can be classified into an early onset, classical, and a later-onset, non-classical phenotype [9, 10, 13]. A recently conducted observational longitudinal cohort study in 213 FD patients confirmed the heterogeneity of cardiac disease manifestations in FD [14]. Male patients with classical FD (cFD) over the age of 45 years, invariably suffered a cardiovascular event. In contrast, only a subset of females with cFD developed cardiovascular events and with a highly variable age of onset [14]. For male patients with cFD there is no debate about the need for Fabry specific treatment and recent studies suggest that early initiation (specifically of enzyme replacement therapy (ERT) has a better effect on suppressing disease progression) [15, 16]. For women with cFD there is much more uncertainty about the need for treatment of an individual patient (since not all patients will develop complications) and even more about the optimal timing of treatment initiation. Thus, there is a need to identify female patients at risk for symptomatic cardiac disease (and thus in need of intensive monitoring) versus those who are unlikely to develop major cardiac complications.

LVH on CMR is a commonly used clinical marker for presence of cardiac manifestation in FD and thus the need to initiate therapy [15]. However, in those that develop LVH this usually occurs in later stages of the disease and some female patients develop cardiac FD complications in the absence of LVH [10, 17]. Hence, identification of biomarkers (other than age, sex and left ventricular mass) that can reliably detect cardiac involvement at an earlier, asymptomatic, disease

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stage are important to be able to limit further progression of cardiac disease in FD patients. ECG parameters might be suitable to track this progression.

Previous cross-sectional and longitudinal studies with a relatively small sample size show that early ECG abnormalities in FD patients include a short PR-interval and bradycardia [18, 19]. A long P-wave duration, prolonged QRS-duration, QTc, high QRS-amplitude, T-wave inversion and left frontal QRS- axis deviation may occur in later disease stages [19-21]. There are no longitudinal studies to show electrophysiological development over time in different patient groups (e.g., men versus women with FD) and how this differs from age related changes in ECG parameters in healthy individuals. This knowledge is needed to guide timing of treatment initiation (especially in women). But also to be able to detect the effect of (new) FD treatments on cardiac disease progression, since overt clinical complications take decades to develop [22, 23], far surpassing the duration of clinical trials.

We hence conducted a retrospective study in the FD patient cohort under follow-up at the Amsterdam University Medical Centres (AUMC), to establish the course of electrophysiological parameters in male and female FD patients, to compare them to apparently healthy control subjects and to study their relationship to left ventricular mass and the presence of fibrosis on CMR. This study is unique in terms of both sample size and length of systematic ECG follow-up of FD patients.

Primary aims of the study are:

1. Describing the evolution of alterations in ECG parameters in patients with classical FD and comparing these features to those of an apparently healthy control group;
2. Comparing the evolution of ECG alterations in men versus women with classical FD.

And the secondary aim is:

3. Investigating the relationship between ECG features and left ventricular mass and the presence of late gadolinium enhancement on CMR in classical FD patients.

Methods

Study population and design

FD patients
This retrospective cohort study was conducted at the FD centre of excellence in the Netherlands (AUMC, location University of Amsterdam). All available ECGs from adult (≥ 18 years) patients with classical FD obtained between February 1996 and July 2018 were included. The ECGs were obtained as part of the routine clinical follow-up (outpatient clinical visits are every 6 to 12 months, depending on age, sex and treatment status of the patient) or during hospital admissions. All patients of whom ECGs were included had a definite diagnosis of classical FD. The diagnosis and phenotype assignment were based on alpha-galactosidase A in leucocytes, family history (for women), classical FD symptoms and the levels of a deacylated form of Gb3 (Globotriaosylsphingosine (LysoGb3)) **(supplemental figure 1)** [9, 10, 13, 14].

Control group
ECGs of apparently healthy subjects of Dutch origin were collected between January 2011 and November 2015 as part of the baseline data collection of the 'HELIUS' study (Healthy Life in an Urban Setting – a large prospective cohort study in Amsterdam, The Netherlands) [24]. HELIUS is a multi-ethnic study, including roughly equally sized groups of Surinamese (South-Asian and African), Ghanaian, Turkish, Moroccan and Dutch origin participants, and was designed to study the (causes of the) unequal burden of disease across ethnic groups. To increase comparability with the FD cohort, of which the vast majority is of Dutch origin, only HELIUS participants with Dutch origin were selected as controls. In addition, age was restricted to 18-65 for men, and 18-71 for women, since this was the age range in FD patients. Baseline measurements of HELIUS included a single ECG during a physical examination at the research location, collection of biological samples and questionnaires [24, 25].

Individuals with and without cardiovascular risk factors (including smoking, hypertension, obesity, diabetes mellitus and the use of anti-lipaemics, full list and definitions can be found in **table 1**) were included, but they had to be free from apparent cardiovascular disease (myocardial infarction or stroke) as determined by self-report, medication use and ECG evaluation. We chose a control group in which cardiovascular risk factors were present since these same risk factors were also present in a significant number of the included FD patients **(table 1)**.

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Ethics approval
This work was conducted following the Declaration of Helsinki [26]. Because of the retrospective nature of this study, the need for informed consent for the use of data from the FD patients was waived by the Medical Ethics Committee of the Amsterdam UMC. The HELIUS study was approved by the same Medical Ethics Committee and all participants provided written informed consent.

ECG processing and analysis
Standard 12-lead supine digital resting ECGs were recorded in FD patients and control subjects (GE MAC5500, 500 samples/sec). The obtained ECGs were processed with the Modular ECG Analysis System (MEANS) program, which determines P-wave, QRS and T-wave onsets and offsets for all 12 leads together on one representative averaged beat [27]. All on- and offsets were manually checked and adjusted when needed. Thereafter, various ECG parameters were automatically computed, including P-wave duration, PR-interval, QRS-duration, QTc, QRS minimum and maximum amplitudes for each lead, Spatial QRS-T angle, Frontal QRS-axis and Frontal T-axis. In case of ventricular pacing all parameters were excluded from the analysis and in case of atrial fibrillation or atrial pacing P-wave duration and PR-interval were selectively excluded from analysis.

Cardiovascular risk factors and clinical characteristics
For FD patients, cardiovascular risk factors (smoking, hypertension, obesity, diabetes mellitus and dyslipidemia, full list and definitions can be found in **table 1**) were assessed at the first outpatient visit. With the exception of dyslipidemia, the same cardiovascular risk factors were surveyed in the control group. As an alternative for dyslipidemia evaluation in the control subjects we reported the use of antilipaemics.

Data on estimated glomerular filtration rate (eGFR) and the presence of albuminuria were collected. For FD patients, these data were extracted from the clinical records for the date closest to the last included ECG (maximum 1 year earlier or later).

Renal function was estimated by calculating the estimated glomerular filtration rate (eGFR) using the CKD-EPI formula. Microalbuminuria in FD patients was defined as $\geq$30 mg albumin in collected 24 hours urine samples. For the control subjects, microalbuminuria was defined as $\geq$20 mg/l albumin in a urine portion, as no 24-hour urine samples were available.

ECG and CMR characteristics in FD patients

To assess the relation between ECG parameters and cardiac imaging parameters, CMR data (left ventricular mass indexed to body surface area (LVMi) and the presence of late gadolinium enhancement (LGE)) were extracted from the patients records for the date closest to the last included ECG (maximum 1 year earlier or later).

Cardiovascular events in FD patients

Cardiac events from birth to last outpatient clinic visit were recorded in FD patients (the majority of the patients included in the current study were also included in our recently published study on cardiovascular events in FD [14]). Recorded cardiac events were major cardiovascular events (MACE) (combined endpoint including cardiovascular death, heart failure hospitalization, sustained ventricular arrhythmias and myocardial infarction) (for definitions of these events see: **supplemental table 3**).

Statistical analysis

R Studio (version 4.0.3) was used for Statistical analysis. Data are presented as proportions or median and minimum/maximum ranges. Differences in the prevalence of cardiovascular risk factors between FD patients and the control group were tested by the 2x2 Fisher exact test. Generalized linear mixed- effect models (GLM) were used to evaluate the effect of age at the time of obtaining an ECG, type of study subject (FD patient or control subject) and sex on seven ECG parameters: P-wave duration, PR- interval, QRS-duration, QTc, Cornell Index (voltage sum of R in aVL and S in V3), spatial QRS-T angle, and frontal QRS-axis. A random intercept and slope were introduced into all mixed models, taking inter-patient differences into account. Model assumptions were checked and met [28]. Because of the assumption that the effect of age on ECG parameters would be different between men and women but also between FD patients and controls, we tested for three-way interactions (age * type of the study subject (FD patient or control subject) * sex) in all models. Subgroups were defined as: men with classical FD, women with classical FD, controls- men and controls- women. From the resulting model, regression lines per subgroup were obtained following the standard regression equation for a linear model: $y = a + \beta*X$, with the intercept (a) and slope (β) specified for each subgroup. Similarly, the differences between two given subgroups were calculated. The slopes of these regression equations (β per 10 years increase in age) were compared to study the difference in evolution of a given ECG parameter over time between subgroups. In a GLM subanalysis, the untreated FD patients were excluded to investigate if there was a difference in ECG parameters' increment between the ERT treated patients only and the complete study cohort, that also contains a minority of untreated FD patients. The frontal T-axis showed too much nonlinear variation for a valid GLM approach.

For that reason, only the raw data on frontal T-axis over time were visually displayed in a polar plot.

The GLM assumes a linear change in ECG parameters over time, and is therefore unsuitable for making an accurate statement about the age at which an ECG parameter in FD patients start to deviate from those in control subjects. To study the timeframe in which ECG alterations occur in FD, the differences in absolute values for each ECG parameter between FD patients and the control subjects for each decade of adult life were assessed by a Wilcoxon rank test. For these comparisons, a Bonferroni adjusted p- value is displayed to correct for multiple testing. For this comparison of absolute values, only the last available ECG per patient per decade was selected to ensure that the influence of repeated measurements in single individuals was limited.

By using a Spearman correlation analysis, the correlation between the seven electrophysiological parameters on the last obtained ECG and LVMi on the corresponding CMR was evaluated. A Wilcoxon signed-rank was used to study if the absolute values of the parameters on the last ECG were different between patients with and without LGE on the corresponding CMR (LGE assesses the presence of myocardial fibrosis). We regarded a *p-value ≤0.05* as statistically significant.

Results

Participants' characteristics
Serial ECGs of a total of 133 patients with classical FD (36% men and 64% women) were included averaging 15 ECGs per patient (range: 4-66), totaling 1,995 ECGs. For the FD patients, the median age at last obtained ECG was 48 years (range: 19-82). The HELIUS cohort consisted of 3,893 control subjects (43% men and 57% women) with a median age of 46 years (range: 18-71). Full description of the participants' characteristics can be found in **table 1**.
Eighty percent (106/133) of the included FD patients were treated with ERT, during a median period of 7 years (range: 0-17). ERT initiation decisions were based on the presence of FD symptoms [29] or on recommendations by the European Fabry Working Group after these became available in 2015 [15]. See supplemental table 4 for the GLA mutations characteristics of the FD patients.

Table 1: Participants' characteristics

Number of patients, n (%)	All 133 (100%)	Men 48 (36%)	Women 85 (64%)
General			
Number of ECGs	1,995	819	1,176
Number of ECGs per patient, median (range)	15 (4-66)	18 (4-66)	15 (4-47)
Age at first ECG (years), median (range)	38 (18-68)	27 (18-58)	41 (18-68)
Age at last ECG (years), median (range)	48 (19-82)	37 (19-65)	50 (20-82)
Follow-up duration (years), median (range)	9 (0-18)	8 (0-18)	9 (0-17)
Enzyme replacement therapy (ERT)			
Patients on ERT, n (%)	106 (80%)	45 (94%)	61 (72%)
Duration of ERT treatment (years), median (range)	7 (0-17)	9 (0-17)	7 (0-14)
Cardiovascular comorbidities			
Smoking†, n (%)	47 (35%)	16 (33%)	31 (37%)
Hypertension†, n (%)	22 (17%)	7 (15%)	15 (18%)
Obesity†, n (%)	15 (11%)	0 (0%)	15 (18%)
Diabetes mellitus†, n (%)	2 (1.5%)	0 (0%)	2 (2%)
Dyslipidemia†, n (%)	6 (5%)	0 (0%)	6 (7%)
Laboratory findings at last ECG			
eGFR- CKD EPI formula (ml/min), median (range)	87 (15-144)	101 (15-144)	85 (22-131)
Urine albumin (mg/ 24 hours), median (range)	42 (3-3498)	54 (4-3498)	42 (3-1175)
Presence of microalbuminuria**, n (%)	65/115 (57%)	25/40 (63%)	40/75 (53%)
Cardiac MRI findings at last ECG			
Left ventricular mass index (g/m^2), median (range)	63 (32-141)	78 (43-141)	60 (32-117)
Presence of late Gadolinium enhancement, n (%)	60/118 (51%)	17/40 (43%)	43/78 (55%)
Cardiovascular events during follow-up			
Major adverse Cardiovascular events, n (%)	22 (17%)	11 (23%)	11 (13%)
Age at MACE (years), median (range)	55 (33-70)	55 (33-66)	66 (34-70)
Number of controls, n (%)	All 3893 (100%)	Men 1667 (43%)	Women 2226 (57%)
General			
Age at ECG, median (range)	46 (18-71)	45 (18-65)	46 (18-71)
Cardiovascular comorbidities			
Smoking*, n (%)	2377 (61%)	1049 (63%)	1328 (60%)
Hypertension*, n (%)	983 (25%)	514 (31%)	469 (21%)

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Table 1: Participants' characteristics (continued)

Number of controls, n (%)	All 3893 (100%)	Men 1667 (43%)	Women 2226 (57%)
Obesity, n (%)	345 (9%)	139 (8%)	206 (9%)
Diabetes mellitus, n (%)	102 (3%)	57 (3%)	45 (2%)
Antilipaemics, n (%)	164 (4%)	76 (5%)	88 (4%)
Laboratory findings			
eGFR- CKD EPI formula (ml/min), median (range)	97 (24-146)	99 (25-146)	95 (24-137)
Presence of microalbuminuria**, n (%)	162/3879 (4%)	69/1660 (4%)	93/2219 (4%)

† cardiovascular risk factors were for FD patients assessed at first outpatient clinic visit:
- Obesity: Body Mass Index ≥ 30 kg/m2
- Smoking: ever smoked
- Hypertension: antihypertensive medication use or systolic blood pressure of >140 mmHg and/or diastolic blood pressure of >90 mmHg, measured at least twice
- Dyslipidemia: elevated levels of total cholesterol (>6.5 mmol/l) or low density lipoprotein (LDL) cholesterol (>2.5 mmol/l) or triglycerides (>3.0 mmol/l) , or low levels of high-density lipoprotein (HDL) cholesterol (men: <1.0 mmol/l, women <1.2 mmol/l), or medication prescribed for the indication dyslipidemia
- Diabetes mellitus: type I or type II if reported by a medical doctor in the medical chart or when the patient is using anti-diabetic medication.
* The prevalence of smoking and hypertension in the control group was higher than in FD patients.
** Microalbuminuria in FD patients was defined as ≥ 30 mg albuminuria in the collected 24 hours urine sample. In controls, microalbuminuria was defined as ≥ 20 mg/l albumin in a urine portion.

ECG parameters

The reported conduction times—P-wave duration, PR-interval, and QRS-duration—represent structural modifications to the conduction system. Repolarisation problems are characterized by the QTc and frontal T-axis. Additionally, the Cornell index and frontal QRS-axis are indicative of anatomical alterations in the LV myocardium. Lastly, The relationship between myocardial depolarisation and repolarisation is well depicted by the spatial QRS-T angle. The modelled increment of each ECG parameter per 10 years was not different between the ERT treated patient group (N=106) and the complete study cohort, which included 27 untreated patients (N=133) (see **supplemental table 1C-D**). Thus, excluding the untreated patients did not alter the observed changes in the ECG parameters. For this reason, we re-port further results for the complete study cohort only.

The results of the GLM are presented in **tables 2A-2B** and **supplemental tables 1**. **Figures 1-2** display the modelled course of each ECG parameter, while raw longitudinal ECG data are presented in **supplemental figures 2-5**. Boxplots and the descriptive statistics of the absolute values of ECG parameters per age decade are displayed in **figure 3-4** and **supplemental table 2**, respectively.

Table 2A: Conduction times- estimated regression coefficients (β) per 10 years increase in age in each study participants subgroup with 95%-CI (based on the GLM)

Participants subgroup	P-wave duration (ms)	PR-interval (ms)	QRS-duration (ms)	QTc (ms)
FD men	2.9 (1.7-4.2)***	2.3 (-1.0-5.5)	10.5 (8.9-12.0)***	20.8 (18.1-23.5)***
Controls- men	3.2 (2.7-3.6)***	3.6 (2.7-4.5)***	-0.6 (-1.1- -0.2)**	4.4 (3.4-5.3)***
FD women	5.0 (4.0-5.9)***	10.4 (7.9-12.9)***	9.3 (8.0-10.5)***	12.6 (10.5-14.8)***
Controls- women	2.6 (2.2-2.9)***	3.3 (2.6-4.0)***	0.3 (-0.1-0.7)	4.4 (3.7-5.1)***
Estimated differences in regression coefficients (β) per 10 years increase in age between study participants subgroups with 95%-CI				
FD men minus FD women	-2.1 (-3.6- -0.5)*	-8.2 (-12.3- -4.1)***	1.2 (-0.8-3.2)	8.2 (4.7-11.6)***
FD men minus Controls- men	-0.2 (-1.6- 1.1)	-1.3 (-4.7- -2.1)	11.1 (9.4- 12.7)***	16.4 (13.6-19.3)***
FD women minus Controls- women	2.4 (1.4-3.4)***	7.1 (4.5-9.7)***	9.0 (7.7-10.2)***	8.2 (6.0-10.5)***
Controls- men minus Controls- women	0.6 (0.001-1.1)*	0.3 (-0.9- 1.4)	-0.9 (-0.15- -0.3)**	0.0 (-0.11-0.12)

* p< 0.05, ** p< 0.01, ***p<0.001

Table 2B: Other LV ECG parameters- estimated regression coefficients (β) per 10 years increase in age in each study participants subgroup with 95%-CI (based on the GLM)

Participants subgroup	Cornell index (mm)	Spatial QRS-T angle (°)	Frontal QRS-axis (°)
FD men	2.6 (1.9-3.4)***	29.4 (26.1-32.6)***	-15.5 (-19.7- -11.2)***
Controls- men	0.0 (-0.2-0.3)	4.3 (3.3-5.3)***	-9.4 (-10.7- -8.1)***
FD women	1.8 (1.3-2.4)***	27.7 (25.2-30.2)***	-11.4 (-14.6- -8.2)***
Controls- women	0.6 (0.4-0.8)***	3.7 (2.9-4.5)***	-7.4 (-8.4- -6.3)***
Estimated differences in regression coefficients (β) per 10 years increase in age between study participants subgroups with 95%-CI			
FD men minus FD women	0.8 (-0.1-1.7)	1.7 (-2.4-5.8)	-4.1 (-9.4- 1.3)
FD men minus Controls- men	2.6 (1.8-3.4)***	25.1 (21.7-28.5)***	-6.1 (-10.5- -1.6)**
FD women minus Controls- women	1.2 (0.7-1.8)***	24.0 (21.4-26.6)***	-4.0 (-7.4- -0.6)*
Controls- men minus Controls- women	0.6 (0.3-0.9)***	0.6 (0.7-1.9)	-2.0 (-3.7- -0.4)*

* p< 0.05, ** p< 0.01, ***p<0.001

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Figure 1: Based on the GLM, the estimated effect plots with the 95%-CI of A. P-wave duration, B. PR-interval, C. QRS-duration and D. QTc for each study participants.
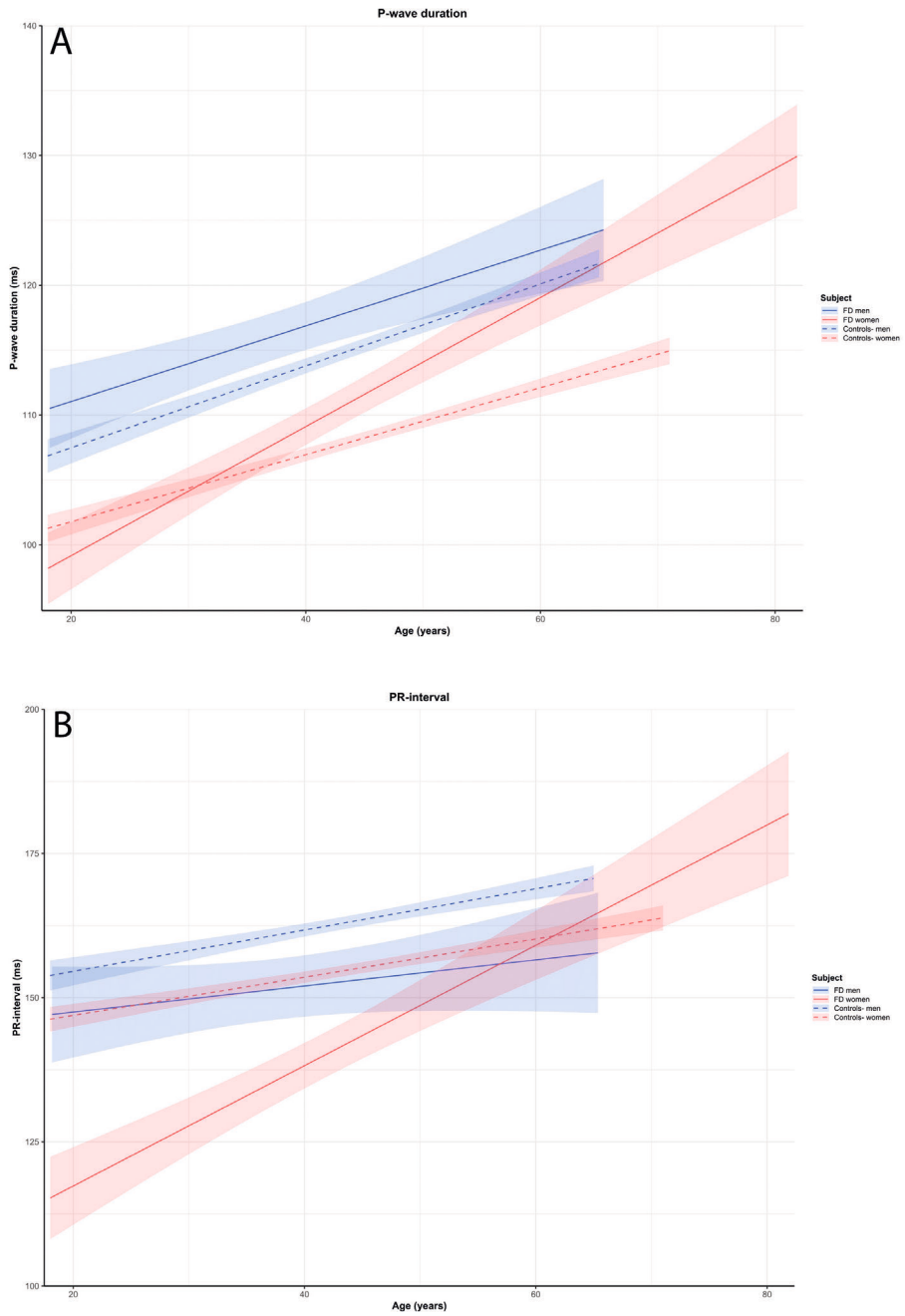
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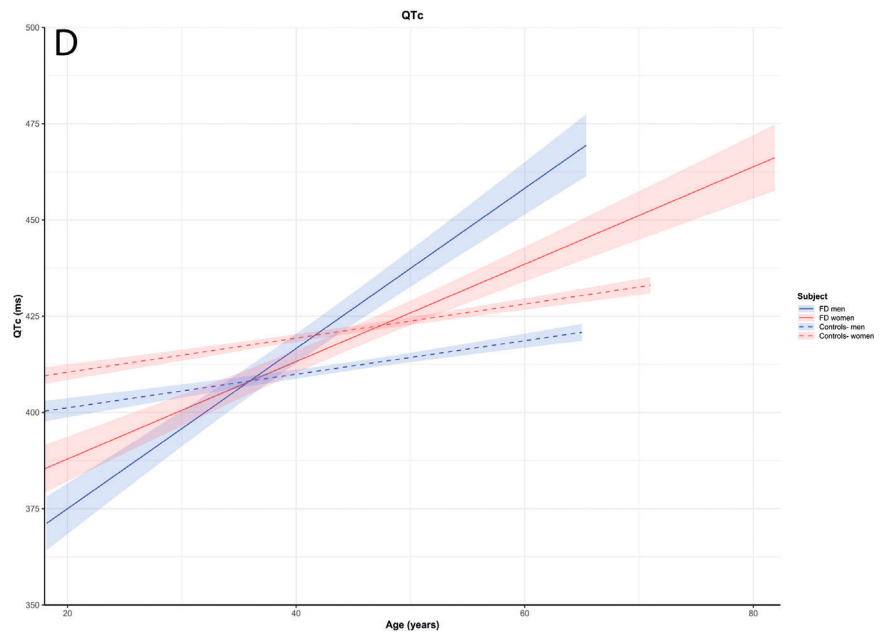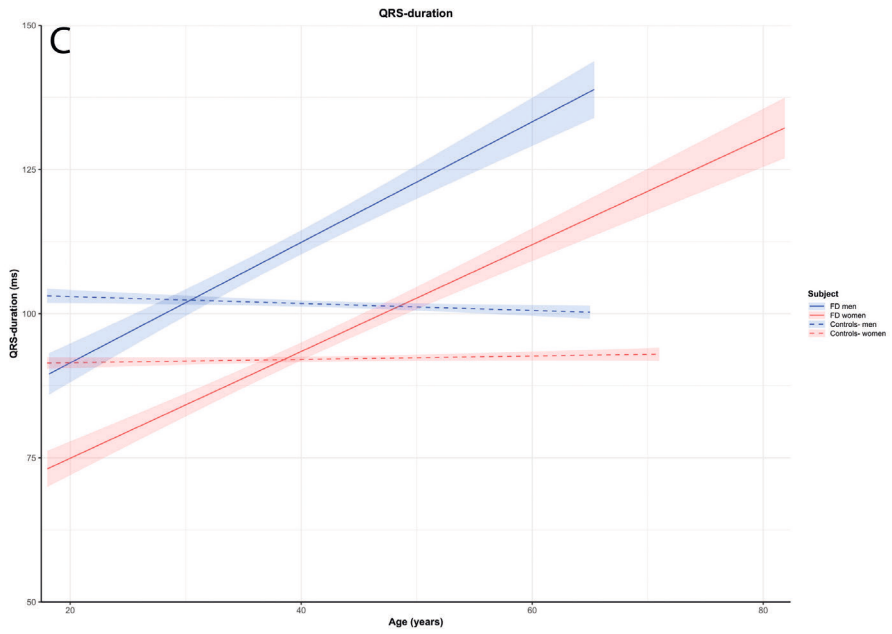
Figure 2: Based on the GLM, the estimated effect plots with the 95%-CI of A. Cornell index, B. Spatial QRS-T angle and C. Frontal QRS axis for each study participants subgroup.
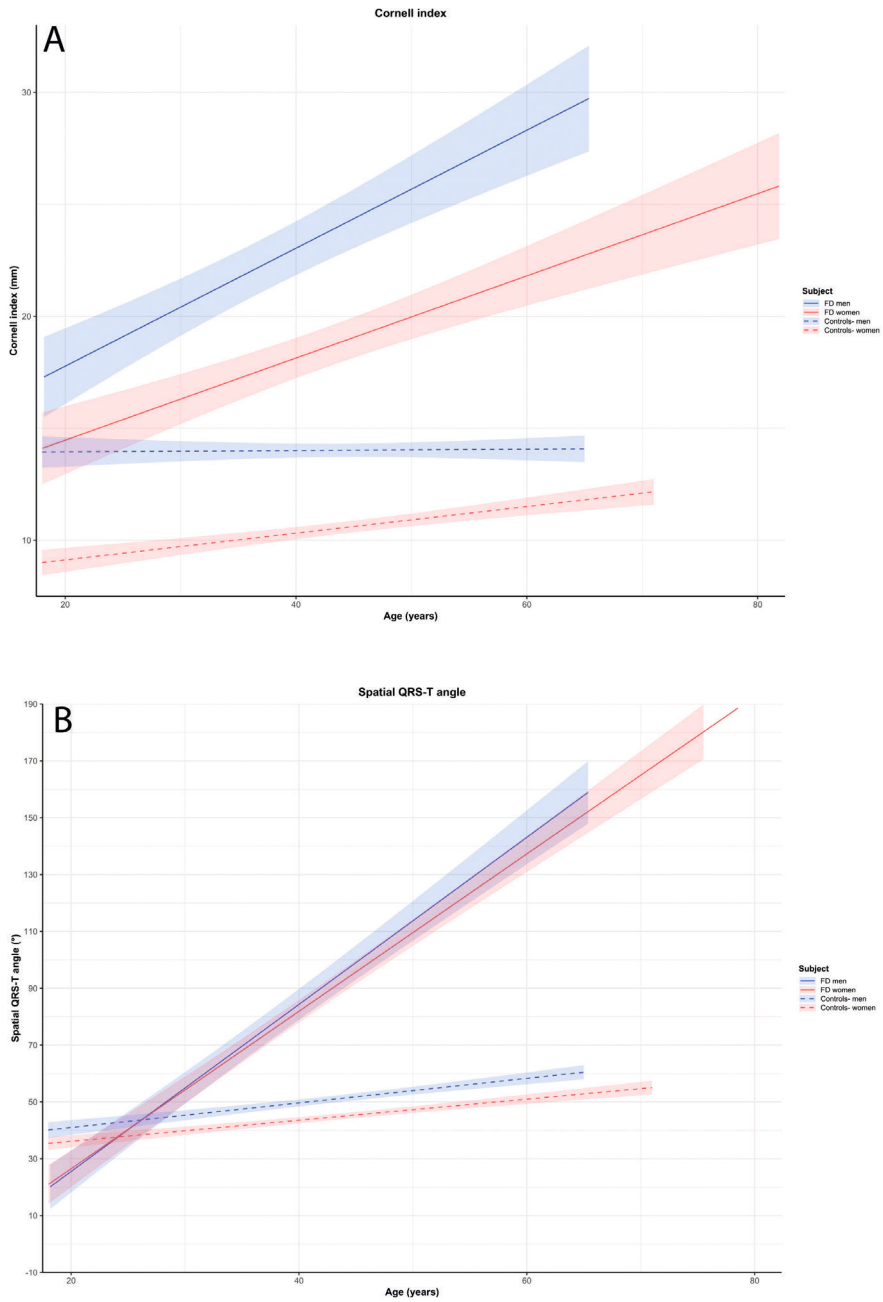
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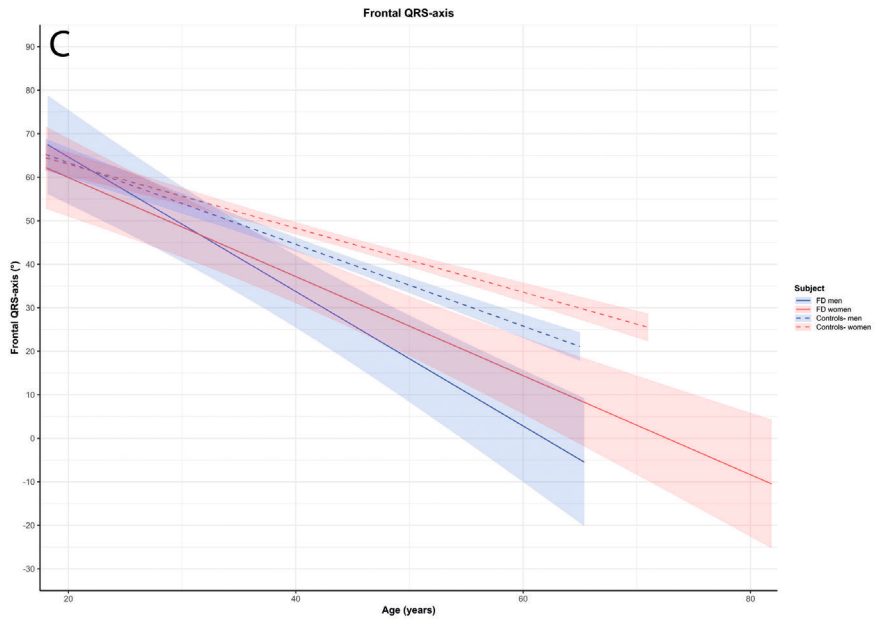
Figure 3: Boxplots of A. P-wave duration, B. PR-interval, C. QRS-duration and D. QTc per age decade in Fabry patients and controls. Numbers inside the boxes are the numbers of the analyzed ECGs. The last available ECG per FD patient per decade was selected to ensure that the influence of repeated measurements was limited. The horizontal lines represents the reference ranges of each ECG parameter, based on the literature. *p< 0.05, ** p< 0.01, ***p<0.001.

Figure 3: (continued)

C — QRS duration in men / QRS duration in women

D — QTc in men / QTc in women
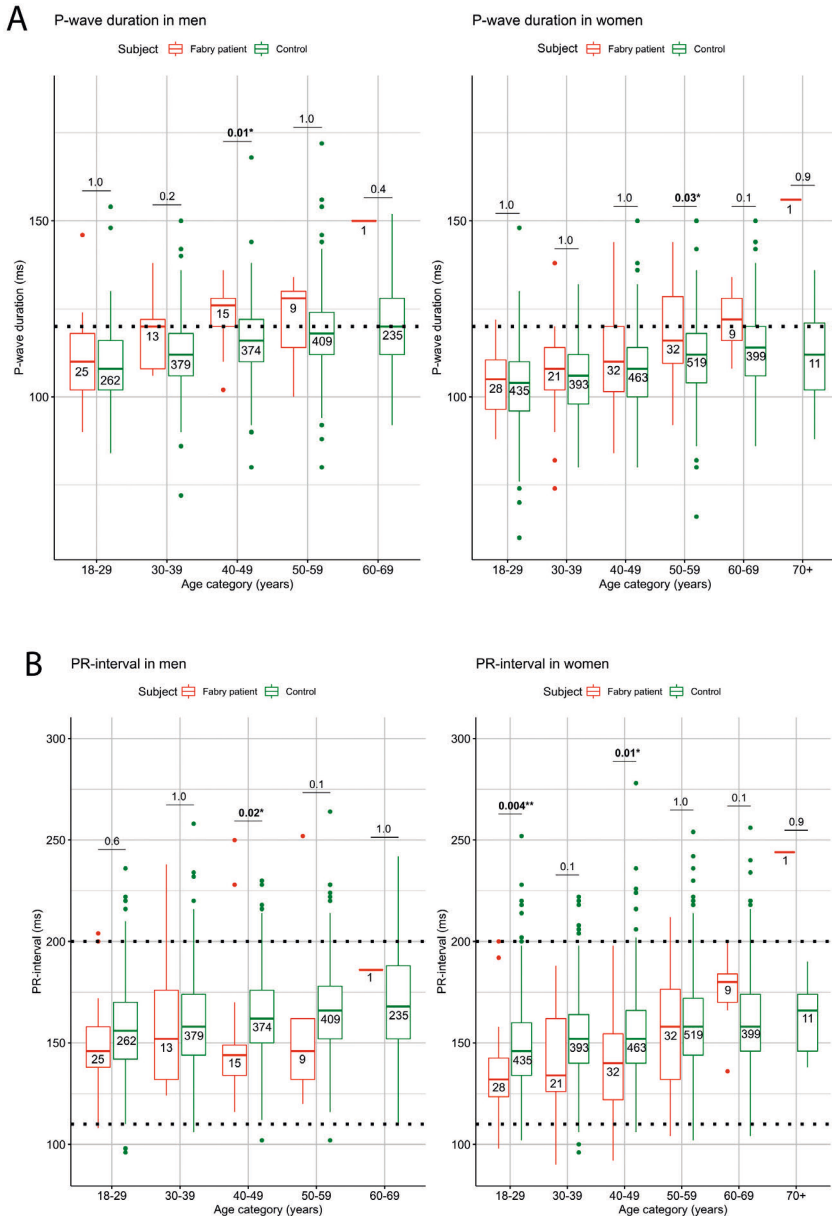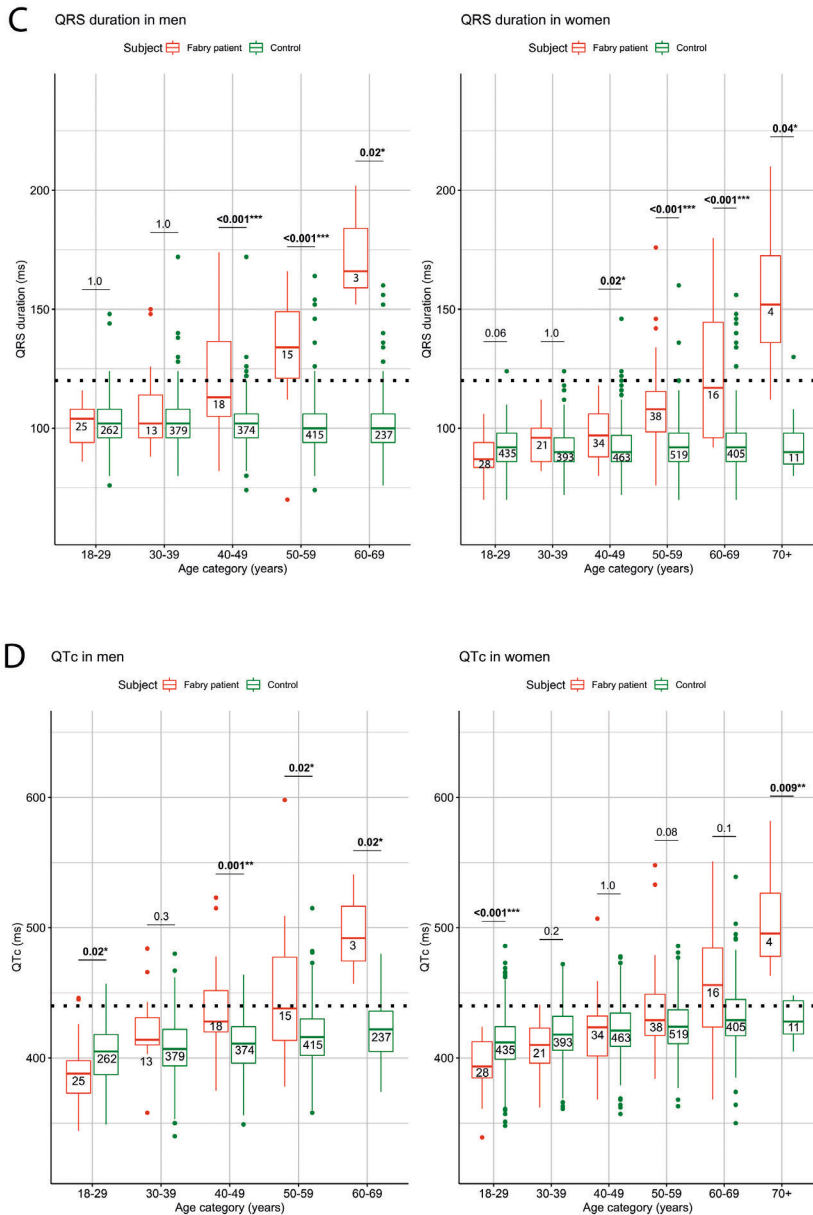
Figure 4: Boxplots of A. Cornell index, B. Spatial QRS-T angle and C. Frontal QRS-axis per age decade in Fabry patients and controls. Numbers inside the boxes are the numbers of the analyzed ECGs. The last available ECG per FD patient per decade was selected to ensure that the influence of repeated measurements was limited. The horizontal lines represents the reference ranges of each ECG parameter, based on the literature. *p< 0.05, ** p< 0.01, ***p<0.001.

Figure 4: (continued)

C   Frontal QRS-axis in men / Frontal QRS-axis in women
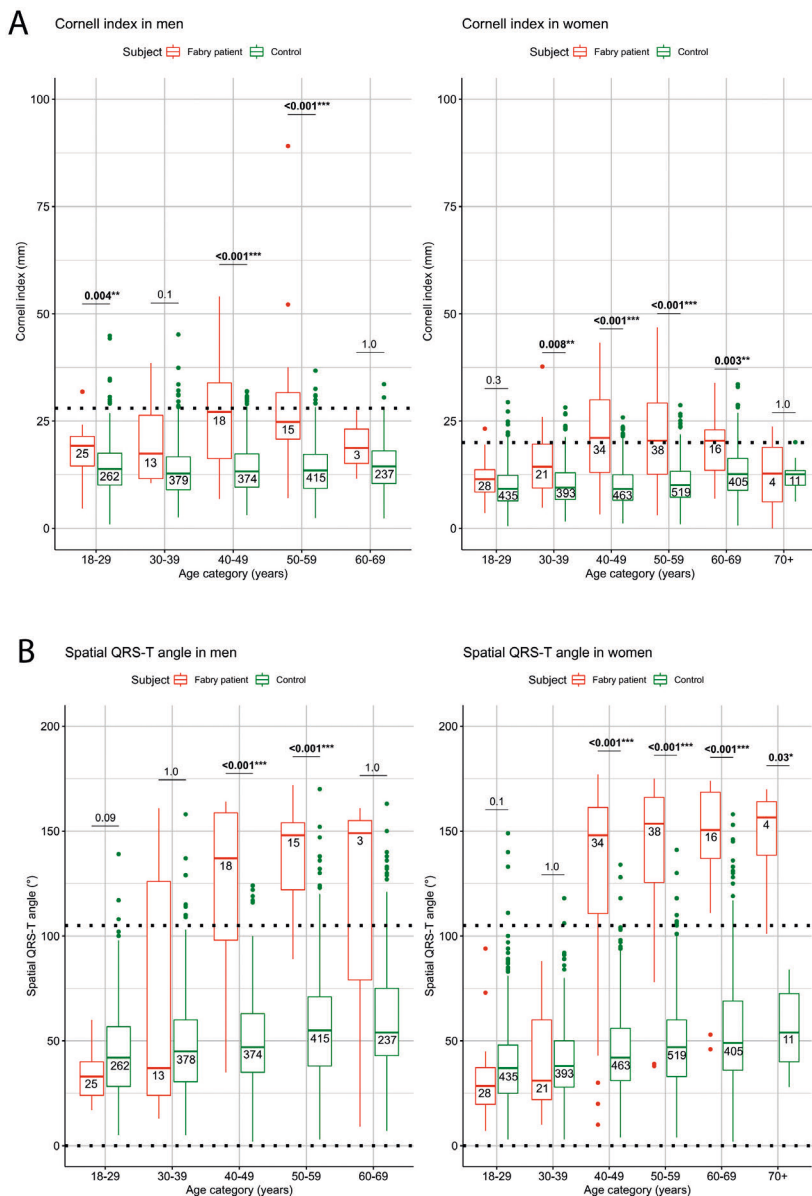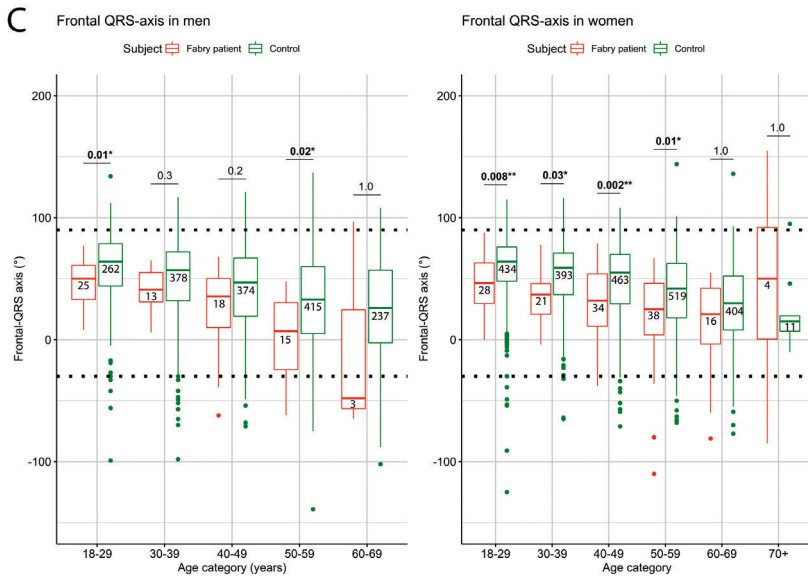
P-wave duration and PR-interval

In all subgroups, P-wave duration increased significantly with increasing age. Women with FDshowed a greater increment in P-wave duration with age compared to women controls (women: FD minus healthy controls β=2.4 ms per decade; 95%-CI 1.4-3.4) and compared to men with FD (FD: women minus men β=2.1 ms per decade; 95%-CI 0.5-3.6). In men, the increase in P-wave duration with ageing was similar for FD patients and control subjects **(table 2A, figure 1A)**. In patients with FD, the absolute values for P-wave duration were significantly longer in men between age 40-50 years and in women between age 50-60 years compared to control subjects. These differences were not significant in the decades thereafter, but the number of observations in these later decades was low **(figure 3A)**. P-wave duration showed median values above the normal range of 120 ms from 30-40 years in FD men and 50-60 years in FD women onwards **(supplemental table 2, figures 3A)**.

PR-interval significantly increased with ageing in both control groups and in women with FD, but not in men with FD **(table 2A)**. In women, PR-interval showed a significantly faster prolongation with age in women with FD as compared to healthy controls (women: FD- controls β=7.1 ms per decade; 95%-CI 4.5-9.7), whilst in men with FD vs men controls this difference was not significant. In line with this observation, women with FD had a significantly faster prolongation with age compared to men with FD (FD: women-men β= 8.2

ms per decade; 95%-CI 4.1-12.3) **(table 2A, figure 1B)**. PR-interval in women with FD was significantly lower compared to control subjects up to the age of 50 years. Notably, the PR-interval remained within the normal range of 120-200 ms in FD patients in all age categories [30] **(figure 3B)**.

QRS-duration and QTc
QRS-duration showed a significant prolongation over time in patients with FD, in contrast to controls in whom QRS-duration was similar at all ages. This was reflected by a significant difference in the increment of QRS-duration in FD patients as compared to controls (men: FD minus controls: β= 11.1 ms per decade; 95%-CI 9.4-12.7, women: FD patients minus controls β= 9.0 ms per decade; 95%-CI 7.7-10.2) **(table 2A, figure 1C)**. The increment in QRS- duration with ageing did not differ between men and women with FD (FD: men minus women β= 1.2 ms per decade; 95%-CI -0.8-3.2), but QRS- duration was significantly longer in male patients with FD compared to female patients during the entire follow-up period (p=0.001) **(figure 1C)**. Up until 40 years, the absolute values for QRS- duration were not different in FD patients compared to control subjects, while after 40 years, QRS- duration became significantly longer both in male and female FD patients. The QRS-duration reached a threshold of 120 ms or higher between age 40-50 years in males with FD, whilst this point in FD females was reached between age 60-70 years **(supplemental table 2, figure 3C)**.

QTc showed significant prolongation with increasing age in all four subgroups, but progression was greater in FD patients (men: FD minus controls: β= 16.4 ms per decade; 95%-CI 13.6-19.3 and women: FD minus controls: β= 8.2 ms per decade; 95%-CI 6.0-10.5, respectively). The difference in QTc could not be explained by difference in heart rate between FD patients and the healthy controls **(supplemental figure 6-7)**. In addition, the increment in QTc with ageing was more pronounced in men with FD compared to women with FD (FD: men minus women: β= 8.2 ms per decade; 95%-CI 4.7-11.6) **(table 2A, figure 1D)**. In accordance with the model, the differences in the absolute value of QTc between FD patients and controls became more pronounced throughout adult life. The QTc reached a threshold of 440 ms or higher between age 40-50 years in men with FD, while abnormal QTc values in women with FD were observed from 50 years of age onwards **(supplemental table 2, figure 3D)**.

Cornell index, Spatial QRS-T angle, and Frontal QRS-axis
In patients with FD, Cornell index values showed a significant increase with ageing compared to controls where it remained stable (in men) or showed a less pronounced increase (in women) **(table 2B)**. The increment in Cornell index did not differ between men and women with FD (FD: men minus women: β= 0.8 ms per decade; 95%-CI -0.1-1.7), but Cornell index was higher in men compared to

women (both control subjects and patients) at all ages **(figure 2A)**. Compared to control subjects, FD patients had a significantly higher Cornell index from a young age onwards (from 18-29 years in men with FD and 30-39 years in women with FD) **(supplemental table 2, figure 4A)**.

Spatial QRS-T angle increased with ageing in all subgroups, but the increases were much greater in patients with FD as compared to control subjects (men: FD minus controls β=25.1° per decade; 95%-CI 21.7-28.5, females: FD-controls: β= 24.0° per decade; 95%-CI 21.4-26.6) **(table 2B)**. There was overlap between the sexes in FD, both in absolute value and increment with ageing, in spatial QRS-T angle **(figure 2B)**. Compared to controls, both men and women with FD had a higher spatial QRS-T angle from 40 years onwards coinciding with this parameter exceeding the upper range of normal (105°) [31] **(supplemental table 2, figure 4B)**.

With ageing, the frontal QRS-axis became progressively more negative in all subgroups (i.e. left ward axis deviation), with progression being more pronounced in FD patients as compared to control subjects (men: FD minus controls: β= -6.1° per decade; CI -10.5 - -1.6, women: FD minus controls: β= -4.0° per decade; CI -7.4 - -0.6). There was no significant difference between men and women with FD with respect to the absolute value or change in QRS-axis deviation **(table 2B, figure 2C)**. The absolute values of the frontal QRS-axis tended to be horizontal in patients with FD compared to a more normal QRS- axis in controls, but nonetheless remained within normal limits **(figure 4C)**.

Frontal T- axis
The majority of control subjects remained within the normal range of 15°-75° regardless of age [32]. On the other hand, patients with FD developed a divergent frontal T-axis from approximately 30 years (men) and 40 years (women) onwards **(supplemental figure 5)**.

Electrocardiographic and CMR imaging properties in FD patients
For the 133 included FD patients, a total of 119 CMRs (90%) were available that could be linked to the last obtained ECG during follow-up. LVMi and LGE data were reported for 101 (76%) and 118 (99%) of the 119 CMRs, respectively. See **table 1** for detailed descriptive statistics on LVMi and LGE. Based on the Spearman analyses, we found statistically significant correlations between the seven main ECG parameters (P-wave duration, PR-interval, QRS-duration, QTc, Cornell index, spatial QRS-T angle and frontal QRS axis) and LVMi **(supplemental figure 8)**. The absolute values of P- wave duration, QRS-duration, QTc, Cornell index and spatial QRS-T angle were significantly divergent in patients with LGE vs patients without LGE. The ECG parameter that best

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distinguishes patients with and without fibrosis was the spatial QRS-T angle, where 51 out of 53 FD patients (96%) without LGE had a normal QRS-T angle (between 0 ° and 105 °) **(supplemental figure 9)**.

Discussion

This is the first long-term longitudinal study in classical FD patients that assessed the evolution of electrophysiological depolarisation, repolarisation and their interaction. In addition, we could include ECGs from a large cross-sectional sample of apparently healthy control subjects, enabling a comparison across a wide age range.

The results show that for the studied ECG parameters, the differences between FD patients and controls increase with ageing. These parameters differ from the included control cohort, both in terms of rate of progression as well as absolute values, but they often fall within generally accepted reference ranges and may therefore not be recognized as abnormal. This is particularly true for the PR-interval and frontal QRS-axis. PR-interval has long been considered a hallmark of FD [33], but both in this study, as well as the study by Namdar et al, the absolute values of PR-intervals for most FD patients fall within the reference range (120-200 ms) [30] [34].

Perhaps the most important result of this study is that for all ECG parameters studied, it is not so much the absolute value, but the rate of change over time that clearly distinguishes the FD patients (especially at younger age) from the control subjects **(figure 1-4).** This likely represents progression of cardiac disease since: a) the course is very different from that of controls with cardiovascular risk factors, b) there is a clear association between the ECG parameters and other established markers of cardiac disease (left ventricular hypertrophy and the presence of fibrosis as assessed by CMR) and c) in studies in both the general population [31, 35-37] and patients with other cardiac disorders [38-40] abnormal values of ECG parameters are known to be associated with higher risk of cardiac complications.

Considering the described differences in the rate of change between FD patients and controls from the general population, we suggest that monitoring the rate of change in FD patients with still apparently 'normal' ECGs might be a suitable way, in combination with echocardiography, CMR and biochemical markers, to detect early signs of cardiac involvement in FD.

Surprisingly, for several parameters the rate of change in female patients was comparable and sometimes even more pronounced compared to male patients

(e.g. P-wave duration, PR-interval, QRS- duration, Cornell index, spatial QRS-T angle and frontal QRS-axis) (**figure 1-2**). Since not all female patients will develop cardiac events of FD, it is especially important to be able to detect development of cardiac disease in these patients. As can be deducted from **supplemental figure 2-5** changes in ECG parameters do not occur in all patients and a proportion of the female patients follows a trajectory over time that is similar to control subjects. This suggests that these parameters may indeed be suitable to differentiate between those patients who develop progressive cardiac disease and those who do not. The parameter that best distinguishes patients with from those without LGE is the spatial QRS-T angle, which is likely to be a much more reproducible measurement compared to other ECG parameters [41] to quantify the interaction between ventricular depolarisation and repolarisation. This study shows, for the first time, the increment in spatial QRS-T angle in FD patients. Previous studies have shown that this vectorcardiographic parameter can independently predict cardiac events in the general population and patients with other heart disease (e.g., heart failure and myocardial infarction) [31, 39, 40]. More longitudinal studies are required to investigate whether the spatial QRS-T angle is a useful parameter to predict cardiovascular events in FD patients.

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What is needed to firmly establish these parameters as prognostic biomarkers is an established link between early ECG changes and clinical outcomes. This study shows that men with classical FD showed differences in ECG parameters compared to control subjects from age 20 years onwards, with the Cornell index as the earliest marker to change. In women with classical FD this occurs from age 30 years onwards (**figure 4A**). Whether these ECG alterations predict cardiac complications or the occurrence of LVH and LGE, that are known to occur approximately 20 years later [14, 20] could not be determined because of: a) the relatively small number of cardiac events in this cohort and the absence of cardiac events in younger patients and b) the missing long-term ECG data prior to an event for patients who were diagnosed after their first cardiovascular event (especially women without a positive FD family history or classical FD symptoms). To investigate the predictive utility of ECG markers in FD patients, it is essential to conduct multicenter studies that can provide these missing data. The current study provides insight which parameters can be studied to assess their predictive value for the development of cardiovascular complications in FD patients.

This study has several limitations. First, survival (premature death) and treatment bias (exclusion of ECGs of patients with externally paced rhythm) attribute to selection bias. This is in particular relevant for older men with classical FD, since it has led to under-recording of ECGs of these severely affected patients. The unavoidable selection of ECGs from patients over the age of 50 with a relatively

mild phenotype (severely affected patients have by then died or received a pacemaker) could incorrectly suggest that with advanced age, male patients have a similar electrocardiographic phenotype as female FD patients. Second, in FD patients, changes in electrophysiological parameters reflect time- dependent alterations of each parameter (longitudinally collected ECGs). However, for the control subjects, the data were obtained in a cross-sectional study and no changes within the included subjects can be analyzed. Third, the gross majority of the included patients were treated with enzyme replacement therapy (ERT) for at least part of the follow-up and the electrophysiological changes depicted do not represent the natural disease course. This effect of treatment (ERT) on the electrophysiological parameters cannot be studied given the small untreated patient sample, the treatment indication bias and the vast difference in age at which treatment was started between patients. Previous studies indicated that cardiac disease may progress despite ERT [19, 42], this is confirmed by the current study. The data from the current study, can, however, be used to analyze the effect of new FD therapies to see if they outperform current treatment in preventing progression of cardiac disease.

Conclusions

In FD, several ECG parameters show progressive alterations during adult life. The frontal QRS-axis is already significantly different in both male and female FD patients aged 18-29 years and is thus the earliest marker of cardiac disease identified in this study. For male patients this is also true for the Cornell index, with female patients following a decade later. For the other ECG parameters, specifically the rate of change throughout adulthood, more than the absolute values, is grossly different in FD patients from that in apparently healthy individuals. Tracking the rate of change could be a good way to detect disease progression in early adulthood, guiding treatment initiation in those that show significant changes. At later disease stages the absolute values for all ECG parameters, except for PR-interval, become abnormal, in comparison to both the controls and the reference values. All studied ECG parameters showed a positive correlation with left ventricular mass index on CMR. P-wave duration, QRS-duration, QTc, Cornell index and spatial QRS-T angle were different in patients with cardiac fibrosis compared to those without and the spatial QRS-T angle could be used to identify those requiting CMR follow up of fibrosis development.

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SUPPLEMENTAL MATERIAL

Supplemental table 1A: Generalized linear mixed model of the conduction times

Fixed effects	P-wave duration (ms) β	(95% CI)	p value	PR-interval (ms) β	(95% CI)	p value	QRS-duration (ms) β	(95% CI)	p value	QTc (ms) β	(95% CI)	p value
Model 1 (age, Type and Sex separated)												
Age	0.28	0.25 – 0.31	<0.001	0.38	0.32 – 0.43	<0.001	0.059	0.02 – 0.0	0.001	0.54	0.49 – 0.60	<0.001
Type (FD patient)	1.40	0.02 – 2.77	0.046	-14.65	-18.00 – -11.30	<0.001	2.92	1.35 – 4.48	<0.001	-1.18	-3.82 – 1.46	0.381
Sex (Male)	6.94	6.21 – 7.67	<0.001	8.54	7.09 – 9.99	<0.001	9.93	9.23 – 10.62	<0.001	-8.84	-10.33 – -7.36	<0.001
Model 2 (Controls and FD patients combined groups- with age*Type*Sex interactions)												
(Intercept)	96.62	95.00 – 98.23		140.30	136.94 – 143.66		90.91	89.30 – 92.53		401.60	398.23 – 404.97	
Age	0.26	0.22 – 0.29	<0.001	0.33	0.26 – 0.40	<0.001	0.03	-0.01 – -0.07	0.114	0.44	0.37 – 0.51	<0.001
Type (FD patient)	-7.407	-12.02 – -2.7	0.002	-43.83	-55.51 – -32.14	<0.001	-34.51	-39.91 – -29.12	<0.001	-38.99	-49.24 – -28.74	<0.001
Sex (Male)	4.55	1.95 – 7.15	0.001	7.10	1.75 – 12.46	0.009	13.27	10.70 – 15.83	<0.001	-9.09	-14.48 – -3.70	0.001
Interactions												
Age*Type (FD patient)	0.24	0.14 – 0.34	<0.001	0.71	0.45 – 0.97	<0.001	0.90	0.77 – 1.02	<0.001	0.82	0.60 – 1.05	<0.001
Age*Sex (Male)	0.06	0.00 – 0.11	0.045	0.03	-0.09 – 0.14	0.653	-0.09	-0.15 – -0.03	0.002	-0.01	-0.12 – -0.11	0.904
Type (FD patient)*Sex (Male)	11.44	4.29 – 18.59	0.002	39.40	21.08 – 57.71	<0.001	0.89	-7.56 – 9.34	0.837	-20.13	-36.05 – -4.20	0.013
Age*Type (FD patient)*Sex (Male)	-0.26	-0.43 – -0.10	0.002	-0.84	-1.27 – -0.42	<0.001	0.21	0.00 – 0.42	0.049	0.82	0.46 – 1.19	<0.001

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Supplemental table 1B: *Generalized linear mixed model of the other LV related ECG parameters*

	Cornell index (mm)			Spatial QRS-T angle (°)			Frontal QRS-axis (°)		
	β	(95% CI)	p value	β	(95% CI)	p value	β	(95% CI)	p value
Model 1 (age, Type and Sex separated)									
Fixed effects									
Age	0.04	0.02 – 0.05	<0.001	0.53	0.47 – 0.60	<0.001	-0.84	-0.92 – -0.77	<0.001
Type (FD patient)	8.22	7.40 – 9.03	<0.001	35.91	32.10 – 39.73	<0.001	-6.82	-12.06 – -1.58	0.011
Sex (Male)	3.39	2.98 – 3.80	<0.001	5.07	3.33 – 6.80	<0.001	-3.93	-6.05 – -1.80	<0.001
Model 2 (Controls and FD patients combined groups- with age*Type*Sex interactions)									
(Intercept)	7.94	7.05 – 8.82		28.73	25.17 – 32.30		77.72	72.99 – 82.44	
Age	0.06	0.04 – 0.08	<0.001	0.37	0.29 – 0.45	<0.001	-0.74	-0.84 – -0.63	<0.001
Type (FD patient)	2.87	0.22 – 5.51	0.034	-57.61	-69.01 – -46.21	<0.001	5.00	-10.16 – 20.16	0.518
Sex (Male)	5.95	4.54 – 7.37	<0.001	3.63	-2.08 – 9.34	0.213	4.42	-3.11 – 11.96	0.250
Age*Type (FD patient)	0.12	0.07 – 0.18	<0.001	2.40	2.14 – 2.66	<0.001	-0.40	-0.74 – -0.06	0.020
Age*Sex (Male)	-0.06	-0.09 – -0.03	<0.001	0.06	-0.07 – 0.19	0.344	-0.20	-0.37 – -0.04	0.017
Type (FD patient)*Sex (Male)	-4.25	-8.33 – -0.17	0.041	-8.01	-25.69 – 9.68	0.375	8.42	-15.54 – 32.39	0.491
Age*Type (FD patient)*Sex (Male)	0.14	0.04 – 0.23	0.005	0.11	-0.32 – 0.54	0.619	-0.20	-0.76 – 0.36	0.477

Supplemental table 1C: Conduction times - estimated regression coefficients (β) per 10 years increase in age in ERT treated versus ERT treated and untreated FD patients with 95%-CI (based on the GLM)

ERT treated	P-wave duration (ms)	PR-interval (ms)	QRS-duration (ms)	QTc (ms)
FD men	2.7 (1.5-4.0)***	2.1 (-1.2-5.4)	10.9 (9.3-12.4)***	20.8 (18.0-23.5)***
FD women	5.3 (4.2-6.4)***	12.4 (9.5-15.3)***	10.1 (8.7- 11.5)***	13.8 (11.3-16.2)***
ERT treated and untreated	**P-wave duration (ms)**	**PR-interval (ms)**	**QRS-duration (ms)**	**QTc (ms)**
FD men	**2.9 (1.7-4.2)***	2.3 (-1.0-5.5)	**10.5 (8.9-12.0)***	**20.8 (18.1-23.5)***
FD women	**5.0 (4.0-5.9)***	**10.4 (7.9-12.9)***	**9.3 (8.0-10.5)***	**12.6 (10.5-14.8)***

* p< 0.05, ** p< 0.01, ***p<0.001

Supplemental table 1D: Other LV ECG parameters - estimated regression coefficients (β) per 10 years increase in age in ERT treated versus ERT treated and untreated FD patients with 95%-CI (based on the GLM)

ERT treated	Cornell index (mm)	Spatial QRS-T angle (°)	Frontal QRS-axis (°)
FD men	**2.8 (2.0-3.5)***	29.7 (26.4-33.0)***	-16.2 (-20.6- -11.8)***
FD women	**1.6 (1.0-2.2)***	28.5 (25.6- 31.4)***	-11.9 (-15.6- -8.1)***
ERT treated and untreated	**Cornell index (mm)**	**Spatial QRS-T angle (°)**	**Frontal QRS-axis (°)**
FD men	**2.6 (1.9-3.4)***	29.4 (26.1-32.6)***	-15.5 (-19.7- -11.2)***
FD women	**1.8 (1.3-2.4)***	27.7 (25.2-30.2)***	-11.4 (-14.6- -8.2)***

* p< 0.05, ** p< 0.01, ***p<0.001

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Supplemental table 2: ECG descriptives per age decade (the last available ECG per FD patient per decade was selected to ensure that the influence of repeated measurements was limited)

P-wave duration in men (ms)					
Age category	Available ECGs in Fabry men, n	Fabry men Median (IQR)	Available ECGs in Controls-men, n	Controls- men Median (IQR)	P- value
18-29	25	110 (102-118)	262	108 (102-116)	1.0
30-39	13	120 (108-122)	379	112 (106-118)	0.2
40-49	15	126 (120-128)	374	116 (110-122)	**0.01***
50-59	9	128 (114-130)	409	118 (112-124)	1.0
60-69	1	150 (-)	235	121 (112-128)	0.4
70+	-	-	-	-	-

PR-interval in men (ms)					
Age category	Available ECGs in Fabry men, n	Fabry men Median (IQR)	Available ECGs in Controls-men, n	Controls- men Median (IQR)	P- value
18-29	25	146 (138-158)	262	156 (142-170)	0.6
30-39	13	152 (132-176)	379	158 (144-174)	1.0
40-49	15	144 (134-149)	374	162 (150-176)	**0.02***
50-59	9	146 (132-162)	409	166 (152-178)	0.1
60-69	1	186 (-)	235	168 (152-188)	1.0
70+	-	-	-	-	-

QRS-duration in men (ms)					
Age category	Available ECGs in Fabry men, n	Fabry men Median (IQR)	Available ECGs in Controls-men, n	Controls- men Median (IQR)	P- value
18-29	25	104 (94-108)	262	102 (96-108)	1.0
30-39	13	102 (96-114)	379	102 (96-108)	1.0
40-49	18	113 (105-137)	374	102 (96-106)	**<0.001***
50-59	15	134 (121-149)	415	100 (94-106)	**<0.001***
60-69	3	166 (159-184)	237	100 (94-106)	**0.02***
70+	-	-	-	-	-

QTc in men (ms)					
Age category	Available ECGs in Fabry men, n	Fabry men Median (IQR)	Available ECGs in Controls-men, n	Controls- men Median (IQR)	P- value
18-29	25	388 (373-398)	262	405 (387-418)	**0.02***
30-39	13	414 (410-431)	379	407 (394-422)	0.3
40-49	18	428 (420-452)	374	411 (396-424)	**0.001****
50-59	15	438 (414-478)	415	416 (402-430)	**0.02***
60-69	3	492 (475-517)	237	422 (405-436)	**0.02***
70+	-	-	-	-	-

P-wave duration in women (ms)					
Age category	Available ECGs in Fabry women, n	Fabry women Median (IQR)	Available ECGs in Controls-women, n	Controls-women Median (IQR)	P- value
18-29	28	105 (97-111)	435	104 (96-110)	1.0
30-39	21	108 (102-114)	393	106 (98-112)	1.0
40-49	32	110 (102-120)	463	108 (100-114)	1.0
50-59	32	116 (110-129)	519	112 (104-118)	**0.03***
60-69	9	122 (116-128)	399	114 (106-120)	0.1
70+	1	156 (-)	11	112 (102-121)	0.9
PR-interval in women (ms)					
Age category	Available ECGs in Fabry women, n	Fabry women Median (IQR)	Available ECGs in Controls-women, n	Controls-women Median (IQR)	P- value
18-29	28	132 (124-143)	435	146 (134-160)	**0.004****
30-39	21	134 (126-162)	393	152 (140-164)	0.1
40-49	32	140 (122-155)	463	152 (140-166)	**0.01***
50-59	32	158 (132-177)	519	158 (144-172)	1.0
60-69	9	180 (170-184)	399	158 (146-174)	0.1
70+	1	244 (-)	11	166 (146-174)	0.9
QRS-duration in women (ms)					
Age category	Available ECGs in Fabry women, n	Fabry women Median (IQR)	Available ECGs in Controls-women, n	Controls-women Median (IQR)	P- value
18-29	28	87 (84-94)	435	92 (86-98)	0.06
30-39	21	96 (86-100)	393	90 (86-96)	1.0
40-49	34	97 (88-106)	463	90 (86-97)	**0.02***
50-59	38	108 (99-116)	519	92 (86-98)	**<0.001*****
60-69	16	117 (96-145)	405	92 (86-98)	**<0.001*****
70+	4	152 (136-173)	11	90 (85-98)	**0.04***
QTc in women (ms)					
Age category	Available ECGs in Fabry women, n	Fabry women Median (IQR)	Available ECGs inControls-women, n	Controls-women Median (IQR)	P- value
18-29	28	394 (385-413)	435	412 (399-424)	**<0.001*****
30-39	21	410 (396-423)	393	418 (406-432)	0.2
40-49	34	424 (402-432)	463	421 (409-435)	1.0
50-59	38	429 (417-449)	519	424 (411-437)	0.08
60-69	16	456 (424-485)	405	429 (417-445)	0.1
70+	4	496 (478-527)	11	428 (419-444)	**0.009****

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Supplemental table 2: (continued)

Cornell index in men (ms)					
Age category	Available ECGsin Fabry men, n	Fabry men Median (IQR)	Available ECGs in Controls-men, n	Controls-men Median (IQR)	P- value
18-29	25	19 (15-22)	262	14 (10-18)	**0.004****
30-39	13	17 (12-26)	379	13 (9-17)	0.1
40-49	18	27 (16-34)	374	13 (10-17)	**<0.001*****
50-59	15	25 (21-32)	415	14 (9-17)	**<0.001*****
60-69	3	19 (15-23)	237	14 (11-18)	1.0
70+	-	-	-	-	-
Spatial QRS-T angle in men (°)					
Age category	Available ECGs in Fabry men, n	Fabry men Median (IQR)	Available ECGs in Controls-men, n	Controls-men Median (IQR)	P- value
18-29	25	33 (24-40)	262	42 (28-57)	0.09
30-39	13	37 (24-126)	379	45 (31-60)	1.0
40-49	18	137 (98-159)	374	47 (35-63)	**<0.001*****
50-59	15	148 (122-154)	415	55 (38-71)	**<0.001*****
60-69	3	149 (79-155)	237	54 (43-75)	1.0
70+	-	-	-	-	-
Frontal QRS-axis in men (°)					
Age category	Available ECGs in Fabry men, n	Fabry men Median (IQR)	Available ECGs in Controls-men, n	Controls- men Median (IQR)	P- value
18-29	25	50 (33-61)	262	64 (44-79)	**0.01***
30-39	13	41 (31-55)	378	57 (32-72)	0.3
40-49	18	36 (10-50)	374	47 (19-67)	0.2
50-59	15	7 (-25-31)	415	33 (5-60)	**0.02***
60-69	3	-48 (-57-25)	237	26 (-4-57)	1.0
70+	-	-	-	-	-

*p<0.05, ** p<0.01, ***p<0.001

Cornell index in women (ms)					
Age category	Available ECGs in Fabry women, n	Fabry women Median (IQR)	Available ECGs in Controls-women, n	Controls-women Median (IQR)	P- value
18-29	28	12 (9-14)	435	9 (6-12)	0.3
30-39	21	14 (9-20)	393	10 (7-13)	**0.008****
40-49	34	21 (13-30)	463	9 (7-13)	**<0.001*****
50-59	38	20 (13-29)	519	10 (7-13)	**<0.001*****
60-69	16	20 (14-23)	405	13 (9-16)	**0.003****
70+	4	13 (6-19)	11	13 (10-14)	1.0
Spatial QRS-T angle in women (°)					
Age category	Available ECGs in Fabry women, n	Fabry women Median (IQR)	Available ECGs in Controls-women, n	Controls-women Median (IQR)	P- value
18-29	28	29 (20-37)	435	37 (25-48)	0.1
30-39	21	31 (22-60)	393	38 (28-50)	1.0
40-49	34	148 (111-161)	463	42 (31-56)	**<0.001*****
50-59	38	154 (126-166)	519	47 (33-60)	**<0.001*****
60-69	16	151 (137-169)	405	49 (36-69)	**<0.001*****
70+	4	157 (139-164)	11	54 (40-73)	**0.03***
Frontal QRS-axis in women (°)					
Age category	Available ECGs in Fabry women, n	Fabry women Median (IQR)	Available ECGs in Controls-women, n	Controls-women Median (IQR)	P- value
18-29	28	47 (30-63)	434	64 (48-76)	**0.008****
30-39	21	37 (21-46)	393	59 (37-71)	**0.03***
40-49	34	32 (11-53)	463	55 (30-70)	**0.002****
50-59	38	25 (4-46)	519	42 (18-63)	**0.01***
60-69	16	21 (-4.0-42)	404	30 (8-52)	1.0
70+	4	50 (1-92)	11	15 (7-20)	1.0

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Supplemental table 3: Definitions of cardiac events [1]

Events	Definition
Major adverse cardiovascular events (MACE): composite of cardiovascular death, heart failure hospitalization, sustained ventricular arrhythmias (SVA) and myocardial infarction	
1. Cardiovascular death	Death as a result of one of the following diseases/ syndromes: - Acute coronary syndrome - Sudden cardiac death (SCD) - Hypertensive crise - Ischemic or hemorrhagic stroke - Cardiomyopathy - Other cardiovascular cause such as: pulmonary embolism, peripheral vascular disease
2. Heart failure hospitalization	Hospital admission (at least one night) with the following clinical manifestations of heart failure: dyspnea, reduced exercise tolerance, fluid retention in peripheral and/ or splanchnic vessels, seen as peripheral edema
3. Sustained ventricular arrhythmia (SVA)	composite of sudden cardiac death (SCD), sudden cardiac arrest (SCA), sustained ventricular tachycardia (VT) including appropriate ICD shock, and ventricular fibrillation (VF)
4. Myocardial infarction	Acute myocardial injury with clinical evidence of acute myocardial ischemia. Definition according to the Fourth Universal Definition of Myocardial Infarction

1. El Sayed, M., et al., *Influence of sex and phenotype on cardiac outcomes in patients with Fabry disease.* Heart, 2021.

Supplemental table 4: GLA mutations frequencies

GLA mutation at DNA level	Frequency in FD- men (N)	Frequency in FD- women (N)
c.1025G>A	8	13
c.1040dupT	0	2
c.1074_1075del	1	3
c.1118G>A	1	2
c.1124_1176del	2	0
c.1156C>T	2	1
c.1246C>T	1	1
c.136C>T	0	1
c.157_160delAACC	2	1
c.215T>G	1	0
c.269G>A	0	1
c.334C>T	1	1

Supplemental table 4: GLA mutations frequencies (continued)

GLA mutation at DNA level	Frequency in FD- men (N)	Frequency in FD- women (N)
c.400delT	0	2
c.406G>T	5	4
c.422C>T	1	1
c.53T>C	1	11
c.548G>A	1	3
c.606T>G	0	1
c.62T>C	0	3
c.639+1G>A	0	1
c.658C>T	5	4
c.677G>A	2	3
c.679C>T	2	2
c.680G>A	1	2
c.728_744del	0	4
c.779G>A	0	1
c.803T>C	2	1
c.898C>T	0	4
c.901C>T	3	2
c.908_910delTCA	0	1
c.955_969delinsTTGC	2	1
c.963_964delinsCA	1	2
c.996_999del	0	2
c.997C>T	0	1
Duplication exon 3+4	1	0
IVS6-2A>T (c.1000-2A>T)	1	2
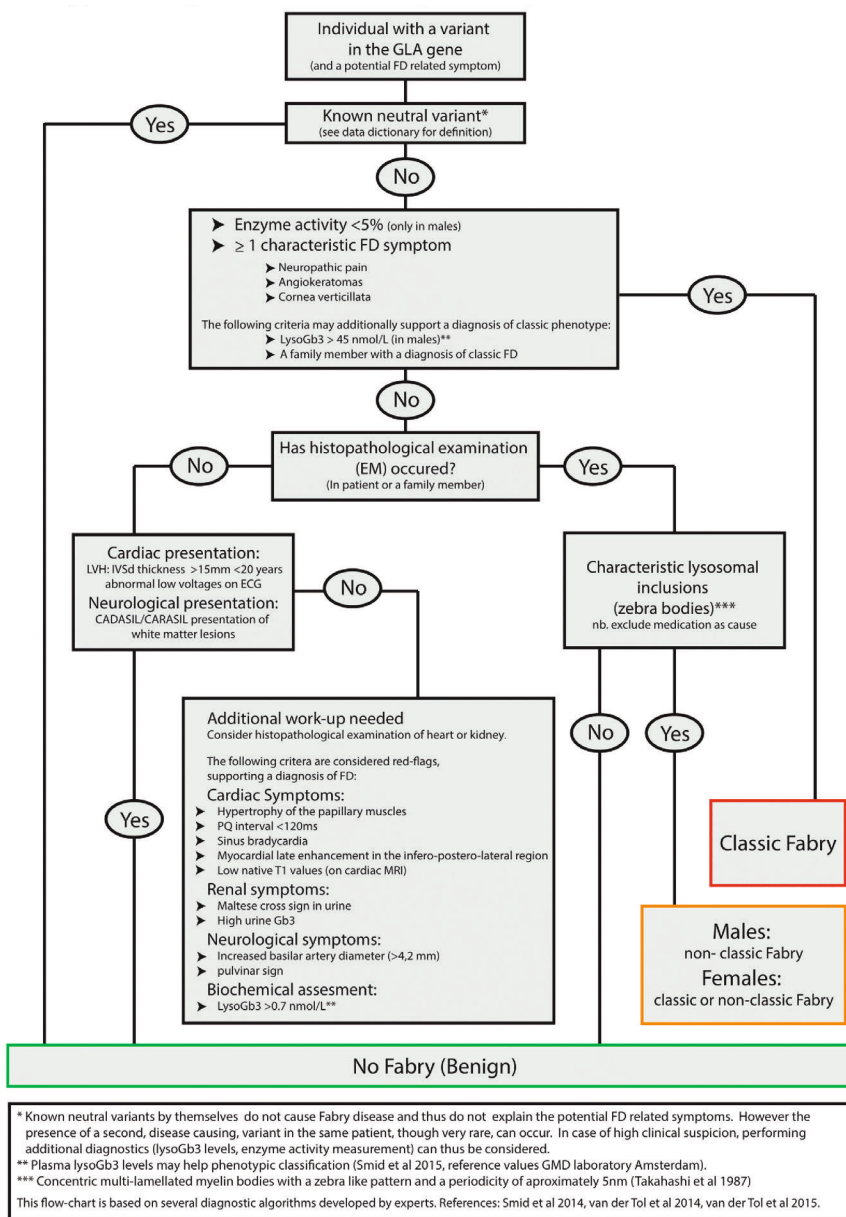
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Supplemental figure 1: Flowchart for the diagnosis and phenotype allocation in FD; cerebral autosomal dominant arteriopathy with subcortical infarcts and leucoencephalopathy (CADASIL); cerebral autosomal recessive arteriopathy with subcortical infarcts and leucoencephalopathy (CARASIL); electron microscopy (EM); galactosidase alpha (GLA); diastolic interventricular septum thickness (IVSd) [1].

Supplemental figure 2: Source raw data of P-wave duration, PR-interval, QRS-duration and QTc for each study participants subgroup. The shaded areas represent the 95%-CI for the GLM fitted curves.

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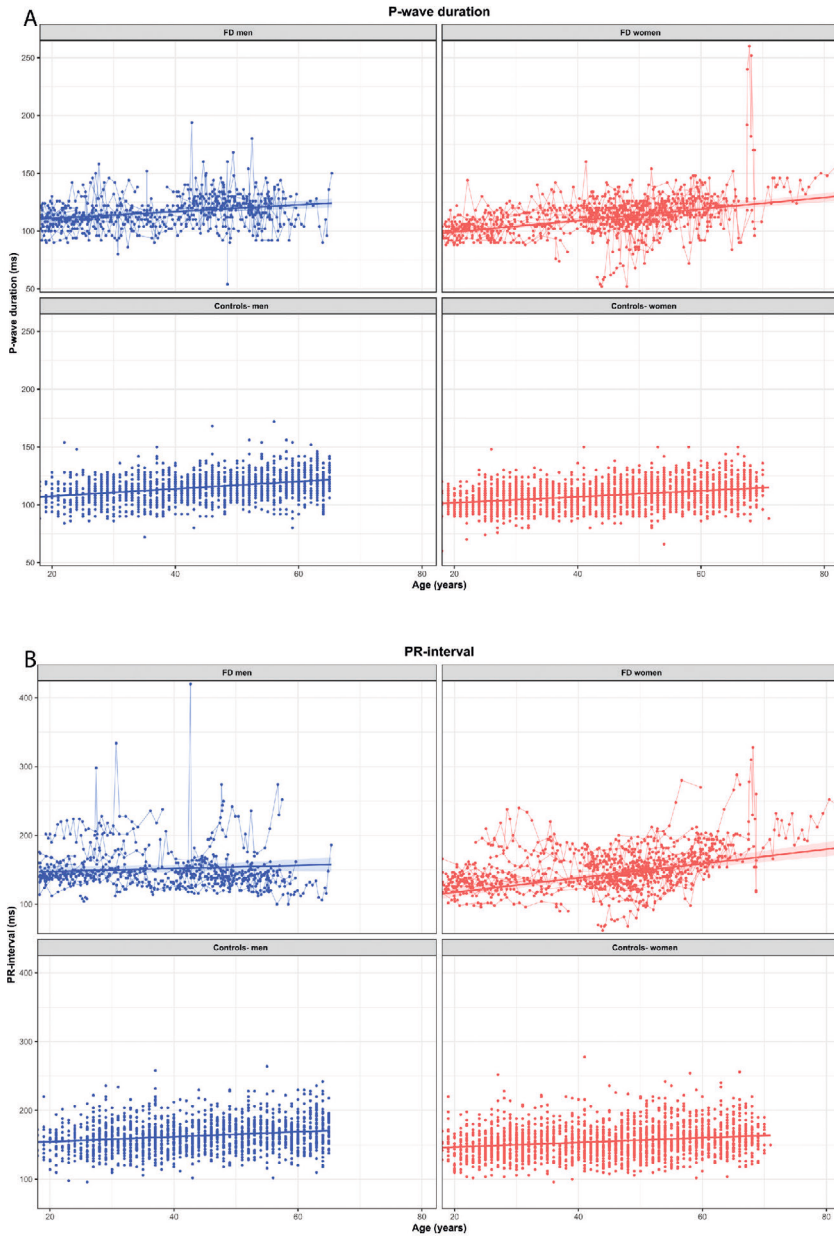
Supplemental figure 2: (continued)
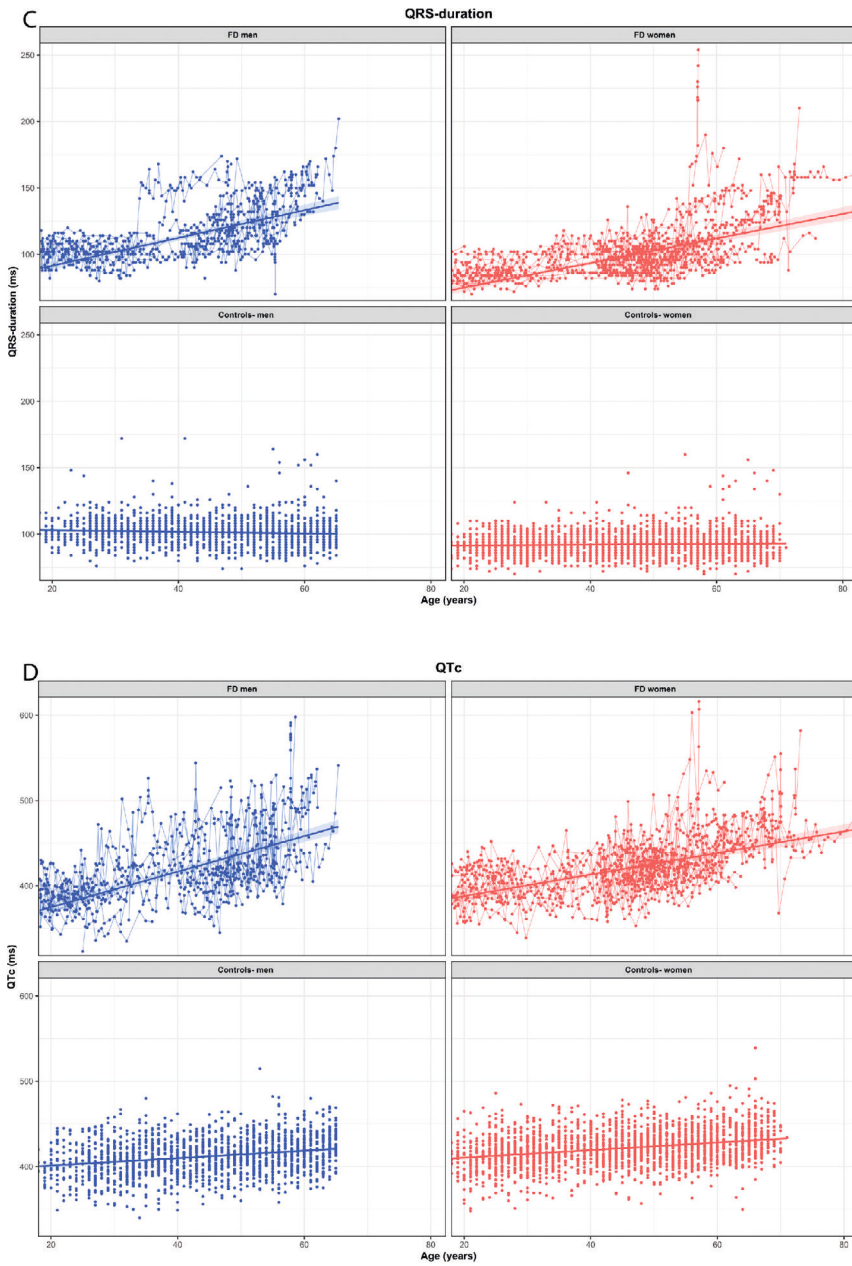
Supplemental figure 3: Source raw data of Cornell index, Spatial QRS-T angle and Frontal QRS-axis for each study participants subgroup. The shaded areas represent the 95%-CI for the GLM fitted curves.
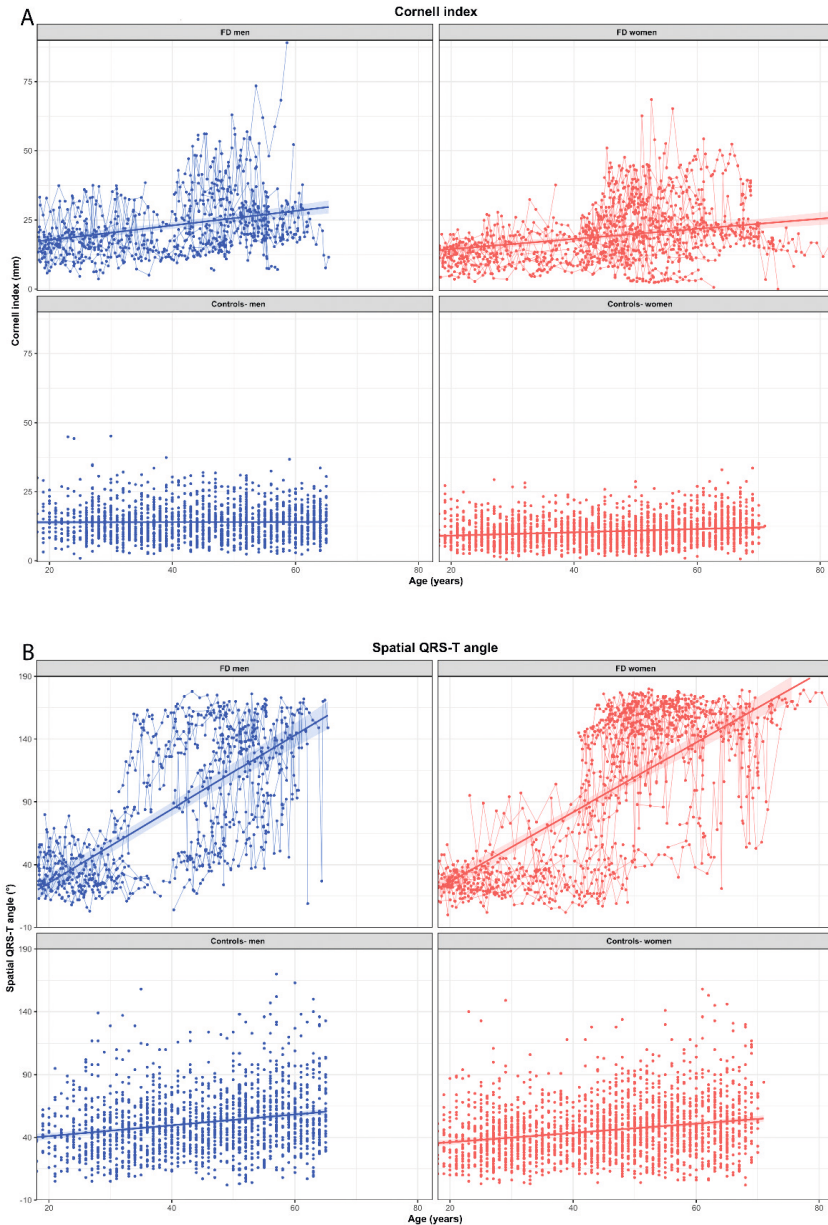
Supplemental figure 3: (continued)
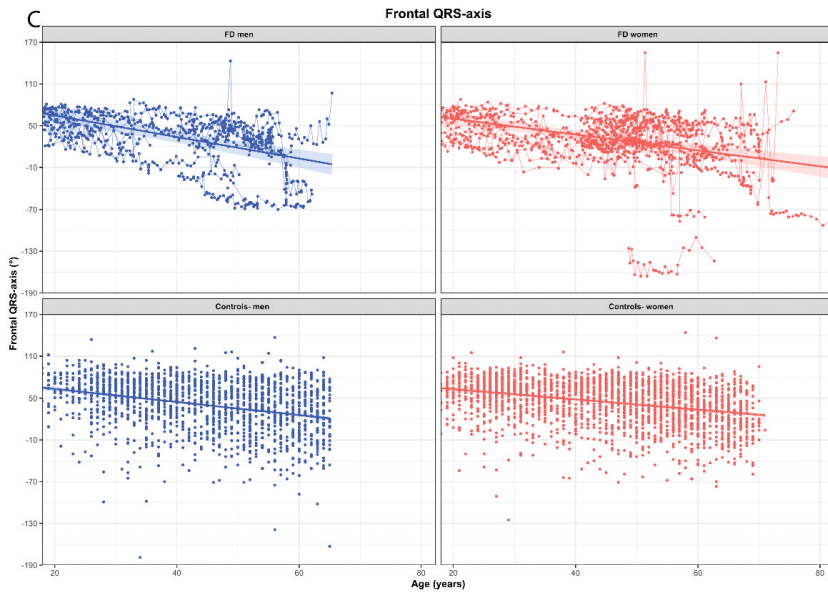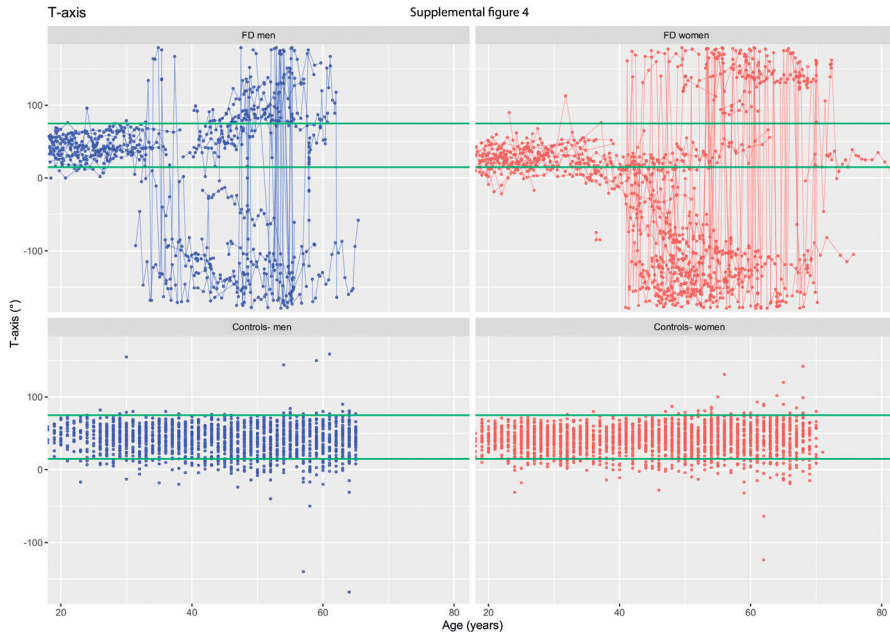
Supplemental figure 4: Source raw data of the frontal T-axis for each study participants subgroup. The T-axis' normal value (15°-75°) is represented by the green lines [2].
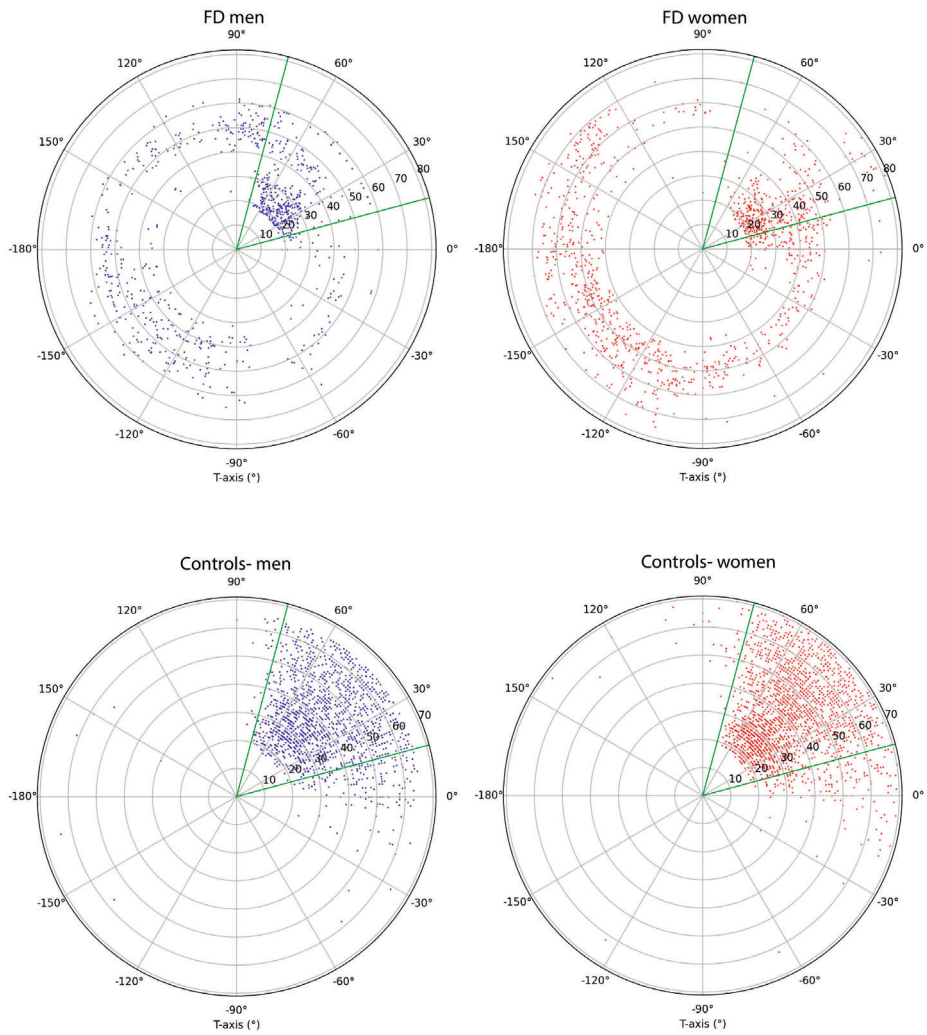
Supplemental figure 5: Polar plot graphs showing the absolute values of the frontal T-axis for each study participants subgroup. The numbered shells represent the age at which an ECG was obtained. The T-axis' normal value (15°-75°) is represented by the green lines [2].
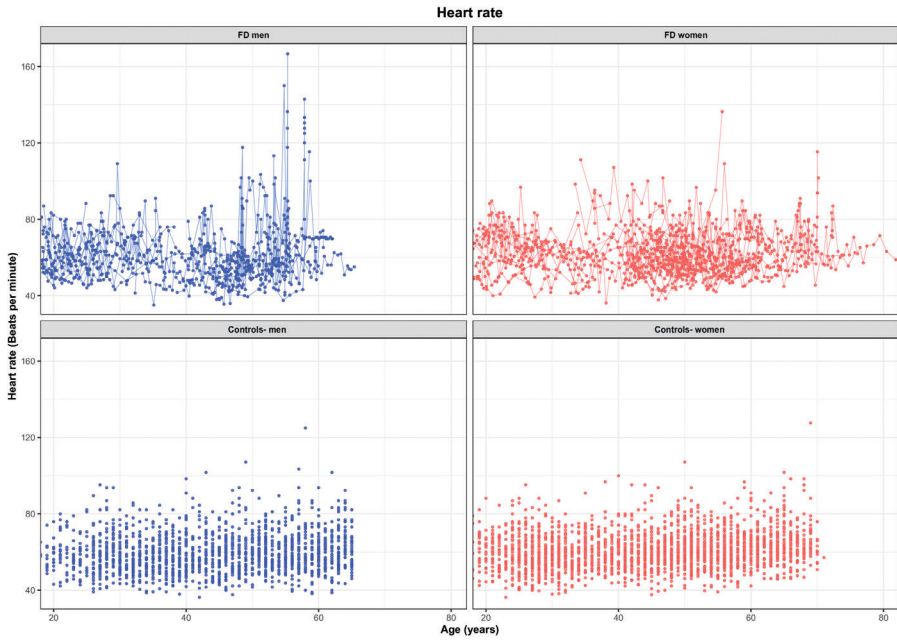
Supplemental figure 6: Source raw data of the heart rate for each study participants subgroup.
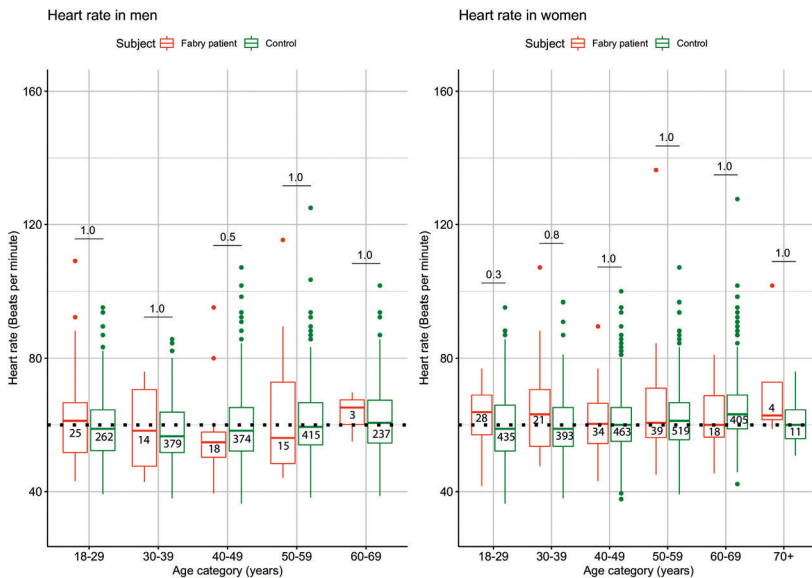
Supplemental figure 7: Boxplots of the heart rate per age decade in Fabry patients and controls. Numbers inside the boxes are the numbers of the analyzed ECGs. The last available ECG per FD patient per decade was selected to ensure that the influence of repeated measurements was limited. The horizontal lines represents the reference range, based on the literature.
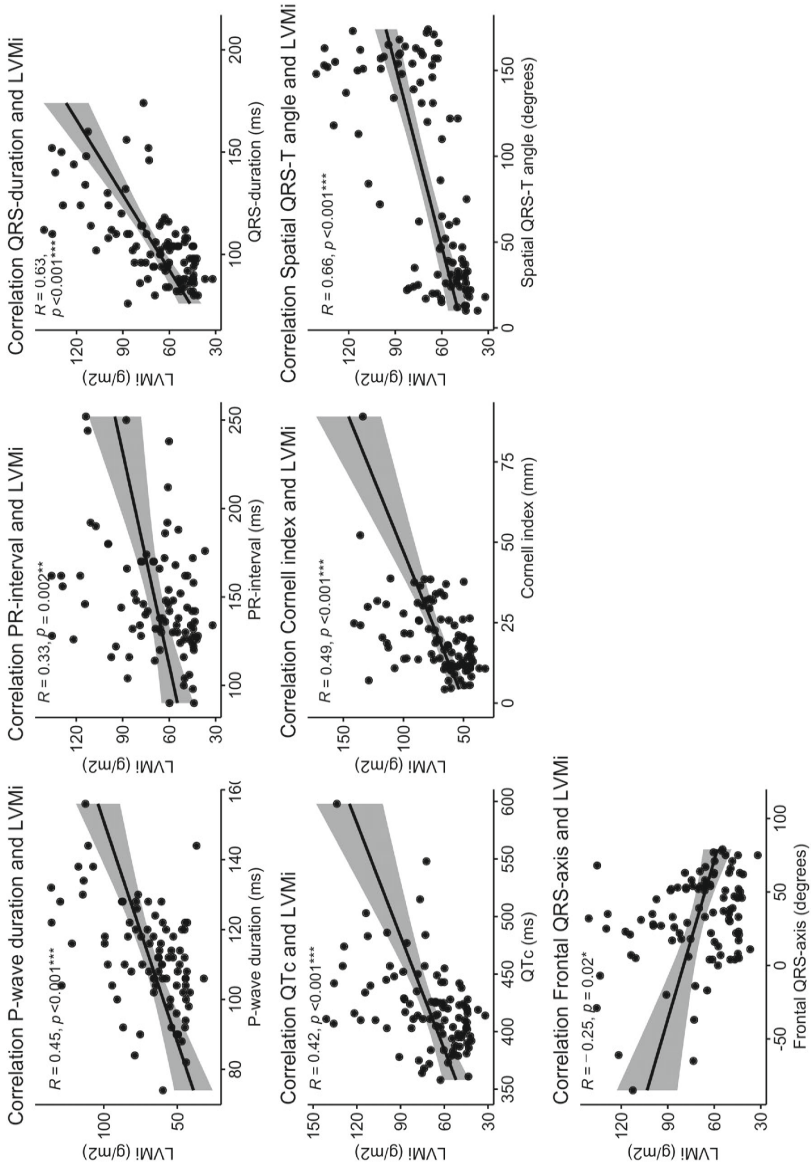
Supplemental figure 8: Scatter plot displaying the Spearman correlation between ECG markers obtained from the last ECG and corresponding LVMi on CMR. The shaded areas represent the 95%- CI for the fitted curve.

Supplemental figure 9: Boxplots of the ECG parameters obtained from the last ECG versus the corresponding scored LGE on CMR.

Supplemental figure 9: (continued)

QRS-duration and LGE

QTc and LGE

Supplemental figure 9: (continued)

Cornell index and LGE · Spatial QRS-T angle and LGE · Frontal QRS-axis and LGE
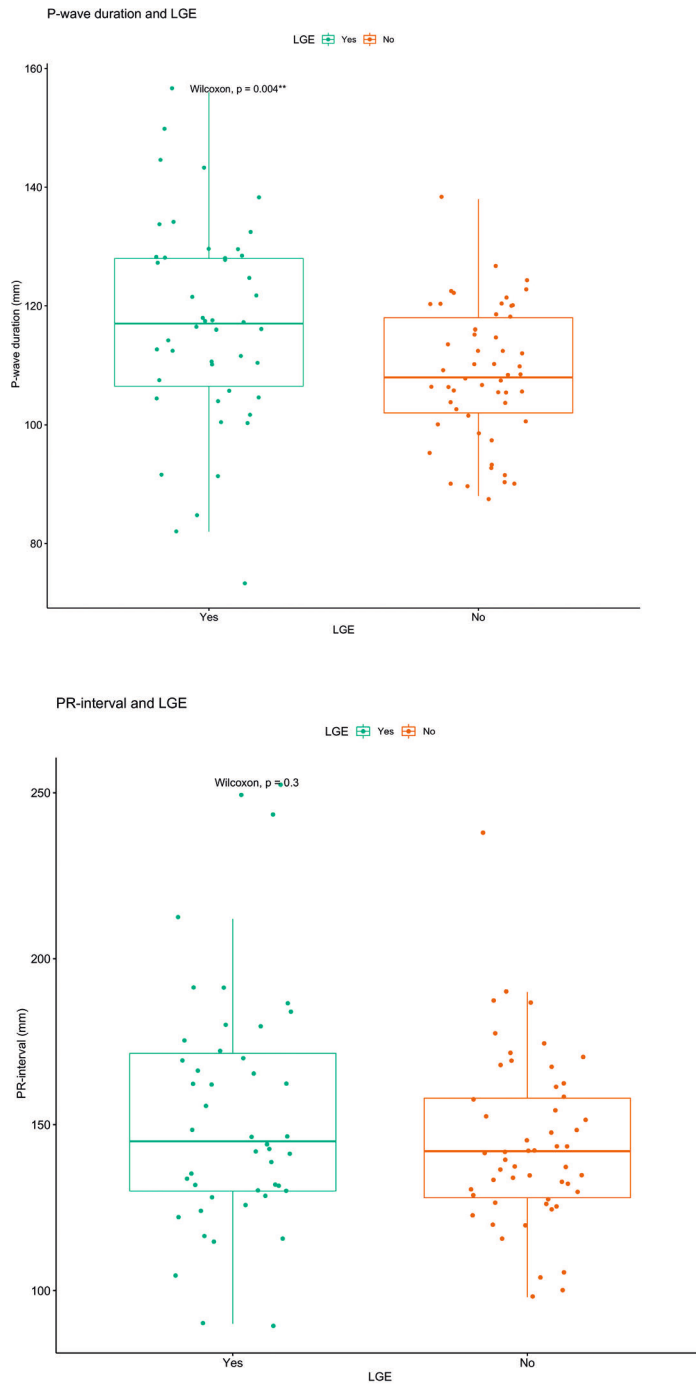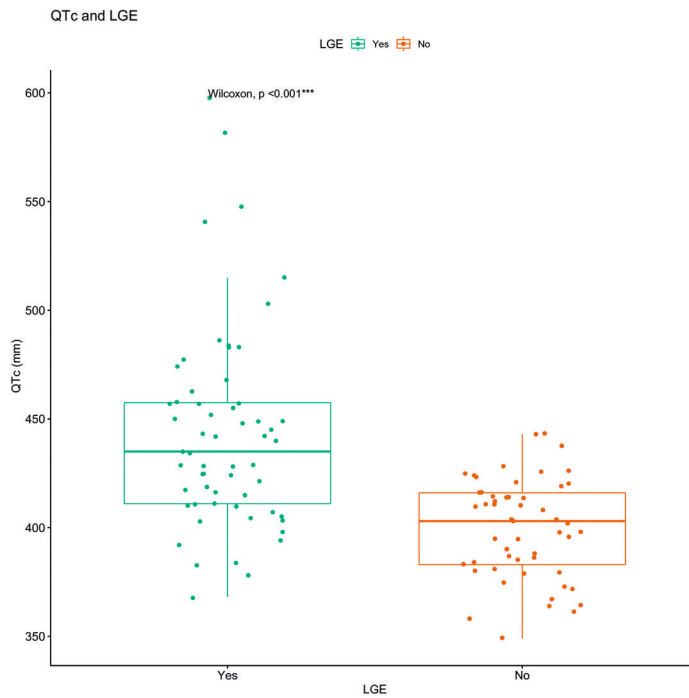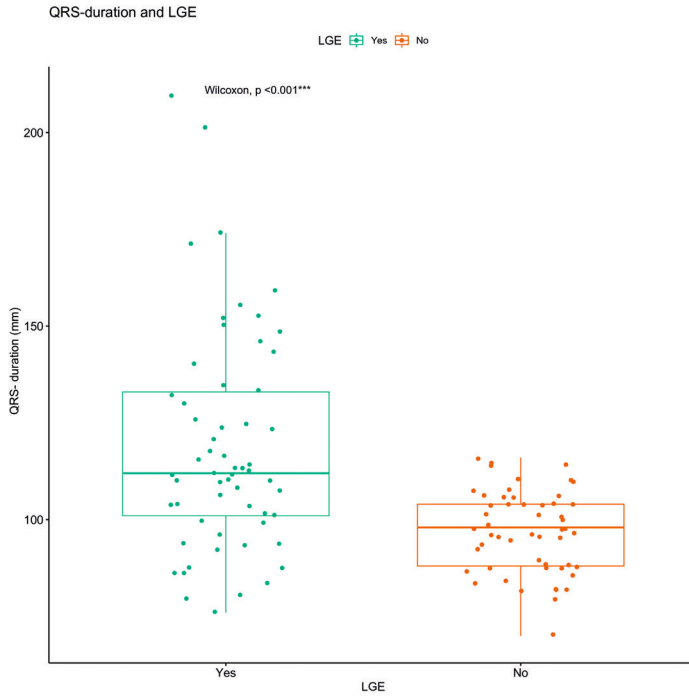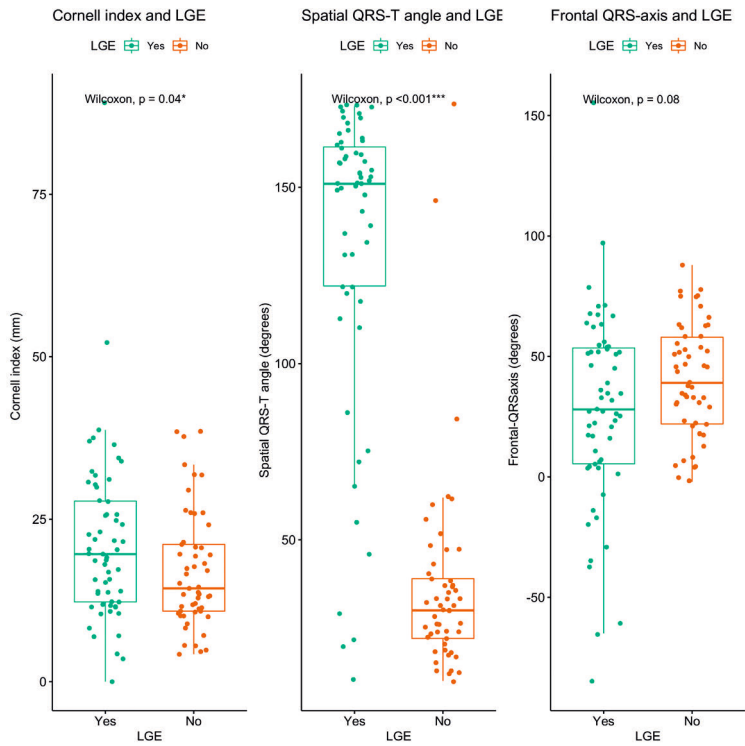
References

1. El Sayed, M., et al., *Influence of sex and phenotype on cardiac outcomes in patients with Fabry disease.* Heart, 2021.
2. Kors, J.A., et al., *T axis as an indicator of risk of cardiac events in elderly people.* The Lancet, 1998. **352**(9128): p. 601-605.
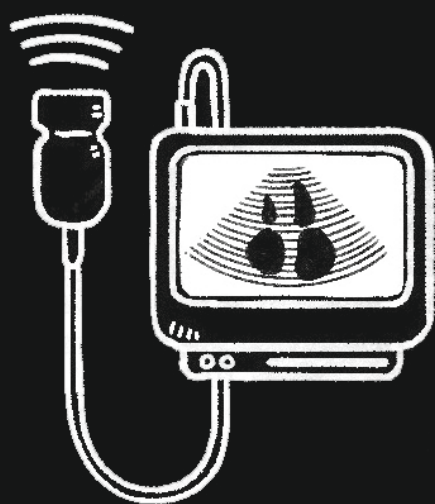
Chapter 4

Early echocardiography markers of Fabry cardiomyopathy identified in a multi-decade longitudinal cohort study

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Abstract

Background
Morphological and functional abnormalities that can be detected by echocardiography in Fabry disease (FD) include left ventricular (LV) hypertrophy, left atrial dilatation and parameters of LV systolic and diastolic dysfunction. Currently, the progression of echocardiographic features of FD over long periods of follow-up in larger cohorts of FD patients is unknown.

Methods
In this study the two echocardiograms, with the longest follow-up period per patient, of 92 patients with classical FD (cFD) were re-assessed and analyzed (34 men, echo's, median 12 years apart, 92% treated with ERT). Results were compared to data from 147 echocardiograms of healthy individuals (age and sex matched on a group level, cross-sectional data). Linear mixed-effect models (GLM) were used to analyze the effect of FD, age and sex on five echocardiographic parameters: end- diastolic interventricular septum thickness (IVSd), relative wall thickness (RWT), left ventricular mass index (LVMi), left atrium volume index (LAVI) and the ratio of early diastolic mitral inflow velocity/ early diastolic septal tissue mitral annulus velocity (E/e'). The regression slope and absolute values per decade for each echocardiographic parameter were compared between FD patients and healthy control subjects. A Cox proportional-hazard model studied the relationship between the first echocardiogram and subsequent development of Atrial fibrillation (AF).

Results
The absolute values for IVSd and RWT in both men and women and E/e' in men were significantly different between FD patients and healthy individuals ($p < 0.05$) from age 18 onwards. Rate of change, rather than absolute values of other echocardiographic parameters were different in FD patients compared to healthy controls ($p < 0.05$ for all comparisons). Increased IVSd, RWT and E/e' on the first echocardiogram were associated with an increased hazard ratio for AF later on (HRs: 1.31, 1.50 and 1.20, respectively, $p < 0.001$).

Conclusions
The earliest signs of cardiac involvement of FD on echocardiography are increased values for IVSd and RWT in all cFD patients and E/e' in men. Early detection of cardiac disease is especially important in women with cFD, since not all of them will develop cardiac symptoms of the disease. During adult life IVSd, RWT, LVMi, LAVI and E/e' increase at a significantly higher rate in FD patients compared to healthy individuals. These parameters may be used for treatment decisions, monitoring disease progression and evaluating the effect of new therapeutic interventions.

Introduction

Fabry disease (FD) is a rare X-linked inherited lysosomal function disorder caused by mutations in the galactosidase alpha (GLA) gene (OMIM 301500), leading to reduced activity of the enzyme alpha- galactosidase A [1, 2]. The main enzyme substrate, globotriaosylceramide (Gb3) and its derivatives accumulate in different tissues causing progressive dysfunction of, most prominently, kidneys, brain and heart [3, 4]. This study focuses on the cardiac manifestations of the disease and how they can be tracked by the echocardiogram. Initial cardiac manifestations of FD may include bradycardia, a low native T1 value on cardiovascular magnetic resonance (CMR) and left ventricular hypertrophy (LVH). As the disease progresses, conduction abnormalities, (supra)ventricular arrhythmias, ischemic heart disease, left ventricular dysfunction and ultimately overt heart failure (HF) leading to cardiac death may develop [3-6].

Since FD has an X-linked inheritance pattern, the disorder is generally more severe in men than in women. FD can be classified into a classical phenotype with early onset of manifestations, or a non- classical, more attenuated (late-onset) phenotype. Even within disease groups stratified by sex and phenotype, there is large heterogeneity in cardiac disease severity and age of symptom onset [5-7]. This heterogeneous disease course (especially in women with classical FD) [6, 8] creates a significant challenge in distinguishing between patients at risk of developing cardiac events, requiring intensive monitoring and treatment from those who are unlikely to develop cardiac complications. Identification of patients at risk is critical, as an increasing number of studies suggest that Fabry specific therapy should be instigated at an early age in order to have impact on disease development [9, 10].

Potentially, early cardiac manifestations as detected by echocardiography might be helpful for identifying FD patients at risk of a severe disease course. Also, changes in echocardiographic markers may be useful to monitor whether or not the development or progression of FD cardiomyopathy is halted by new FD specific therapies [11].

One of the main advantages of the echocardiogram is that it is an easily accessible and affordable diagnostic tool, especially when FD patients are unable to undergo CMR due to an implantable cardiac device or because of claustrophobia.

Initial echocardiographic functional alterations in FD are subtle, such as a reduced global longitudinal strain (GLS) [12, 13]. During adult life, LVH, left atrial dilatation and diastolic dysfunction, mainly with a preserved ejection fraction, can develop. At this stage there may also be secondary heart valve

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disease [8, 14-17]. Previous studies have shown that some morphological (Left Ventricular Mass) and functional (ratio of early diastolic mitral inflow velocity/ early diastolic septal tissue mitral annulus velocity (E/e') and GLS) echocardiographic abnormalities predict the occurrence of cardiac complications in FD patients [18-23]. However, long-term longitudinal studies reporting progression of echocardiographic parameters in men and women with different FD phenotypes, and how this differs from healthy individuals, are lacking [18]. Comparing these echocardiographic features in FD patients with those of a healthy population, helps to recognize echocardiographic abnormalities that are typical for FD at both an early disease stage and later on in the disease course. Once we know how these features change over time and how they are related to known cardiac disease markers (e.g. plasma N-terminal prohormone of Brain Natriuretic Peptide (NT-proBNP) and troponin T) and the development of cardiac complications, these features can be used for decision making regarding treatment initiation and evaluation of therapeutic effectiveness.

To establish the longitudinal echocardiographic changes in FD, we conducted a retrospective study in the FD patient cohort under follow-up at the Amsterdam University Medical Centre (AUMC) and compared the findings to those of a large healthy population study. The size of the FD patient cohort, length of follow-up and systematic review of all echocardiograms make this study unique, providing precise information on echocardiographic changes over time in FD patients.

Aims of the study are:

Primary:
1. Describing the echocardiographic features and their changes over time in men and women with classical FD and comparing these features to those of a healthy control group;
2. Comparing the changes in echocardiographic features between men and women with classical FD.

Secondary:
3. Investigating the relation between echocardiographic features in FD patients and plasma NT- proBNP and Troponin-T levels;
4. Exploring the association between echocardiographic parameters and the subsequent development of cardiac complications.

Methods

Study setting
Amsterdam UMC, location AMC is the national reference centre for FD. Outpatient clinic follow-up visits for classical FD patients are scheduled semiannually, annually or biannually, depending on sex, age and treatment status of the patient. The protocolized follow-up visits include blood tests (among which plasma creatinine, globotriaosylsphingosine (LysoGb3), high-sensitivity Troponin T (hs-TnT) and NT-proBNP), urine analysis (24 hour creatinine and albumin excretion), imaging (CMR and echocardiography) and electrocardiography.

Study population

FD patients
Classification into classical or non-classical FD phenotype is based on the presence of classical FD symptoms (cornea verticillata, acroparesthesia or angiokeratoma), family history (in women), known mutation-phenotype associations, residual enzyme activity and the levels of a deacylated form of Gb3 (Globotriaosylsphingosine (LysoGb3)) [5-7]. See **supplemental figure 1** for the flowchart for FD diagnosis and phenotype allocation [8].

Patients in whom two echocardiograms were obtained with a minimum follow-up time between the first and last echocardiogram of five years were included in this study. The study includes patients with classical FD only. The number of patients with non-classical FD with two echocardiography examinations with a minimum of two years between them was too small.

Healthy volunteers
To assess the difference between echocardiographic features in FD patients and the general population, we used cross-sectional data from a general population study that was previously conducted at the Erasmus MC in Rotterdam, The Netherlands. This study included healthy volunteers who were $\geq$ 18 years of age. Exclusion criteria were: (prior) cardiovascular disease, systemic disease, the use of medication for cardiac conditions, abnormalities during the physical examination pointing towards cardiac disease or cardiovascular risk factors (hypertension: systolic and diastolic blood pressure >140/80mmHg, diabetes mellitus and hypercholesterolemia). Professional athletes, morbidly obese participants (BMI >40 kg/m2) and women who were pregnant or had breast implants were excluded as well [24].

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Ethics

The study was conducted following the Declaration of Helsinki [25]. Because of the retrospective character, using data obtained in the context of regular FD patient care, the Amsterdam University Medical Centres Medical ethics committee confirmed that the Medical Research Involving Human Subjects Act does not apply to the used data of FD patients. Before data collection in the healthy Rotterdam cohort, the local ethics committee at the Erasmus MC approved the protocol and all subjects signed informed consent.

Data collection

Echocardiographic assessments

Between March 2004 and February 2021, FD patients underwent echocardiograms (Vivid 7 or E95, GE Healthcare, Milwaukee, WI, USA) obtained by experienced technicians, according to a standard outpatient clinic protocol. For each patient we selected the two echocardiograms with the longest period between them. All echocardiographic images were re-assessed and re-measured if deemed necessary, by a single observer (*MES*). The healthy volunteers underwent an echocardiogram (Philips iE33 and EPIQ7 ultrasound systems) during a one day visit between 2014 and 2015.

The following echocardiographic features were assessed: end-diastolic interventricular septum thickness (IVSd), end-diastolic left ventricular posterior wall thickness (LVPWd), left ventricular end- diastolic diameter (LVEDd), left atrium volume index (LAVI), early diastolic mitral inflow velocity (E), peak velocity flow in late diastole caused by atrial contraction (A), early diastolic septal tissue mitral annulus velocity (septal e'), biplane left ventricular ejection fraction (LV EF), Tricuspid Annular Plane Systolic Excursion (TAPSE) and global longitudinal strain (GLS). The Relative wall thickness (RWT) (IVSd + LVPWd/ LVEDd) and Left ventricular mass index (LVMi in g/m^2)= $(0.8\{1.04[([LVEDd + IVSd + LVPWd]^3 - LVEDd^3)]\} + 0.6)/(weight^{0.425} * length^{0.725} * 0.007184)$ [26] were calculated using the LV dimension parameters.

GLS was measured only in the last available echocardiogram of each FD patient, since the image quality of the earlier ultrasounds was often insufficient to produce accurate measurements.The maximal tricuspid regurgitation velocity (TR Vmax) and sinus of Valsalva (SoV) diameter were not available of the healthy control subjects and thus only reported for the FD patients.

To get an overall impression of the left ventricular (LV) diastolic function in FD patients, this was scored on the last available echocardiogram.

LV diastolic function was defined as normal, dysfunction (grade I, II and III) or indeterminate on the basis of Doppler mitral inflow pattern parameters, including early (E) and late (A) LV filling velocities, E/A ratio, and tissue Doppler imaging–derived septal e' according to the European Society of Cardiology recommendations published in 2016 [27].

Clinical characteristics and cardiovascular events in FD patients
Cardiac enzymes in plasma (NT-proBNP and hs-TnT), kidney function and microalbuminuria data were extracted from the medical records for the time points closest to the echocardiogram date (with a range of ±1 year for kidney function and microalbuminuria and ±3 months for the cardiac enzymes).

Renal function was approximated by calculating the estimated Glomerular filtration rate (eGFR) using the CKD-EPI formula [28]. Microalbuminuria was expressed as the amount of albumin in the collected 24 hours urine sample.

The presence of cardiovascular risk factors (smoking, obesity, diabetes mellitus, dyslipidemia and hypertension) and the use of cardiovascular (preventive) medication in FD patients were assessed between the time points at which the echocardiograms were performed. The occurrence of major cardiovascular events (MACE) (combined endpoint cardiovascular death (CVD), heart failure (HF) hospitalisation, sustained ventricular arrhythmias (SVAs) and myocardial infarction) and atrial fibrillation (AF) after the first echocardiogram was recorded by data extraction from medical records [8].

Statistical methods
For statistical analysis, R (version 4.0.3) was used. The general clinical characteristics are presented as proportions or medians and minimum/ maximum ranges. Descriptives of the echocardiographic features show the median with an interquartile range (IQR) for each participant subgroup (men with FD, women with FD, healthy men and healthy women) for each age decade.

Generalized Linear mixed-effect models (GLM) were used to evaluate the effect of age at the time of an echocardiogram, study subject type (FD patient or healthy control) and sex on changes in five main echocardiographic morphological and functional features: IVSd, RWT, LVMi, LAVI and E/e'.

All GLM included a random intercept and slope to account for inter-patient variations. The model assumptions were validated and met [29]. Because of the assumption that the effect of age on echocardiographic parameters would be different between men and women and between FD patients and healthy controls, we tested for three-way interactions (age * type (Fabry patient or Healthy control)

* sex) in all models. From the resulting model, regression lines per subgroup could be obtained following the standard regression equation for a linear model: y= a + β*X, with the intercept (a) and slope (β) specified for each subgroup.

The slopes (β) of these regression equations were compared between the patient groups to illustrate the estimated difference in the evolution of a specific echocardiographic parameter over a 10-year period.

To study which of the abnormalities in the echocardiography features occur when (early or late in disease evolution), the differences in absolute values of the echocardiographic parameters between FD patients and the healthy control subjects per age decade were assessed by a Wilcoxon rank test. For these comparisons, an adjusted p-value using the Bonferroni method was displayed to correct for multiple testing. For the comparison of these absolute values, only the last available echocardiogram per patient was selected to ensure that the influence of repeated measurements in single individuals was absent.

Values of the five main echocardiographic parameters on the first echocardiogram of each patient were assessed for their potential relation to development of AF during the follow-up period using a Cox proportional-hazards model, that corrected for unequal follow-up duration for each patient.

To determine the relation of the echocardiographic changes in FD patients to other markers of myocardial injury and dysfunction we assessed the relation between three comprehensive echocardiographic features (LVMi, E/e' and GLS) and the levels of cardiac enzymes in plasma (NT- proBNP and hs-TnT), using a Spearman correlation analysis. We regarded a p-value ≤0.05 as statistically significant in all analyses.

For the following echocardiographic variables only descriptive statistics were given: LV EF, TAPSE (too much nonlinear variation in measurements of FD patients for a valid GLM approach), TR Vmax, SoV (data only available for FD patients) and GLS (different measurement algorithms in FD patients vs healthy controls, which makes the comparison between these two groups invalid).

Results

Participants' characteristics

Ninety-two classical FD patients (34 men and 58 women) were included **(table 1)**. For each FD patient two echocardiograms were assessed (184 in total), with a median of 12 years (range 5-16 years) between them. The median age of FD patients at the first and the last obtained echocardiogram was 39 years (range: 18-72) and 51 years (range: 24-82) respectively. Ninety-two percent of the included FD patients have been treated with ERT, during a median period of 11 years (range: 1-21). ERT initiation decisions were based on FD symptoms [30] or recommendations by the European Fabry Working Group once available from 2015 onwards [31].

The subset of the Rotterdam study [24] consisted of 147 healthy control subjects (single measurement per subject) (50% men and 50% women) with a median age of 44 years (range: 20-72) at time of echocardiography.

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Table 1: FD patients' characteristics

	All	Men	Women
Number of patients, n (%)	92 (100%)	34 (37%)	58 (63%)
General characteristics FD patients			
Age at FD diagnosis (years), median (range)	28 (0.03-67)	20 (5-53)	36 (0.03-67)
Clinical diagnosis, n (%)	25/87 (29%)	18/31 (58%)	7/56 (12%)
Diagnosis through family screening, n (%)	62/87 (71%)	13/31 (42%)	49/56 (88%)
Age at first echocardiogram (years), median (range)	39 (18-72)	27 (18-56)	42 (18-72)
Age at last echocardiogram (years), median (range)	51 (24-82)	39 (25-62)	53 (24-82)
Cumulative follow up (years), median (range)	12 (5-16)	10 (5-16)	12 (5-15)
Enzyme replacement therapy (ERT)			
Number of patients on ERT, n (%)	85 (92%)	34 (100%)	51 (88%)
Age at start ERT (years), median (range)	41 (13-71)	28 (13-54)	44 (16-71)
ERT duration (years), median (range)	11 (1-21)	12 (1-21)	10 (1-16)
Laboratory findings			
Available NT-proBNP results, n samples / n echocardiograms (%)	164/184 (89%)	58/68 (85%)	106/116 (91%)
Available hs-TnT results, n samples / n echocardiograms (%)	111/184 (60%)	42/68 (62%)	69/116 (60%)
Untreated baseline plasma LysoGb3 (nmol/l), median (range)	11 (3-149)	98 (31-149)	8 (3-24)
eGFR- CKD EPI at first echocardiogram (ml/min), median (range)	107 (17-143)	112 (17-143)	105 (46-138)
eGFR- CKD EPI at last echocardiogram (ml/min), median (range)	84 (15-148)	89 (15-148)	82 (22-130)
Albuminuria/ 24 hours at first echocardiogram (mg/24 hours), median (range)	53 (0-4,236)	73 (4-4,236)	36 (0-2,598)
Albuminuria/ 24 hours at last echocardiogram (mg/24 hours), median (range)	41 (3-3,498)	58 (3-3,498)	37 (5-1,331)
Occurrence of Atrial fibrillation (AF) after the first echocardiogram			
Atrial fibrillation (AF), n (%)	14 (100%)	6 (43%)	8 (57%)
Age at first AF episode (years), median (range)	55 (41-67)	49 (41-61)	63 (48-67)

Table 1: FD patients' characteristics (continued)

	All	Men	Women
Cardiovascular risk factors†			
Smoking, n (%)	41 (45%)	17 (50%)	24 (41%)
Obesity, n (%)	16 (17%)	4 (12%)	12 (21%)
Diabetes, n (%)	1 (1%)	0 (0%)	1 (2%)
Dyslipidemia, n (%)	20 (22%)	7 (21%)	13 (22%)
Hypertension, n (%)	28 (30%)	9 (27%)	19 (33%)
Cardiovascular (preventive) medication†			
ACE inhibitors or Angiotensin receptor blockers , n (%)	59 (64%)	22 (65%)	37 (64%)
Thiazide diuretics, n (%)	15 (16%)	5 (15%)	10 (17%)
Loop diuretics, n (%)	17 (19%)	6 (18%)	11 (19%)
Potassium-sparing diuretics, n (%)	10 (11%)	3 (9%)	7 (12%)
Beta-blockers, n (%)	24 (26%)	7 (21%)	17 (29%)
Calcium channel blockers, n (%)	19 (21%)	6 (18%)	13 (22%)
Statins, n (%)	20 (22%)	8 (24%)	12 (21%)

† cardiovascular risk factors or the use of Cardiovascular (preventive) medication at any time during follow-up:
- Obesity: Body Mass Index ≥ 30 kg/m2
- Smoking: patients who have ever smoked
- Hypertension: use of antihypertensive medication or systolic blood pressure of >140 mmHg and/ or diastolic blood pressure of >90 mmHg, present at least two measurements
- Dyslipidemia: elevated levels of total cholesterol (>6.5 mmol/l) or low density lipoprotein (LDL) cholesterol (>2.5 mmol/l) or triglycerides (>3.0 mmol/l) or low levels of high-density lipoprotein (HDL) cholesterol (men: <1.0 mmol/l, women <1.2 mmol/l), or medication prescribed for the indication dyslipidemia
- Diabetes mellitus: type I or type II if reported in the medical chart or the use of anti-diabetic medication

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Echocardiographic results

Descriptives on the five main echocardiographic parameters (IVSd, RWT, LVMi, LAVI and E/e') are presented in **supplemental table 1**. **Figure 1** displays the modeled course of each of these parameters. The results of the GLM for these parameters are displayed in **table 2** and **supplemental table 2**.

Figure 2 shows the boxplots of the five parameters per age decade in FD patients versus healthy controls. In **supplemental table 3**, the descriptives of the other echocardiography markers (LVPWd, LVEDd, Septal e' velocity, E/A ratio, GLS, Biplane LV EF and TAPSE) per age decade are displayed. **Supplemental figures 2-5** show raw data of all studied echocardiographic parameters.

Figure 1: Based on the GLM, the estimated effect plots with the 95%-CI of: A. IVSd, B. RWT, C. LVMi, D. LAVI and E. E/e' for each study participants subgroup.

Figure 1: (continued)

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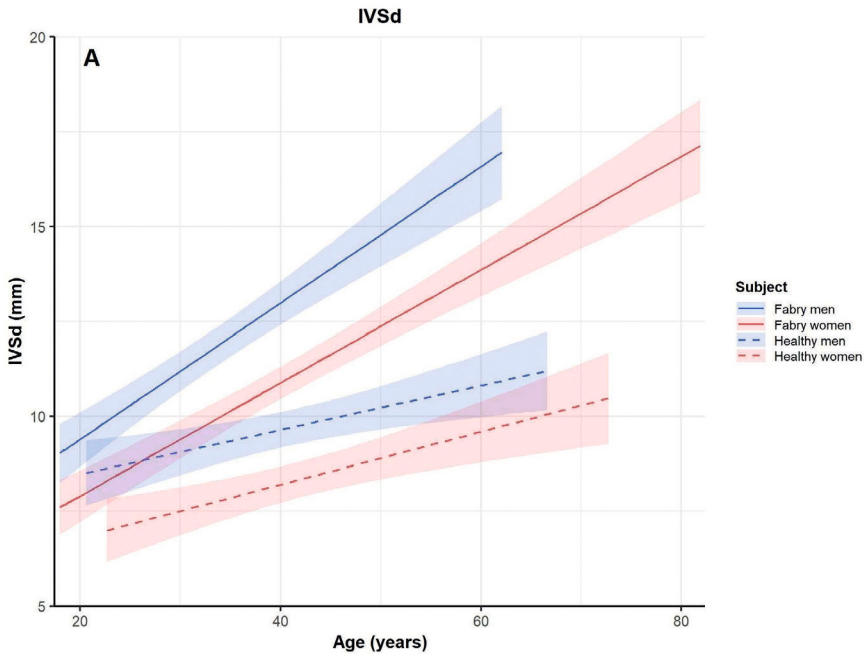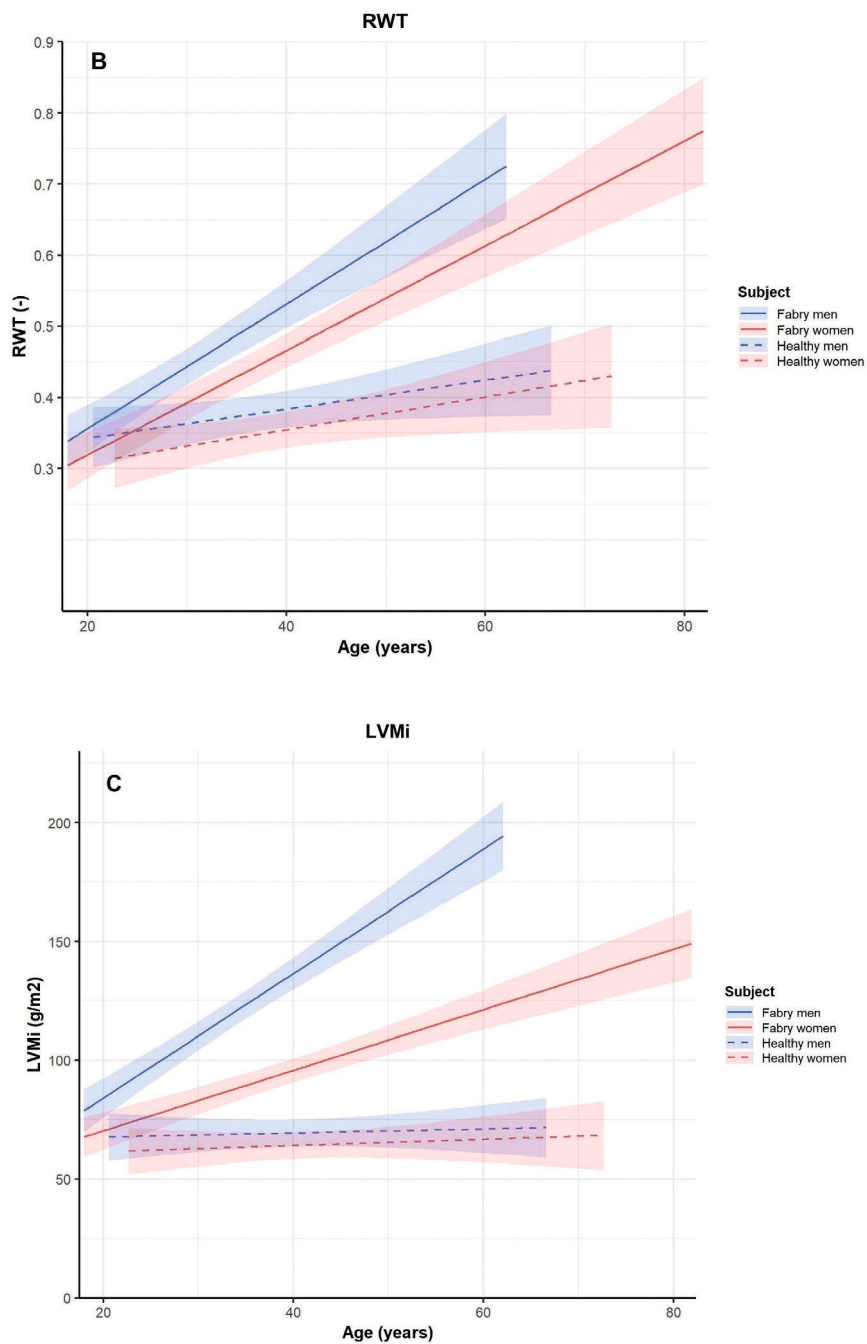
Figure 1: (continued)
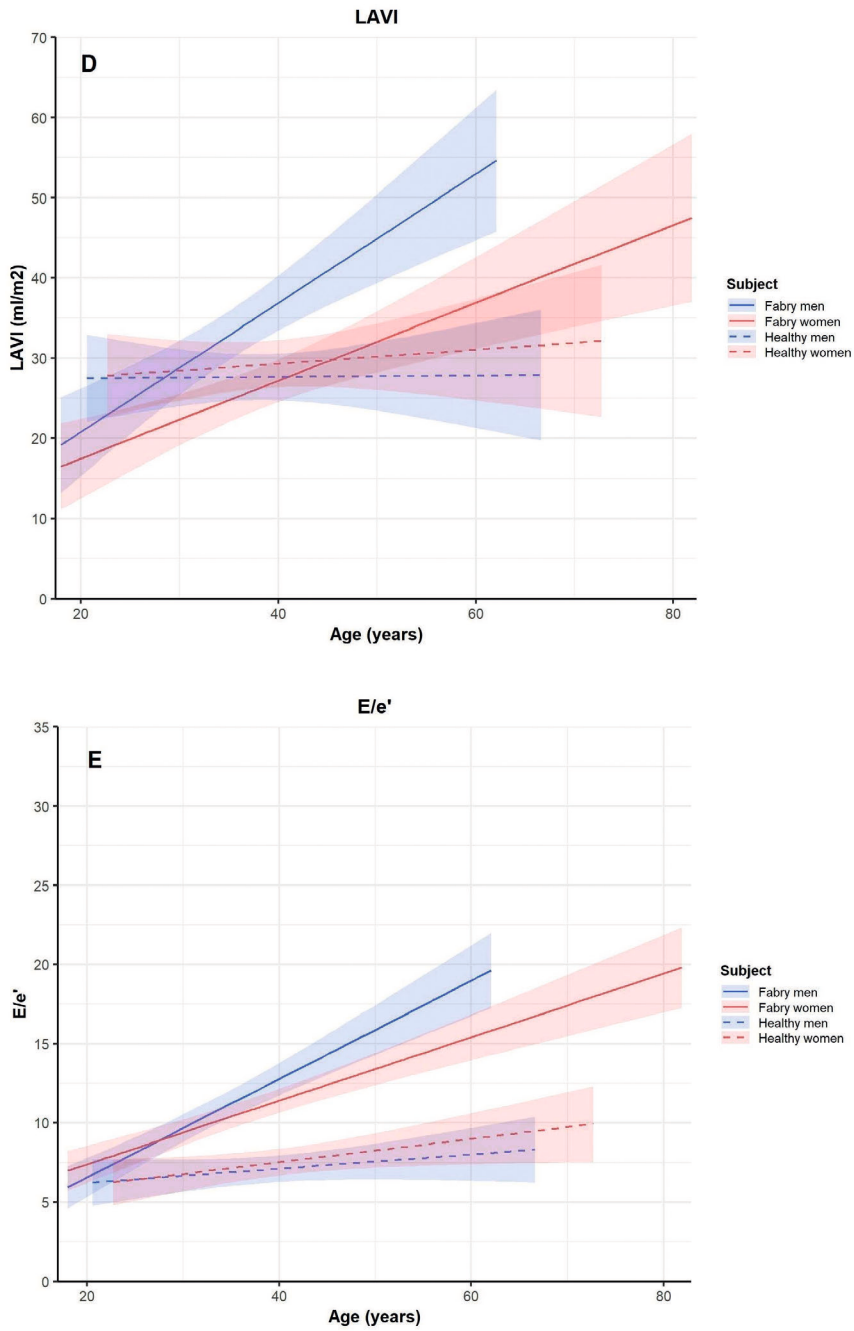
Table 2: Echocardiographic morphological and functional parameters - estimated regression coefficients (β) per 10 years with 95%-CI

Subgroups	IVSd (mm)	RWT (-)	LVMi (g/m²)	LAVI (ml/m²)	E/e' (-)
Fabry men	1.8 (1.4-2.2)***	0.1 (0.1-0.1)***	26.2 (21.6-30.8)***	8.0 (5.0-11.1)***	3.1 (2.4-3.9)***
Healthy men	0.6 (0.2-0.9)**	0.02 (0.002-0.04)*	0.8 (-3.4-5.1)	0.1 (-2.6-2.8)	0.4 (-0.2-1.1)
Fabry women	1.5 (1.2-1.8)***	0.1 (0.1-0.1)***	12.7 (9.5-16.0)***	4.9 (2.5-7.2)***	2.0 (1.5-2.6)***
Healthy women	0.7 (0.3-1.1)***	0.02 (0.003-0.04)*	1.3 (-3.0-5.6)	0.9 (-1.8-3.6)	0.7 (0.04-1.4)*
Differences in regression coefficients (β) per 10 years between subgroups with 95%-CI					
Fabry men minus Fabry women	0.3 (-0.2-0.8)	0.014 (-0.01-0.04)	13.5 (7.8-19.1)***	3.2 (-0.7-7.1)	1.1 (0.2-2.0)*
Fabry men minus Healthy men	1.2 (0.7-1.7)***	0.1 (0.03-0.1)***	25.4 (19.1-31.6)***	8.0 (3.9-12.0)***	2.7 (1.6-3.7)***
Fabry women minus Healthy women	0.8 (0.3-1.2)**	0.1 (0.02-0.1)***	11.4 (6.0-16.8)***	4.0 (0.4-7.6)*	1.3 (0.4-2.2)**
Healthy men minus Healthy women	-0.1 (-0.6-0.4)	0.0 (0.0-0.0)	-0.5 (-6.5-5.6)	-0.8 (-4.6-3.1)	-0.3 (-1.3-0.7)

*$p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Diastolic interventricular septum thickness (IVSd), relative wall thickness (RWT), left ventricular mass index (LVMi), left atrium volume index (LAVI) and the ratio of early diastolic mitral inflow velocity/ early diastolic septal tissue mitral annulus velocity (E/e').

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Figure 2: Boxplots of A. IVSd, B. RWT, C. LVMi, D. LAVI and E. E/e' per age decade in Fabry patients and Healthy controls. Numbers under the boxes are the numbers of the analyzed echocardiograms. The last available echo per FD patient per decade was selected to ensure that the influence of repeated measurements was limited. The horizontal lines represents the reference ranges of each echocardiographic parameter, based on the literature. *$p < 0.05$, ** $p < 0.01$, ***$p < 0.001$.
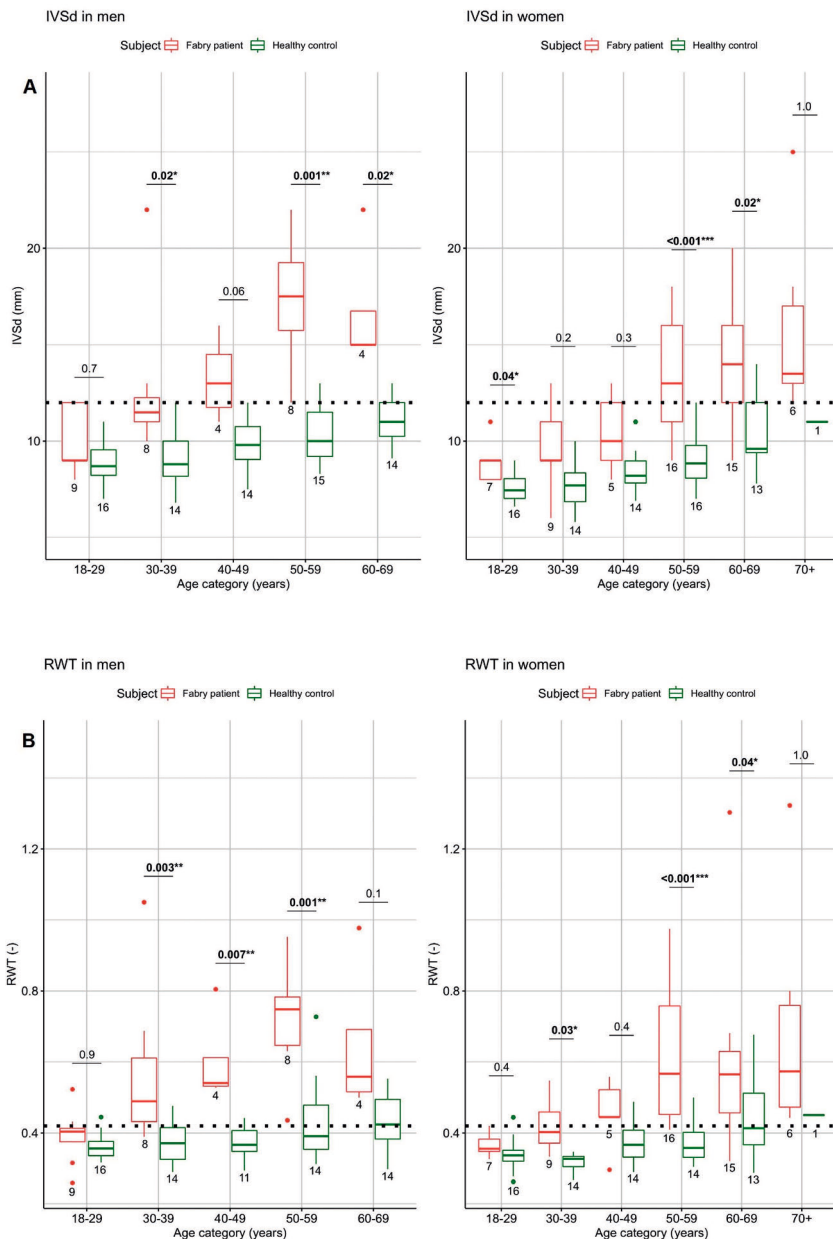
Figure 2: (continued)
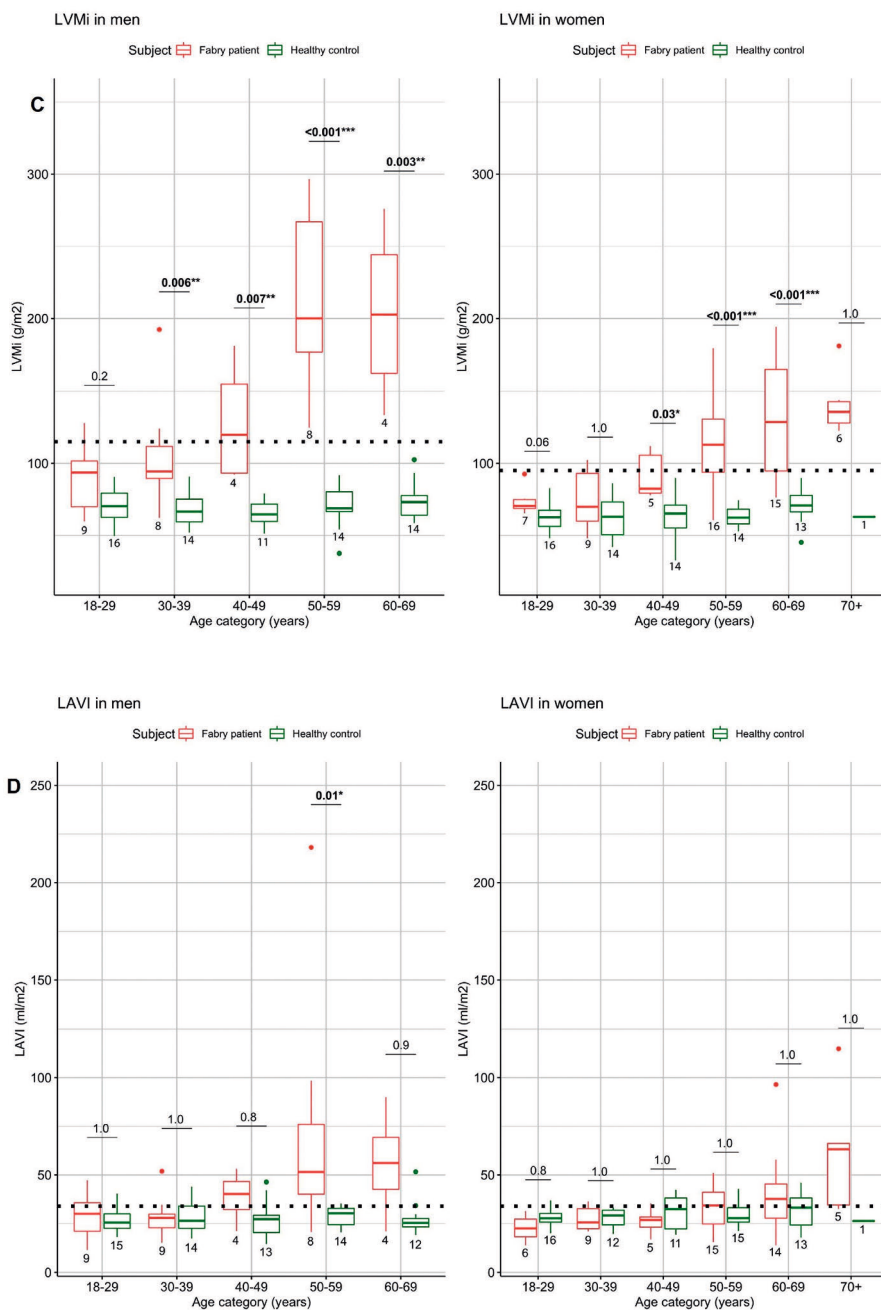
Figure 2: (continued)
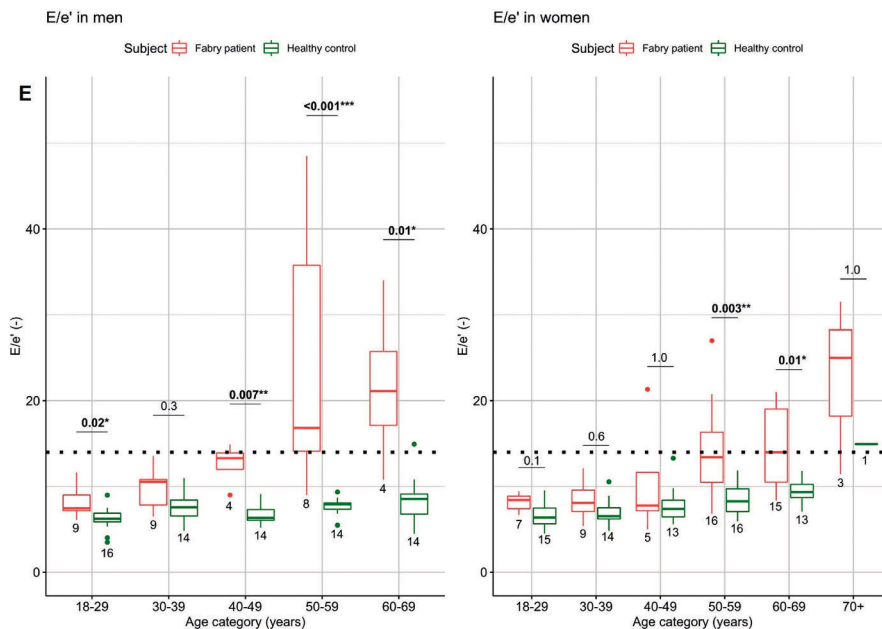
Morphological echocardiographic features

IVSd and RWT

IVSd and RWT increased significantly faster in FD patients compared to healthy control subjects (men: FD minus healthy controls β-IVSd= 1.2 mm per decade; 95%-CI 0.7-1.7, β-RWT= 0.1 per decade; 95%-CI 0.03-0.1, women: FD minus healthy controls β-IVSd= 0.8 mm per decade; 95%-CI 0.3-1.2, β-RWT= 0.1 per decade; 95%-CI 0.02-0.1) **(table 2, figure 1A-1B)**.

The increment in IVSd and RWT over time did not differ between men and women with FD, but male patients showed overall significantly higher IVSd values throughout follow-up **(figure 1A-1B).**

From 30-39 years onwards, the absolute values for IVSd and RWT in men with FD became significantly higher compared to healthy men **(figure 2)**. Women with FD showed a significantly higher value for IVSd from 18-29 years onwards as compared to healthy women, whilst this occurred a decade later for RWT. In men with FD, values increased to levels above references ranges (RWT> 0.42 and IVSd> 12 mm) from 30 years (RWT) and 40 years (IVSd) onwards, this occurred approximately a decade later in women with FD **(figure 2A-2B, supplemental table 1)**.

LVMi and LAVI

In men and women with FD, LVMi and LAVI increased significantly over time, whilst remaining stable in healthy subjects **(table 2, figure 1C-1D)**.

When comparing men and women with FD, the increase in LVMi was greater in men compared to women (FD: men minus women β=13.5 g/m^2 per decade; 95%-CI 7.8-19.1). The change over time in LAVI did not differ between men and women with FD (FD: men minus women β= 3.2 ml/m^2 per decade; 95%-CI -0.7-7.1), but the value for LAVI was overall higher in FD men compared to women with FD throughout follow-up **(figure 1D)**.

Compared to healthy controls, men with FD had a higher LVMi from 30 years onwards, whilst in women this point was reached from 40 years onwards. The absolute value for LAVI was significantly different in men with FD compared to healthy men from 50-59 years onwards. For women the absolute values for LAVI were not significantly different between FD patients and healthy control subjects **(figure 2D, supplemental table 1)**.

LVMi showed median values above the normal range from age 40 years onwards in men with FD and from age 50 years onwards in women with FD. Values outside the reference range for LAVI (> 34 ml/m^2) were present approximately ten years later **(figure 2C-2D, supplemental table 1)**.

Functional echocardiographic features

E/e' increased considerably with time in men and women with FD, in contrast to healthy individuals in whom E/e' was similar at all ages **(table 2) (**men: FD minus healthy controls β= 2.7 per decade; 95%- CI 1.6-3.7 and women: FD minus healthy controls β= 1.3 per decade; 95%-CI 0.4-2.2).

The increment in E/e' over time was greater in men with FD compared to women with FD (FD: men minus women β= 1.1 per decade; 95%-CI 0.2-2.0) **(figure 1E)**.

When comparing age subgroups, the absolute values for E/e' in men with FD are significantly higher as compared to healthy control subjects throughout adult life, while this parameter differs significantly between women with FD compared to healthy control subjects from 50 years onwards. Values for E/e' increase to levels outside of the relevant references range (> 14) [27] in men with FD from the age of 50 years onwards, while this occurs approximately a decade later in women with FD **(figure 2E, supplemental table 1)**.

Grading of the LV diastolic function was possible for 84 out of 92 FD patients (91%) using the last available echocardiograms (echocardiography images

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incomplete for 8 patients). Patients with FD showed LV diastolic dysfunction mainly from 40 years of life onwards and the proportion of patients with diastolic dysfunction increased over the following decades **(figure 3).**

Both men and women with FD showed an increase in GLS evolution over time. Though formal comparison is not possible because of the use of different measurement algorithm in healthy control subjects and FD patients, GLS seems to increase more rapidly with ageing in FD patients compared to healthy control subjects **(supplemental figure 5, supplemental table 3).**

Data on other echocardiographic features (biplane LV EF, TAPSE, SoV diameter and TR Vmax) can be found in the **supplemental results** section and **supplemental figure 3-4.**
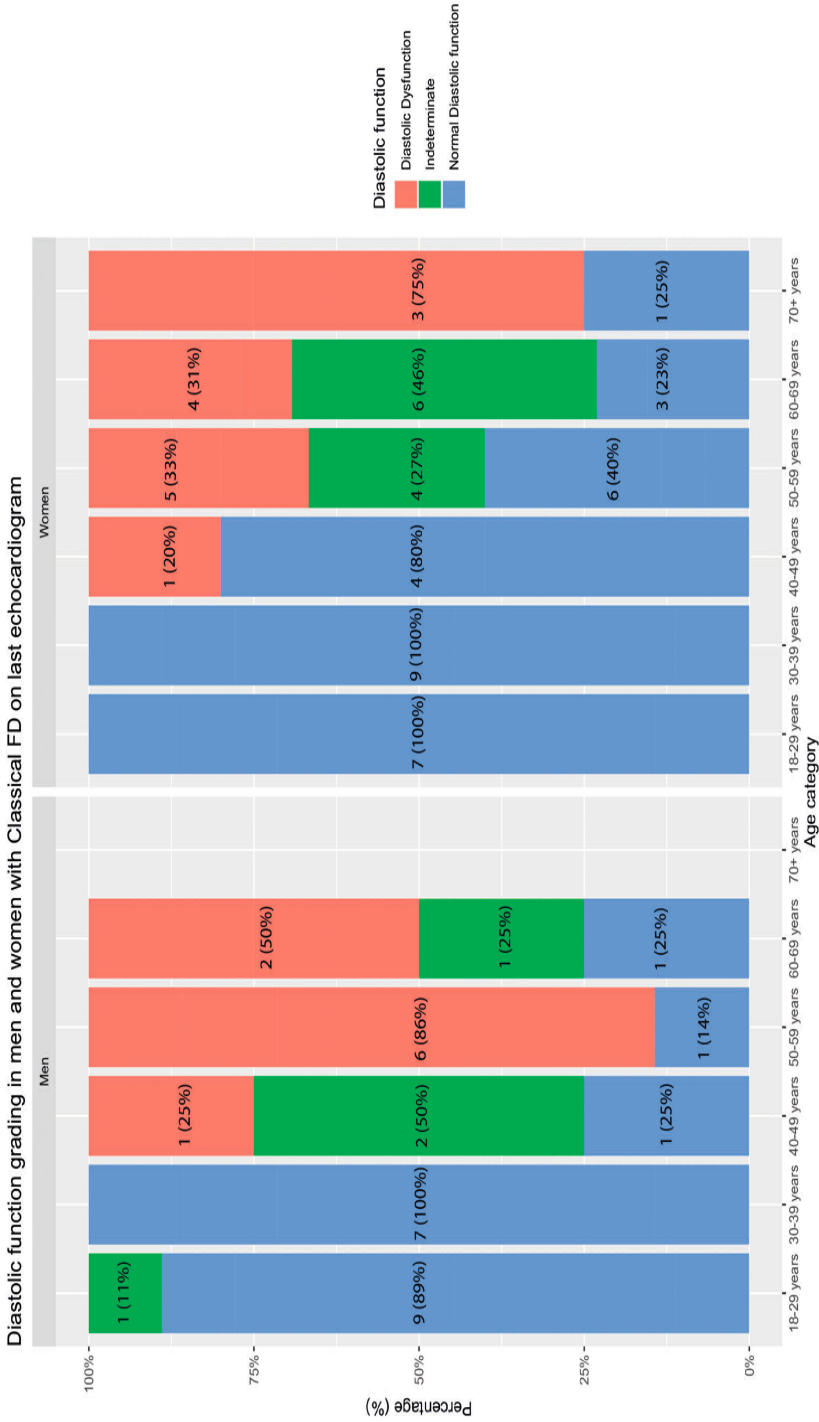
Figure 3: Stacked barplots representing the determination of LV diastolic function in Classical FD patients at different age categories, based on the algorithm proposed by the ESC Guidelines in 2016 [27].

Relationship of echocardiographic features and cardiac enzymes in FD patients

164 NT-plasma NT-proBNP and 111 hs-TnT values were available to be linked to data from the concomitant echocardiograms. There was a significant but moderate correlation (R= between 0.50- 0.69) between the height of the plasma NT-proBNP and hs-TnT levels and LVMi, E/e' and GLS values **(supplemental figure 6)**.

Echocardiographic features and development of atrial fibrillation in FD patients

Seven patients developed a MACE after the first echocardiography, this number was too small to study the relationship between the echocardiographic markers and MACE. Fourteen patients developed AF after the first echocardiogram. For all the 5 parameters analyzed a higher value was associated with AF (Hazard ratio's (HR) between 1.02 and 1.50). The strongest relation was for RWT and IVSd: patients with a higher RWT on the first echocardiogram had a HR of 1.50 (95%CI: 1.25- 1.80) for the development of AF, for a higher IVSd this was 1.31 (95%CI: 1.15-1.49). See **supplemental table 4** for the results on the Cox regression analyses.

Discussion

This is the first long-term longitudinal study that assessed the evolution of echocardiographic features during adult life in classical FD patients versus healthy control subjects. It shows that the progressivecourse of the disease can be documented with serial echocardiography. For early detection of cardiac disease in FD patients three markers appear particularly useful: the morphological markers RWT and IVSd and the functional marker E/e'. RWT is higher in both male and female FD patients from the age of 30 years onwards and potentially even earlier, but the number of patients aged 18-29 years old was too small to confirm this.

It is noteworthy to emphasize that RWT and IVSd in early adulthood were only higher in FD patients when compared to age and sex control specific ranges, not when compared to the usual reference values. Thus, the use of age and sex specific reference ranges is key for detecting the early, subtle changes in these parameters **(supplemental table 1)**.

E/e', an echocardiographic parameter of left atrial pressure, is a particularly relevant marker of early disease in male patients with classical FD, in whom it is already significantly increased in the first decade of adult life. E/e' is significantly associated with both plasma NT-proBNP and troponin T, showing its potential

relevance as a marker of myocardial dysfunction in FD. IVSd, RWT and E/e' at baseline were associated with the development of atrial fibrillation later on, showing the relationship of these markers to the development of clinical cardiac disease. It is possible to use the HRs of the Cox regression analysis to estimate the risk of developing AF per decade for each patient subgroup **(supplemental table 4)**. For example, IVSd increases by 1.8 mm per decade in men with FD **(table 2)**, which means that the atrial fibrillation HR for this increase is 1.6 (exp(1.8*0.27)).

In addition to evaluating single values of these three parameters, progression over time can also be useful to determine which patients are at risk for the development of clinical cardiac disease later on (see **figure 1** and **supplemental figure 2**). This is especially relevant in female patients, since in contrast to male classical FD patients, they do not all develop symptomatic disease and thus not always require treatment [6, 8]. The European Fabry Working Group recommends starting ERT with an IVSd above 12 mm in women with classical FD [31]. The current study shows that that threshold, based on general reference values, is reached relatively late in the disease development in many women (see figure 2B). Yet when female patients were compared to aged matched control subjects, much lower values for IVSd were significantly different, at a much younger age (figure 2B). In addition, not only abnormal values for IVSd, but also for RWT, as well as the rate of change for LVMi, LAVI and E/e' (if longitudinal data are available) can be used to detect early cardiac disease development. We therefore suggest to amend the guideline, using a composite score for early cardiac disease, based on the Z scores of the Fabry patients for each parameter when compared to the reference range of the age and sex matched control group (choosing a cut off of for example plus or minus 1.5). In addition to the Z scores for the echocardiography markers, those for ECG parameters could also be included [32]. This composite score should ideally be validated as valid for prognostication of clinically relevant cardiac disease in a second FD patient cohort. The first limitation of the current study is the fact that the data presented in this study come from patients treated with enzyme replacement therapy (92% of the cohort) for at least a part of the follow- up. Though there is clear progression of cardiac disease in this cohort, this may only partially represent the natural disease course. Because of the wide range in patient ages and disease stage at which ERT was initiated in the current patient cohort, the effect of early treatment with ERT cannot be assessed using this data set. However, the current dataset provides a good benchmark for future therapy assessment, for both earlier (e.g. during adolescence) initiation of current therapies in those at risk, as well as for new therapies.

One of the major problems with evaluating the effect of treatment on cardiac manifestations in FD is that cardiac complications (e.g. arrhythmia, heart failure) occur relatively late in the disease course. In men with classical FD

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this occurs mainly from the fifth decade onwards and for women from the sixth decade onwards [8, 33]. At the same time, there is evidence that early treatment (as early as dolescence in male patients with classical FD) is needed to prevent development of disease manifestations [10, 34]. Thus, evaluation of therapeutic effectiveness based on clinical cardiac endpoints can only be done after approximately two decades of treatment. In this study we show that serial measurements of echocardiographic parameters reflecting both morphological and functional changes can be used to determine whether or not new treatments prevent cardiac disease development. IVSd, RWT, LVMi, LAVI and E/e' all show a greater increase in men and women with FD compared to healthy control subjects. The goal of new therapies should be to normalize the rate of change of the parameters to that of healthy control subjects.

A second limitation of the current study is the fact that the echocardiograms of the FD patients and the healthy control subjects have been performed on different ultrasound systems and obtained by different technicians in two different centers. In addition, the calculated slopes cannot be used to predict the exact course for the different echocardiography parameters in individual FD patients, because of the non-uniform linear course of some of the parameters and, the retrospective nature of the study prevented the analysis of some of the parameters on the older images. Lastly, the analysis examining the association between echo markers and future AF development did not correct for the age of AF development because the number of events was too low to allow for multiple determinants in the model. Patients who developed AF were significantly older than patients who did not (49.7 vs 33.6 years), which represents the effect of age, but also an effect of longer exposure to the genetic condition.

Conclusion

In FD, echocardiography parameters reflecting LV and atrial morphology and LV diastolic function can be used to document cardiac disease progression over time. In the first decades of adult life, the absolute values for IVSd and RWT in all classical FD patients and E/e' in male patients are significantly different from those of healthy individuals. During adult life IVSd, RWT, LVMi, LAVI and E/e' increase at a higher rate in FD patients compared to healthy individuals. Assessing the absolute values (compared to a matched control cohort) and increment of these echocardiographic features can be used to determine the presence of early cardiac involvement, monitor disease progression, estimate the risk of development of atrial fibrillation and evaluate the effect of new and/ or earlier therapeutic interventions.

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SUPPLEMENTAL MATERIAL

Supplemental results

Biplane LV EF and TAPSE
Between 50-60 years, a minority of the patients with FD (4/31 (13%) men and 4/53 (8%) women) developed on the last echocardiogram a LV EF< 50%. For the gross majority of FD patients, TAPSE remained within the normal range (>1.6 cm). On the last echocardiography, 2/31 (7%) men with FD had an abnormal TAPSE after 40 years, while women with FD developed these abnormalities at a comparable frequency (5/57 (9%)) about 10 years later. See **supplemental figure 3 and supplemental table 3** for the detailed data visualization and descriptives, respectively.

SoV diameter and TR Vmax (only assessed in FD patients)
A slight increase in SoV diameter over the ages was observed throughout the follow-up in both men and women. Values for SoV diameter increase to levels outside of the relevant references range (> 40 mm in men and >36 mm in women) after 20 years in men and after 40 years in women. The TR Vmax did not show a clear increasing or decreasing trend in time, wherein the majority of the FD patients also revealed a normal TR V max (<2.8 cm/s), even at an older age. See **supplemental figure 4 and supplemental table 3** for the detailed data visualization and descriptives.

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Supplemental table 1: Echocardiographic parameters' descriptives per age decade

IVSd in men (cm)

	Fabry men		Healthy men		
Age category	Number of observations	Result (IQR)	Number of observations	Result (IQR)	P- value
18-29	9	0.9 (0.9-1.2)	16	0.9 (0.8-1.0)	0.7
30-39	8	1.2 (1.1-1.2)	14	0.9 (0.8-1.0)	**0.02***
40-49	4	1.3 (1.2-1.5)	14	1.0 (0.9-1.1)	0.06
50-59	8	1.8 (1.6-1.9)	15	1.0 (0.9-1.2)	**0.001****
60-69	4	1.5 (1.5-1.7)	14	1.1 (1.0-1.2)	**0.02***
70+	-	-	-	-	-

RWT in men (-)

	Fabry men		Healthy men		
Age category	Number of observations	Result (IQR)	Number of observations	Result (IQR)	P- value
18-29	9	0.40 (0.38-0.41)	16	0.36 (0.34-0.38)	0.9
30-39	8	0.49 (0.43-0.61)	14	0.37 (0.33-0.42)	**0.003****
40-49	4	0.54 (0.53-0.61)	11	0.37 (0.35-0.41)	**0.007****
50-59	8	0.75 (0.65-0.78)	14	0.39 (0.35-0.47)	**0.001****
60-69	4	0.56 (0.52-0.69)	14	0.42 (0.38-0.50)	0.1
70+	-	-	-	-	-

LVMi in men (g/m^2)

	Fabry men		Healthy men		
Age category	Number of observations	Result (IQR)	Number of observations	Result (IQR)	P- value
18-29	9	94 (70-102)	16	70 (63-79)	0.2
30-39	8	94 (90-112)	14	67 (59-75)	**0.006****
40-49	4	120 (93-155)	11	65 (60-72)	**0.007****
50-59	8	200 (177-267)	14	69 (67-80)	**<0.001*****
60-69	4	203 (162-244)	14	73 (64-78)	**0.003****
70+	-	-	-	-	-

LAVI in men (ml/m^2)

	Fabry men		Healthy men		
Age category	Number of observations	Result (IQR)	Number of observations	Result (IQR)	P- value
18-29	9	30 (21-36)	15	26 (23-30)	1.0
30-39	9	28 (23-30)	14	27 (23-34)	1.0
40-49	4	40 (32-47)	13	27 (21-29)	0.8
50-59	8	52 (40-76)	14	30 (25-33)	**0.01***
60-69	4	56 (43-69)	12	25 (23-28)	0.9
70+	-	-	-	-	-

E/e' in men (-)

	Fabry men		Healthy men		
Age category	Number of observations	Result (IQR)	Number of observations	Result (IQR)	P- value
18-29	9	8 (7-9)	16	6 (6-7)	**0.02***
30-39	9	11 (8-11)	14	8 (7-8)	0.3
40-49	4	13 (12-14)	14	6 (6-7)	**0.007****
50-59	8	17 (14-36)	14	8 (7-8)	**<0.001*****
60-69	4	21 (17-26)	14	9 (7-9)	**0.01***
70+	-	-	-	-	-

*$p<0.05$, ** $p<0.01$, ***$p<0.001$*

IVSd in women (cm)

Age category	Number of observations	Result (IQR)	Number of observations	Result (IQR)	P- value
	Fabry women		**Healthy women**		
18-29	7	0.9 (0.8-0.9)	16	0.8 (0.7-0.8)	**0.04***
30-39	9	0.9 (0.9-1.1)	14	0.8 (0.7-0.8)	0.2
40-49	5	1.0 (0.9-1.2)	14	0.8 (0.8-0.9)	0.3
50-59	16	1.3 (1.1-1.6)	16	0.9 (0.8-1.0)	**<0.001***
60-69	15	1.4 (1.2-1.6)	13	1.0 (0.9-1.2)	**0.02***
70+	6	1.4 (1.3-1.7)	1	1.1 (-)	1.0

RWT in women (-)

Age category	Number of observations	Result (IQR)	Number of observations	Result (IQR)	P- value
	Fabry women		**Healthy women**		
18-29	7	0.36 (0.35-0.38)	16	0.34 (0.32-0.35)	0.4
30-39	9	0.40 (0.34-0.45)	14	0.33 (0.31-0.33)	**0.03***
40-49	5	0.44 (0.44-0.52)	14	0.37 (0.33-0.41)	0.4
50-59	16	0.57 (0.45-0.76)	14	0.36 (0.33-0.40)	**<0.001***
60-69	15	0.57 (0.46-0.63)	13	0.41 (0.37-0.51)	**0.04***
70+	6	0.57 (0.47-0.76)	1	0.45 (-)	1.0

LVMi in women (g/m²)

Age category	Number of observations	Result (IQR)	Number of observations	Result (IQR)	P- value
	Fabry women		**Healthy women**		
18-29	7	71 (69-75)	16	63 (56-68)	0.06
30-39	9	70 (60-93)	14	63 (51-73)	1.0
40-49	5	82 (79-106)	14	65 (55-71)	**0.03***
50-59	16	113 (94-131)	14	62 (58-68)	**<0.001***
60-69	15	129 (95-165)	13	71 (66-78)	**<0.001***
70+	6	136 (128-143)	1	63 (-)	1.0

LAVI in women (ml/m²)

Age category	Number of observations	Result (IQR)	Number of observations	Result (IQR)	P- value
	Fabry women		**Healthy women**		
18-29	6	23 (18-27)	16	28 (26-30)	0.8
30-39	9	25 (21-32)	12	29 (24-32)	1.0
40-49	5	27 (23-29)	11	33 (22-38)	1.0
50-59	15	34 (25-41)	15	28 (26-33)	1.0
60-69	14	38 (28-45)	13	33 (24-38)	1.0
70+	5	63 (35-66)	1	26 (-)	1.0

E/e' in women (-)

Age category	Number of observations	Result (IQR)	Number of observations	Result (IQR)	P- value
	Fabry women		**Healthy women**		
18-29	7	8 (7-9)	15	6 (6-8)	0.1
30-39	9	8 (7-10)	14	7 (6-8)	0.6
40-49	5	8 (7-12)	13	7 (7-8)	1.0
50-59	16	13 (11-16)	16	8 (7-10)	**0.003****
60-69	15	14 (11-19)	13	9 (9-10)	**0.01***
70+	3	25 (18-28)	1	15 (-)	1.0

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Supplemental table 2A: GLM of the echocardiographic morphological parameters

	IVSd (mm)			RWT (-)			LVMi (g/m²)			LAVI (ml/m²)		
Fixed effects	β	(95% CI)	p value	β	(95% CI)	p value	β	(95% CI)	p value	β	(95% CI)	p value
Model 1 (age, subject type and sex separated)												
Age	0.09	0.07 – 0.11	<0.001	0.004	0.003 – 0.005	<0.001	0.51	0.21 – 0.80	0.001	0.30	0.16 – 0.44	<0.001
Type (FD patient)	2.39	1.77 – 3.01	<0.001	0.08	0.05 – 0.11	<0.001	33.13	26.31 – 39.95	<0.001	0.10	-2.90 – 3.09	0.950
Sex (Male)	1.17	0.57 – 1.78	<0.001	0.02	-0.01 – 0.05	0.110	11.68	4.40 – 18.95	0.002	2.29	-0.71 – 5.28	0.134
Model 2 (healthy and FD patients combined groups- with age*subject type*sex interactions)												
(Intercept)	5.41	3.84 – 6.99	<0.001	0.26	0.18 – 0.34	<0.001	58.81	40.29 – 77.34	<0.001	25.88	15.06 – 36.71	
Age	0.07	0.03 – 0.11	<0.001	0.002	0.0002 – 0.004	0.027	0.13	-0.30 – 0.56	0.549	0.09	-0.18 – 0.36	0.528
Type (FD patient)	-0.50	-2.45 – 1.46	0.616	-0.09	-0.19 – 0.01	0.081	-14.07	-37.02 – 8.89	0.229	-18.14	-32.39 – -3.88	0.013
Sex (Male)	1.88	-0.31 – 4.08	0.092	0.04	-0.08 – 0.15	0.497	7.11	-18.72 – 32.94	0.588	1.42	-13.74 – 16.57	0.854
Interactions												
Age*Type (FD patient)	0.08	0.03 – 0.12	0.001	0.01	0.002 – 0.01	<0.001	1.14	0.60 – 1.68	<0.001	0.40	0.04 – 0.76	0.030
Age*Sex (Male)	-0.01	-0.06 – 0.04	0.668	-0.0002	-0.003 – 0.002	0.854	-0.05	-0.65 – 0.56	0.881	-0.08	-0.46 – 0.31	0.690
Type (FD patient)*Sex (Male)	-1.01	-3.85 – 1.84	0.486	-0.03	-0.18 – 0.12	0.679	-20.19	-53.58 – 13.20	0.235	-4.49	-25.44 – 16.45	0.673
Age*Type (FD patient)*Sex (Male)	0.04	-0.028 – 0.11	0.240	0.0016	-0.002 – 0.006	0.402	1.39	0.56 – 2.22	0.001	0.40	-0.15 – 0.94	0.153

Supplemental table 2B: GLM of the echocardiographic functional parameters

E/e' (-)			
Model 1 (age, subject type and sex separated)			
Fixed effects	β	(95% CI)	p value
Age	0.12	0.08 – 0.16	<0.001
Type (FD patient)	2.88	1.97 – 3.78	<0.001
Sex (Male)	-0.60	-1.54 – 0.34	0.210
Model 2 (healthy and FD patients combined groups- with age*subject type*sex interactions)			
(Intercept)	4.59	1.71 – 7.47	
Age	0.07	0.00 – 0.14	0.038
Type (FD patient)	-1.21	-4.80 – 2.37	0.506
Sex (Male)	0.74	-3.24 – 4.72	0.715
Interactions			
Age*Type (FD patient)	0.13	0.04 – 0.22	0.005
Age*Sex (Male)	-0.03	-0.13 – 0.07	0.560
Type (FD patient)*Sex (Male)	-3.77	-8.95 – 1.42	0.154
Age*Type (FD patient)*Sex (Male)	0.14	0.00 – 0.27	0.044

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Supplemental table 3A: other echocardiographic morphological and functional parameters' descriptives

LVPWd in men (cm)

Age category	Fabry men		Healthy men	
	Number of observations	Result	Number of observations	Result
18-29	9	1.0 (0.9-1.0)	16	0.8 (0.8-0.8)
30-39	8	1.1 (1.0-1.2)	14	0.8 (0.7-0.9)
40-49	4	1.4 (1.2-1.6)	11	0.7 (0.7-0.8)
50-59	8	1.9 (1.5-2.0)	15	0.8 (0.8-1.0)
60-69	4	1.6 (1.4-1.7)	14	0.9 (0.7-0.9)
70+	-	-	-	-

LVPWd in women (cm)

Age category	Fabry women		Healthy women	
	Number of observations	Result	Number of observations	Result
18-29	7	0.8 (0.8-0.8)	16	0.8 (0.7-0.8)
30-39	9	0.8 (0.7-1.0)	14	0.7 (0.7-0.8)
40-49	5	1.1 (1.0-1.1)	14	0.8 (0.7-0.8)
50-59	16	1.2 (1.0-1.5)	14	0.7 (0.7-0.8)
60-69	15	1.2 (1.0-1.4)	13	0.8 (0.7-0.9)
70+	6	1.3 (1.1-1.4)	1	0.7 (-)

LVEDd in men (cm)

Age category	Fabry men		Healthy men	
	Number of observations	Result	Number of observations	Result
18-29	9	5.1 (4.6-5.4)	16	4.7 (4.6-4.9)
30-39	8	4.7 (4.1-4.8)	14	4.8 (4.4-5.0)
40-49	4	4.4 (4.2-4.8)	14	4.7 (4.5-4.8)
50-59	8	4.8 (4.6-5.2)	14	4.6 (4.4-4.9)
60-69	4	5.0 (4.7-5.4)	14	4.5 (4.3-4.7)
70+	-	-	-	-

LVEDd in women (cm)

Age category	Fabry women		Healthy women	
	Number of observations	Result	Number of observations	Result
18-29	7	4.6 (4.5-4.8)	16	4.5 (4.3-4.7)
30-39	9	4.5 (4.2-4.9)	14	4.6 (4.4-4.9)
40-49	5	4.5 (4.5-4.6)	14	4.4 (4.2-4.7)
50-59	16	4.1 (4.0-4.6)	16	4.5 (4.4-4.5)
60-69	15	4.7 (4.5-4.7)	13	4.3 (3.9-4.5)
70+	6	4.6 (4.1-4.9)	1	4.0 (-)

Septal e' velocity in men (cm/s)

Age category	Fabry men		Healthy men	
	Number of observations	Result	Number of observations	Result
18-29	9	12 (11-13)	16	12 (11-13)
30-39	9	11 (7-12)	14	10 (9-11)
40-49	4	6 (5-7)	14	9 (8-10)
50-59	8	4 (3-6)	14	8 (7-8)
60-69	4	4 (4-6)	14	8 (6-9)
70+	-	-	-	-

Septal e' velocity in women (cm/s)

Age category	Fabry women		Healthy women	
	Number of observations	Result	Number of observations	Result
18-29	7	13 (12-14)	15	13 (12-14)
30-39	9	10 (10-12)	14	10 (10-12)
40-49	5	9 (8-13)	13	9 (8-10)
50-59	16	6 (4-7)	16	9 (7-10)
60-69	15	6 (4-8)	13	7 (5-8)
70+	3	5 (4-6)	1	4 (-)

Supplemental table 3A: other echocardiographic morphological and functional parameters' descriptives (continued)

E/A ratio in men (-)

Age category	Fabry men		Healthy men	
	Number of observations	Result	Number of observations	Result
18-29	9	2.1 (1.5-2.1)	16	1.9 (1.7-2.2)
30-39	9	1.5 (1.3-1.7)	14	1.8 (1.6-2.2)
40-49	4	1.1 (0.9-1.2)	14	1.4 (1.3-1.7)
50-59	8	1.7 (1.0-2.7)	15	1.0 (0.9-1.3)
60-69	3	2.8 (2.0-3.0)	14	1.0 (0.8-1.1)
70+	-	-	-	-

E/A ratio in women (-)

Age category	Fabry women		Healthy women	
	Number of observations	Result	Number of observations	Result
18-29	7	1.9 (1.8-2.0)	16	2.5 (2.0-2.9)
30-39	9	1.8 (1.6-2.2)	14	1.8 (1.5-2.1)
40-49	5	1.4 (1.2-1.4)	13	1.3 (1.2-1.4)
50-59	14	1.2 (0.9-1.3)	16	1.2 (1.1-1.5)
60-69	15	1.1 (0.8-1.4)	13	1.0 (0.7-1.2)
70+	3	2.5 (1.6-3.9)	1	0.6 (-)

GLS in men (%)

Age category	Fabry men		Healthy men	
	Number of observations	Result	Number of observations	Result
18-29	9	-18 (-17,-19)	15	-20 (-19, -21)
30-39	6	-17 (-16,-18)	14	-20 (-20, -22)
40-49	2	-15 (-16,-14)	14	-20 (-19, -21)
50-59	7	-9 (-8,-14)	14	-20 (-19, -22)
60-69	4	-13 (-11,-15)	12	-19 (-19, -21)
70+	-	-	-	-

GLS in women (%)

Age category	Fabry women		Healthy women	
	Number of observations	Result	Number of observations	Result
18-29	5	-21 (-21,-22)	16	-21 (-21, -22)
30-39	9	-22 (-20,-22)	14	-22 (-21, -24)
40-49	3	-18 (-17,-20)	12	-22 (-19, -23)
50-59	13	-17 (-20,-12)	16	-22 (-21, -23)
60-69	11	-17 (-13,-19)	13	-21 (-19, -22)
70+	4	-13 (-11,-13)	1	-19 (-)

Biplane LV EF in men (%)

Age category	Fabry men		Healthy men	
	Number of observations	Result	Number of observations	Result
18-29	9	54 (53-59)	15	61 (57-65)
30-39	7	58 (56-63)	14	60 (60-62)
40-49	4	59 (55-64)	14	59 (57-60)
50-59	7	52 (45-58)	14	60 (56-64)
60-69	4	55 (50-56)	13	55 (55-59)
70+	-	-	-	-

Biplane LV EF in women (%)

Age category	Fabry women		Healthy women	
	Number of observations	Result	Number of observations	Result
18-29	6	56 (54-58)	16	59 (58-61)
30-39	9	64 (62-67)	14	64 (57-68)
40-49	4	57 (56-60)	13	60 (58-63)
50-59	14	67 (65-71)	16	64 (61-67)
60-69	14	57 (56-64)	13	61 (58-62)
70+	6	54 (49-61)	1	65 (-)

4

Supplemental table 3A: other echocardiographic morphological and functional parameters' descriptives (continued)

| | TAPSE in men (cm) | | | | | TAPSE in women (cm) | | | |
| | **Fabry men** | | **Healthy men** | | | **Fabry women** | | **Healthy women** | |
Age category	Number of observations	Result	Number of observations	Result	Age category	Number of observations	Result	Number of observations	Result
18-29	8	2.5 (2.3-2.9)	16	2.7 (2.5-3.0)	18-29	7	2.6 (2.3-2.7)	16	2.6 (2.3-2.8)
30-39	8	2.4 (2.2-2.6)	14	2.7 (2.6-2.9)	30-39	9	2.6 (2.5-2.9)	14	2.7 (2.5-2.9)
40-49	4	2.2 (2.1-2.4)	14	2.5 (2.3-2.6)	40-49	5	2.4 (2.2-2.4)	11	2.4 (2.2-2.7)
50-59	7	2.0 (1.4-2.2)	15	2.4 (2.2-2.6)	50-59	16	2.4 (2.0-2.7)	16	2.7 (2.7-2.9)
60-69	4	2.1 (2.0-2.3)	14	2.6 (2.2-2.8)	60-69	14	2.2 (1.7-2.5)	13	2.7 (2.4-2.9)
70+	-	-	-	-	70+	6	1.7 (1.4-2.0)	1	2.1 (-)

Results are displayed as median with its Interquartile range (IQR)

Supplemental table 3B: Other echocardiographic morphological and functional parameters' descriptives, that are only available in FD patients

Aortic root diameter- Valsalva (cm)					
Fabry men			**Fabry women**		
Age category	Number of observations	Result	Age category	Number of observations	Result
18-29	9	3.7 (3.5-3.8)	18-29	7	2.8 (2.7-3.0)
30-39	9	4.2 (3.7-4.2)	30-39	9	2.9 (2.7-3.2)
40-49	4	4.1 (3.8-4.5)	40-49	5	2.7 (2.6-2.9)
50-59	8	4.1 (3.8-4.4)	50-59	16	3.5 (3.1-3.8)
60-69	4	3.8 (3.8-4.0)	60-69	15	3.1 (2.9-3.2)
70+	-	-	70+	6	3.5 (3.2-3.6)
TR Vmax (cm/s)					
Fabry men			**Fabry women**		
Age category	Number of observations	Result	Age category	Number of observations	Result
18-29	9	2.2 (2.2-2.4)	18-29	5	2.3 (2.1-2.3)
30-39	7	2.1 (2.1-2.4)	30-39	7	2.3 (2.1-2.3)
40-49	4	2.2 (2.2-2.4)	40-49	4	2.4 (1.9-2.7)
50-59	4	2.2 (2.1-2.3)	50-59	12	2.3 (2.2-2.6)
60-69	4	2.6 (2.5-2.7)	60-69	10	2.5 (2.4-2.7)
70+	-	-	70+	6	2.5 (2.3-2.6)

Results are displayed as median with its Interquartile range (IQR)

Supplemental table 4: Effect of the echocardiographic parameters on the first obtained echocardiogram on Atrial fibrillation during follow-up in FD patients based on a Cox proportional hazard model

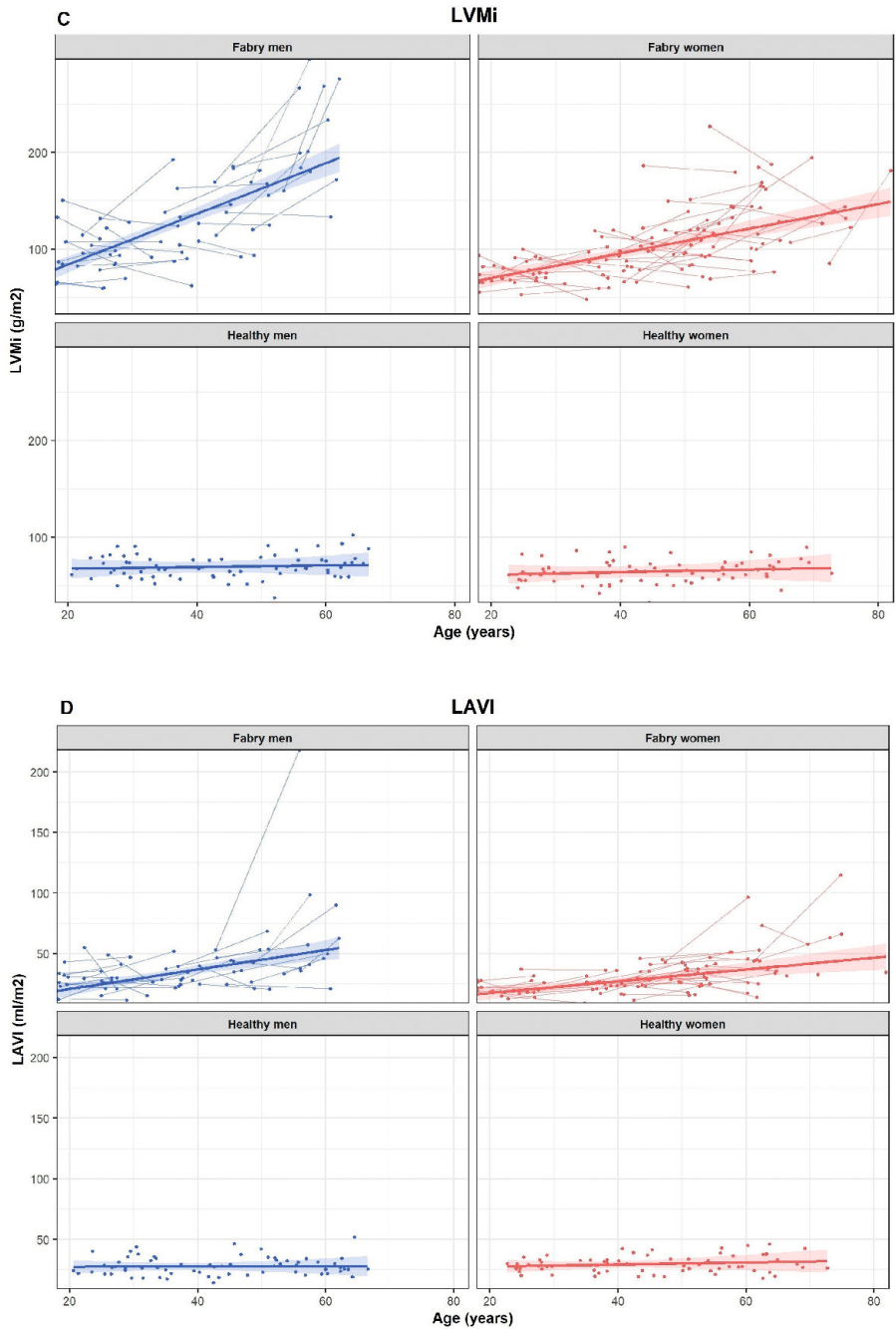| Echocardiographic parameter	β	HR- Atrial fibrillation (exp(β))	95% CI- p value
IVSd	0.27	1.31	1.15-1.49 **(0.00005)*****
RWT	0.41	1.50	1.25-1.80 **(0.00001)*****
LVMi	0.02	1.02	1.01-1.03 **(0.0004)*****
LAVI	0.08	1.08	1.04-1.12 **(0.0003)*****
E/e'	0.18	1.20	1.10-1.32 **(0.0001)*****

*p<0.05, ** p<0.01, ***p<0.001
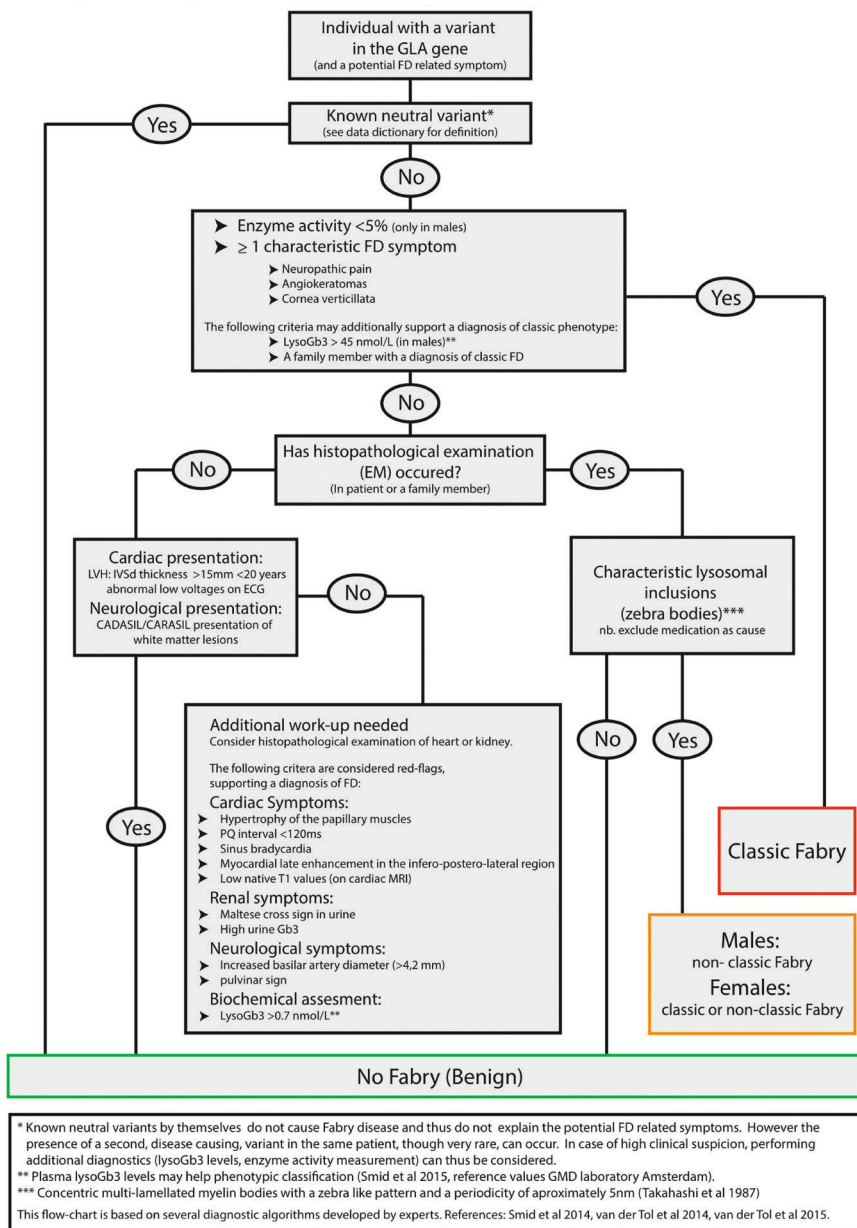
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Supplemental figure 1: Flowchart for the diagnosis and phenotype allocation in FD; cerebral autosomal dominant arteriopathy with subcortical infarcts and leucoencephalopathy (CADASIL); cerebral autosomal recessive arteriopathy with subcortical infarcts and leucoencephalopathy (CARASIL); electron microscopy (EM); galactosidase alpha (GLA); diastolic interventricular septum thickness (IVSd).
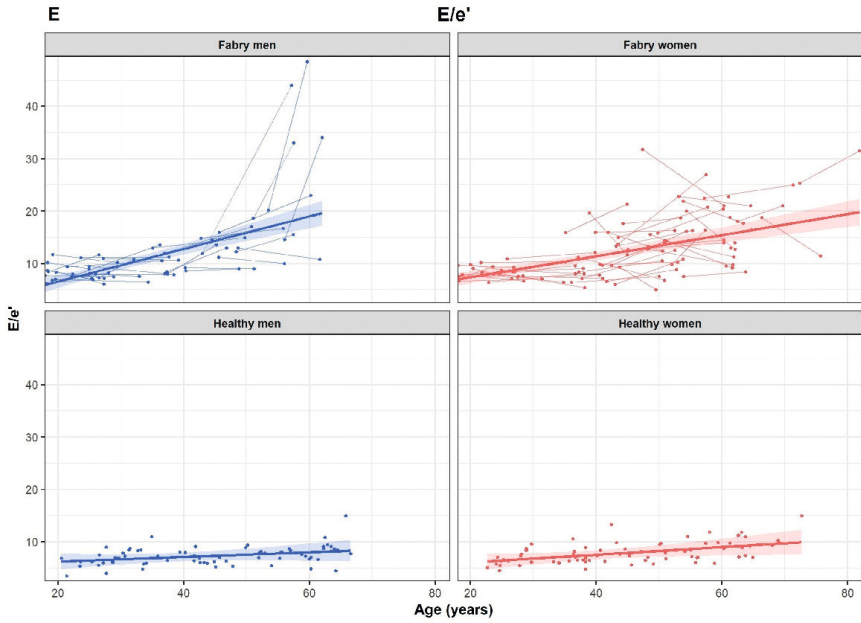
Supplemental figure 2: Source raw data of IVSd, RWT, LVMi, LAVI and E/e' for each study participants subgroup. The shaded areas represent the 95%-CI for the GLM fitted curves.
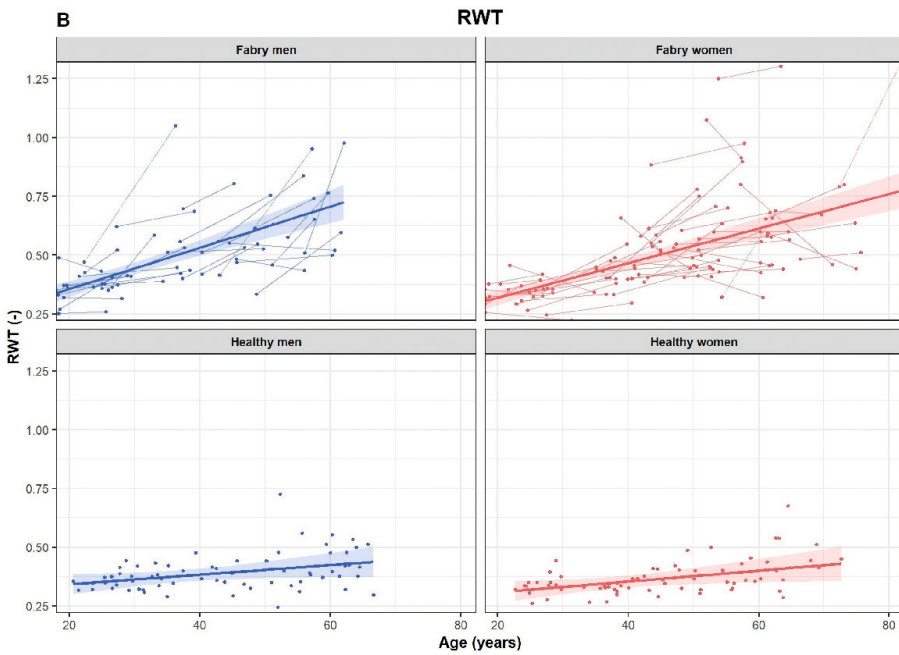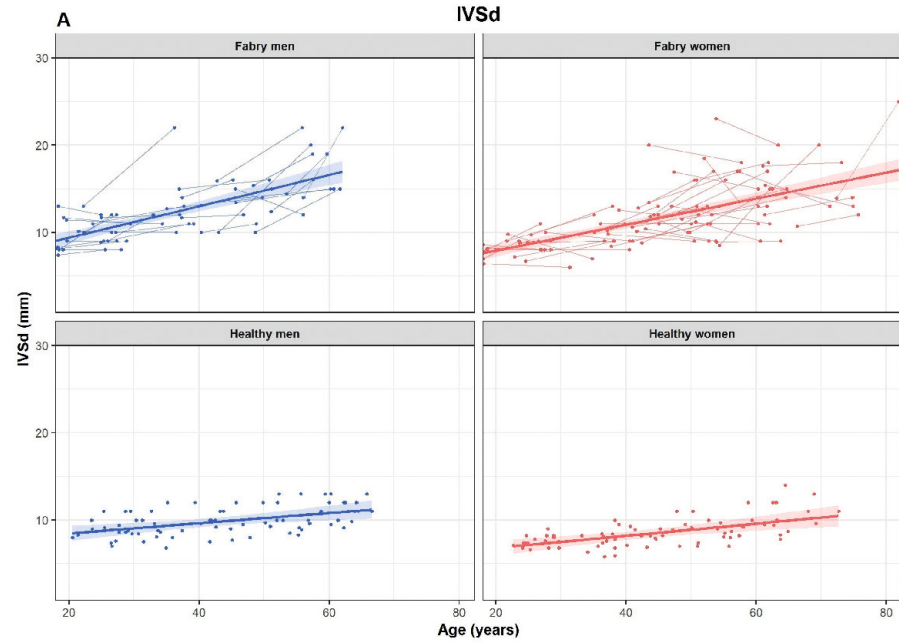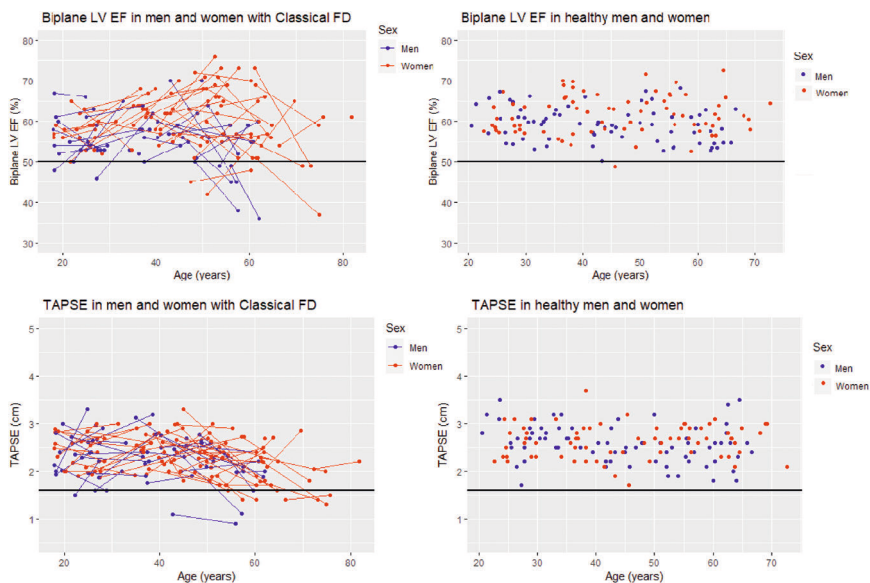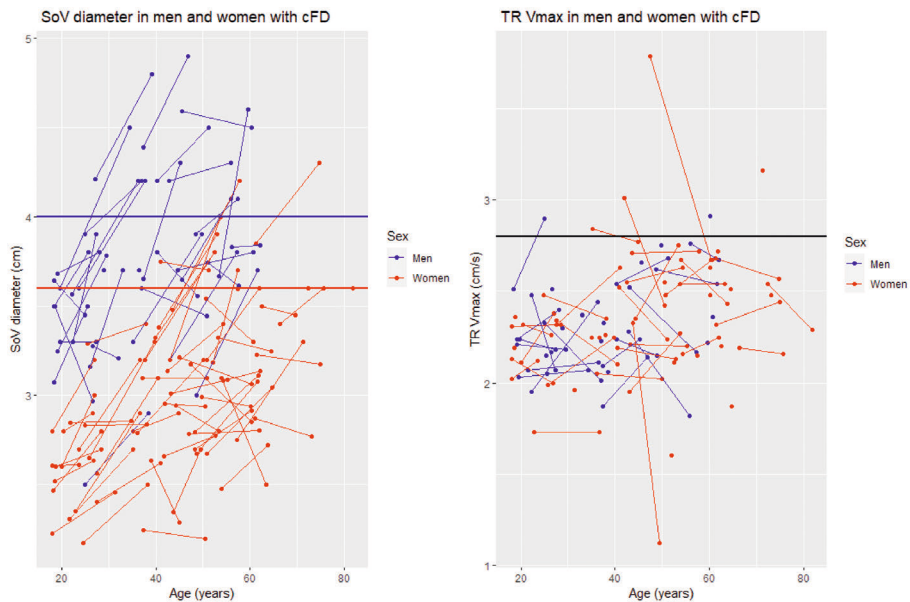
Supplemental figure 2: (continued)

Supplemental figure 2: (continued)

Supplemental figure 3: Source raw data of Biplane LV EF and TAPSE for each study participants subgroup. The horizontal lines represents the reference ranges of each echocardiographic parameter, based on the literature.

Supplemental figure 4: Source raw data of SoV diameter and TR Vmax in men and women with classical FD. The horizontal lines represents the reference ranges of each echocardiographic parameter, based on the literature.

Supplemental figure 5: Scatter plot of GLS with a fitted regression line in FD patients and healthy volunteers. Shaded areas represent the 95%-CI for the fitted curves. The normal value of GLS (-16%, based on the literature) is represented by the horizontal line.
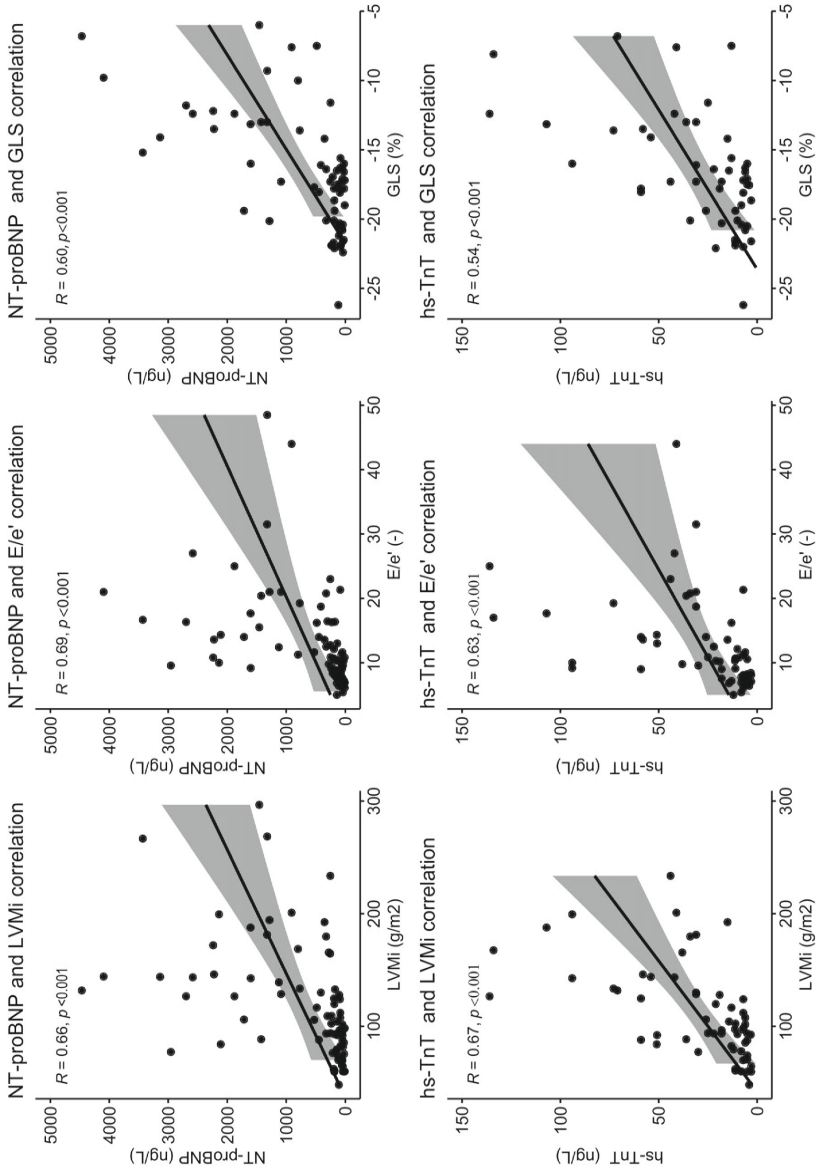
Supplemental figure 6: Scatter plot displaying the Spearman correlation between echocardiographic markers obtained from the last echo and corresponding plasma NT-proBNP and hs-TnT. The shaded areas represent the 95%-CI for the fitted curve.

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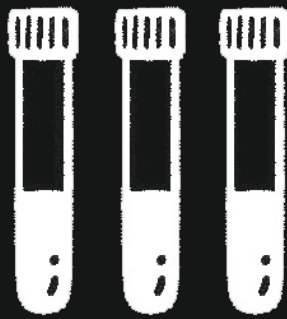
Chapter 5

Early risk stratification for natural disease course in Fabry patients using plasma globotriaosylsphingosine levels

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Significance statement

The phenotypical spectrum of Fabry disease ranges from patients with minimal complications and a normal life expectancy to patients with severe morbidity leading to premature death. Increased genetic screening in milder and asymptomatic individuals vastly increased the number of patients with milder forms of FD and an uncertain disease course. As disease manifestations may take decades to develop and the window of opportunity to start targeted treatment is limited, all diagnosed FD patients are currently under life-long follow up. We found that the biomarker lysoGb3 is stable over time in the plasma of untreated Fabry patients and is significantly associated with disease progression. This enables early risk stratification of patients and could reduce over-medicalization and improve efficiency of clinical care.

Abstract

Background
Fabry disease is a very heterogeneous X-linked lysosomal storage disease. Renal, cardiac and cerebral disease manifestations differ greatly, even between patients of the same sex and with the same disease classification (classical or non-classical). A biomarker with a strong predictive value for the development of disease manifestations is needed to determine the need for Fabry-specific treatment and appropriate frequency of follow-up, since clinical manifestations of the disorder take decennia to develop.

Methods
We investigated the levels of plasma lysoGb3 levels over time and its association with disease - manifestations and -course in 237 untreated FD patients using linear mixed effect models.

Results
LysoGb3 levels are stable over time in plasma of untreated Fabry patients. Higher levels of lysoGb3 were associated with steeper decline in estimated glomerular filtration rate (eGFR, $p=0.04$) and a faster increase in albuminuria (measured as the urinary albumin to creatinine ratio, UACR, $p<0.001$), left ventricular mass (LVMi, measured on echocardiography, $p<0.001$), left atrial volume index (LAVI, $p=0.003$) and Fazekas score ($p=0.003$). Additionally, regardless of age, higher lysoGb3 levels were associated with higher relative wall thickness (RWT, $p<0.001$) and unfavorable functional markers on echocardiography, including septal mitral annular early diastolic velocity (e', $p<0.001$) and the ratio of early trans mitral velocity (E) to e' (E/e', $p=0.001$).

Conclusion
LysoGb3 is a static, individual FD trait with a close relationship to clinical disease severity. Since it reaches stability well before clinical disease manifestations occur, measuring lysoGb3 at diagnosis provides insight into the expected natural disease course, facilitating clinical decision making.

5

Introduction

Fabry disease (FD; OMIM 301500) is a rare X-linked lysosomal storage disease, caused by pathogenic mutations in the alpha-galactosidase A (GLA) gene. Depending on the mutation, FD patients can either have total or partial alpha-galactosidase A (αGalA) deficiency, which roughly corresponds with either a classical (more severe) or a non-classical (attenuated) disease phenotype [1]. Due to the X-linked inheritance, female patients develop a milder disease phenotype compared to male patients and some female patients can remain without clinical events up to the 8th decade of life [1, 2]. Due to the αGalA deficiency, the lipid Globotriaosylceramide (Gb3) cannot be broken down properly and accumulates in the lysosomes of the cell. The intracellular accumulation of Gb3 and its derivatives is thought to set in motion several pathophysiological processes, ultimately resulting in a variety of clinical symptoms. Left ventricular hypertrophy and myocardial fibrosis often occurs and may result in (diastolic) heart failure and arrhythmias [2], podocytes loss and fibrosis in the kidneys can cause proteinuria and renal failure [3, 4] and vascular dysfunction may result in the development of white matter lesions (WMLs), transient ischemic attacks (TIAs) and strokes [5]. Patients with the most severe phenotype (e.g. male patients with a classical disease type), always become symptomatic and seem to benefit from early treatment initiation with enzyme replacement therapy (ERT) [6-8]. Starting ERT in patients with more advanced disease (e.g. with advanced myocardial fibrosis or impaired renal function), no longer seems to alter disease course [9-12]. This supports early treatment initiation. However, while there is no discussion whether or not male patients with classical disease need treatment, the remaining groups contain both patients at risk for complications, as well as those with a (near) normal life expectancy, who will develop no or minimal FD related clinical symptoms [1, 2, 13]. It's unlikely that this last group would significantly benefit from lifelong biweekly ERT infusions. This poses an important clinical dilemma; not all patients will develop clinically relevant symptoms of FD warranting targeted treatment, but those who will become symptomatic, seem to benefit from early treatment initiation [6]. In addition, for patients presenting with new GLA variants and minor and/or nonspecific symptoms, it's not always clear whether the variant is disease causing- or benign, especially in women, in whom enzyme activity can be normal. Therefore, in current clinical practice, many patients are in routine follow up to identify early signs of organ manifestations so that the window of opportunity for treatment will not be missed. In order to reduce over-medicalization of patients and to improve efficiency of clinical care, there is a need for a tool to stratify FD patients by their risk for developing complications.

Levels of Globotriaosylsphingosine (lysoGb3), the water soluble, deacylated form of Gb3, are strongly associated with both disease type (classical or non-classical) and sex of the patient [14, 15]. Additionally, lysoGb3 was found to be associated with several disease severity parameters in small studies, including left ventricular mass [16, 17], white matter lesions [17] and overall disease severity measured using the Mainz Severity Score Index (MSSI) [14, 16, 17]. Interestingly, even in patients that share the same *GLA* variant, those with higher lysoGb3 levels were more severely affected compared to those with lower plasma lysoGb3 levels [16]. In a more recent study, high lysoGb3 levels were linked to an increased risk for the development of clinically relevant endpoints, including kidney replacement therapy, ICD/pacemaker implantation and cerebrovascular events [18]. However, most studies were small, cross sectional and some included both treated and untreated patients. It is currently unknown whether plasma lysoGb3 levels are stable throughout life and represent an individual patient trait that can be assessed at any time, or that it increases/decreases with age. We hypothesized that, in untreated FD patients, plasma lysoGb3 remains stable and reflects how severely an individual FD patient is or will be affected. In this study, we investigated whether or not plasma lysoGb3 levels remained stable over time in untreated FD patients, we defined optimum cut-off values to classify patients (classical vs non-classical) and we used linear mixed models to test the relation of plasma lysoGb3 levels to disease manifestations and progression.

Materials and Methods

Patients

This study was conducted in accordance with the principles of the Helsinki Declaration, as revised in 2013. Data were collected retrospectively from clinical records at the Amsterdam University Medical Center (Amsterdam UMC), location AMC, the national referral center for FD in the Netherlands. All included patients had a confirmed FD diagnosis and all patients and/or legal guardian signed informed consent. Data included basic diagnostic data, clinical and biochemical parameters, medication use, and the presence of comorbidities and cardiovascular risk factors. The risk factor 'Smoking' was scored as present in case of current or former smoking. Obesity was defined as a body mass index >30kg/m^2 at time of lysoGb3 analyses. Hypertension was defined as either a previous diagnosis of hypertension treated with anti-hypertensive medication or an increased systolic or diastolic blood pressure measured on at least two occasions (i.e. 140/90 mmHg).

Patients were classified as having a classical or non-classical phenotype as previously described by Arends et al [1]. A flow chart used to phenotype classification in clinical practice is added in the **supplemental material (SM2)**.

To study the association of plasma lysoGb3 with the natural disease course, only data obtained before the start of ERT or any other Fabry specific treatment were included in this study.

Laboratory measurements

Plasma lysoGb3 (nmol/l) was measured by tandem mass spectrometry, as previously described [19]. For reproduction purposes, a detailed description of our assay is added as supplemental material (**SM3**). All available untreated lysoGb3 values were used to assess stability in individual patients over time. Only the last available untreated value of lysoGb3 was used to assess its association with markers of disease severity. For the sake of visualization only (**figures 1-6**) patients were grouped in 4 different groups. Groups are not included in any of the statistical models. The cutoffs of 2.3 nmol/L and 40 nmol/L were chosen as they differentiate between classical and non-classical disease in female and male patients respectively. 7.3 nmol/L was chosen to split the remaining groups (predominately men with non-classical FD and women with classical FD) into equal halves to visualize the effect within patient groups with the same disease phenotype. A separate table of patient characteristics for each visual group is added in the supplemental material (**SM1**).

Glomerular filtration rate was estimated (eGFR) in mL/min/1.73m^2 using CKD-EPI formula. Patients <18 years were excluded from this analysis as the formula can be used in adults only. (Micro)albuminuria was assessed using the urinary albumin to creatinine ratio (uACR, mg/mmol). For analyses, uACR was included as a continuous variable. If urinary albumin was below the level of detection, uACR was set to be 0. The following patients were excluded from the analysis of all renal outcome parameters: patients with a renal transplantation at time of presentation (n=5), as well as patients (n=3) with a confirmed second renal disease that was considered to be the main reason for the decline in renal function (i,e, renal artery stenosis; acute glomerulonephritis and severe bilateral kidney atrophy of uncertain origin).

Echocardiograms

Left ventricular mass index (LVMI) was calculated using the Devereux formula and was corrected for body surface area (BSA) using the Dubois formula. For the calculation of LVMI and relative wall thickness (RWT), longitudinal data, extracted from clinical echocardiography reports, were used. In a subset of patients (those with at least two echocardiograms with a minimum of 5 years between them) extensive re-evaluation of a large number of echocardiography markers was performed (as part of a different study on the development of cardiac manifestations in FD) by a single observer (MES). If Fabry specific treatment was started between the first and last echocardiogram in this study, only the

data of the first echo were used for analysis. From this dataset we selected the following markers of diastolic dysfunction: e' (as a marker of diastolic relaxation), E/e' (As a marker of left atrial filling pressure) and LAVI (as a marker for the duration of the increased LA pressure).

Cerebral MRI
Cerebral MRI data obtained at our site for a previous study were used, see [13] for more detailed information. All MRI data were obtained using 3T scanners. Scans were assessed by an independent neuroradiologist blinded for all identifying data as well as scan order. WMLs were defined as hyper intensities on axial T2-weighted and fluid-attenuated inversion recovery-weighted (FLAIR-weighted) imaging without cavitation. Assessment was done visually using the Fazekas scale [20]. Each scan was given a score between 0 and 6, depending on the severity of WML at 2 different locations: periventricular and deep. Both locations were attributed a score between 0 (no WML) and 3 (severe confluent WMLs).

Statistics
For statistical analysis and model building, R (version 4.0.3) was used. Packages 'ggplot2' and 'ggpubr' were used for visualization, packages 'data.table', 'tidyverse' and 'lubridate' were used to organize data. 'lme4' and 'lmerTest' were used to perform linear mixed effect model analyses. Optimal cutoffs for phenotyping were determined visually and checked with sensitivity and specificity calculations. All other analyses are done using Linear mixed effect models correcting for multiple measurements using patient ID as a random variable (random intercept). LysoGb3 was transformed (Log10) to improve fit of the models. Models were build using manual forward selection of variables. For each variable the interaction between lysoGb3 and age were tested to calculate the effect of lysoGb3 on slope. Next we tested the effect of sex (male/female) and the presence of any cardiovascular risk factor (e.g. one or more of the following risk factors were present: hypertension, obesitas, smoking). Only variables that significantly influenced the model (p<0.05) were included in the final model.

Assumptions for linear regression were checked. To check if the observations in male patients with classical FD were not the sole factor driving the associations, all analyses were repeated after removing classical male FD patients from the dataset. For some variables (functional markers on echocardiogram and Fazekas score on MRI) insufficient data of untreated male patients with classical FD above the age of 30 were available (since they are usually diagnosed young and start treatment early). For these variables analyses were only performed without this group. A complete summary of the analyses and outcomes are presented in **Table 2**.

Results

Patient characteristics
Patient characteristics of the 237 included FD patients are outlined in **table 1**.

Table 1:. Baseline patient characteristics of the 237 included untreated FD patients

	Male FD (N=89)	Female FD (N=148)
Disease classification		
classical	54 (61%)	108 (73%)
non-classical	35 (39%)	40 (27%)
Mutatation type		
Nonsense/frameshift	24 (27%)	44 (30%)
missense	60 (67%)	98 (66%)
others	4 (5%)	6 (4%)
Plasma lysoGb3 (nmol/L)	82 (1 - 178)	5 (0.4 - 41)
Log10(1+lysoGb3)*	1.9 (0.3-2.3)	0.8 (0.1-1.6)
Age of last untreated lysoGb3	40 (3 - 71)	44 (13 - 76)
Plasma lysoGb3 categories**		
< 2.3 nmol/L (n=42)	7 (8%)	35 (24%)
2.3 – 7.3 nmol/L (n=71)	5 (5%)	66 (44%)
7.3 – 40 nmol/L (n=70)	24 (27%)	46 (31%)
> 40 nmol/L (n=54)	53 (60%)	1 (1%)
Cardiovascular risk factors		
Smoker (former or current)	38 (43%)	57 (39%)
Hypertension	22 (25 %)	32 (22 %)
Body mass index >30 kg/m^2	7 (8 %)	30 (20 %)
Any of the above present	50 (56%)	82 (55%)

Data are presented as number (percentage) or median and range, as appropriate.
Missing data: mutation type (n=1), male patient with clear clinical and biochemical FD characteristics but no mutation found in the coding GLA sequence; smoking (n=26); hypertension (n=6).
* Transformed lysoGb3 values as they are used in the statistical models.
** Patients were divided into groups for visualization purposes only. Groups are not included in any of the statistical models.

Stability of plasma lysoGb3 over time in individual untreated patients
In individual FD patients not treated with FD specific therapy, plasma lysoGb3 levels remain stable over time throughout life (p=0.6 for change over time, **fig.1**). Median follow up time is 3.7 years (range 0.1-16 years). The exact age at which lysoGb3 stabilizes could not be determined because of the low number of measurements in very young patients.

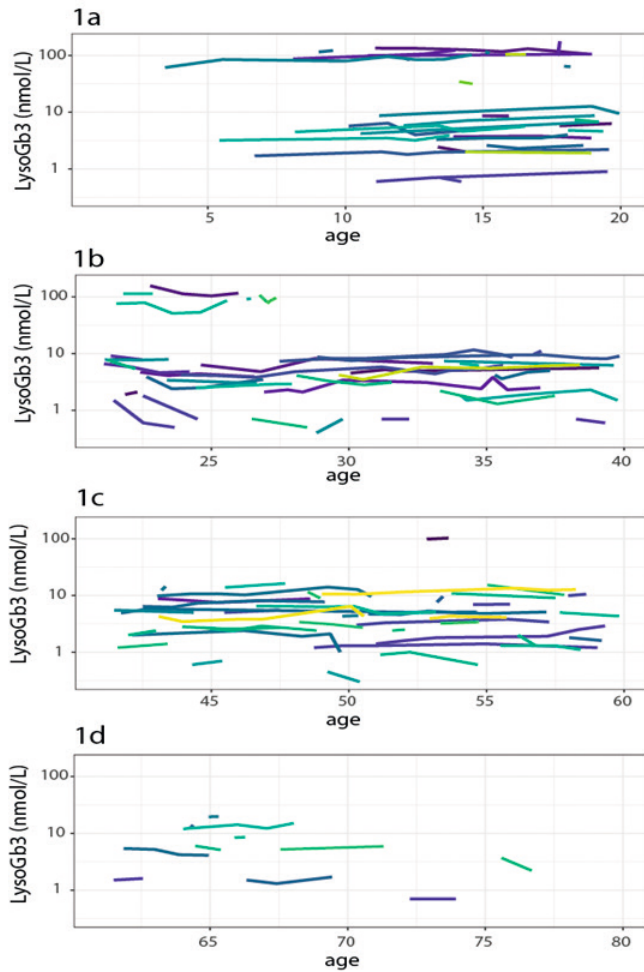**Figure 1:** LysoGb3 in individual untreated FD patients over time. Only patients with at least 2 separate untreated measurements are displayed to visualize change over time. The earliest measurements were at the age of 3 years.
(a) Patients aged 3-20 years (n= 36). (b) Patients aged 21-40 years (n=36). (c) Patients aged 41-60 years (n=40).
(d) Patients aged 61-80 years (n=11). Colored lines represent individual patients.

Untreated lysoGb3 in plasma as a marker for disease phenotype

All but one of the 237 FD patients had plasma lysoGb3 levels above the reference range (0.3-0.5 nmol/L). The one female patient with a normal plasma lysoGb3 level (0.3 nmol/L) had a pathogenic GLA variant associated with a non-classical phenotype in her male relatives. At her last evaluation (aged 39) she had no clinical signs or symptoms associated with FD. Plasma lysoGb3 levels >40 nmol/L separated male patients with classical disease from male patients with non-classical disease with 98 % sensitivity and 100 % specificity (P<0.001, PPV= 98%, NPV= 100%, **fig 2**). Plasma LysoGb3 levels > 2.3 nmol/L separated female patients with classical disease from female patients with non-classical disease with 98% sensitivity and 83% specificity (P<0.001, PPV=94%, NPV= 94%).
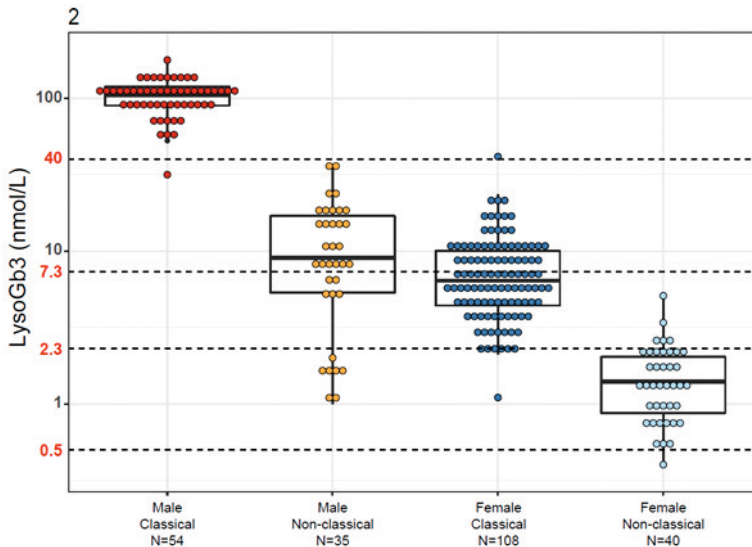
Figure 2: Plasma lysoGb3 levels in Fabry patients with different phenotypes. Dotted lines represent upper range of normal (0.5 nmol/L), best cut off value to differentiate between classical and non-classical FD in female patients (2.3 nmol/L) and the best cut off value to differentiate between classical and non-classical FD in male patients (40).

Association of plasma lysoGb3 with renal manifestations in untreated FD patients

Higher lysoGb3 levels were significantly associated with steeper eGFR slopes in all FD patients of 18 years and older (n= 202, median numbers of measurements 2 per patient, range 1-20) (**fig 3a**, p=0.04). As male patients with classical FD are known to be most at risk for accelerated decline in renal function, analyses were also performed after exclusion of this patient group. The association remained statistically significant (p=0.005). Higher lysoGb3 levels were also significantly

associated with a faster increase in albuminuria (n=198, median 2 measurements per patient, range 1-16) (**fig 3b**, p<0.001), though this association seemed to be driven largely by male patients with a classical phenotype as the association was no longer statistically significant after exclusion of this group (p=0.09). Adding sex of the patient or the presence of cardiovascular risk factors to either of the models did not significantly impact the outcome and was thus not included in the final analyses. For full results see **table 2**.
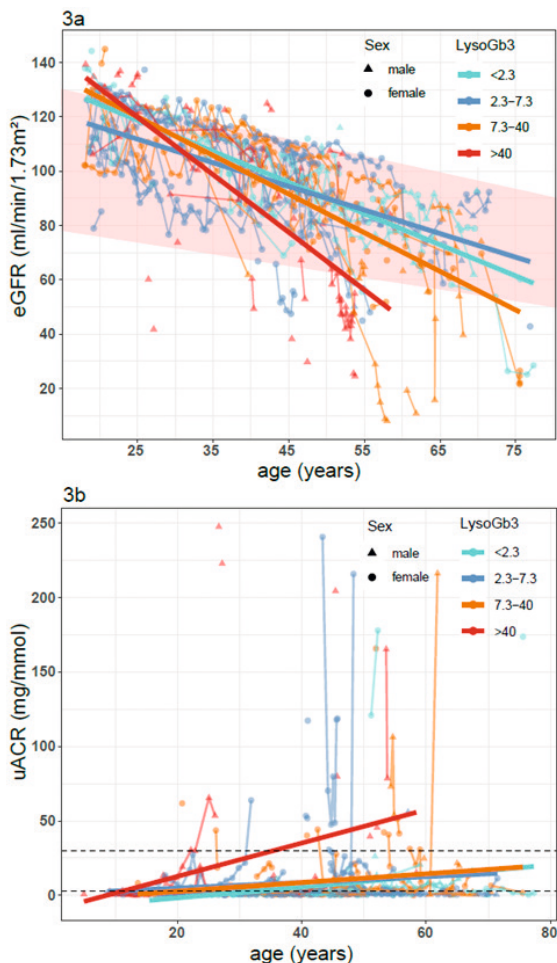
Figure 3: Relation between lysoGb3 and the progression of renal manifestations in untreated Fabry patients. All analyses are performed with actual plasma lysoGb3 levels (after log10 transformation). Grouping is done for visualization purposes only. (a) Association between plasma lysoGb3 and eGFR slope in untreated patients, thepink reference area visualizes the approximate 95th percentile eGFR range in healthy subjects, extrapolated from Baba et all [21]. (b) Association between plasma lysoGb3 levels and progression of albuminuria (uACR) in untreated Fabry patients.

Association between plasma lysoGb3 and left ventricular mass and relative wall thickness on echocardiography

In untreated FD patients, plasma lysoGb3 levels were significantly associated with the increase in left ventricular mass over time (indexed to body surface area, LVMi) on echocardiogram (p<0.001, n=192, median number of measurements: 1 per patient, range 1-12, **fig 4a**). Aside from lysoGb3 levels, having one or more cardiovascular risk factors (hypertension, smoking or obesity) was an independent risk factor for higher LVMi (p=0.003). Using the estimates in **table 2**, we can calculate that left ventricular mass of a patient with a plasma lysoGb3 value of 1 increases with 0.78g/m^2 every year, compared to a yearly increase of 2.5g/m^2 for a patient with a lysoGb3 of 100 nmol/L. Removing male patients with classical FD from the analyses did not change the outcome (p<0.001 for lysoGb3, p=0.005 for cardiovascular risk factor). Higher lysoGb3 levels were significantly associated with higher relative wall thickness (RWT), as a sign for concentric remodeling, at any age (p<0.001, **fig. 4b**). No association between the plasma lysoGb3 levels and the rate of increase of RWT was found. Female patients had a significantly higher RWT compared to male patients (p=0.04). Removing male patients with classical FD from the analyses did not change the outcome (p=0.001).
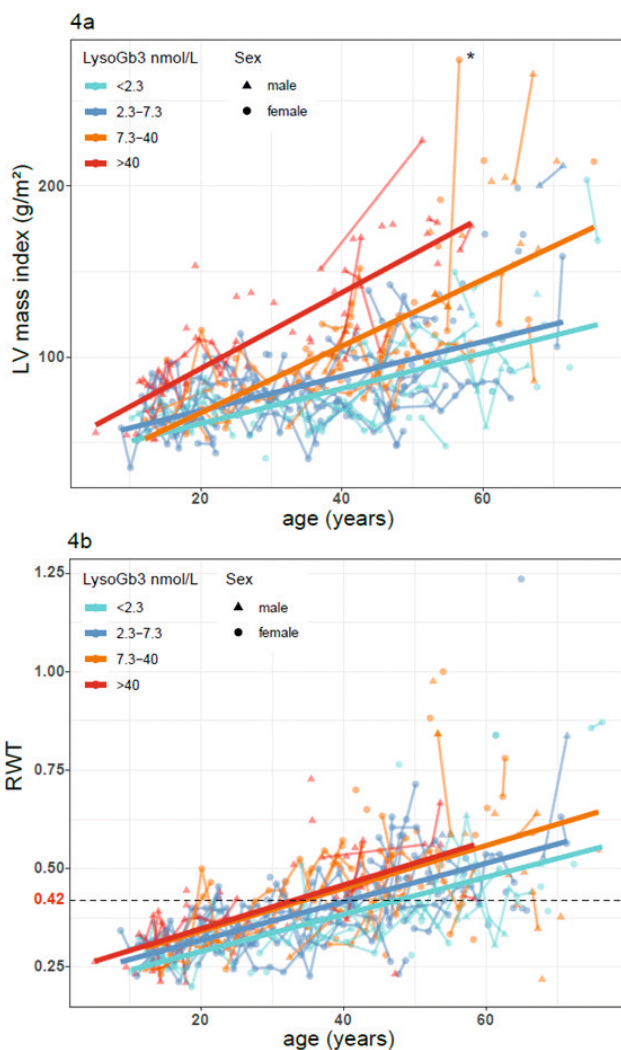
Figure 4: (a) Relation between lysoGb3 and the increase (slope) of left ventricular mass (LVMi) over time in untreated FD patients. Reference ranges of normal LVMi are 49-115 g/m² for males and 43-95 for females, >149g/m² is considered grossly abnormal in males and >122 g/m² for females [22]. (b) Association of plasma lysoGb3 with RWT on echocardiography in untreated Fabry patients. RWT was higher in patients with higher lysoGb3 values, but there was no difference in slope over time. Values above >0.42 suggest concentric remodeling [22]. All analyses are performed with actual plasma lysoGb3 levels (after log10 transformation). LysoGb3 levels are grouped for visualization purposes only as described in the legend.

*Patient suffered severe cardiac decompensation between first and last assessment. Patient was repeatedly admitted to ICU after last assessment and passed away 2 years later (due to heart failure). In addition, quality of the echocardiograms in this patient were described as suboptimal. Removing the patient from analyses did not change the outcome of the analysis.

Plasma lysoGb3 and the functional parameters on echocardiography
Functional parameters on echocardiography were assessed in a subset of patients. Insufficient data of male patients with classical FD, especially above the age of 30, were available to be able to include them in these analyses on the relationship between plasma lysoGb3 levels and these parameters.

In the remaining patient groups (145 patients, median number of measurements per patient was 1, range 1-2), we studied the relationship between plasma lysoGb3 levels and functional echocardiography parameters related to diastolic dysfunction and heart failure with preserved ejection fraction (HFpEF) [23]. Higher plasma lysoGb3 levels were significantly related to lower e' (mitral annular early diastolic velocity, $p<0.001$, **fig 5a**) as well as higher E/e' (the ratio between early mitral inflow velocity and mitral annular early diastolic velocity, $p=0.008$, **fig 5b**) at any age. No significant association of lysoGb3 on progression over time (slope) for these markers was found. Higher plasma lysoGb3 levels were significantly related to a faster increase in left atrial volume index (LAVI, $p=0.001$) over time.

Figure 5: Association between plasma lysoGb3 levels and functional parameters on echocardiography in untreated FD patients. (a) Association of plasma lysoGb3 with e' ($p<0.001$). e' represents the velocity of mitral annular motion during early diastole, and is a marker for myocardial relaxation. Patients with higher lysoGb3 values had lower e' (suggesting stiffer LV) at any age, there was no difference in slope (b) Association of plasma lysoGb3 with E/e' ($p<0.001$). E/e' indicates the ratio between mitral inflow velocity during early diastole (E) and e', and represents a marker for left atrial filling pressure. Patients with higher lysoGb3 values had higher E/e' (suggesting higher filling pressure) at any age, there was no difference in slope (c) Association of plasma lysoGb3 and left atrial volume index (LAVI). Higher lysoGb3 levels were associated with a faster increase over time ($p=0.003$). The dotted lines in every figure represent the cutoff values for diastolic dysfunction as recommended by the European Association of Cardiovascular Imaging/American Society of Echocardiography (EACVI/ASE) [24]. In a healthy heart, relaxation of the LV causes a high velocity of the mitral annulus during early diastole (high e') resulting in blood being 'sucked' from the LA into the LV. Under these circumstances, E/e' is low, usually <8. In the presence of diastolic dysfunction due to LV hypertrophy and stiffening, the LV does not relax properly (e' becomes lower), and as a result E/e' increases. E/e' >14 is highly suggestive of elevated filling pressures. Chronic increase of LA pressure resulting LA dilatation, as indicated by an increased LAVI. All analyses are performed with actual plasma lysoGb3 levels (after log10 transformation). LysoGb3 levels are grouped for visualization purposes only as described in the legend.
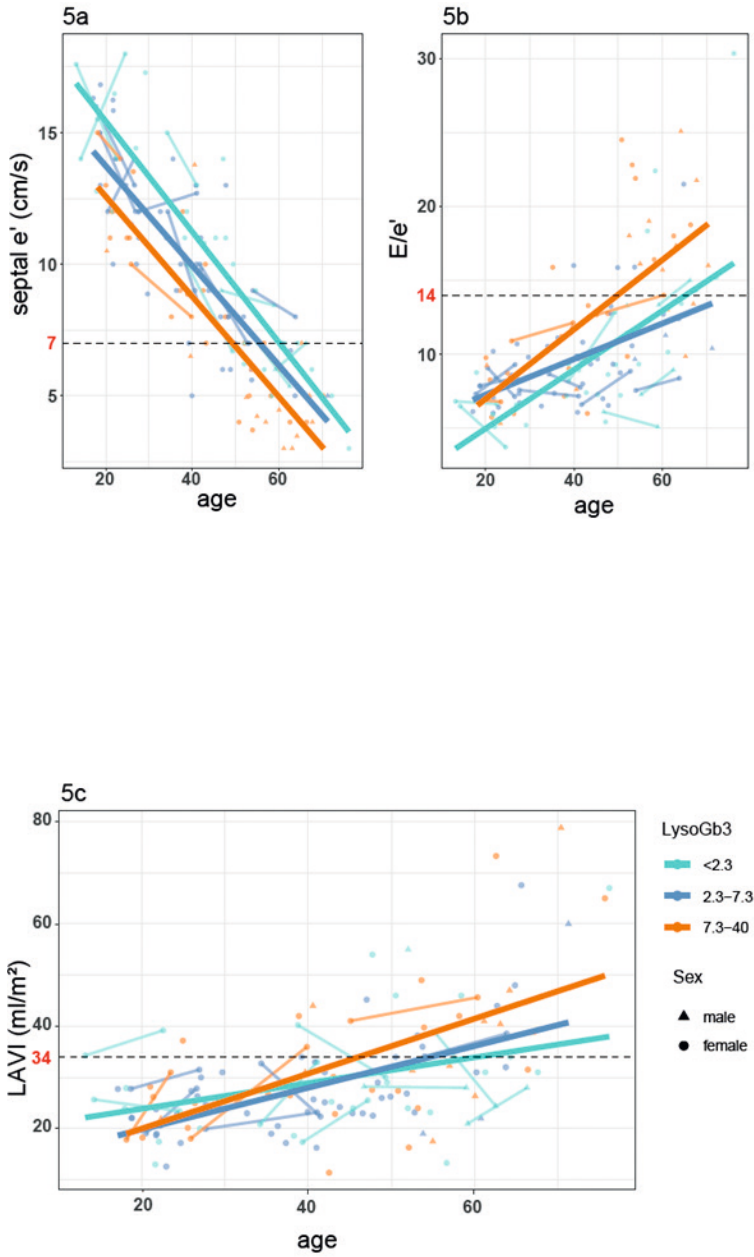
Correlation between plasma lysoGb3 and Fazekas score on brain MRI

We studied the association between plasma lysoGb3 levels and Fazekas score (1-6) on cerebral MRI, a reflection of the white matter lesion load (WML), in untreated FD patients (n=77, median measurements 2 per patient, range 1-7). Higher plasma lysoGb3 levels were significantly associated with a faster increase in WML over time (**fig 6**, p=0.003).
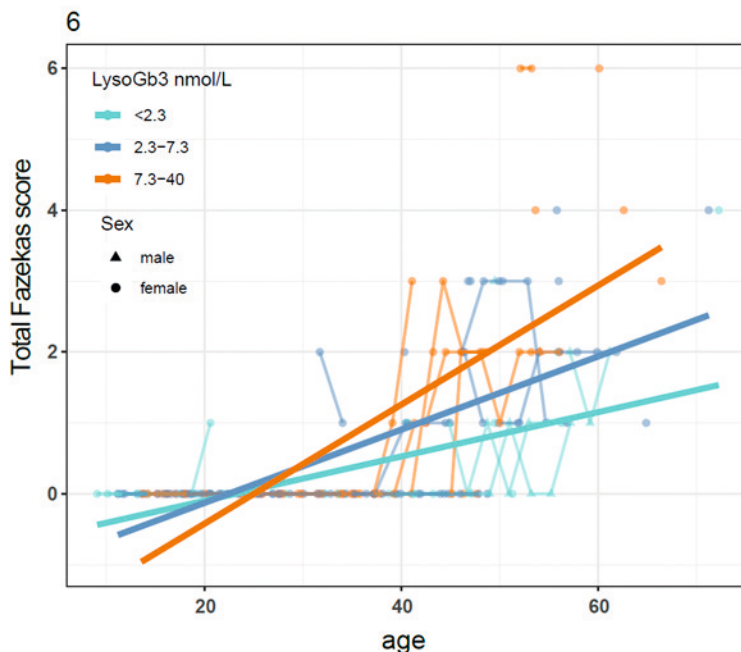

Figure 6: Association of plasma lysoGb3 with the progression of white matter lesions (measured using Fazekas score) on MRI in untreated patients (n=77).

Table 2: Summary of the performed statistical analyses for every included disease manifestation of FD

FD disease manifestation	Included variables In analyses	Estimate Of effect	Confidence Interval (95%) Min	Max	P value
eGFR (mL/min/1.73m2)					
- Including CMFD*	Log10(LysoGb3)	3.64	-6.95	14.1	0.494
	Age (years)	-1.08	-1.35	-0.83	**<0.001**
	Log10(LysoGb3)*Age	-0.26	-0.511	-0.01	**0.037**
- Excluding CMFD*	Log10(LysoGb3)	18.6	-1.78	39.1	0.074
	Age (years)	-0.85	-1.17	-0.53	**>0.001**
	Log10(LysoGb3)*Age	-0.58	-0.98	-0.18	**0.005**
Log10(uACR*)					
- Including CMFD*	Log10(LysoGb3)	-0.17	-0.42	0.08	0.190
	Age (years)	-0.001	-0.008	0.006	0.852
	Log10(LysoGb3)*Age	0.013	0.006	0.019	**<0.001**
- Excluding CMFD*	Log10(LysoGb3)	0.82	0.204	1.433	**0.009**
	Age (years)	0.02	0.006	0.026	**0.002**
	Log10(LysoGb3)*Age	-0.01	-0.023	0.002	0.087
LVMi (g/m²)					
- Including CMFD*	Log10(LysoGb3)	-2.839	-16.23	10.39	0.674
	Age (years)	0.471	0.077	0.858	**0.017**
	Log10(LysoGb3)*Age	1.022	0.682	1.366	**<0.001**
	Any CV risk factor	11.09	3.760	18.50	**0.003**
- Excluding CMFD*	Log10(LysoGb3)	-23.61	-57.53	10.11	0.170
	Age (years)	0.075	-0.500	0.643	0.794
	Log10(LysoGb3)*Age	1.582	0.893	2.276	**<0.001**
	Any CV risk factor	12.20	3.834	20.68	**0.005**
RWT					
- Including CMFD*	Log10(LysoGb3)	0.079	0.037	0.120	**<0.001**
	Age (years)	0.006	0.005	0.007	**<0.001**
	Sex (male)	-0.053	-0.102	-0004	**0.035**
- Excluding CMFD*	Log10(LysoGb3)	0.102	0.040	0.163	**0.001**
	Age (years)	0.006	0.005	0.007	**<0.001**
	Sex (male)	-0.045	-0.102	0.011	0.115
e' (cm/s)					
- Excluding CMFD*	Log10(LysoGb3)	-2.780	-0.209	-0.171	**<0.001**
	Age (years)	-0.190	-3.717	-1.844	**0.001**
E/e'					
- Excluding CMFD*	Log10(LysoGb3)	0.176	1.269	5.160	**0.001**
	Age (years)	3.212	0.138	0.214	**<0.001**

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Table 2: Summary of the performed statistical analyses for every included disease manifestation of FD (continued)

FD disease manifestation	Included variables In analyses	Estimate Of effect	Confidence Interval (95%) Min	Max	P value
LAVI (ml/m²)					
- Excluding CMFD*	Log10(LysoGb3)	-18.69	-35.61	-1.778	**0.031**
	Age (years)	0.014	-0.266	0.294	0.921
	Log10(LysoGb3)*Age	0.534	0.190	0.879	**0.003**
Fazekas score					
- Excluding CMFD*	Log10(LysoGb3)	-1.612	-3.434	0.177	0.079
	Age (years)	0.011	-0.021	0.043	0.485
	Log10(LysoGb3)*Age	0.062	0.021	0.103	**0.003**

All analyses are done using linear mixed effect models correcting for multiple measurements using patient ID as a random variable (random intercept). LysoGb3 was transformed (Log10) to improve fit of the models. For each analyses the variables 'sex' and 'presence of cardiovascular risk factors' (e.g. one or more of the following risk factors were present: hypertension, obesity, smoking) were tested alongside age and individual lysoGb3 values (lysoGb3 was used as a continues variable). Apart from age and lysoGb3 value, only variables that significantly influenced the model (p<0.05) were included in the final model. **P values below 0.05 are underlined.**

*CMFD classical male Fabry patients, To assess if the effect remains after excluding male patients with classical FD analyses are performed with and without this group. For some variables, we lacked sufficient data of untreated classical male patients above the age of 30.

*uACR was measured as mg/mmol before transformation to logscale (log10).

Discussion

In this study, we show for the first time that plasma lysoGb3 stays stable over decades in individual FD patients from childhood onwards (**figure 1**). Additionally, we showed that plasma lysoGb3 levels are associated with either the severity or progression of nearly all measured FD manifestations. Combined, these findings suggest that plasma lysoGb3 reaches a stable level early on, and indicates which disease burden can be expected later in life. This is in contrast to Gb3 accumulation in -for example- podocytes, which has been shown to slowly increase over time [25]. Interestingly, after start of ERT, plasma lysoGb3 levels reach a new equilibrium within a year after start of treatment (in the absence of anti-drug antibodies) [26]. Once this new level is established, it only changes if the ERT dose is changed [26] or treatment is stopped. Why plasma lysoGb3 remains stable in the untreated state and rapidly reaches a new equilibrium during treatment is currently unknown.

Clinically, FD is a slowly progressive disease and children/young adults do not always show clinical signs of the disorder yet. Our data suggest that measuring

plasma lysoGb3 at the time of diagnosis could help predict the disease course in an individual patient, irrespective of the age at diagnosis. The strict association of plasma lysoGb3 with disease type (e.g. classical or non-classical disease) − regardless of the age of analysis− further supports this observation (**figure 2**, [14, 17, 27, 28]). In fact, we showed that plasma lysoGb3 levels can differentiate between the classical and non-classical phenotype with 99% accuracy in male patients and 92% accuracy in female patients and could thus be used as the decisive parameter to determine the disease type in an individual with a GLA variant of unknown significance.

We confirmed the reverse association of plasma lysoGb3 with decline in renal function (**figure 3a**, [14, 16]) and verified that this association was not mainly driven by male patients with the classical disease type (who are known to have more renal involvement compared to patients with the other phenotypes). A similar effect was found when looking at albuminuria: higher lysoGb3 levels in plasma were associated with a faster increase in uACR (**figure 3b**). The strongest association we found, is that between plasma lysoGb3 levels and increase in LVMi (**figure 4a**, [16, 17]). In non-FD patients, LVH is strongly associated with overall mortality, myocardial infarction and stroke [29]. In FD patients, higher LVMi on echocardiography was also associated with higher clinical event rate [9]. A new finding is the association of plasma lysoGb3 with markers of diastolic (dys)function, measured by echocardiography (e', E/e' and LAVI). This is particularly relevant since heart failure with preserved ejection fraction (HFpEF), the clinical syndrome caused by diastolic dysfunction, is a major cause of heart failure in FD [30] and heart failure is currently the leading causes of symptoms and death in FD patients [2]. In many other diseases, higher e', E/e' and LAVI values are strongly associated with increased mortality and increased occurrence of clinical cardiac endpoints [31-34]. The associations indicate that lysoGb3 does not only have a relationship with cardiac morphology, but also with cardiac function and the risk of developing HFpEF. Lastly, we found that higher lysoGb3 levels were associated with a faster progression of white matter lesions in the brain on MRI.

One of the main drawbacks of this study was that this was not a prospective study with longitudinal data collection after an initial lysoGb3 measurement. This represents the real world situation in which many patients were started on treatment and long term untreated data of new patients are simply not available. Strictly speaking, this makes our dataset unfit to be used for prediction modelling. However, we showed the stability of lysoGb3 in individual (untreated) patients, suggesting that the measured lysoGb3 value represents a static individual FD trait which justifies its use in prognostication.

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In conclusion, we showed that plasma lysoGb3 values can further fine-tune clinical phenotyping. We confirmed that in patients with lysoGb3 levels below 2.3 nmol/L (with a reference range 0.3-0.5) renal function remains within the normal range (**figure 2**) and cardiac morphological and functional parameters show slow progression over time and become abnormal only late in life (from the 7th decade of life onwards, **figure 4 and 5**). These findings are consistent with earlier observations that the risk of cardiovascular complications in female patients with a non-classical disease type (in whom lysoGb3 levels are usually below 2.3 nmol/L) is low and if they do occur it is late in life [2]. The seven male (non-classical) FD patients with lysoGb3 levels below 2.3 in this study showed a similar benign disease course. In our opinion this group is unlikely to benefit from Fabry specific therapy and may not even require routine follow up before the sixth decade of life. Within the group of patients with a plasma lysoGb3 between 2.3 and 40 nmol/L (mostly male patients with non-classical FD and female patients with classical FD), measuring plasma lysoGb3 can help to give additional insight into the expected clinical course. In patients with relatively low lysoGb3 levels and no- or minor clinical FD manifestations, prolonging the interval between clinical evaluations seems justified and –in our opinion- the potential effect of treatment should be weighed against the burden of lifelong infusions in consultation with the patient. While in patients with higher lysoGb3 levels, more rigorous follow up is warranted and treatment may be started with less restraint. Further multicenter studies are needed to confirm these findings and identify other biomarkers to improve clinical risk stratification of FD patients.

Based on the results from this study, we propose that measuring plasma lysoGb3 at time of diagnosis can be a useful tool to help diagnose and classify FD. Additionally, the early stabilization of lysoGb3 in plasma over time makes it a suitable marker to aid clinical decision making, more specifically to help determine the needed frequency of follow and the timing of treatment initiation in asymptomatic patients.

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SUPPLEMENTAL MATERIAL

SM1: Patient characteristics per visualized lysoGb3 group

LysoGb3 group:	Low (n=42)	Low mid (n=71)	High mid (n=70)	High (n=54)
Sex				
- Male	7 (17%)	5 (7%)	24 (34%)	53 (98%)
- female	35 (83%)	66 (93%)	46 (66%)	1 (2%)
Phenotype				
- classical	2 (5%)	59 (83%)	47 (67%)	54 (100%)
- non-classical	40 (95%)	12 (17%)	23 (33%)	0 (0%)
Age	49 (19 - 76)	44 (13 - 76)	48 (14 - 70)	27 (3 - 58)
Mutatation type				
- nonsense	1 (2%)	19 (27%)	23 (33%)	25 (46%)
- missense	41 (98%)	47 (66%)	45 (64%)	25 (46%)
- other	0 (0%)	4 (5%)	2 (3%)	4 (8%)
LysoGb3 (nmol/L)	1.3 (0.4 – 2.2)	4.8 (2.4 - 7.2)	11 (7.3 - 36.2)	105 (38 - 166)
Additional risk factors				
- ever smoked	20 (53%)	26 (43%)	29 (45%)	20 (42%)
- Hypertension*	13 (32%)	16 (24%)	17 (24%)	8 (15%)
- BMI >30 kg/m2	10 (24%)	17 (24%)	8 (11%)	2 (4%)
Any known risk factor	28 (67%)	38 (54%)	41 (59%)	25 (47%)

Supplemental table 1. Baseline characteristics of included FD patients per visualized group. Grouping was done for visualization purposes only. Low group (<2.3 nmol/L) and high group (>40 nmol/L) were chosen based on the best cut-off value for classification (classical vs non-classical disease in females and males respectively). The remaining group was cut in half at the median of 7.3 nmol/L to create 2 equally dividedgroups. Data are presented as number (percentage) or median and range, as appropriate.

SM2: Current flowchart used to diagnose and phenotype FD at the AUMC

Individual with a variant in the GLA gene
(and a potential FD related symptom)

Known neutral variant
(see data dictionary for definition)

+

**No FD
(Benign)***

−

- Enzyme activity <5% (only in males)
- ≥1 characteristic FD symptom
  - Neuropathic pain
  - Angiokeratoma
  - Cornea verticillata

The following criteria may additionally support a diagnosis of classic phenotype:
- LysoGb3 >50 nmol/L (in males)
- A family member with a diagnosis of classic FD

*NB known neutral variants by themselves do not cause Fabry disease and thus not explain the potential FD related symptoms. However the presence of a second, disease causing, variant in the same patient, though very rare, can occur. In case of high clinical suspicion, performing additional diagnostics (lysoGb3 levels, enzyme activity measurement) can thus be considered

**in males to determine whether they have a benign variant or non-classical disease, in females to determine whether they have a benign variant, classical or non-classical disease

*** height of lysoGb3 in plasma may help phenotypic classification (Smid et al 2015)

**** concentric multi lamellated myelin bodies with a zebra like pattern with a periodicity of approximately 5 nm (Takahashi et al 1987)

−

Additional
work-up needed**

+

**Classic
phenotype**

Has histopathological examination
been performed(EM)?
(in a family member)

+

Characteristic lysosomal inclusions
(zebra bodies)****
exclude medication as cause

−

Cardiac presentation:
LVH: IVSd thickness >15mm <20 years
Abnormally low voltages on ECG
Neurological presentation
CADASIL/CARASIL presentation of white matter lesions

−

**No FD
(Benign)***

+

Male:
**Non-classical
Fabry**
Female:
**Classical or
Non-classical
Fabry*****

+

**No FD
(Benign)***

−

Additional work-up needed for determination of disease status and phenotype**
consider histopathological examination of heart or kidney

The following criteria are considered red-flags supporting a diagnosis of FD:
Cardiac symptoms:
- PQ interval <120ms
- sinus bradycardia
- hypertrophy of the papillary muscles
- myocardial late enhancement in the infero-postero-lateral region
- low native T1 values on cardiac MRI
Renal symptoms:
- Maltese cross sign urine
- high urine Gb3
Neurological symptoms:
- increased basilar artery diameter (>4,2 mm)
- pulvinar sign
Biochemical assesment:
- plasma lysoGb3 >0.7 nmol/L (reference values Lab GMD Amsterdam)***

This flow-chart is based on several diagnostic algorithms developed by experts.
References: Smid et al 2014, van der Tol et al 2014, van der Tol et al 2015.

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SM3: Detailed procedure of Lab GMD (Amsterdam UMC) lysoGb3 assay
LysoGb3 is measured using LC-MS/MS ((Xevo TQ MS, Waters Inc.) in multiple reaction monitoring(MRM) mode.

Sample preparation:
- Pipette 50 µL plasma into a 2 mL vial.
- Add 25 µL Internal Standard (IS) (concentration: 0.1 µM).
- Add 25 µL of MilliQ water.
- Add 300 µL MeOH and 150 µL CHCl3 and vortex for 30 sec.
- Centrifuge at 16060 g (14000 rpm) at 4°C for 10 min.
- Transfer the supernatant to a 2 mL vial.
- Add 150 µL CHCl3 and 225 µL MilliQ water and vortex for 30 sec.
- Centrifuge at 16060 g (14000 rpm) at 4°C for 2 min.
- Take 400 µL of the supernatant and transfer to a 1.5 mL screw cap vial.
- Evaporate to dryness at 35°C under N2.
- Take up the residue in 60 µL MeOH by vortexing (30 sec) and sonicating (1 min) in a sonication bath.
- Centrifuge for 10 min at 16060 g at 4°C.
- Transfer 50 µL to a gilson vial and cap and add 50 µL MilliQ water.
- Mix by vortexing briefly.

(Internal) standards and LC-column
- Internal standard: Gly-lysoGb3 (Matreya, 1530)
- Standard: LysoGb3 (Sigma, G9534)
- LC-column: Acquity BEH C18 200x2.1 mm, 1.7µm particles (Waters, 186002350)

Liquid chromatography settings

Run time (min):	5.50			
Solvent selection A:	A1			
Solvent selection B:	B1			
Low pressure limit (bar):	0			
High pressure limit (bar):	134			
Solvent name A:	Water			
Solvent name B:	Methanol			
Seal wash (min):	5.0			
Gradient start:	At injection			
Gradient table:				
Time (min)	Flow rate	%A	%B	Curve
Initial	0.250	100.0	0.0	Initial
2.50	0.250	0.0	100.0	6
4.00	0.250	0.0	100.0	6
5.00	0.250	100.0	0.0	6
5.50	0.250	100.0	0.0	6

MS/MS settings

Type:		MRM						
Ion mode:		ES+						
Inter channel delay (sec):		- 1.000						
Inter scan time (sec):		- 1.000						
Span (Da):		0.0						
Start time (min):		0.0						
End time (min):		5.5						
Ch	Parent (Da)	Daughter (Da)	Dwell (sec)	Cone (V)	Coll (eV)	Delay (s)	Compound	
1	786.40	282.30	0.100	45.00	35.00	- 1.000	Lyso-CTH	

Type:		MRM						
Ion mode:		ES+						
Inter channel delay (sec):		- 1.000						
Inter scan time (sec):		- 1.000						
Span (Da):		0.0						
Start time (min):		0.0						
End time (min):		5.5						
Ch	Parent (Da)	Daughter (Da)	Dwell (sec)	Cone (V)	Coll (eV)	Delay (s)	Compound	
1	843.5	264.40	0.100	45.00	55.00	- 1.000	Gly-lyso-CTH	

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NB.
- Lyso-CTH=LysoGb3
- Gly-Lyso-CTH=gly-lysoGb3 (IS)

Calculation

Concentrations are calculated in Targetlynx (Waters Inc.) using a lysoGb3 standard line prepared inplasma: (concentrations: 0, 2, 5, 10, 20, 50, 100, 200 nmol/L.

Chapter 6

Summary and general discussion

Summary

Although the exact pathophysiological mechanism is still not completely understood, it is widely accepted that Globotriaosylceramide (Gb3) accumulation in cardiac tissue is the culprit leading to progressive cardiac dysfunction and ultimately early death in Fabry disease (FD). Following the launch of enzyme replacement therapies, an increasing number of studies has been published describing the cardiac manifestations of FD. However, extensive longitudinal studies that reveal the exact clinical course of Fabry cardiomyopathy in distinct groups divided by phenotype and sex were lacking. The hypothesis is that in FD gradual alterations in electrophysiological, echocardiographic and biochemical markers arise with increasing age of the patient, some of which will be present prior to clinical manifestations. Once we know which and when these biomarkers start to change in different patient groups and how they are related to the occurrence of cardiac events, it will be possible to:

1) Early identify and treat FD patients at risk for developing cardiac events, which may delay irreversible myocardial damage and improve prognosis.
2) Identify clinically relevant electrophysiological, echocardiographic and biochemical markers that help clinicians in the evaluation of Fabry specific and supportive treatment effects.
3) Develop tailored follow-up protocols taking these prognostic markers into account, in combination with sex, phenotype and age of the patient.

The studies performed within this thesis included observational longitudinal retrospective studies in a group of well-phenotyped FD patients followed at the Amsterdam University Medical Centre (Amsterdam UMC), the Netherlands. Data on cardiac outcomes, electrophysiological, imaging (cardiac MRI (CMR) and echocardiography) and biochemical parameters were collected. For comparison to the general population, data from healthy population cohorts (the Healthy Life in an Urban Setting (HELIUS) and Rotterdam Navigator cohort) were used. By comparing the electrophysiological and echocardiographic characteristics of FD patients, to those of healthy individuals cardiac features that are typical for FD, at both an early and late disease stage, could be recognized.

In **chapter 2**, we recorded the prevalence and timing of cardiac events from birth to the last outpatient clinic visit in a large cohort of 213 FD patients with classical and non-classical FD (average follow-up time: 50 years). In men with classical FD, events occurred mainly from the fifth decade of life onwards. In women with classical FD and men with non-classical FD, events were observed on average one decade later, with a larger variation in the age of complications' onset and in a smaller proportion of patients.

The study also aimed to describe the effect of sex and disease phenotype on the occurrence of cardiac events in FD. The risk of major adverse cardiovascular events (MACE) differed significantly between the patient groups classified by phenotype and sex. The risk for developing a MACE was, as expected, the greatest for men with classical FD, intermediate in women with classical FD and men with non- classical FD and even absent in women with non-classical FD. Of note, none of the patients in the latter group developed a MACE. Interestingly, heart failure (HF), not sudden cardiac death, as was indicated in earlier studies, was most often identified as cause of death (42% of all deaths). More than half of the patients who developed sustained ventricular arrhythmias did so in the context of either a 'structurally' damaged myocardium (after myocardial infarction) or evident HF. These findings shed new light on the cardiac course of FD within different phenotypes and emphasize the need for diagnostic and therapeutic strategies to detect and treat HF in FD patients.

In **chapter 3**, we sought to identify electrocardiogram (ECG) markers that reflect early cardiac manifestations of FD and markers of disease progression. A total of 1,995 ECGs from 133 patients with classical FD (80% treated with Enzyme replacement therapy (ERT)), spanning 20 years of follow-up, were compared to ECGs from 3,893 apparently healthy individuals from the HELIUS cohort.

We assessed how age, FD and sex affected seven ECG parameters (P-wave duration, PR-interval, QRS-duration, QTc, Cornell index, Spatial QRS-T angle and Frontal QRS-axis).

Cornell index was greater and frontal QRS-axis more negative in FD patients than in controls before age of 40. For the other ECG parameters, in early adulthood, the rate of change, more than the absolute value, was greater in FD patients compared to controls. From the fifth decade (men) or sixth (women) onwards, absolute values for P-wave duration, QRS-duration, QTc and spatial QRS-T angle were longer and higher in FD patients than controls. The LVMi on CMR was correlated with each of the examined ECG parameters. Additionally, patients with cardiac fibrosis had a longer and higher P- wave duration, QRS-duration, QTc, Cornell index and spatial QRS-T angle than those without fibrosis. These findings demonstrate that the ECG abnormalities indicative of FD vary with age and sex, e.g. higher Cornell index in early adulthood and prolonged QRS-duration in late adulthood.

Tracking the rate of change in ECG parameters could be a good way to detect disease progression in early adulthood, guiding treatment initiation in those that exhibit significant ECG changes and lack alterations on conventional cardiovascular imaging. In addition, monitoring ECG changes in FD can be

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used to evaluate the effectiveness of treatment, with the current study providing reference data for comparison of new treatments.

Chapter 4 describes the morphological and functional echocardiographic alterations in patients with classical FD versus healthy controls. We conducted this study because 1) the progression of echocardiographic features of FD over long follow-up periods was largely unknown, and 2) insight into the evolution of echocardiographic parameters and their relation with cardiac events may aid in therapeutic decision-making.

The two echocardiograms, with the longest follow-up period between them, per patient were re- assessed and analyzed for 92 patients with classical FD (58 women, echo's median 12 years apart, 92% treated with ERT). Results were compared to data from 147 echocardiograms of healthy individuals (age and sex-matched on a group level, cross-sectional data). The effect of FD, age and sex on end-diastolic interventricular septum thickness (IVSd), relative wall thickness (RWT), left ventricular mass index (LVMi), left atrium volume index (LAVI) and the ratio of early diastolic mitral inflow velocity/ early diastolic septal tissue mitral annulus velocity (E/e') was analyzed. As a secondary study aim, the relation between the first echocardiogram and subsequent development of Atrial fibrillation (AF) in FD was tested. The earliest signs of cardiac involvement of classical FD on echocardiography were increased values for IVSd and RWT in all FD patients and E/e' in men.

Increased absolute values of these markers on the first echocardiogram were associated with an increased risk for AF later. During adult life, IVSd, RWT, LVMi, LAVI and E/e' increase significantly in FD patients compared to healthy individuals. These findings suggest that echocardiographic parameters reflecting left ventricular (LV), atrial morphology and LV diastolic function can represent disease progression over time in classical FD patients. Comparing the absolute values of echocardiographic parameters in FD patients with age and sex-specific reference ranges or evaluating the increment over time can be used to determine the need for treatment initiation, monitor disease progression and evaluate the effect of therapeutic existing and new future interventions.

Finding a biomarker with a substantial predictive value for disease course is crucial to establish the need for Fabry-specific treatment and the right follow-up frequency for an individual FD patient. In **Chapter 5**, we investigated whether levels of plasma Globotriaosylsphingosine (lysoGb3) were stable over time in untreated FD patients and how these levels relate to cardiac and non-cardiac FD manifestations by studying 237 untreated FD patients. Plasma lysoGb3 levels remained stable over time in these untreated FD patients, confirming its

usefulness as an individual disease trait. The strongest association observed, was that between plasma lysoGb3 levels and the LVMi on the echocardiogram. The association of plasma lysoGb3 with echocardiographic markers of diastolic (dys)function (RWT, LAVI, Septal e' and E/e') was a novel finding. This is particularly relevant since diastolic dysfunction is the major contributor to HF in FD, which we showed to be the leading cause of death in FD patients (**this thesis- Chapter 2**). Higher plasma lysoGb3 levels were associated with a greater LAVI. For RWT, septal e' and E/e', there was a significant association for absolute levels, but no difference was found in slope over time. These results indicate that lysoGb3 does not only have a relationship with altered cardiac morphology (LVMi) but also with cardiac function. Additionally, the plasma lysoGb3 was closely related to markers of renal and cerebral disease. We may conclude that measuring lysoGb3 at the time of diagnosis gives information into the expected natural cardiac and non-cardiac disease course, facilitating rational treatment and follow-up protocols in FD patients.

General discussion

Fabry cardiomyopathy, towards early diagnosis and rational follow-up
The disease course in patients with FD is very heterogeneous. Studies conducted by our team and others have shown significant differences in age at symptom onset and disease progression between men and women and patients with classical and non-classical FD [1-3]. The X-linked inheritance and the variety in mutation severity can account for, at least a large part, of this heterogeneity. Since the disease can also differ between male members of the same family, other factors, such as genetic, epigenetic or environmental factors, must be at play as well [4]. To be able to understand the natural history and the effect of interventions it is crucial to establish criteria to separate particular FD phenotypes with comparable symptoms and severity.

Even though there seems to be consensus regarding the criteria used to distinguish the classical and non-classical (sometimes referred to as cardiac variant or late onset) FD phenotypes, many authors still report on FD cohorts as a single patient group [5-7]. This makes the interpretation of treatment outcome data almost impossible, since the difference in symptom free survival between male FD patients with classical FD and non-classically affected males or classically affected females on average is 10 years [1, 2]. We strongly believe that a minimum requirement for FD cohort studies is to optimize the classification between classical and non-classical disease and to distinguish between sexes. Only when this is performed properly, new markers can come and would enable a further differentiation between patients. Thus, in all studies performed within this thesis we consistently distinguished between the sexes and between classical and non-

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classical FD. First, we used this basic classification for a detailed description of early and late cardiac complications of FD. Next, we looked for widely applicable and easily obtainable clinical markers to diagnose Fabry cardiomyopathy early and identify individual patients who may be at risk of developing cardiac complications. These insights may then become essential to 1) initiate early treatment so that disease progression can be halted through supportive cardiac and non-cardiac interventions (e.g. medication, cardiac implantable devices or tailored physiotherapy programs, etc.) and 2) design rational follow-up practices specific for each patient group to limit unnecessary diagnostics and interventions.

Fabry cardiomyopathy and enzyme replacement therapy: knowledge gaps
The heart in Fabry disease is the most uniformly affected organ amongst all phenotypes. Left ventricular hypertrophy (LVH) and myocardial fibrosis are the main signs of cardiac involvement in FD. This fibrosis nearly often develops in men with classical FD who also have developed LVH before. It is interesting to note that on cardiovascular imaging, women with classical FD can develop myocardial fibrosis without prior signs of LVH [8], making LVH a less suitable marker for an early diagnosis of Fabry cardiomyopathy in this group. The described early cardiac manifestations of FD in earlier literature includes a shortened PR- interval, higher E/e' and LAVI [5, 6, 9]. Later on, adults may develop symptoms of cardiac disease such as conduction abnormalities, arrhythmias, ischemic heart disease and HF, which frequently result in cardiac mortality [2]. However, the course and sequence of (early) cardiac biomarkers leading to the development of these cardiac complications remained unknown.

Enzyme replacement therapy (ERT) has been authorized for clinical use in patients with FD since 2001 [10]. According to the current clinical recommendations, ERT in men with classical FD (cFD) should be started at the age of 16 years, regardless of the presence of symptoms. However, given the recent insights in the effect of earlier treatment initiation, the start of ERT can be considered from 10 years onwards in male patients with cFD [11]. Thus, although there is some remaining uncertainty about the optimal timing, there is no doubt about the need for FD specific treatment in these patients. While in this group of classically affected males a protective effect on renal function is important, involvement of the kidney is much less relevant in other phenotypes [12]. For the entire cohort of Fabry patients, ERT effects are specifically relevant to improve or prevent cardiac disease.

In women with cFD, initiation of ERT in the current clinical practice is contingent upon the manifestation of cardiac disease such as LVH, cardiac events, or subtle myocardial fibrosis [11, 13]. Selecting women with cFD who will benefit from receiving ERT is challenging, as some women display a cardiac disease

course that is comparable to the general population. At the same time, some women carry a cardiac phenotype resembling that of men with a severe classical phenotype. Ideally, within this group, we should select individual patients at risk for developing cardiac complications so that we can treat them with Fabry-specific or supportive therapy earlier, as multiple studies have shown that initiating therapy at the onset of cardiac fibrosis is less effective than in patients in whom therapy is started prior to fibrosis [14]. Studies that have examined the therapeutic effect of ERT suggest that the application of ERT can stabilize left ventricular mass (LVM) or septal thickness or even cause a reduction of LV mass in both men and women with cFD. Other studies claim that patients receiving ERT show a longer median survival time compared to untreated patients [15]. These findings, in our opinion, should be interpreted with great caution because of following three main reasons:

1) The conducted studies often have a retrospective design with relatively short follow-up periods and do not consider the significant differences in the diagnosis's timing and treatment initiation among FD patients. For example, a patient diagnosed at 50 years of age through family screening and treated because of subtle cardiac manifestations will have a very different cardiac outcome compared to a patient who is diagnosed at the same age and has already experienced multiple cardiac events with significant cardiac damage. In our opinion it is invalid to compare two groups of patients who have not been treated with ERT and who have vastly different baseline conditions.
2) Retrospective studies with short follow-up periods and, for example, only two measurements of LV mass are difficult to interpret because of the high variability in the measures, especially if echocardiography is used. In studies that show a stabilizing effect on LVM, it is still being determined whether this stable LVM is simply an age-related effect within the general population, as control groups from the general population are often lacking.
3) Studies in women with cFD often focus on the effect of ERT on cardiac mass, however women with cFD can develop cardiac complications and fibrosis in absence of an increased LV mass [8]. Therefore, LVM is not an ideal marker for response to therapy, especially in women with cFD.

Fabry cardiomyopathy and ERT: use of clinical markers
As mentioned above, previously published data shows that early treatment with ERT, i.e. before irreversible damage has occurred, may improve renal and cardiac outcomes [16, 17]. Males with classical FD over 45 years of age invariably suffer a cardiovascular event [2]. For this reason, there is, again, no debate about the need for Fabry-specific treatment in the classically affected male group. On the other hand, we have shown in **chapter 2** that only a subset of women with classical FD (cFD) developed cardiovascular events, with a highly variable age

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of onset. Consequently, there is still uncertainty about the need and timing of treatment for an individual female patients with (cFD) [13, 18, 19]. The previously mentioned minimal distinction between classical and non-classical and males and females has shown that the effect of therapy is most clear in classically affected males [20]. The natural history of classically affected males is also more uniform than the disease course in men with non-classical FD and classically affected females. At the other end of the spectrum, the non-classically affected females, the disease course is also better understood: in fact in this sub-group, the risk for complications related to FD is minimal. What is left, is the more heterogeneous group of "in between" patients: the classically affected females and non-classically affected males. Specifically classically affected females are a challenge, since random X-inactivation plays a role as well [21], contributing to the heterogeneity and thus the unpredictability of the disease course. Identifying those patients that will develop clinically significant organ involvement and dysfunction in this subgroup is therefore the most challenging. The hypothesis, is that some women with cFD exhibit progressive changes on, for example, the ECG or echocardiogram, but in another subset, little or no changes in disease markers were observed. From this perspective, it is crucial to determine when and which markers become progressive in this group in order to identify the patients who are at risk for disease progression and probable cardiovascular events.

In the studies outlined in **chapter 2-4**, we identified electrophysiological and echocardiographic markers of FD cardiomyopathy (other than left ventricular mass) which are incorporated into a disease development model in **figure 1 and 2**. The model will primarily aid in detecting cardiac involvement at an earlier, asymptomatic disease stage in women with cFD. Secondarily, it will help evaluate the effect of (new) FD treatments [22] on cardiac disease progression in both men and women with cFD since overt clinical complications take decades to develop, far surpassing the duration of clinical trials.

Implications of the findings in classically affected females for males with non-classical FD
In the study described in **chapter 2**, men with non-classical FD formed 12% of the Fabry population at the AUMC at the time of the investigations [2]. This group is not included in the in the studies in **chapter 3-4**, which deal with early disease markers because these patients are often diagnosed later in life and the number of investigations prior to development of disease complications in these patients was too low for a longitudinal study. We do have information on the development of cardiac complications over time in this patient group (**chapter 2**). In agreement with previous studies, we found, that men with non-classical FD have an almost identical disease course as women with cFD [1, 23]. It could therefore be argued that the identified cardiac markers in the women with cFD

are likely also applicable to men with non-classical FD. However, international cooperation is required to collect more data of men with non-classical FD (identified through family screening) so that a specific cardiac course model can be developed for this patient group. In addition, since women with non-classical FD did not experience MACE, they were not included in the model below. Furthermore, only a small proportion of these patients (9%) experienced non-major cardiac problems (AF and conduction abnormalities) after the seventh decade [2]. Given the low likelihood of the development of cardiac complications in this group compared to other FD patients, it is questionable whether these few events are truly related to the genetic trait (carrying a GLA variant) or whether other cardiovascular risk factors may have contributed more in these patients. Future research should determine whether a non- classical variant in women may be considered as a cardiovascular risk with a minor contribution to the overall morbidity and mortality.

As outlined in the beginning of this discussion, it is our ultimate goal to develop more differentiated guidelines for initiation and follow-up of treatment based upon additional markers, besides phenotype and sex. To this end we attempted to compile a cardiac disease model. This requires a thorough analysis of the course of the cardiac markers, which is discussed below.

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Proposed ECG course in classical FD patients
Figure 1 is based on comparing absolute values of the ECG parameters of FD patients versus control subjects from the general population **(chapter 3)**. To ensure a valid comparison, a group with a comparable number of CV risk factors as the FD cohort was chosen. With this approach, we gained more insight into the more Fabry-specific ECG changes [24].

The ECG alterations related to changes in the LV myocardium (negative frontal QRS-axis and increased Cornell index) were found to be the earliest altered ECG parameters in FD in patients. In a substantial proportion of patients with an increased Cornell index, LVMi on CMR was still normal, indicating that an abnormal Cornell index could be a precursor to visible LV thickening on conventional imaging and that this specific alteration may be related to subtle ultra-anatomical LV myocardial cell changes or that the reference values for LVH detection need to be adjusted for each sex and age group [25].

Accelerated AV-conduction time (shortened PR-interval) was observed in female patients up to the fifth decade, whereas in male patients the absolute value or increment in PR-interval was not different from controls. This suggests that the rate of change in atrial depolarization time in adult male patients is less suitable for monitoring heart disease development in FD, and a short PR-interval in an adult female patient could indicate the onset of cardiac FD involvement.

As opposed to the common idea that a pathognomonic shortened PR-interval is present in FD, the absolute values of the PR-interval in the studied FD patients were often within the normal range of 120-200 milliseconds [26]. This finding was in agreement with the results reported by Namdar et al., who showed that a shortened PR-interval was not a frequent electrocardiographic finding (14%) in newly diagnosed adult patients (age: 40 ± 14 years) [27]. It can thus be concluded that a normal PR- interval does not exclude cardiac involvement in adult FD patients (particularly in male patients with FD and female FD patients over 50 years of age).

Later in life, PR-interval shortening disappears in women with FD, and is replaced by a prolonged P- wave duration, which is an indicator of atrial enlargement [28]. Other abnormalities in LV conduction times (QRS-duration and QTc) and depolarization-repolarization interaction (frontal T-axis and spatial QRS-T angle) were observed in women with FD from the fifth decade onwards, and in men from the fourth decade onwards. It can be deduced that abnormalities in ECG parameters reflecting early LV myocardium alterations appear two decades before the occurrence of cardiac complications and that markers representing LV depolarisation-repolarisation become apparent one decade prior cardiac complications' onset.
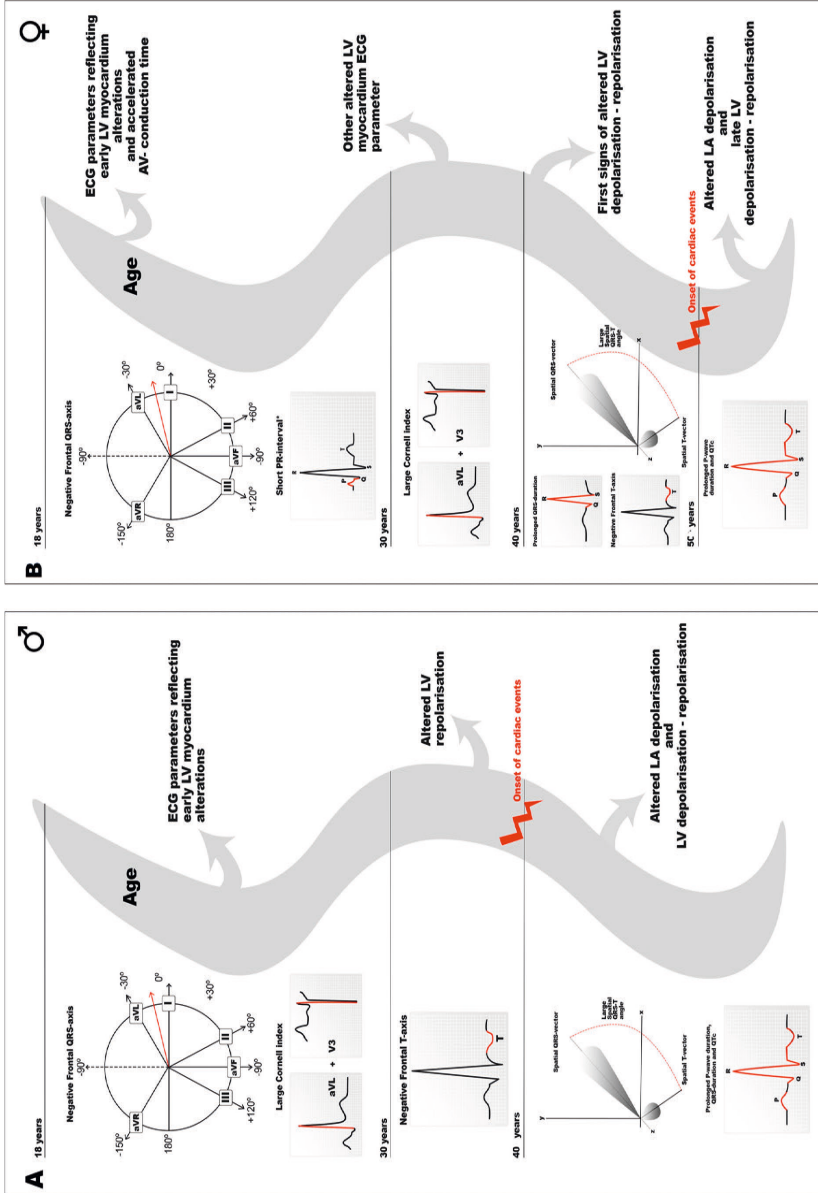
Figure 1: Proposed ECG stages in A. men and B. women with cFD.

Proposed echocardiographic course in classical FD patients

In **chapter 2**, the median age at onset of reduced LV EF and valvular disease was investigated and presented in **figure 2**. Additionally, the figure includes information from **chapter 4**, which compared the absolute values of echocardiographic markers between FD and healthy controls. As the comparison of absolute values within these two groups was not possible for sinus of Valsalva (SoV) and Global longitudinal strain (GLS), we took the age at which these abnormalities developed when the first patients exceeded the generally accepted reference value.

In the first decades of adult life, IVSd and RWT were found to be higher in FD patients than in healthy controls. Surprisingly, an increase in RWT, a marker indicating LV remodeling, was observed in women with FD from the fourth decade onwards, whereas differences in LVMi were only observed ten years later. This suggests that for female FD patients, the course of LV cardiac disease follows a different path than in males with FD, with LV concentric remodeling prior to the development of concentric LVH. Thus, RWT may be a suitable measure for detecting women with early onset cardiac manifestations of FD, whereas LVMi may be a less ideal indicator of cardiac disease at a younger age. Previous studies have described sex dimorphism in FD [29] and sarcomeric hypertrophic cardiomyopathy (HCM) [30], which is an autosomal dominant disorder. This implies that, in addition to the sex dimorphism in FD (X-linked inheritance), the less marked LVH in female FD patients could be related to a different response on Gb3 storage.

Other early and late ultrasound markers shown in the model (**figure 2**) demonstrate that morphological and functional echocardiographic abnormalities in women with FD manifest 10-20 years later than in men with FD, indicating again that men with classical FD have a more severe phenotype. However, the disease course in women is varied, with some women exhibiting a clinical, electrocardiographic and echocardiographic phenotype similar to men.
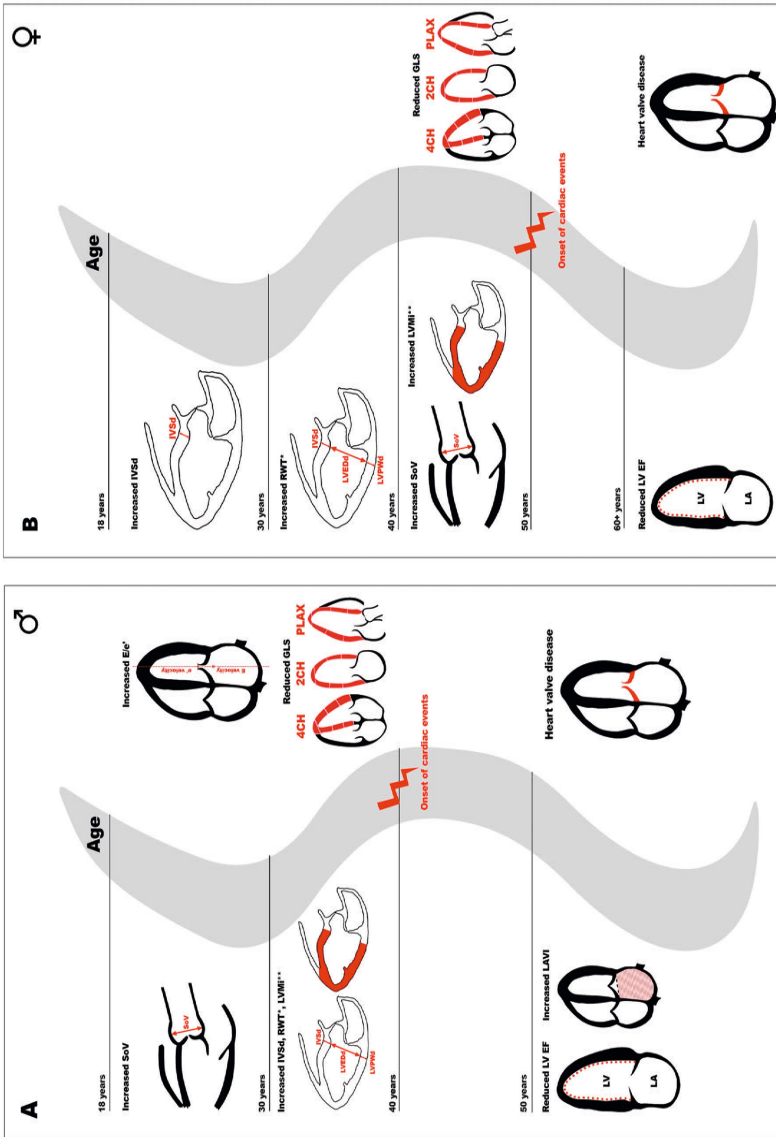
Figure 2: Proposed echocardiographic stages in A. men and B. women with cFD. Note that only a minority of the patients with cFD (19% men and 8% women) develop a reduced LV EF [2]. *RWT= (IVSd + LVPWd/ LVEDd) . ** (LVMi g/m2)= (0.8{1.04[([LVEDd + IVSd +LVPWd]3 − LVEDd3)]} + 0.6)/ (weight0.425 * length0.725 * 0.007184) [31] .

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Fabry cardiomyopathy and lysoGb3

Besides the extensively studied ECG and echocardiography markers, overall disease markers can be of help to predict the disease course and aid in decision making for treatment initiation. The best plasma marker available in FD is lysoGb3 (a water-soluble deacylated form of Gb3). Of note, we have recently shown that in a single patient, plasma lysoGb3 reaches an equilibrium early in life and does not change subsequently which makes it a suitable marker of disease severity, independent of age (**chapter 5**). In this thesis, we connected these data and investigated the relationship between plasma lysoGb3 and cardiac morphological and functional markers, that are related to cardiac complications such as cardiovascular death, sudden cardiac death, life-threatening arrhythmias, ischemic heart disease and heart failure hospitalization in previous studies [32-35]. We found that plasma lysoGb3 can accurately distinguish between men and women with classical versus non-classical FD with very high accuracy. In men with cFD, the plasma lysoGb3 is almost always above 40 nmol/L. A lysoGb3 between 2.3-40 nmol/L is found in female patients with cFD or male patients with non-classical FD. A lysoGb3 < 2.3 nmol/L is characteristic for women with non-classical FD. We found that the higher the plasma lysoGb3 level, the more severe the cardiac morphological and functional changes were.

This suggests that, for example, in a female patient with cFD with a relatively low lysoGb3 level (e.g. 3.5 nmol/L), the risk for development of cardiac complications early in life is low and this can weigh in when determining the follow-up frequency and the consideration of start of Fabry specific therapy. Thus, measuring the plasma lysoGb3 and the described ECG and echo parameters can help to identify women at risk for cardiac complications within the heterogeneous group of female patients with a cFD phenotype.

In the group of patients with a lysoGb3 level < 2.3 nmol/L echocardiographic markers only deviate from the static reference ranges from the seventh decade onwards. This may not differ significantly from the course in the general healthy population, once risk factors such as obesity and hypertension are taken into account.

Fabry cardiomyopathy: revising guidelines to start treatment for cardiac manifestations

We propose a two-track approach to detect early cardiac involvement and later deterioration in FD patients. This method would involve assessing both the rate of change and absolute values of ECG and echocardiographic markers and comparing them to reference values from the same sex and age category in healthy controls. The revision of the guidelines [13] by incorporating a composite score for the assessment of early cardiac disease in Fabry patients is proposed.

The score would be based on the Z scores of various ECG and echocardiographic parameters, as determined by comparison with a reference range for an age and sex matched control group, using a cut-off of 1.5 as an example. To ensure its validity for prognostication of clinically significant cardiac disease in Fabry patients, it is recommended that the composite score be validated through application to a second cohort of patients with FD.

An important area of further research as well is that the investigated ECG and echo markers in this thesis usually remain within standard reference limits, which are the same for adults of all ages. Hence we also discovered that there is a need for improved age and sex specific ECG reference values to be able to detect early changes in Fabry cardiomyopathy and other genetic cardiomyopathies [36].

Although the current thesis provides insight into which parameters can be studied to assess their predictive value for the development of Fabry cardiomyopathy, future studies should focus on developing a prediction model in which a combination of clinical and readily available imaging and electrophysiological data will predict the risk of developing ventricular arrhythmias, heart failure or cardiovascular death in FD patients. This knowledge will help clinicians make better decisions regarding the administration of ERT and future emerging therapies.

Fabry cardiomyopathy: revising guidelines for follow-up
Given our observations that the rate of change of ECG and echocardiographic markers is low (e.g. increase/decrease of a few milliseconds or millimeters), it is advisable to increase the intervals of cardiac imaging evaluations in order to:
1. Make more accurate statements about the manifestation and progression of cardiac disease;
2. Reduce measurement variation; and
3. Decrease unnecessary frequent examinations, which will result in lowering the pressure on potentially overloaded national healthcare systems.

The frequency of ECG evaluations may be higher, as it is a relatively simple, quick and inexpensive examination that can give the clinician an impression of cardiac morphology and function dynamics. Second, assessing the ECG can be used to justify the need for supportive therapies such as the placement of a cardiac device or the intensification of cardiac imaging frequency.

Further studies should formally investigate the exact cardiac course of women with non-classic FD. If this is different from the general population, these patients do not require the extensive follow-up or even specific FD treatment, including repeated cardiac imaging, that they are currently receiving.

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Another outcome may be that carrying a non-classical genetic GLA variant in women only increases the risk of cardiovascular complications in the presence of additional risk factors, e.g. hypertension, dyslipidemia or smoking. In that case, management of general cardiovascular risk factors, with a lower threshold for treatment compares to the general population, may be more important than FD specific treatment. This approach will hopefully prevent the over-medicalization of women with non-classical FD and reduce therapy costs considerably. Also, the lysoGb3 will help clinicians in genetic counselling and discussions about expected disease severity in offspring.

Below we propose a follow-up protocol per FD patient group:

Patient group	Outpatient clinic visit and ECG frequency, every	Cardiovascular imaging (echocardiography and CMR) frequency, every**
Treated FD patients with FD specific treatment or patients in whom the cardiac disease is too extensive for FD specific treatment	1 year	3 years
Untreated patients with plasma lysoGb3: 2,3-40 nmol/L	1 year	2 years, intervals may be increased if no changes are observed
Untreated patients with plasma lysoGb3 < 2,3 nmol/L	3 years from age 40§	5 years from age 50§

** The frequency at which cardiovascular imaging evaluations are performed can be adjusted individually if there is a reason, based on ECG, medical history and laboratory tests, to carry out the examination earlier, later, or not at all.
§ The necessity of this recommendation will need to be reassessed once more data become available.

Finally, we propose that the current guidelines should be amended to include a composite score for early cardiac disease detection, incorporating electrocardiographic parameters, echocardiographic and plasma LysoGb3. This composite score should ideally be validated for prognosticating clinically relevant cardiac disease in a second cohort of FD patients.

Future perspectives
An important limitation of the studies in this thesis is that the studied changes in ECG and echocardiographic markers could not predict the occurrence of major adverse cardiovascular events (MACE) because: a) for patients who were diagnosed with FD after their first MACE we did not have ECG or echocardiography data prior to this event, and b) the younger FD patients did not develop a MACE during follow-up. To provide answers to the prognostic value question even longer follow-up in larger international FD cohorts is needed, to

reach the necessary event rate to assess the predictive value of the parameters discussed above. Since heart failure with preserved ejection fraction (HFpEF) is the predominant cardiac phenotype in FD [37, 38], paying attention to the history and physical examination indicating chronic heart failure is essential. This is important because there is new supportive treatment. In HFpEF patients, regardless of the cause of heart failure, the administration of SGLT2 inhibitors in acute HF hospitalized patients led to reduced 90-day morbidity and mortality, compared to patients receiving a placebo (EMPULSE trial) [39]. Whether this treatment will also impact disease outcomes in patients with chronic HFpEF in the context of FD requires a randomized control trial, in which FD patients treated with an SGLT2 inhibitor on top of the regular recommended heart failure medication (diuretics) [40] will be compared with patients only receiving regular care.

The majority of patients with FD report exercise intolerance. Patients describe this as (muscle) fatigue, lack of endurance capacity or breathlessness. These symptoms limit the patients' ability to participate in work and daily activities, leading to reduced mobility and independence.

Simultaneously, it is one of the features of FD that is most difficult to tackle, as the origin of the exercise intolerance is often not clear, limiting the possibility of giving individualized medical advice (e.g. early treatment of heart failure, training/exercise advice, rehabilitation programs and psychosocial interventions). Lack of interventions results in the persistence of the problem and more immobility, resulting in a vicious cycle. The relationship between complaints and cardiac dysfunction is not always apparent, especially in younger patients that show a structural and functionally normal or only subtly abnormal heart on routine clinical tests (e.g. ECG, echocardiography and blood tests). It is essential to note that these clinical tests are performed at rest, whereas the complaints occur during exercise.

Exercise intolerance symptoms in FD can be related to cardiac dysfunction [41]. However, it may also result from lung or skeletal muscle function or deconditioning changes. Pulmonary dysfunction, especially bronchial obstruction, was frequently observed in FD [42, 43]. A histopathological study in 12 FD patients suggests that in skeletal muscle, accumulation of glycosphingolipids can occur, in both vascular endothelium and myocytes, resulting in skeletal myopathy [44]. Recent studies have shown that lysosomal storage disorders such as FD cause mitochondrial dysfunction [45-47]. Whether this also occurs in cardiac and skeletal muscle in FD and contributes to exercise intolerance in FD has not yet been determined. By performing incremental cardiopulmonary exercise (CPX) tests in our ongoing Fabry Exercise Intolerance STudy (FEISTY), more insight

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can be gained into the aetiology of this exercise intolerance (ClinicalTrials.gov ID: NCT05413876). Among other parameters, maximum oxygen uptake (V'O2 max) is measured and compared to individuals of identical sex, age and weight from a reference database. However, the accuracy, reproducibility and relevance of an incremental CPX test is less than optimal, as it is not representative for exercise during daily activities, in which sudden bouts of maximal activity do not regularly occur. In the FEISTY, an intermittent CPX test is an extension of the 'block workload test', in which a step-change from rest to a relatively low constant workload is studied and provides a view in the dynamic properties of gas transport during exercise [48].

This study is an attempt to identify functional outcome parameters, related to the patient's ability to participate in everyday life. These are needed to be improve in FD research, since current outcome parameters used to assess for example the effectiveness of ERT, such as cardiac mass, do not reflect the patients ability to participate in daily life. In the FEISTY, we will evaluate exercise capacity using a new CPX protocol and evaluate if the protocol can be used to evaluate existing ERT and future FD treatments, with a better relation to functional outcome than currently used parameters.

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Appendices

Dutch summary (Nederlandse samenvatting)

Fabry cardiomyopathie, naar een vroege diagnose en rationele follow-up

Hoewel een exact pathofysiologisch mechanisme nog onvoldoende is opgehelderd, is het bekend dat Globotriaosylceramide (Gb3) accumulatie in het hartweefsel progressieve hartfunctiestoornissen bevordert en bijdraagt aan vroegtijdige sterfte bij patiënten met de ziekte van Fabry (FD). Met de introductie van enzymvervangende therapie verschenen er meer studies die de cardiale manifestaties van FD beschreven. Echter, ontbraken de longitudinale studies die het klinisch verloop van Fabry- cardiomyopathie per fenotype en geslacht onderzochten. Een fenotype is het totaal van alle waarneembare eigenschappen van een ziekte. Binnen FD, maken wij onderscheid tussen het klassieke en het niet-klassieke fenotype. De hypothese is dat met veroudering geleidelijke veranderingen in elektrofysiologische, echocardiografische en biochemische markers ontstaan bij FD patiënten. Deze markers kunnen al afwijkend zijn vóór de klinische ziektemanifestatie. Zodra we weten wanneer en welke biomarkers beginnen te veranderen bij de verschillende patiëntengroepen en hoe deze gerelateerd zijn aan het optreden van cardiale complicaties, is het mogelijk om:

1. FD-patiënten die een risico lopen op het ontwikkelen van cardiale complicaties vroegtijdig te identificeren en te behandelen. Zo kan het ontstaan van onomkeerbare hartspier schade worden vertraagd, wat weer leidt tot het verbeteren van de ziekte prognose.
2. Klinisch relevante elektrofysiologische, echocardiografische en biochemische markers te identificeren, die clinici zullen ondersteunen in het evalueren van therapie effecten.
3. Follow-up protocollen te ontwikkelen, die rekening houden met deze prognostische markers, in combinatie met geslacht, fenotype en leeftijd van de patiënt.

De uitgevoerde studies in dit proefschrift omvatten observationele longitudinale retrospectieve studies bij een groep FD-patiënten, geclassificeerd op basis van geslacht en ziekte ernst (klassiek, niet- klassiek) binnen het Amsterdam Universitair Medisch Centrum (AUMC), Nederland. Er werden data verzameld die ondersteunde in de beschrijving van de cardiale complicaties, elektrofysiologische, beeldvormende (MRI en echocardiografie) en biochemische parameters bij FD patiënten.

Elektrofysiologische en echocardiografische gegevens van twee gezonde populatiecohorten (de Healthy Life in an Urban Setting (HELIUS) en de Rotterdam Navigator-cohort) werden ter vergelijking met FD patiënten gebruikt

om hiermee cardiale kenmerken, die typisch zijn voor FD, zowel in een vroeg als laat ziektestadium te herkennen.

In **hoofdstuk 2** werd de prevalentie en het tijdstip waarop hartklachten ontstaan geregistreerd bij 213 FD-patiënten met klassieke en niet-klassieke FD (gemiddelde follow-up tijd: 50 jaar). Bij mannen met klassieke FD vonden de klachten voornamelijk plaats vanaf het vijfde decennium van het leven. Bij vrouwen met klassieke FD en mannen met niet-klassieke FD werden klachten gemiddeld 10 jaar later waargenomen, met een grotere variatie in de leeftijd waarop de complicaties optraden en bij een kleiner percentage van de patiënten.

Tevens, beschreven wij het effect van geslacht en fenotype op het optreden van cardiale complicaties bij FD patiënten. Het risico op een belangrijke, majeure, cardiovasculaire complicatie (MACE) was, zoals verwacht, het grootst voor mannen met klassieke FD, gemiddeld voor vrouwen met klassieke FD en mannen met niet-klassieke FD en laag bij vrouwen met niet-klassieke FD. Belangrijk om te noemen is dat geen van de patiënten in deze laatste groep een MACE ontwikkelde. Het interessante is dat hartfalen (HF), en niet acute hartdood, zoals in vorige studies werd aangegeven, het meest werd geïdentificeerd als doodsoorzaak (42% van alle sterfgevallen). Meer dan de helft van de patiënten met ventriculaire ritmestoornissen vertoonde deze aritmieën in kader van een 'structureel' beschadigde hartspier (na een myocardinfarct) of evident hartfalen. Deze bevindingen werpen nieuw licht op de cardiale ziekte evolutie in FD en benadrukken het belang van nieuwe diagnostische en therapeutische strategieën, om zo HF bij FD-patiënten vroeg op te sporen en te behandelen.

In **hoofdstuk 3** onderzochten wij elektrocardiogram (ECG) markers, die vroege en late hartziekte manifestaties bij klassieke FD reflecteren. In totaal werden 1.995 ECG's van 133 patiënten met klassieke FD (maximale follow-up: 20 jaar, 80% behandeld met enzymvervangende therapie (ERT)), vergeleken met 3.893 ECG's van 'schijnbaar' gezonde controles uit het HELIUS-cohort. Er werd beoordeeld wat het effect is van leeftijd, FD en geslacht op zeven ECG-parameters (P-golfduur, PR- interval, QRS-duur, QTc, Cornell-index, ruimtelijke QRS-T-hoek en frontale QRS-as). De Cornell- index was hoger en de frontale QRS-as meer negatief bij FD-patiënten dan bij controles van 40 jaar of jonger. Voor de andere ECG-parameters bij jongvolwassen FD patiënten was de veranderingssnelheid, en niet de absolute waarde meer afwijkend ten opzichte van gezonde controles. Vanaf het vijfde decennium (mannen) of zesde (vrouwen) waren de absolute waarden voor de P-golfduur, QRS-duur, QTc en ruimtelijke QRS-T-hoek langer en hoger bij FD-patiënten dan bij controles. De geïndexeerde linker ventrikel massa (LVMi) op de cardiale MRI was gecorreleerd met elk van de onderzochte ECG- parameters. Bovendien hadden patiënten met myocardiale

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fibrose een langere en hogere P-golfduur, QRS-duur, QTc, Cornell-index en ruimtelijke QRS-T-hoek dan degenen zonder fibrose. Deze bevindingen tonen aan dat de ECG-abnormaliteiten die kenmerkend zijn voor FD variëren met leeftijd en geslacht, bijvoorbeeld een hogere Cornell-index in de jongvolwassenen en een verlengde QRS-duur op latere leeftijd. Het volgen van de veranderingssnelheid in ECG-parameters kan een goede manier zijn om ziekteprogressie te detecteren bij patiënten die subtiele ECG-veranderingen vertonen, maar nog geen veranderingen tonen op andere conventionele cardiovasculaire beeldvorming. Bovendien kan monitoring van ECG-veranderingen bij FD worden gebruikt om de behandel effectiviteit te evalueren, waarbij de huidige studie een referentiekader biedt voor vergelijking van nieuwe behandelingen.

Hoofdstuk 4 beschrijft de morfologische en functionele echocardiografische veranderingen bij patiënten met klassieke FD in vergelijking met gezonde controles. Dit onderzoek werd uitgevoerd omdat 1) de progressie van echocardiografische kenmerken van FD over lange follow-up periodes grotendeels onbekend was en 2) inzicht in de evolutie van echocardiografische parameters en hun relatie met cardiale complicaties kan helpen bij therapeutische besluitvormingen.

Voor 92 patiënten met klassieke FD werden de twee echocardiogrammen per patiënt met de langste follow-up duur opnieuw beoordeeld en geanalyseerd (58 vrouwen, mediane duur tussen de echo's: 12 jaar, 92% behandeld met ERT). De resultaten werden vergeleken met gegevens van 147 echocardiogrammen van gezonde personen (cross-sectionele onderzoek data). Er werd onderzocht wat het effect is van leeftijd, FD en geslacht op: de interventriculaire septumdikte in eind-diastole (IVSd), de relatieve wanddikte (RWT), de geïndexeerde linker ventrikel massa (LVMi), de geïndexeerde linker atrium volume (LAVI) en de verhouding van de vroeg diastolische mitralis inflow snelheid / vroeg diastolische septale weefsel mitralis annulus snelheid (E/e'). Voor de secundaire onderzoeksvraag werd de relatie tussen het eerste echocardiogram en de latere ontwikkeling van atriumfibrilleren (AF) bij FD getest. De vroegste tekenen van cardiale betrokkenheid op echocardiografie bij klassieke FD patiënten waren: verhoogde waarden voor IVSd en RWT bij alle FD-patiënten en E/e' bij de mannelijke patiënten. Verhoogde waarden van deze markers op het eerste echocardiogram waren geassocieerd met een verhoogd risico op AF op latere leeftijd. Ten opzichte van gezonde personen en gedurende het volwassen leven, namen IVSd, RWT, LVMi, LAVI en E/e' significant toe bij FD-patiënten. Deze bevindingen suggereren dat de progressie in de metingen die de linkerventrikel (LV), linker atrium morfologie en LV-diastolische functie weergeven, cardiale FD activiteit representeren. Het vergelijken van de absolute waarden van echocardiografische metingen bij FD-patiënten met leeftijds- en geslacht specifieke referentiewaarden

kan tevens worden gebruikt om de noodzaak van behandeling te bepalen, de voortgang van de ziekte te meten en het effect van bestaande en toekomstige interventies te evalueren.

Het vinden van een biomarker met een voorspellende waarde kan ondersteunen in het maken van Fabry-specifieke behandelbeslissingen en het vaststellen van rationele follow-up frequenties.

In **hoofdstuk 5** onderzochten wij of plasma-globotriaosylsphingosine (lysoGb3) niveaus stabiel bleven bij onbehandelde FD-patiënten. Ook werd er onderzocht of deze lysoGb3 niveaus gerelateerd zijn aan de cardiale en niet cardiale ziekte manifestaties binnen 237 onbehandelde FD-patiënten. Het vinden van een biomarker die evident verband heeft met het ziekteverloop ondersteund in de beslisvorming rondom het starten van Fabry-specifieke behandelingen, maar kan ook helpen in het vaststellen van een rationele follow-up frequentie voor een individuele FD-patiënt. Bij onbehandelde FD patiënten, bleef het plasma lysoGb3-niveau stabiel over de tijd, wat bevestigd dat het bruikbaar is als een ziekte indicator. De sterkste associatie die werd waargenomen, was die tussen plasma lysoGb3-niveaus en de LVMi. De associatie tussen plasma lysoGb3 en de echocardiografische markers die diastolische (dys)functie (RWT, LAVI, Septal e' en E/e') weergeven was een nieuwe bevinding. Hogere plasma lysoGb3-niveaus waren ook geassocieerd met een steilere helling in LAVI veranderingen. Deze resultaten zijn met name relevant, gezien het feit dat diastolische dysfunctie de belangrijkste bijdrage levert aan HF bij FD, en wat momenteel de grootste doodsoorzaak is bij FD-patiënten (Hoofdstuk 2 van dit proefschrift). Deze studie ondersteund dat lysoGb3 een relatie heeft met de veranderde cardiale morfologie (LVMi), en ook met de hartfunctie. Bovendien was het plasma lysoGb3 nauw verbonden met markers van nier- en hersenziekte. We concluderen dat het meten van plasma lysoGb3 tijdens de diagnose informatie geeft over de verwachte natuurlijke ziekteverloop van (hart)klachten, waarmee besluitvormingen rondom de behandeling en vervolgonderzoek bij FD-patiënten vergemakkelijken.

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Arabic summary

موجز الرسالة

اعتلال عضلة القلب في مرض فابري، نحو تشخيص مبكر و متابعة منطقية

من المعروف أن تراكم الدهون من نوع غلوبوتريوسيلسراميد (Gb3) في أنسجة القلب يؤدي إلى خلل تدريجي في وظائف القلب والموت المبكر في المرضى المصابين بمرض فابري. على الرغم من العدد المتزايد في الدراسات التي تصف الأعراض القلبية لمرض فابري، إلا أن الدراسات طويلة المدى التي تكشف مسار المرض التفصيلي ما زالت مفقودة. من المفترض أن هناك تغييرات تدريجية في الفيزيولوجيا الكهربية للقلب وسونار القلب والعلامات البيوكيميائية تنشأ مع زيادة عمر مريض فابري والتي بعضها يكون موجودًا قبل ظهور أعراض القلب. بمجرد أن نعرف الوقت الذي تتغير فيه هذه المؤشرات الحيوية عند مرضى فابري وكيف ترتبط بحدوث النوبات القلبية، فمن الممكن ان:

1) نشخص المرضى المعرضين لخطر الإصابة بنوبات قلبية وعلاجهم في وقت مبكر، مما يؤخر تلف عضلة القلب ويحسن التقييم المستقبلي للمرض.
2) نحدد المؤشرات الحيوية التي قد تساعد الأطباء في تقييم الآثار العلاجية.
3) نضع بروتوكولات متابعة مخصصة حسب جنس و عمر المريض.

الدراسات التي تم إجراؤها ضمن هذه الرسالة قائمة على الملاحظة في مجموعة من مرضى يعانون من مرض فابري والذين توبعوا في المركز الطبي الاكاديمي المتخصص بجامعة أمستردام بهولندا. تم جمع بيانات عن الحالة القلبية والفيزيولوجيا الكهربية للقلب والتصوير الطبي للقلب والمعايير البيوكيميائية، وتم مقارنتها ببيانات لمجموعات سكانية تتمتع بصحة جيدة. مقارنة هذه الخصائص الكلينكية لمرضى مرض فابري، بخصائص الأفراد الأصحاء، تتيح باب التعرف على السمات القلبية النموذجية لمرض فابري، في مرحلة المرض المبكرة والمتأخرة.

في الفصل الثاني، سجلنا معدلات حدوث النوبات القلبية وتوقيتاتها في مجموعة كبيرة مكونة من ٢١٣ مريض فابري (متوسط وقت المتابعة: ٥٠ سنة). في الرجال الذين يعانون من مرض فابري المبكر، حدثت النوبات بشكل أساسي من العقد الخامس من العمر. في النساء المصابات بمرض فابري المبكر والرجال الذين يعانون من مرض فابري المتأخر، لوحظت النوبات بعد العقد السادس من العمر.

هدفت هذه الدراسة أيضًا إلى وصف تأثير جنس المريض والحالة الظاهرية للمرض على حدوث نوبات قلبية. اختلفت مخاطر الآثار السلبية الكبيرة على القلب والأوعية الدموية (اختصار: MACE) بشكل ملحوظ بين مجموعات المرضى المصنفة حسب الحالة الظاهرية والجنس. كان أكبر خطر لحدوث MACE في الرجال المصابين بمرض فابري المبكر. وكان الخطر متوسطًا في النساء المصابات بمرض فابري المبكر والرجال المصابين بمرض فابري المتأخر. بينما كان الخطر منخفضًا في النساء المصابات بمرض فابري المتأخر، حيث لم يحدث أي نوبة MACE في هذه المجموعة من المرضى. ومن المثير للاهتمام أن قصور القلب وليس السكتة القلبية المفاجئة (كما أشير في دراسات سابقة) يعد اكبر مسبب لوفاة مرضى فابري (٤٢٪ من جميع الوفيات). أكثر من نصف المرضى الذين عانوا من عدم انتظام ضربات القلب البطيني توفوا إما بسبب عضلة القلب التالفة "من الناحية الهيكلية" (بعد ذبحة قلبية) أو بسبب قصور قلبي واضح. هذه النتائج تلقي ضوءًا جديدًا على المسار القلبي لمرض فابري وتؤكد على الحاجة المستمرة لايجاد أساليب تشخيصية وعلاجية جديدة لكشف القصور القلبي في مرض فابري و معالجته.

في الفصل الثالث، سعينا إلى تحديد علامات رسم القلب التي تعكس الخصائص القلبية المبكرة لمرض فابري وعلامات تطور المرض. تمت مقارنة ١٩٩٥ رسومات قلب على مدى ٢٠ عامًا في ١٣٣ مريضًا يعانون من مرض فابري (٨٠ ٪ عولجوا بالاستبدال الإنزيمي) لرسومات قلب في ٣٨٩٣ فردًا يتمتعون بصحة جيدة. قمنا بتقييم كيفية تأثير العمر ومرض فابري و جنس المريض على سبع مؤشرات رسم قلب (طول مدة الموجة P - الفاصل الزمني PR - مدة QRS - مؤشر كورنيل - QTc - زاوية QRS-T - محور QRS الأمامي).

قبل سن الاربعين كان مؤشر كورنيل أكبر وكان محور QRS الأمامي أكثر سلبية في مرضى فابري مقارنتاً بالافراد الصحيين. في بداية العمر كان معدل التغير الزمني لمؤشرات رسم القلب وليس القيمة المطلقة اكثر تغيرًا في مرضى فابري مقارنتاً بالافراد الصحيين. من العقد الخامس (في الرجال) أو السادس (في النساء)، كانت القيم المطلقة لمدة الموجة P ومدة QRS و QTc وزاوية QRS-T أطول وأعلى في مرضى فابري مقارنتاً بالافراد الصحيين. وجدنا علاقة بين كتلة البطين الأيسر على الرنين المغناطيسي و كل مؤشرات رسم القلب التي تم فحصها. بالإضافة إلى ذلك كان المرضى الذين يعانون من تليف القلب لهم قيم مدة موجة P ومدة QRS و QTc ومؤشر كورنيل وزاوية QRS-T أطول وأعلى من أولئك الذين لا يعانون من تليف قلبي. توضح هذه النتائج أن تشوهات رسم القلب التي تشير إلى مرض فابري تختلف باختلاف عمر وجنس المريض. على سبيل المثال كان مؤشر كورنيل أعلى في المرحلة المرضية المبكرة و كانت مدة QRS مطولة في أواخر مراحل المرض. من الممكن أن تتبع معدل التغيير في مؤشرات رسم القلب قد يكشف عن تطور المرض في مرحلة المرض المبكر مما قد يساعد في بدء العلاج الذين يظهرون تغيرات دقيقة في رسم القلب مع عدم وجود تغيرات في التصوير التقليدي للقلب. بالإضافة إلى ذلك يمكن استخدام تغيرات رسم القلب لتقييم فعالية العلاج حيث توفر الدراسة الحالية بيانات مرجعية لمقارنة العلاجات الجديدة.

الفصل الرابع يصف التغيرات المورفولوجية والوظيفية في سونار القلب في المرضى الذين يعانون من مرض فابري مقابل الضوابط الصحية. لقد أجرينا هذه الدراسة لأن ١) تطور خصائص سونار القلب لمرض فابري خلال فترات المتابعة الطويلة غير معروف إلى حد كبير، و ٢) النظرة الثاقبة لتطور مؤشرات سونار القلب وعلاقتها بالنوبات القلبية قد تساعد في اتخاذ القرار العلاجي السليم.

تم إعادة تقييم اول و اخر سونار القلب - مع أطول فترة متابعة بينهم - لكل مريض وتحليلهما لـ 92 مريضًا يعانون من مرض فابري في (٥٨ امرأة ، متوسط المتابعة الزمنية ١٢ عامًا، ٩٢٪ عولجوا بالاستبدال الإنزيمي). تمت مقارنة النتائج بـ١٤٧ سونار قلب لأفراد أصحاء. تم تحليل تأثير مرض فابري والعمر والجنس على سمك الحاجز الانبساطي بين البطينين (IVSd) وسمك الجدار النسبي (RWT) ومؤشر كتلة البطين الأيسر (LVMi) ومؤشر حجم الأذين الأيسر (LAVI) ونسبة سرعة التدفق التاجي الانبساطي المبكر / سرعة الحلقة التاجية لنسيج الحاجز الانبساطي المبكر ('E/e).

تم اختبار العلاقة بين سونار القلب الأول والتطور اللاحق للرجفان الأذيني (AF) في مرض فابري كهدف دراسة ثانوية. كانت العلامات المبكرة القلبية لمرض فابري في سونار القلب هي زيادة قيم IVSd و RWT في جميع مرضى فابري و 'E/e عند الرجال. ارتبطت القيم المطلقة المتزايدة لهذه العلامات في سونار القلب الأول بزيادة خطر الإصابة بالرجفان الأذيني لاحقًا. خلال حياة البالغين، يزداد IVSd و RWT و LVMi و LAVI و 'E/e بشكل ملحوظ في مرضى فابري مقارنة بالافراد الأصحاء. تشير هذه النتائج إلى أن مؤشرات سونار القلب التي تعكس البطين الأيسر (LV) والوظيفة الانبساطية للبطين الأيسر يمثلوا تطور المرض في مرضى

نتبع معدل التغيير بمرور الوقت لتحديد الحاجة إلى بدء العلاج ومراقبة تطور المرض وتقييم تأثير التدخلات العلاجية الحالية والمستقبلية الجديدة.

في الفصل الخامس، درسنا ما إذا كانت مستويات بلازما جلوبوتريوسيل سفينغوزين (LysoGb3) مستقرة بمرور الوقت في مرضى فابري غير المعالجين وكيف ترتبط هذه المستويات بأعراض فابري القلبية وغير القلبية من خلال دراسة ٢٣٧ مريضى فابري غير المعالجين. يُعد العثور على علامة حيوية ذات قيمة تنبؤية كبيرة أمرًا بالغ الأهمية لإثبات الحاجة إلى علاج خاص بمرض فابري وتكرار المتابعة الصحيحة لمريض مصاب بمرض فابري.

ظلت مستويات البلازما lysoGb3 مستقرة بمرور الوقت في مرضى فابري غير المعالجين مما يؤكد فائدتها كصفة مرضية فردية. أقوى ارتباط لوحظ كان بين مستويات البلازما lysoGb3 ومؤشر كتلة البطين الأيسر (LVMi). كان ارتباط البلازما lysoGb3 بعلامات سونار القلب الوظيفة الانبساطية (RWT و LAVI و Septal e' و E/e') بمثابة اكتشافًا جديدًا. هذا مهم بشكل خاص لأن الخلل الوظيفي الانبساطي هو المساهم الرئيسي في حدوث قصور قلبي لمرضى فابري والذي يعد حاليا السبب الرئيسي للوفاة في مرضى فابري (هذه الرسالة - الفصل الثاني). تشير هذه النتائج إلى أن lysoGb3 ليس له علاقة فقط بمورفولوجيا القلب المتغيرة (LVMi) ولكن أيضًا مع وظيفة القلب. بالإضافة إلى ذلك كانت البلازما lysoGb3 مرتبطة بشكل وثيق بأعراض أمراض الكلى والمخ. قد نستنتج أن قياس lysoGb3 في وقت التشخيص يعطي معلومات عن الدورة الطبيعية المتوقعة للأمراض القلبية و غيرالقلبية.

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Portfolio

Name PhD student: Mohamed El Sayed
PhD period: June 2018-July 2023
Names of PhD supervisor & co-supervisor: Prof. dr. C.E.M. Hollak and Dr. M. Langeveld

	Year	ECTS
Courses, seminars and masterclasses		
Clinical Epidemiology 1: RCT, Graduate School, Amsterdam UMC, AMC	2018	0.6
eCRF development in OpenClinica	2018	0.6
e-BROK, Graduate School, Amsterdam UMC, AMC	2019	1.0
Computing in R, Graduate School, Amsterdam UMC, AMC	2020	0.4
Writing a Scientific Article, Graduate School, Amsterdam UMC, AMC	2020	1.5
Observational Epidemiology: Effects and Effectiveness, Graduate School, Amsterdam UMC, AMC	2021	0.6
Didactical skills, Graduate School, Amsterdam UMC, AMC	2021	0.4
Weekly research meeting, dept of inherited metabolic diseases, AMC	2018-2022	4.0
Presentations		
The MODIFY study- annual meeting of the Fabry Support and Information Group, The Netherlands, Almere	2018	0.5
Cardiac events in Fabry disease- annual meeting of the Fabry Support and Information Group, The Netherlands, Almere	2019	0.5
Cardiac events in Fabry disease- symposium of the Dutch and Belgian Inherited metabolic disease Group, The Netherlands Utrecht	2019	0.5
Cardiac events in Fabry disease- symposium of the Society for the Study of Inborn Errors of Metabolism, The Netherlands, Rotterdam	2019	1.25
The role of cardiopulmonary exercise testing in Fabry disease (FEISTY) proposal- annual meeting of the Fabry Support and Information Group, The Netherlands, Almere	2021	0.5
The Fabry electro and echocardiography study-annual meeting of the Fabry Support and Information Group, The Netherlands, Almere	2022	0.5
Two posters, The Fabry electro and echocardiography study- symposium of the Society for the Study of Inborn Errors of Metabolism, Freiburg, Germany	2022	1.0
Two posters, The Fabry electro and echocardiography study- annual international Fabry meeting, Würzburg, Germany	2022	1.75
(Inter)national conferences		
Symposium of the Dutch and Belgian Inherited metabolic disease Group, The Netherlands Utrecht	2018	0.25
Annual international Fabry meeting, Prague, Czech Republic	2019	0.75

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Portfolio (continued)

	Year	ECTS
Teaching and supervising		
Master student Amina Aljic	2020	1.0
Mentor meetings- Professional development for Bachelor Medicine students, Amsterdam UMC, AMC	2019-2020	1.0
Clinical reasoning lessons for Bachelor Medicine students, Amsterdam UMC, AMC	2020	0.6
Organization skills Organized SPHINX research presentations, location AMC	2018-2022	0.5
Clinical skills Working as a medical doctor at the Fabry outpatient clinic and preparing multidisciplinary meetings	2018-2022	5.5
Other Writing a research protocol and setting up the Fabry Excersice Intolerance STudy (FEISTY)	2019-2022	3.0

Curriculum Vitae

Mohamed El Sayed is op 03 Januari 1993 geboren in Port-Said, Egypte. In 2009 behaalde Mohamed zijn General Certificate of Secondary Education (GCSE) in Port-Said. Hierna startte hij met een VASVU (voorbereidingsjaar voor anderstalige studenten aan de Vrije Universiteit) traject, dat hem klaarstoomde voor een Universitaire studie in Nederland.

In 2010 begon Mohamed met een Werktuigbouwkunde studie aan de TU Eindhoven. Geneeskunde bleek later beter bij hem te passen, waarna hij in 2011 werd ingeloot voor de Bachelor Geneeskunde aan de Vrije Universiteit in Amsterdam. Gedurende zijn studie heeft Mohamed zich geïnteresseerd in de Interne Geneeskunde.

Na zijn afstuderen in 2017, heeft Mohamed als arts-assistent Interne Geneeskunde in het BovenIJ ziekenhuis te Amsterdam gewerkt. In Juni 2018 begon hij met een promotietraject bij de Interne Geneeskunde- erfelijke stofwisselingsziekten aan het Amsterdam UMC, locatie AMC. Daar deed Mohamed onderzoek naar de klinische kenmerken van Fabry cardiomyopathie. Dit deed hij onder leiding van Prof. dr. C.E.M. Hollak en Dr. M. Langeveld. Naast het promotietraject werkte Mohamed voor verschillende medische NGO's, waaronder Dokters van de Wereld, Stichting Bootvluchteling en de Kruispost.

In Juni 2022, startte Mohamed een opleiding tot internist in regio Amsterdam/Tergooi MC.

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