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Target organ damage in Fabry disease

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Target organ damage in Fabry disease

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Editorial: Target organ damage in Fabry disease

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Editorial on the Research Topic
Target organ damage in Fabry disease

Introduction

The body of research dedicated to Fabry Disease (FD) has grown significantly in the last 25 years, concurrent to the availability of therapies for the disease. Currently, consolidate therapies include enzyme replacement and chaperone therapy. Experimental therapeutics, such as gene therapy and substrate supplementation, are in the pipeline to be deployed in the clinical realm.

With greater awareness among clinicians and researchers, the number of diagnosed cases has risen, along with the identification of putative pathogenic mutations in the GLA gene (encoding GalA). To date, more than 1,500 such mutations have been reported, and the number continues to grow. Some mutations have been shown to have a clear impact on the disease, while others remain of uncertain clinical relevance (1).

As more clinical cases are studied, the heterogeneity in the disease's clinical presentation has become increasingly apparent. The relationship between genotype and phenotype appears to be less straightforward than initially assumed, and target organs might be affected differently despite the presence of similar or even the very same mutation, in particular with variants of unknown significance. Notably, since the GLA gene is located on the X chromosome, significant sex-based differences in clinical manifestation have been observed (2). Males typically exhibit a more severe disease phenotype, with greater target organ involvement and shorter survival, while females often present with a milder clinical course and longer life expectancy (3). This calls for different management strategies between male and female Fabry patients, as pointed out by Tuttolomondo et al.

All of the above suggests that the mechanisms of FD might be more complicated than originally thought. In a classical vision, the damage of target organs such as the heart, kidney, brain, bowels, and vasculature is due to the intracellular accumulation of globotriaosylceramide (Gb3), which leads to cellular dysfunction and therefore loss of organ function (4). Such a vision, though, implies an important GB3 intracellular accumulation that in some cases is barely present or even missing entirely. Although lysosomal dysfunction can be considered the beginning of cellular dysfunction, other mechanisms need to be taken into account that might accelerate or delay the onset of the target organ damage associated with the disease (5) (Figure 1).

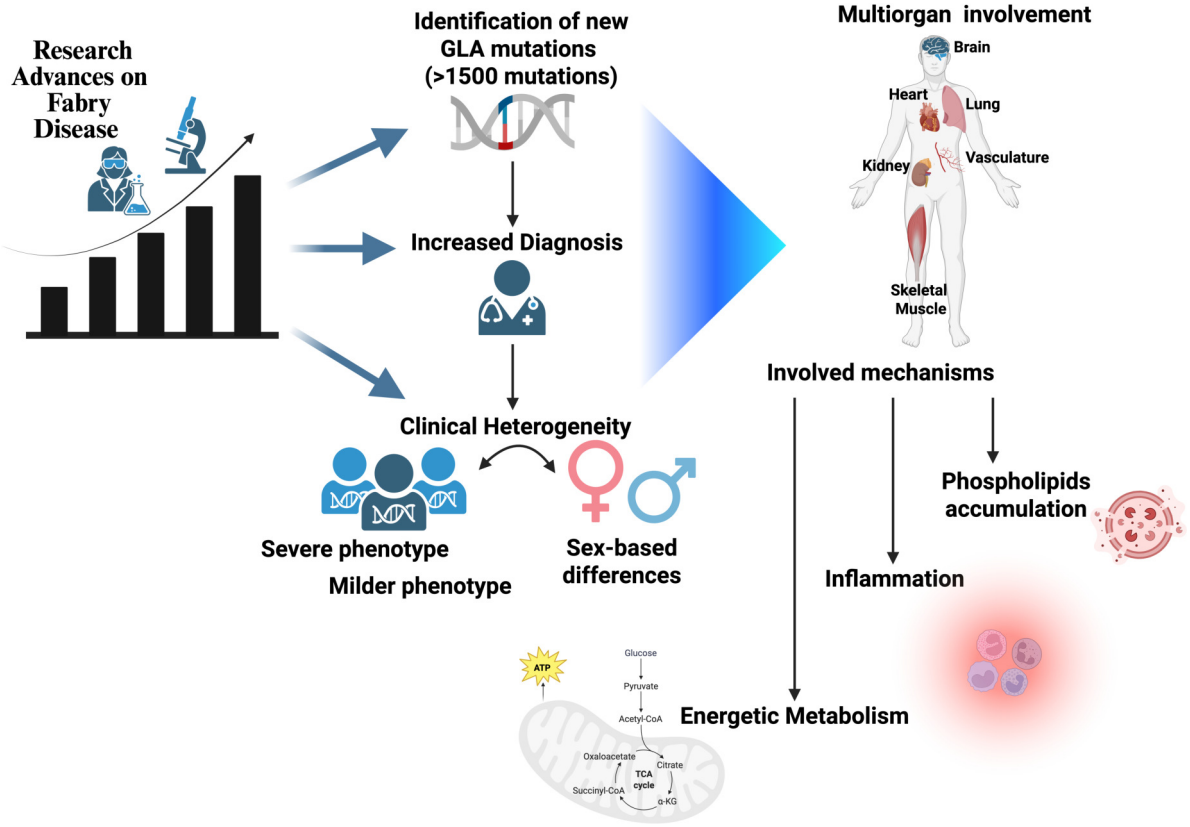

FIGURE 1
An increased volume of papers in recent years has revealed the complexity of Fabry disease, which presents with intermediate phenotypes, different involvment of target organs, and a non-univocal relationship between genotype and phenotype. Sex is one determinant of such variability; novel mechanisms of disease can also be considered as possible modifiers.

In this Research Topic dedicated to the Target Organ Damage (TOD) in FD, we focused on the current interpretation of clinical manifestation.

Twelve papers, with over 100 authors and more than 18,000 total views at the time this editorial is written, confirm the renewed interest in this condition.

The heart

The heart is the target organ of FD that has been most often investigated. In this RT, it is clear that the cardiac ultrasounds (cardiac US) remains the easiest and most accessible way to assess cardiac damage in FD. All cardiac structures, such as the left ventricle and the left atrium, the aorta, the right sections, and the heart valves, can be affected by morphological and functional abnormalities. Conte et al. state that standard echocardiography has a crucial role in the characterization of FD cardiomyopathy and provide a comprehensive review on the topic. Furthermore, echocardiographic evaluation is an essential imaging method to support the physician in follow-up and risk stratification. Spinelli et al. suggest that techniques such as tissue Doppler imaging and speckle-tracking echocardiography (STE) allow detection of subclinical changes in left ventricular (LV) systolic and diastolic function, particularly reductions in global longitudinal strain and circumferential strain gradients. These techniques are also valuable for evaluating right atrial and ventricular involvement, often preceding hypertrophy. Lillo et al. performed STE analysis of the right atrium and revealed impaired strain values of this structure and all RA strain phases in patients with FD. When considering FD patients without left ventricle hypertrophy (LVH), RA reservoir and contractile strains were significantly reduced. The right atrium is therefore another candidate parameter to monitor cardiac TOD in FD.

Indeed, the most advanced technology for the analysis of cardiac structural abnormalities is the MRI, which can provide insight into cardiac hypertrophy features of the Fabry patient. Tondi et al. show that Papillary Muscle hypertrophy is more pronounced in FD, and mitral valve anatomy alterations progressively worsen with advancing FD stages. The findings highlight papillary muscle hypertrophy and mitral valve anatomy abnormalities as potential early markers of cardiac involvement in FD and recommend their routine assessment during cardiac magnetic resonance (CMR) in patients with hypertrophic cardiomyopathies.

Finally, FD often associates with arrhythmias that need to be monitored. To this aim, Roy et al. demonstrate that implantable loop recorders might help to explore the impact of the disease, irrespective of LVH.

The kidney

Kidney failure is another typical manifestation of the TOD in FD. Rozenfeld et al. focus on Renal fibrosis and consider it as the end result of multiple damages, including inflammation, cell migration, differentiation, and increased extracellular matrix production. In Fabry nephropathy, these effects occur within the kidney tubule, following initial damage caused by lysosomal dysfunction and disruption of Gb3–LysoGb3 activity. Concurrent to mitochondrial failure, kidney tubular cells requiring healthy energetic metabolism to function are the first cells to be damaged in the kidney. Therefore, the contribution of the tubular epithelial cells and the interstitial space to Fabry nephropathy is important from its initiation to its progression and may contribute to the pathogenesis of renal injury.

Fatigue

Energetic metabolism is also considered a mechanism to fatigue and reduced exercise tolerance in FD, according to De Marco et al. and to Gambardella et al. Fatigue, often an early and independent symptom, can be due to cardiac and pulmonary dysfunction as well as impairments in skeletal muscle. In FD, skeletal muscle bioenergetic alterations such as mitochondrial impairment, metabolic inflexibility, and increased glycolysis might explain the fatigue. Other mechanisms such as inflammation, muscle atrophy, and vascular and neuronal dysfunction further contribute. Cardiopulmonary exercise testing and biomarkers like lactate and mitomiRs might help in the stratification of the clinical condition of patients with FD diagnosis. Enzyme replacement therapy offers limited relief, while personalized exercise programs might offer a more tailored approach to improve patient care and quality of life.

Inflammation and endothelium

Inflammation is an emerging mechanism for TOD in FD. Reading the review of Kurdi et al., the main activator of inflammation is the accumulation of sphingolipids, resulting from the deficiency of alpha galactosidase, which triggers cellular stress. Acute, chronic, and resolving stages are a continuum rather than strictly distinct, although each stage has a recognized hallmark. There are also overlaps between the innate and adaptive immune responses, suggestive of an autoinflammatory component to FD. Inflammation is the possible mechanism behind endothelial dysfunction in FD, which can be monitored through serum levels of VEGF. According to Lund et al. this parameter correlates with markers of renal and cardiac damage in FD patients and can be considered a useful biomarker for endothelial dysfunction.

Conclusion

Research into FD still has many questions to answer, although it has opened new fields of investigation. The success of this RT shows the need for new papers for "Frontiers in Research topic: Target Organ Damage in Fabry Disease 2.0".

Author contributions

GI: Conceptualization, Writing – review & editing, Writing – original draft. FG: Supervision, Writing – review & editing.

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Clinical utilisation of implantable loop recorders in adults with Fabry disease—a multi-centre snapshot study

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Fabry disease (FD) is an X-linked deficiency of alpha-galactosidase-A, leading to lysosomal storage of sphingolipids in multiple organs. Myocardial accumulation contributes to arrhythmia and sudden death, the most common cause of FD mortality. Therefore, there is a need for risk stratification and prediction to target device therapy. Implantable loop recorders (ILRs) allow for continual rhythm monitoring for up to 3 years. Here, we performed a retrospective study to evaluate current ILR utilisation in FD and quantify the burden of arrhythmia that was detected, which resulted in a modification of therapy. This was a snapshot assessment of 915 patients with FD across three specialist centres in England during the period between 1 January 2000 and 1 September 2022. In total, 22 (2.4%) patients underwent clinically indicated ILR implantation. The mean implantation age was 50 years and 13 (59%) patients were female. Following implantation, nine (41%) patients underwent arrhythmia detection, requiring intervention (six on ILR and three post-ILR battery depletion). Three patients experienced sustained atrial high-rate episodes and were started on anticoagulation. Three had non-sustained tachyarrhythmia and were started on beta blockers. Post-ILR battery depletion, one suffered complete heart block and two had sustained ventricular tachycardia, all requiring device therapy. Those with arrhythmia had a shorter PR interval on electrocardiography. This study demonstrates that ILR implantation in FD uncovers a high burden of arrhythmia. ILRs are likely to be underutilised in this pro-arrhythmic cohort, perhaps restricted to those with advanced FD cardiomyopathy. Following battery depletion in three patients as mentioned above, greater vigilance and arrhythmia surveillance are advised for those experiencing major arrhythmic events post-ILR monitoring. Further work is required to establish who would benefit most from implantation.

KEYWORDS

Fabry disease, arrhythmia, stroke, implantable loop recorder, sudden death

Introduction

Fabry disease (FD) is an X-linked lysosomal storage disorder manifesting in multi-organ accumulation of sphingolipids, such as globotriaosylceramide (Gb3), resulting in cardiac, renal, and cerebral manifestations (1). Sphingolipids accumulate because of alpha-galactosidase-A enzyme deficiency (2). Alpha-galactosidase-A is responsible for the breakdown of terminal galactose from Gb3, resulting in lysosomal accumulation of Gb3 (1). Cardiac sphingolipid accumulation occurs in all cardiac cell types, resulting in left ventricular hypertrophy (LVH), myocardial inflammation, fibrosis, and scarring (3). These effects contribute to the high prevalence of arrhythmia in FD, and it is noteworthy that sudden cardiac death (SCD) is the most common form of FD mortality (4, 5). Studies indicate that the rate of frequency of malignant ventricular arrhythmia may be as high as 30% (6). Atrial fibrillation (AF), bradyarrhythmia, ventricular tachyarrhythmia, and SCD may indeed be the first manifestations of cardiomyopathy before the occurrence of clinical or imaging abnormalities (6). However, in published literature, it is shown that the true prevalence of arrhythmia is dependent on both the stage of the disease and the duration of monitoring, with high rates detected on implantable loop recorders (ILRs) in individuals with advanced cardiomyopathy (7). The rates of atrial high-rate episodes (AHREs) detected on implanted cardiac devices are also high (8). This is associated with a higher prevalence of stroke events (9). However, the benefits of anticoagulation for AHREs in the absence of electrocardiogram (ECG) evidence in the general population are outweighed by bleeding risk (10).

To date, research into arrhythmia in FD has been limited to single-centre studies using 12-lead ECG or short-term (<48 h) Holter monitoring, with no evidence from large multi-centre collaborative studies. Current guidelines in the general population recommend ILR implantation in patients who suffer recurrent unexplained symptoms potentially related to arrhythmia after initial assessment that includes patient history, examination, ECG, and transthoracic echocardiography (TTE) (11). Although there are no specific guidelines for treating FD, ILRs have the added benefit of long-term, continual rhythm monitoring for up to 3 years. This may be particularly advantageous in FD cardiomyopathy, which is insidious. Given the potential benefit of ILRs as a tool for arrhythmia detection in FD, we performed a multi-centre retrospective clinical cross-sectional study on the use of ILRs in FD. Our aims were as follows:

1. To provide a comprehensive evaluation of the clinical utilisation of ILRs in FD and
2. To quantify the detected arrhythmia burden, which resulted in a modification or initiation of therapy.

Methods

This was an observational, retrospective cross-sectional snapshot review of cardiovascular data collected from adults over 18 years of age with genetic or enzymatic confirmation of FD who were referred to three large UK centres managing adults with FD (Queen Elizabeth Hospital, Birmingham; Salford Royal Hospital, Salford; and Royal Free Hospital, London). These centres offer a one-stop service whereby patients undergo detailed clinical assessment including 12-lead ECG, TTE, and cardiac magnetic resonance (CMR) imaging, according to current guidelines (11). All patients were screened between 1 January 2000 and 1 September 2022, and those with prior or current ILRs were included in the analysis. Data were extracted from investigations performed within the preceding 12 months.

This study was approved by local clinical governance committees (CARMS-13350) and it conformed to the principles of Good Clinical Practice guidelines. Ethical approval was obtained for the conduct of this study (IRAS 325613 23/WM/0180). The inclusion and exclusion criteria are detailed below.

Inclusion criteria were as follows:

1. Adults over 18 years of age.
2. Genetic or enzymatic confirmation of FD.
3. History of prior or current ILR implantation on clinical grounds.

Exclusion criteria were as follows:

1. ILR implantation on research grounds.

Electrocardiography

Standard 12-lead ECGs were acquired according to current standardised guidelines for acquisition and interpretation (12).

Transthoracic echocardiography

TTE (iE33/EPIC, Phillips; and Vivid, GE) was performed by an accredited sonographer in accordance with the minimum data set guideline of the British Society of Echocardiography (13). The chamber size and function were measured according to current standard guidelines (14). Parameters for assessment of diastolic function were acquired according to the general principles for TTE assessment established by the American Society for Echocardiography in association with the European Association of Cardiovascular Imaging (15). Diastolic function was graded by an experienced cardiologist specialising in TTE according to current guidelines (15).

Cardiac magnetic resonance imaging

Contrast-enhanced CMR (1.5 T Avanto, Siemens Healthcare, Erlangen, Germany) was performed in line with standard protocols to obtain left ventricular (LV) dimensions, volumes, and mass (16). A steady-state free precision single breath-hold modified Looker Locker inversion recovery (MOLLI) sequence was used for T1 mapping in the basal and mid LV short-axis levels and horizontal long axis, before and 15–20 min after administration of the gadolinium-based contrast agent (17). Imaging for assessment of late gadolinium enhancement (LGE),

regional and global T1, and myocardial extracellular volume was performed as previously described (18).

Statistical analysis

The baseline demographics of the cohort were summarised, with continuous variables reported as means ± standard deviations (SDs). Where two continuous variables were being compared, normality was assessed using the Shapiro–Wilk test. Where data were normally distributed, an unpaired *t*-test was performed. When comparing the relationships between two quantitative and independent variables, a simple linear regression analysis was performed. A p-value < 0.05 was deemed to be indicative of statistical significance throughout. All analyses were performed using GraphPad Prism version 9.3.1, GraphPad Software, San Diego, CA, USA (www.graphpad.com).

Results

In this snapshot assessment, 915 patients with FD across three specialist treatment centres in England were identified between 1 January 2000 and 1 September 2022. Of these, 22 (2.4%) patients underwent clinically indicated ILR implantation. The mean age at the time of implantation was 49.6 ± 9.9 years and 13 (59%) patients were female. The mean systolic blood pressure (BP) level was 127 ± 24 mmHg and diastolic BP level was 75 ± 13 mmHg. The mean body mass index (BMI) was 27 ± 8 kg/m^2. BMI and systolic BP significantly increased with age ($p = 0.0434$ and $p = 0.0287$, respectively) as illustrated in **Figure 1**. At the time of implantation, six (27%) patients experienced hypertension, three (14%) had renal impairment defined at an estimated glomerular filtration rate <90 ml/min, six (27%) had a prior cerebrovascular accident (CVA), and 18 (82%) were on enzyme replacement therapy (ERT). Cohort characteristics are described in **Table 1**.

At the time of implantation, 21 (96%) patients had a sinus rhythm and one patient had AF. The mean heart rate, PR interval, QRS duration, and QTc were in the normal range. Altogether, 21 (95%) patients had TTE imaging data available. The mean left ventricular ejection fraction (LVEF) was in the upper/normal range at 64% ± 6% and the mean maximum wall thickness (MWT) was elevated at 16 ± 18 mm. The mean left atrial (LA) volume was in the normal range at 44 ± 18 ml. Among the patients whose diastolic function could be assessed, 14 (67%) had evidence of diastolic dysfunction.

TABLE 1 Cohort characteristics.

Variable	Number	Mean	SD
Demographics			
Age at implant	22	49.60	9.920
HR (bpm)	16	72.40	11.000
Systolic BP (mmHg)	16	127.00	23.900
Diastolic BP (mmHg)	16	74.90	12.600
Height (m)	21	1.68	0.085
Weight (kg)	21	75.50	18.700
BMI	16	26.80	7.900
Electrocardiogram			
PR interval (ms)	21	149.00	21.800
QRSd (ms)	22	105.00	20.300
Cardiac magnetic resonance imaging			
LVMi (g/m^2)	13	123.00	53.500
MWT (mm)	16	18.20	5.860
LVEDVi (ml/m^2)	16	67.90	16.200
LVESVi (ml/m^2)	16	20.20	8.020
LVSVi (ml/m^2)	15	37.40	18.300
LVEF (%)	18	70.40	5.280
Septal T1 (ms)	13	898.00	98.800
Transthoracic echocardiogram			
LVEF (%)	20	64.20	6.110
MWT (mm)	19	15.60	3.750
LA volume (ml)	17	44.10	17.800

LVSVi, indexed left ventricular stroke volume.

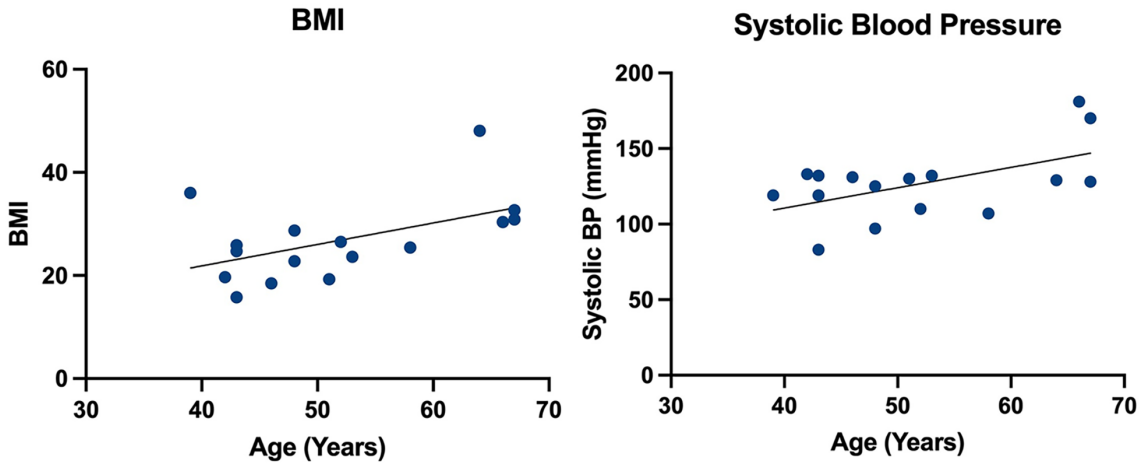

FIGURE 1
Simple linear regression of age vs. BMI and systolic blood pressure.

Eighteen (82%) patients had CMR imaging data available. On CMR, 16 (89%) patients had evidence of LVH, with the mean indexed left ventricular mass (LVMi) elevated at 123 ± 54 g/m^2 with an MWT of 18 ± 6 mm. The mean indexed left ventricular end-diastolic volume (LVEDVi) was 68 ± 16 ml/m^2 and indexed left ventricular end-systolic volume (LVESVi) was 20 ± 8 ml/m^2. The mean LVEF was supranormal at 70% ± 5%. Nine (50%) patients had reduced septal T1 relaxation times, indicative of sphingolipid accumulation with a mean septal T1 of 898 ± 99 ms. Fourteen (78%) patients had evidence of LGE, indicative of myocardial fibrosis.

Indications for ILR implantation varied. Twelve (55%) patients underwent implantation for recurrent and persistent symptoms despite normal ECG and Holter monitoring. Of these, eight patients had palpitations and four reported syncopal episodes. Five (23%) were asymptomatic but underwent implantation to seek undiagnosed AF as an aetiology following cryptogenic CVA. Four (18%) were asymptomatic but underwent implantation because of abnormal Holter monitoring [one non-sustained ventricular tachycardia (VT) under 5 s, two sinus pauses between 1 and 2 s, and one AF detection under 10 s]. None of the arrhythmias were detected by Holter-mandated therapy. These patients were all deemed high risk for arrhythmia with evidence of LVH and LGE on CMR with a broad QRSd on ECG. One (4%) patient was asymptomatic with a normal Holter monitor result but underwent ILR implantation and was deemed high risk for arrhythmia with LVH and LGE on CMR with a broad QRSd on ECG.

Following ILR implantation, arrhythmia was detected in nine (41%) patients, who required the initiation of or a change in therapy. Of these nine, six episodes of arrhythmia were detected on ILRs and three were detected within 1 year following ILR battery depletion. Of these patients, one was in the group of four with documented arrhythmia on Holter monitoring; the remaining three did not develop arrhythmia on ILRs or post-ILRs. Of the six patients with arrhythmia on ILRs, three had recurrent and prolonged AHREs for over 6 hours. Given the duration and frequency of these AHREs, they were started on anticoagulation therapy. None of these patients had documented arrhythmia prior to ILR implantation. One had a high burden of symptomatic ventricular ectopy (11%) and was initiated on a beta blocker. This patient had non-sustained VT on Holter prior to implantation. Two had symptomatic non-sustained VT and were also started on beta blockers. Of the three with arrhythmia that occurred 1 year after ILR battery depletion, one was admitted with acute syncope and was found to have prolonged pauses and complete heart block, requiring isoprenaline. The three subsequently underwent dual-chamber implantable cardiac defibrillator (ICD) implantation. Two were admitted with syncope and were found to have bursts of sustained VT on inpatient telemetry. Both these patients underwent dual-chamber ICD implantation. None of the three patients with documented arrhythmia post-ILRs showed evidence of arrhythmia prior to Holter monitoring.

By comparing the characteristics in the arrhythmia cohort vs. those without, it was found that those in the arrhythmia group had a significantly shorter PR interval (although still normal) on 12-lead ECG than those without ($p = 0.0402$), as illustrated in Figure 2, with no other ECG changes. Otherwise, no significant differences were observed between the two groups.

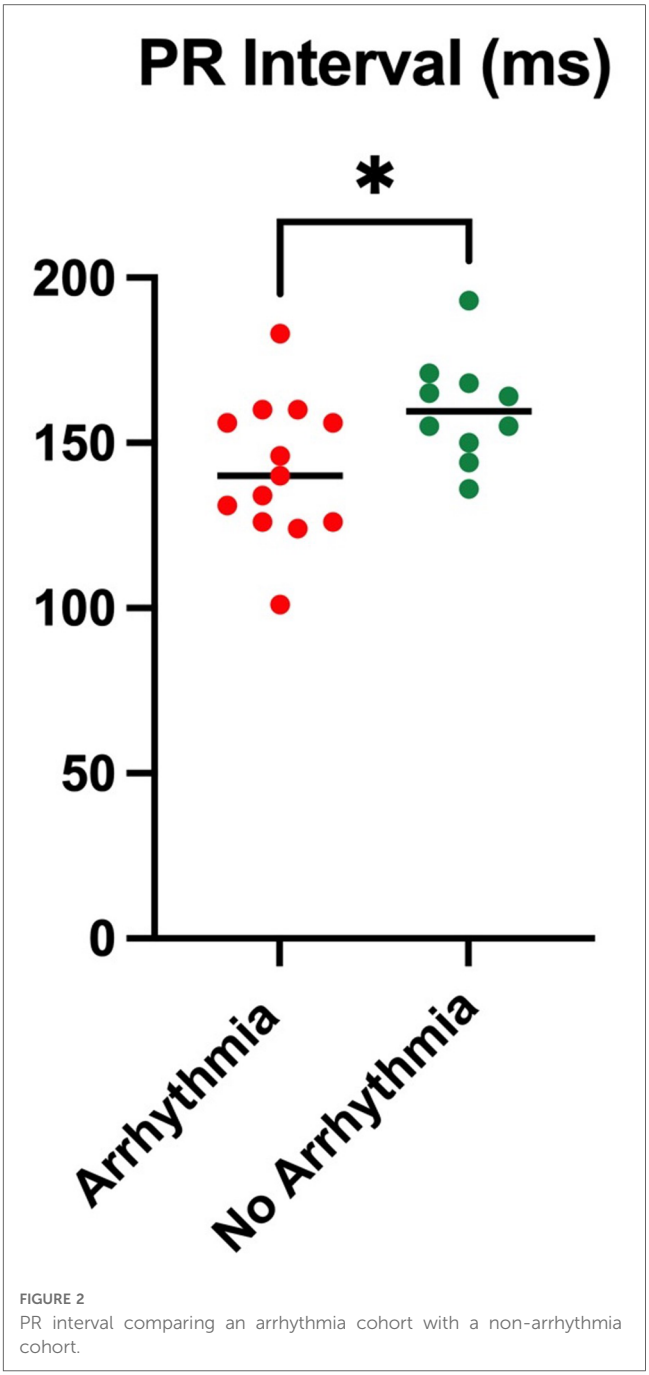

FIGURE 2
PR interval comparing an arrhythmia cohort with a non-arrhythmia cohort.

Discussion

This multi-centre snapshot assessment is the first of its kind to report the clinical utilisation of ILRs in FD. We demonstrated that the current UK practice of ILR implantation in FD uncovers a higher-than-expected burden of arrhythmia, requiring initiation or modification of therapy. Our findings are in keeping with

those from a previously published single-centre study in adults with advanced FD cardiomyopathy, who demonstrated a high burden of arrhythmia on ILR but no arrhythmia on Holter monitoring (7). However, the patients in that study were included on the basis of advanced disease and did not undergo ILR implantation on clinical grounds. The burden of arrhythmia identified in this study was higher than observed in a previous single and multi-centre study in adults without FD who underwent ILR implantation. In the single-centre study population (post-cryptogenic CVA with no history of arrhythmia), no patients with ILRs implanted developed arrhythmia, and in the multi-centre study population (aged 70–90 years with at least one additional CVA risk factor but no history of CVA), 31.8% developed arrhythmia (19, 20).

Our findings suggest that patients undergoing ILR implantation on clinical grounds have advanced disease, which is evidenced by an increased indexed left ventricular mass, MWT, and supranormal LVEF, and the majority of them have evidence of LGE on CMR indicative of myocardial fibrosis. Unsurprisingly, because of this, the arrhythmia detection rate is high, as these factors are shown to predispose to tachy- and bradyarrhythmia in FD (6, 21). There were no differences in mass, MWT, LVEF, and presence of LGE in those with or without arrhythmia, and this largely reflects the heterogeneity of the cohort, with the majority of patients already having advanced disease at the time of ILR implantation. Furthermore, we also demonstrated that the BMI of patients in this cohort increases with age. The effects of elevated BMI and obesity alter cardiac hemodynamics and probably contribute to the development of LVH in this cohort through systemic inflammation, insulin resistance, and subsequent cardiac remodelling, termed obesity cardiomyopathy (22).

ILRs allow for continual recording and can record episodes of AHRE, which we have demonstrated. As these may resemble AF, clinicians may initiate anticoagulation therapy in the presence of risk factors for stroke (9). This is particularly relevant in FD where the risk of stroke is greater than in the general population. However, a recent large multi-centre study was conducted comparing anticoagulation vs. no anticoagulation in adults with AHRE and at least one risk factor for stroke. This study of 2,536 patients demonstrated that anticoagulation resulted in a higher rate of major bleeding and death compared with placebo, and did not significantly reduce the incidence of stroke, systemic embolisation, and cardiovascular death (10). These findings suggest that even if additional risk factors are present, as is the case in FD, anticoagulation for AHREs without ECG confirmation of AF may not reduce stroke prevalence but rather cause an additional risk of bleeding. At the time when these data were collected and decisions were made about therapy, it was thought that the risk of anticoagulation was outweighed by its potential benefit given the frequency of AHREs (10). A recently published study has highlighted that this may not be the case. Therefore, it is unclear whether these patients should or should not be anticoagulated.

Published outcome data suggest that cardiac symptoms are reported to be more than 60% in men and 45% in women, with symptoms including angina, palpitations, dyspnoea, and syncope (23). Within our cohort, we report a disproportionately low rate of ILR implantation, given the high prevalence of cardiac symptoms reported in FD. With the frequency of symptoms in the FD population and the known risk of arrhythmia, ILR implantation seems to be overly restricted to those with the most advanced stages of FD cardiomyopathy.

PR interval changes are commonly reported in FD, and PQ interval shortening may indeed be one of the earliest features of cardiac involvement. Changes in PQ interval and *P*-wave duration are presumed to be secondary to atrial sphingolipid deposition, which may take place prior to the onset of LVH (24). We demonstrated that in those with arrhythmia detected on ILRs, the PR interval was shorter, although still within the normal range, compared with the arrhythmia-free cohort. This may suggest that the effects of sphingolipid accumulation alter the conduction pathways between the sinoatrial and the atrioventricular nodes, which has been demonstrated previously (25).

Interestingly, after 3 years of ILR monitoring, a proportion of the cohort subsequently suffered major adverse arrhythmic events, all of whom subsequently required dual-chamber ICD therapy. None of the patients underwent repeat ILR implantation following battery depletion. Among those who underwent Holter monitoring post-ILRs, none of them demonstrated arrhythmia. This highlights the fact that despite the effectiveness of a 3-year continual cardiac rhythm monitoring with ILRs, vigilance is advised in patients with FD. In line with local guidance, patients with FD should continue to undergo frequent ambulatory monitoring, especially given the progressive nature of FD cardiomyopathy with the subsequent arrhythmic risk increasing with age and disease progression (6).

Limitations

Given the fact that FD is a rare disease, the inevitable limitation of this study is its relatively small sample size, despite the pooling of data from three of the UK's largest centres for managing patients with FD. Although the number of patients analysed was small, this largely reflects the low utilisation of ILRs, as the total of 915 patients represents one of the largest adult cohorts available in FD. It is also an accepted fact that centres have various reporting criteria and different parameters for the investigations conducted, which can limit the pooling of data for comparison, particularly with T1 mapping on CMR.

Four patients underwent ILR implantation with previously documented arrhythmia on Holter monitoring. They underwent implantation for prolonged monitoring to assist with decision-making regarding therapeutic cardiac device therapy and/or further medical therapy. These patients will be pre-selected for an arrhythmia substrate and therefore are more likely to experience arrhythmia compared to those without documented arrhythmia prior to ILR implantation.

It is also important to note that three patients developed arrhythmia post-ILR battery depletion. This suggests that although ILRs allow for prolonged continuous screening,

arrhythmia may still be a case of missed diagnosis. A select group of patients with high arrhythmic risk may be considered for re-implantation of ILRs following battery depletion.

Conclusion

This study demonstrates a high arrhythmia burden detected in adults with FD using ILRs compared to the non-Fabry population, which is in keeping with published literature. ILR implantation is undertaken in patients with advanced FD cardiomyopathy at a stage when their arrhythmic risk is high, and therefore, it is probable that ILRs will be implanted on them at a later stage. ILRs allow for prolonged monitoring and should be considered at an early stage in FD to detect ventricular arrhythmia, conduction disease, and AF. This may reduce the prevalence of SCD and stroke in FD. However, a large-scale study is needed in this area to understand the subgroup of patients who would benefit most from ILR implantation.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material; further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by West Midlands—South Birmingham Research Ethics Committee (23/WM/0180 IRAS 325613). The studies were conducted in accordance with the local legislation and institutional requirements. The Ethics Committee/institutional review board waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because data were acquired from a research database using routinely collected clinical data for the purpose of research.

Author contributions

AR: Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Resources, Validation, Writing – original draft, Writing – review & editing. RV: Investigation, Methodology, Validation, Writing – review & editing. HK: Writing – review & editing. CO: Writing – review & editing. PW: Writing – review & editing. MK: Writing – review & editing. AJ: Writing – review & editing. CM: Writing – review & editing. JM: Writing – review & editing. DH: Writing – review & editing. TG: Writing – review & editing. RS: Writing – review & editing.

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Conflict of interest

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Fatigue as hallmark of Fabry disease: role of bioenergetic alterations

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Fabry disease (FD) is a lysosomal storage disorder due to the impaired activity of the α-galactosidase A (GLA) enzyme which induces Gb3 deposition and multiorgan dysfunction. Exercise intolerance and fatigue are frequent and early findings in FD patients, representing a self-standing clinical phenotype with a significant impact on the patient's quality of life. Several determinants can trigger fatigability in Fabry patients, including psychological factors, cardiopulmonary dysfunctions, and primary alterations of skeletal muscle. The "metabolic hypothesis" to explain skeletal muscle symptoms and fatigability in Fabry patients is growing acknowledged. In this report, we will focus on the primary alterations of the motor system emphasizing the role of skeletal muscle metabolic disarrangement in determining the altered exercise tolerance in Fabry patients. We will discuss the most recent findings about the metabolic profile associated with Fabry disease offering new insights for diagnosis, management, and therapy.

KEYWORDS

Fabry, metabolism, fatigue, skeletal muscle, exercise intolerance

1 Introduction

Fabry disease (FD) is an inherited X-linked lysosomal storage disorder due to the impaired activity of the α-galactosidase A (GLA) enzyme. The partial or total inactivity of this enzyme, essential to metabolize glycosphingolipids (GB3), induces the deposition of GB3 in lysosomes of cells in several organs and tissues evolving progressively toward multiorgan dysfunction (1, 2). The molecular pathogenesis of FD encompasses several pathologic mechanisms involving mitochondrial dysfunction, lysosomal dysfunction, GB3 accumulation, endothelial dysfunction, and autophagy abnormalities (3, 4). Today, more than 1,000 GLA gene variants have been identified in the chromosomal region Xq22.1, including splicing alterations, deletions, translocations, complex gene rearrangement, and point missense variants (5, 6), but an exact genotype-phenotype correlation in FD cannot be established. The signs and symptoms of FD are heterogeneous with high variability among patients (7). Males carrying the defective gene will develop the pathology with often severe clinical manifestations while females, once thought to be just asymptomatic carriers, could develop the disease with mild to severe signs (8). The complete loss of enzyme function is usually associated with the

"classic" phenotype with severe symptoms appearing in childhood while the residual enzyme activity may lead to a slow progression of the disease (the "late-onset" phenotype) with milder symptoms occurring in adults. Pain, and gastrointestinal (9) ocular, ear, or skeletal manifestations are the earliest symptoms of Fabry disease, which are not always immediately associated with FD. If left untreated, this multisystemic disease is progressive with cardiac and renal involvements as severe complications (10–12). FD may also manifest with mild nonspecific symptoms frequently affecting the musculoskeletal system (13).

2 Therapies and management of FD

As a multiorgan pathology, FD management requires multidisciplinary care involving cardiology, nephrology, gastroenterology, neurology, psychology, and genetics, aiming to treat the specific symptoms by available current therapies, ameliorate the quality of life, manage pain, and give psychosocial support. Guidelines for the diagnosis and treatment of FD are available for both adults (14, 15) and children (16), establishing the inclusion and exclusion criteria for treatments. The disease presents a variable expression of symptoms, which are also gender and age-dependent, therefore, their association with FD is very difficult. To identify Fabry patients and avoid delay or lack of diagnosis, it is essential to consider all potential conditions that could generate "clinical suspicion" and trigger the diagnostic process as soon as possible.

The FD diagnosis is mainly based on the genetic screening of GLA gene mutations and activity, especially for women as enzymatic levels of the female heterozygote could not associate with the pathologic manifestations. Such diagnosis is then confirmed by the analysis of urine Gb3 and plasma lyso-Gb3 levels.

Available treatments are based on the administration of intravenous/oral specific drugs and adjuvant therapies (renal, cardiac, neurological therapies) aiming to treat symptoms and prevent the progression toward organ complications. The enzymatic replacement therapy (ERT) with agalsidase alfa (Replagal) and agalsidase beta (Fabrazyme), was the first to be identified in 2001 and is administered once every 2 weeks by intravenous injections. Clinical data suggest that treatment is improved with early ERT initiation (12, 13). The oral chaperone Migalastat was approved in Europe in 2016 for the treatment of patients carrying amenable mutations. It is a well-tolerated therapy that can be administered orally every other day with a promising impact on both primary and secondary endpoints of FD.

3 Exercise intolerance and fatigue in FD: the clinical relevance

Fatigue is the impairment of the ability to carry out the usual daily activities, and is different from muscle weakness, due to myopathy or a neurological disorder. The main symptoms of fatigue are listlessness, lack of energy, exhaustion, tiredness, early fatigability, sleepiness, a tendency to fall asleep during the day, physical weakness, or a feeling of running on empty. Fatigue represents the most bothersome symptom for most pathological conditions, including Fabry disease.

Exercise intolerance and fatigue are frequent and early findings in FD patients and were previously considered a consequence of diastolic heart failure or related to peripheral nerve involvement. Today, several reports suggest that the reduced tolerance to physical activity in patients with FD is not a secondary effect of diastolic heart failure, but a self-standing clinical phenotype that occurs independently from chronic renal or cardiac dysfunction (17–19). The involvement of the skeletal muscle leads to muscular cramps in the early phase and muscular pain, fatigability, and asthenia, in the advanced phases of the disease which involve walking and motor performance (19). The resulting chronic fatigue has a significant impact on quality of life, affecting patients' daily activities. This mainly concerns children's participation in after-school activities, and alterations of concentration and memory at work, with resulting negative impacts also on psychosocial attitudes.

4 Fatigue management

Since FD is a multiorgan disease, the treatment is focused not only on reactivating the enzymatic activity of GLA but also on the specific management of symptoms, which vary among patients. Chronic fatigue occurs in over half of FD patients but is also the most difficult FD symptom to manage since current therapies are not effective in reducing fatigue and ameliorating the quality of life (20). Moreover, no alternative treatments are available considering the scarce knowledge of its pathogenetic mechanism. Pain medications and changes in lifestyles, including stress reduction and exercise, could ameliorate energy levels.

A cardiopulmonary exercise test is a good tool to evaluate cardiac function and aerobic fitness and to determine how the heart responds to exercise-induced stress (21) but despite muscular symptoms in FD, just a few studies evaluated cardiopulmonary fitness with exercise testing in Fabry patients (21–24). This test is instead essential to assess functional capacity and aerobic fitness level and could also be useful to perform disease diagnosis, determine symptoms severity, and monitor the effects of treatments, including exercise programs. The few recent findings suggest that Fabry patients have impaired cardiopulmonary exercise capacity and failed to reach maximum heart rate both before and after ERT treatment (21). This suggests that alterations in the exercise performance of Fabry patients cannot be improved by ERT. Similarly, Migalastat therapy cannot revert the muscular phenotype in FD even if an 18-month treatment was associated with a "trend" towards an improvement in exercise tolerance (25). In our cohort of Fabry patients, we observed a significant reduction of aerobic exercise tolerance alongside an accumulation of stress-induced lactate production (26). Interestingly, this phenomenon occurs also in young and female patients, in which the classical Fabry phenotypes were not still obvious (kidney and cardiac symptoms). Moreover, in a small pool of our FD population, we

retrospectively tested the effects on exercise tolerance of available FD therapies. After one year, in non-treated patients, exercise duration was comparable to the baseline, whereas in the treated subgroup we observed a significant improvement in exercise endurance (26). This finding suggests that the reduced tolerance to exercise is a specific and sensitive hallmark of FD which might be included in the panel of clinical parameters useful to establish the starting point of the therapy and to monitor its efficacy. In this perspective, exercise-induced blood lactate accumulation could also be considered a potentially useful biomarker for monitoring muscular phenotype in Fabry patients. Nevertheless, further dedicated studies are needed to definitely establish the benefits of available FD therapies on fatigue and exercise intolerance in FD patients.

In some cases, exercise intolerance in FD patients could result from physical inactivity suggesting the clinical benefits of an exercise program (27), and the possibility to include FD in the plethora of chronic pathologies within the Adapted Physical Activity (AFA) programs. However, there are no specific guidelines for exercise prescription in the FD population. Certainly, the complexity of the phenotype requires a thorough assessment of the clinical phenotype before prescription. The general guidelines for exercise and physical activity by the American College of Sports Medicine (ACSM) might be adapted to the FD population as previously tested by Schmitz and colleagues (27). Specifically, they evaluated the effects of an adapted strength/circuit exercise program on a small population of FD patients. The results indicated that regular training for 12 months was able to improve exercise capacity, muscle strength, and the general well-being of FD participants. The study by Schmitz et al. is the only one to present data on a prospective training intervention in patients with FD. Hence, there is an urgent need for further studies exploring the effects of exercise programs on a larger FD population. With more data, it will be possible to draft a specific FD-AFA program and also to establish the impact of disease severity in order to determine which FD patients will benefit from certain training interventions.

5 Pathogenetic mechanisms of fatigue in FD patients

Fatigue is a very complex symptom and, as such, it is highly subjective and difficult to define, evaluate, and quantify. Broadly, fatigue can be described as an overwhelming sense of tiredness, lack of energy, and feeling of exhaustion (28, 29). Despite the high impact on patient's quality of life, fatigue is an under-recognized and undertreated condition (30). It is a common feature of a wide variety of disorders including infective, neurological, psychiatric, neoplastic, metabolic diseases, and myopathies (31–33). Fatigue can be classified as "mental fatigue", which refers to the cognitive or perceptual aspects of fatigue, and "physical" or muscular fatigue, which refers to the performance of the motor system (34). Specifically, muscle fatigue is defined as the inability of muscles to generate an expected power or to maintain the required force for a given task (35). Muscle fatigue, in turn, can be distinguished in central or peripheral according to which level of the motor system is affected. Central fatigue originates in the central nervous system (CNS), with a decreased neural drive to the muscle. Peripheral fatigue arises from the muscle and predominately involves muscle bioenergetics or excitation-contraction mechanism alterations.

Fatigue is commonly experienced by Fabry patients and despite the exact pathogenetic mechanisms remain unknown, different components are suggested to contribute to its generation. Cognitive impairment and psychological components like depressive symptoms have been associated with pain, negative health perception, and "mental fatigue" in Fabry patients (36). However, "muscle fatigue" in Fabry disease seems to be more relevant and it is attracting a growing interest. Organ damage could surely contribute to chronic muscle fatigue, considering that kidney and heart damage can lead to anemia, and lung alterations can affect breathing (37). However, several Fabry patients without organ complications still suffer from muscle fatigue (17) suggesting that the motor system is primarily affected and muscle fatigue is an independent symptom in FD.

Very little data has been published regarding the mechanisms altered in Fabry disease and their specific impact on chronic fatigue. In the next sections, we provide a summary of the evidence available in the literature, and of their hypothesized clinical implications. Specifically, we will focus on the primary alterations of the motor system, without excluding that other mechanisms could indirectly trigger fatigability in Fabry patients like pulmonary, and cardiac alterations as well as anhidrosis, frequently described in Fabry patients (37–40). Figure 1 summarizes the main contributors to Fatigue in Fabry patients, emphasizing the role of skeletal muscle alterations.

6 The "metabolic hypothesis"

The "metabolic hypothesis" to explain skeletal muscle symptoms and fatigability in Fabry patients is growing acknowledged. It takes ground from the biochemistry of the Fabry Muscle which appears to be very different from that of non-Fabry patients.

6.1 Relevance of skeletal muscle bioenergetics

Muscular work must be supported by a high supply of ATP energy. Indeed, skeletal muscle is the district with the highest energetic demand, and as such, it is the main determinant of the whole body's metabolic rate. In particular, skeletal muscle can vary its metabolism to a greater extent than any other tissue through high metabolic flexibility, shifting its reliance between anaerobic glycolysis with lactate production, and aerobic oxidation of pyruvate or lipid (41, 42). Disruptions of metabolic flexibility of skeletal muscle are associated with several pathological conditions characterized by exercise intolerance and fatigue (42, 43). Growing evidence supports significant metabolic

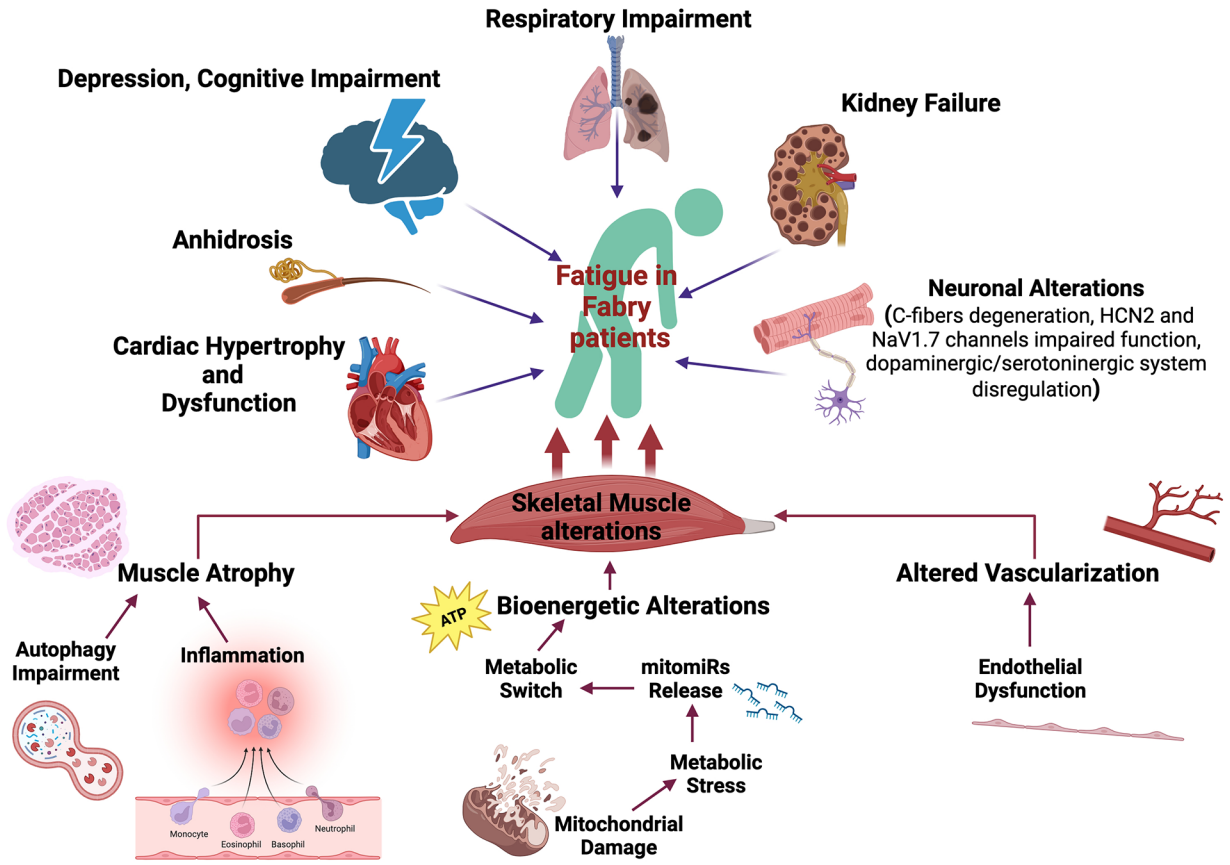

FIGURE 1
Fatigue determinants in Fabry disease. Several mechanisms are potentially involved in the fatigability experienced by Fabry patients including cardiopulmonary alterations, kidney failure, anhidrosis, cognitive impairment, and skeletal muscle disarrangement. Specifically, primarily skeletal muscle alterations that can strongly impact physical performance have been described: (i) inflammation can potentially drive muscle atrophy; (ii) endothelial dysfunction can induce altered muscle vascularization with impaired oxygen and nutrient supply; (iii) putative mitochondrial dysfunction can induce bioenergetic alterations. The metabolic stress triggers the release of specific mitomiRs which can drive a maladaptive metabolic reprogramming of skeletal muscle. The illustration has been created by Biorender (License number EW26AUO200).

alterations of skeletal muscle in Fabry disease. We have recently shown a muscle fibers switch in a mouse model of Fabry Disease resulting in a higher abundance of Type II/glycolytic fibers. Accordingly, we recorded high glycolytic rate and lactate overproduction, in line with reduced exercise tolerance and fatigability in Fabry mice and patients (26). The mechanism of this metabolic remodeling involves miR-17-mediated HIF-1 upregulation, which in turn induces the high expression of the key enzymes of lactacid metabolism. As we have shown in another recent report, the recruitment of miR17 alongside other metabolism/mitochondria-related miRNAs (mitomiRs) is likely induced by the intrinsic metabolic stress of Fabry cells (44). Specifically, mitomiRs regulating fundamental aspects of mitochondrial homeostasis and fitness, including expression and assembly of the respiratory chain, mitogenesis, antioxidant capacity, and apoptosis are significantly dysregulated in FD patients. Accordingly, oxidative stress biomarkers including [Advanced Oxidation Protein Products (AOPP), Ferric Reducing Antioxidant Power (FRAP), and thiolic groups] are altered in FD patients already at an early stage of the disease (45). This evidence suggests the early involvement of mitochondrial dysfunction as a mechanism of metabolic and bioenergetic stress in Fabry disease, according to the reduced expression of respiratory chain complexes and the decline of ATP production in Fabry patients (44, 46). In this view, the lactacid metabolic switch of Fabry skeletal muscle could represent an adaptative mechanism to the failure of mitochondrial energetic production, a stress response driven by miR17. This phenomenon occurs at the expense of physical performance and fatigability of Fabry patients, thus representing a maladaptive response that requires monitoring and specific treatments.

In chronic fatigue syndrome (CFS), a targeted, broad-spectrum metabolomics performed on plasma from patients has indicated significant abnormalities in 20 metabolic pathways, including sphingolipid, phospholipid, and mitochondrial metabolic pathways (47). This evidence further supports the "metabolic hypothesis" in the explanation of muscle fatigue in Fabry disease. Supporting data was also derived from the metabolomic and lipidomic analysis that we conducted in a Fabry population (26). Specifically, we have shown a metabolic remodeling in Fabry

patients characterized by reduced levels of acetyl-carnitine, fatty acids, and diacyl glycerol, alongside triglycerides accumulation, confirming the altered mitochondrial oxidation of lipids. A similar metabolic profile has been described for *metabolic myopathies*, a group of genetic myopathies characterized by an increased rate of anaerobic glycolysis, exercise intolerance, blood lactate accumulation, and reduction of circulating Acylcarnitine (48, 49). Taken together, these considerations request a redefinition of Fabry disease as a metabolic disorder where the skeletal muscle is a key involved district, and exercise intolerance and fatigue are specific and primary symptoms to be managed.

6.2 Muscle atrophy

The atrophy of muscle mass could be another reason for compromised physical performance and fatigability in Fabry patients. Indeed, among the primary myopathic changes in FD, areas of muscle atrophy have been described as granules (19, 50, 51). The immobilization, and sedentary lifestyle are important factors inducing muscle atrophy, and loss of strength (52). However, in Fabry disease, more specific mechanisms able to actively trigger myosin loss could be involved. Among them, pro-inflammatory signaling activation with TNF-α and IL-6 production has been indicated as a potent trigger of ubiquitin ATP-dependent proteasome (UPP response) in the skeletal muscle causing muscular loss (53). Fabry disease was associated with elevated serum levels of IL-6 and TNF-α, and their levels strongly correlated with MSSI scores reflecting a greater disease burden (54).

Lysosomal dysfunction and autophagy dysregulation in Fabry disease could be another mechanism inducing muscle atrophy in Fabry patients. Indeed, autophagy in skeletal muscles is finely tuned under both, physiological conditions and metabolic stress. Altered autophagy activity characterized by either increased formation of autophagosomes or inhibition of lysosome-autophagosome fusion has been identified as one of the major causes of muscle loss in several skeleton muscle disorders (55).

6.3 Neuronal contribution

Degeneration of small fibers (C-fibers) and impaired function of ion channels like HCN2 and NaV1.7 sodium channel has been associated with widespread pain and chronic muscle fatigue in Fabry patients, alongside nausea, constipation or diarrhea, and itching (56, 57). Specifically, the pattern of small fiber dysfunction in Fabry disease is similar to other diseases characterized by peripheral neuropathy, including diabetes mellitus (58).

Central neurotransmitters, including serotonin and dopamine, play an important role during whole-body exercise and fatigue. The so-called central fatigue hypothesis states that exercise affects the concentration of these neurotransmitters (within the CNS, or proximal to the neuromuscular junction) thus generating fatigue (59). From a neuroimaging study with 18F-DOPA PET scans, a presynaptic dopaminergic disruption emerges in Fabry patients (60). Moreover, an imbalance between cholinergic and dopaminergic activity in the basal ganglia, which has a central role in movement disorders, has been suggested also for Fabry disease. Alterations of the serotonergic system have been hypothesized as well since a large Gb3 accumulation in serotonergic nuclei of Fabry patients was described (61).

6.4 Vasculature and blood flow alterations: reduced O_2 muscular delivery

An adequate blood flow ensures an adequate oxygen delivery to the working muscle necessary for aerobic ATP production and for removing waste products of metabolic processes, thus playing an important role in the maintenance of force output. Indeed, the occlusion of blood flow to a working muscle induces a reduction of the time to exhaustion and a decline in generated force indicating the importance of blood flow in fatigue prevention (62, 63). Vascular remodeling has been described in Fabry patients. Specifically, a considerable accumulation of glycosphingolipid occurs in vascular smooth muscle and endothelial cells disturbing peripheral endothelial function and promoting intima-media thickening (64). Indeed, high levels of plasmatic endothelial biomarkers have been detected in Fabry patients, like VCAM-1, indicative of considerable vascular dysfunction (65). Inflammation-mediated endothelial dysfunction seems to be also involved; the release of heparanases, a marker of chronic inflammation, appears to be responsible for the degradation of the endothelial glycocalyx, contributing to endothelial dysfunction in FD (66).

Some data suggest a contribution of vasculature alterations in the reduced physical performance and muscle fatigue in Fabry patients. Indeed, a study of histologic examination of Fabry skeletal muscle indicated that muscle myocytes were unaffected, whereas muscle vessels showed the presence of mild glycosphingolipid accumulation in endothelial and smooth muscle cells (19). Moreover, evidence of a perfusion change in the vasa nervorum could also contribute to the dysfunctional processing of sensory information, which likely occurs under physical stress and generates fatigue sensation (67).

7 Future directions

FD has a serious impact on the quality of life, morbidity, and mortality of affected people. Great advances have been made in the last decade to identify both early markers of pathology and more effective treatments but several issues in the management of this pathology remain to be solved. First of all, genetic testing for FD should be improved to identify more affected patients and properly start the treatment. The genetic identification of the pathology is just done when the worse complications are manifested and the therapy could be less effective at this stage. Only family members of identified patients are recommended for genetic counseling and benefit from an early diagnosis and treatment. Also, an optimal time to start therapy should be

evaluated as well as the possibility of combined therapies to ameliorate patients' quality of life. For chronic fatigue, in particular, specific research is essential to establish the triggering mechanisms and potential specific treatments that could impact both body and mind of Fabry patients. In this view, the cardiopulmonary exercise test and exercise tolerance test should be used as diagnostic and follow-up tools in the clinic management of Fabry patients, to identify exercise intolerance and to monitor the effectiveness of treatments. Also, establishing precise and individual exercise programs for Fabry patients based on their clinical features and exercise tolerance could not only ameliorate physical conditions but also have a psychosocial effect with a great impact on the quality of life. Exercise intolerance is a sensitive and early disease manifestation that could be used also to monitor FD females, which are often undertreated basing the decision on classic phenotypes (cardiac and renal dysfunction). Future studies will be specifically dedicated to FD females evaluating the potential use of exercise testing for monitoring disease stage and for deciding the starting point of the treatment. The assessment of metabolic alterations should also be considered as part of the diagnostic and monitoring iter of Fabry patients. In particular, a panel including lactate, mitomiRs, TG, and Acylcarnitine could be employed to evaluate the entity of metabolic dysregulation, potentially useful to risk stratify Fabry patients and to design a specific therapeutic plan for early intervention. Especially, stress-induced lactate production represents an easy-to-detect, low-cost biomarker of skeletal muscle involvement and muscular metabolic dysregulation, which assessment should attend the exercise tolerance test in Fabry patients routinely.

Author contributions

JG: Conceptualization, Writing – original draft, Writing – review & editing. ER: Writing – original draft, Writing – review & editing. AB: Writing – original draft. FA: Writing – original draft. FC: Writing – original draft. AB: Writing – original draft. TDR: Writing – original draft. AV: Writing – original draft. RA: Writing – original draft. AP: Funding acquisition, Writing – review & editing. DS: Conceptualization, Writing – original draft, Writing – review & editing. GI: Conceptualization, Funding acquisition, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Overexpression of VEGFα as a biomarker of endothelial dysfunction in aortic tissue of α-GAL-Tg/KO mice and its upregulation in the serum of patients with Fabry's disease

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Introduction: Fabry's disease is an X-linked lysosomal storage disorder caused by reduced activity of α-galactosidase A (GAL), leading to premature death on account of renal, cardiac, and vascular organ failure. Accumulation of the GAL substrate globotriaosylceramide (Gb3) in endothelial and smooth muscle cells is associated with early vascular cell damage, suggesting endothelial dysfunction as a driver of cardiorenal organ failure. Here, we studied the vascular expression of the key angiogenic factors, VEGFα and its antagonist angiostatin, in Fabry α-GAL-Tg/KO mice and determined circulating VEGFα and angiostatin serum levels in patients with Fabry's disease and healthy controls.

Methods: Cryopreserved aortic vessels from six α-GAL-Tg/KO and six wild-type (WT) mice were obtained and VEGFα and angiostatin levels were determined by performing Western blot analysis. VEGFα expression was visualized by an immunohistochemical staining of paraffin aortic rings. In addition, VEGFα and angiostatin serum levels were measured by using an enzyme-linked immunosorbent assay in 48 patients with genetically verified Fabry's disease (50% male) and 22 healthy controls and correlated with disease severity markers such as lyso-Gb3, albuminuria, NTproBNP, high-sensitive troponin T (hsTNT), and myocardial wall thickness.

Results: It was found that there was a significant increase in VEGFα protein expression (1.66 ± 0.35 vs. 0.62 ± 0.16, $p = 0.0009$) and a decrease in angiostatin expression (0.024 ± 0.007 vs. 0.053 ± 0.02, $p = 0.038$) in aortic lysates from α-GAL-Tg/KO compared with that from WT mice. Immunohistochemical staining revealed an adventitial VEGFα signal in α-GAL-Tg/KO mice, whereas no VEGFα signal could be detected in WT mice aortas. No differences in aortic angiostatin expression between α-GAL-Tg/KO- and WT mice could be visualized. The serum levels of VEGFα were significantly upregulated in patients with Fabry's disease compared with that in healthy controls (708.5 ± 426.3 vs. 458.5 ± 181.5 pg/ml, $p = 0.048$) and positively

associated with albuminuria ($r = 0.82$, $p < 0.0001$) and elevated NTproBNP ($r = 0.87$, $p < 0.0001$) and hsTNT values ($r = 0.41$, $p = 0.048$) in male patients with Fabry's disease. For angiostatin, no significant difference was found between patients with Fabry's disease and healthy controls (747.6 ± 390.3 vs. 858.8 ± 599.3 pg/ml).

Discussion: In conclusion, an overexpression of VEGFα and downregulation of its counter player angiostatin in aortic tissue of α-GAL-Tg/KO mice support the hypothesis of an underlying vasculopathy in Fabry's disease. Elevated VEGFα serum levels were also observed in patients with Fabry's disease and were positively associated with elevated markers of organ manifestation in males. These findings suggest that angiogenetic markers, such as VEGFα, may be potentially useful biomarkers for the detection of endothelial dysfunction in classical Fabry's disease.

KEYWORDS

Fabry's disease, vasculopathy, endothelial dysfunction, angiogenic markers, VEGF, angiostatin, aortic vessels, transgenic knockout model

Introduction

Fabry's disease is an X-linked lysosomal storage disorder caused by reduced or absent α-galactosidase A (GAL) activity resulting from mutations in the *GLA* gene (1). Subsequently, globotriaosylceramide (Gb3) and its deacylated form, globotriaosylsphingosine (lyso-Gb3), accumulate in different cells and tissues throughout the body causing various clinical manifestations.

Endothelial and smooth muscle cells are the preferential target of Gb3 storage (2). Gb3 induces a variety of angiogenesis factors such as VEGF, VEGFR2, TGFß, and FGF-2 in these cells (3). Endothelial dysfunction was described in experimental models of Fabry's disease (4, 5) and may also play a pivotal role in affected patients with Fabry's disease (6). Endothelial dysfunction (6) and vascular damage reflected by increased intima-media thickness (IMT) and decreased brachial flow–mediated dilation (7) also appear to contribute to organ dysfunction in patients with Fabry's disease, including chronic kidney disease, cardiomyopathy, and stroke (8).

VEGFα is a specific endothelial cell mitogen acting as a potent pro-angiogenic factor (9). Its antagonist, angiostatin, attenuates VEGF expression, resulting in the inhibition of extracellular matrix formation and migration (10, 11). VEGFα is activated in a variety of kidney, heart, cerebral, and cutaneous diseases associated with angiogenesis (12–15). In this context, prior work from our group suggests altered markers for vascular dysfunction and shear stress in the blood of patients with Fabry's disease (16).

Here, we studied the vascular expression pattern and localization of VEGFα and angiostatin in aortic vessels of Fabry R301Q transgenic/KO (α-GAL-Tg/KO) mice. In addition, VEGFα and angiostatin concentrations were determined in the serum of patients with Fabry's disease and healthy controls and were found to be associated with biomarkers of disease severity.

Materials and methods

Mouse model

Experiments were carried out in C57BL/6 (wild-type, WT) and hR301Q α-GAL-Tg/KO mice. Specifically, these mice are homozygous for endogenous GLA knockout and express the human transgene GLA carrying the R301Q mutation under the transcriptional control of the human GLA promoter. These mice were kindly provided by AMICUS Therapeutics and used as a model of Fabry's disease (17–19). The mice were housed in the animal facility of the Department of Translational Medical Sciences of Federico II University of Naples (Italy) in a 12-h light–dark cycle and provided access to a commercial mouse diet and water *ad libitum*. Animal husbandry and other experiments were conducted under Institutional Animal Care and the use of committee-approved protocols at Federico II University (Prot. n. 971/2016-PR). Aortas were collected from 9-month-old mice. Mice were euthanized, and aortas were quickly removed, rinsed in cold phosphate-buffered saline (PBS), and processed for successive analyses: one part was immediately frozen in liquid nitrogen for a biochemistry analysis, and another part was fixed in 4% paraformaldehyde for histological analysis.

Tissue preparation (aortic vessels)

Cryopreserved aortic vessels from α-GAL-Tg/KO mice and WT mice were pulverized and homogenated on ice in a RIPA buffer supplemented with protease inhibitors by repeated sonication and incubation for 1 h. The homogenates were centrifuged for 30 min at 13,000 rpm at 4°C and protein content in the supernatants was measured using a BCA assay (Thermo Fisher).

Western blotting

The total protein extracts (20 µg) were separated on TGX 4%–20% precast gradient gels (Bio-Rad, Germany) and transferred onto nitrocellulose membranes. The membranes were blocked in 5% milk (for anti-angiostatin antibody) or 3% milk (for anti-VEGFα and anti-α-actinin antibodies). The membranes were then incubated with the following primary antibodies:

Polyclonal rabbit-a-angiostatin (Abcam, ab2904) 2 µg/ml in 5% milk, overnight at 4°C; monoclonal mouse-a-VEGFα (Abcam, ab1316), 5 µg/ml in tris-buffered saline (TBS)/T, 1 h at room

temperature; rabbit-a-α-actinin (Cell Signaling, 3134), 1:1,000 in 5% milk, overnight at 4°C. After washing with TBS/T, the membranes were incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies (sheep anti-mouse, Jackson ImmunoResearch 515-035-003, 1:20,000 in 3% milk; goat anti-rabbit, Jackson ImmunoResearch JIM-111-035-003, 1:10,000 in 5% milk), washed again, and specific signals were detected using the ECL or Femto system (Thermo Fisher) according to the manufacturer's protocol. A densitometric analysis of the protein bands was done using ImageLab (Bio-Rad).

Immunohistochemistry

Paraffin sections (3 µm) of aortic vessels from WT and α-GAL-Tg/KO mice were deparaffinized and incubated in a 10 mM citrate buffer at 95°C for 40 min for antigen retrieval. Afterward, the sections were incubated for 10 min in 0.5% Triton/TBS for permeabilization, washed in TBS, and quenched in a 0.25% Sudan black solution for 30 min on a shaker in the dark. After repeated washing, the sections were blocked in 3% bovine serum albumin (BSA) in TBS for 1 h at room temperature. The sections were then incubated with primary antibodies [anti-angiostatin (Abcam ab2904), 1:20 in 3% BSA or anti-VEGFα (Abcam, ab1316), 1:400 in 3% BSA] overnight at 4°C, washed in TBS, and incubated with secondary antibodies [donkey anti-mouse Alexa Fluor 488 (A21202 life tech) or donkey anti-rabbit Alexa Fluor 488 (21206 life tech), 1:500 in 3% BSA] for 2 h at room temperature. After repeated washing in TBS, the slides were mounted with DAPI Fluoromount G (Biozol) and fluorescence signals were descriptively analyzed using a Zeiss Axiovert M200 with ApoTome.

Study population

In this retrospective cross-sectional study, a total of 48 patients with genetically verified Fabry's disease was included and compared with 22 healthy control patients. Participants were enrolled during routine visits at the outpatient clinic of the University Heart and Vascular Center Hamburg between January 2014 and December 2020. A medical history of coronary artery disease (CAD), diabetes mellitus, arterial hypertension, and stroke was confirmed by self-report or the use of corresponding medication. A diagnosis of atrial fibrillation (AF) was established by a positive history and electrocardiogram (ECG) documentation within the last 5 years prior to examination. Cardiac symptoms were classified according to the New York Heart Association (NYHA) classification. All participants received a 12-lead surface ECG, a transthoracic echocardiography, and a routine blood test including a measurement of hsTNT (high-sensitive troponin T) and NTproBNP (brain natriuretic peptide), creatinine, GFR, and albumin in urine. The serum levels of NTproBNP were assessed by using the Atellica® IM NT-proBNP assay (Siemens Healthcare, Erlangen, Germany). For the measurement of serum hsTNT levels, the Elecsys Troponin T hs STAT assay (Roche Diagnostics, Rotkreuz, Schweiz) was used. In 40 patients with Fabry's disease and in all healthy controls, cardiac magnetic resonance (CMR) imaging was performed to determine the extent of fibrosis as a marker of Fabry-associated cardiomyopathy. The study was conducted in compliance with the principles outlined in the Declaration of Helsinki and was approved by the local ethics committee (Ethikkommission der Ärztekammer Hamburg, Nr.: PV4056). All study participants gave their written informed consent.

Cardiac imaging

All study subjects underwent a comprehensive transthoracic echocardiographic examination (Philips iE33 system, Philips Healthcare, Best, Netherlands) including M-mode, two-dimensional, pulsed, and continuous-wave Doppler and tissue Doppler imaging. Structural and functional imaging parameters were measured according to the current recommendations of the American Society of Echocardiography and diastolic dysfunction was classified according to current guidelines (20). Images were analyzed using the commercially available software Syngo Dynamics (Siemens Healthcare, Erlangen, Germany).

CMR was performed on a 1.5-T scanner (Achieva, Philips Healthcare, Best, Netherlands). The imaging protocol included cine imaging and late gadolinium enhancement (LGE) imaging. LGE images were acquired using a standard phase-sensitive inversion recovery (PSIR) sequence at least 10 min after a bolus injection of gadoterate meglumine (Dotarem, Guerbet, Sulzbach, Germany) in three long-axis orientations (2CH, 3CH, and 4CH views) and a stack of short-axis slices. Typical imaging parameters were as follows: voxel size 0.98 $mm^3 \times 0.98$ $mm^3 \times 8$ mm^3, echo time = 2.39 ms, time to repetition = 4.97 ms, and flip angle = 15°. The presence of LGE was assessed by using the commercially available software cvi42 (Circle Cardiovascular Imaging Inc., Calgary, Alberta, Canada).

Quantification of VEGFα and angiostatin in patients' serum

Protein serum levels in samples from patients with Fabry's disease and healthy controls were quantified using enzyme-linked immunosorbent assay (ELISA) kits. For measuring and analyzing VEGFα serum levels, the ELISA kit BMS277-2TEN (Invitrogen) was used, and for determining angiostatin serum levels, the ELISA kit #ELH-Angiostatin (RayBio) was used, respectively, in accordance with the manufacturer's instructions.

Statistical analysis

A statistical analysis was performed using IBM SPSS Statistics (Version 28.0, Statistical Package for the Social Sciences, International Business Machines, Inc., Armonk, NY, USA). Continuous data are given as mean and standard deviation (SD). Categorical data are given as frequencies and percentages. Outliers were identified via evaluation of the standardized residues and included whenever measurement errors could be

excluded. Statistical comparisons between the two groups were performed using the Student's *t*-test. Associations with disease severity parameters were analyzed by using Pearson's correlation tests and corrected for age- and sex-related differences. Logistic regression was performed to investigate the relationship between clinical and laboratory markers [albuminuria, lyso-Gb3, NTproBNP, hsTNT, and interventricular septal wall diameter (IVSd)] and VEGFα and angiostatin serum levels, respectively. **p*-value ≤0.05 was considered statistically significant.

Results

Protein expression of VEGFα and angiostatin in aortic rings of α-GAL-Tg/KO mice

Western blots with aortic lysates from six α-GAL-Tg/KO and six WT mice revealed a significant increase in VEGFα protein levels in α-GAL-Tg/KO mice compared with that in WT mice (signal intensity = 1.66 ± 0.35 vs. 0.62 ± 0.16); **p* = 0.0009), as shown in Figure 1.

Angiostatin expression showed a decrease in aortic tissue from α-GAL-Tg/KO mice compared with that from WT mice (signal intensity = 0.024 ± 0.007 vs. 0.053 ± 0.02, **p* = 0.038), as shown in Figure 2.

Immunohistochemical staining of VEGFα in aortic rings of α-GAL-Tg/KO mice

Immunohistochemical staining of aortic rings showed an adventitial VEGFα signal in α-GAL-Tg/KO mice. No VEGFα signal could be detected in WT mice aortas (Figure 3). In

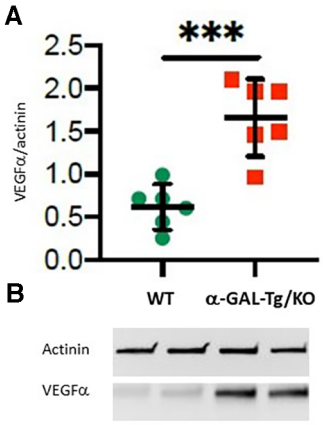

FIGURE 1
VEGFα protein expression in the aortic rings of α-GAL-Tg/KO mice. (**A**) Protein expression by Western blotting of VEGFα in the aortic rings of α-GAL-Tg/KO (*n* = 6) and WT mice (*n* = 6). The expression level of VEGFα was normalized to the internal control actinin and represented as the expression ratio. **p*-value <0.05 was considered statistically significant, ****p* < 0.001. (**B**) Characteristic Western blots for VEGFα expression compared with actinin as control.

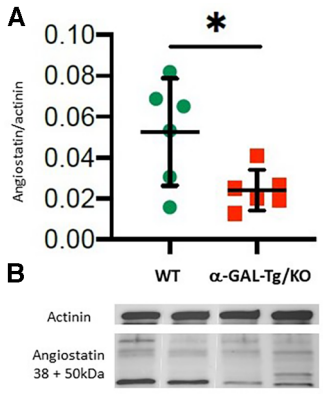

FIGURE 2
Angiostatin protein expression in the aortic rings of α-GAL-Tg/KO mice. (**A**) Protein expression by Western blotting of angiostatin in the aortic rings of α-GAL-Tg/KO (*n* = 6) and WT mice (*n* = 6). The expression level of angiostatin was normalized to the internal control actinin and represented as the expression ratio. **p*-value <0.05 was considered statistically significant. (**B**) Characteristic Western blots for angiostatin expression compared with actinin as control.

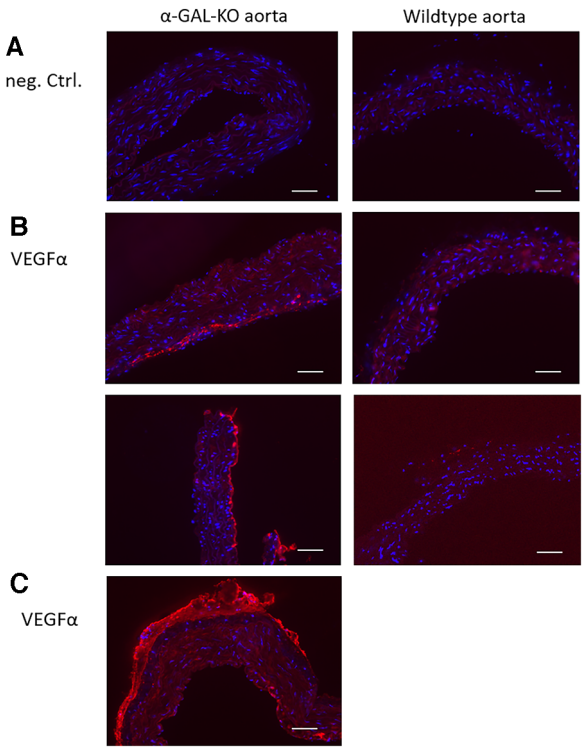

FIGURE 3
Immunohistochemical VEGFα staining of aortic rings from α-GAL-Tg/KO- and WT mice. A specific VEGFα signal could be detected in the adventitial layer of α-GAL-Tg/KO aortas. No VEGFα signal could be detected in the aortic rings from WT mice. (**A**) Negative control (staining without a primary antibody). (**B**) VEGFα signal in the aortic rings from α-GAL-Tg/KO mice (left) and WT mice (right). (**C**) An overview of a half VEGFα-stained aortic ring from a α-GAL-Tg/KO mouse. A similar VEGFα signal distribution pattern was detectable in all other aortic preparations from α-GAL-Tg/KO mice (not shown). Scale bars = 50 µm.

contrast to the results of the Western blot analysis, there were no detectable differences in angiostatin expression levels between α-GAL-Tg/KO and WT mice (not shown).

VEGFα- and angiostatin serum levels in Fabry patients

Baseline characteristics

A total of 48 patients with Fabry's disease with genetically confirmed mutations in the *GLA* gene [c.1277del (3×), c.500T>C, c.718del (4×), IVS1-6, p.717del (2×), p.A143T (3×), p.A230del (2×), p.A389V, p.C94S, p.E341K (2×), p.I384N (2×), p.I91T (2×), p.K213M (2×), p.L310V, p.L311P, p.L89del (2×), p.N139S, p.N215S (6×), p.205 T, p.Q327l (2×), p.R118C, p.R227Q, p.R227X (4×), p.R342, p.S247P] and 22 healthy controls were included in the study. Of these, 26 patients with Fabry's disease presented with signs of cardiomyopathy, such as left ventricular hypertrophy combined with elevated serum markers of NTproBNP and/or hsTNT and/or positive LGE in CMR. In 22 patients, Fabry-associated kidney disease was detected. This was defined by biopsy and/or albuminuria without a potential other cause. Table 1 summarizes the baseline characteristics for male and female patients with Fabry's disease and for the control group. As expected, patients with Fabry's disease enrolled into this study suffered from a significantly increased rate of arrhythmias and dyspnea, resulting in a higher NYHA class, and presented with higher levels of cardiac and renal markers as the control group patients. Male patients with Fabry's disease showed higher lyso-Gb3 levels than female patients, indicating a more severe phenotype in male patients, which was reflected by a higher percentage of male patients with cardiomyopathy and renal dysfunction. In accordance with this, the parameters of Fabry-associated cardiomyopathy and nephropathy were more prominent in male patients than in female patients.

VEGFα and angiostatin serum levels in patients with Fabry's disease compared with those in healthy controls

The serum levels of VEGFα were significantly upregulated in all patients with Fabry's disease compared with that in healthy controls (708.5 ± 426.3 vs. 458.5 ± 181.5 pg/ml, $^*p = 0.048$), as shown in Figure 4. This effect was more pronounced in male patients with Fabry's disease (721.1 ± 420.5 pg/ml) than in female patients (695.9 ± 153.5 pg/ml), although a gender-specific analysis revealed no statistical significance.

An analysis of angiostatin serum levels revealed no statistical significance (747.6 ± 390.3 vs. 858.8 ± 599.3 pg/ml) either in male or in female patients with Fabry's disease compared with those in controls (Figure 5). However, in female patients, there was a trend toward reduced angiostatin concentrations (581.6 ± 153.5 pg/ml), whereas this trend was not observed in male patients (913.6 ± 623.1 pg/ml).

Association of VEGFα and angiostatin serum levels with markers of disease severity in patients with Fabry's disease

VEGFα levels were positively associated with increased albuminuria and elevated serum concentrations of NTproBNP and hsTNT in male patients with Fabry's disease, whereas this effect could not be detected in female patients. Lyso-Gb3 and IVSd were not associated with higher VEGFα concentrations in these patients. No relationship could be detected between angiostatin serum concentrations and disease severity markers (Table 2).

No statistically significant influence of concomitant medication on VEGF and angiostatin serum levels could be detected in patients with Fabry's disease. Also for enzyme replacement therapy (ERT), there was no significant effect on VEGF and angiostatin concentrations, although there was a trend toward lower VEGF concentrations in patients with Fabry's disease under ERT compared with those in therapy-naive patients with Fabry's disease (data not provided).

Discussion

Main findings

In this study, we found increased endothelial VEGFα and reduced angiostatin levels in the aortic rings of mice with genetically defective α-GAL activity, as well as increased serum levels of VEGFα in patients with Fabry's disease, highlighting the role of endothelial cell activation and oxidative stress in Fabry's disease.

Overexpression of VEGFα

Our data demonstrate for the first time a significant increase in VEGFα protein expression in aortic lysates from α-GAL- Tg/KO mice compared with those from WT mice. In Fabry's disease, storage of Gb3, as a result of deficient α-GAL A activity, was identified to induce oxidative stress in endothelial cells (21, 22), leading to an activation of inflammatory and pro-angiogenic reactions (23) *in vitro* and *in vivo*, including changes in the pattern of circulating miRNA (24). In this context, we analyzed the expression of the most potent pro-angiogenic factor VEGFα and its counter player angiostatin in the aortic rings of the Fabry R301Q transgenic/KO mouse model and compared this with the expression in the serum of patients with Fabry's disease and healthy controls. In line with our data, treatment of bovine aortic endothelial cells with Gb3 induced the expression of angiogenic factors such as TGfβ, VEGFR2, VEGFα, and FGF2 (3, 25), demonstrating a pivotal role of angiogenetic factors in Gb3-induced vasculopathy. Further studies using the α-GAL-Tg/KO mouse model revealed a renal overexpression of VEGF and TGFβ in the presence of Fabry nephropathy (3).

In addition, immunohistochemical staining for VEGFα was performed in the aortic rings of α-GAL-Tg/KO and WT mice, which showed a higher degree of staining of VEGFα in α-GAL-Tg/KO mice. Although smooth muscle cells have been shown to be the major source of VEGFα expression and secretion (26), and accordingly in a model of hypertensive rats, VEGFα expression

TABLE 1 Baseline characteristics of patients with Fabry's disease and healthy controls.

n (%) or mean ± SD	Fabry's disease	Fabry's disease	Fabry's disease	Control
	Total	Male	Female	M = 22/F = 2
n	48	24 (50%)	24 (50%)	22
Age (years)	41.3 ± 11.4*	39.6 ± 12.3	42.9 ± 10	30.8 ± 8.7
BMI (kg/m^2)	24.4 ± 4.1	24.6 ± 3.9	24.2 ± 4.4	23.8 ± 1.8
Fabry nephropathy	22 (46%)**	15 (63%)	7 (29%)	0
Albuminuria <30 mg/L	26 (54%)	9 (38%)	17 (71%)	n.d.
Albuminuria 30–300 mg/L	13 (27%)	6 (25%)	7 (29%)	n.d.
Albuminuria >300 mg/L	9 (19%)	9 (38%)***	0	n.d.
Fabry cardiomyopathy	26 (54%)*	17 (71%)***	9 (38%)	0
Clinical presentation				
NYHA I	31 (65%)*	13 (54%)***	18 (75%)	22 (100%)
NYHA II	13 (27%)*	7 (29%)	6 (25%)	0
NYHA III	4 (8%)*	4 (17%)***	0	0
NYHA IV	0	0	0	0
Syncope	6 (13%)*	4 (17%)***	2 (8%)	0
ECG				
QTc (ms)	415 ± 24.3**	409.8 ± 27.3	420.7 ± 21.5	388 ± 15.5
Atrial fibrillation	5 (10%)*	4 (17%)***	1 (4%)	0
nsVT	5 (10%)*	3 (13%)***	2 (8%)	0
Laboratory values				
Lyso-Gb3 (ng/ml)	20.7 ± 20.9	35.9 ± 27.1****	5.4 ± 3.4	n.d.
NTproBNP (ng/L)	956.5 ± 1,427.2*	1,549 ± 2,489.1	364 ± 425.3	34.7 ± 16.8
hsTNT (pg/ml)	21.9 ± 21.7*	25.4 ± 22.1	18.3 ± 20.7	4.6 + 2
GFR (CKD) (ml/min)	78.7 ± 28.3	77.7 ± 35.5	79,7 ± 18	95.9 + 21
Creatinine (mg/dl)	1.2 ± 0.6	1.6 ± 0.9***	0.9 ± 0.2	1 + 0.1
Albuminuria (mg/L)[b]	557.2 ± 596.9	860.5 ± 729***	64 ± 33.5	n.d.
Cardiac imaging				
IVSd (mm)	13.1 ± 3.6**	14.7 ± 3.4***	11.4 ± 3.0	10.1 ± 0.7
PWd (mm)	12 ± 2.8**	13.5 ± 2.6***	10.4 ± 2.4	8.9 ± 1.1
LVEF >60%	40 (83%)	19 (79%)	21 (88%)	22 (100%)
LVEF 46%–60%	6 (13%)	4 (17%)	2 (8%)	0
LVEF <45%	1 (2%)	1 (4%)	0	0
LA (mm)	38.3 ± 6.5*	41.9 ± 6.5***	34.8 ± 6.2	34.4 ± 4.6
E/A	1.6 ± 0.4	1.5 ± 0.4	1.6 ± 0.5	1.8 ± 0.3
E/e'	10.7 ± 4.5*	12.9 ± 5.2***	8.6 ± 2.8	7.1 ± 1.2
LGE in CMR[a]	20 (50%)**	13 (65%)	7 (35%)	0
Medication				
ERT	29 (60%)	19 (79%)****	10 (21%)	0
Migalastat	1 (2%)	1 (4%)	0	0
ACE inhibitors/AT-1 blocker	18 (38%)	13 (54%)***	5 (21%)	0
Beta blockers	8 (17%)	5 (21%)	3 (13%)	0
Statins	9 (19%)	7 (29%)***	2 (8%)	0
Diuretics	8 (17%)	5 (21%)	3 (13%)	0
Concomitant diseases				
CAD	5 (10%)*	5 (21%)***	0	0
Diabetes	1 (2%)	1 (4%)	0	0
Hypertension	17 (35%)**	11 (46%)***	6 (25%)	0
Stroke	5 (10%)*	3 (13%)	2 (8%)	0

A, peak late transmitral filling velocity; ACE inhibitors, angiotensin-converting enzyme inhibitors; AT-1R, angiotensin I-receptor; BMI, body mass index; E, peak early transmitral filling velocity; e, early mitral annulus velocity; eGFR, estimated glomerular filtration rate; F, female; LA, left atrial; LVEF, left ventricular ejection fraction; M, male; *n*, total number; nsVT, non-sustained ventricular tachycardia; PWd, posterior wall diameter.

Values for continuous data are given as mean ± SD. Values for categorical data are given as counts and percentage of the total column number.

[a]LGE in *n* = 40 receiving CMR.

[b]Albuminuria in *n* = 22 with Fabry-associated nephropathy.

*p-value ≤0.05 vs. control.

**p-value ≤0.001 vs. control.

***p-value ≤0.05 male vs. female patients with Fabry's disease.

****p-value ≤0.001 male vs. female patients with Fabry's disease.

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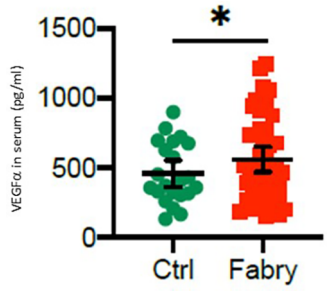

FIGURE 4
VEGFα serum level in patients with Fabry's disease. Serum concentrations of VEGFα in 48 patients with Fabry's disease and 22 healthy controls determined by using an enzyme-linked immunosorbent assay. *p-value <0.05 was considered statistically significant.

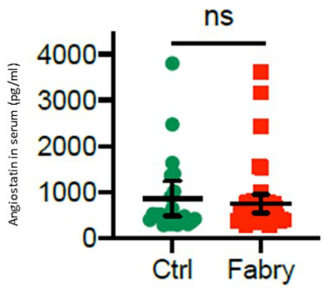

FIGURE 5
Angiostatin serum level in patients with Fabry's disease. Serum concentrations of angiostatin in 48 patients with Fabry's disease and 22 healthy controls determined by using an enzyme-linked immunosorbent assay.

was mainly distributed in the outer to middle layers of the media (27), our data indicate that the VEGFα signal in mice aortic tissue was mainly increased in the adventitial layer. Whether this is a species-specific effect is not known. However, our observations are supported by a study in human atherosclerotic arteries analyzing the localization of different VEGF isoforms, also showing the most prominent staining for VEGFα in the adventitia along with some staining of the media (28).

To reveal the relevance of VEGFα expression seen in this *in vitro* model, we also measured VEGFα concentrations in the serum of 48 patients with Fabry's disease and 22 healthy controls, which showed significantly higher VEGFα concentrations in the Fabry's disease cohort. In line with other studies demonstrating VEGFα overexpression in patients with Fabry's disease with cutaneous and systemic manifestations, this effect was more pronounced in male patients (29). This may be explained by the fact that male patients presented with a more severe clinical phenotype and a higher lyso-Gb3 level, which is usually attributed to lower enzyme activities in male patients compared with female patients. In this context, a proteomics-based analysis recently showed a correlation of VEGFα with lyso-Gb3 and residual enzyme activity in classical Fabry's disease (30). Although an association with a higher lyso-Gb3 level could not be confirmed in our cohort, we show that VEGFα was positively associated with albuminuria, NTproBNP, and hsTNT, as markers for organ manifestation in Fabry's disease. However, this relationship was solely seen in male patients with Fabry's disease.

No statistically relevant influence of co-medication on VEGFα levels could be found, although there was a trend toward lower VEGFα serum concentrations in patients with Fabry's disease under ERT compared with those in the small group of untreated patients with Fabry's disease. This may indicate a potentially beneficial effect of ERT, which has to be further evaluated in a larger cohort of patients with Fabry's disease.

TABLE 2 Association of different biomarkers for organ manifestation with VEGFα and angiostatin serum levels.

	Fabry's disease Total ($n = 48$)		Fabry's disease Male ($n = 24$)		Fabry's disease Female ($n = 24$)	
	r	p-value	r	p-value	r	p-value
VEGFα (pg/ml) correlated to						
Lyso-Gb3 (ng/ml)	0.11	0.47	0.23	0.28	−0.19	0.37
NTproBNP (ng/l)	0.51	≤0.001	0.87	≤0.0001	0.2	0.34
hsTNT (pg/mL)	0.05	0.75	0.41	0.048*	−0.13	0.54
Albuminuria (mg/L)	0.47	≤0.001	0.82	≤0.0001	−0.16	0.46
IVSd (mm)	−0.03	0.84	0.1	0.63	−0.15	0.48
Angiostatin (pg/ml) correlated to						
Lyso-Gb3 (ng/ml)	0.28	0.051	0.19	0.38	0.2	0.34
NTproBNP (ng/L)	−0.002	0.99	−0.04	0.85	0.1	0.64
hsTNT (pg/ml)	−0.03	0.84	−0.15	0.48	0.12	0.59
Albuminuria (mg/L)	0.15	0.3	0.09	0.68	−0.1	0.64
IVSd (mm)	0.10	0.48	0.01	0.96	0.05	0.83

r, Pearson's correlation coefficient.
Results from the correlation tests of laboratory values and markers of organ manifestation in 48 patients with Fabry's disease. All values were corrected for age- and sex-related differences.
*p-value ≤0.05 was considered statistically significant.

Reduced expression of angiostatin

Angiostatin is an internal fragment of plasminogen and is generated by proteolytic cleavage through matrix metalloproteinases (MMP) and urokinase plasminogen activator (uPA) (31, 32). It acts as a potent inhibitor of angiogenesis and attenuates VEGF expression, resulting in the inhibition of extracellular matrix formation and migration (10, 11). Recently, we showed altered MMP9 and angiostatin levels in the serum of patients with Fabry's disease compared with healthy controls, suggesting a higher extracellular matrix turnover in Fabry's disease (16). Accordingly, we found a suppressed angiostatin expression in aortic rings of the α-GAL-Tg/KO mouse model. In the serum of patients with Fabry's disease, we also observed a trend toward reduced angiostatin serum levels; however, this did not reach statistical significance.

Conclusions

An overexpression of VEGFα in aortic tissue of the Fabry mouse model and a corresponding higher serum level of VEGFα, especially in male patients with a progressive state of Fabry's disease, support the hypothesis of an underlying vasculopathy. There is growing evidence that vascular damage induced by lipid storage or substrate-independent mechanisms is an underlying pathophysiologic feature in the development of progressive organ manifestation. In this context, our findings call for further research determining whether oxidative stress and endothelial cell activation are suitable therapeutic targets in Fabry's disease.

Strengths and limitations

One of the strengths of the study lies in the translational approach quantifying VEGFα in the vascular rings of an established murine model of Fabry's disease and in the serum of genotyped patients with Fabry's disease. However, a limitation is that data were obtained only from a single center, which necessitates independent validation. The other limitations are as follows: patients with Fabry's disease were approximately 10 years older than the control group patients and only two female patients were included in the control group. Therefore, age- and sex-related influences on VEGFα and angiostatin levels could not be sufficiently validated. Also, residual enzyme activity α-GAL was not explicitly measured in our cohort.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Ethikkommission der Ärztekammer Hamburg. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Ethical approval was not required for the studies on animals in accordance with the local legislation and institutional requirements because only commercially available established cell lines were used.

Author contributions

NL: Formal Analysis, Writing – original draft, Data curation, Methodology. HW: Data curation, Formal Analysis, Methodology, Writing – review & editing. LFi: Data curation, Writing – review & editing. NM: Writing – review & editing, Supervision, Validation. FB: Validation, Writing – review & editing, Funding acquisition, Resources, Visualization. TH: Validation, Writing – review & editing, Supervision. DS: Writing – review & editing, Data curation, Methodology. GI: Methodology, Writing – review & editing, Funding acquisition, Supervision, Validation. KM: Supervision, Validation, Writing – review & editing, Data curation. ET: Data curation, Validation, Writing – review & editing, Methodology. GA: Validation, Writing – review & editing, Supervision. PK: Supervision, Writing – review & editing, Project administration. LFa: Supervision, Writing – review & editing. MP: Funding acquisition, Conceptualization, Formal Analysis, Validation, Visualization, Writing – original draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The authors declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

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The role of tubular cells in the pathogenesis of Fabry nephropathy

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The pathophysiology of Fabry nephropathy (FN) is induced by galactosidase A deficiency with a chronic exposure of glycolipids to every lineage of renal cells. Tissue damage is attributed to the activation of molecular pathways, resulting in tissue fibrosis and chronic kidney disease. Podocytes have been the primary focus in clinical pathophysiological research because of the striking accumulation of large glycolipid deposits observable in histology. Yet, the tubular interstitium makes up a large portion of the whole organ, and therefore, its role must be further considered in pathogenic processes. In this review, we would like to propose Fabry tubulopathy and its ensuing functional effects as the first pathological signs and contributing factors to the development of FN. We will summarize and discuss the current literature regarding the role of tubular cells in Fabry kidney pathophysiology. Starting from clinical and histological evidence, we will highlight the data from animal models and cell cultures outlining the pathophysiological pathways associated with tubular interstitial injury causing renal fibrosis in Fabry nephropathy.

KEYWORDS

Fabry disease, nephropathy, tubular cells, pathogenesis, fibrosis

Introduction

Fabry disease (MIM 301500) is an X-linked lysosomal disorder caused by the deficient activity of the enzyme alfa galactosidase A (GLA) (EC:3.2.1.22), which leads to an intracellular deposition of complex sphingolipids caused by pathogenic variants in the *GLA* gene. The disease is phenotypically described in patients as "classic" or "late onset" (1). The kidney is one of the predominantly affected organs in Fabry disease. Patients with classic Fabry disease suffer from progressive kidney affection, resulting in mild proteinuria and a deterioration of renal function with the development of chronic renal failure. Patients with late-onset disease develop Fabry-associated organ damage mostly during adulthood, which is commonly restricted to one or two organ systems (2).

Structural changes involve all compartments of the kidney, including the tubular system and interstitium. The pathophysiology of Fabry nephropathy (FN) is attributed to tissue damage induced by GLA deficiency with a chronic exposure of globotriaosylceramide (Gb3) and its deacylated form globotriaosylsphingosine (LysoGb3) to cells and tissues. Every lineage of renal cells, glomerular epithelial cells (podocytes and parietal cells), and mesangial, vascular, and tubular cells show involvement in these pathways, leading to cellular decay and a chronic activation of inflammation, ultimately resulting in tissue fibrosis and chronic kidney disease (3).

Podocytes have been the primary focus in clinical research aiming to better understand the progressive renal failure associated with Fabry disease. Glycolipid deposits in podocytes are striking on Fabry kidney histology. Podocytes are long-lived, terminally differentiated cells with a limited capacity of replication (4) and separated from the bloodstream by the basement membrane (after birth) (5, 6). These features may be one of the bases for which these cells present with the highest amount of Gb3 inclusions and for the high resistance to the clearance of Gb3 in treated patients. Their dysfunction and loss are closely associated with glomerular injury, proteinuria, and declining renal function.

On the other hand, renal tubular cells are also affected by Fabry disease. The tubular–interstitial involvement attracts less attention perhaps because of mild clinical signs such as polyuria or urine concentration defects. The tubular interstitium makes up a large portion of the whole organ, and therefore, its role must be further considered in pathogenic processes (7). Tubular cells are dividing cells with a high metabolic expenditure and energy consumption. Their main function is the reabsorption of the bulk of the glomerular ultrafiltrated and secretion of solutes into the urine. Beyond this, they are among the cells with the highest number of mitochondria per cell in the body.

In this review, we would like to propose Fabry tubulopathy and its ensuing functional effects as the first pathological signs and contributing factors to the development of FN. We will summarize and discuss the current literature regarding the role of tubular cells in Fabry kidney pathophysiology. Starting from clinical and histological evidence, we will highlight the data from animal models and cell cultures outlining the pathophysiological pathways associated with tubular interstitial injury causing renal fibrosis in Fabry nephropathy.

Clinical manifestations and histologic features

In males with the classic Fabry disease, signs of renal involvement appear during the second decade of life; patients develop mild proteinuria (<1 g/24 h) and a progressive reduction of renal function. End-stage renal disease occurs in most untreated patients around the age of 40. In the late-onset variant, patients have a less severe nephropathy (8, 9).

The intracellular deposition of Gb3 and LysoGb3, caused by the reduction of GLA activity, takes place in all lineages of renal cells. The histological changes in FN start early when clinical signs are absent or minimal. Tøndel et al. reported podocyte foot process effacement and intracellular Gb3 inclusions occurring very early in life. They occur when proteinuria is mild or even in the normal range (10, 11). Over time, podocyte injury leads to the detachment and loss of these cells into the urine, with consequent podocyturia (6, 12). In light microscopy, the visceral and parietal epithelium shows the typical, but not pathognomonic, aspect of vacuolized cells (foamy appearance), while glomerular tufts can depict focal segmental sclerosis. The mesangium expands and presents with hypercellularity. The vasculature can develop increased wall thickness with a hyperplasia of pericytes and smooth muscle, resulting in a remodeling of its structure (12). In tubular epithelial cells, there is a pronounced preference for deposition in the epithelium of the distal tract (10), which may also occur in proximal tubules (13).

The difference between histological levels of deposits between podocytes and tubules could be attributed to the distinct turnover rate of these cells. Podocytes are long-lived non-dividing, terminally differentiated cells that accumulate Gb3 throughout the organism's lifetime. In contrast, tubular cells are dividing cells, and as a consequence, lower deposition levels are found in kidney biopsies.

Trimarchi (14) recently demonstrated that proximal tubules are functionally affected by the decrease of megalin, cubilin, and electrogenic chloride/proton exchanger ClC-5, which may affect the activity of ClC-5, a Cl^-/H^+ antiporter channel mainly located in early endosomes where it is involved in the acidification process of lysosomes. Furthermore, a reduced expression of uromodulin, Na^+-K^+-ATPase, and Na^+-K^+-2Cl cotransporter in thick ascending limb cells has been demonstrated, which could be responsible for the occurrence of polyuria in patients with Fabry disease (15).

Tubular functional alterations are not extensively reported. This is attributed to the fact that the clinical symptoms or complaints related to these alterations are minimal and do not draw the attention of clinicians and patients. In addition, the tests to investigate tubular alterations are cumbersome and therefore are not commonly performed in nephrology units. Nevertheless, concentration defects and polyuria are commonly reported symptoms. This is underlined by the fact that fibrotic lesions such as tubular atrophy and interstitial fibrosis can be present starting from the early phases of FN (3). In more advanced stages of FN, glomeruli are obliterated by global sclerosis, and tubular atrophy is associated with interstitial fibrosis in different phases (8).

The effects of specific therapy on tubular epithelial cells have been minimally investigated. While we have enough data on the cellular clearance of Gb3 from glomerular and endothelial cells, limited data are available for tubular cells. In an early paper on the effect of agalsidase beta on cellular lipid inclusions, Thurberg et al. described the clearance of Gl-3 from distal tubular epithelial cells after 11 months of therapy was initiated in 50% of patients. This value is lower than expected, considering the high turnover of the tubular epithelial cells. We can speculate that specific treatment effectively reduces Gb3 deposition, but the clearance could not be complete (16–18).

Therefore, with regard to the tubular alterations in FN, we have a limited clinical description despite extensive histological evidence and the growing knowledge on the mechanisms by which tubular interstitial fibrosis often leads to end-stage renal disease.

In vitro studies

In vitro studies have predominantly investigated the molecular pathomechanisms in Fabry podocytopathy. These delineated the direct effects of LysoGb3 on podocytes triggering a profibrotic effect through transforming growth factor (TGF)-β1 and Notch1 signaling with fibronectin and Collagen 4 deposition (19, 20).

Likewise, Kim reported on decreased podocyte survival upon LysoGb3 treatment due to RIPK3-mediated necroptosis (21). Beyond this, GLA deficiency resulted in decreased mTORC1 activity with a subsequent increase in autophagy (22). To what extent these effects are solely dependent on Gb3 deposition was recently put into question, as Gb3 clearance of Fabry podocytes using enzyme replacement therapy failed to restore aberrant autophagy and Notch 1 signaling (23). This could also indicate that there is a point of no return in the pathogenesis, especially once profibrotic signaling is triggered. Another explanation for this can be substrate-independent mechanisms such as other lysosomal proteins accumulating and aggravating lysosomal dysfunction, such as the recently reported alpha-synuclein (24).

Jehn et al. (22) identified the acid sphingomyelinase ASAH1 to be upregulated in tubular cells of a patient with Fabry disease. This is particularly interesting, as decreased sphingomyelin, e.g., due to increased ASAH1 expression was detected as a feature of murine kidney aging. (25). In Fabry podocytes and patient-derived urinary cells, there was an increased abundance of lysosomal proteins under GLA-deficient conditions (26). These aberrations could be crucial, as lysosomes are implicated in multiple cellular processes affecting even the structure and function of the endoplasmic reticulum (ER) and mitochondria (27). Likewise, misfolded GLA proteins can trigger direct effects on cellular organelles such as ER stress (28, 29).

Tubular cells collected from the urine samples of patients with Fabry disease revealed an impairment of mitochondrial morphology and increased oxygen consumption rate reflecting mitochondrial dysfunction (30), a pathologic trait also reported in Fabry fibroblasts (31). In line with this, siRNA-mediated downregulation of GLA protein abundance in human proximal tubular cells (HK-2) leads to increased autophagy in a transcription factor EB (TFEB)-dependent manner, in turn, resulting in increased ROS production and proapoptotic signaling (32). In kidney organoids, GLA-KO leads to a decreased expression of multiple nephron markers with increased ROS production and decreased signals for mitochondria with the accumulation of intracellular calcium (33). As the tubular system of the kidney is highly dependent on mitochondrial function to uphold the transcellular transport of solutes and active secretion of compounds into the urine, a dysfunction of the central energy metabolism can have widespread effects. Gb3 and LysoGb3 exposure of HK-2 furthermore results in a wide range of transcriptional abnormalities indicative of epithelial-mesenchymal transition (EMT) (34) that is dependent on an increased expression of TGF-β, N-Cadherin, and alpha-smooth muscle actin (α-SMA) and phosphorylation of the PI3K/AKT pathway.

Overall, the current *in vitro* data point toward mitochondrial dysfunction as a central mechanism in Fabry tubulopathy, which could be the driving force behind profibrotic signaling and EMT through increased ROS production and decreased cellular metabolism.

Animal models of Fabry disease

In the late 1990s, Ohshima et al. presented the first Fabry mouse model, GLA-KO, generated by deleting the *GLA* gene (35). Surprisingly, GLA-KO mice clinically exhibited a normal phenotype despite a progressive accumulation of Gb3 in the kidneys, ranging from 3 to 5 times the levels of the wild type (36). The failure to represent the phenotype of the full human disease can be explained by the fact that the metabolism of glycolipids differs in mice, with Gb3 levels being much higher in murine liver than in the kidneys, while the opposite relationship is observed in patients (37).

A histopathological analysis of a GLA-KO mouse kidney by hematoxylin–eosin staining revealed the presence of inclusions in proximal and distal tubular epithelial cells, and in the glomeruli, parietal epithelial cells revealed the highest amount of cytoplasmic inclusions with only small and inconspicuous podocyte inclusions (38). Strikingly, the electron microscopy of GLA-KO kidney tissue revealed electron-dense concentric lamellar structures only in tubular cells.

Since the limited development of the Fabry phenotype could be due to a lower capacity for synthesizing and accumulating Gb3 per tissue mass than humans, a symptomatic Fabry model mice was developed by cross-breeding the GLA-KO mice with a Gb3 synthase transgenic mice (GLA-KO-Tg). These mice presented higher Gb3 levels in serum and organs as compared to the GLA-KO mice. In addition, serum LysoGb3 was detected at higher levels in GLA-KO-Tg (39).

Electron microscopy revealed electron-dense concentric lamellar structures in the proximal and distal convoluted tubules and collecting ducts, with a small number of lipid inclusions in podocytes (15). The evaluation of renal function in GLA-KO-Tg revealed a deficiency of concentrated urine, the presence of albuminuria, and increased blood urea nitrogen. GLA-KO-Tg mice showed early lethality associated with the loss of body weight, neurological abnormalities, and progressive renal impairment characterized by polyuria, polydipsia, and decreased urine osmolality, which resulted in water- and salt-loss phenotypes, without remarkable glomerular damage (40). The treatment of these mice with recombinant agalsidase resulted in Gb3 clearance from organs, low serum lysoGb3 levels, and reduction in urine albumin concentration.

A decreased ability to concentrate urine, leading to polyuria, is thought to be the first symptom of Fabry disease (41) that could be attributed to distal tubular dysfunction. Moreover, albuminuria might be caused by decreased protein reabsorption at the proximal tubules. These findings indicate that the renal impairment seen in GLA-KO-Tg mice may correspond to the initial steps of renal involvement in patients with Fabry disease (42).

We could assume from this model that the first signs of kidney involvement mainly affect tubular cells instead of podocytes or other glomerular cells. In this model, tubular cells contained the most pronounced lamellar bodies, rounded mitochondria, and disorganized, flattened infoldings. On a molecular and histological level, the tubular–interstitium compartment showed signs of inflammation, oxidative stress, and focal fibrosis, alongside macrophage infiltration. As this pattern occurred without causing profound podocyte injury, it may be important to note the occurrence of tubule injury in addition to podocyte injury in human FN.

Further insights can be gained from a model of unilateral ureteral obstruction (UUO) in GLA-KO mice. This resulted in increased fibrosis and an increased number of tubular apoptotic cells, suggesting that Fabry disease is associated with enhanced tubular susceptibility to apoptosis (42).

The GLA-KO rat model further reinforces the proposition of tubular cell affection as an early sign of FN (43). Fabry rats developed proximal tubular disease that manifested as increased urine flow rate, decreased osmolarity, and increased urine calcium with age. A decline of the estimated glomerular filtration rate also occurred at a later stage. Functional analysis correlated to the histological data, with the proximal tubule cells of KO rats appearing more vacuolated with the accumulation of several large, circular inclusions.

Additional insights into the pathology of FN will definitely be shaped by animal models beyond rodent systems. The first evidence for this is presented through a zebrafish model lacking the GLA homolog. Despite missing Gb3 deposition, due to the fact that zebrafish do not express Gb3 synthase, the model developed increased creatinine levels and proteinuria, pointing toward mechanisms beyond substrate accumulation (44).

Although Fabry disease models to date have failed to represent the full Fabry disease classical phenotype as present in patients, they inform us that a minimal biochemical alteration associated with glycolipid deposits may have an impact on tubular kidney cells that could be related to the development of fibrosis. They reinforce the notion that exposure to low levels of Gb3 early in the disease can trigger a cascade of events. Moreover, of the many different cell types in the kidneys, the tubule could be the most affected one at low deposit levels and is responsible, at least in part, for the development of fibrosis.

Pathogenetic pathways of fibrosis in nephropathies

Based on clinical and histological features, and taking into account animal and cell culture studies, we propose a decisive role for the tubular epithelium and interstitium in causing renal fibrosis in Fabry nephropathy.

Renal fibrosis involves the deposition of the extracellular matrix that determines glomerulosclerosis, vessel arteriolosclerosis, tubular atrophy, and interstitial fibrosis (45). In particular, it needs to be pointed out that the expansion of interstitial fibrosis is the best marker for the progression of renal disease, outperforming the assessment of glomerular damage (46).

Cell damage causes the activation of inflammatory processes with the infiltration and activation of immune cells: neutrophils, macrophages, and dendritic cells. Fibrogenesis results from a long-lasting activation of the pathways and a wound-healing response to tissue injury not resolving (47). This process is marked by inflammation, myofibroblast activation, migration, and matrix deposition (48). The activated immune cells and/or modified epithelial cells stimulate the release of profibrotic cytokines such as TGF-β, platelet- derived growth factor (PDGF), among others. Among the recruited cells, myofibroblasts are detected in the interstitium, the arterioles, and the mesangium. Kuppe et al. demonstrated that pericytes and fibroblasts are the primary cellular source of myofibroblasts (49). α-SMA is the marker of myofibroblast, and it produces filaments anchoring the myofibroblast to the matrix during the process of reorganization and healing (48). In Fabry nephropathy, Rozenfeld et al. demonstrated a pivotal role of myofibroblasts. On renal tissue from biopsies of Fabry patients, proximal tubular cells produce TGF-β, inducing the activation of myofibroblasts in the vessels and glomeruli, and stimulate tissue fibrosis (50).

A recent yet growing body of evidence suggests that these processes are, in part, propagated by an increased systemic inflammatory response. Early depiction of this phenotype reported dendritic cells and monocytes to be involved through direct Gb3 action via TLR4 (51). Both endogenous Gb3 and exogenous LysoGb3 can contribute to these effects (52). Beyond this, TNFa and MCP-1 were increased in patients with Fabry disease, coinciding with elevated CCR2 levels on monocytes (53). Furthermore, an involvement of T cells and an effect on their ratios upon enzyme replacement therapy (ERT) were shown (54, 55), while there were reports of decreased Il-4 production by invariant NKT cells (56). In whole peripheral blood mononuclear cells (PBMCs), differentially altered expressions of TNF and TLR4 were detected (57), and several studies showed higher IL6 and TNFa levels in patient sera (58, 59). The same reports, however, showed conflicting data on Il-1beta. Most recently, first evidence was presented on the therapy-resistant activation of the complement system, further indicating a systemic response (60). In solid tissue, increased monocyte adhesion to Fabry endothelial cells was shown, as also an improvement of this immune attraction through anti-inflammatory drugs (61).

Such chronic inflammation and dysregulated matrix production impair blood flow and cause reduced availability of oxygen to mitochondria. This hypoxia alters mitochondrial function with an increase in reactive oxygen species and collagen synthesis (62). As reported in the previous section, An et al. (32), who studied lysosomes, found significant alterations in number, energy, and fuel consumption. The levels of oxidative stress were high, and oxidative phosphorylation was upregulated. Disturbed fatty acid oxidation has already been linked to interstitial renal fibrosis (63).

TGF-β is a master regulator of the fibrotic process, and its production increases with chronic inflammation in chronic renal disease. In an *in vitro* model of Fabry nephropathy, Jeon et al. (64) demonstrated that under the exposition of Gb3, the human renal proximal tubular epithelial cells undergo EMT driven by TGF-β upregulation. Moreover, lasting damage results in a cell-cycle arrest of tubular cells with subsequent senescence and apoptosis, followed by tubular atrophy and interstitial fibrosis (65). TGF-β can also activate extracellular matrix protein deposition by podocytes *in vitro*. However, TGF-β production is not revealed in the glomeruli of kidney biopsies from patients with Fabry disease (50). This difference suggests that although podocytes are cells with the highest levels of Gb3 deposits, they may not be the ones that create a TGF-β-associated profibrotic environment.

The deposition of Gb3 in the Notch1 system seems to trigger the NF-kappa-B signaling pathway stimulating the synthesis of

chemokine production (19, 20). There is evidence in the literature that the activation of Notch1 determines fibrosis through the activation of the EMT transcriptional program (3, 66).

Moreover, metalloproteinases (MMP) affect the process of fibrosis. MMP-7 can activate EMT, TGB-β signaling, and ultimately the deposition of the extracellular matrix (67). In tubular cell cultures from mice and patients with Fabry disease treated with Gb3 and LysoGb3, an upregulation of the metalloproteinase 9 (MMP9) gene has been demonstrated. MMP-9 degrades extracellular matrix proteins and activates cytokines and chemokines to regulate tissue remodeling (68).

Li et al. (69) reported that renal fibrosis starts focally in specific sites defined as "niches." The niches include kidney residents, infiltrated cells, and the extracellular matrix. The niches have a specific microenvironment that activates fibroblasts. There is a progressive diffusion of fibrotic processes driven by a specialized network that includes tenascin C, connective tissue growth factor, fibrillin 1, and periostin, resulting in an activation of all significant steps causing tissue fibrosis.

The above processes determine fibrosis when they last over time and the disease progresses. However, the fibrosis seems potentially reversible, and the interstitial matrix can resolve renal fibrosis (70). Some evidence indicates that macrophages, ADAMTS13, metalloproteinase, and other proteins can be exploited to tip the balance between production and degradation of the extracellular matrix toward resolution (71).

Lastly, a significant factor further aggravating tubulointerstitial fibrosis occurs as soon as glomerular damage leads to constant (micro-)albuminuria. Currently, our pathophysiologic understanding still points toward a role for albumin as a mediator of tubular damage and renal fibrosis (72, 73). Therefore, future research efforts need to focus on both the clinical and the histological benefits that novel strategies of lowering albuminuria and renal protection bring to patients with Fabry disease (74).

Conclusions

All this reported evidence demonstrates that renal fibrosis is the final result of an insult of different origin with the activation of inflammation, cellular migration, and differentiation, and an increase in the extracellular matrix. In Fabry nephropathy, these processes are present early in the tubular compartment following the primary injury of lysosomal dysfunction and a derangement of the metabolism of Gb3-LysoGb3. As evidence suggests that a mitochondrial phenotype is a partial pathogenic process, tubular cells with their energy needs and consumption may be one of the primary and early cells affected in the kidney. Hence, the contribution of tubular epithelial cells and the interstitial compartment to Fabry nephropathy could be substantial, from the starting point to a progressive and continuous development and contribution of pathological mechanisms leading to renal affection and failure (Figure 1).

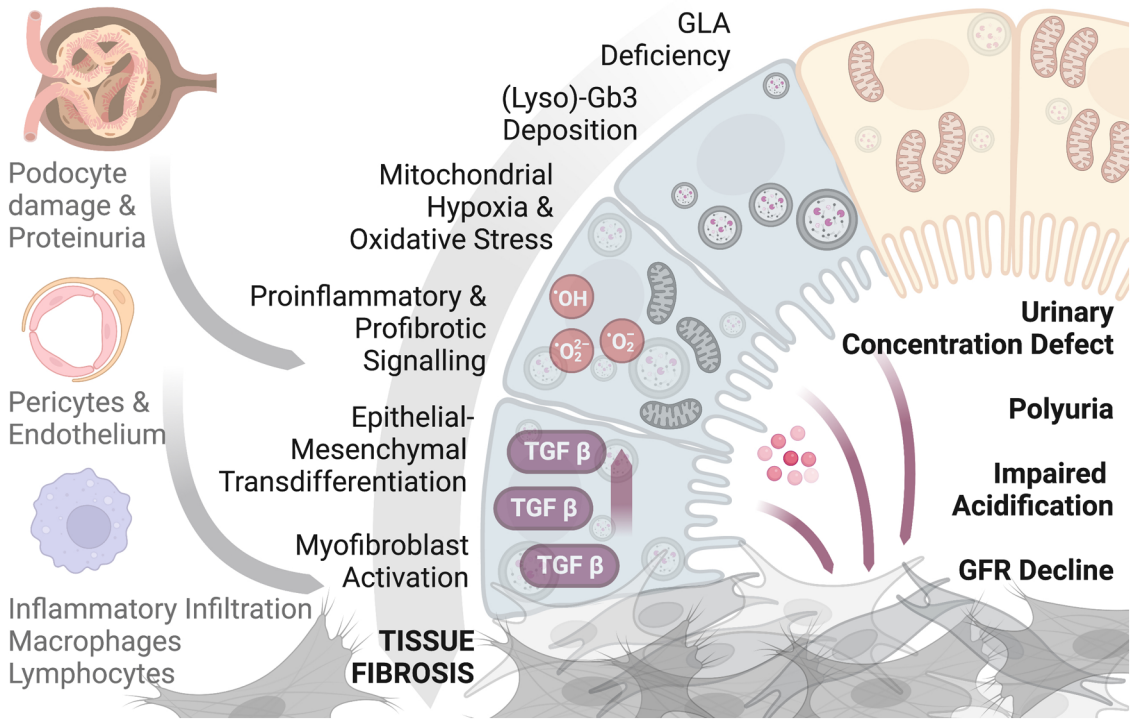

FIGURE 1
A graphical scheme showing the contribution of tubular epithelial cells and the interstitium compartment to Fabry nephropathy. Figure produced with biorender.com.

Author contributions

PR: Writing – original draft, Writing – review & editing, Conceptualization, Project administration, Supervision. SF: Conceptualization, Project administration, Supervision, Writing – original draft, Writing – review & editing. FB: Writing – original draft, Writing – review & editing, Conceptualization, Project administration, Supervision, Visualization.

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Conflict of interest

PR received research grants and travel/accommodation expenses for lectures from Takeda and PintPharma. SF received research grants and travel/accommodation expenses for lectures from Takeda, Sanofi, Amicus, and Otsuka. FB received research grants and travel/accommodation expenses for lectures from Amicus, research grants from Chiesi, and travel/accommodation expenses for lectures from Takeda and Sanofi.

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Cardiopulmonary determinants of reduced exercise tolerance in Fabry disease

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Fabry disease (FD), also known as Anderson-Fabry disease, is a hereditary disorder of glycosphingolipid metabolism, caused by a deficiency of the lysosomal alpha-galactosidase A enzyme. This causes a progressive accumulation of glycosphingolipids in tissues and organs which represents the main pathogenetic mechanism of FD. The disease is progressive and multisystemic and is characterized by early symptoms and late complications (renal, cardiac and neurological dysfunction). Fatigue and exercise intolerance are early common symptoms in FD patients but the specific causes are still to be defined. In this narrative review, we deal with the contribution of cardiac and pulmonary dysfunctions in determining fatigue and exercise intolerance in FD patients.

KEYWORDS

Fabry disease, fatigue, exercise intolerance, cardiac dysfunction, pulmonary dysfunction

1 Introduction

Fabry disease (FD) is a rare and progressive genetic disorder characterized by the deficiency of α-galactosidase A enzyme, leading to the accumulation of glycosphingolipids in various tissues and organs. This multisystemic condition causes symptoms in many districts, including the skin, kidney, heart, and neurological system (1–5).

FD patients frequently experience fatigue, and numerous studies demonstrate that FD subjects have lower exercise tolerance than healthy controls, especially patients with peripheral neurological abnormalities (6). However, the precise causes of fatigue in FD and how it affects the effectiveness of exercise are still unknown (6).

Fatigue is a complex phenomenon resulting from internal homeostasis breakdown in response to increased energetic demand by external stimuli. The mechanisms of fatigue are multifactorial as it can be influenced by a great variety of aspects. Some of these factors are modifiable, including lifestyle, while others are nonmodifiable, such as genetics, and sex. Indeed, fatigue susceptibility impacts men and women differently (7). In FD population, a wide spectrum of factors could synergically contribute to chronic fatigue. Primary muscle and metabolic alterations play an essential role in Fabry-related fatigue, alongside cardiac and respiratory dysfunctions which also have a strong impact on

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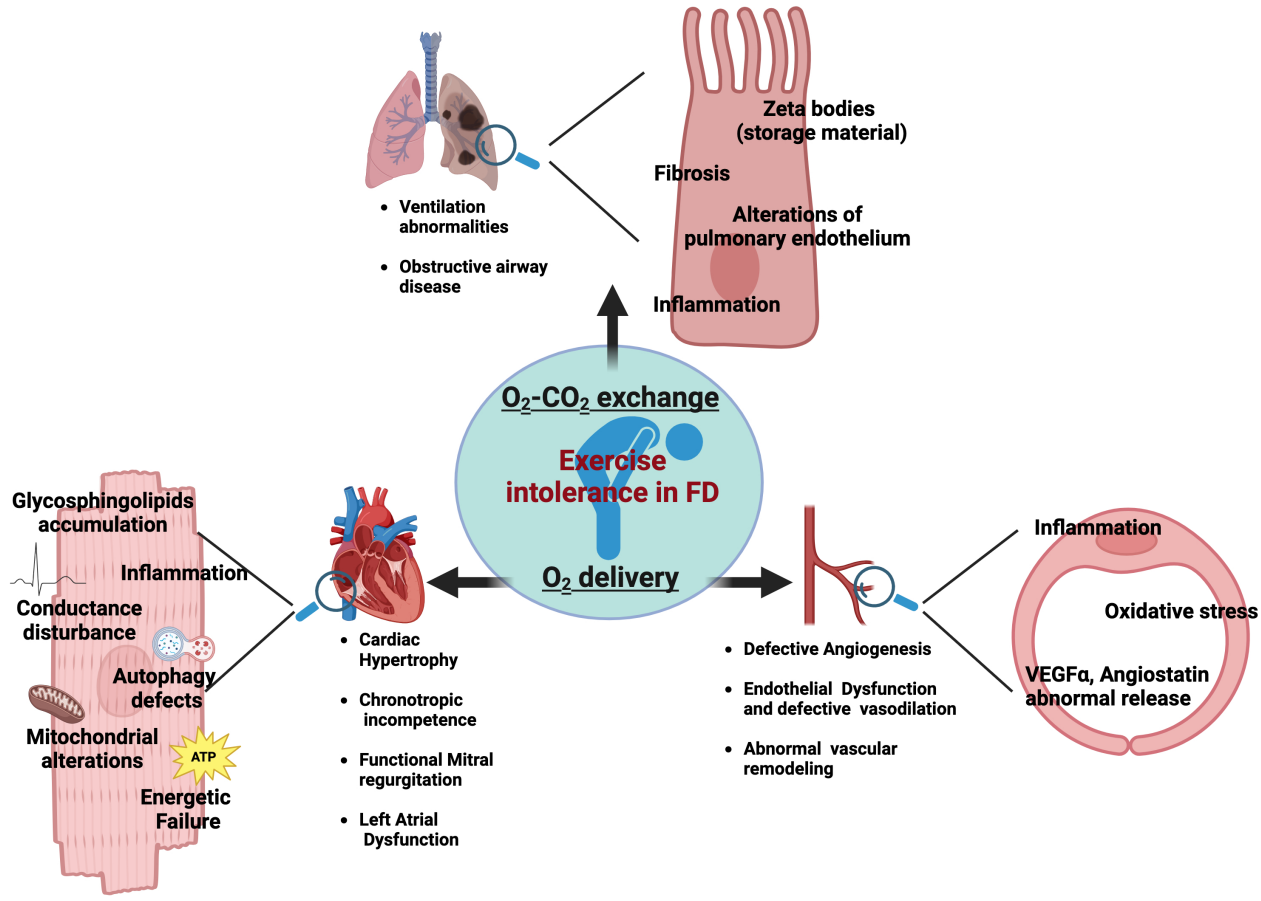

FIGURE 1
Main cardiopulmunary determinants of exercise intolerance in Fabry patients.

exercise tolerance and fatigability of FD patients. The detrimental impact of chronic fatigue on patients' quality of life highlights the need for further research to understand the underlying mechanisms and their synergic effects. In this issue, we specifically focus on the contribution of cardiac and pulmonary abnormalities in determining fatigue and exercise intolerance in FD patients (Figure 1). By a critical revision of the literature, we provide useful insights to diagnose and promptly manage fatigue in FD patients.

2 Exercise intolerance and fatigability in FD patients

An adequate exercise capacity is a complex response that requires the optimal interaction among heart, lung, vasculature, and skeletal muscle. Specifically, during physical effort the orchestration of the following processes is needed: (i) an adequate exchange of O_2 and CO_2 through pulmonary ventilation; (ii) an optimal function of the heart and vascular system to supply oxygenated blood at a sufficient flow rate to meet the metabolic demands of working muscles; (iii) efficient O2 diffusion, nutrients extraction and utilization in skeletal muscle (8). Alterations in any of these critical steps contribute to exercise intolerance and susceptibility to fatigue.

Fatigue and pain especially during effort is an emerging hallmark of FD (9). Commonly, this phenotype has an early onset and can precede FD-related organ damage (kidney, heart, lung) suggesting primary abnormalities of the motor system. However, the cardiovascular and pulmonary alterations also can contribute to exercise intolerance and fatigability in FD, especially in the late stage of the disease requiring specific prevention, management, and treatments. Indeed, although the exact underlying mechanisms are still unknown, it is certain that the reduced exercise capacity in FD is multifactorial and the specific vascular, cardiac and lung alterations described in FD patients could be potential contributors.

In the sections below we annotated the main cardiac and pulmonary morpho-functional alterations which can potentially contribute to exercise capacity limitation in FD.

2.1 Vascular determinants

Peripheral vascular function, especially endothelial homeostasis, is a key determinant of O2 delivery to muscle during exercise. Indeed, the impairment of endothelial dependent-vasodilation in response

to aging and chronic illness is responsible for a reduction in systemic O2 delivery aggravating fatigue and dyspnea (10). Vascular tree alterations have been extensively described in FD patients. Specifically, endothelial and smooth muscle cells are among the main target of Gb3 storage (11), and drive the vascular manifestations in FD patients, including the basilar artery remodeling, increased intima-media thickness and decreased brachial flow–mediated dilation (12, 13). The vascular dysfunction strongly contributes to organ damage in FD exasperating kidney disease, cardiomyopathy, cerebral lesions and likely exercise capacity of skeletal muscle (9, 12, 14, 15). Systemic inflammation, oxidative stress and the abnormal release of angiogenic factors, including VEGFα and angiostatin, are considered the underling mechanisms of endothelial dysfunction in FD (16–18). Data about a direct correlation among endothelial function and exercise tolerance in FD patients are missing. However, already in the early stage of the disease, histological examination shows a significant presence in muscle vessels of glycosphingolipid accumulation (19). On this ground, we cannot exclude that the altered vessel homeostasis could affect O2 delivery, exacerbating muscle incompliance to physical effort. In this scenario, the well-established beneficial effects of physical activity on vessel homeostasis (20) further support the potential therapeutic power of an adapted physical activity program for FD patients.

2.2 Cardiac determinants

In health subjects, the increase of venous return during exercise is matched by an increased cardiac output through elevation in HR, contractility, and lusitropy and not in cardiac filling pressures (21). Specifically, the increased contractility in combination with vasodilation determines an enhanced end-diastolic volume and reduced end-systolic volume guaranteeing the match between systemic perfusion and muscle metabolic needs (8). An insufficient increase in cardiac output during effort leads to lactic acidosis and muscular fatigue limiting exercise and functional capacity (21, 22). In FD patients, cardiac alterations are not the exclusive but key determinants of exercise intolerance (8). More than 50% of FD patients show cardiac involvement including left ventricular hypertrophy, heart failure with preserved ejection fraction, chest pain, and arrhythmias (23, 24). FD register shows that cardiac damage is the first cause of death in FD patients and despite the late clinical onset, cardiac alterations start early in the life and progress sub-clinically (25). The buildup of glycosphingolipids and Gb3 occurs in all cardiac cell types including myocytes, endothelial and smooth muscle cells of intramyocardial vessels, endocardium, valvular fibroblasts, and conduction tissue probably culminating in inflammation, necrosis, fibrosis, and hypertrophic myocardial disarray (26, 27). However, Gb3 accumulation in the heart is *per se* not sufficient to explain the whole spectrum of cardiac manifestations and it has emerging the hypothesis that the primary enzymatic defect in FD triggers other processes that result in biochemical and functional alterations including autophagy alterations, mitochondrial defects, and energetic failure (25, 28). The energy depletion may activate pro-hypertrophic pathways, common to other hypertrophic cardiomyopathies, and may affect cardiac responsiveness to stress. Accordingly, the increase of energetic demand during physical effort represents a stress condition revealing the unsuitable energetic metabolism of the FD heart.

Chronotropic incompetence is another cardiac symptom frequently recorded in the FD population that could contribute to the exercise intolerance of FD patients. In healthy humans, during aerobic exercise, the VO_2 increases approximately 4-fold, mainly through a significant increase in heart rate (2.2-fold) (29). Chronotropic incompetence, broadly defined as the inability of the heart to increase its rate in response to increased demand, is therefore, among the primary contributors of exercise intolerance (30). Approximately, 18%–20% of FD patients show chronotropic incompetence and/or sinus node dysfunction or severe atrioventricular block (31). In a cohort of 38 Australian patients with FD, 70% of subjects had resting bradycardia, with impaired ability to increase heart rate during exercise (32). Electrical alterations and conduction impairment could be involved in this phenomenon. Specifically, it has been proposed that glycosphingolipids accumulation may alter ion channel expression and/or cell membrane trafficking, affecting the electrical properties of cardiac cells (33). It should not be excluded that also energetic depletion can affect the functionality of ATP-dependent pumps, ionic homeostasis maintenance, and conductance capacity (34).

Functional mitral regurgitation as well is a key determinant of exercise intolerance in the general population. Despite the valve abnormalities are not the major limitations for cardiac function in the FD population, mild left ventricular valve regurgitations are commonly reported in FD patients (35). Indeed, the postmortem examination of Fabry hearts revealed that the greatest concentrations of glycosphingolipids were in the mitral valve (36). The most recent study revealed that in classic- FD, the prevalence of valvular disease, from moderate to severe, was 10%, with mitral and tricuspid regurgitation being the most common (37, 38). Beyond the glycosphingolipids accumulation specifically at valvular levels, other phenomenon could be involved in FD valve dysfunction, including thickening of the sub-valvular apparatus (35) geometric distortion of the atria, valvular annulus, or aortic root dilatation (37).

Emerging evidence implicates left atrial dysfunction as an important pathophysiologic mechanism of exercise intolerance (39). Specifically, it has been reported that LA stiffness is independently associated with impaired exercise tolerance and quality of life and may be an important therapeutic target in patients with heart failure with preserved ejection fraction. In FD population, studies of speckle-tracking echocardiography reveal that the left atrial reservoir, conduit, and contractile functions are all affected (40). Also, cardiac MRI studies show an impairment of left atrial function and morphological parameters already in the early stage of the disease (41), when effort tolerance is affected as well.

2.3 Lung determinants

While the main determinant in exercise tolerance is considered the heart, it is essential to recognize that limitation

in lung function also contributes to the overall exercise capacity. Aerobic exercise increases oxygen and ventilation demands, leading to rapid and deep breathing, which can cause airway smooth muscle stretch, bronchodilation, and airway caliber maintenance (42). The multiorgan compromission in FD also includes the lung. Specifically, the pulmonary involvement in FD emerges as alterations of functional parameters, including the increase in resting dead space or ventilation abnormalities (43). Symptoms like coughing, wheezing, and shortness of breath are commonly reported in FD population, however, they could be also influenced by external factors such as smoking habits and age (44–46). Obstructive airway disease has been observed in a range of 27%–36% of FD patients across various cohorts, which is a higher prevalence compared to the general population (47).

The underlying mechanisms responsible for pulmonary function decrease in FD are still not fully elucidated. One hypothesis suggests that the accumulation of sphingolipids in the lung tissue, which in turn triggers an inflammatory response, may be responsible for mechanical damage and small airway disease (48). Rather, recent evidence suggests that pulmonary involvement in FD is indirect, linked to the lipid deposits occurring in vascular endothelium and bronchial smooth muscle, with subsequent obstruction of small airways. Also, in this case, the inflammatory process triggered by sphingolipids could serve as a crucial mechanism for mediating obstructive events (49). Even, Svensson et al. posited that the glycosphingolipids accumulation could activate a maladaptive remodeling of the bronchial tree with interstitial fibrosis and chronic airway limitation (27). Specifically, the obstructive events observed in FD patients could stem from either airway constriction due to smooth muscle hyperplasia or the accumulation of glycosphingolipids directly within bronchial cells (50, 51).

The electron microscopy analysis of sputum and lung biopsy samples from FD patients, revealed the presence of "myeloid-like" inclusions within ciliated cells (52). Additionally, lamellar inclusion bodies known as "Zebra bodies" were detected within the cytoplasm of ciliated bronchial epithelial cells (53). These inclusions were also found in bronchiolar/arteriolar smooth muscle cells and endothelium (54).

Overall, the pulmonary involvement in FD includes micro and macro-structural alterations producing a complex dysfunctional phenotype which still needs further research to be better characterized, as well as, the precise mechanisms responsible for lung function decline need to be delineated. Moreover, poor data are available on the effects of therapies on lung phenotype. Such studies including the report by Brier et al., showed an improvement in pulmonary function with ERT treatment (55), and the same results have also been shown in case reports with more critical situations (56).

2.4 The use of CPET to assess cardiopulmonary involvement in FD

The assessment of exercise tolerance is crucial to establish the overall health and fitness level of a subject. Cardiopulmonary exercise testing (CPET) is a comprehensive diagnostic test used to assess the integrated function of cardiovascular and respiratory systems during exercise. It involves incremental exercise, typically on a treadmill or stationary bike, while continuously monitoring various physiological parameters. These parameters include oxygen consumption, heart rate, blood pressure, ventilation, gas exchange (oxygen and carbon dioxide levels), and other relevant data (57–60). By measuring and integrating these data at different levels of physical effort, CPET provides valuable insights into an individual's exercise capacity, cardiorespiratory fitness, and any abnormalities or limitations in the cardiopulmonary system (61–64). Specifically, CPET provides parameters like heart rate, blood pressure, and cardiac output, allowing to identify alterations in anaerobic threshold and cardiac limitations. Contextually, Pulmonary limitations are detected through the analysis of oxygen uptake, ventilation, and gas exchange parameters, unveiling conditions like COPD or interstitial lung disease. Muscular fatigue could also be assessed by monitoring exercise capacity and an early onset of anaerobic metabolism. Overall, The CPET provides joint data analysis that allows complete assessment of the cardiovascular, respiratory, muscular and metabolic systems during exertion (57).

Therefore, the CPET is a useful tool to assess the impairment of cardiopulmonary homeostasis and function in FD patients. Specifically, the first consideration is that reduced exercise tolerance and fatigue are not only detectable in male patients where the clinical signs of FD are obvious (renal and cardiac dysfunction) but also in heterozygous female patients. Hence, CPET could unveil early and preclinical signs of cardiopulmonary dysfunction. For instance, Wang et al. investigated women with FD and recorded a reduced quality of life alongside fatigue in 58.5% and exercise intolerance in 82.5% of the participants. From this study, it emerges that a decrease in diastolic blood pressure greater than or equal to 10 mmHg was associated with exercise intolerance, while reduced maximal oxygen consumption correlated with fatigue. Moreover, during the stress test, the exercise intolerance reflected the decrease in maximal heart rate (65). A similar phenomenon was also described by Bierer et al. Their research revealed that approximately 46% of individuals with FD experienced a notable decline in diastolic blood pressure during exercise, especially among female patients (66).

To understand the relative involvement of heart and lung in reduced exercise tolerance, Spinelli et al. examined 16 patients with FD compared to control subjects, performing a radionuclide myocardial perfusion at rest and during exercise, tissue Doppler echocardiography, and magnetic resonance imaging (MRI) at rest. The participants were divided into two groups, according to their left ventricular mass and renal function parameters. The study revealed that patients with more severe organ damage exhibited abnormal stroke volume response to exercise, characterized by decreased end-diastolic volume and not reduced end-systolic volume. Moreover, compared with controls, FD patients had elevated plasma levels of NT-proBNP (a marker of cardiac stress), higher indexed left ventricular mass (LVMi), and altered parameters at tissue Doppler echocardiography,

suggesting an advanced diastolic dysfunction. Overall, the study indicated that left ventricular hypertrophy and interstitial fibrosis play a significant role in affecting stroke volume response during exercise in FD, highlighting the impact of cardiac determinants in exercise intolerance of FD population (67).

The incompetence of the heart in supporting exercise-induced stress also emerged from the study by Réant and colleagues. The authors showed that FD subjects reach a lower mean peak oxygen consumption (VO_2) and a higher VE/VCO_2 slope compared to the general population, again confirming the reduction in cardiac output at peak exercise (68). Accordingly, Powell et al., by using CPET, showed a lower increase in heart rate at peak exercise in FD patients, alongside reduced indexed maximum oxygen consumption and indexed oxygen peak, with both maximal and submaximal testing criteria. The Authors also suggest the involvement of pulmonary circulation since a positive correlation between functional capacity and right ventricular volumes at cMRI was found. Indeed, although static imaging revealed normal systolic function, the impaired right ventricular stroke volume seemed to affect oxygen consumption (VO_2) during exercise (69).

Overall, these findings indicate that CPET is a valuable tool to unveil cardiopulmonary alterations in FD even in the early stage of the disease, when the damage to cardiopulmonary systems is not yet full-blown or bland, for instance in women. The main results of all the studies evaluating exercise tolerance in Fabry patients are summarized in Table 1.

2.5 Therapeutic strategies for impaired exercise tolerance and fatigue in FD

Enzyme Replacement Therapy has proven to be a life-changing treatment for Fabry disease, significantly improving the prognosis and quality of life for affected individuals. It addresses the underlying enzyme deficiency, slows disease progression, and mitigates symptoms and complications associated with the condition (1, 23, 70, 71). However, the impact of ERT on exercise

TABLE 1 Alterations of exercise performance parameters in FD patients and impact of interventions including ERT and exercise prescription.

	Parameters of exercise performance		
	Test performed	Untreated patients	Patients post- intervention
Bierer et al. (55)	CPET	Mean VO2max was 1.462 ± 0.25 L/min and decreased by 0.116 ± 0.44 L/min in untreated patients	In response to ERT: - Mean VO2max increased by 0.459 ± 0.64 L/min - Mean oxygen pulse (VO2/HR) increased by 1.71 - Estimated stroke volume (SV) increased by 10 ml
Lobo et al. (32)	Bicycle stress tests with VO(2) max measurement and once-only 6 min' walk tests	Exercise capacity was reduced in FD compared with that predicted from normative population data.	In response to ERT: - Improvement of Anaerobic threshold - No changes in V02 max (different M/F, organ damage, genetic variants)
Tuan et al. (72)	Symptom-limited cycle ergometry	Peak exercise capacity of FD patients was lower than healthy controls. Peak of metabolic equivalent and of oxygen consumption decreased significantly over a period of 3 years in FD patients with cardiac variant	In response to ERT: - Stabilization of exercise capacity in patients with classic variant - No difference in patients with cardiac variant
Schmitz et al. (78)	Cycloergometry (stationary cycling) and isokinetics (resistance exercise).	Lower relative maximum performance in FD population at baseline	After exercise prescription: - The relative maximum performance increased by 12.1%. - The mean of blood lactate at maximum performance increased from 5.4 (78) $mmol{\cdot}L^{-1}$ to a mean of 7.2 (2.4–10.2) $mmol{\cdot}L^{-1}$ ($p = 0.038$). - Patients reported increased well-being, daily activity and reduced fatigue
Wang et al. (65)	CPET	In FD women: - Fatigability (58.5%, 24/41) - Exercise intolerance (82.5%, 33/40). - Decrease in Diastolic blood pressure ≥10 mmHg - Reduction of Maximal oxygen consumption - Reduction in maximal heart rate during stress test	N/A
Bierer et al. (66)	CPET	Decrease in diastolic blood pressure of about 10 mmHg.	N/A
Powell et al. (69)	Bruce protocol (treadmill), ramp protocol (cycle ergometer) and CPET.	- Impaired cardiopulmonary exercise capacity measured by CPET. - Lower heart rate at peak exercise, max indexed VO2, and peak index oxygen pulse.	N/A

N/A, not applicable.

tolerance in FD patients has been poorly addressed, and the few available data are controversial. Such studies suggest that ERT may positively influence the exercise capacity and cardiopulmonary performance of individuals with Fabry disease, in particular increasing the $V0_2$/HR ratio during physical activity (55). Conversely, from other studies, only a modest improvement in the anaerobic threshold emerges for FD patients under ERT, while the V02 max did not change at all (32). The conflicting results could be explained by differences among the study populations (male vs. female) and/or the entity of organ damage, and genetic variants. In a recent report, Tuan et al. assessed the peak exercise capacity of patients with classic vs. cardiac FD variant. The study revealed that patients with cardiac variant experienced a decrease in peak exercise capacity over time, while patients with classic variant-FD showed no significant changes in exercise capacity during the same period. Moreover, ERT appears to have potential benefits in stabilizing exercise capacity in patients with the classic variant and not for subjects with the cardiac variant (72). These findings confirm that the heart plays a critical role in determining exercise capacity in individuals with FD, and that the cardiac variant may have a more profound impact on the exercise tolerance of FD patients and on their response to ERT.

Non-conventional therapeutic strategies should also be employed to manage exercise intolerance and fatigability in FD patients. Exercise tolerance can be trained by exercise prescription. Indeed, exercise therapy is a widely recognized and evidence-based therapeutic approach that utilizes physical activities and exercises to prevent, manage, and rehabilitate various medical conditions. It plays a pivotal role in improving physical function, reducing pain, and enhancing overall well-being for individuals of all ages and fitness levels. It is utilized in various medical settings, including hospitals, clinics, and outpatient facilities, to optimize physical health and improve the quality of life for patients. Exercise therapy also plays a crucial role in managing chronic diseases such as diabetes (73), heart disease (74, 75), and chronic obstructive pulmonary disease (COPD) (76). Regular physical activity has been shown to improve symptoms, control disease progression, and enhance the overall quality of life for patients with these conditions (77). Only one pilot study evaluated the possibility of prescribing exercise in patients with FD. Over one year, the patients underwent an exercise protocol, and 58% of them reported decreased fatigability. Moreover, the study showed that physical performance improved among the patients, with an approximate load increase of 12% (78). This improvement in physical performance suggests that exercise therapy can be employed to enhance functional capacity and overall physical well-being in individuals with FD. However, further studies are needed to support the exercise training of FD patients and also to design a specific and adapted exercise program for this condition. The effects of intervention (ERT or training) on exercise tolerance in FD patients are reported in Table 1.

3 Conclusions

Exercise intolerance emerges as a phenotypic hallmark of FD. Even with different extensions, reduced exercise capacity, and fatigue are observed in FD patients with classic and cardiac variants as well, in males and females, in the presence or not of full-blown target organ damage. The exercise capacity is the result of a systemic engagement of different districts including heart, lung, vasculature, and skeletal muscle (Table 2). Likely, the alterations of their synergic work and dynamism occur early, preceding the single target organ abnormalities. Hence, the assessment of exercise capacity could be a precious tool to reveal the early signs of dysfunctions. The current challenge in FD management is the detection of the "silent alerts" which can guarantee a tempestive therapeutic decision, especially in female patients who are undertreated. In this scenario, we propose to employ the evaluation of exercise tolerance by CPET in the routine diagnostic and monitoring process of FD patients, including females. Another important gap that the research should overcome is the study of the effects of available therapies on exercise tolerance, even because fitness capacity is a key aspect of patient quality of life. Moreover, in the current report, we also point out the lack of data on the effects of exercise training in FD population. Hence, we underline the urgent need of research studies specifically focused on potential therapeutic effects of an adapted physical activity program in FD patients.

TABLE 2 Main determinants of exercise intolerance in FD patients.

District	Mechanisms of dysfunction
Cardiac determinants	Cardiac hypertrophy, chronotropic incompetence, functional mitral regurgitation, left atrial dysfunction
Pulmonary involvement	Ventilation abnormalities, obstructive airway disease
Skeletal muscle determinants	Altered metabolic capacity, fibers disarrangement
Neurological determinants	Anhidrosis, neuronal alterations, depression

Author contributions

OD: Writing – review & editing, Writing – original draft, Conceptualization. JG: Writing – review & editing, Writing – original draft, Conceptualization. AB: Writing – original draft. AF: Writing – original draft. FC: Writing – original draft. AB: Writing – original draft. RA: Writing – original draft. IC: Writing – original draft. MA: Writing – original draft. TD: Writing – original draft. ER: Writing – original draft. LS: Writing – original draft. AP: Writing – original draft. GI: Writing – review & editing, Writing – original draft. DS: Writing – review & editing, Writing – original draft, Conceptualization.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Publisher's note

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Late-onset renal variant Fabry disease with R112H mutation and mild increase in plasma globotriaosylsphingosine: a case report

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Fabry disease (FD) is an X-linked disorder resulting in a deficiency of α-galactosidase A (GLA) activity. The R112H mutation of GLA is relatively common in Japanese FD patients, characterized by a late-onset phenotype, almost normal to mild lyso-Gb3 elevation, and mild clinical symptoms, despite low GLA activity. This is due to the structural features of the R112H GLA protein. We herein report the case of a 42-year-old male patient with late-onset FD with a R112H mutation. The patient exhibited only renal involvement with no other organ damage and was successfully treated with galactosidase beta and subsequent migalastat for approximately 10 years. Especially, migalastat was clinically effective in normalizing plasma lyso-Gb3 levels and inhibiting the progression of renal damage associated with FD. Therefore, the use of migalastat in the FD patients with R112H mutation is highly recommended based on this case report.

KEYWORDS

Fabry disease, R112H mutation, migalastat, proteinuria, chronic kidney disease

Introduction

Fabry disease (FD) is an X-linked recessive disorder caused by *GLA* mutations that result in deficient lysosomal α-galactosidase A (GLA) activity (1). Enzymatic defects lead to systemic lysosomal accumulation of glycolipids, including globotriaosylceramide (Gb3) and globotriaosylsphingosine (lyso-Gb3) (2). Plasma lyso-Gb3 levels are correlated with the severity of FD (3). FD can be classified into the classical type (no residual enzymatic activity and markedly elevated lyso-Gb3 levels) or the late-onset type (low residual enzymatic activity and mildly elevated lyso-Gb3 levels). Affected men with the classical type show angiokeratoma, acroparasthesia, hypohidrosis, and gastrointestinal disorders during childhood or adolescence. In adults, disease progression leads to cerebrovascular disease, cardiac damage, chronic kidney disease, and premature death (4). Men with the late-onset type develop renal and/or cardiac

disorders in adulthood, without childhood symptoms. Heterozygous women can manifest both classical and late-onset type of FD owing to random X-chromosomal inactivation (2).

R112H (*c.335G > A*) and M296I (*c.888G > A*) are common *GLA* variants in Japanese patients with FD. R112H has been detected in various countries, whereas M296I is unique to Japanese patients with FD. Among FD patients, R112H and M296I are characterized by a late-onset phenotype, almost normal to mild lyso-Gb3 elevation, and mild clinical symptoms, despite relatively low GLA activity (5, 6). Therefore, the extent of FD involvement in clinical manifestations should be confirmed not only by GLA activity but also by plasma lyso-Gb3 levels, histological Gb3 accumulation, and genotypic characterization. In this paper, we report a case of late-onset renal type FD with R112H mutation, which was successfully treated with galactosidase beta and subsequent migalastat for approximately 10 years. We discuss the characteristics of patients with R112H-mutated FD.

Furthermore, available evidence indicates that migalastat is a suitable treatment option for FD in patients with amenable mutations. Experience has shown its potential to improve quality of life, control gastrointestinal symptoms, stabilize the renal function, and reduce cardiac hypertrophy. Given its efficacy, broad tissue penetration, simple oral regimen, and the limited treatment options that are currently available, migalastat can be considered as first-line therapy in patients receiving enzyme-replacement therapy who experience side effects, have poor compliance with chronic intravenous administration, or in patients with an unstable disease status (7). R112H is an amenable mutation, and we herein report the clinical efficacy of migalastat in an FD patient with R112H.

Case presentation

A 42-year-old Japanese man was referred to our department with proteinuria (0.6 g/gCr) and mild renal dysfunction (serum creatinine: 1.2 mg/dL). Proteinuria had been noted at a medical checkup conducted when he was 32 of age, but he had not been examined closely. The patient had no medical history other than proteinuria. The patient was a karate instructor and truck driver (height, 167 cm; weight, 77 kg; body mass index, 27.5). He had smoked 15 cigarettes per day for 10 years since his 20s. His clinical laboratory findings showed no immunoglobulin or complement abnormalities. Mulberry bodies were not detected in his urinary sediments.

Abdominal ultrasonography revealed mild arteriosclerotic changes, but no renal or urinary malformations (Figure 1A). A renal biopsy was performed to investigate underlying cause of proteinuria and mild renal dysfunction. Light microscopy of the kidney specimens showed that the glomeruli had swollen podocytes with significant vacuolation (Figure 1B). Approximately half of the glomeruli showed global or segmental sclerosis, and arteriosclerosis was observed in the vessels. Transmission electron microscopy revealed numerous zebra bodies in podocytes (Figure 1C), a characteristic finding of FD.

Reduced GLA activity in leukocyte and increased lyso-Gb3 levels in plasma confirmed the diagnosis of FD (Table 1, Case 1). The plasma Gb3 level was 2.7 μg/mL. A *GLA* mutation (c.335G > A, p.R112H) was identified. Electrocardiography revealed sinus rhythm at a rate of 56 bpm. Echocardiography revealed no cardiac hypertrophy [interventricular septum (IVS) thickness, 9 mm; left ventricular mass index (LVMI), 88 g/m^2] and no valvular disease, with a normal systolic function (ejection fraction: 62%). He had no acroparesthesia, hypohidrosis, angiokeratoma, or corneal opacity. He also had no symptoms of central nervous system (e.g., dizziness or hearing loss).

Agalsidase beta (1 mg/kg, every 2 weeks) was initiated. He also quit smoking. During the 5 years of treatment with agalsidase beta, he exhibited decreased plasma Gb3 (below the detection limit) and a preserved renal function (serum creatinine: 1.3 mg/dL). The patient was then switched to migalastat treatment to reduce hospital visits. Currently, after 4 years of migalastat treatment, his renal findings have not markedly worsened. Angiotensin II receptor blocker (ARB) treatment was initiated because the patient had proteinuria sometimes exceeding 1 g/gCr along with hypertension, and mild changes in serum creatinine (1.4 mg/dL). No findings were suggestive of cardiac involvement (IVS, 10 mm; LVMI, 112 g/m^2). The patient's plasma lyso-Gb3 level decreased (1.5 ng/mL).

Intrafamily screening revealed that his 65-year-old mother was heterozygous for the R112H mutation but had no symptoms. Her 90-year-old mother had not undergone genetic testing but had been undergoing hemodialysis since 87 years of age. The patient's 37-year-old brother and 34-year-old sister had no genetic mutations.

Discussion

In this presentation, the FD patient with the R112H mutation had a late-onset phenotype, mildly elevated plasma lyso-Gb3 levels, and only mild renal involvement. Migalastat treatment following agalsidase beta treatment successfully prevented marked progression of organ damage for approximately 10 years.

R112H is a relatively common pathogenic variant in Japanese FD patients. Sakuraba reported that in 207 Japanese FD patients, M296I was the most common mutation (allele frequency: 5.8%, 12/207), c.639 + 919G > A and R227* were the second most common mutations (4.3%, 9/207), and R112H, R112C, and R301Q were the third most common mutations (3.9%, 8/207) (6). All five male patients with R112H had the late-onset type. In another study of 236 FD patients from 143 families (12), M296I was the most common (3.5%, 5/143) and R112C was the second most common (2.8%, 4/143), and R112H was the third most common (2.1%, 3/143) as well as the other seven *GLA* variants. Two patients with R112H in the study had renal manifestations (details not available). Furthermore, R112H has been detected in various countries, including Argentina (13), the Czech Republic (14), Austria (15), and Turkey (16), where R112H has been detected in screening tests for patients undergoing hemodialysis or peritoneal dialysis. Thus, R112H has been reported to have a late onset and a tendency to develop renal manifestations. These characteristics are consistent with those observed in the present case.

Table 1 shows the cases of FD patients with R112H whose characteristics are evident in previous reports (8–11). Interestingly, in the R112H mutation, plasma lyso-Gb3 levels were almost normal or only mildly elevated, even though GLA activity was almost as low as in classical mutations. Similar findings were reported by Rombach et al. (17) and Tsukimura et al. (5). All patients, with the exception of case 7, showed only renal involvement. In case 7, it is unclear and controversial whether cardiac manifestations and renal failure requiring hemodialysis

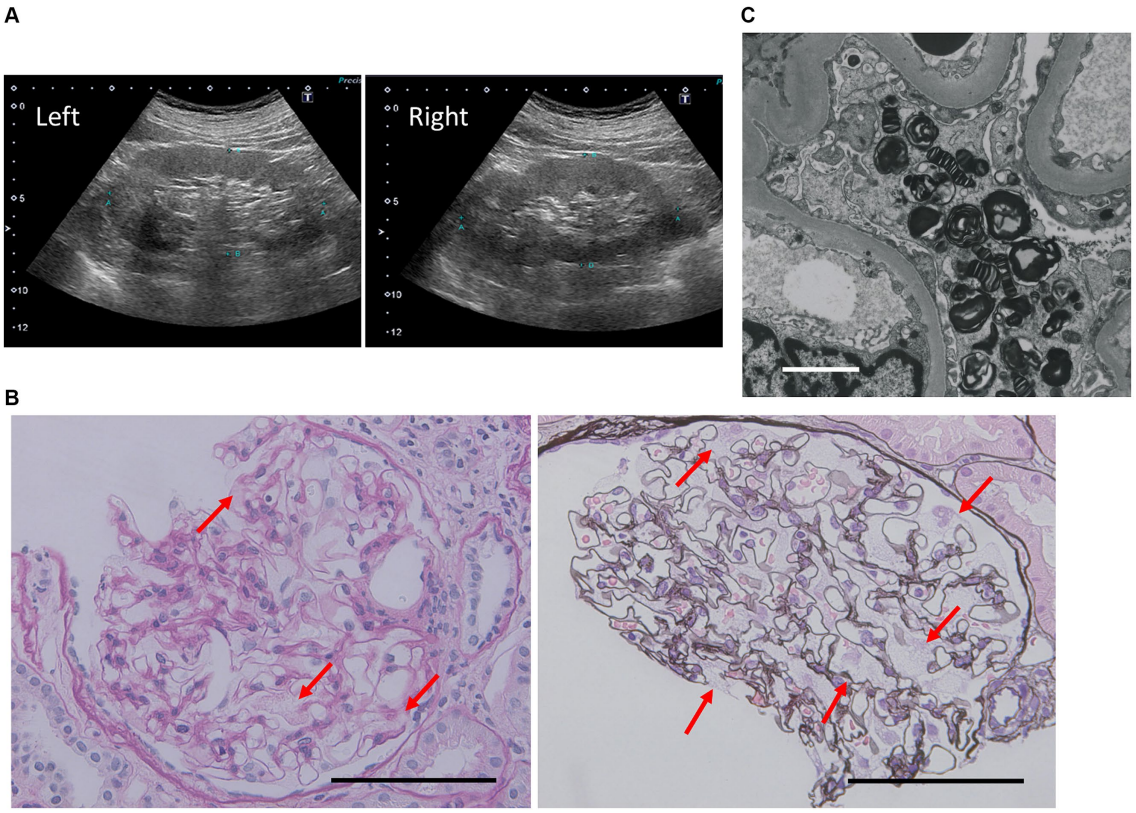

FIGURE 1
The renal findings. **(A)** On abdominal ultrasonography, both kidneys were of normal size and had normal blood flow, with mild atherosclerotic changes. **(B)** Light microscopy images of the kidney section (periodic acid–schiff staining and periodic acid-methenamine-silver staining) showing foamy podocytes with many vacuolations (arrows). Scale bar: 100 μm. **(C)** Transmission electron microscopy images showing zebra bodies in podocytes. Scale bar: 2 μm.

TABLE 1 The characteristics of FD patients with R112H mutation previously reported.

Case	Age	Gender	GLA activity	Standard	Plasma lyso-Gb3 levels	Standard	Kidney	Heart	Symptoms	References
1	42	M	(Leukocyte) 1.0 nmol/mgP/h	49.8–116.4	4.1 ng/mL	<2.0	Proteinuria, mild renal dysfunction	None	None	The present case
2	28	F	—		1 ng/mL		Proteinuria	None	Mild pain	(8)
3	59	M	—		2 ng/mL		None	None	Mild pain	(8)
4	63	M	—		2.4 ng/mL		Proteinuria	None	None	(8)
5	13	M	(Leukocyte) 0.2 nmol/mgP/h	20–80	5.0 nmol/L	<2.0	Proteinuria, mild renal dysfunction (Oligonephropathy)	None	None	(9)
6	21	M	(Leukocyte) <1.0 nmol/mgP/h	17–65	5.3 nmol/L	0.38–0.70	Proteinuria, mild renal dysfunction	None	None	(10)
7	61	M	(Serum) 2.7 nmol/h/mL	4≤	(serum) 3.6 ng/mL	<2.0	End-stage renal failure	LVH, IHD, As	Unknown	(11)

M, Male; F, Female; LVH, Left ventricular hypertrophy; IHD, Ischemic heart disease; and As, Mild aortic stenosis.

could be explained solely by FD. As FD patients age, they are more likely to develop cardiac disorders and progress renal dysfunction from causes other than FD, such as hypertension and atherosclerosis (18). As mentioned above, several cases of renal failure requiring dialysis have been reported in FD patients with R112H, which may have a broad phenotypic spectrum from mild to severe. However, it must be carefully determined whether renal failure requiring dialysis is directly associated with FD severity. In general, FD patients with R112H have mildly elevated plasma lyso-Gb3 levels and mild clinical symptoms.

Tsukimura reported that FD patients with R112H had low GLA activity but substantial amounts of GLA protein, resulting in nearly normal plasma lyso-Gb3 levels (5). R112H is a missense mutation that results in a different amino acid substitution on GLA. R112 is located on the loop comprising the barrel domain of the GLA structure, close to the surface of the molecule, and the amino acid substitution has no effect on the active site (5). This conformational change is thought to affect the stability of the GLA protein, causing partial degradation and denaturation of the mutant GLA protein. The denatured enzyme exhibits slight residual activity, presumably promoting the degradation of lyso-Gb3 in plasma (5). Thus, FD patients with R112H exhibited residual GLA activity, and their plasma lyso-Gb3 levels were lower than those with the other late-onset mutations.

It makes sense to use migalastat, an oral pharmacological chaperone, in patients with the R112H mutation. This is because migalastat would correct the conformational changes in the abundant denatured GLA proteins and greatly increase GLA activity in the R112H mutation. Migalastat has been shown to be responsive to increasing the GLA activity from 2.6 to 14.8% (a 6.7-fold increase) in a GLP-HEK assay of R112H (19). However, the efficacy of migalastat *in vitro* and *in vivo* does not always coincide, and the clinical efficacy of migalastat is only observed in some of the genotypes that meet the criteria for amenable mutations. In patients undergoing migalastat treatment, changes in Lyso-Gb3 levels are not always correlated with increased enzyme activity in leukocytes and Lyso-Gb3 levels may not be correlated with clinical symptoms. As there is no appropriate biomarker for monitoring migalastat treatment, the treating clinician should carefully monitor clinical and laboratory features to confirm the clinical response (20, 21). In this case, migalastat was clinically effective in normalizing plasma lyso-Gb3 levels and inhibiting the progression of renal damage associated with FD. The present case showed a clinical course of renal damage due to atherosclerosis and a better renal prognosis than case 6, which had been untreated for 30 years despite similar GLA activity and lyso-Gb3 levels in plasma (In case 6, serum creatinine was 4–5 mg/dL at age 50). The long-term effects of migalastat are not known, and careful follow-up is needed in the future. Since the latest expert consensus for FD patients states that renoprotective therapies such as ARB and Sodium-glucose transport protein 2 (SGLT2) inhibitors may be effective (22), administration of SGLT2 inhibitors may also be considered in this case.

In conclusion, we reported the case of a patient with a late-onset renal variant of FD with a R112H mutation that responded effectively to migalastat treatment following agalsidase beta treatment. We discussed the pathologic significance of the R112H mutation in FD and the efficacy of migalastat. Therefore, it is important to fully characterize the genotype when considering the clinical course and treatment of FD.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material; further inquiries can be directed to the corresponding authors.

Ethics statement

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

KeT: Writing – original draft. HS: Writing – review & editing. HMo: Writing – review & editing. AO: Writing – review & editing. KaT: Writing – review & editing. HU: Writing – review & editing. HMa: Writing – review & editing. JW: Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Inflammation in Fabry disease: stages, molecular pathways, and therapeutic implications

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Fabry disease, a multisystem X-linked disorder caused by mutations in the alpha-galactosidase gene. This leads to the accumulation of globotriaosylceramide (Gb3) and globotriaosylsphingosine (Lyso-Gb3), culminating in various clinical signs and symptoms that significantly impact quality of life. Although treatments such as enzyme replacement, oral chaperone, and emerging therapies like gene therapy exist; delayed diagnosis often curtails their effectiveness. Our review highlights the importance of delineating the stages of inflammation in Fabry disease to enhance the timing and efficacy of diagnosis and interventions, particularly before the progression to fibrosis, where treatment options are less effective. Inflammation is emerging as an important aspect of the pathogenesis of Fabry disease. This is thought to be predominantly mediated by the innate immune response, with growing evidence pointing towards the potential involvement of adaptive immune mechanisms that remain poorly understood. Highlighted by the fact that Fabry disease shares immune profiles with systemic autoinflammatory diseases, blurring the distinctions between these disorders and highlighting the need for a nuanced understanding of immune dynamics. This insight is crucial for developing targeted therapies and improving the administration of current treatments like enzyme replacement. Moreover, our review discusses the complex interplay between these inflammatory processes and current treatments, such as the challenges posed by anti-drug antibodies. These antibodies can attenuate the effectiveness of therapies, necessitating more refined approaches to mitigate their impact. By advancing our understanding of the molecular changes, inflammatory mediators and causative factors that drive inflammation in Fabry disease, we aim to clarify their role in the disease's progression. This improved understanding will help us see how these processes fit into the current landscape of Fabry disease. Additionally, it will guide the development of more effective diagnostic and therapeutic approaches, ultimately improving patient care.

KEYWORDS

Fabry disease, inflammation, autoinflammatory, innate immunity, adaptive immunity, biomarker, lysosomal storage disorders

Introduction

Fabry Disease (FD) is a rare genetic disorder. It is characterised not just by lysosomal enzyme deficiencies but also by the impact this has on inflammatory pathways. Affecting both males and females, this X-linked disorder arises due to mutations in the galactosidase alpha (GLA) gene (1–3). This results in the accumulation of glycolipids,

globotriaosylceramide (Gb3) and globotriaosylsphingosine (lyso-Gb3) within lysosomes in cells throughout the body. This disrupts normal physiological function including the innate immune response (4, 5), such as antigen presentation (6–8), release of inflammatory mediators, and phagocytosis (6, 7, 9). The substrate accumulation is more than a storage anomaly; it triggers a cascade of inflammatory reactions, disrupting typical cellular functions and setting the stage for multiple systemic manifestations.

Inflammation, the body's innate defence response to harmful stimuli, is central to the pathogenesis of FD. Although inherently protective, this immune response, when awry, can change into a chronic, deleterious state. Innate and adaptive immune responses each contribute uniquely to disease progression and their relationship adds complexity to FD.

The purpose of this review is to chart the course of inflammation as it unfolds in FD. By mapping out the stages, Figure 1, highlighting the consequences of chronic inflammation such as cardiac fibrosis, Figure 2 and delineating the molecular mechanisms at play, Figure 3. By contrasting the roles of adaptive and innate immune responses, we aim to provide a comprehensive overview of inflammatory dynamics in Fabry Disease. Elucidating these mechanisms is pivotal in identifying potential therapeutic interventions, aiming to enhance patient outcomes amidst the complexities of this disorder.

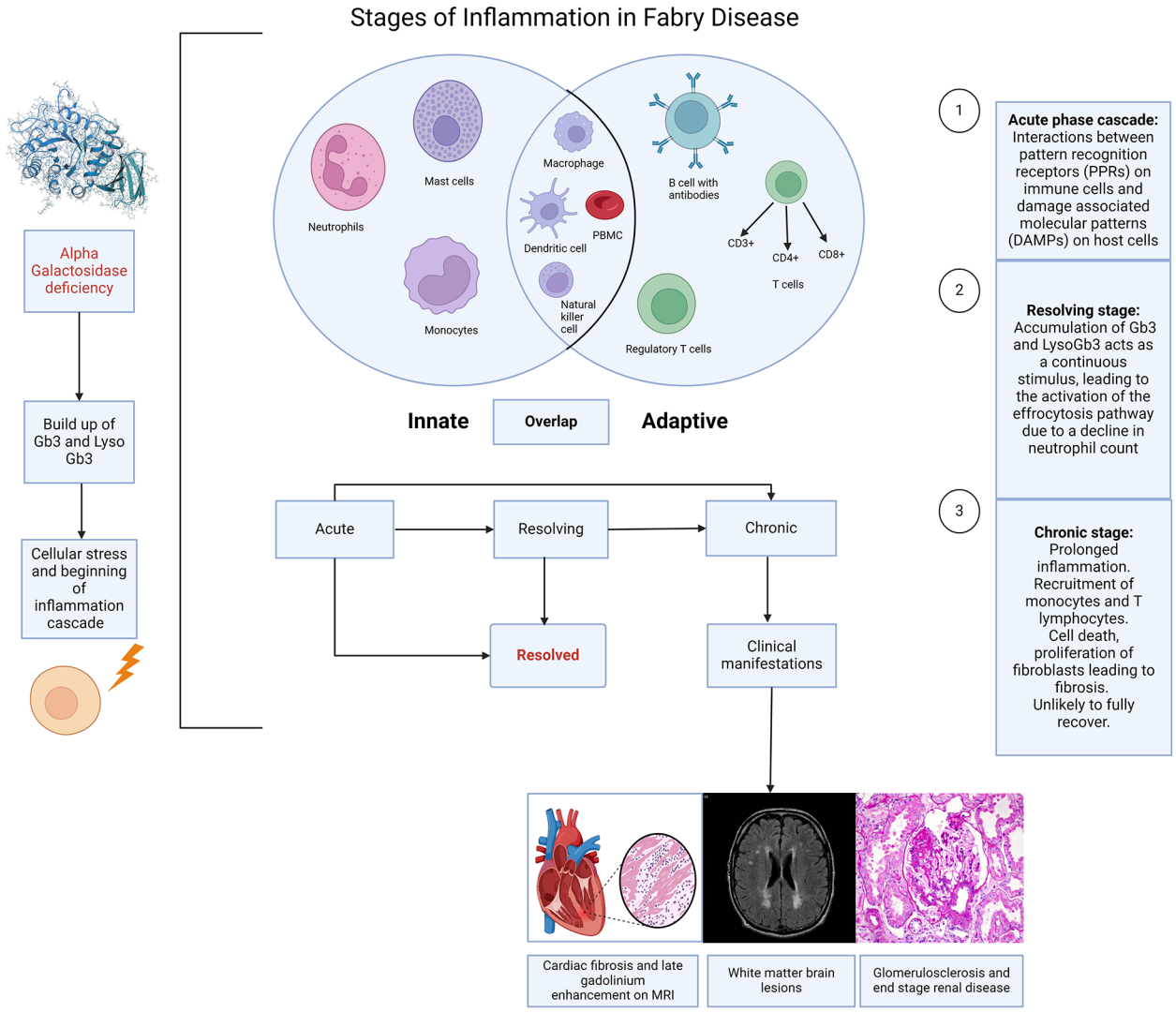

FIGURE 1
Stages of inflammation in Fabry disease. Caption: illustration depicting the different stages of inflammation in Fabry disease (FD) and the interaction of this with the innate and adaptive immune responses. The immune response in FD is largely initiated by the accumulation of sphingolipids, resulting from the deficiency of alpha galactosidase, which triggers cellular stress and the activation of the inflammation cascade. Acute, chronic and resolving stages are a continuum rather than strictly distinct, although each stage has a recognised hallmark. The innate response involves neutrophils, mast cells, monocytes, macrophages, dendritic cells, and natural killer cells. The adaptive response includes T cells (CD3+, CD4+, CD8+), regulatory T cells, and B cells with antibodies. There are also overlaps between the innate and adaptive immune responses, suggestive of an autoinflammatory component to Fabry disease. (Created with Biorender.com).

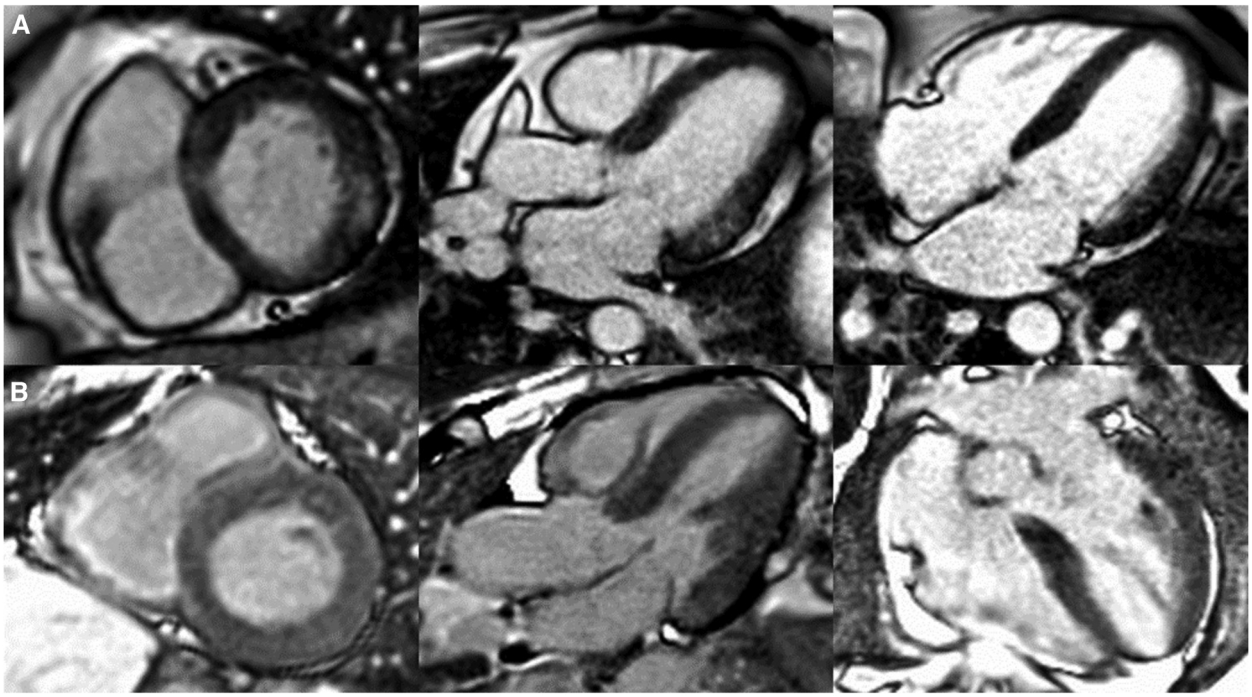

FIGURE 2
Patterns of cardiac late gadolinium enhancement (LGE) via MRI in Fabry disease. Caption: cardiovascular magnetic resonance LGE images captured using phase sensitive inversion recovery sequences with motion correction. Panel (**A**), left to right: basal short axis, 3 chamber and 4 chamber views depicting pathognomonic basal inferolateral wall fibrosis in a female patient (60 years old) with no left ventricular hypertrophy (maximal wall thickness = 7 mm). Panel (**B**), left to right: basal short axis, 3 chamber and 4 chamber views depicting lack of LGE, therefore fibrosis in a male Fabry patient (51 years old) with left ventricular hypertrophy (maximal wall thickness = 17 mm in the basal antero-septum).

Innate vs. adaptive immune response in Fabry disease

The demonstration that the accumulation of Gb3 and lyso-Gb3 activates Toll-like receptors (TLRs) (10–12) and inflammasomes provides clear evidence of stimulation of the innate immune response. This provides immediate, non-specific defence, whereas the humoral, also known as the adaptive response is a slower, more targeted mechanism against specific pathogens. Tissue-resident cells including macrophages, neutrophils, and mast cells become activated in response to the cellular stress caused by this accumulation (13, 14). This has been highlighted by previous histological studies from endomyocardial and renal biopsies demonstrating the presence of these cells in FD (15, 16). However, the limited sample size and diversity in disease stages and severity among these studies, coupled with varying treatment backgrounds [including both enzyme replacement therapy (ERT) naïve and non-naïve patients], complicate the direct correlation of these markers with clinical presentations. This variability presents a challenge in accurately determining disease stages, see Figure 1 and understanding the precise role of these markers in FD.

Consequently, chronic inflammation, also described as an autoinflammatory disorder (17), arises from the maladaptation of the innate immune system, which responds to the recognition of damage-associated molecular patterns (DAMPs) in affected cells (14). However, the roles of the innate and adaptive immune responses overlap in chronic inflammation. This is demonstrated not only by the presence of cells such as macrophages which have a role in both immune responses but also due to the role of cells such as dendritic cells and invariant natural killer (iNK) cells that possess characteristics of both NK cells of the innate immune system and T cells of the adaptive immune system and can recognise antigens. These cells have both been implicated in the pathophysiology of inflammation in FD (13, 18, 19). Furthermore Hayashi et al. have demonstrated the ongoing presence of macrophage related markers (CD68, CD163, CD45) on histology in patients with FD and amyloid compared to conditions such as myocarditis (16). Notably, myocarditis presents a continuum of inflammation severity, ranging from mild to severe and can evolve from acute to chronic stages. These findings underscore that the inflammatory profiles observed in Fabry disease and amyloidosis exhibit greater intensity compared to the varied stages and severity seen in myocarditis. This suggests the presence of an intrinsic factor, likely stemming from glycolipid deposits, sustaining inflammation continuously in Fabry Disease and amyloidosis, unlike the fluctuating nature seen in myocarditis (16). The lysosomal accumulation of glycosphingolipids also disrupts regular cellular operations, leading to cellular stress and apoptosis, which mainly stimulates the innate immune system and instigates inflammation pathways like the inflammasome pathway in macrophages (14). However, the myocarditis patients

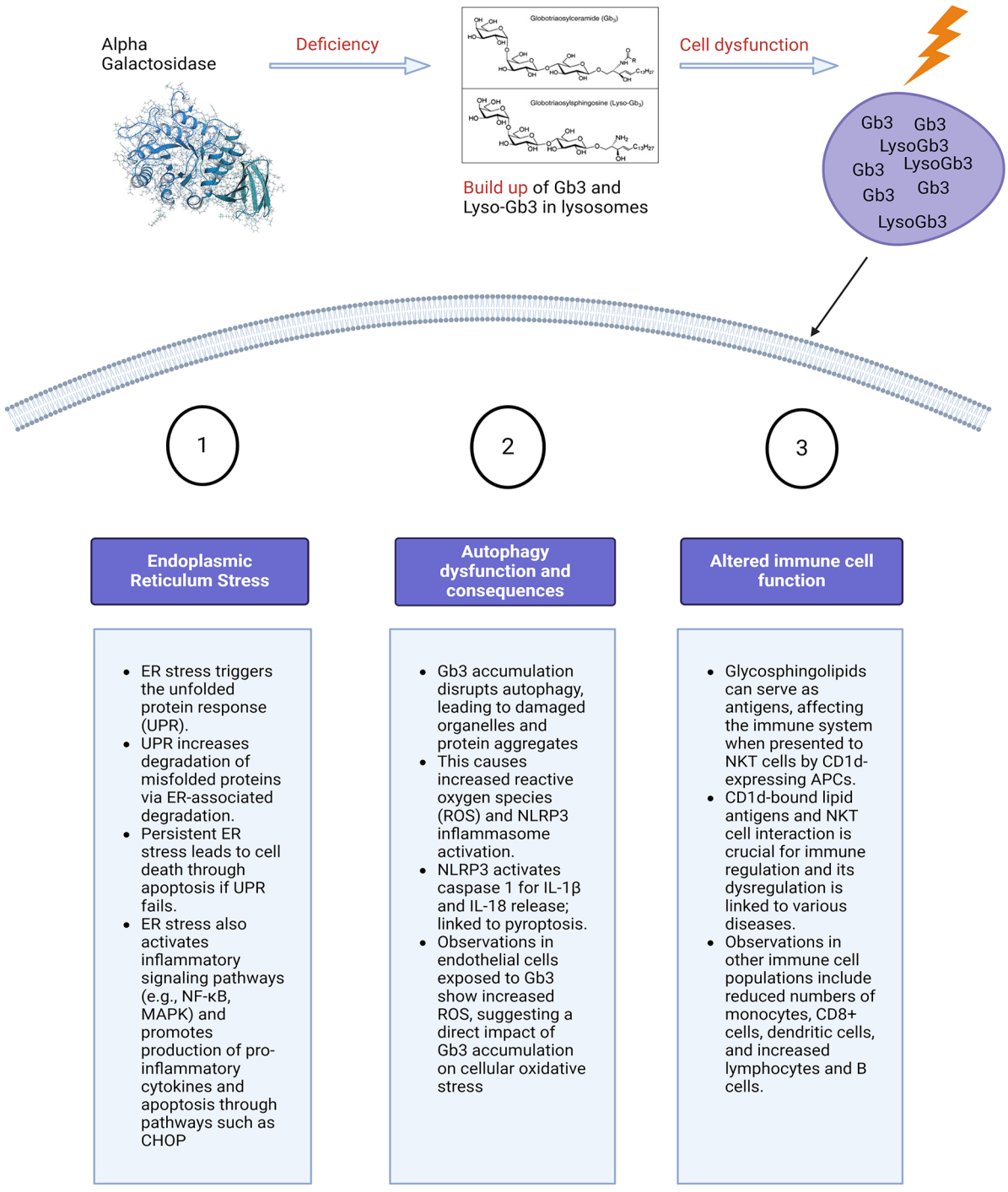

FIGURE 3
Molecular consequences of glycosphingolipid accumulation in Fabry disease. Caption: figure summarising the cellular stress and molecular consequences of inflammation in FD. This figure illustrates the molecular mechanisms resulting from glycosphingolipid (Gb3 and Lyso-Gb3) accumulation due to alpha-galactosidase deficiency. The main pathways affected are ER stress, autophagy dysfunction, and altered immune cell function. ER stress triggers the unfolded protein response (UPR), leading to protein degradation, cell death, and activation of inflammatory pathways. Autophagy dysfunction caused by Gb3 accumulation results in increased reactive oxygen species (ROS) and the likely activation of the NLRP3 inflammasome, leading to cell death. Additionally, glycosphingolipids act as antigens affecting immune regulation through interactions with NKT cells and altering immune cell populations. Glycosphingolipids can also serve as antigens. ER, endoplasmic reticulum; UPR, unfolded protein response; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; MAPK, mitogen activated protein kinase; CHOP, The C/EBP homologous protein; ROS, reactive oxygen species; NLRP3, NLR family pyrin domain containing 3; Gb3, globotriaosylceramide; APC, antigen presenting cells; NKT, natural killer cells.

in this study by Hayashi at al may not represent the full spectrum of myocarditis severity.

In the context of Fabry Disease, Gb3 accumulation also leads to the activation of toll like receptors (TLRs) particularly TLR4 (10–12), which in turn stimulates the production of pro-inflammatory cytokines and chemokines, further amplifying the inflammatory response. TLR4 recognises endogenous molecules exposed during cellular injury. Binding of glycolipids such as lyso-GB3 to TLR4 triggers NOTCH1 signalling, which subsequently activates the nuclear factor kappa B (NF-κB) pathway. This results in the production of pro-inflammatory cytokines, giving rise to both systemic and local inflammatory responses (10, 11). In addition to Fabry Disease, TLR4 has been implicated in other lysosomal storage disorders such as mucopolysaccharidoses (20) and Niemann-Pick Type C (21, 22).

The adaptive immune system, mainly composed of T cells and B cells, typically identifies specific pathogens, and fosters long-term immunological memory was thought to play a less dominant role in inflammation in FD. It is traditionally associated with diseases categorised as autoimmune that involve the adaptive immune system mistakenly recognising the body's own molecules as foreign and mounting an immune response against them. However, in FD, self-neutral glycosphingolipids are recognised as antigens by NK cells by CD1d-bearing antigen-presenting cells (23, 24).

This is more typical of an adaptive immune response and there are some studies that demonstrate an association of FD with autoimmune diseases, most commonly latent endocrine disorders related to the thyroid (25, 26). High levels of antibodies associated with autoimmune diseases such as anti-dsDNA and anti-phospholipid have also been reported in FD patients as well as a higher rate of thrombosis hypothesised to be related to the higher levels of anti-phospholipid (27). However, late presenting endocrine dysfunction such as adrenal insufficiency has been seen in a non-immune capacity in FD (28) and may be related to the degree of glycosphingolipid deposition and underlying genetic mutation (29).

Researchers have investigated NKT cells in patients with Fabry disease, finding varying results. While total numbers of CD8+ NKT cells were consistent across studies, the proportion of these cells differed. One study reported a lower proportion of CD8+ NKT cells in Fabry patients compared to healthy controls (30), while another found no significant difference (18). The variations in the results from these two studies could stem from several factors. For instance, the heterogeneity among patients with Fabry disease, in terms of disease severity, specific GLA gene mutations, age, sex, and other individual health considerations, may lead to differing immune responses even in individuals with the same disease. In addition, methodological differences between the two studies could contribute to the discrepancies observed. The techniques employed for sample collection and preparation, sample size and the use of distinct reagents or protocols for identifying and quantifying NKT cells, and the differing statistical analysis methods may all affect the results.

Furthermore, the treatment status of the patients at the time of the study could also impact the number or proportion of NKT cells, thereby influencing the study outcomes. Additionally, some studies observed alterations in other immune cell populations, including reduced numbers of monocytes, CD8+ cells, and dendritic cells, and increased percentages of total lymphocytes and B cells (30). The clinical implications of these immune cell changes in Fabry disease patients remain unclear. Other important considerations arise when taking into consideration the mouse models used in studies investigating immune pathways and treatment efficacy in FD. The α-GAL-A knockout mouse model is often used (31). Although they accumulate GB3 in their organs, there are limitations that mean directly translating this to human models is challenging. This includes a normal lifespan in the mouse models and lack of FD phenotype (31, 32).

While the adaptive immune system's role in Fabry disease inflammation was previously thought to be relatively peripheral, it is worth highlighting that it may have a larger but not fully understood role. For instance, it may participate in responses to cell death and tissue damage. Additionally, it may respond to enzyme replacement therapy, a common treatment for Fabry disease, generating antibodies against the infused enzymes. In addition, cells such as iNKT have roles that bridge both the innate and adaptive immune response.

Complement activation is a recently emerging subject of research in the field of FD and inflammation (33) and is also known to play a dual role in both immune systems. Whilst being a major component of the innate immune response (34), it has a crucial role in augmenting the adaptive immune response through various mechanisms. Key mechanisms include opsonisation, where proteins like C3b mark pathogens for enhanced phagocytosis, thereby facilitating the processing and presentation of antigens by phagocytes (35).

Additionally, the complement degradation product C3d augments B cell efficiency by binding to antigens and enhancing their uptake through complement receptor 2 (CR2), also known as CD21 (34, 36, 37). Anaphylatoxins such as C3a and C5a directly influence lymphocyte activity, promoting cellular responses including proliferation and differentiation (34, 38, 39). Moreover, complement components help recruit and activate more immune cells, such as dendritic cells, which undergo maturation and are crucial for effective antigen presentation and T cell activation (40).

Through these interactions, complement activation also supports the adaptive immune system, highlighting the ongoing continuum between the two. The role of complement activation will be discussed in more detail under the section "Molecular mechanisms of inflammation in Fabry Disease". Overall, a more thorough understanding of the intricate interplay between the innate and adaptive immune systems in the context of Fabry disease necessitates further research.

Categorising pathways of inflammation to help define stages of disease

The question "What is inflammation?" is fundamental but challenging to address comprehensively within the confines of a review. As we better understand inflammation, its research, and

clinical applications grow clearer. Yet, its definition, especially within lysosomal storage disorders, varies widely. For clarity in disease context, it's useful to categorise inflammation as acute, resolving, and chronic.

Acute inflammation

The acute inflammation phase in FD correlates to the activation of the innate immune response (41). What follows is an intricate interaction between surface receptors present on the host tissue cells, called pattern recognition receptors (PPRs) and two subclasses of molecules. These are pathogen-associated molecular patterns (PAMPs), which are associated with various external pathogens but indistinguishable from the host cells, and damage-associated molecular patterns (DAMPs). DAMPs are associated with local host-related tissue injury and damage (14).

The cascade that follows, makes up the acute inflammatory response. In Fabry disease and other disease processes this results from the processes between PPRs and DAMPs. The main cell types in the acute phase are neutrophils and macrophages, followed by lymphocytes in the sub-acute to chronic phases, Figure 1. The acute inflammatory stage can also be described as two distinct stages based on cellular response: the vascular component and the cellular component. The result is an increase in vascular permeability and a leucocyte mediated extravasation and phagocytosis.

The sustained activation of these cellular and vascular pathways leads to chronic inflammation, a disease state. Acute inflammation by comparison is short lived, it requires constant stimulation to be sustained as the inflammatory mediators involved are quickly degraded and therefore the process ceases once the stimulus is removed (14). If the stimulus is not removed, there is continuous activation of the acute inflammatory cascade and DAMPs leading to cell death and progression to the chronic stage of inflammation.

Chronic inflammation

Chronic inflammation is a slow process that operates in silence, with clinical consequences only apparent at later stages when the damage and resulting clinical sequelae are often irreversible (42). In comparison to acute inflammation, which is time limited, chronic inflammatory responses persist for undefined periods of time, from weeks to years (43). There are also other distinct differences between the two phases. In the acute phase, recruitment of neutrophils from the circulation is the hallmark, whereas the continued recruitment of circulating mono-nuclear leukocytes (including monocytes and populations of T lymphocytes), signal the chronicity of inflammation (43). Accumulation of these cells leads to cell death. Chronic inflammation is also characterised by the proliferation of fibroblasts which by extracellular remodelling and collagen deposition culminating in fibrosis. The emergence of fibrosis can precipitate organ dysfunction, a characteristic of Fabry disease.

Linking inflammation with clinical outcomes presents a challenge. An established hypothesis suggests that accumulated glycolipids in FD instigate a chronic inflammatory response, which subsequently triggers the production of extracellular matrix proteins to aid tissue repair (14). However, if left unchecked, this reparative mechanism can transition into a pathological process, leading to excessive protein deposition and resulting in multi-organ fibrosis. Yet, the direct association between fibrosis and the disease stage remains to be unequivocally demonstrated, confounded by the diverse disease presentations in Fabry disease owing to the varying degrees of residual enzyme activity.

For instance, in females, fibrosis manifests as late gadolinium enhancement (LGE) via cardiac magnetic resonance imaging prior to the development of left ventricular hypertrophy, whereas in males, left ventricular hypertrophy typically precedes fibrosis (44), see Figure 2. LGE is a valuable imaging technique to visualise fibrotic tissues due to their uptake of the gadolinium contrast agent, thereby enabling the identification of the extent and progression of fibrosis. In Fabry disease, LGE serves as an essential tool in disease severity assessment, progression monitoring, and guiding treatment strategies.

Although we will be discussing the inflammatory pathways involved in FD, we will not be discussing these in an organ specific way in the body of this review. However, there are some interesting insights that can be derived from the cardiac manifestations seen in FD.

Cardiac inflammation and fibrosis in Fabry disease are critical factors contributing to the progression of heart dysfunction, with both processes intricately linked to the underlying lysosomal storage disorder and the subsequent immune response. Sphingolipid accumulation leads to activation and infiltration of immune cells (13, 45). This response is influenced by the presence of Gb3 and lyso-Gb3, which serve as antigens, and the activation of the toll-like receptor-4 signalling pathway (13). This leads to local inflammation and promotes pro-fibrotic signalling (13, 14). Additionally, interactions between immune cells and fibroblasts may be critical in promoting the differentiation of myofibroblasts and the subsequent deposition of the extracellular matrix, leading to the cardiac manifestations including hypertrophy and fibrosis (46–48). Endomyocardial biopsy has revealed disorganised and hypertrophied cardiomyocytes, evidence of apoptosis, nitric oxide synthase and accumulation of glycosphingolipids (49). Furthermore, the hypertrophy seen in FD has been linked to ischaemia of the heart tissue (50, 51)' however, more recent advances have highlighted that ischaemia in FD is more likely associated with coronary microvascular dysfunction (52) and some limited evidence that early assessment of coronary flow reserve using cardiac MRI may be an important step in the assessment of the disease; although use of this for prognostication purposes is currently limited due to lack of large scale evidence (53).

The cardiomyopathy seen in FD is more increasingly associated with heart failure with preserved ejection fraction (HFpEF) rather than reduced ejection fraction (HFrEF) (54). In addition, it has been demonstrated that increased levels of pro-inflammatory cytokines such as tumour necrosis factor 1&2, interleukin 6 and matrix metalloprotease (MMP) are seen with increasing severity of cardiac involvement in FD associated with LVH and HFpEF (54, 55) and has differed between FD and healthy volunteers as well as treated FD and non-treated FD. More work is needed on

the impact of inflammation on fibroblast activation and proliferation in Fabry disease, the consequences of which are diastolic dysfunction. Inflammation is also thought to be a key player in the cardiac conduction system malfunction seen in FD (56). Although more studies are needed to further elucidate the processes involved, it has been shown that oxidative damage of cardiomyocytes and DNA leads to cell dysfunction and apoptosis which in turn leads to electrical instability and prolonged refractory periods seen clinically on the ECG as conduction disease (49, 57).

Inflammation also plays a part with regards to immune cells of the heart. This is highlighted by endomyocardial biopsy specimens revealing inflammatory macrophage infiltrates (16), T cell interstitial infiltrates noted on CD3 staining (58) and apoptotic myocytes on caspase 3 positive cytoplasmic staining (58). There has also been recent interest into the role of calpains in contributing to hypertrophy and fibrosis diseases of HFpEF. Calpains are cytosolic calcium activated cysteine proteases. It has been hypothesised that they may play a role in hypertrophy through activation of NF-Kβ and fibrosis through activation of growth factor β. Additional research is required to bring these discoveries from the laboratory to clinical practice (59).

In chronic inflammation, distinct families of chemokines regulate the migration of mononuclear cells in comparison to those involved in the acute phase. There are interactions between innate (mononuclear phagocytes) and adaptive (subsets of T lymphocytes) cells. However, overall chronic inflammation morphologically is characterised by the presence of macrophages, as is also evidenced by macrophage infiltrates seen in biopsies of FD patients as described above (16). Macrophages outnumber other cells, although other mononuclear cells such as monocytes are seen. Cytokines released from macrophages regulate the proliferation and activity of fibroblasts. Depending on the pathology involved and cytokine microenvironment, other cells such as CD4+ T helper cells, activated CD8+ helper cells and plasma cells may also be present in cells with chronic inflammation and have been demonstrated in histology of patients with FD.

Insights into the role of macrophages in Fabry disease have been gleaned from studies investigating the renal system. Examination of inflammatory cells in the kidney has shed light on their connection to fibrosis and provided valuable insights into the pathogenesis of this disease process. Following recruitment to the injured kidney, monocytes differentiate into macrophages influenced by the local microenvironment. Macrophages exhibit two main phenotypes: M1 (classically activated) and M2 (alternatively activated) (60, 61). M1 macrophages, induced by Interferon-γ and lipopolysaccharide (LPS), produce proinflammatory molecules, exacerbating glomerular injury in conditions like crescentic glomerulonephritis. Conversely, M2 macrophages, induced by IL-4, IL-13, and other factors such as IL-10, transforming growth factor β1 (TGF-β1), and glucocorticoids, promote renal fibrosis through various pathways, including profibrotic growth factor production and activation of fibroblasts into myofibroblasts (61).

The balance between M1 and M2 macrophages plays a crucial role in the progression of renal fibrosis. Additionally, the metabolism of arginine influences macrophage polarisation, with M1 macrophages producing toxic nitric oxide (NO) and M2 macrophages contributing to tissue repair and healing through the arginase pathway (60, 61). Furthermore, recent findings suggest that CD163+ macrophages, commonly associated with the M2 phenotype, may serve as relevant mediators of fibrosis in Fabry nephropathy, potentially inducing TGFβ1 production and apoptotic cell death in tubular cells (61). These insights highlight the importance of understanding the functional roles of macrophage subtypes in renal fibrosis and other FD related end organ dysfunction, such as cardiac (62).

In addition, TGFβ1 and vascular endothelial growth factor (VEGF-A) have also been noted to be biomarkers of FD associated cardiomyopathy, which is the chronic stage of the disease as evidenced by left ventricular hypertrophy, fibrosis and arrhythmias (48). Ivanova et al, identified in a study of 45 FD patients categorised by LVH severity, that firstly Lyso-Gb3 levels correlate with TGF-β1 and VEGF-A (63). In addition, there was no gender-related connection between TGF-β1 and LVH. However, VEGF levels were higher in males with FD and LVH. Females who had electrical abnormalities on the ECG but no obvious LVH also showed increased levels of TGF-β1; thus highlighting this as an effective biomarker of early disease (45, 63). The association of TGF-β1 is seen with cardiac end points such as fibrosis and LVH is seen in other lysosomal storage diseases. For instance, in cardiac biopsies obtained from individuals with MPS type I, an excessive activation of TGF-β signalling was observed, as shown by an increase in the levels of phosphorylated SMAD2/3 (64). These findings were associated with cardiac hypertrophy, stenosis in the coronary arteries, and fibrosis.

It has also been demonstrated that Lyso-Gb3 can result in TGF-β1 secretion through vascular endothelial cell activation. However, using a murine model of adventitial fibroblasts, Choi et al. found that lyso-Gb3 could inhibit proliferation, differentiation, and collagen synthesis (65). This highlights that glycosphingolipid accumulation can result in different outcomes according to cell type (45).

In summary, transformation from acute to chronic inflammation is not a continuum, but rather a paradigm shift. Rozenfeld and colleagues hypothesise that FD can be classed as an autoinflammatory disorder (14). This spectrum of disorders is marked by disruptions in the innate immune system, partially as a result of the recognition of DAMPs within injured cells. Autoinflammatory and autoimmune diseases are distinct. Autoinflammatory conditions do not stem from a breakdown in adaptive immune pathways and do not directly influence lymphocyte function. However, both diseases involve the deactivation of negative regulators and an escalation of inflammatory mediators, including chemokines and adhesion molecules.

Resolving inflammation (latent stage)

Resolution of inflammation is now regarded as the third stage of the inflammation pathway. It was initially thought to be a passive process, originating from the depletion of the original injury and therefore the depletion of neutrophils from tissues after the initial infiltration (66). However, distinct biochemical

pathways are actively turned-on during inflammation in the resolution phase (67), providing evidence for the role of active biochemical pathways in resolution and possibilities for new therapeutic targets.

Chiang et al. highlight that there are specialised lipids that promote the active phase of resolution, 9 specialised pro-resolving mediators (SPMs) (68) and have come to be known as immunoresolvents or "resolvins". They promote inflammation resolution by clearance of microbes and promote tissue regeneration via specific cellular and molecular mechanisms. Therefore, these resolvins, their pathways and receptors provide new approaches for treating inflammation associated disease. Pro-resolution pathways promote an adaptive immune response that allows for clearance of infection (68). SPMs are not the only therapeutic targets of interest.

Other pathways of interest include the role of cyclopentenone prostaglandins (69) and those involved in the effrocytosis pathway. Effrocytosis is the pathway by which apoptotic cells are removed by phagocytic cells (70). Driving chronic inflammation down a pro-resolution pathway, inhibiting proinflammatory signals that subvert resolution can be sufficient to trigger spontaneous resolution. This may not apply or be possible in chronic disease where the pathways have been depleted or permanently down regulated. Although these pathways are yet to be studied specifically in the context of Fabry Disease, they are future avenues of consideration, especially as the sub-acute or latent phase of Fabry disease is seen clinically but not well defined.

Molecular mechanisms of inflammation in Fabry disease

In the absence of alpha-galactosidase A, Gb3 and lyso-Gb3 accumulate within the lysosomes of many cells and tissues throughout the body. This leads to cellular damage and inflammation responsible for the multi-system symptoms seen in FD (12, 14). In addition to activation of the innate immune system, there are several other mechanisms by which the accumulation of Gb3 and lyso-Gb3 have been reported to trigger inflammation (13). These will be discussed in the following sub-sections and summarised in Figure 3. The organ specific consequences of direct cellular damage are summarised in Figure 4.

Endoplasmic reticulum stress and unfolded protein response

Gb3 accumulation triggers endoplasmic reticulum (ER) stress and the unfolded protein response (UPR); enhancing molecular chaperones, reducing protein translation, and increasing misfolded protein degradation via ER-associated degradation (ERAD), Figure 3 (71). Persistent ER stress and UPR failure can lead to apoptosis and activation of inflammatory pathways like NF-κB and MAPK, increasing pro-inflammatory cytokine production (72). Chronic ER stress induces apoptosis, activating signalling pathways such as the C/EBP homologous protein (CHOP) (72). Consolato et al. showed certain α-Gal A mutations, such as missense in Fabry Disease cause ER retention and UPR activation, suggesting a new pathogenic pathway (73). Yet, the reproducibility of results in animal cell-related studies has shown varying results, this will be described in the following paragraphs (73, 74). This phenomenon is also dependent on the underlying structural changes caused by the mutation and the residual activity of the enzyme. Changes in the protein structure can affect how it folds, how stable it is, and how it interacts with other molecules, to different extents depending on the type of mutation.

Furthermore, a study by Nikolaenko et al. demonstrated that lyso-Gb3 both at mild and more severe classical phenotype levels led to increased protein ubiquitination, suggesting that Lyso-Gb3 as well as Gb3 accumulation causes ER stress, a feature of the UPR (74). Proteomic analyses showed that lyso-Gb3 affected cellular systems involved in protein ubiquitination and translation, closely linked to the UPR, such as chaperone/heat shock proteins, cytoskeletal proteins, and synthesis/translation proteins such as heat shock protein 60 and the TRiC complex responsible for the correct folding of newly synthesised proteins and the refolding of misfolded proteins (74). Concluding that lyso-Gb3 exposure disrupts protein translation and folding pathways, leading to ER stress and activation of the UPR.

As described, although persistent ER stress and UPR failure can lead to apoptosis and activation of inflammatory pathways, controversial results occur in different experimental models. For example, different results have been seen in FD peripheral blood mono-nuclear cells (75), compared to α-Gal A missense mutations in HEK293 (Human Embryonic Kidney 293 cell line) knock out mice (73). De Francesco et al. demonstrated that mononuclear cells from untreated Fabry patients presented a higher apoptotic state than patients undergoing ERT. They also demonstrated a high level of caspase 3, however no differences in procaspase3 levels between untreated and treated patients, were seen suggesting that ERT may exert its effect downstream to caspase 3 (75). In addition, variable levels of caspase 4 related to ER stress was found as well as no significant differences between the expression of genes relating to ER stress between untreated patients and normal controls, eliminating the possibility that ER stress is involved in apoptotic cell death in Fabry PBMCs.

Impaired autophagy, oxidative stress, and reactive oxygen species

Accumulation of Gb3 in lysosomes interferes with the autophagic process (14, 76), leading to the accumulation of damaged organelles including mitochondria (77) and protein aggregates, triggering inflammation. This leads to increased production of reactive oxygen species (ROS) and activation of the NLRP3 (NOD-, LRR and pyrin domain-containing protein 3) inflammasome. Generation of ROS have been seen in endothelial cells that are exposed to Gb3 (78), and Fabry patients have been noted to have increased oxidative protein damage (79).

The role of the mitochondria in FD pathogenesis and inflammation has been established although the exact mechanisms remain yet to be fully elucidated. Mitochondria play a vital role in cellular energy production, and any disruption to their function can significantly impact conditions like Fabry disease. Lucke et al, were the first to in 2004, report diminished activity in respiratory

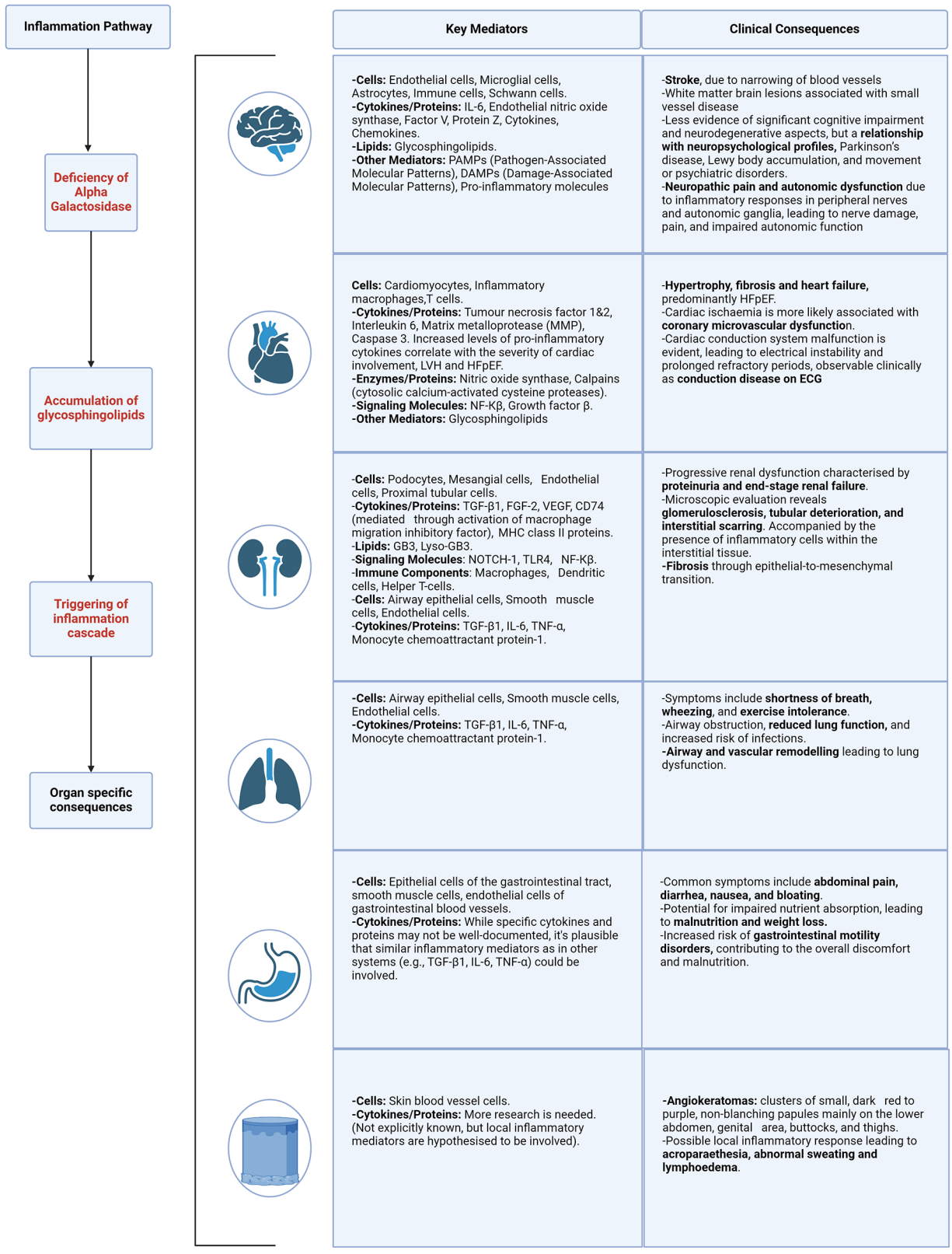

FIGURE 4
Organ specific mediators and clinical consequences of inflammation. Described for the following body systems—brain, heart, kidney, lung, gastrointestinal and skin. Caption: figure demonstrating the clinical consequences and target organ damage resulting from the inflammation cascade and resulting cellular stress. HFpEF, heart failure with preserved ejection fraction; ECG, electrocardiogram; LVH, left ventricular hypertrophy; NF-kB, nuclear factor kappa B; TGF-β1, transforming growth factor beta-1; fibroblast growth factor 2; VEGF, vascular endothelial growth factor; MHC, major histocompatibility complex; TLR4, toll like receptor 4; TNF-α, tumour necrosis factor alpha.

chain complexes using skin fibroblasts from Fabry patients. This compromised energy production was further corroborated by Ivanova et al. in 2019, who observed variations in total ATP levels in peripheral blood mononuclear cells from individuals with Fabry disease and Gaucher disease (80). This was accompanied by impaired autophagy and accumulation of damaged and ageing mitochondrial cells in peripheral blood mononuclear cells (80). More recently, an *in vitro* investigation by Kim SY revealed that lyso-Gb3 can raise ROS levels via a receptor-interacting protein kinase-3 (RIPK3)-dependent pathway, leading to mitochondrial dysfunction by inhibiting respiratory chain complexes I and III (81). These findings suggest that such mitochondrial alterations are due to substrate buildup.

Energy molecules that directly reflect mitochondrial function such as adenosine triphosphate (ATP) and adenosine diphosphate (ADP), as well as indirect biomarkers of mitochondrial function such as phosphocreatine (PCr) have also been implicated in the pathogenesis of FD and other lysosomal storage disorders (82, 83). Studied have demonstrated reduced levels of phosphocreatine and ATP in FD patients, with levels of phosphocreatine partially restored by ERT (84). This has also been demonstrated through phosphorus 31 magnetic resonance spectroscopy; showing a negative correlation between reduced PCr/ATP ratio and increased LV mass in FD (85). The dysfunction of mitochondrial bioenergetics has also been shown in other lysosomal diseases including Pompe (86) and Gaucher disease (87), suggesting a potential unifying mechanisms that may be a viable therapeutic target.

The integrity of the interaction between lysosomes, autophagy, and mitochondria is essential, and its loss plays a critical role in organ damage in AFD. Schumann et al. (88) observed a dysfunction in autophagy, together with the presence of fragmented mitochondria with altered cristae, in renal cells of patients with FD. In addition, Chou et al. observed a decrease in mitochondrial fatty acid oxidation and a pathological shift towards glycolysis-dominant metabolism for ATP synthesis in iPSCs-derived cells (89).

Chou et al. used Induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) obtained from individuals with FD (89). The changes observed were linked to a decrease in the activity of important enzymes responsible for the transportation of fatty acids, specifically mitochondrial carnitine palmitoyltransferase 1 (CPT1) and 2 (CPT2). The alterations were accompanied by an increase in the expression of genes linked to cardiac hypertrophy and a decrease in contractility. Significantly, metabolic disruption continued even after ERT treatment, despite a decrease in Gb3 deposits. This highlights the fact that the development of FD goes beyond just the buildup of sphingolipids and underscores the importance of using additional therapeutic approaches, such as heart failure medication where appropriate alongside ERT. Mitochondria based interventions have been trialled, such as coenzyme Q10 (90) and are discussed in this review by Weissman et al. (45).

Mitochondria also have a large role to play in oxidative stress. Oxidative stress arises when there is an imbalance between the generation of reactive oxygen species (ROS), mainly during mitochondrial oxidative phosphorylation and various enzymatic processes, and the body's ability to detoxify them through its antioxidant system (91). Increased plasma levels of 8-hydroxydeoxyguanosine (8-OHdG), a biomarker for oxidative DNA damage (49, 92) has been found in patients with FD cardiomyopathy. Shen et al. (93) also discovered a lack of tetrahydrobiopterin (BH4), a necessary cofactor for nitric oxide, in heart and kidney biopsies taken from individuals with FD. Other biomarkers of oxidative stress in FD include suppression of superoxide dismutase 2 (SOD2) (94) and glutathione which was found to display sex specific differences in murine models, being downregulated in male mice models more than female (95).

The NLRP3 inflammasome is another emerging area of interest. Given the established link between TLR4 and NLRP3 (96) as well as evidence from Gaucher Disease models (97), highlighting activation of the inflammasome, with subsequent caspase-1 activation, leads to the maturation of IL-1β in Gaucher macrophages and that inflammasome activation in these cells is the result of impaired autophagy. Activation of the NLRP3 is known to result in caspase 1 dependent release of pro-inflammatory cytokines such as IL-1β and IL-18 (98). It has also been linked to gasdermin D mediated cell death termed pyroptosis (99). The discovery of the NLRP3 inflammasome resulted from the uncovering of the gain of function mutation associated with cryopyrin-associated periodic syndrome (CAPS) (100). It has since been implicated in many autoinflammatory diseases that involve chronic inflammation leading to fibrosis and those driven by metabolic dysfunction (101). This includes but is not limited to Alzheimer's disease (102), atherosclerosis (103) and asthma (104). FD appears to have some common inflammation pathways with some of these diseases including cytokine mediated inflammation via IL-1β, mitochondrial dysfunction leading to ROS (105) and lysosomal dysfunction leading to NLRP3 activation (106). FD has been also hypothesised to belong to the "autoinflammatory" class of diseases (14). Targeting the NLRP3 inflammasome and its downstream effects may therefore offer potential therapeutic strategies for managing Fabry disease.

However, in murine models, NLRP3 cytokine blockade still resulted in death (107). Despite the therapeutic targeting of the NLRP3 inflammasome via cytokine blockade, the intervention failed to prevent mortality. This observation underscores the complexity of the NLRP3 pathway and its role in disease pathology. While cytokine blockade effectively reduced IL-1β, it did not address other critical pathways contributing to disease progression, ultimately resulting in death (107). These results highlight the potential limitations of single-target therapies and suggest a need for comprehensive approaches that address multiple aspects of the inflammatory response in these models. It may mean that multiple different approaches to treatment are needed and that these therapies may be best used as adjuvant therapies to existing treatment that replace the defective enzyme, such as ERT or gene therapy.

In addition, Brydges et al. highlight that whilst the major consequence of NLRP3 inflammasome activation is IL-1β

mediated, other factors are likely at play (107). In their CAPS murine models, although a T cell response was evident, it was not necessary for displaying the overt disease phenotype and therefore there is likely an innate immunity component to the CAPS syndrome of diseases that may extend to other autoinflammatory diseases, of which FD, as described, is hypothesised to belong to (14).

Complement activation

The role of complement activation in the pathogenesis of inflammation in Fabry Disease is an emerging area of research interest. As discussed, it plays a major role in the innate immune system, but also bridges and enhances aspects of the adaptive immune response. A pubmed search using the key words complement and Fabry Disease reveals only 2 relevant studies to date (33, 108). A wider search using lysosomal storage disorders reveals insights into the complement pathway through Gaucher Disease (109–111) and to a lesser extent Niemann-Pick C disease in a murine liver model (112).

The complement system is a vast network of complex cascading protein interactions (34), that are triggered via distinct, yet interconnecting pathways comprised of the classical, alternative and lectin (113). A detailed explanation and analysis of the roles of the complement pathway in health and disease is beyond the scope of this review. However, in 1997, Pandey et al. demonstrated that complement activation in Gaucher Disease (GD), driven by glucosylceramide (GC)-specific IgG autoantibodies formed in the absence of glucocerebrosidase (GCase), leads to C5a production, an anaphylatoxin peptide. This in turn binds to and activates the G protein coupled receptor, C5aR1 (the C5a receptor) which affects the balance of GC synthesis and degradation, promoting further GC storage and triggering immune cell activation and recruitment (109). Thus highlighting the direct role of the complement system in GD and C5Ar1 as a potential therapeutic target. More recently, Laffer et al, have demonstrated the activation of the complement pathway in FD, demonstrated in patients with missense and nonsense mutations, both before and after ERT (33). This study brings to the fore some interesting observations with regards to the relationship between FD genetic mutation type, ERT and complement. A reduction in Lyso-Gb3 compared to baseline was seen with ERT in all groups, suggesting a good treatment response. Patients with nonsense mutations displayed a notable increase in levels of C3a and C5a complement components, suggesting a heightened activation of the complement system. This could be linked to the more severe loss of enzyme function typically associated with these mutations, leading to more intense disease manifestations and immune responses. The relationship between mutation type and response to ERT, is discussed in more detail in the section "enzyme replacement therapy and inflammation".

Interestingly, in this study by Laffer et al. (33), the presence of anti-drug antibodies (ADAs) is also predominantly observed in patients with nonsense mutations, who also display the elevated levels of C3a and C5a (33). This correlation implies that ADAs may exacerbate immune responses, further stimulating complement activation. The development of ADAs in response to ERT highlights the immune system's reaction to the therapeutic proteins, potentially impacting treatment effectiveness and patient outcomes. Conversely, patients with missense mutations, who generally retain some enzyme function and are less likely to develop ADAs, display variable levels of C3a and C5a in this study (33). This variability suggests a less uniform response to disease and treatment, influenced by the degree of residual enzyme activity and individual immune reactions.

These findings stress the importance of considering genetic and immunological profiles in managing FD. By identifying the type of mutation and ADA status, clinicians can better predict how a patient might respond to ERT and tailor treatment plans accordingly. Additionally, monitoring complement levels could serve as a biomarker for assessing the inflammatory impact of therapy and the overall effectiveness of the treatment regimen.

Dysregulation of the complement system is associated with a myriad of clinical conditions, including haematological—paroxysmal nocturnal haemoglobinuria (114, 115), ocular—age related macular degeneration (116), many renal pathologies—including, but not limited to, C3 glomerulopathy, autoimmune haemolytic uraemic syndrome (HUS), IgA nephropathy, antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis and lupus nephritis (117, 118). As well as the SARS-COV-2 virus (119, 120). This is summarised by Mastellos et al, as well as the current complement derived therapeutic targets and their approval status (34). This includes the approved for clinical use the C5AR1 anatagonist, Avacopan, used in ANCA associated vasculitis (34). Overall, the complement pathway provides new avenues of research and therapeutic targets in lysosomal storage disorders including FD.

Exosomes in inflammation

Exosomes are nano-vesicles derived from endosomes; on binding to the plasma membrane, they are excreted into the extracellular space (121, 122). They serve as conduits for the transfer of proteins, lipids, and nucleic acids from donor to recipient cells, thereby modulating their metabolic activities. They are made by many types of immune cells including dendritic cells, T and B lymphocytes, macrophages, mast cells, and reticulocytes, as well as tumour cells and neurons. The exosome cargo is also varied and not only composed of proteins and lipids but also nucleic acids such as miRNA. This includes several inflammatory cytokines including TNF-α (121), which is shown to contribute to inflammation in FD and in particular, myocardial inflammation (54, 55).

Exosomes contain little snapshots of the metabolic material of their origin cell and are present in saliva, blood and urine making them attractive potential biomarkers and targets for therapeutic intervention (121). They are not only mediators of intracellular communication but also have been associated with many inflammatory pathways associated with disease including autoinflammatory conditions such as obesity and Type II diabetes (123, 124), as well as autoimmune disease such as rheumatoid arthritis (125, 126), neurodegenerative disorders such as Alzheimer's disease (127) and cancer (128).

From the observations made from these diseases, advances in the understanding of the role of exosomes in modulating the immune system have made great strides over the last decade. Exosomes that carry cytokines, either within their structure or attached to their surfaces, have the capability to regulate the host immune system. They achieve this by activating B and T cells, enhancing, or inhibiting the production of cytokines in various cells, initiating or suppressing inflammatory responses in specific cells, and encouraging the movement of granulocytes to areas of inflammation.

In this way, it was identified that exosomes could not only act as disease diagnostic and prognostic markers but also as potential avenues for therapeutic approaches. Drawing on these insights, researchers have adapted these concepts to investigate the potential roles of exosomes in Fabry disease, both for enhancing diagnostic accuracy and developing novel therapeutic strategies. Certain microRNAs (miRNAs) in urinary extracellular vesicles (uEVs) showed changes that suggest they play significant roles in the disease's early progression (121). Specifically, miR-21-5p and miR-222-3p were upregulated in patients with stable renal function, while miR-30a-5p, miR-10b-5p, and miR-204-5p were downregulated in those with progressive nephropathy, indicating a connection to key signalling pathways involved in nephropathy development (129). Additionally, miR-126-3p levels were found to increase in plasma EVs of FD patients, with levels rising with age. This miRNA was also linked to premature senescence and increased levels due to glycosphingolipid accumulation in cell studies (130). Another key finding was that exosomal secretion was significantly higher in a FD cell model, and following enzyme replacement therapy with agalsidase-beta, this secretion decreased notably. This change in secretion was interpreted as a cellular response to mitigate the toxic buildup of Gb-3, involving mechanisms like enhanced p53 expression (121, 131).

Although these studies provide interesting insights into the role of exosomes in FD and their potential use as disease biomarkers, there are several limitations that may hinder their translation in to mainstream clinical practice. In the study by Levstek et al, the sample size was very small comprising of 10 FD patients and 10 matched controls. All of these were on disease modifying agents of which the impact on the investigated miRNAs is unknown. Lo Curto et al (130), have a larger cohort of 90 patients but have used treatment naïve patients, highlighting that the generalisability of these study findings across the entire FD cohort is limited due to the wide variations in the population groups. The current treatment avenues exploring exosomes in FD will be discussed under section "Therapeutic targets, future perspectives and challenges".

Enzyme replacement therapy and inflammation

Enzyme replacement and oral pharmacological chaperone therapy are now the cornerstones of treatment in Fabry disease with evidence of organ involvement. Exogenous recombinant enzyme preparations such as agalsidase alpha or beta partially stabilise FD by arresting further accumulation of glycosphingolipids in organs; preventing disease progression and deterioration. It is unclear if ERT also modulates the inflammatory mediators seen in cells and tissues described in this review. A consensus on this is made harder by the different methodologies used in the studies attempting to investigate further. This includes different enzyme preparations, patient populations (sex, age, underling coronary artery disease), treated and untreated FD, stage of disease and non-classical phenotypes that are yet to be fully understood.

Some studies have reported elevated levels of proinflammatory cytokines (79, 132), after ERT treatment and others a reduction (132, 133). However, interpretation of these results is challenging especially due to confounding factors such as concurrent treatment with other anti-inflammatory medication such as statins. New ERT therapies are emerging that provide sustained plasma concentrations, such as Pegunigalsidase (134). However, the nature of the complex interplay between this ERT and the immune system is yet to be determined. As of the time of writing this review there are no studies investigating the use of oral chaperone therapy and its effects on the immune system in FD, apart from indirectly by Braunstein et al. who demonstrated that the UPR could be alleviated by migalastat in transgenic flies expressing mutant α GAL-A variants (135).

Proteomic investigations have evaluated the immunomodulatory impacts of ERT in both animal models and human research. Agalsidase beta was found to normalise the expression of genes related to inflammation, vascular function, and renal function in a study using mouse models (136). Whereas a study conducted on human FD urine proteome demonstrated that agalsidase beta resulted in a decrease in the synthesis of pro-inflammatory proteins, including uromodulin and prostaglandins (137). A further study found that the irregularities in urine proteome indicators of female FD patients were rectified using agalsidase alfa and beta, although the study sample size was small.

De Francesco et al, noted a reduced level of apoptosis in FD PBMCs after agalsidase alfa (75). However, a different study noted no change in lymphocytes, CD19+ cells, CD8+ cells or myeloid dendritic cells between those receiving and not receiving ERT, although these levels were significantly higher in FD patients compared to controls (30). Agalsidase alfa, was shown to normalise upregulated expression of hepatic serum amyloid A1, S100 calcium-binding proteins A8 and A9, and lipocalin 2 in a FD murine model (136). Matafora et al, also showed a reduced level of urinary inflammatory markers such as uromodulin and prostaglandin-H2 isomerase with agalsidase beta treatment (137). A small study by Ko et al. ($n = 6$) demonstrated that agalsidase beta is linked to the upregulation of inflammation-related pathways (innate/adaptive immune pathway, lymphocyte proliferation and leukocyte proliferation were actively regulated under ERT), negative regulation of apoptosis, and activation of the innate immune system, T cell receptor, P38MAPK, IL2RB, and T cell receptor signalling pathways. Conversely, it is associated with the downregulation of the oxidative phosphorylation pathway (138).

Studies have also looked at cardiac parameters of FD, such as the presence of LVH and how this is affected by ERT. In a study by Chen et al, ERT reduced serum levels of IL-6, IL-2, IL-1β, TNF-α, MCP-1, ICAM-1, and sVCAM, and also reduced parameters such as left ventricular mass and indexed left

ventricular mass determined by echocardiography (55). This not only emphasises the role of ERT in inflammation but also adds weight to the involvement of these immune complexes in FD associated LVH. All the aforementioned studies highlight that the impact of ERT on inflammatory pathways is varied and complex, also made harder to interpret by the different pathways and immune modulators that are investigated in the different studies.

Although the role of therapy in modulating immune pathways in FD is poorly understood, the recognition of neutralising anti-drug antibodies is an important step in tailoring treatment for maximum efficacy. ERT, which entails infusing a foreign recombinant protein intravenously, can trigger an immune response, leading to the development of specific antibodies against the enzyme (139). Studies show that individuals lacking any endogenous enzyme, termed "cross-reactive immunologic material" (CRIM) negative, are at high risk of such immune reactions following ERT initiation (139–141). Anti-drug antibody formation has been found to be an increased risk in males without any endogenous enzyme (139), higher levels of lyso-GB3 (140, 142, 143) and in some literature the use of agalsidase beta, suggesting there is a link to the increased dosage of agalsidase beta compared to agalsidase alpha (144). The timely identification of ADAs is important as it may lead to a reduction in ERT efficacy and poorer patient outcomes. These findings may also explain why patients with nonsense mutations, and therefore more severe loss of enzyme function had the highest level of the complement anaphylatoxins, C3a and C5a (33).

It is also important to note that this is a separate entity from infusion related reactions (139), which appear to be largely due to IgE independent rather than IgE mediated Type I hypersensitivity reactions (139, 145). Future treatment strategies will focus on further identifying those at risk of anti-drug antibodies by further developing existing laboratory methods to improve sensitivity, including enzyme-linked immunosorbent (ELISA) and electrochemiluminescence (ECL) immunoassays (139). This will allow for more effective screening, confirmation, titration and characterisation of ADAs, thereby enabling the delivery of impactful individualised patient treatment.

Therapeutic targets, future perspectives, and challenges

The management of FD has significantly evolved in recent years, and emerging therapies like substrate reduction therapy (SRT) and gene therapy promise further transformation. Understanding the links between inflammation and FD pathophysiology is crucial for identifying therapeutic targets and developing novel treatment strategies to mitigate inflammation-associated risks and improve patient outcomes.

SRT aims to reduce glycosphingolipid production by inhibiting their synthesis, potentially alleviating the inflammatory response in FD. Instead of replacing the missing enzyme (as with enzyme replacement therapy), SRT uses small molecules to inhibit the synthesis of glycosphingolipids. This leads to less glycosphingolipids produced, thereby reducing the burden on the cells, and slowing or potentially even stopping the pathological accumulation of these lipids; thereby dampening the inflammation response. One such molecule is Lucerastat (146), an oral glucosylceramide synthase inhibitor, that is under investigation and has shown promising early results. However, in a study on Sandhoff disease, a disorder caused by lysosomal enzyme deficiency, SRT treatment improved survival and protected brain cells in mice without reducing glycosphingolipid buildup. This highlights our limited understanding of glycosphingolipidosis and the urgent need for a symptomatic mouse model for better preclinical testing of Fabry disease therapies.

Gene therapy introduces a functional GLA gene to restore α-Gal A enzyme activity. Gene therapy and ERT both serve to address the root issue of Fabry disease—a deficiency in the α-Gal A enzyme. However, the mechanisms through which they accomplish this differ substantially, and these differences may impact their respective effects on the inflammatory component of Fabry disease.

Enzyme replacement therapy involves the administration of the functional α-Gal A enzyme to a patient. The infused enzyme can reduce the accumulation of glycosphingolipids in the body, hence alleviating symptoms. However, ERT is not a cure, and its benefits are temporary. It needs to be repeatedly administered because the body continually clears the infused enzyme. ERT might not reach all the affected cells effectively due to challenges in crossing biological barriers (e.g., blood-brain barrier, intracellular compartments), which may limit its overall therapeutic efficacy. Recent studies are investigating different administration methods, including subcutaneously, although this far this has only been demonstrated in mouse models (147).

Gene therapy, on the other hand, introduces a functional copy of the GLA gene into the patient's cells. This allows the cells to produce the functional α-Gal A enzyme. Currently most vectors target the liver resulting in expression of alpha galactosidase A from liver cells and secretion into the circulation not dissimilar to exogenously infused ERT. However, novel vectors may allow tissue specific expression. For example, Lentiviral vectors are capable of transducing both dividing and non-dividing cells, offering a durable therapeutic effect. By manipulating the viral envelope proteins, scientists can create lentiviral vectors that target specific cell types (148). Additionally, incorporating tissue-specific promoters into the lentiviral DNA can ensure that the therapeutic gene is only activated in desired tissues. Gene therapy may therefore potentially be expressed by tissues and cells which ERT may not easily penetrate, such as the central nervous system. Furthermore, gene therapy may be more convenient than regular bi-weekly infusions.

While gene therapy has shown promise for treating a range of genetic disorders, it is not without potential risks and challenges. The delivery vectors used in gene therapy, often modified viruses such as adeno-associated viruses (AAVs), have been implicated in cases of immune responses such as haemolytic uraemic syndrome (HUS) (149). When the body detects the viral vector, it may respond as it would to an infection. This can lead to inflammation, as the immune system mobilises to

neutralise what it perceives as an invasive pathogen. This inflammatory response can potentially interfere with the effectiveness of the therapy. For example, if the immune system mounts a response strong enough to eliminate the vector, this could limit the duration of the therapeutic effect unless immune modulator therapy is considered. This highlights the importance of careful vector selection. Recent advances in viral vector-based gene therapy have shown promise in preclinical models, with clinical trials underway to assess safety and efficacy in FD patients (77).

Targeting dysregulated cytokines and chemokines, such as TGF-β1, TNF-α, IL-6, and monocyte chemoattractant protein-1, also represent a promising therapeutic avenue. This is a new avenue for research in FD with limited clinical applications currently. However, this approach has been used in other disease states with good clinical success and have now become mainstay of treatment. This includes the use of TNF-α blockers, such as infliximab and adalimumab in Rheumatoid arthritis and Crohn's Disease. Developing inhibitors or modulators of these mediators could alleviate inflammation across various organ systems affected by FD. However, these approaches are not themselves without side effects, which include increased risk of infection due to neutropenia, infusion reactions and anti-drug antibodies (150).

Exosomes have also been explored as potential therapeutic targets in FD, mostly in the role of enzyme delivery. Exosomes are a subpopulation of extracellular vesicles (EVs) (151) which have been investigated as a method for delivering GLA (152). Studies conducted both *in vitro* and *in vivo* demonstrated that these GLA-loaded EVs (EV-GLA) were quickly absorbed and efficiently transported directly to the lysosomes, where they restored enzyme function more effectively than ERT with agalsidase-alfa (152). Moreover, these EVs proved to be well-tolerated and distributed extensively throughout major organs, including the brain, offering a significant benefit over enzyme replacement therapy (ERT), which does not cross the blood brain barrier effectively (153). Additionally, a single dose of EV-GLA administered intravenously successfully reduced Gb-3 levels in key organs such as the kidneys and brain (153).

However, although these new techniques are promising they are currently only in the cell stages of investigation and have yet to be trialled in patients. Whereas the current studies use native exosomes, there is some research ongoing in other diseases using engineered exosomes that may be extrapolated to the FD population in the near future (154). In addition, there are limitations that have been observed from developing EVs for other disease including cancer that are also likely to apply here in addition to the Fabry related heterogeneity of disease.

The isolation and purification of EVs are complex due to their diversity and the presence of other particles in bodily fluids, which is crucial for maintaining purity and avoiding biological effects from contaminants. Additionally, there are no standardised methods for EV production and characterisation, resulting in variability that can impact the effectiveness and safety of these delivery systems.

Efficiently loading EVs with GLA, ensuring their stability during storage and transport, and enhancing their targeting to specific cells affected by Fabry Disease are key technical hurdles. Scalability of production and cost-effectiveness are also major concerns, alongside potential immunogenicity, and safety issues, particularly with EVs derived from allogeneic sources.

Moreover, understanding the *in vivo* behaviour and pharmacokinetics of EVs is limited, complicating their clinical application. Regulatory challenges are significant as well, with guidelines for EV-based therapeutics still under development. Addressing these challenges will be critical for advancing EVs from a novel concept to a practical therapeutic option in clinical settings.

Conclusion

Fabry disease, characterised by progressive organ damage and increased inflammation due to α-Gal A enzyme deficiency, presents a complex interplay between glycosphingolipid accumulation, inflammation, and organ damage. Despite progress in understanding its pathophysiology, many aspects remain unclear. This review has highlighted the need for comprehensive research and clinical strategies to address inflammation in FD, emphasising the importance of innovative biomarkers, new therapeutic approaches, and integrated care for improving patient outcomes. While current treatments like enzyme replacement and pharmacological chaperone therapy have advanced, the exploration of novel therapies such as substrate reduction, gene therapy, and anti-inflammatory drugs opens new avenues.

However, challenges such as understanding the link between glycosphingolipid accumulation, inflammation, and stage of disease, and developing more effective treatments persist. Fabry disease may possess autoinflammatory characteristics or share autoimmune features that are not yet fully understood. In summary, inflammation plays a critical role in FD, requiring ongoing research to refine treatment and management strategies, with the potential for future breakthroughs in understanding and treating this complex condition.

Author contributions

HK: Writing – review & editing, Writing – original draft, Conceptualization. LL: Writing – review & editing. JM: Writing – review & editing, Supervision. DH: Writing – review & editing, Writing – original draft, Supervision, Conceptualization.

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Conflict of interest

DH has fees for speaking and advisory boards from Takeda, Sanofi, Amicus, Idorsia, Chiesi, Freeline, UniQure, administrated through UCL Consultants used in part to fund Fabry related research.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Cardiac involvement in Anderson–Fabry disease. The role of advanced echocardiography

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Anderson–Fabry disease (AFD) is a lysosomal storage disorder, depending on defects in alpha galactosidase A activity, due to a mutation in the galactosidase alpha gene. Cardiovascular involvement represents the leading cause of death in AFD. Cardiac imaging plays a key role in the evaluation and management of AFD patients. Echocardiography is the first-line imaging modality for the identification of the typical features of AFD cardiomyopathy. Advanced echocardiography that allows assessment of myocardial deformation has provided insights into the cardiac functional status of AFD patients. The present review highlights the value and the perspectives of advanced ultrasound imaging in AFD.

KEYWORDS

Anderson–Fabry disease, cardiac function, myocardial strain, speckle-tracking echocardiography, tissue doppler imaging

Introduction

Anderson–Fabry disease (AFD) is a lipidosis caused by deficient αGLA (α-galactosidase A) enzyme activity due to a mutation in the galactosidase alpha gene leading to progressive lysosomal accumulation of complex sphingolipids in vascular endothelial and smooth-muscle cells throughout the body and in the cells of kidney, nervous system, eyes, and heart (1, 2). Cardiac involvement represents the main cause of impaired quality of life and of reduced life expectancy (3). In the heart, accumulation of globotriaosylceramide (Gb3) affects all cell types, including myocytes, endocardium, valvular fibroblasts, and specific myocardium cardiomyocytes. Imaging represents a key tool in the diagnostic and therapeutic approaches to AFD cardiac manifestations (4, 5). Two-dimensional (2D) transthoracic echocardiographic assessment is the first step in to detail morphologic and functional aspects of heart in AFD, namely: left ventricular (LV) concentric hypertrophy, preserved ejection fraction, disproportionate hypertrophy of papillary muscles and, often, right ventricular (RV) hypertrophy. Advancement in cardiac ultrasound imaging allows to quantify myocardial deformation in the different spatial directions offering an innovative evaluation of LV function. Tissue Doppler strain rate curves and speckle tracking echocardiography (STE) unrevealed an impairment of myocardial function in AFD patients with preserved ejection fraction. Given its angle dependency, tissue Doppler is limited in assessing LV apex. Owing the ability to assess myocardial deformation in all segments of LV walls, STE allows to overcome such limitations. Myocardial deformation measurements by 2D STE have been validated against both sonomicrometry and 2D-tagged cardiac magnetic resonance imaging (6). Further technological advancement of real-time three-dimensional (3D) echocardiography has developed software that tracks the motion of speckles irrespective of their direction and

allows to obtain a homogeneous spatial distribution of all three components of the myocardial displacement vector. Myocardial strain can be analyzed from full-volume acquisitions, potentially overcoming the out-of-plane loss of speckles associated with 2D STE analyses. Thus, a series of studies revealing the impairment of LV function caused by AFD flourished during the last two decades. New insights have been provided in subclinical detection of AFD-related abnormalities as well as in disease staging and in prognostication. Given that AFD is a rare disease, most studies offered insights into small cohorts of patients. This paper aims to provide a comprehensive review of current knowledge and of ongoing research into the evaluation of AFD cardiomyopathy with use of advanced echocardiography.

Left ventricular systolic function

The heart in AFD patients presents a phenocopy of hypertrophic cardiomyopathy with preserved LV ejection until the late stages of disease. Strain imaging revealed that the AFD patients may have an impairment of LV systolic function, despite an ejection fraction within the normal range. Studies using tissue Doppler echocardiography demonstrated subclinical LV dysfunction even in early stages of disease (7, 8). Weidemann et al. found out that both peak systolic strain rate and systolic strain were significantly reduced in either the radial or longitudinal direction in 16 AFD patients compared with controls (9). In 2007, the same group described a double peak sign in tissue Doppler strain rate curves in myocardial segments with late gadolinium enhancement by magnetic resonance imaging (10) and demonstrated that a pattern-based analysis was more sensitive and more specific for detecting fibrosis than peak strain (11). Studies by STE showed a decrease in LV longitudinal strain (12–14) involving mainly basal segments (15–17) although apical segments were not completely spared, unlike amyloidosis related cardiomyopathy (15, 18). Moreover, AFD patients with LV hypertrophy were found to have a worse longitudinal function than patients with non-obstructive hypertrophic cardiomyopathy (14). By using the quantitative measurement of myocardial fibrosis with magnetic resonance imaging, Kramer et al, demonstrated an association between the impairment of longitudinal strain and the amount of myocardial replacement fibrosis (19). Interestingly, measuring time-to-peak longitudinal strain unveiled a high prevalence of intraventricular dyssynchrony in AFD patients with LV hypertrophy (20). Cardiac sympathetic denervation has been described in AFD related cardiomyopathy (21–24). It has been found that the presence of denervated areas affects segmental longitudinal strain yielding reduction of global LV function (25). Several studies have highlighted the reduction of LV global longitudinal function before the occurrence of LV hypertrophy, suggesting that myocardial functional impairment is an intrinsic feature of disease and not a consequence of increased LV mass (13, 17, 26, 27). In AFD patients, cardiomyocyte glycosphingolipid storage causes myofibrillolysis and myofilament derangement resulting in a detrimental functional effect (28). A study including a quite large cohort of patients with late- onset cardiac variant showed that AFD patients without LV hypertrophy still had a reduced global longitudinal strain when compared to healthy subjects, despite similar LV mass and morphology (29). It has been suggested that basal longitudinal strain should be considered when screening for cardiac involvement in AFD, particularly in female AFD patients with normal LV wall thickness (17). Reduction in longitudinal strain was found associated with low native T1 in AFD patients without LV hypertrophy (30, 31). Furthermore, in females carrying α-Gal A mutation and without LV hypertrophy, LV global longitudinal strain was impaired in presence of focal myocardial inflammation, identified as focal ^{18}F-luorodeoxyglucose uptake by cardiac positron emission tomography (32).

There are limited data on the impairment of LV circumferential strain in AFD (13, 14, 19, 33). Circumferential strain refers to mid-wall fibers, the same myocardial portion where fibrosis finds its most typical distribution in AFD. Shanks et al. did not find any difference in circumferential function between AFD patients and age- and gender matched healthy subjects (13), while other studies by echocardiography (14, 21, 33) or cardiac magnetic resonance (34) demonstrated that, alongside with impairment in longitudinal function, AFD patients experienced the decrease of global circumferential strain and the loss of base to apex gradient irrespectively of LV geometry (14). Conversely, patients with nonobstructive hypertrophic cardiomyopathy compensated the decrease in longitudinal function with an increase in global circumferential strain and preservation of the base-to-apex gradient (14). Thus, the loss of base to apex gradient seems to be specific to AFD cardiomyopathy and could be caused by the greater impairment of subepicardial fibers, which are mainly responsible for circumferential strain (35).

The data on LV radial strain are even more scarce. Color Doppler myocardial imaging demonstrated an impairment in radial strain rate of posterior wall in AFD patients with LV hypertrophy as well as in female patients with normal LV mass and evidence of late gadolinium enhancement by cardiac magnetic resonance (9, 36). Studies by 2D STE reported conflicting findings (13, 37). While an early study showed normal values of radial strain (13), another study including a larger population demonstrated an early deterioration in LV radial strain, affecting even patients without clear-cut wall hypertrophy (37). Interestingly, global longitudinal strain was significantly associated to LV mass whereas, radial strain was not. However, a recent study by 3D echocardiography has shown an inverse correlation between LV mass and radial strain in 75 AFD patients (51% with LV hypertrophy or concentric remodeling). The use in 3D analysis of a different method for radial strain assessment that was based on volume conservation might account for the different results (38). However, among the various myocardial deformation components, global longitudinal strain has shown the best ability in detecting subclinical LV systolic dysfunction. Figure 1 shows representative examples of longitudinal strain in AFD patients (panel 1). Nevertheless, longitudinal strain is influenced by loading conditions. Myocardial work derived by pressure-strain analysis is a novel non-invasive method to characterize myocardial

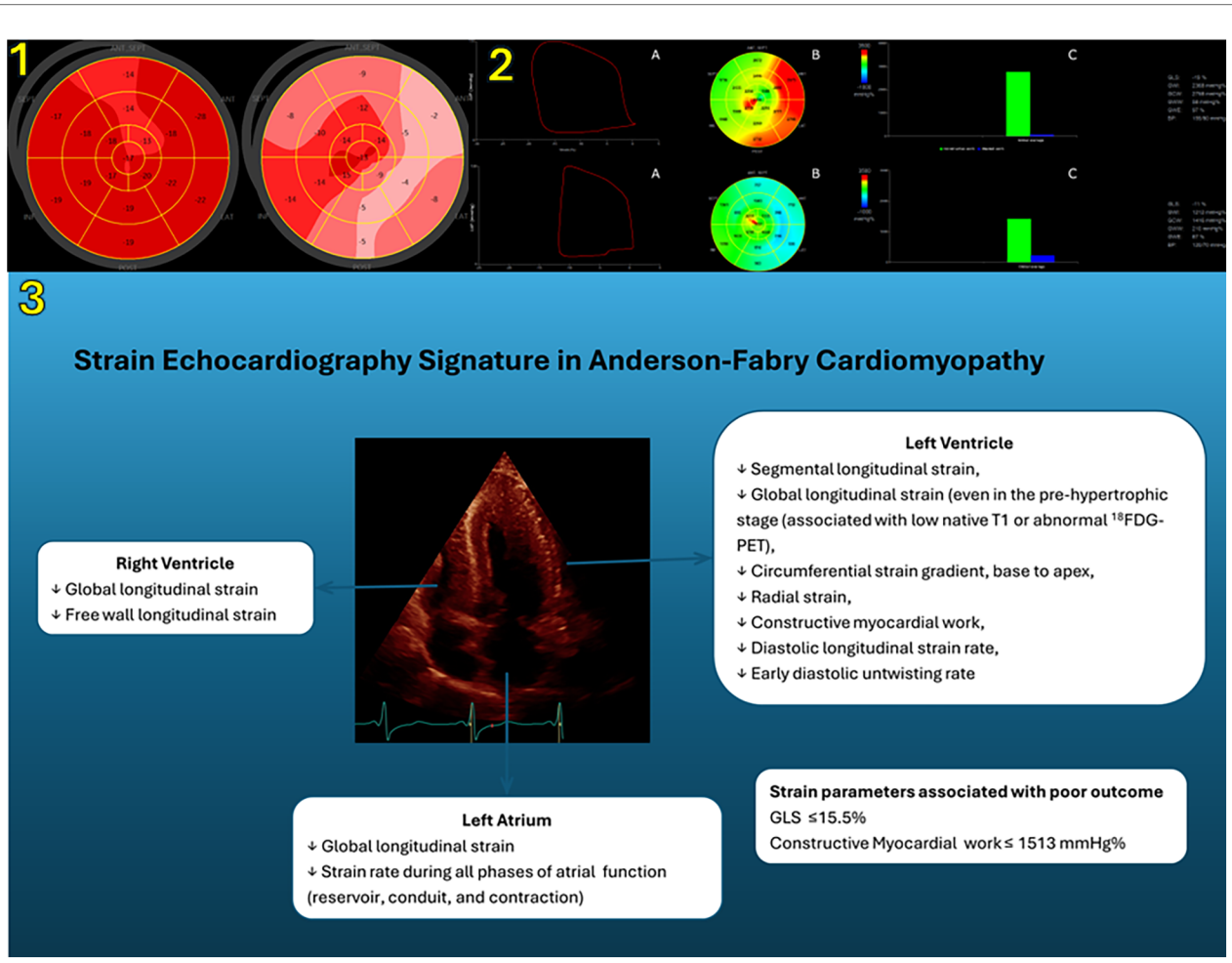

FIGURE 1
Panel 1. Left ventricular longitudinal strain measurements by means of two-dimensional speckle tracking echocardiography. Bull's-eye rendering of LV longitudinal strain from a 42-year-old female AFD patient without LV hypertrophy (left) and a 53-year-old male AFD patient with LV hypertrophy (right). Panel 2. (**A**) Representative example of pressure–strain loops by estimated LV pressure and LV longitudinal strain; (**B**) Bull's eye plot showing segmental LV myocardial work index in a 17-segment model; (**C**) constructive work (green column) and wasted work (blue column) values from the same patients as in the panel 1 (*upper*, female patient without LV hypertrophy; *lower*, male patient with LV hypertrophy). Panel 3 shows the role of strain echocardiography in the characterization of cardiac involvement in AFD. LV, left ventricular; AFD, Anderson–Fabry disease; SEPT, septal; LAT, lateral; ANT, anterior; INF, inferior; POST, posterior; LVP, left ventricular pressure; GLS, global longitudinal strain; GWI, global work index; GCW, global constructive work; GWW, global wasted work; GWE, global work efficiency; BP, blood pressure; ^{18}F-FDG PET, ^{18}F-luorodeoxyglucose uptake by cardiac positron emission tomography.

deformation in relation to afterload conditions (39). Early findings indicate that myocardial work may have an additive value in the functional assessment of AFD cardiomyopathy (40, 41). In the Figure 1, representative examples of myocardial work from AFD patients are shown (panel 2).

Left ventricular diastolic function

Progressive LV hypertrophy with preserved ejection fraction and diastolic dysfunction have been described as the major echocardiographic features of AFD cardiomyopathy (42). There is a growing awareness of diastolic dysfunction being an early sign of cardiac involvement in AFD. It has been suggested that the tissue Doppler derived diastolic index, namely early diastolic mitral annulus velocities (i.e., e′) could provide satisfactory preclinical evidence for diastolic dysfunction in patients with AFD (7). Yet, this was not found in subsequent studies (8, 29). The diastolic strain rate measured by 2D STE emerged as a sensitive tool in detecting diastolic dysfunction, better than conventional diastolic indices (43). Shanks et al. showed that longitudinal strain rate parameters, particularly those measured during early diastole, identify AFD patients from healthy controls, independent of LV hypertrophy and in a more specific manner than tissue Doppler measurements (13). A recent study confirmed the impairment in diastolic longitudinal strain rate of AFD patients without clear-cut LV wall hypertrophy (44). LV diastolic longitudinal strain rate is attenuated by myocardial fibrosis, a typical feature of AFD cardiomyopathy (45). Similarly, LV diastolic rotational mechanics may be also impaired in AFD patients. Indeed, reduced early diastole untwisting rate has been demonstrated associated to myocardial sympathetic denervation (21).

Left atrial function

Histopathological findings demonstrated the accumulation of Gb3 in the left atrium (LA) of AFD patients supporting atrial myopathy (42, 46, 47). However, few studies have investigated the effects of AFD on LA size and mechanical function. LA acts as a blood reservoir during ventricular systole, as a passive conduit during the passage of blood from the pulmonary veins to the left ventricle during early diastole and as a contractile chamber to increase ventricular filling during atrial systole. Strain and strain rate imaging allows to assess atrial function via the analysis of the cardiac cycle. Boyd et al. used tissue Doppler imaging with a four-point segmental approach to assess LA strain and strain rate and demonstrated that LA systolic strain and early diastolic strain rate were selectively reduced in AFD patients with LV hypertrophy. Interestingly, LA enlargement and reduced atrial compliance were found in the subgroup without LV hypertrophy, despite a normal diastolic function with e′ values like those in controls (48). Almost all studies explored LA function using 2D STE, as the technique allows a complete assessment of endocardial strain. Morris et al. could detect LA myocardial dysfunction in AFD patients, even when LA volume was normal. However, in their study data on conduit function were not reported (12). Notwithstanding, a retrospective study comparing 50 AFD patients with 50 healthy control subjects demonstrated that LA reservoir, conduit, and contractile functions were all affected in AFD patients (49). Saccheri et al. analyzed LA function in AFD patients with LV hypertrophy in comparison with patients with hypertrophic cardiomyopathy and found out that both disorders exhibited a severe functional impairment, although LA volume was lower in AFD (50). Conversely, a lower LA volume and a lower impairment of all three phases of LA mechanics have been detected in AFD patients than in patients with cardiac amyloidosis (51, 52). Several data suggest that differential echocardiographic diagnostic work-up of unclear LV hypertrophy can be improved by integrating LA strain analysis. Frumkin et al. analyzed patients with AFD cardiomyopathy and patients with LV hypertrophy due to other causes and found that LA conduit strain showed the highest diagnostic accuracy to discriminate AFD, superior to the posterolateral strain impairment and papillary muscle hypertrophy pattern (53). Likewise, in a recent study by cardiac magnetic resonance imaging the impairment in LA reservoir strain performed better than the established approach using LV mass index and low native T1 in identifying early disease (54). Although none of the above parameters has so far been validated as independent predictor in large enough cohorts, deformation analysis by means of advanced echocardiography or other cardiac imaging modality has the potential to provide valuable insights into LA functional status of AFD patients. Bradyarrhythmia are common manifestations of cardiac involvement in AFD, often requiring pacemaker implantation. It has been demonstrated that LA reservoir dysfunction can be a useful marker associated to bradyarrhythmia (55). Atrial fibrillation is a possible complication of AFD occurring in about 13% of patients. The risk factors for atrial fibrillation hitherto identified are limited to age, LV hypertrophy and atrial dilatation. Few data suggest an association between the impairment of LA strain parameters with the occurrence of atrial fibrillation and stroke in FD patients (49). However, the role of LA dysfunction as a risk factor for atrial fibrillation needs to be addressed in large studies. Quite common features of central nervous system involvement in AFD are non-specific periventricular and deep white matter lesions along with silent lacunar infarctions of the brain. In a small cohort of AFD patients, Esposito et al. found that LA function expressed as peak atrial longitudinal strain was inversely associated with the presence of non-specific white matter lesions (56).

Right ventricular function

Anatomopathological findings demonstrated that structural changes such as the accumulation of Gb3 take place also in the right ventricle (RV). RV hypertrophy, defined as wall thickness >5 mm, is more frequent with increasing age, and the extent is correlated with the degree of coexisting LV hypertrophy (57–61). When assessed by conventional echocardiography, indices of RV systolic function may be found within the normal range, even when severe RV hypertrophy is present (12, 60). Indeed, only in the late disease stages RV involvement progresses to severe systolic and diastolic RV dysfunction (57). Morris et al. (12) evaluated longitudinal systolic strain peak from the free and septal wall (i.e., RV strain) and just from the free wall of the RV (i.e., RV FW strain) and unveiled systolic dysfunction in 20% of patients (61, 62). Lillo found out that the physiologic difference between the RV-FW strain and the global RV strain was preserved regardless of the presence of overt cardiomyopathy (62). Compared to patients with hypertrophic cardiomyopathy, AFD patients showed worse RV FW longitudinal strain, despite comparable conventional parameters (52, 61). Conversely, a minimal involvement of RV function has been documented in AFD compared to cardiac amyloidosis (51). According to 2D echocardiography, RV involvement seems to be a late phenomenon of the disease as RV strain is preserved in the pre-hypertrophic phase (62). Nevertheless, a pilot study by 3D STE showed an early subclinical functional damage (63). Nevertheless, the putative 3D imaging advantages that derive from the independency from the through-plane phenomenon and the ability to provide real information on volume and wall deformation with no need for geometric assumptions (64), still need to be confirmed in larger patient cohorts.

Advanced echocardiography and prognosis

Identifying patients who are at risk of adverse cardiac outcome may facilitate more evidence-based treatment guidance (65–67). The assessment of LV function by longitudinal strain has become widely adopted, but its prognostic value in AFD remains unclear. Early findings indicated a link between alterations in LV global longitudinal function and symptomatic status and prognosis (12). Interestingly, also basal longitudinal strain reduction was found

associated with major adverse cardiovascular events (17). In a cohort of 96 AFD patients, global longitudinal strain showed an incremental prognostic value over clinical factors, LV mass index and diastolic function, during a median follow-up of 5.2 years (40). The prognostic value of LV global longitudinal strain has been confirmed by other studies including one by 3D echocardiography (38, 68, 69). Mechanical dispersion STE has been proposed as an additional risk marker (70). The only study utilizing strain derived myocardial work for the assessment of LV function in AFD suggested a higher accuracy of myocardial work in comparison with global longitudinal strain (GLS) in predicting event free survival has been observed, with constructive myocardial work being the best performing index (40).

Advanced echocardiography and the effects of therapy

While there is a growing acceptance of the role of strain imaging in early detecting of cardiac involvement of AFD and thus, in determining the candidacy to disease- specific therapy, there is a lack of findings regarding its usefulness in assessing the effects of therapy Already in 2003, Weidemann et al. demonstrated a significant improvement in longitudinal and radial strain values by tissue Doppler after 1 year of enzyme replacement therapy (9), whereas the presence of myocardial fibrosis did not benefit from therapy over a period of 3 years (71). A significant decrease of longitudinal systolic strain rate at basal-mid level of LV lateral wall was observed in AFD patients treated prospectively with enzyme replacement therapy for 6 years (72). There are findings suggesting that enzyme replacement therapy may delay the onset of cardiac involvement, thus, supporting the initiation of therapy at an earlier stage of the disease (73). Recently, a significant improvement in apical circumferential strain was observed during enzyme replacement therapy (44) The effects of therapy on LA function have been scarcely investigated. Pichette et al. reported an improvement in LA reservoir strain and in some cases in conduit and contractile strains after 12 months of treatment (49). However, therapy was able to improve LA strain, but not to reduce LA volume (62). A recent study demonstrated no improvement, rather a stabilized LA strain in patients treated with enzyme replacement therapy as well as in those receiving chaperone therapy (74). Finally, therapy seems to have no direct impact on RV morphology and function (59).

Advanced echocardiography: benefits and pitfalls

The diagnosis AFD is based on signs and symptoms suggestive of a systemic disease, family history, an absent or reduced (<5% of normal) leukocyte α-GalA activity level (in men) and is confirmed by genotype testing. Standard and advanced echocardiography are not enough to confirm diagnosis of AFD, but provide essential insights in the functional evaluation AFD, unrevealing myocardial dysfunction in patients with LV hypertrophy and preserved ejection fraction. Once a diagnosis of AFD disease has been established, the presence of abnormal global or segmental strain in an otherwise normal heart may be suggestive of early involvement and should trigger closer clinical follow-up. In the Figure 1, the role of strain echocardiography in identifying the features of heart involvement in AFD is shown (panel 3). When comparing standard and advanced echocardiography to other morphological analysis such as cardiac magnetic resonance, it has to be kept in mind that the information is often additive, more than alternative. Indeed, cardiac magnetic resonance can provide a precise evaluation of heart morphology and tissue characteristics. Native T1 mapping allows early detection of cardiac involvement in a pre-hypertrophic stage and can discriminate between control subjects and AFD patients without LV hypertrophy. Moreover, low myocardial T1 values in pre-hypertrophic stage correlate with reduced global longitudinal strain (30). However, its wide adoption is hampered by the lack of standardized cut-off values for T1 mapping as the analysis is influenced by imaging equipment and protocols. In this perspective, echocardiography has the advantage of being widely spread, less expensive and easily repeatable. Nevertheless, cardiac imaging findings, either by advanced echocardiography or by cardiac magnetic resonance, are not specific nor pathognomonic of AFD. Some feature can help diagnosis: in the setting of LV hypertrophy the presence of a typical pattern of midmyocardial late gadolinium enhancement in the basal to mid inferolateral wall may aid in differential diagnosis (10). Longitudinal strain

TABLE 1 Strain echocardiography features of Anderson–Fabry disease and other conditions of left ventricular hypertrophy in adults.

	Left ventricle	Left atrium	Right ventricle
Anderson–Fabry disease	Reduced longitudinal strain in the basal posterior-lateral wall; reduced GLS, inversely and independently associated with LV mass (12–17), impaired subepicardial longitudinal strain at multilayer strain analysis (35), loss of normal circumferential strain base-to-apex gradient (12, 33, 34), reduced radial strain (9, 36–38), reduced constructive work (40, 41).	Reduced left atrial strain/ strain rate parameters (48–52)	Reduced longitudinal systolic right ventricle strain and right ventricle free wall strain (12, 58, 59, 61–63)
Hypertrophic cardiomyopathy	Reduced longitudinal, circumferential and radial strain (75) reduced constructive work (76).	Reduced phasic left atrial strain (50, 53)	Reduced longitudinal systolic right ventricle strain and right ventricle free wall strain (61)
Cardiac amyloidosis	Reduced longitudinal strain with relative apical sparing pattern (18) and increased EFSR (77); reduced radial strain (78), reduced global constructive work and work efficiency (41, 79).	Impairment of left atrial strain more severe than in AFD (51, 52).	Reduced longitudinal systolic right ventricle strain and right ventricle free wall strain with increased apical ratio (51, 80).

GLS, global longitudinal strain; LV, left ventricular; EFRS, ejection fraction longitudinal systolic strain ratio.

has proven to be less useful in distinguishing AFD patients from other conditions associated with LV hypertrophy (14, 18, 50–52, 61, 75–80). Loss of base to apex gradient of LV circumferential strain, irrespectively of the increase in LV wall thickness, seems to be specific for AFD (12, 33, 34). Table 1 summarizes myocardial strain characteristics of AFD and of other forms of cardiac hypertrophy such as nonobstructive hypertrophic cardiomyopathy and cardiac amyloidosis.

Conclusions

Advanced cardiac imaging has played a crucial role in defining features of the unique cardiac involvement due to AFD. Strain imaging by cardiac ultrasound is involved in many aspects: the initial diagnostic suspicion of AFD in case of evidence of unexplained heart damage, the differential diagnosis with other cardiomyopathies, the early detection of heart involvement in patients with already diagnosed AFD, the decisions regarding the initiation of chaperone or enzyme replacement therapy. Further large studies are warranted to ascertain the prognosticator value of LV longitudinal strain in defining patient risk profile and monitoring evolution of AFD cardiomyopathy. Research should be prompted to verify whether and at what extent advanced echocardiography may provide insights into the impact of disease-specific therapy on the heart of AFD patients.

Author contributions

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Cardiovascular magnetic resonance insights into anomalies of the mitral valve apparatus in Fabry cardiomyopathy and hypertrophic cardiomyopathy

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Background and aims: Despite different etiopathogenesis, Fabry Disease cardiomyopathy (FDc) and sarcomeric hypertrophic cardiomyopathy (HCM) share a similar hypertrophic phenotype, including anomalies of the mitral valve apparatus (AMVA). Some of these anomalies have also been described in the pre-hypertrophic stage of both diseases. This cardiovascular magnetic resonance (CMR) study aimed to: (i) compare AMVA between FDc and HCM with a similar degree of left ventricular hypertrophy (LVH), to add new insights into differential diagnosis; (ii) assess whether AMVA represent an early and progressive alteration in FDc; (iii) propose simple and potentially reproducible measurements of AMVA.

Methods: This observational, retrospective study enrolled: (i) 80 Fabry patients, divided into three groups with increasing severity of cardiac phenotype (20 patients LVH-/normal T1, 20 patients LVH-/low T1 and 40 patients LVH+), and (ii) 40 patients with HCM. All patients underwent CMR. The LVH + FDc and the HCM groups were matched for age, sex, body surface area and left ventricular (LV) mass. The following AMVA were measured on cine images: papillary muscles (PMs) hypertrophy (maximal diameter (Dmax) of anterolateral (Al) and posteromedial (Pm) PM), apical displacement, anteriorization of Al PM and anterior mitral valve leaflet (AMVL) elongation. Reference values for defining AMVA were derived from a matched healthy control group ($n = 40$).

Results: Both HCM and FDc LVH + patients showed PMs hypertrophy, with a greater degree in the FDc LVH + group [Dmax Al PM 16 ± 3.4 vs. 15 ± 3.1 mm, p 0.017; Dmax Pm PM 14 ± 4.0 vs.12 mm (10.0–14.0), p 0.039] As compared to controls, both HCM and FDc LVH + patients showed PMs apical displacement (HCM 83% vs. healthy volunteers 8%, $p < 0.001$; FDc LVH + 65% vs. healthy volunteers 8%, $p < 0.001$), with a greater prevalence in HCM. Anteriorization of Al PM was only evident in HCM (15 ± 6.2 vs. healthy controls 21 ± 5.3 mm, $p < 0.001$). Elongation of AMVL

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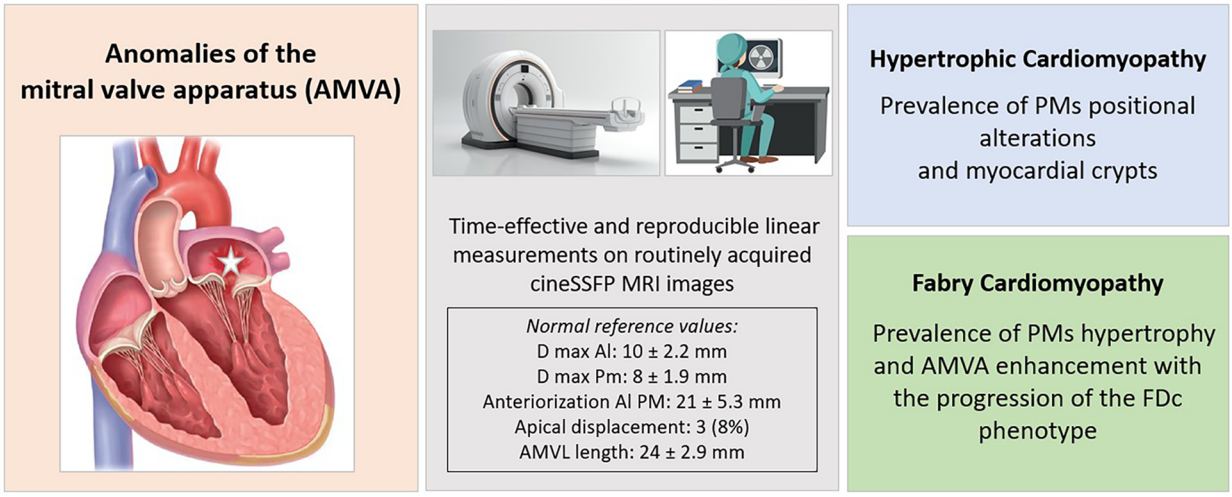
was detected both in HCM and FDc with LVH + (HCM 29 ± 4.0 vs. healthy volunteers 24 ± 2.9 mm, $p < 0.001$; FDc LVH + 27 ± 4.0 vs. healthy volunteers 24 ± 2.9 mm, $p < 0.001$) without significant differences between the two phenocopies. The prevalence of myocardial crypts was higher among HCM patients than in FDc LVH + patients (75% vs. 48%, p 0.012).

Conclusions: we report greater PMs hypertrophy in FDc and a higher prevalence of PMs positional alterations (anterior and apical displacement) and myocardial crypts in HCM. All these AMVA became more pronounced with the progression of the FDc phenotype. We suggest the systematic inclusion of the analysis of AMVA by simple linear measurements on cine images in the CMR assessment of hypertrophic cardiomyopathies, to help in the differential diagnosis between HCM and FDc and to facilitate early detection of cardiac involvement in FDc.

KEYWORDS

cardiovascular magnetic resonance, hypertrophic cardiomyopathy, Fabry cardiomyopathy, mitral valve apparatus abnormalities, myocardial hypertrophy, papillary muscles



GRAPHICAL ABSTRACT

The figure presents the study's main findings, focused on the characterization of AMVA. The study proposes simple and reproducible measurements of mitral valve apparatus parameters on routinely acquired cine SSFP sequences. The central box in grey displays normal values of these measurements, derived from the cohort of healthy controls. Patients with HCM showed a prevalence of PMs positional abnormalities, while in FDc patients PMs hypertrophy prevailed, and AMVA enhanced with the progression of the disease phenotype. Al PM, anterolateral papillary muscle; AMVL, anterior mitral valve leaflet; cineSSFP, cine steady-state free precession; Dmax, maximum diameter; FDc, Fabry cardiomyopathy; FD, Fabry disease; HCM, hypertrophic cardiomyopathy; LVH, left ventricular hypertrophy; Pm PM, posteromedial papillary muscle.

Background

Anomalies of the mitral valve apparatus (AMVA), including hypertrophy of the papillary muscles (PMs), anterior displacement, apical displacement, and elongation of the anterior mitral valve leaflet (AMVL), have frequently been observed in association with left ventricular hypertrophy (LVH), as seen in Anderson-Fabry cardiomyopathy (FDc) (1) and hypertrophic cardiomyopathy (HCM) (2–6). These two phenocopies stem from different pathophysiological backgrounds. In FDc, LVH is triggered by the intracellular lysosomal accumulation of glycosphingolipids (globotriaosylceramide, Gb3) in all cardiac cell types, due to a partial or total deficiency in alpha-galactosidase A enzyme activity. Conversely, in HCM, primary dysfunction of the sarcomere leads to impaired excitation-contraction coupling (7), resulting in cardiomyocyte disarray and LVH.

In FDc, PMs exhibit disproportionate hypertrophy (8). This feature is more pronounced in FD compared to other hypertrophic phenotypes (9) and can be detected even in the absence of LVH (10, 11).

Cardiac Magnetic Resonance (CMR) plays a pivotal role in assessing cardiomyopathies, as it combines volumetric and functional evaluation with tissue characterization, and provides good spatial resolution to define the morphology of small

structures such as PMs and mitral leaflets. However, current literature lacks standardization of measurements and normality ranges for the evaluation of the mitral valve apparatus, making its characterization arbitrary.

The aims of the present study are to: (i) compare AMVA between FDc and HCM with a similar degree of LVH, which may be useful for differential diagnosis between the two phenocopies; (ii) assess whether AMVA represent an early and progressive alteration in the cardiomyopathic spectrum of FDc; (iii) propose simple and potentially reproducible linear measurements of AMVA, applicable to commonly acquired CMR cine steady-state free precession (cineSSFP) sequences, in a time-saving manner.

Methods

Study population

This single-centre, observational and retrospective study enrolled: (i) 80 patients with genetically confirmed Fabry disease, divided into three groups according to the increasing severity of cardiac phenotype (20 patients LVH-/normal T1, 20 patients LVH-/low T1 and 40 patients LVH+), and (ii) 40 patients with HCM. The study includes only FD patients with pathogenetic or likely pathogenetic variants, while variants of unknown significance (VUS) have been excluded. All patients were referred for CMR to the Multimodality Cardiac Imaging Unit of IRCCS Policlinico San Donato (San Donato Milanese, Milan Italy), from 2016 to 2023.

In FD, LVH was defined as a maximal wall thickness (MWT) $\geq$ 13 mm in one or more left ventricular (LV) myocardial segments (12, 13) and/or increased LV mass index (14). In HCM LVH was defined according to the 2023 Guidelines of the European Society of Cardiology (15), as a MWT $\geq$ 15 mm which cannot be explained by abnormal loading conditions, or $\geq$13 mm in consideration of other features including family history, genetic findings, and electrocardiographic abnormalities.

The LVH + FDc and the HCM groups were matched for age, sex, body surface area (BSA) and LV mass. Defining subgroups based on LVH would be arbitrary since there are no specific cut-off values of LV mass or MWT for grading LVH. Given the extremely heterogeneous LVH pattern exhibited by HCM and FD patients, we choose to match them for LV mass, rather than for MWT, since myocardial mass might better reflect the progression of LVH compared to an isolated and regional MWT value, which has also proven to be a poorly reproducible parameter (16). Since in our FDc cohort no patients exhibited isolated apical LVH, HCM patients with apical phenotype were excluded, in order to identify the prevalence and type of AMVA between phenocopies with comparable LVH patterns. Of note, a higher prevalence of apical PMs displacement has been recently reported in apical HCM (17). Other exclusion criteria were prior surgical myectomy/alcohol septal ablation, age <18 years, unwillingness/inability to provide informed consent, any contraindication to CMR and poor image quality.

Patients were compared to a group of 40 gender, age and BSA-matched controls, sourced from a local database of healthy volunteers, without cardiovascular disease or significant comorbidities, with non-pathological electrocardiogram and CMR parameters within normality ranges (14).

The research protocol was approved by the local Ethics Committee (Protocol identification number 109/int/2019) and complied with the Declaration of Helsinki. Informed consent was obtained from all participants.

CMR protocol and image analysis

CMR was performed on a 1.5 T magnet (MAGNETOM Aera, Siemens Healthcare, Erlangen Germany). Each scan included: (i) scout images (ii) balanced cineSSFP images in LV short-axis and at least 3 long-axis views. Sequence parameters were slice thickness, 8.0 mm; no gap; flip angle, 60°–80°; repetition time, 3.8 ms; echo time, 1.7 ms; typical readout field of view, 350 mm; phase resolution matrix, 75%; voxel size 1.4 × 1.4 mm; mean temporal resolution ~33 ms; (iii) Shortened Modified Look-Locker inversion recovery sequence (ShMOLLI; Work-in-Progress # 780B VD13A-SP4; basal, mid-ventricular and apical short axis, 3 long-axis views,) before and 15 min after 0.1 mmol/kg of contrast (Gadovist, Bayer Schering Pharma, Berlin, Germany) for T1 mapping; (iv) two-dimensional gradient echo inversion recovery LGE images in LV short-axis and long-axis views, acquired 8–10 min after contrast administration.

LV volumes, mass and ejection fraction (EF) were calculated from cineSSFP images and indexed to BSA using the thresholding method on a commercially available software (Qmass, MR version 6.2.1; Medis Medical Imaging Systems, Leiden, The Netherlands). Both PMs and trabeculae were included in the computation of global LV mass. According to the AHA 16 segments model the MWT was measured in cineSSFP images for each myocardial segment. Late gadolinium enhancement (LGE) was quantified as % of LV mass using the standard deviations (SDs) method with a 5 SDs cut-off. Inline-generated T1 maps were analyzed using Argus software (Siemens Healthcare, Erlangen, Germany). In HCM patients two regions of interest (ROI) were manually drawn in two sites of LGE-negative myocardium, on pre- and post-contrast maps, favouring the basal and mid-interventricular septum; pre and post-contrast T1 values were obtained by averaging the two ROIs measurements. In FDc patients two ROIs were systematically drawn, one in the basal interventricular septum and the other in the basal infero-lateral wall, the latter being the usual location of fibrosis/inflammation. Extracellular volume (ECV) of remote myocardium (r-ECV) was calculated as ECV = (1-Hct)[ΔR1 myocardium]/[ΔR1 blood]. Blood samples for hematocrit were obtained at the time of CMR. Site-specific upper reference limits were 956 ± 34 ms for native T1 and 27 ± 2% for ECV, measured on ShMOLLI sequences.

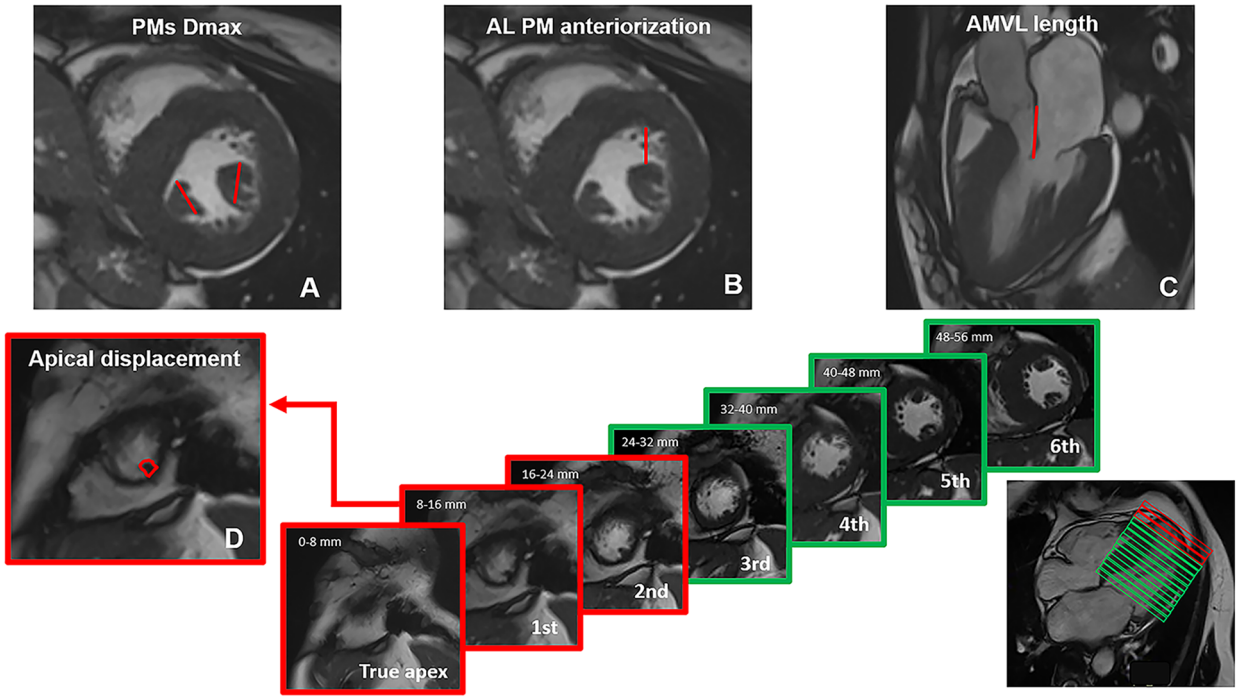


FIGURE 1
Methods used for measuring mitral valvular apparatus anomalies on CMR cine images. **(A)** papillary muscles (PMs) hypertrophy was expressed as the maximum diameter (Dmax) of anterolateral (Al) and posteromedial (Pm) PMs on short-axis images; **(B)** anteriorization of Al PM was described as the distance in mm between the Al PM and the anterior interventricular junction; **(C)** anterior mitral valve leaflet (AMVL) length measurement was performed in 3-chamber images, with maximally extended leaflets parallel to the anterior septum and LV free wall during diastole; **(D)** apical displacement was classified by counting the number of short axis slices, starting from the apex, where the first distal insertion of the PM appeared; based on the healthy control cohort, distal PMs insertion was considered normal when it could be detected in or above the third slice. All measurements were acquired in diastole.

Evaluation of the anomalies of the mitral valve apparatus

The methods used for measuring AMVA are depicted in Figure 1.

PMs measurements were performed in diastole on short-axis cineSSFP images, and included the following parameters: (i) PMs hypertrophy was expressed as the maximum diameter (Dmax) of antero-lateral (Al) and postero-medial (Pm) PMs; (ii) apical displacement was classified by counting the number of slices, starting from the apex, where the first distal insertion of the PM appeared; (iii) anteriorization of Al PM was described as the distance in mm between the Al PM and the anterior interventricular junction.

AMVL length measurement was performed in diastole, in 3-chamber cineSFFP images, with maximally extended leaflets parallel to the anterior septum and LV free wall (4). Myocardial crypts were defined as narrow, deep blood-filled invaginations penetrating >50% of the thickness of the adjoining myocardium during diastole (6).

Due to the lack of normality ranges for AMVA, the matched healthy control group was used as the reference.

Two expert operators (L.T. and A.C., Level 3 EACVI CMR Certification), blinded to clinical data, analyzed CMR images and performed intra and inter-observer reproducibility analysis for mitral valve apparatus anomalies on a subset of 30 randomly chosen patients. For intra-observer variability the same operator reanalyzed the same data set weeks apart and blinded from the first measurements; for inter-observer variability, the second operator independently and blindly analyzed the same images.

Statistical analysis

Statistical analyses were performed using SPSS version 24.0 (IBM SPSS, IBM Corporation, Armonk, NY, USA). Categorical variables were shown as count (n) and percentage (%) and compared with the $\chi 2$ test. The Kolmogorov–Smirnov test was used to assess the normal distribution of data collected. Normally distributed variables were expressed as mean (M) ± standard deviation (SD) and compared with the T student test and analysis of variance; non-normally distributed variables were expressed as median and interquartile range and compared with Mann Whitney *U*-test and non-parametric analysis of variance (Kruskal Wallis test).

Intra- and inter-observer reproducibility of mitral valve apparatus anomalies measurements were assessed using

TABLE 1 Characteristics of the study population.

	Healty volunteers (40)	FDc LVH+ (40)	FDc LVH-Low T1 (20)	FD LVH-Normal T1 (20)	HCM (40)	*p*-Value
Demographic data						
Age (years)	53 ± 11.8[d]	54 ± 10.1	32 ± 10.1[b]	28 ± 13.1	54 ± 14.8	<0.001*
Male, *n* (%)	27 (67)	27 (67)	14 (70)	5 (25)	27 (67)	0.080
BSA (m^2)	1.9 ± 0.1[d]	1.8 ± 0.3	1.8 ± 0.2	1.6 (1.6–1.8)[c]	1.9 ± 0.2	0.001*
CMR data						
LVEF (%)	66 ± 7.1[d]	72 ± 7.5	70 ± 5.4	70 ± 6.5	73 ± 8.7	<0.001*
LVEDVi (ml/m^2)	73 ± 11.9	69 ± 14.8[a]	80 ± 12.9[b]	73 ± 11.4	62 (53.3–73.5)	<0.001*
LVESVi (ml/m^2)	25 ± 6.5	18 (14.3–24.7)	24 ± 6.5[b]	22 ± 6.2	17 ± 7.9	<0.001*
SV (ml)	91 ± 20.3	89 (70–108)	98 ± 17.5	86 ± 11.9[c]	83 (72–96)	0.079
LV Mass i (g/m^2)	60 ± 11.6	136 ± 40.4	70 ± 5.4[b]	56 ± 9.3[c]	127 (104.3–161.9)	<0.001*
LV MWT (mm)	–	17 (14.3–21.0)[a]	10 ± 1.3[b]	7 (7.0–8.0)[c]	20 (17.0–21.8)	<0.001*
Crypts, *n* (%)	8 (20)	19 (47%)[a]	3 (15%)[b]	2 (10%)	30 (75)	<0.001*
LGE (% LVmass)	0	4 (1.3–8.1)	0[b]	0	6 (2.0–13.0)	<0.001*
Septal T1 (ms)	–	848 ± 50.9[a]	872 ± 45.3	969 ± 25.6[c]	970 ± 34.4	<0.001*
Remote ECV (%)	–	26 ± 2.5[a]	26 ± 2.7	28 ± 2.2[c]	29 ± 3.3	<0.001*

Data are presented as mean ± SD, median and interquartile range or *n* (%).

BSA, body surface area; ECV, extracellular volume; FDc, Fabry cardiomyopathy; FD, Fabry disease; HCM, hypertrophic cardiomyopathy; LGE, late gadolinium enhancement; LV, left ventricular; LVEDV i, left ventricular end-diastolic volume index to BSA; LVEF, left ventricular ejection fraction; LVESV i, left ventricular end-systolic volume index to BSA; LVH, left ventricular hypertrophy; LV Mass i, left ventricular mass indexed to BSA; LVWT, maximum left ventricular wall thickness; SV, stroke volume.

[a]$p < 0.05$ FDc LVH + vs. HCM

[b]$p < 0.05$ FDc LVH + vs. FDc LVH-/lowT1

[c]$p < 0.05$ FDc LVH-/low T1 vs. FDc LVH-/normal T1

[d]$p < 0.05$ FDc LVH-/normal T1 vs. healthy controls.

*$P < 0.05$ considered statistically significant.

Bland–Altman plots and intraclass correlation coefficients (ICCs) for continuous variables, and Coehn's K coefficient for dicotomic variables. Significance was defined as a *P*-value < 0.05.

Results

Study population

The study population included 160 subjects: 40 healthy volunteers (age 53 ± 11.8, 67% males), 40 patients with FDc LVH + (age 54 ± 10.1, 67% males), 20 patients with FDc LVH-/low T1 (age 32 ± 10.1, 70% males), 20 patients with genetic diagnosis of FD and no CMR signs of cardiac involvement (LVH-/normal T1, age 28 ± 13.1, 25% males) and 40 patients with HCM (age 54 ± 14.8, 67% males).

Supranormal LV EF was the only distinguishing parameter between healthy controls and Fabry patients without signs of cardiac involvement (FD LVH-/normal T1 70 ± 6.5% vs. healthy volunteers 66 ± 7.1%, $p < 0.001$). Our data confirm that progressive myocardial involvement in FD encompasses the lowering of native T1, the development of LVH with increasing LV mass index and the appearance of LGE.

Patients with hypertrophic FDc showed lower native septal T1 and ECV (native T1: 848 ± 50.9 ms vs. 970 ± 34.4 ms, $p < 0.001$; ECV: 26 ± 2.5% vs. 29 ± 3.3%, $p < 0.001$) than HCM patients. The prevalence of myocardial crypts was higher among HCM patients than in FDc LVH + patients (75% vs. 48%, *p* 0.012).

Reference values for defining AMVA derive from the healthy volunteers' cohort. Regarding apical displacement, we observed that in the healthy controls the distal insertion of the PMs typically appeared in or above the third slice. For reference, we counted the true apex as slice 0, which is defined as the slice cutting the apex without the left ventricular (LV) cavity visible in diastole. Since there is no gap between slices and each one is 8 mm thick, we defined apical displacement as the appearance of the distal insertion of PMs between 0 and 16 mm from the apex (i.e., 2 slices) (Figure 1). Baseline characteristics of the study populations are detailed in Table 1.

Anomalies of the mitral valve apparatus in Fabry cardiomyopathy and hypertrophic cardiomyopathy

Both HCM and FDc LVH + patients showed PMs hypertrophy (Dmax Al PM HCM 15 ± 3.1 vs. healthy controls 10 ± 2.2 mm, $p < 0.001$; Dmax Pm PM HCM 12 (10.0–14.0) vs. healthy controls 8 ± 1.9 mm, $p < 0.001$; Dmax Al PM FDc LVH + 16 ± 3.4 vs. healthy controls 10 ± 2.2 mm, $p < 0.001$; Dmax Pm PM FDc LVH + 14 ± 4.0 vs. healthy controls 8 ± 1.9 mm, $p < 0.001$). Of note, in the FDc LVH + group, a greater degree of PMs hypertrophy was observed compared to HCM (Dmax Al PM 16 ± 3.4 vs. 15 ± 3.1 mm, *p* 0.017; Dmax Pm PM 14 ± 4.0 vs. 12 mm (10.0–14.0), *p* 0.039). As compared to controls, both HCM and FDc LVH + patients showed PMs apical displacement (HCM 83% vs. healthy volunteers 8%, $p < 0.001$; FDc LVH + 65% vs. healthy volunteers 8%, $p < 0.001$). Comparing

the two phenocopies, the prevalence of PMs apical displacement was higher in the HCM cohort, although it did not reach statistical significance (p 0.075).

Anteriorization of Al PM was only evident in HCM (15 ± 6.2 vs. healthy controls 21 ± 5.3 mm, $p < 0.001$) and a significant difference was observed between the two phenocopies (HCM 15 ± 6.2 vs. FDc LVH + 18 mm (15.0–21.8), p 0.020).

Elongation of AMVL was detected both in HCM and FDc with LVH + (HCM 29 ± 4.0 vs. healthy volunteers 24 ± 2.9 mm, $p < 0.001$; FDc LVH + 27 ± 4.0 vs. healthy volunteers 24 ± 2.9 mm, $p < 0.001$); no significant differences were observed between the two phenocopies (27 ± 4.0 vs. 29 ± 4.0, p 0.078).

All results are reported in Table 2 and illustrated in Figure 2.

TABLE 2 Comparison of mitral valve apparatus anomalies in Fabry cardiomyopathy and hypertrophic cardiomyopathy.

	Healty volunteers (40)	FDc LVH+ (40)	HCM (40)	Overall *p*-Value
Dmax Al PM (mm)	10 ± 2.2[b,c]	16 ± 3.4[a]	15 ± 3.1	<0.001*
Dmax Pm PM (mm)	8 ± 1.9[b,c]	14 ± 4.0[a]	12 (10.0–14.0)	<0.001*
Anteriorization of Al PM (mm)	21 ± 5.3[c]	18 (15.0–21.8)[a]	15 ± 6.2	<0.001*
PM apical displacement *n*, (%)	3 (8)[b,c]	26 (65)	33 (82)	<0.001*
AMVL length (mm)	24 ± 2.9[b,c]	27 ± 4.0	29 ± 4.0	<0.001*

Data are presented as mean ± SD, median and interquartile range or *n* (%).
Al PM, antero-lateral papillary muscle; AMVL, anterior mitral valve leaflet; Dmax, maximum diameter; FDc, Fabry cardiomyopathy; HCM, hypertrophic cardiomyopathy; LVH, left ventricular hypertrophy; Pm PM, postero-medial papillary muscle.
[a]FDc LVH + vs. HCM.
[b]FDc LVH + vs. healthy controls.
[c]HCM vs. healthy controls.
*$P < 0.05$ considered statistically significant.

Anomalies of the mitral valve apparatus across Fabry cardiomyopathy

With the increasing severity of cardiac involvement across FD groups, we observed a parallel progression in the magnitude of AMVA (i.e., Dmax Al PM, Dmax Pm PM, PMs apical displacement and AMVL length) (Figure 3). No significant differences were observed between healthy volunteers and FD patients without detectable storage, although a trend towards an increased prevalence of apical displacement was observed (8% vs. 25%, p. 0.060). The first alterations in mitral valve apparatus emerge in FDc LVH-/low T1 and become overt in the FD LVH + cohort. Of note, PMs hypertrophy and apical displacement are appreciable in pre-hypertrophic FD patients with the lowering of native T1 (FDc LVH-/lowT1 vs. healthy controls: Dmax Al PM 13 ± 2.8 vs. 10 ± 2.2, $p < 0.001$, Dmax Pm PM 11 ± 2 vs. 8 ± 1.9, $p < 0.001$, apical displacement 45 vs. 8%, $p < 0.001$). All results are detailed in Table 3.

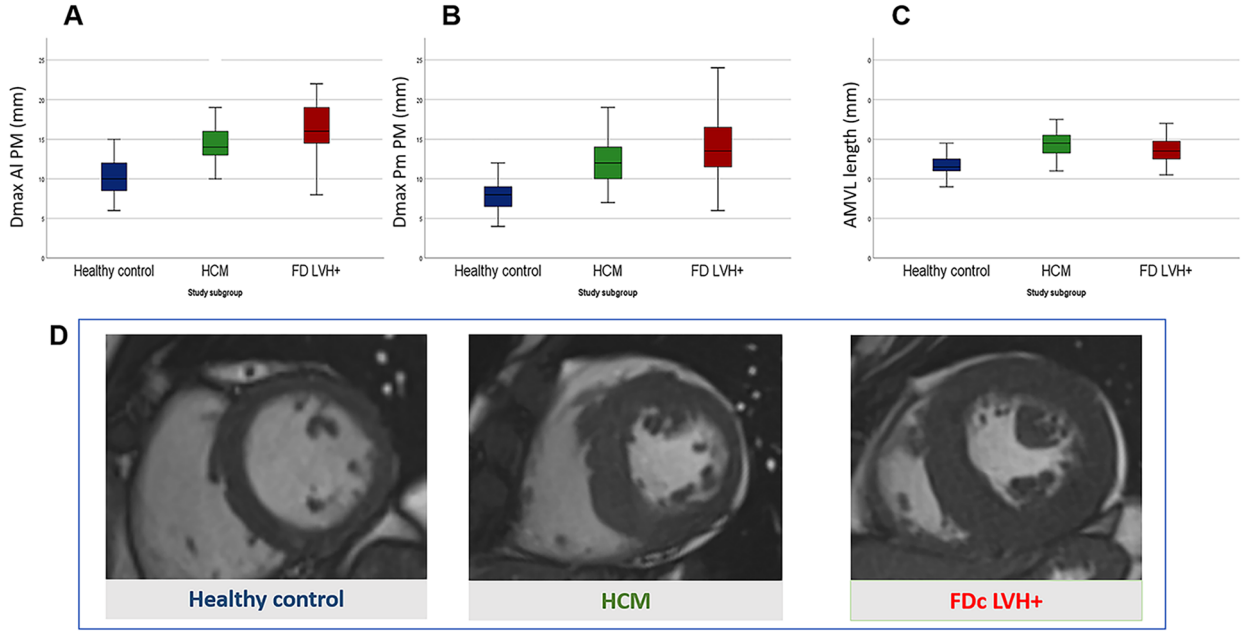


FIGURE 2
Comparison of mitral valve apparatus anomalies between Fabry disease cardiomyopathy (FDc) and hypertrophic cardiomyopathy (HCM) with similar degree of LVH. Box plots in **(A–C)** show measurements of the mitral valve apparatus in healthy controls (blue), HCM (green) and FDc (red) patients. Measurements are expressed in millimetres and are respectively in **(A)** the maximal diameter of posteromedial papillary muscle (Dmax Pm PM), in **(B)** the maximal diameter of anterolateral papillary muscle (Dmax Al PM) and in **(C)** the length of the anterior mitral leaflet (AMVL length). **(D)** Short axis cineSSFP images in diastole of a healthy control (blue), HCM (green) and FDc LVH + (red) patients.

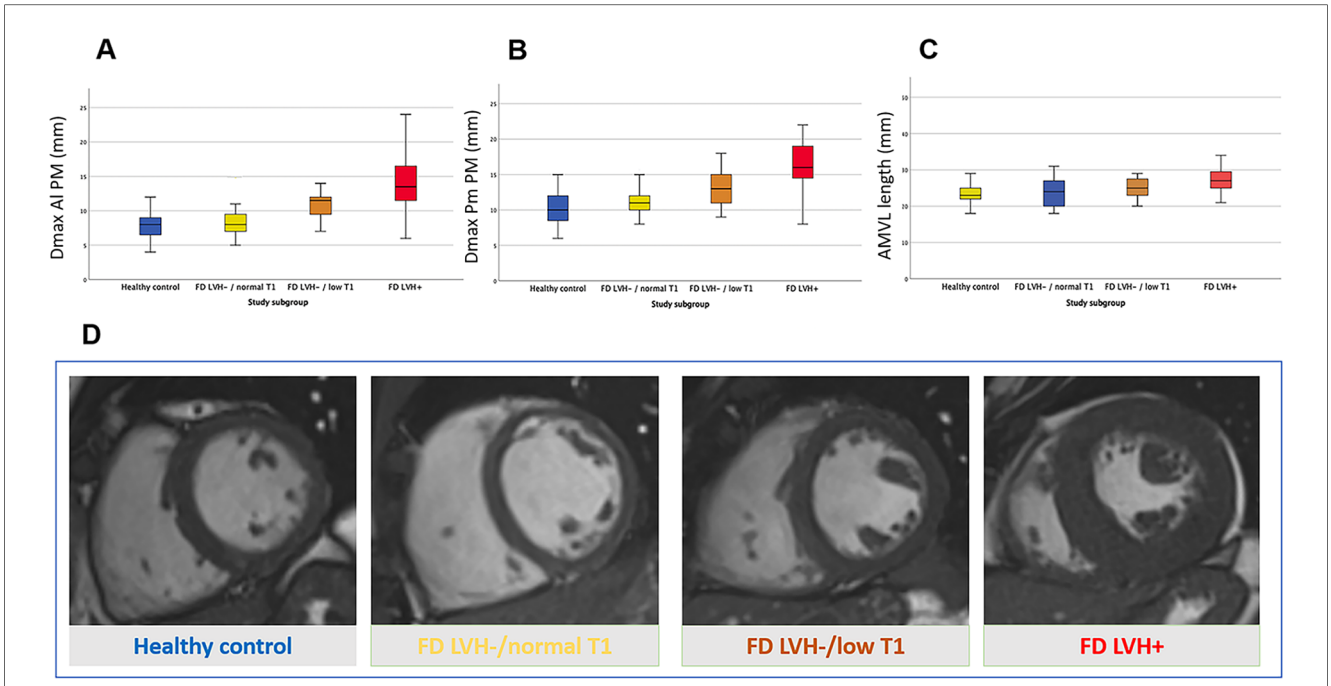


FIGURE 3
Anomalies of the mitral valve apparatus across Fabry cardiomyopathy: Fabry disease without left ventricular hypertrophy and normal myocardial T1 (FD LVH-/normal T1), Fabry disease without left ventricular hypertrophy and with low myocardial T1 suggestive for cardiac storage disease (FD LVH-/lowT1) and Fabry disease with cardiac hypertrophy (FD LVH+). Box plots in **(A–C)** show the progressive increase of mitral valve apparatus measurements alongside the worsening of FD cardiomyopathy; the measurements are expressed in millimetres and are respectively in **(A)** the maximal diameter of posteromedial papillary muscle (Dmax Pm PM), in **(B)** the maximal diameter of anterolateral papillary muscle (Dmax Al PM) and in **(C)** the length of the anterior mitral leaflet (AMVL length). **(D)** Short axis cineSSFP images in diastole of a healthy control (blue), FD LVH-/normal T1 (yellow), FD LVH-/reducedT1 (orange) and FD LVH + (red) patients.

TABLE 3 Anomalies of the mitral valve apparatus across Fabry cardiomyopathy.

	Healty volunteers (40)	FD LVH- Normal T1 (20)	FDc LVH-Low T1 (20)	FDc LVH+ (40)	Overall *p* Value
Dmax Al PM (mm)	10 ± 2.2	11 (10.0–12.0)	13 ± 2.8[b,c]	16 ± 3.4[a]	<0.001*
Dmax Pm PM (mm)	8 ± 1.9	8 (7.0–9.8)	11 ± 2.0[b,c]	14 ± 4.0[a]	<0.001*
Anteriorization of AL PM (mm)	21 ± 5.3	19 ± 3.2	20 (16.0–22.0)	18 (15.0–21.8)	0.237
PM apical displacement *n*, (%)	3 (8)	5 (25)	9 (45)[c]	26 (65)	<0.001*
AMVL length (mm)	24 ± 2.9	24 ± 3.9	25 ± 2.8	27 ± 4.0[a]	<0.001*

Data are presented as mean ± SD, median and interquartile range or *n* (%).
Al PM, antero-lateral papillary muscle; AMVL, anterior mitral valve leaflet; Dmax, maximum diameter; FDc, Fabry cardiomyopathy; FD, Fabry disease; LVH, left ventricular hypertrophy; Pm PM, postero-medial papillary muscle.
[a]FDc LVH + vs. FDc LVH-/lowT1.
[b]FDc LVH-/low T1 vs. FDc LVH-/normal T1.
[c]FDc LVH-/low T1 vs. healthy controls.
*$P < 0.05$ considered statistically significant.

Intra and interobserver reproducibility of AMVA measurements on cine images

Intra ed interobserver reproducibility of AMVA measurements on cine images (Dmax PMs, AMVL length and anteriorization of Al PM) was good to excellent, with small biases and ICC ranging from 0.89 to 0.98. Intra and interobserver reproducibility results for AMVA measurements are reported in Table 1 of Supplementary Files.

Discussion

The main findings of the present study are: (i) in the presence of LVH of a similar degree, PMs hypertrophy is more pronounced in the FDc group, whereas PMs positional alterations (anterior and apical displacement), and myocardial crypts, are more evident in the HCM group; (ii) PMs hypertrophy and apical displacement, AMVL elongation and myocardial crypts become increasingly apparent with the progression of the FDc phenotype; (iii) the

proposed method for assessing AMVA is time-effective and demonstrates good intra- and inter-observer variability. The main findings are presented in the Graphical abstract.

AMVA have been reported both in HCM and in FDc, likely due to secondary common pathways activated by a different initial trigger represented by abnormal sarcomere protein function and glycosphingolipid storage, respectively. In FDc, disproportionate hypertrophy of the PMs has been described (8, 9), exceeding that seen in HCM and significantly affecting the estimation of LV mass. Even in patients with HCM the PMs are frequently hypertrophied, and the hypertrophy of PMs correlates with LV wall thickness and myocardial mass (18). The distance between the PMs and the septum and PMs apical displacement are also points of interest in patients with HCM, with a potential impact on dynamic outflow tract obstruction (19). Elongation of AMVL was first observed in HCM as a part of a subclinical cardiac phenotype, alongside the presence of myocardial crypts (20). Following studies demonstrated the same abnormalities in FDc (21, 22).

To our knowledge, this is the first CMR study to evaluate AMVA with a head-to-head comparison between FDc and HCM with similar degrees of LVH. We confirmed that FDc LVH+ patients exhibit greater PMs hypertrophy (9). On the other hand, HCM patients display more evident PMs positional abnormalities (apical displacement and anteriorization of Al PM). Finally, in comparing the two phenocopies, the prevalence of myocardial crypts is significantly higher in HCM patients than in FDc, where it is known as an early marker of disease (23). The different alterations in AMVA observed in the two phenocopies could be interpreted based on their different etiopathologies. As a storage disease, in FD, the glycosphingolipid accumulation could explain the predominant and progressive hypertrophy of PMs. Conversely, despite the two cohorts sharing comparable LV mass, HCM patients more frequently show asymmetry of the parietal hypertrophy, associated with greater anatomical distortion of the left ventricle, and this could explain the higher prevalence and extent of positional anomalies of the PMs.

It must be acknowledged that differences in AMVA have a limited impact on the differential diagnosis between HCM and FDc when considered in isolation, and native T1 remains the cornerstone for this purpose (24). However, given the drastic implications of differential diagnosis on clinical management, we strongly support the integration of all the information deriving from CMR in a multiparametric evaluation to differentiate between FDc and HCM with similar degrees of LVH (25).

In the field of FD, detailing AMVA may also be useful for the early detection of heart damage, which is the main driver of prognosis (26, 27). Prompt identification of cardiac involvement in FD is a major challenge, entailing relevant therapeutic and prognostic implications (21, 28). In this regard, our group has already described early-onset morpho-functional and electrocardiographic alterations (29–31), showing progressive enhancement throughout the spectrum of FDc. PMs hypertrophy has been previously reported to precede overt LVH in patients with FDc, as demonstrated by echocardiography in a population of FD LVH- patients (32). This study, however, did not include the evaluation of native T1 to distinguish between the presence or absence of detectable storage. Interestingly, when subtyping the FD LVH- population, we confirmed that PMs hypertrophy is already present in LVH-/low T1 patients, suggesting it as an early morphological marker of the disease. On the other hand, PMs hypertrophy was not evident in patients without non-invasively detectable myocardial storage (LVH-/normal T1). Previous findings in this latter population are discordant. Kozor et al. (8) observed the presence of PMs hypertrophy, expressed as % of LV mass, even in LVH-/normal T1 patients, while Nordin et al. (11) found no significant differences in PMs mass between healthy volunteers and FD patients without detectable storage. Indeed, in our population, we noted an increasing trend in the diameter of the anterior Al PM, although it did not reach statistical significance. Across FDc groups, we also observed a trend towards a progressive increase in the prevalence of PM apical displacement, myocardial crypts and AMVL elongation. Overall, the concept that in FDc mitral valve apparatus anomalies advance with the progression of damage remains consistent across all studies; discrepancies in results may depend on different methodologies used for measurement. This study confirms that in FDc women exhibit a lesser degree of LVH compared to men. Having shown that valvular apparatus abnormalities follow the structural evolution of the disease, we expect that these abnormalities might be less evident in women as well. Of note, despite the lesser degree of hypertrophy in women, it has been shown that this does not imply a better cardiovascular prognostic profile (33).

Finally, while reference standards exist for tissue parameters and major morpho-structural indices, AMVA are usually reported descriptively, hence arbitrarily, in daily practice. There is no standardization for quantification nor ranges of normal values. Indeed, various studies in the literature report different methodologies (9, 10, 17, 32, 34) for AMVA analysis. This work proposes a simple, reproducible and time-effective method that allows the evaluation of these parameters, using linear measurements on cineSSFP images, which are routinely acquired in any CMR examination.

In conclusion, when comparing FDc and HCM, we report greater PMs hypertrophy in FDc and a higher prevalence of PMs positional alterations (anterior and apical displacement) and myocardial crypts in HCM. All these anomalies become more pronounced with the progression of the FDc phenotype. We suggest the systematic inclusion of the analysis of AMVA in the CMR assessment of cardiomyopathies with a hypertrophic phenotype using simple and reproducible linear measurement on cineSSFP images. This approach not only aids in the differential diagnosis between HCM and FDc but also facilitates the early detection of cardiac involvement in FD, thereby definitively impacting clinical and therapeutic management.

Limitations

In addition to the single-centre and retrospective design of the study, the following limitations must be mentioned. The exclusion

of cases with pure apical HCM from the analysis: (i) prevents extending the current findings to this subgroup of patients and (ii) likely led to an underestimation of the real prevalence of PM anomalies in the HCM group, known to be more frequent in the apical phenotype (17). Given the sample size, a specific gender-based analysis within the Fabry population was not achievable. Information about genetic testing in the HCM cohort is not available for all patients. The lack of longitudinal follow-up hinders the interpretation of the prognostic impact of AMVA in FDc and HCM.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Comitato etico Ospedale San Raffaele. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

LT: Conceptualization, Data curation, Formal Analysis, Supervision, Writing – original draft, Writing – review & editing. GD: Data curation, Formal Analysis, Supervision, Writing – original draft. PD: Data curation, Writing – review & editing. AA: Data curation, Formal Analysis, Writing – review & editing. GG: Conceptualization, Writing – review & editing. FP: Writing – review & editing. GD: Data curation, Writing – review & editing. MC: Writing – review & editing. GC: Writing – review & editing. MP: Writing – review & editing. PS: Writing – review & editing. ML: Conceptualization, Supervision, Writing – review & editing. AC: Conceptualization, Supervision, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2024.1458705/full#supplementary-material

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Role of standard echocardiography in Anderson–Fabry disease

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Cardiac involvement strongly impacts prognosis in patients with Anderson–Fabry disease (AFD). All cardiac structures, such as the left ventricle and the left atrium, the aorta, the right sections, and the heart valves can be affected by morphological and functional abnormalities. Standard echocardiography has a crucial role in the characterization of AFD cardiomyopathy. Being a diffuse, non-invasive, easily reproducible, and inexpensive investigation, echocardiography represents the most appropriate tool for screening AFD cardiomyopathy. Furthermore, echocardiographic evaluation is the essential imaging method to support the physician also in the follow-up and risk stratification of AFD patients. Therefore, echocardiography is useful in all stages of the disease, both to reveal the first signs of cardiac involvement and to guarantee timely treatment in the preclinical stage and to estimate the extent of cardiac involvement, define possible complications, and evaluate the response to treatment in patients with established cardiomyopathy. The latest advanced echocardiographic techniques, such as speckle-tracking analysis, are offering new insights into the early detection of AFD cardiac involvement, thus suggesting a promising role for echocardiography in selecting appropriate candidates for treatment. In this review, we will examine the role of standard echocardiography in AFD, focusing on its use in screening for cardiac involvement, detailed characterization of AFD cardiomyopathy, and risk stratification of AFD patients.

KEYWORDS

Anderson–Fabry disease, echocardiography, cardiomyopathy, risk stratification, cardiac imaging

Introduction

Anderson–Fabry disease (AFD) is a rare X-linked lysosomal storage disorder caused by a deficiency of the α-galactosidase A lysosomal enzyme (α-Gal A), resulting in pathological accumulation of lysosomal globotriaosylceramide (Gb3) and related globotriaosylsphingosine (lyso-Gb3) in several tissues, leading to multiorgan involvement and high morbidity and mortality (1).

Two major clinical phenotypes are described: the type 1 classic phenotype and the milder, type 2 later-onset phenotype. Type 1 is prevalent in male patients and typically

occurs in childhood or adolescence with early symptoms including acroparesthesias, angiokeratomas, hypohidrosis, and a characteristic corneal dystrophy. Type 2 later-onset AFD typically occurs later and often primarily involves the heart.

Type 1 phenotype is associated with a higher risk of multiorgan failure and premature mortality (2, 3).

Cardiac involvement strongly impacts on prognosis (4). Echocardiography is the first-line imaging tool in AFD, with an essential role in screening, clinical management, and prognostic stratification (5). Echocardiography has many advantages as it is widely diffused and reproducible, fast, non-invasive, and low-cost. Thus, AFD patients routinely undergo echocardiographic examinations, and evaluation of the images over time allows physicians to early detect abnormal structural changes and timely treat disease progression and possible complications.

In this review, we will deeply analyze the role of standard echocardiography in AFD, focusing on screening for cardiac involvement, AFD cardiomyopathy evaluation, and AFD risk stratification (Figure 1).

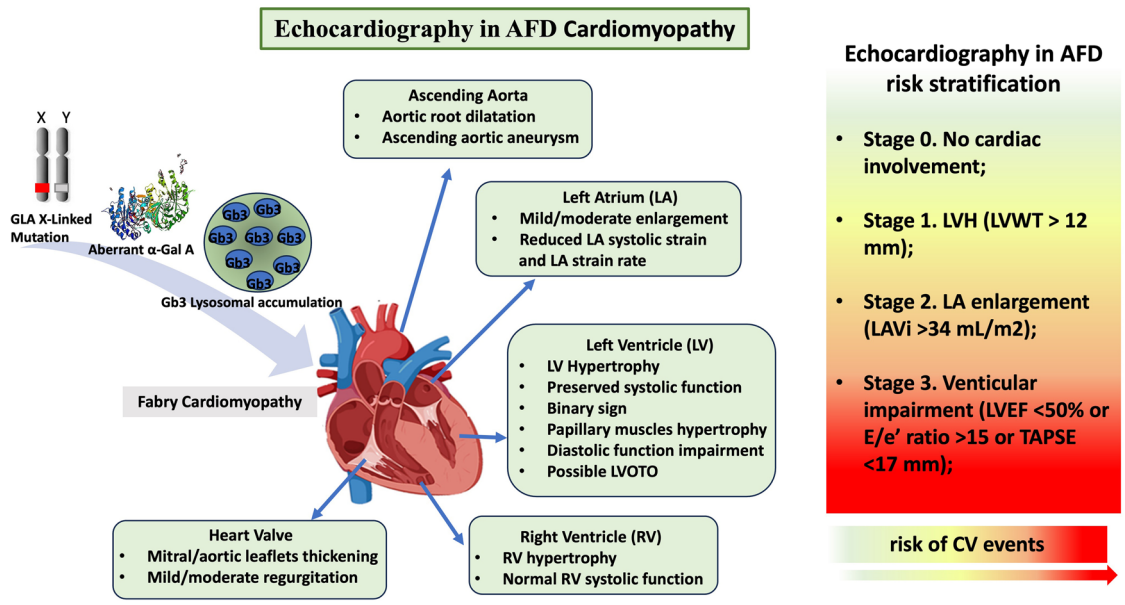
Echocardiography in AFD screening

AFD cardiac involvement is the result of the accumulation of lysosomal globotriaosylceramide (Gb3) and related globotriaosylsphingosine (lyso-Gb3) in the heart, which in turn activate a chronic inflammatory process and autoimmunity (6–8). Following the introduction of enzyme replacement therapy (ERT), early recognition of AFD has become crucial to limit disease progression (9).

In these terms, echocardiography has a key role in the detection of early abnormalities. Recent guidelines from the European Society of Cardiology state that AFD should be suspected in patients with left ventricular hypertrophy (LVH) and additional cardiac and extracardiac red flags (10) (Figure 2). However, LVH is unlikely to be seen in patients aged <20 years, where the diagnosis is usually based on family history or other extracardiac symptoms.

In carriers of pathogenic variants, tissue Doppler imaging (TDI) and speckle tracking allow early detection of cardiac involvement independently of LVH (11). TDI strain and strain rate were reduced in AFD patients compared with normal control subjects (12). Zamorano et al. (13) showed, on both male and female patients with AFD, that TDI velocities were inversely related to left ventricular (LV) mass and demonstrated that abnormal TDI velocities were evident prior to the development of LVH in serial echocardiographic evaluations.

More recently, the speckle-tracking strain has been shown to identify AFD independently of LVH with greater sensitivity and specificity than TDI (14). The reduction of global longitudinal strain (GLS) is usually due to a regional decrease in longitudinal strain in the basal inferolateral region (15, 16). Of interest, the regional strain impairment has been shown to correlate in the same myocardial regions with late gadolinium enhancement (LGE) at cardiac magnetic resonance (CMR). Circumferential



AFD: Anderson-Fabry disease; ***α-Gal A***: alpha galactosidase A; ***GLA***: α-Gal A gene; ***Gb3***: lysosomal globotriaosylceramide; ***LVOTO***: LV outflow-tract obstruction; ***LVWT***: LV wall thickness; ***LAVi***: LA Volume index; ***LVEF***: LV ejection fraction; ***TAPSE***: Tricuspid Annular Plane Systolic Excursion.

FIGURE 1
The role of standard-echocardiography in characterization of Anderson-Fabry disease (AFD)-cardiomyopathy and risk stratification. Standard echocardiography evaluation and its crucial role in AFD patients: echocardiography supports physician in the diagnosis, follow up and risk stratification of AFD patients.

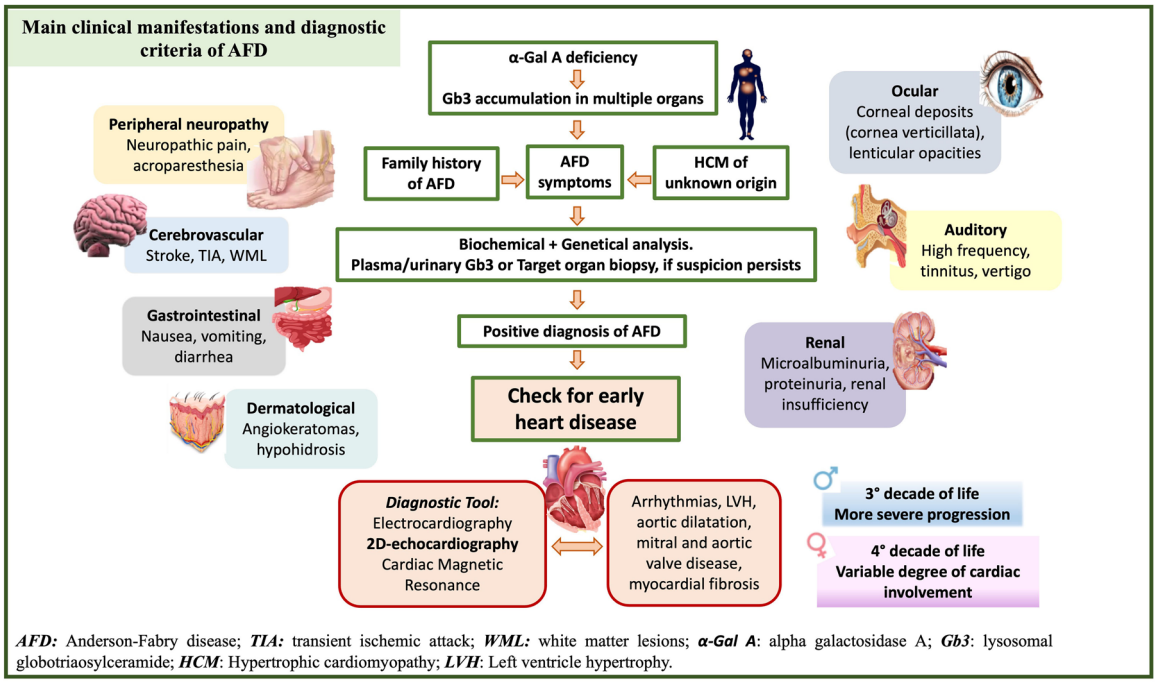


FIGURE 2
Clinical and diagnostic criteria of Anderson Fabry (AFD) disease and early detection of AFD-cardiomyopathy. Clinical manifestation, main signs and symptoms and diagnostic tools to support physicians in the diagnosis of AFD patients.

gradient strain reduction and a loss of the normal base-to-apex circumferential gradient were evident in both AFD patients with and without LVH (16).

These pieces of evidence support the role of echocardiographic TDI and speckle tracking in the early detection of AFD cardiac involvement and suggest a promising role for echocardiography in selecting appropriate candidates for treatment.

Echocardiography in AFD cardiomyopathy

Echocardiography is an essential tool in AFD patients and provides a detailed characterization of AFD cardiomyopathy. Cardiac alterations in AFD include structural and functional abnormalities of both the left and right ventricles (RV), left atrium (LA), aorta, and heart valves (Table 1; Figure 3).

Left ventricle

Hypertrophy

The echocardiographic study of the LV allows the evaluation of LV mass. An increased LV mass index (defined as >95 g/m^2 for women and >115 g/m^2 for men) identifies LVH (17). AFD represents an under-recognized cause of LVH, and an integrated approach is required for differential diagnosis, including clinical evaluation and a multi-imaging approach (Table 2).

According to the current guidelines, AFD disease should be suspected in patients with LVH and additional cardiac and extracardiac red flags (10). LVH, commonly concentric (18), can be asymmetric with maximal wall thickness at the septal or apical level. However, at the early stages of the disease, LVH may be limited to the posterolateral basal wall (19, 20).

Differential diagnosis of LVH etiology is sometimes challenging, especially in the presence of other comorbidities that increase LV afterload. In these cases, a multi-imaging approach, including CMR, along with a careful clinical evaluation is required to guide the diagnosis and management.

CMR is often necessary for accurate volumetric and functional analysis and detailed myocardium characterization. CMR has a high spatial resolution, ideal for evaluating LV wall thickening patterns. Deva et al. (21) studied the spectrum of morphological phenotypes and CMR myocardial scar patterns in AFD. They demonstrated that concentric thickening and inferolateral mid-myocardial scarring are the most common manifestations of AFD; however, they reported a percentage of cases mimicking the morphological manifestations of hypertrophic cardiomyopathy, with apical and asymmetric septal hypertrophy. These phenotypes have more apical and midventricular LV scarring. CMR is also useful in the early diagnosis of AFD. Recent studies have identified non-contrast T1 mapping as an early marker of cardiac involvement in AFD, with high sensitivity and specificity (22–24).

TABLE 1 Echocardiographic features of AFD cardiomyopathy according to disease stage.

Structure	Early stages	Overt cardiomyopathy
Left ventricle	No LVH Preserved systolic function No Regional wall motion abnormalities Mild diastolic dysfunction Impaired TDI velocities and GLS	Increased wall thickness: concentric (most common), asymmetric septal, eccentric, apical Binary sign Papillary muscle thickening and hyperechogenicity Preserved systolic function until late stages Diastolic function often impaired Abnormal TDI findings (often before increase in wall thickness) Reduced GLS (often in the basal posterior/lateral segments) LVOTO or midventricular obstruction (not uncommon)
Left atrium	Abnormal TDI findings LA strain impaired	Mild to moderate atrial enlargement Increased atrial reversal velocities Abnormal TDI findings Reduced LA systolic strain and strain rate
Valves	No significant structural and functional abnormalities	Leaflet thickening and redundancy (especially in mitral and aortic valves) Valvular regurgitation (often mild, rarely significant)
Aorta	Arterial remodeling (increased intima–media thickness)	Aortic dilatation at the sinus of Valsalva and ascending aorta
Right ventricle	No RVH Normal TDI findings Normal TAPSE value Normal RV-GLS and RV-FWS	RVH Generally preserved systolic function, but severe dysfunction can occur Abnormal TDI findings RV-GLS and RV-FWS impaired despite normal systolic function

AFD, Anderson–Fabry disease; LVH, left ventricular hypertrophy; TDI, tissue Doppler imaging; GLS, global longitudinal strain; LV, left ventricle; LVOTO, left ventricular outflow tract obstruction; LA, left atrium; RVH, right ventricular hypertrophy; RV, right ventricle; TAPSE, tricuspid annular plane systolic excursion; RV-FWS, strain of RV free wall.

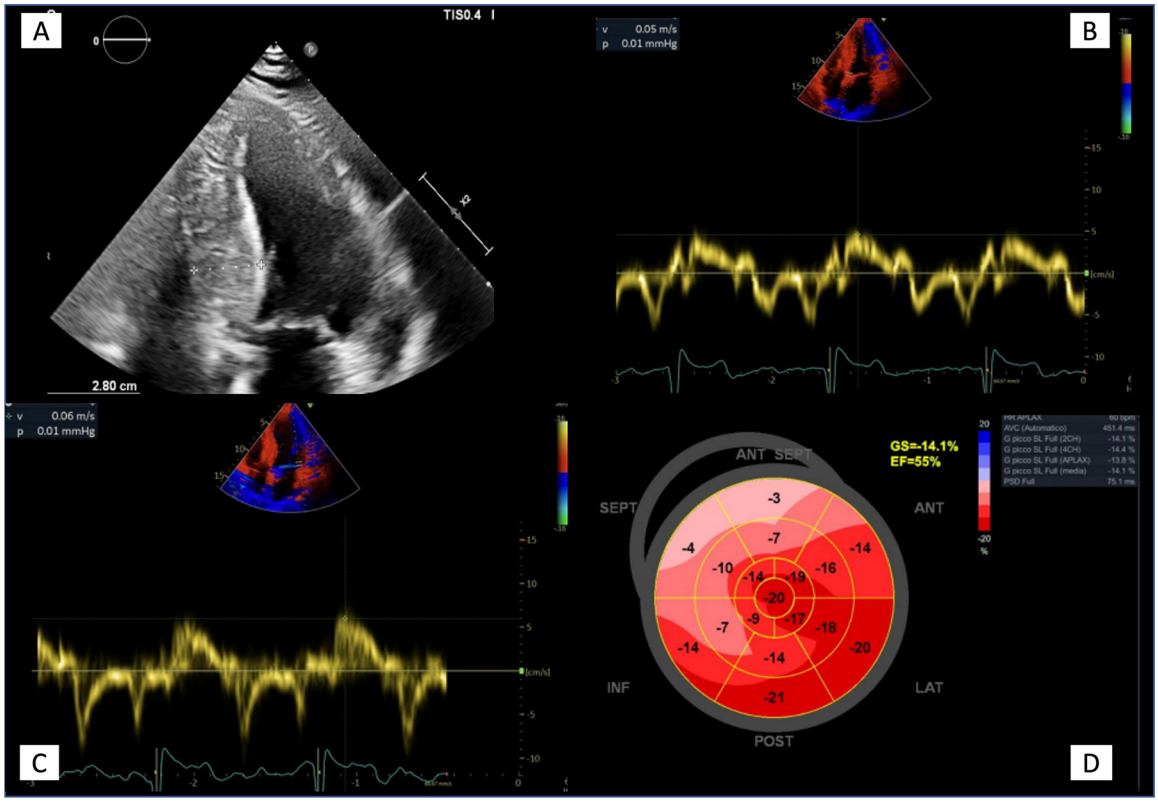


FIGURE 3
Echocardiographic features in advanced Anderson-Fabry disease cardiomyopathy. **(A)** Apical four chambers view showing the maximal wall thickness (28 mm) and the typical binary sign; **(B, C)** Tissue Doppler mitral annular velocities at septal and lateral corners respectively, showing low systolic velocities (5 and 6 cm/s respectively); **(D)** 2D speckle tracking analysis bull's eye plot, showing reduced LV GLS value (–14.1%).

TABLE 2 Clinical findings and multi-imaging approach for differential diagnosis in patients with LVH.

Disease	Clinical presentation	Echocardiography	ECG	CMR LGE	Histology
Fabry disease	- Young age at presentation (male: 11 ± 7 years; female: 23 ± 16 years) - Neuropathic pain - Impaired sweating - Skin rashes	- Symmetrical increase in LV and RV wall thickness (most common) - Preserved EF	- Increased or normal QRS complex voltage - Abnormal PR interval	- Focal - Midwall - Inferolateral wall	- Enlarged myocytes - Glycolipid deposits in lysosomes
Hypertrophic cardiomyopathy	- Young age at presentation - (17–18 years) - Asymptomatic - Angina - Dyspnea - Syncope - Sudden death	- Asymmetrical hypertrophy - Small LV cavity - LVOT obstruction - Preserved EF	- Increased QRS complex voltage - Pseudo-delta wave - Giant T-wave inversion	- Patchy - Midwall - Junctions of the ventricular septum and RV	- Myocyte hypertrophy - Myofibrillar alteration - Fibrosis
Hypertensive heart disease	- Adults - History of hypertension	- Symmetrical hypertrophy - Mild LV dilation - Preserved EF	- Increased QRS complex voltage - Non-specific ST-T-wave changes	- No pattern - Mainly subendocardial	- Enlarged myocytes - Increased myocyte nuclei concentration`

LVH, left ventricular hypertrophy; LV, left ventricle; RV, right ventricle; EF, ejection fraction; LVOTO, left ventricular outflow tract obstruction.

LV systolic function

In the early stages of cardiac involvement, no impairment of systolic function is observed, and the LV ejection fraction (LVEF) is usually preserved or even supranormal (25). In the advanced stages of the disease, a reduction in LVEF can be observed, which is associated with a worse prognosis (26). Generally, there are no regional wall motion abnormalities, especially in the early stages of the disease. However, hypokinesia or akinesia of the LV posterior and inferior walls is described in some patients, as an expression of myocardial fibrosis.

Binary sign

Pieroni et al. (27) identified a specific echocardiographic feature of AFD called "binary sign" matching echocardiographic and histologic findings. They defined the binary sign as "the appearance of a clear black and white interface of the LV myocardium due to the adjacency of a bright, hyperechogenic region to a relatively low echo intensity region." At histology, this finding was associated with endomyocardial glycosphingolipid compartmentalization, reflecting a peculiar feature of AFD. However, other authors raised doubts about the clinical utility of the binary sign as this feature can be non-specific. Kounas et al. (28) reported the binary sign in 21% of hypertrophic cardiomyopathy (HCM) patients without AFD. Also, Koskenvuo et al. (29) reported a lack of sensitivity and specificity of binary signs in AFD patients. Overall, the heterogenicity of AFD phenotypic variation makes applicability of binary sign of limited utility, but it can have a role in the differential diagnosis of unexplained LVH.

Papillary muscle hypertrophy

Echocardiographic findings of papillary muscle (PM) hypertrophy and trabecular complexity support the diagnosis of AFD cardiac involvement (30). However, PM hypertrophy is not specific to AFD and is present also in Friedreich ataxia, amyloidosis, and hypertensive patients (31). In the parasternal short axis, both PMs can be seen simultaneously. Through the measurement of both PM areas (manually traced from a short-axis view at the papillary muscle level) and LV cavity circumference using the endocardial border, it is possible to calculate the PM/LV ratio. AFD patients present a significantly higher PM/LV ratio than hypertensive, amyloidosis, and Friedreich patients (31). In hypertensive patients, the high wall stress leads also to a progressive LV cavity dilation, so relative PM/LV does not increase over time. Also in Friedreich ataxia, the end-diastolic wall thickness is not more than 14 mm, and PM size does not increase as much (32). Thus, in AFD cardiomyopathy, LV and papillary hypertrophy in combination with a small LV cavity lead to an increased absolute and relative PM/LV ratio which results in a useful, although non-specific marker.

LV outflow tract obstruction (LVOTO)

In contrast to hypertrophic cardiomyopathy, LVOTO has a very low incidence in patients with AFD (33). In AFD patients, LVOTO appears to be the result of a small LV cavity and PM hypertrophy and can be diagnosed during exercise echocardiography. Calcagnino et al. (34) studied exercise-induced LVOTO in AFD symptomatic patients. LVOTO was measured using continuous-wave Doppler in the apical five-chamber view with a latent obstruction defined as a peak LV outflow tract (LVOT) gradient >50 mmHg during or after exercise. This was the first report of a provocable LVOTO in symptomatic AFD patients. Graziani et al. (35) reported a case series of patients with advanced cardiac involvement at diagnosis who developed LVOTO during follow-up, despite optimal therapy. They described that LVOTO was mainly due to the extension and distribution of the LVH, rather than asymmetric septal basal hypertrophy and mitral valve abnormalities.

Left atrium and diastolic function

Fibrosis and increased LV wall thickness, typical of AFD cardiomyopathy, are associated with progressive impairment of diastolic function (36), although restrictive pathophysiology is

observed rarely and only in the most advanced stages of the disease. In AFD, the accumulation of glycolipids occurs intracellularly, and not in the interstitium, as happens in infiltrative disorders, such as amyloidosis. This different pathophysiology would explain why restrictive physiology is a frequent feature in infiltrative disorders but uncommon in AFD (37).

Assessment of myocardial relaxation with TDI function is a reliable method for early identification of preclinical AFD cardiomyopathy, even before the development of LVH (38). AFD patients, regardless of LVH, have a significant reduction of systolic (S'), early diastolic (e'), and late diastolic (a') velocities, compared with normal control subjects. In AFD patients, the Ea/Aa ratio is reduced, and the E/e' ratio is higher compared to control subjects (39).

Diastolic dysfunction, related to myocardial fibrosis, is frequent in patients with AFD. Septal E/e' ratio has been described as the best echocardiographic marker suggestive of LGE at CMR. At the same time, LGE is detected even in the absence of measurable cardiac functional impairments, suggesting that diastolic dysfunction would not be a prerequisite for LGE in AFD (40).

Histological evaluation of the atrial myocardium demonstrated glycolipid accumulation, which appears related to LA dilation, dysfunction, and arrhythmias (41).

Recent studies have highlighted an increase in LA size in AFD patients, even before the development of LVH, and offered new insights into LA functional impairment in AFD patients. In a study by Boyd et al., LA volume was increased in AFD patients without LVH and normal diastolic function. This evidence suggests that the early stages of the pathological process may be associated with alterations in atrial myocardial properties and that measurements of LA size and function may be useful in the early diagnosis of AFD, before the development of LVH (42). Therefore, atrial myopathy may be independent of diastolic dysfunction and LVH.

In a retrospective cohort study, LA strain, strain rates, and phasic LA volumes were studied in 50 patients with AFD and compared with 50 healthy control subjects. In AFD, the LA reservoir, conduit, and contractile functions by speckle-tracking echocardiography were all found to be affected (43).

Although there are no robust data on the role of LA strain, some authors (44) highlighted the usefulness of left atriopathy in discriminating AFD and amyloidosis, both cardiomyopathies with a hypertrophic phenotype. These authors demonstrated that transthyretin cardiac amyloidosis is characterized by more advanced LA structural and functional remodeling compared to patients with AFD and similar degree of LVH, suggesting that left atriopathy is less important in AFD than in amyloidosis when comparing patients with the same degree of LVH.

Valvular heart disease

It is increasingly recognized that cardiomyopathies and valvular heart diseases may share different pathophysiological mechanisms. Several genetic or acquired diseases, such as storage or immune-mediated disorders, can affect both the myocardium and the valves, with important prognostic and therapeutic implications (45).

The glycolipid deposition typical of AFD has also been found at the level of the heart valves, and valvular infiltration leads to leaflets thickening and deformation (46).

Although all the cardiac valves can be affected, Linhart et al. (18) reported that structural abnormalities most frequently involve the mitral and aortic valves. The mitral valve is the most affected, especially in young patients which usually has mild regurgitation and leaflets thickening. After the age of 40 years, alterations also occur in the aortic valve. Aortic valve involvement and the presence of valve abnormalities are associated with a more advanced stage of AFD.

Mild aortic and mitral regurgitation is frequently observed, especially in advanced stages of AFD. Lillo et al. (47) raised the hypothesis of deposition of glycolipids also in the subvalvular apparatus and reported a case of isolated chordal rupture without valve leaflet prolapse. Evidence of aortic stenosis is a very rare finding in AFD. Vlachou et al. (48) analyzed at histology of the aortic valve of an AFD patient, reporting a loss of valve architecture with edema, myxoid degeneration, and calcification. Giustino et al. (49) first reported a case of low gradient aortic valve stenosis in an elderly patient with AFD later undergoing transcatheter aortic valve implantation (TAVI).

Nowadays, few cases of patients with AFD treated surgically for severe aortic stenosis have been reported in the literature; nonetheless, all agree on the clinical benefit of treatment even during enzyme therapy. Overall, valve involvement in AFD appears to be frequent but rarely requires surgical intervention (48, 49).

Aortic dilation

Aortic root dilation is listed among the clinical features of AFD (50). The excessive accumulation of glycolipids in the aortic media and in the small arterioles causes degenerative changes in the vessel wall. The hypertrophy of the media and the marked vacuolization of the smooth muscle cells also determine the involvement of the aortic root in patients with AFD, as demonstrated by biochemical studies carried out postmortem (51).

Echocardiographic dilation of the aortic root is often found in patients with AFD (52). Ascending aortic dilatation appears to be independent of other cardiovascular risk factors (53); however, in patients with dilatation of aortic root, the ascending aorta dilation is associated with LVH, suggesting a relationship between an advanced stage of AFD and vascular remodeling.

Barbey et al. (54) assessed that ascending aortic dilation occurred frequently in AFD male patients compared with the normal population. Female patients with AFD developed dilation of the sinus of Valsalva and ascending aorta less frequently than males, and approximately 15–20 years later. These authors did not observe dilation at the descending aorta in any patient.

The most serious complications of aneurysms of the ascending aorta are dissection and rupture; although no cases of aortic emergencies have been reported in AFD patients, the presence of aortic dilation must be closely monitored to prevent such events (36).

Right sections

Several studies investigated the extent of RV involvement in AFD patients and reported that the RV is frequently and progressively affected, with a prevalence ranging between 31% and 71% (55, 56).

Niemann et al. performed standard echocardiography in 75 genetically confirmed consecutive AFD patients and described RV hypertrophy in 53 patients (71%). The authors found a significant positive correlation between LV and RV wall thickness. Interestingly, the degree of RV involvement correlated with the stage of LV cardiomyopathy (56).

In a study conducted by Kampmann et al. (55), 28% of patients with RV hypertrophy had RV dysfunction, expressed by tricuspid annulus movement <10 mm and a prolonged RV pre-ejection period/pulmonary ejection time ratio or pseudo-normal or restrictive RV filling patterns. Interestingly, in these patients, the severity of RV dysfunction correlated with the extent of LVH.

In line with this evidence, Palecek et al. (57) found RV hypertrophy in 40% of AFD patients, with similar prevalence in both genders. They identified the right ventricular hypertrophy (RVH) feature with preserved systolic but impaired diastolic function as the typical RV structural change in AFD. Indeed, they described RV systolic dysfunction in only 4.3% of FD patients with RVH, while diastolic dysfunction was highlighted in 47% of examined patients.

RVH therefore does not appear to significantly influence RV systolic function, although a slight reduction in RV TDI systolic velocity values can be observed in patients with RVH (58). Systolic velocities at TDI might be helpful in the differential diagnosis of infiltrative heart disease: AFD patients have better RV systolic function compared with those with cardiac amyloidosis with similar levels of RV thickness (58).

Interestingly, the tricuspid annular plane systolic excursion (TAPSE), a parameter for the evaluation of global RV function, was found to be normal even in the advanced stages of AFD cardiomyopathy, making it not very useful for the evaluation of RV involvement in this population (56).

Although conflicting evidence, RV speckle-tracking echocardiography and RV strain may highlight initial RV involvement in AFD patients, even when conventional ultrasound parameters are within normal ranges, with worst RV systolic function parameters in overt cardiomyopathy (59–61).

So far, there is limited evidence regarding right atrial dysfunction in AFD patients. However, it has been reported that right atrial strain values are slightly decreased in AFD patients. Mattig et al. (62) found that alterations in the strain parameters of the right heart, both atrial and ventricular, were more pronounced in patients with cardiac amyloidosis compared to those with AFD, as already highlighted for the LV. Thus, the integration of structural and functional parameters of the right and left heart could be useful to discriminate AFD and amyloidosis.

To date, there are no consistent data on the clinical implications of RV involvement in AFD. A recent study (63) evaluated a possible correlation between RV hypertrophy and dysfunction and major clinical events, showing a significant association between RV hypertrophy and systolic function with clinical outcomes in AFD. However, the authors highlighted that only proteinuria and LVH emerged as independent predictors of outcome in AFD patients, thus suggesting that RV involvement may represent a useful marker of advanced disease but does not influence prognosis in AFD patients.

Gender differences in Anderson–Fabry disease

Gender differences in AFD are primarily due to the nature of X-linked inheritance. Males, who are hemizygous for the mutated gene, typically experience more severe disease manifestations than females, who are heterozygous and may exhibit a wider range of clinical phenotypes. Sex differences in cardiac manifestations are described. Heterozygote female carriers have been reported to be affected by both cardiac and extracardiac manifestations, and it therefore appears that AFD should be considered an X-linked dominant disease (64). Males experience an earlier onset and more severe progression of cardiomyopathy, such as LVH, diastolic dysfunction, and valvular abnormalities. Because of X-chromosome inactivation, female carriers can have variable disease expression, with later symptoms and a variable degree of cardiac involvement, often without significant LVH and only mild echocardiographic changes, with less GLS involvement (3, 18). Regarding LVH, in females, the severity of LVH is strongly correlated with increasing age (65).

A cross-sectional study by Niemann et al. (66) enrolled a large AFD cohort, including 58 female and 46 male patients. In this population, LVH, regional myocardial deformation, and myocardial fibrosis were assessed by standard echocardiography, strain rate imaging, and LGE-CMR. No significant differences have been reported in the ejection fraction and diastolic parameters between female and male patients and respective sex-matched controls. The LV wall thickness was significantly higher in AFD male patients. While only 17% of female patients showed LVH, 65% of male patients had LVH. LGE was detected in 48% of male patients, interestingly all with LV wall thickness >12 mm. In contrast, in females, LGE was detected in 33% of women, consistently in the posterolateral basal wall and in the absence of increased LV wall thickness, suggesting that female patients may develop fibrosis without showing LVH. Therefore, assessment of replacement fibrosis should be included in the clinical management of AFD patients (66).

Gender differences have also been described in vascular remodeling and aortic dilation, which is experienced less frequently in women and typically about 15–20 years later (54).

Role of standard echocardiography in risk stratification

LVH is a major phenotypic expression of cardiac involvement in AFD and has been considered a risk marker

for cardiovascular events and heart failure (67, 68). LVH is considered the strongest prognostic marker in AFD cardiomyopathy and the main cardiac marker to monitor the treatment efficacy (69, 70). More recently, a comprehensive echocardiographic evaluation has been used to classify AFD cardiac involvement into four stages associated with cardiovascular outcomes. In 314 patients with genetically confirmed AFD, Meucci et al. (5) proposed the following staging: Stage 0, patients without cardiac involvement; Stage 1, LVH, defined as a maximal LV wall thickness >12 mm; Stage 2, LA enlargement, defined as LA volume index >34 mL/m^2; and Stage 3, LV systolic or diastolic dysfunction, defined as LV ejection fraction <50% or diastolic dysfunction measured by E/e' ratio ≥15 or TAPSE <17 mm. The study endpoint was the composite of all-cause death, hospitalization for heart failure, new-onset atrial fibrillation, major bradyarrhythmias or tachyarrhythmias, and ischemic stroke. Interestingly, although worsening stages of cardiac damage were accompanied by greater LVH, the association with cardiovascular events was more robust with the use of the proposed stages than maximal LV wall thickness alone. These findings suggest that a comprehensive echocardiography evaluation has a significant role in AFD patient risk stratification.

Sometimes it is useful to integrate the diagnostic process with CMR. Morphological and functional study of the heart with CMR and GLE seems to be useful in identifying AFD patients who are at high risk of adverse cardiac events, regardless of established clinical risk factors. Thus, CMR seems to be an important tool for the risk stratification of this population (67). CMR is also important for therapeutic strategies, as the presence of GLE is a major biomarker of AFD disease and is one of the indicators that allows the financed enzyme replacement therapy (71).

Role of standard echocardiography in monitoring the effects of treatment

Intravenous enzyme replacement therapy (ERT) with agalsidase-alfa, agalsidase-beta, or oral chaperone therapy (migalastat) represents the specific treatments for AFD, to reduce symptoms and improve survival (72).

However, the benefit of treatment seems to be significant only in the early stages of the disease. Therapy started later may only slow the progression of disease, without affecting already existing organ damage, such as cardiomyopathy, which may become irreversible. Advanced AFD cardiomyopathy, defined by the coexistence of LVH, myocardial fibrosis, and severely reduced regional LV function, indicates a poor response to therapy. Thus, echocardiography represents a main tool, together with other indicators, for monitoring the response to AFD-specific treatment (73).

One of the most used parameters to monitor the effects of ERT is the change in LV mass (74). A reduction in LV mass and an improvement in LV function has been described after 1 year of ERT in a small cohort of AFD patients, thus highlighting a short-term effect of the treatment (12). The long-term effects of ERT on AFD cardiomyopathy appear to be related to the extent of myocardial fibrosis at the time of treatment initiation. In patients without detectable fibrosis and mild hypertrophy at baseline, normalization of LV wall thickness and mass during ERT was observed, as well as improvement to normalization of LV radial and longitudinal septal function. These patients experienced benefits also in terms of exercise capacity.

Strain rate imaging seems to be superior to global parameters such as ejection fraction in monitoring ERT treatment. An increase in radial strain rate after 1 year of ERT appears to predict long-term improvement in regional myocardial function. In contrast, a reduction in lateral longitudinal function is an unfavorable sign in the advanced stages of the disease and predicts an adverse outcome (73).

Conclusions

Standard echocardiography evaluation has a crucial role in AFD patients. Echocardiography is a readily available and diffuse imaging methodology that supports physicians in the diagnosis, follow-up, and risk stratification of AFD patients.

Author contributions

MC: Writing – original draft, Writing – review & editing. GC: Writing – review & editing. MR: Writing – review & editing. LP: Writing – review & editing. EP: Writing – review & editing. PP: Writing – review & editing. VM: Writing – review & editing. VR: Writing – review & editing. RL: Writing – review & editing. RA: Writing – review & editing. DL: Conceptualization, Supervision, Writing – review & editing. VP: Conceptualization, Supervision, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

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Right atrial strain in Anderson–Fabry disease

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Background: To date, only limited data are available on right atrium (RA) morphofunctional remodeling in Fabry disease (FD).
Purpose: We aimed to investigate RA structural and functional remodeling in patients with FD vs. healthy controls using 2D speckle tracking echocardiography (STE) and to explore whether any differences exist in FD patients with and without left ventricular hypertrophy (LVH).
Methods: We prospectively enrolled patients with FD and controls matched for age, sex, and cardiovascular risk factors. Patients with FD were divided in two groups according to the presence/absence of LVH (LVH+: left ventricular wall thickness >12 mm). All patients underwent standard echocardiography and STE analysis investigating the mechanics of all cardiac chambers, including RA reservoir, contractile and conduit strain.
Results: A total of 64 patients with FD (50% males; mean age 50 ± 17 years; 51.5% LVH+) and 64 control patients were included in the study. Focusing on right chambers, RA and right ventricular (RV) dimensions were similar between FD and controls. No differences were found for tricuspid annular plane systolic excursion ($p = 0.073$) and RV fractional area change ($p = 0.461$), while RV systolic Tissue Doppler velocity was reduced in patients with FD ($p = 0.041$). STE analysis revealed impaired strain values for all cardiac chambers in FD vs controls, specifically: left ventricular global longitudinal strain (LV-GLS, $p < 0.001$), left atrial (LA) reservoir strain ($p = 0.001$), conduit strain ($p = 0.012$), and contractile strain ($p < 0.001$), RV-GLS and RV free wall strain ($p < 0.001$). Similarly, all RA strain phases were significantly reduced in patients with FD compared with control patients (RA reservoir 27.4 ± 11.1 vs. 41.9 ± 8.3%, $p < 0.001$; RA contractile 9.9 ± 5.1 vs. 18.0 ± 4.9%, $p < 0.001$; RA conduit 19.1 ± 8.1 vs. 24.1 ± 8.1%, $p = 0.001$). When comparing FD patients without LVH to controls, it was found that RA reservoir and contractile strains were significantly reduced in the former ($p < 0.001$). In multivariable linear regression analyses, LA reservoir strain ($p = 0.010$) and LV-GLS ($p = 0.044$) emerged as independent correlates of RA mechanics after adjustments were made for RA dimensions, RV systolic function parameters and hypertrophy, and LV maximal wall thickness.
Conclusions: In FD impaired RA strain is a common finding. RA reservoir and contractile strains are reduced in FD patients even before LVH ensues, as compared to controls. LA reservoir strain and LV-GLS show an independent correlation with RA reservoir strain.

KEYWORDS

Anderson–Fabry disease, right atrium, speckle tracking echocardiography, strain, cardiomyopathy

Introduction

Anderson–Fabry disease (FD) is a rare X-linked lysosomal storage disorder caused by pathogenic variants of the α-galactosidase A gene, resulting in complete or partial deficiency of α-galactosidase A (α-Gal A) enzyme activity and consequent globotriaosylceramide (Gb3) accumulation (1). Overt cardiac involvement is usually defined by the presence of left ventricular hypertrophy (LVH) (2), but histological studies have proved that all cardiac chambers are affected by Gb3 storage (3, 4).

Two-dimensional speckle tracking echocardiography (2D-STE) is a powerful tool for the assessment of cardiac chamber mechanics, and the evaluation of LV global longitudinal strain (LV-GLS) is widely used in clinical practice. Also right ventricle (RV) and left atrium (LA) strains are emerging as novel markers of dysfunction in cardiomyopathies (5–8), and their impairment is linked to prognosis (9, 10). In the context of Fabry cardiomyopathy, LV (11) and RV strains (5) have already been investigated, as also LA strain (7, 12). Specifically, LA deformation can be impaired even before the occurrence of LVH and overt diastolic dysfunction (13). To date, only limited data are available on right atrium (RA) remodeling in FD. Therefore, our aim in this study is to assess RA structural and functional remodeling in patients with FD compared with sex and age-matched healthy controls (HCs) and to explore whether any differences exist in FD patients with and without LVH.

Materials and methods

Study population

This is a prospective study performed at the Cardiac Rare Disease Outpatient Clinic, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy, between July 2020 and March 2024. All patients with a diagnosis of FD were screened ($n = 78$). We excluded patients whose speckle tracking analysis was not feasible due to poor image quality ($n = 14$). FD was diagnosed by measuring plasma and leucocyte alfa-galactosidase A enzyme activity in males and confirmed by sequencing the GLA gene in all patients (14). All patients underwent a comprehensive clinical and echocardiographic evaluation. Patients with FD were divided in two groups according to the maximal LV wall thickness: those with LVH (defined as LV wall thickness >12 mm, LVH+) and those without LVH (LV wall thickness ≤12 mm, LVH−), who were regarded as genetic positive but phenotype negative (6). HCs identified from the students, nurses, and doctors of our center and matched for age, sex, and cardiovascular risk factors were included.

This study complied with the ethical principles of the Declaration of Helsinki, and it was approved by our local ethics committee. Informed written consent was obtained from all patients to participate in the study.

Echocardiography

Comprehensive two-dimensional echocardiography was performed in accordance with current guidelines (15), and as previously described (7), by experienced cardiologists (MCM and GI). STE analysis was performed by experienced cardiologists (FG and RL), blinded to patients' clinical characteristics, using the commercially available software, 2D Cardiac Performance Analysis© by TomTec-Arena TM (TomTec Imaging Systems, Unterschleissheim, Germany). A speckle tracking analysis was performed in all cardiac chambers in accordance with the latest recommendations (16–18). Briefly, to take the measurements of LV-GLS, images from apical four-, two,- and three-chamber views, zoomed on the LV and acquired with frame rates >50 frames/s, were used. The LV endocardial border was traced from an end-systolic frame and automatically tracked throughout the cardiac cycle by the software. The adequacy of tracking was manually verified and the region of interest properly adjusted. LV-GLS was obtained by averaging all segmental strain values and later by averaging the values calculated in each view. For the RV strain analysis, the average values of the longitudinal peak systolic strain from the three segments of the free wall (RV-FWS) and from all six segments of the free wall and septal wall of the RV (RV-GLS) were calculated (16).

LA strains were measured from the apical four-chamber view, and RA strains were measured from the RV-focused apical four-chamber view (16). The three components of atrial strain were identified from the created curves, as follows: reservoir strain was measured as the peak value during the cardiac cycle; contractile strain was assessed during the peak atrial contraction; conduit strain (strain during passive LV filling) was calculated as the difference between reservoir and contractile strains (16). In patients with atrial fibrillation (AF), measurements were obtained by averaging three consecutive cardiac cycles; in this group, atrial strain analysis was limited to the investigation of atrial reservoir and conduit strains, as recommended by guidelines (16) and performed in other studies (7, 19). Strain values are reported as absolute numbers throughout the text. According to current evidence, RA reservoir <25% was considered impaired (20).

Statistical analysis

Normally distributed continuous variables were presented as mean ± standard deviation and compared between two groups using an unpaired Student's t-test, whereas non-normally distributed data were expressed as median and interquartile range and compared using the Mann–Whitney U test. Categorical data were presented as frequencies and percentages, and a comparison between groups was performed by using χ^2 test or Fisher's exact test, as appropriate. One-way analysis of variance with Bonferroni *post-hoc* tests was used to compare three groups (LVH+, LVH−, and HCs) when continuous variables were normally distributed. Alternatively, the Kruskal–

Wallis test was performed when continuous variables were not normally distributed, with Bonferroni *post-hoc* correction.

Univariable and multivariable linear logistic regression analyses were performed to identify the clinical and echocardiographic determinants of RA reservoir strain in the overall population. Variables with a significant correlation in the univariable analysis ($p < 0.05$) were further investigated by using a multivariable model. All tests were two-sided, and *p*-values <0.05 were considered statistically significant. All analyses were performed using SPSS statistical software (SPSS version 23, Inc., Chicago, IL, USA).

Results

Clinical characteristics

A total of 64 patients with FD and 64 HCs were enrolled in the study. The main clinical characteristics of the overall FD population are reported in Table 1, while Supplementary Table S1 reports the differences between two groups of FD patients, LVH+ and LVH−. As shown, 50% of patients were male, and the mean age was 50 ± 17 years. The majority of the patients had a classic phenotype (71.8%) and were on specific therapy (57.8%). Roughly, a quarter of them were hypertensive (28.1%), only 8% had chronic kidney disease, and three patients previously underwent kidney transplantation. All but six patients (9.4%) were in sinus rhythm at the time of evaluation.

Standard and speckle tracking echocardiography

Echocardiography showed LVH in 33 patients (LVH+, 51.5%). Table S2 shows a comparison between echocardiographic measurements of the FD population vs. HCs. As expected, several differences emerged among them. Patients with FD had increased LV wall (12 IQR: 9–16 mm vs. 9 IQR: 8–10.9 mm, $p < 0.001$) and RV wall thickness values (4.9 ± 1.9 mm vs. 3.4 ± 0.5 mm, $p < 0.001$); 26 patients (40.6%) had right ventricular hypertrophy (RVH), all of whom were in the LVH group. With regard to the atria, patients with FD had increased left atrial volume index (LAVi) values ($p < 0.001$), while RA dimensions were similar between the two groups defined by RA area (15.3 ± 4 vs. 14.3 ± 2.3 cm^2, $p = 0.098$) or volume index (23.0 ± 8.1 vs. 20.7 ± 5.2 ml/m^2, $p = 0.056$).

The Tissue Doppler analysis revealed lower systodiastolic function indices in patients with FD compared with control patients, while LVEF values were similar in both groups. With regard to RV function, RV systolic velocity was lower in patients with FD (12.3 IQR: 11.0–13.7 cm/s vs. 13 IQR: 12–14 cm/s, $p = 0.041$), while tricuspid annular plane systolic excursion (TAPSE) values did not significantly differ between the FD population and HCs ($p = 0.073$), as also right ventricular fractional area change (RVFAC) values ($p = 0.461$). Pulmonary artery systolic pressure (PASP) was higher in patients with FD (even if mostly within normal range, $p = 0.031$), while TAPSE/PASP values were lower ($p = 0.042$). STE analysis revealed impaired values of all chambers strains in patients with FD compared to controls. Specifically with regard to the atria, patients with FD showed lower LA strain (LA reservoir $p = 0.001$, LA conduit $p = 0.012$, LA contractile $p < 0.001$) and RA strain values (RA reservoir: 27.4 ± 11.1% vs. 41.9 ± 8.3%, $p < 0.001$; RA contractile: 9.9 ± 5.1% vs. 18.0 ± 4.9%, $p < 0.001$; RA conduit 19.1 ± 8.1% vs. 24.1 ± 8.1%, $p = 0.001$) when compared with controls (Figure 1); in 27 patients with FD (42.1%), RA reservoir strain was <25%.

Even if FD patients with AF were excluded, all chambers strains were significantly reduced in these patients when compared with controls, as reported in Supplementary Table S3.

The main echocardiographic findings in LVH+ vs. LVH− vs. controls are summarized in Table 2.

Among the LVH+ group of patients, the median maximal wall thickness was 16 mm (IQR: 15–20) and, as expected, several differences emerged between the three groups (LVH+ vs. LVH− vs. controls), as shown. Of interest, the LVH− patients differed from controls for lower values of LV-GLS ($p = 0.025$), lateral e' ($p = 0.010$), septal and lateral a' ($p < 0.001$), RA reservoir (31.3 ± 9.6 vs. 41.9 ± 8.3%, $p < 0.001$), and contractile (8.8 ± 4.4 vs. 18.0 ± 4.9%, $p < 0.001$) strains. Figure 2 shows an example of RA strain assessment in a patient with FD LVH+, in a patient with LVH−, and in an HC.

Supplementary Table S4 reports a univariable linear regression analysis for echocardiographic predictors of RA reservoir strain, while a multivariable analysis is presented in Table 3. LA reservoir strain ($p = 0.010$) and LV-GLS ($p = 0.044$) emerged as independent correlates of RA mechanics after adjustments were made for the parameters of RA dimensions, RV systolic function and hypertrophy, and LV maximal wall thickness. No independent predictors of RA reservoir strain have been identified among clinical variables, as reported in Supplementary Table S5.

TABLE 1 Clinical characteristics of the Fabry population.

Variable	Overall Fabry population (*n* = 64)
Age (years)	50 ± 17
Female, *n* (%)	32 (50.0)
Classic phenotype, *n* (%)	46 (71.8)
Sinus rhythm, *n* (%)	58 (90.6)
Hypertension, *n* (%)	18 (28.1)
Diabetes, *n* (%)	1 (1.5)
Dyslipidemia, *n* (%)	9 (14.0)
Ischemic HD, *n* (%)	7 (10.9)
NYHA class ≥2, *n* (%)	20 (31.2)
CKD, *n* (%)	5 (7.8)
RRT, *n* (%)	3 (4.6)
AF, *n* (%)	6 (9.3)
PM, *n* (%)	8 (12.5)
Specific therapy, *n* (%)	37 (57.8)
Previous HF, *n* (%)	2 (3.1)
Previous stroke, *n* (%)	3 (4.6)

HD, heart disease; CKD, chronic kidney disease; RRT, renal replacement therapy; AF, atrial fibrillation; PM, pacemaker; HF, heart failure.

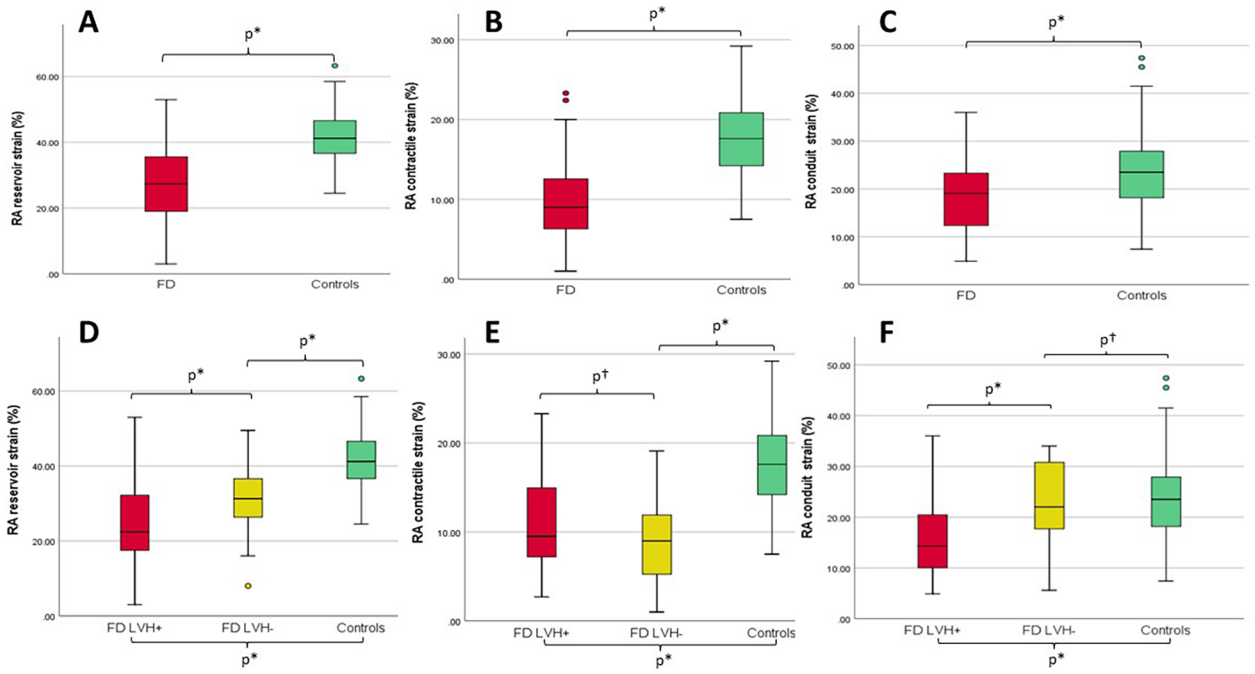

FIGURE 1
(A–C) Box plots with median and interquartile ranges of RA reservoir, contractile, and conduit strains for patients with FD (overall population, red) compared with controls (green), respectively. (D–F) Box plots with median and interquartile ranges of right RA reservoir, contractile, and conduit strains for FD patients with LVH (LVH+, red) vs. FD patients without LVH (LVH–, yellow) vs. controls (green), respectively. *$p \leq 0.05$; †p = not statistically significant; RA, right atrium; LVH, left ventricular hypertrophy.

Discussion

In this study we investigated RA structural and functional remodeling in patients with FD vs controls. We found that in patients with FD, RA strains were reduced, while RA dimensions were similar between the two groups. Interestingly, RA reservoir and contractile strains were also significantly reduced in patients with FD LVH– when compared with healthy subjects.

Fabry cardiomyopathy is a pan-cardiac disease, as Gb3 accumulates in the lysosomes of all cardiac cellular types (21–23). Indeed, histologic studies have shown that glycosphingolipid deposition also affects atrial cardiomyocytes (24), causing atrial enlargement, impaired function, and a predisposition to supraventricular arrhythmias (25). During the last few decades, studies on patients with FD have mainly focused on *left* atrial involvement, showing that the mean LA size on echocardiography is greater than in age-matched control subjects (2). STE analysis showed that atrial deformation can be impaired even before the occurrence of LVH and diastolic dysfunction, and in patients with overt cardiomyopathy, LA mechanics impairment correlates with the degree of LVH (13, 18).

However, to date, only limited data are available on RA remodeling in FD. Recently, Mattig et al. (19) retrospectively analyzed the diagnostic accuracy of right heart and LA strain parameters to distinguish cardiac amyloidosis (CA) from FD. The authors found that atrial strain parameters were impaired in both patients with CA and those with FD, with patients with CA demonstrating significantly lower LA and RA strain values. Moreover, the authors demonstrated that a combination of standard and STE imaging, including RA strain (together with age, basal RV diameter, and global RV strain), showed the best diagnostic accuracy to distinguish the two diseases.

To the best of our knowledge, this is the first study specifically comparing RA strain in FD patients with and without LVH and controls. The factors that contribute to the impairment of RA mechanics in FD cannot be deduced by the limited data of our study, but we might speculate that the following may have a role: (1) the intrinsic involvement of the RA myocardium, which may affect RA compliance and contractility; (2) the RV systodiastolic dysfunction, affecting RA pressure and right "atrial afterload"; (3) hemodynamic factors influencing RA pressure reliant on LV and LA involvement (26). With regard to the last-mentioned point, the potential role of "left-sided" cardiomyopathy in RA mechanics is supported by the results of the present work, since LA reservoir strain and LV-GLS emerged as independent correlates of RA mechanics after adjustments for the parameters of RA dimensions, RV systolic function and hypertrophy, LV wall thickness.

The findings of this study add new data for the complex understanding of right-sided cardiac involvement in FD. As we demonstrated previously (27, 28), in FD, RV involvement parallels LV structural changes. Indeed, RVH is a feature of advanced disease (14), as suggested by the fact that it is detected in those with concomitant LVH and is associated with the LV

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TABLE 2 Echocardiographic characteristics of Fabry patients with and without left ventricular hypertrophy and controls.

Variable	LVH+ (n = 33)	LVH− (n = 31)	Controls (n = 64)	p-value[a]	p-value[b]	p-value[c]
Septal WT (mm)	15 (14–19.5)	8.6 (8–10)	9 (8–10.6)	**<0.001**	**<0.001**	0.999
Posterior LVWT (mm)	13.4 (12.2–15.0)	8 (8–9)	8 (7–9)	**<0.001**	**<0.001**	0.825
Maximal LVWT (mm)	16 (15–20)	9 (8–10)	9 (8–10.9)	**<0.001**	**<0.001**	0.999
LVEDD (mm)	48.5 ± 12.3	44.9 ± 5	43.6 ± 4.1	0.253	**0.018**	0.999
LVEF (%)	61.6 ± 6.7	63.3 ± 4.3	61.5 ± 3.5	0.473	0.999	0.285
LV-GLS (%)	15.9 (13.8–18.6)	22 (20–23)	23 (22–24)	**<0.001**	**<0.001**	**0.025**
Septal S' (cm/s)	6 (4.6–7)	8 (7.3–8.8)	9 (8–10)	**< 0.001**	**<0.001**	0.378
Septal e' (cm/s)	5.6 ± 2.2	11.3 ± 2.6	10.2 ± 2.9	**<0.001**	**<0.001**	0.189
Septal a' (cm/s)	8 (7–9)	8 (6.5–9)	10 (9–12)	0.999	**0.001**	**<0.001**
Lateral S' (cm/s)	6.7 (5.6–8.2)	9 (8.1–10.5)	10 (9–11.3)	**<0.001**	**<0.001**	0.102
Lateral e' (cm/s)	8.3 (7–10.5)	16.2 (13.8–19)	11.1 (10–16.7)	**<0.001**	**<0.001**	**0.010**
Lateral a' (cm/s)	9 (7.3–10)	7 (6.5–9.4)	10 (8–12)	0.477	0.079	**<0.001**
E velocity (cm/s)	70 (60.5–80.3)	90 (80–100)	73.5 (66–86)	**<0.001**	0.833	**0.001**
A velocity (cm/s)	71 (62–86)	55 (50–73)	65 (51.2–78)	0.083	0.152	0.999
E/A	0.9 (0.76–1.22)	1.4 (1.2–1.7)	1.1 (0.8–1.4)	**<0.001**	**0.013**	**0.019**
Average E/e'	10 (7.5–14.5)	7 (6–8)	6.3 (5.5–8)	**<0.001**	**<0.001**	0.999
LAVi (ml/m^2)	44.2 (28.3–66.8)	27 (21.3–30.4)	24.9 (21–28.8)	**<0.001**	**<0.001**	0.250
LA reservoir strain (%)	23.2 (15.9–28.2)	38.8 (28.6–44.0)	35.1 (28.1–44.3)	**<0.001**	**<0.001**	0.999
LA contractile strain (%)	10.3 (7.2–15.5)	8.5 (6.8–16)	15.4 (11.5–22.4)	0.999	**0.004**	**0.002**
LA conduit strain (%)	12.7 (10.0–16.8)	16.4 (14–25)	19.2 (13.1–25.3)	**0.014**	**0.001**	0.999
LL/2 (mm)	29.3 ± 5.2	29.8 ± 4.4	28.3 ± 3.9	0.999	0.925	0.402
TAPSE (mm)	19.9 ± 3.6	23.5 ± 2.9	22.6 ± 2.8	**<0.001**	**<0.001**	0.768
RV S' (cm/s)	11.7 (9.5–13)	12.5 (12–14)	13 (12–14)	**0.024**	**0.004**	0.999
RVFAC (%)	42 ± 4.9	43.5 ± 5.3	42. 0 ± 4.1	0.616	0.999	0.539
RV-FWS (%)	19.4 (16.1–25.3)	23 (26–29.2)	23.4 (21.4–29.7)	**0.009**	**<0.001**	0.484
RV-GLS (%)	17.3 (15.6–22.2)	23.1 (20.1–26.5)	23.4 (21.4–28)	**0.001**	**<0.001**	0.999
RAA (cm^2)	17.0 ± 4.7	13.5 ± 2.1	14.3 ± 2.3	**<0.001**	**<0.001**	0.667
RAVi (ml/m^2)	25.2 ± 8.3	20.7 ± 7.3	20.7 ± 5.2	**0.026**	**0.006**	0.999
RA reservoir strain (%)	23.8 ± 11.4	31.3 ± 9.6	41.9 ± 8.3	**0.007**	**<0.001**	**<0.001**
RA contractile strain (%)	11.2 ± 5.7	8.8 ± 4.4	18.0 ± 4.9	0.197	**<0.001**	**<0.001**
RA conduit strain (%)	15.4 ± 6.9	22.5 ± 7.7	24.1 ± 8.1	**0.002**	**<0.001**	0.999
PASP (mmHg)	28 (25–30)	25 (20–30)	25 (20–25)	0.763	**0.045**	0.894
TAPSE/PAPS (mm/mmHg)	0.72 ± 0.18	0.95 ± 0.23	0.94 ± 0.18	**0.009**	**0.002**	0.999
RV WT (mm)	6.4 ± 1.8	3.4 ± 0.4	3.4 ± 0.5	**<0.001**	**<0.001**	0.999

WT, wall thickness; LVWT, left ventricular wall thickness; LVEDD, left ventricular end diastolic dimension; LVEF, left ventricular ejection fraction; LV-GLS, left ventricular global longitudinal strain; LA, left atrium; LAVi, left atrial volume index; LL/2, right ventricle mid diameter; TAPSE, tricuspid annular plane systolic excursion; RV, right ventricle; RVFAC, RV fractional area change; RV-FWS, three-segment right ventricular free wall strain; RV-GLS, six-segment right ventricular global longitudinal strain; RAA, right atrial area; RAVi, right atrial volume index; PASP, pulmonary artery systolic pressure; RV WT, right ventricular wall thickness; S', Tissue Doppler systolic velocity; e', Tissue Doppler early diastolic velocity; a', Tissue Doppler late diastolic velocity.

[b]Bold values indicate statistically significant p-values (<0.05).

[a]p-value for the comparison between Fabry patients with LVH (LVH+) and those without (LVH−).

[b]p-value for the comparison between Fabry patients with LVH (LVH+) and healthy controls.

[c]p-value for the comparison between Fabry patients without LVH (LVH−) and healthy controls.

mass index and Mainz Severity Score Index (27). Moreover, in our previous work (5), we found that the conventional parameters of RV systolic function, namely, TAPSE, RVFAC, and tissue Doppler imaging systolic velocity, are usually normal even when RVH is present, while 2D-STE is a more sensitive tool to unveil *subtle* RV systolic dysfunction, which turns out to be a common finding even when standard parameters are within normal ranges. Interestingly, in the present work, FD patients without LVH had impaired RA reservoir and contractile strain when compared with controls. Thus, we can speculate that RA strain impairement is an early sign of right-sided FD cardiac involvement. These pieces of evidence support the importance of 2D-STE for a comprehensive echocardiographic evaluation in this disease and the importance of assessing all cardiac chambers strains, including RA.

Limitations

The main limitation of this work is the small sample size, which carries relevant statistical implications; however, this is a common disadvantage for studies on rare diseases. Moreover, the short follow-up interval and the limited number of major cardiac events did not allow us to assess the clinical and prognostic value of RA strain in FD. Previous studies (29) showed that LA strain is a predictor of AF and LA strain can be more useful in patients

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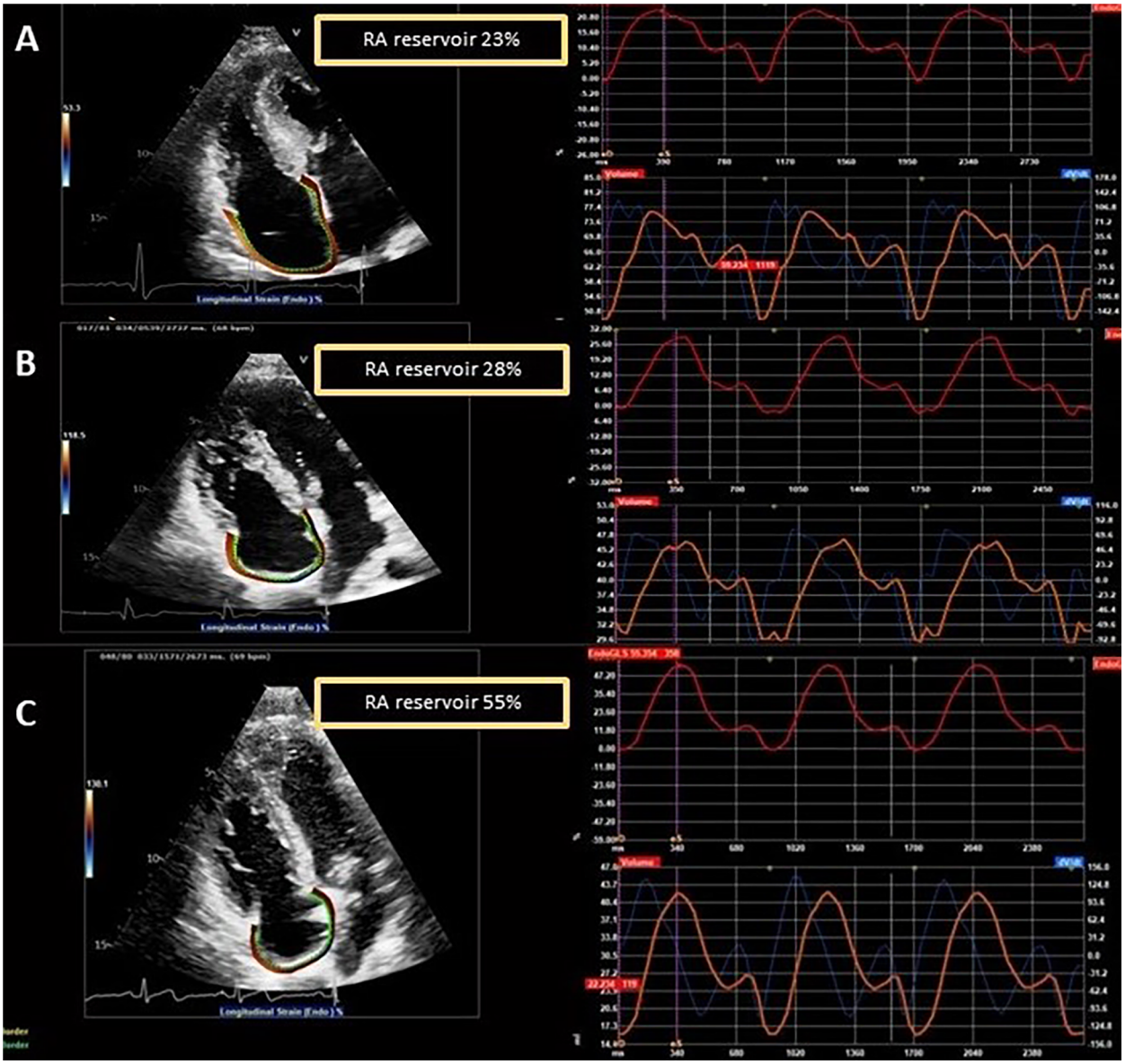

FIGURE 2
Examples of RA strain assessment in a patient with FD and left ventricular hypertrophy (LVH+, A), in a patient with FD without left ventricular hypertrophy (LVH–, B), and in a healthy control (C).

TABLE 3 Determinants of right atrial reservoir strain in multivariable linear regression analysis.

	Multivariable	
	Beta	*p*-value
RV WT	−1.291	0.193
RV S'	0.624	0.145
RAA	−0.494	0.104
Maximal LV WT	0.168	0.680
LA reservoir strain	0.227	**0.010**
LV-GLS	0.769	**0.044**

RV WT, right ventricular wall thickness; S', Tissue Doppler systolic velocity; RAA: right atrial area; LA, left atrium; LV WT, left ventricular wall thickness; LV-GLS, left ventricular global longitudinal strain.
Bold values indicate statistically significant *p*-values (<0.05).

with normal LA volumes when compared with those with LA dilatation. In this context, future specifically designed studies aiming to assess the clinical value of RA strain in FD patients with and without RA dilatation could be of great interest.

In the present study, we decided to also include patients with AF and those who had undergone pacemaker implantation previously, with the aim to investigate RA mechanics in a real-world population that included patients with arrhythmias. In the AF group, the investigation was limited to atrial reservoir and conduit strains as recommended by guidelines (16) and performed in other studies on cardiomyopathies (7, 19). As expected, cases of AF and previous pacemaker implantation were found only in FD patients with LVH. Both supraventricular

arrhythmias and pacing may alter atrial mechanics, and thus, these factors may have a role in influencing our results.

Lastly, cardiac magnetic resonance data obtained within 1 year from an echocardiographic evaluation were available only in a minority of patients, and hence, the correlation between RA mechanics and tissue characterization could not be assessed.

Conclusions

In FD, impaired RA strain is a common finding. The results of this study reveal that RA reservoir and contractile strains are reduced in FD patients even before LVH ensues, as compared to controls. LA reservoir strain and LV-GLS show an independent correlation with RA reservoir strain.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, and further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by Comitato Etico Fondazione Policlinico universitario "Agostino Gemelli"-Università Cattolica del Sacro Cuore, Largo Gemelli 8 – 00168 Roma. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

RL: Data curation, Investigation, Writing – original draft, Formal Analysis, Methodology. AC: Data curation, Investigation, Writing – original draft, Conceptualization. MM: Conceptualization, Project administration, Writing – original draft. GI: Data curation, Methodology, Writing – original draft. CD: Data curation, Formal Analysis, Visualization, Writing – review & editing. FT: Conceptualization, Data curation, Investigation, Writing – review & editing. MM: Data curation, Investigation, Validation, Writing – review & editing. GL: Resources, Supervision, Validation, Writing – review & editing. AL: Investigation, Methodology, Supervision, Validation, Writing – review & editing. FB: Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing. FG: Investigation, Methodology, Resources, Software, Supervision, Writing – original draft.

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Conflict of interest

RL has received advisory board fees from Sanofi Genzyme, Takeda, and Shire; she has also received travel support from Amicus Therapeutics. FG has received research grants and advisory board/speaker fees from Takeda, Shire, Amicus Therapeutics, and Sanofi Genzyme.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2025.1496534/full#supplementary-material

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Females with Fabry disease: an expert opinion on diagnosis, clinical management, current challenges and unmet needs

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Females with Fabry disease (FD) often have a milder phenotype, later symptom onset, and slower disease progression than males, causing delayed diagnosis and undertreatment. A survey was conducted at nine Italian FD centers to evaluate routine management of females with FD; results were discussed at a meeting of eleven Italian specialists and recommendations developed. Of the 227 females managed by the physicians surveyed, 85% were diagnosed through family screening and 38.5% were symptomatic at presentation. Female patients usually underwent cardiac, renal, and neurologic monitoring, and measurement of plasma lyso-globotriaosylsphingosine (Gb3) levels at 6- or 12-month intervals. Treatment was initiated in 54%, mostly enzyme replacement therapy. Experts recommended screening all female relatives of index cases and evaluating all potentially affected organ systems. Diagnosis should be based on genetic analysis. Individualized monitoring of asymptomatic females must balance the need to detect organ damage while maintaining adherence. Treatment decisions should be based primarily on signs/symptoms of FD, but age, family screening results, *GLA* mutations, Gb3/lyso-Gb3 accumulation, and organ damage should be considered in asymptomatic females. More research on FD in females is needed and physicians should be aware of differences in the diagnosis, monitoring, and management of females vs. males with FD.

KEYWORDS

alpha-galactosidase A, enzyme replacement therapy, Fabry disease, female, genetic testing, heterozygote

1 Introduction

Fabry disease (FD) is a rare X-linked lysosomal storage disorder caused by the lack or absence of lysosomal alpha-galactosidase A (α-Gal A) activity secondary to mutations in the α-Gal A gene (*GLA*) at Xq22.1 (1). This deficiency in α-Gal A leads to the progressive accumulation of its substrate globotriaosylceramide (Gb3) and its deacylated form lyso-Gb3, resulting in a multisystemic disease that mainly affects the kidneys, heart, and nervous system (1–3).

FD is often categorized into the classical phenotype, which develops in early childhood, is marked by absent or severely reduced α-Gal A activity, and has a severe outcome, and the non-classical phenotype, characterized by residual enzyme activity, variable disease course, age of onset, and manifestations, and has less severe outcomes (2, 4). Genomic screening has led to the identification of pathogenic and non-pathogenic variants, as well as *GLA* variants of unknown significance (VUS) (5).

The phenotype varies depending on the *GLA* variant, residual α-Gal A activity, age, gender, and, in heterozygous females, X chromosome inactivation (XCI) (5, 6). This phenotypic heterogeneity complicates FD diagnosis, particularly when a VUS is present (5). The increasing number of these variants highlights the need to clarify the pathogenic role of VUS when making treatment decisions.

The X-linked inheritance of FD means that hemizygous males are typically more severely and consistently affected, whereas heterozygous females present a variety of symptoms, ranging from asymptomatic to severe (4). Overall, females with FD tend to have a milder phenotype, later onset of symptoms, and slower disease progression than males (4, 7). This variability in females depends on the pathogenic variant and the XCI profile (8, 9). XCI is random, dependent on the cell type, and frequently non-uniform across the silenced chromosome (10), leading to different patterns of Gb3 accumulation.

As a multisystemic disease, the diagnosis, monitoring, and management of FD should involve a multidisciplinary clinical team, including an internist, neurologist, nephrologist, cardiologist, medical geneticist, genetic counsellor, psychologist, and nurse. At present, four therapies are approved in Europe for FD: three enzyme replacement therapies [ERTs; i.e., agalsidase alpha (agalsidase-α, Replagal®, Takeda UK, Ltd), agalsidase beta (agalsidase-β, Fabrazyme®, Sanofi), and pegunigalsidase-α (Elfabrio®, Chiesi Farmaceutici S.p.A.)], and one pharmacologic chaperone [i.e., migalastat (GalafoldTM, Amicus Therapeutics)].

There are many challenges and unmet needs in the diagnosis and clinical management of females with FD. FD diagnosis is often missed in females, who are still sometimes considered as mere carriers of a defective *GLA* gene, particularly when paucisymptomatic (11). Thus, many female patients remain undiagnosed for more than 10 years after they first experience symptoms (12, 13). Furthermore, there is evidence that female patients are undertreated in spite of having major organ involvement, such as left ventricular hypertrophy (LVH) and stage 3 chronic kidney disease (CKD) (13–16). Moreover, recommendations from an international panel of FD specialists specify that males with classical FD should receive treatment regardless of symptoms, whereas females with classical FD should only receive treatment following the appearance of major organ injury (17). Thus, the appropriate timing of treatment in female FD patients remains an unanswered question, as does the optimal follow-up and monitoring strategy.

An Italian advisory board meeting of expert clinicians was held on 7 July 2022 to discuss the challenges and unmet needs in the diagnosis and clinical management of females with FD based on the results of a survey conducted at nine Italian FD centers. The survey was conducted to understand how female patients with FD are treated in clinical practice today and to compare real-life approaches with what is documented in the literature. The purpose of the meeting was to highlight the specific features of FD in females pertaining to presentation and disease progression, and the consequent diagnostic, therapeutic, and follow-up requirements, aiming to promoting greater awareness and better management of female patients with FD. The main conclusions of the meeting are summarized here.

2 Survey questionnaire and advisory board meeting

Eleven Italian clinicians (four cardiologists, one neurologist, one internist/neurologist, four nephrologists, and one geneticist) provided their expert opinion, based on the survey results, and shared their clinical experiences; these clinicians are the authors of this article. The advisory board meeting was sponsored by Sanofi.

The survey questionnaire was designed by the Sanofi medical team based on the topics to discuss at the meeting (clinical presentation, and disease progression and monitoring). The survey questionnaire was reviewed Dr Mignani and Prof. Tuttolomondo and then emailed to the survey participants between 20 and 28 June 2022. Physicians at participating centers were asked to provide information about the characteristics and management of female patients with FD at their center. The questionnaire included nine items regarding the screening and diagnosis of female patients, disease characteristics, type and frequency of disease monitoring after FD diagnosis, factors considered for treatment initiation, and their treatment status (Supplementary Material). Nine of 11 participating Italian FD centers responded to the survey and provided data on 227 female patients with FD who were being treated or monitored if untreated. The survey sample included both symptomatic and asymptomatic cases of females with FD, as well as patients whose symptoms were initially not recognized as related to FD. Data were collected and analyzed by the Sanofi medical team in collaboration with Prof. Tuttolomondo.

3 Survey findings and analysis

3.1 Genetic and biochemical aspects of FD in females and diagnostic implications

3.1.1 Survey findings

Among the 227 female patients with FD in the survey, 85% were diagnosed through family screening and diagnosis was

based on clinical manifestations in the other 15%. All centers used molecular genetic testing for the identification of *GLA* mutations encoding an absent or evidently dysfunctional α-Gal A. While many centers perform enzymatic assays for α-Gal A activity quantification and measure Gb3 or lyso-Gb3 concentrations, none of the centers rely on these parameters for the diagnosis of FD in females. Instead, these parameters are used to inform treatment decisions. XCI evaluation, as a predictive marker of disease progression, was not performed in any of the centers.

3.1.2 Analysis

It is well known that male patients with FD can be diagnosed by α-Gal A activity testing alone, but in female patients, it is necessary to demonstrate a disease-causing mutation in the *GLA* gene as the plasma α-Gal A activity is usually normal or highly variable due to skewed XCI (9). Genetic diagnosis in females with FD can be even more challenging in the case of deletions or duplications, usually associated with severe phenotype, as this type of mutation cannot be assessed by direct DNA sequencing but only by specific techniques such as multiplex ligation-dependent probe amplification (MLPA) (18).

In males with FD disease where there is only one X chromosome, genetic diagnosis cannot be missed. However, in females with two X chromosome copies (one normal and the other affected in different proportions in different tissues), these types of mutation (deletions and duplications) cannot be detected with routine genetic tests (19). Heterozygosity of the X chromosome in females makes it important to combine routine sequencing analysis with allelic dosage assays, such as MLPA, to more reliably exclude or confirm FD (18).

FD diagnosis based on lyso-Gb3 levels is sometimes unreliable, as lyso-Gb3 levels vary depending on the disease phenotype, increasing significantly in classical FD but increasing slightly in non-classical FD, while in VUS, lyso-Gb3 level at diagnosis is quite variable but mostly normal (20, 21). Therefore, lyso-Gb3 levels can be a useful tool for the diagnosis of classical vs. non-classical FD in females (20, 22). Moreover, baseline lyso-Gb3 level predicts disease severity over time and is associated with important clinical events (23). Lyso-Gb3 levels can also be useful to assess treatment response in females as it usually decreases upon initiation of ERT or chaperone therapy (23).

Although this survey reported no epigenetic evaluation, the XCI profile may guide treatment decisions in female patients (17). XCI in females is an epigenetic mechanism occurring during embryonic development to ensure X-linked dosage compensation between cells of females (XX karyotype) and males (XY) (24). During XCI, one of the X chromosomes in each cell is randomly silenced and converted into a Barr body, resulting in the mosaic expression of X-linked genes in different organs/tissues. This is facilitated by X-inactive specific transcript (Xist), a non-coding RNA, which coats its chromosome of origin, recruits heterochromatin factors and silences gene expression.

An estimated 15%–30% of the genes within Barr bodies escape inactivation in a constitutive (10%) or variable (90%) manner, leading to biallelism, which may be beneficial for females (25). The maternal vs. paternal X-inactivation ratio is generally 50:50, but imbalances may occur due to genetic mechanisms, such as XIST gene mutations. X chromosome imbalance increases with age in all women (26), leading to worsening disease manifestations in those with FD (9). Analysis of XCI imbalance in different tissues previously showed a correlation between the XCI patterns of blood and other tissues, and significant differences in residual α-Gal A levels, severity scores, progression of cardiomyopathy, and deterioration of kidney function depending on the direction (random XCI, wild-type allele or mutant allele expression) and degree of XCI imbalance (9). This led to the conclusion that XCI significantly impacts the phenotype and natural history of FD in females, and that monitoring and therapeutic intervention depends on the predominantly expressed allele (i.e., wild-type GLA expression leads to mild phenotype and minimal disease progression, whereas mutant GLA expression leads to severe phenotype, rapid progression with age, and poorer prognosis) (9).

Because XCI influences FD disease severity, its early characterization may help identify asymptomatic patients at risk of developing severe disease and requiring early medical attention (27). However, in contrast with previous studies, a 2021 meta-analysis showed no correlation between XCI imbalance and phenotype in female FD carriers (19).

The HUMan Androgen Receptor gene Assay (HUMARA) test is the most widely used method for analyzing XCI imbalance (28). However, because HUMARA only tests a single locus, methylation status of only a few CpG islands are assessed; therefore, it does not reflect which allele has a pathogenic variant and it cannot predict clinical severity as it does not evaluate mRNA expression (29).

More recently, ultra-deep targeted RNA sequencing has been introduced, using next-generation sequencing (NGS) to examine locus-specific methylation within a single cell of a tissue (30). The benefits of NGS include detection of all the CpG islands and the degree of methylation and expression of genes at any given time, and the direct measurement of mutated-to-wild-type alleles ratio in the *GLA* gene at the mRNA level, and consequently, the influence of XCI on clinical manifestations (29). However, these data on epigenetic patterns data need to be statistically validated (31).

Methylation-based assessments are still not widely used in Italy; most centers perform the HUMARA test because it is simple and cost-effective. Despite being complex, ultra-deep RNA sequencing with NGS can be simplified for creating family trios and will be relatively affordable if performed on selected cohorts of patients. However, at present, only *GLA* gene sequencing using the Sanger method is being performed. The experts agreed that there was a lack of reliable evidence linking genotype and phenotype in females with FD and stressed the need for a reliable molecular method based on available evidence.

A direct method for XCI characterization involves family trio-based integrated whole-exome and mRNA sequencing, which can identify potentially pathogenic genetic mutations and XCI ratio using phased and unphased allele-specific expression analysis (32). Working with family trios can reduce genetic variability, improve the evaluation of genotype-phenotype correlation, and facilitate biomarker detection (32).

Extensive research has been conducted to identify novel markers that can potentially be used as screening/diagnostic tools

and for the assessment of treatment response. An ideal biomarker should be easily evaluable, reliable, reproducible, organ-specific, able to accurately diagnose FD and assess disease expression early (before organ damage begins), and characterized by prognostic power (33). Clinical assessment requires the selection of appropriate tissue for testing (34); for example, podocyte testing and blood testing will generate different results. Podocytes can be examined by studying urinary extracellular vesicles, which are easily accessible and contain genetic material that is an informative source of cell-cell interactions and epigenetic modifications in renal cells (35). Exosomes within other bodily fluids may allow similar assessments of changes in the heart or other tissues that are not directly accessible, meaning that blood is no longer the only tissue studied.

3.2 Clinical manifestations and disease monitoring

3.2.1 Survey findings

According to the survey, 38.5% of the 227 female patients with FD were symptomatic at presentation, and the most common manifestations were cardiac, neurologic, renal, and gastrointestinal (GI) symptoms (Figure 1). More participating centers performed cardiac monitoring at 12-month than at 6-month intervals (Figure 2A). An electrocardiogram (ECG), echocardiogram, and cardiac magnetic resonance imaging (MRI) were used to monitor the heart, depending on clinical need. Cardiac MRI was performed every 24 months in 50% of the centers. In contrast, renal function was more commonly monitored at 6-month than at 12-month intervals, using daily proteinuria and estimated glomerular filtration rate (eGFR) assessments (Figure 2B). In 50% of centers, renal biopsy was considered during monitoring, in the case of sudden renal function deterioration or onset of overt proteinuria. Involvement of the peripheral nervous system (PNS) was monitored by assessing pain, heat or cold sensitivity, and dysautonomic symptoms, mostly every 12 months (in 75% of the centers; Figure 2C). Skin biopsy was performed at 62.5% of the centers in selected patients.

Central nervous system (CNS) symptoms were monitored using brain MRI in 57% of the centers, and echo-doppler examinations of the supra-aortic trunks (DESAT) or transcranial doppler ultrasound (TDU) in 25% of centers, each at 12-month intervals, but not at 6 months (Figure 2D). Brain MRI was performed every 24 months in 43% of the centers. DESAT and TDU were also performed at diagnosis in 25% of centers each. Plasma lyso-Gb3 levels were monitored in 38% of centers at 6-month intervals and in 62% at 12- month intervals (Figure 2E).

At the time of this survey, 82% of the female patients were in a stable condition, while 18% had disease progression (Figure 3A). In patients with progressive disease, the most commonly affected organs were the heart (in 55% of patients), kidney (34%), nervous system (9%) and GI tract (3%). Among patients who progressed, 45% were receiving treatment, while the remaining 55% were still without treatment. In patients with stable disease, 60% were receiving therapy and 40% were untreated (Figure 3B).

3.2.2 Analysis

Life expectancy in untreated patients is approximately two decades longer in females with FD compared with males (36). The most common cause of death among both sexes is cardiovascular disease (36), with arrhythmias being the most frequent cardiac event (37). While approximately 50% of untreated male patients have cardiac rhythm disturbance by the age of 45 years, these events also affect 20%–45% of female patients aged 45–65 years (37).

Males and females with FD have similar clinical manifestations and organ damage, but they differ in the frequency, intensity, and onset of symptoms (16). However, with recent advances in molecular techniques, family screening, and better clinical characterization, clinicians are able to detect the non-classical forms of FD, and importantly, able to diagnose more female patients.

Data indicate that males with classical FD have a much higher risk of developing cardiac, renal, or cerebral events than those with non-classical FD and female patients with either phenotype (2). Females with classical disease are also at higher risk of developing complications compared with those with non-classical

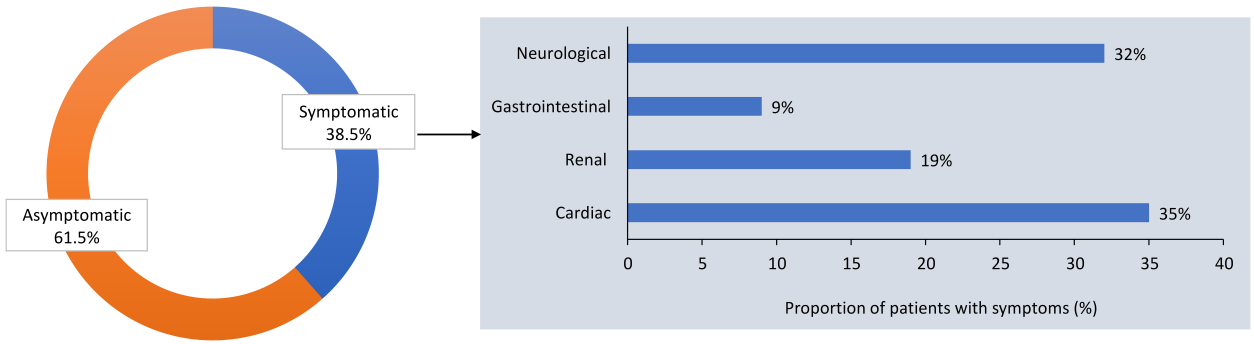

FIGURE 1
Proportion of asymptomatic and symptomatic females with Fabry disease at presentation, and clinical manifestations of Fabry disease in symptomatic females in the survey of Italian centers.

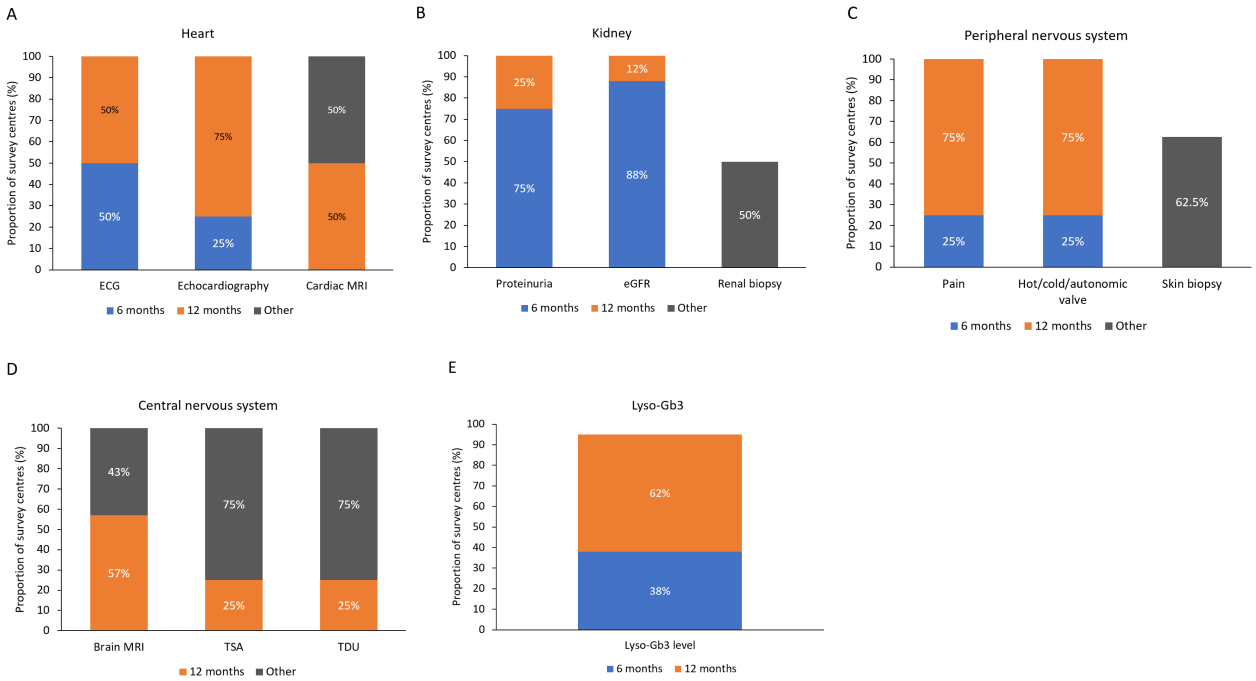

FIGURE 2
Monitoring of **(A)** heart, **(B)** kidney, **(C)** peripheral nervous system, **(D)** central nervous system, and **(E)** plasma lyso-Gb3 levels at different time intervals at the nine Italian centers participating in the survey. ECG, electrocardiogram; eGFR, estimated glomerular filtration rate; lyso-Gb3, globotriaosylsphingosine; MRI, magnetic resonance imaging; TDU, transcranial Doppler ultrasound; TSA, transcortical sensory aphasia.

FD; however, the difference in risk in females is lower than for male patients with classical vs. non-classical FD (2).

Cerebral small vessel disease is often observed in patients with FD. In a case-control study (43% of the total cohort was female), the prevalence of impaired cerebral autoregulation was assessed using TDU as a biomarker for cerebral small vessel disease, with the finding that impaired cerebral autoregulation was more prevalent in patients with FD than in healthy controls (38). However, the indices of this impairment did not show independent association with white matter hyperintensities; in addition, the indices had low-to-moderate predictive ability for discriminating FD patients with and without white matter hyperintensities.

Strategies for disease monitoring should include an assessment of pathologic, metabolic, and clinical phenotypes; however, this is not always practiced in female patients. There is currently a lack of data about symptoms specific to female patients. Typical symptoms of FD should be proactively investigated in females, who tend to ignore their symptoms, continuing to live with them for years before reporting them to their primary care physician.

Data suggest that more females than males experience pain in their hands and feet (39% vs. 29%) and abdomen (30% vs. 22%) (39, 40). The frequency and severity of diarrhea, the second most common GI symptom after abdominal pain, are more variable; 20% of FD patients report diarrhea, which is more common in males (26%) than females (17%) (41).

Renal biopsy can be a helpful diagnostic and monitoring tool for females, especially when there is uncertainty about the actual presence of the disease (42). Historically, renal biopsy was thought to be of little value in females because of the possible presence of mosaicism and VUS; however, it is now known that renal histology is similar in male and female patients, with frequent accumulation of Gb3 inclusions in affected renal podocytes. Tissue damage precedes the presentation of signs and symptoms and, therefore, the Gb3 inclusions may not necessarily be associated with proteinuria or renal dysfunction (43, 44). This accumulation of Gb3 and subsequent podocyte injury progresses with age, indicating that renal biopsy could help to identify females with a greater risk of CKD progression (45).

Evidence indicates that cardiac biopsy can also be useful in making a diagnosis. Cardiac symptoms in females are often misunderstood or neglected, even in the general population, but it is important to pay attention to the earliest signs of cardiac damage in females with FD to avoid progression to microvascular ischemia. FD patients often have structural myocardial changes and altered ECG parameters even before they have developed LVH or detectable sphingolipid storage on MRI (46).

The pathogenesis of cardiac damage may differ between males and females with FD. One study found that in males with FD, the development of hypertrophy was always followed by the development of myocardial fibrosis, whereas in females with FD, myocardial fibrosis sometimes occurred in the absence of hypertrophy (47). A study of 34 female patients with late-onset hypertrophic cardiomyopathy found that 12% had FD (48). The biopsies of females with FD showed patchy myocardial

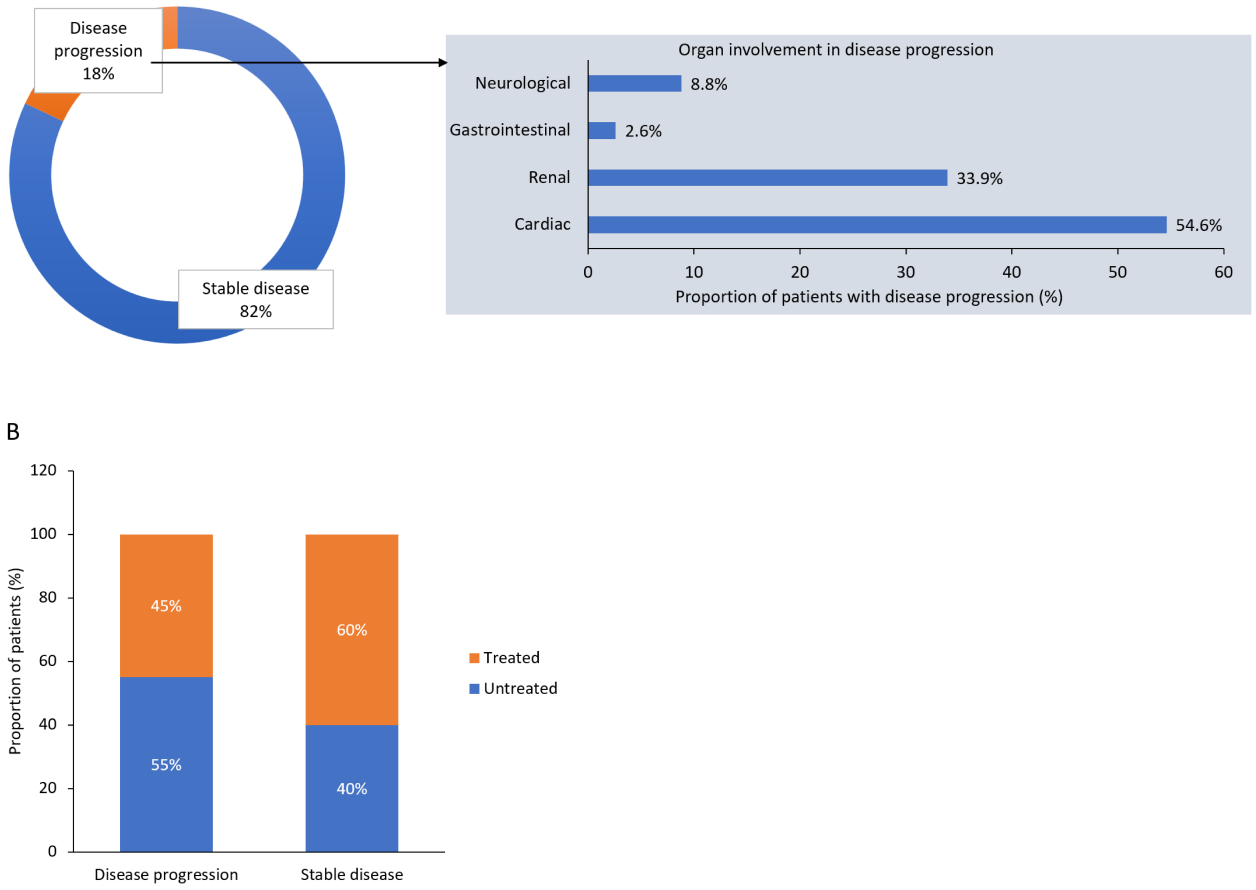

FIGURE 3
Disease status and organs involved in **(A)** disease progression, and **(B)** disease progression according to treatment status: results from the survey of Italian centers.

distribution of cells containing glycolipid inclusion vacuoles as clearly distinguishable islands of affected cells within areas of normal cells, whereas in males with FD, glycolipid-laden cells were diffusely distributed (48). Another study revealed that unaffected myocardial cells can also be abnormal, showing cell hypertrophy that correlated with the increase in left ventricular maximal wall thickness (49). This suggests that affected cells may have a negative paracrine effect on healthy neighboring cells, stimulating cellular hypertrophy that can contribute to the progression and severity of FD cardiomyopathy (49). Therefore, endomyocardial biopsy can provide valuable information for the diagnosis and treatment of FD in females, especially if VUS are present or when the only organ involved is the heart. The experts, therefore, suggest that cardiac biopsy should be considered in female patients with cardiac symptoms, even in the absence of LVH or other FD clinical manifestations, to clearly define myocardial involvement.

Skin biopsy may also be useful for the diagnosis of FD, although histologic skin changes tend to be less marked in female than male patients (50). Electron microscopic analysis of punch biopsy skin samples from patients with FD showed abnormal glycolipid storage in fibroblasts, but the number of affected fibroblasts differed between the sexes, with males showing an average of 90% affected and females an average of 15% (50). Similar differences between the sexes have been shown in skin biopsy samples analyzed using immunofluorescence; samples from patients with FD showed more Gb3 deposition compared with samples from healthy controls, but the magnitude of Gb3 deposition was greater in males than females with FD (51). Thus, cutaneous biopsy may be advisable in selected females with suspected FD and those with VUS.

The greatest decision-making issues for clinicians are choosing how often to monitor, and when to initiate therapy in, female patients with FD who are completely asymptomatic and identified by family screening or for reasons other than symptoms. For example, the unconventional progression of heart damage in females with FD raises the question of whether treatment should be started early or delayed until all biomarkers are identified. Early biomarkers of disease progression are crucial for treatment decision-making and in preventing organ damage in patients with actual risk of progression. The combination of MRI tissue mapping sequences and ECG appears promising to

detect early myocardial changes (46), because currently it is not possible to use the development of hypertrophy or fibrosis as a biomarker for the prevention of cardiac damage.

Proteomics have been used to search for novel plasma biomarker signatures to improve disease prognosis; Hollander and colleagues reported sex-specific proteomic signatures (52), whereas L'Imperio and colleagues reported phenotype-specific proteomic signatures, but no sex-specific differences (53). This phenotypic-specific signature is a promising approach in helping the correct classification of FD phenotypes and interpreting undetermined mutations or VUS (53).

The role of single nucleotide polymorphisms (SNPs) in the monitoring of FD has historically been neglected, but SNPs could help in risk stratification, provide valuable information on prognosis and help personalize disease management. For example, an SNP upstream of the promoter of the *GAL* gene (−1° C→T) in patients with FD reduces the transcription of the gene and the residual expression of α-Gal A compared with the effect of a *GAL* mutation alone, and increases the patient's predisposition to have a transient ischemic attack (TIA) (54). While *GAL* mutations cause FD, SNPs increase the risk of developing organ damage during the disease course, but it is difficult to assess the impact of SNPs because of similarities between the effects of mutations and polymorphism. Therefore, SNPs should be considered in association with *GAL* mutations. Neurologic manifestations can occur in patients with FD (55) and are characterized by stabbing pain and burning paresthesias in the extremities that are triggered by temperature changes and are often severe. Unfortunately, standard neurophysiologic procedures are inadequate to accurately assess the peripheral and autonomic nervous systems of FD patients with these symptoms. Alternative methods to determine the extent of neurologic dysfunction have been developed and include assessment of impaired temperature perception, vibratory perception, sudomotor and sweat gland function, blood flow, and vasoreactivity of the limbs and superficial skin using thermal provocation tests, the quantitative sudomotor axon reflex test, and venous occlusion plethysmography (56).

Peripheral neuropathic manifestations present later in females than in males (39, 57), but the burden of neurologic disability increases with age in both sexes (58). Targeted neurologic tests at 20–30 years of age, before Gb3 accumulation in renal or cardiac cells, could allow for earlier initiation of therapy, with the hope of preventing the disease from worsening. The experts also suggested that assessing anhidrosis may be specifically useful, because this sign of autonomic function changes after starting therapy (i.e., patients with FD start sweating after starting treatment) (59), and can be easily performed in all clinics. There are scales to measure autonomous dysfunction, such as the Scales for Outcomes in Parkinson's disease (SCOPA) score (60), but such specific assessment scales are generally used only at second-level centers. These targeted neurologic tests should also be performed in patients with certain types of VUS that have been reported to be linked to peripheral neuropathic pathology (61) or in patients with VUS and peripheral neurologic symptoms.

Assessment of α-Gal A activity in females with suspected FD is not useful for diagnostic purposes, but may help to assess disease progression when used in conjunction with plasma lyso-Gb3 levels (62, 63). It is important to remember that, while increased plasma lyso-Gb3 levels are suggestive of FD, normal levels cannot exclude the disease, especially in females with VUS (21). In asymptomatic females with VUS and no family history of FD, histologic evaluation of the kidney or lyso-Gb3 levels may aid in treatment initiation decision making (64). In female patients with classic FD, Gb3 accumulation could also occur in renal and cardiac cells in childhood (65, 66).

The attending physician should actively investigate all signs and symptoms, but unfortunately, in clinical practice, investigation of medical history tends to vary depending on the specialty of the physician. It is, however, difficult for any specialist to link symptoms/events in a specific organ with the occurrence of a rare disease. For example, GI symptoms in FD are nonspecific and may be misdiagnosed as irritable bowel syndrome or colitis. There is still uncertainty about the best way to monitor female patients with FD (17), and there is no evidence that one approach is better than another. There is also no evidence that monitoring progression in females using the same tests and controls as are used in male patients is useful/necessary or whether it may be preferable to use assessments based on sex and disease phenotype.

A further challenge in monitoring asymptomatic female patients with FD is that they are often lost to follow-up due to lack of clinical manifestations. However, a long period without clinical manifestations does not rule out significant organ damage (e.g., fibrosis) or high risk of acute symptoms (e.g., stroke).

3.3 Treatment initiation and therapeutic efficacy in females

3.3.1 Survey findings

All survey centers used the following parameters for treatment initiation: plasma lyso-Gb3 levels in asymptomatic patients; signs and symptoms of damage to the heart, kidney, CNS, PNS, or GI tract; family screening; and *GLA* mutation genotype/pathogenicity. Among the 227 patients of the physicians surveyed, 54% were receiving therapy; of these patients, 87% were using ERT and 13% were treated with chaperone therapy (Figure 4).

3.3.2 Analysis

Treatment decisions should be undertaken by a multidisciplinary team. The experts agreed that all clinical criteria for treatment initiation should be taken into account because, when considered individually, the only relevant criterion is the finding of signs and symptoms of FD, while evidence from family screening, Gb3/lyso-Gb3 accumulation or the presence of *GLA* mutation alone is not sufficient to initiate treatment. The genotype is related to the pathogenicity of the *GLA* mutation, for example, if there is a mutation in the stop-codon, Gb3 and lyso-Gb3 levels will increase and their accumulation will occur, which as per recommendations is sufficient to initiate treatment (17).

There is evidence that ERT is underused in females, even in symptomatic cases. According to the 2008 Fabry Registry Annual Report, at least 63% of females with FD did not receive ERT (16).

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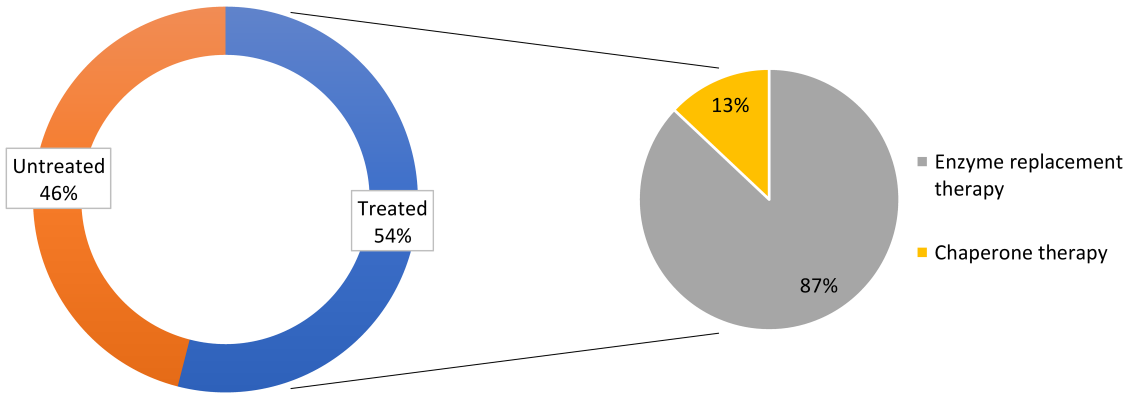

FIGURE 4
Treatment status of females with Fabry disease in the survey of Italian centers.

In a multicenter clinical survey in Germany including 224 female patients with FD, approximately 34% of female patients did not receive ERT, despite fulfilling the criteria for ERT initiation (67). Among those who did receive ERT, 42% had two or three different manifestations (i.e., renal, cardiac, neurologic, or GI) and all of them had missense or nonsense *GLA* mutations (67). The Spanish Fabry women study reported that 57% of female patients did not receive treatment despite major organ involvement (15).

The experts agreed that a considerable proportion of female patients with stable disease do not receive treatment. However, they were unable to explain the reason why 46% of female patients in this survey were not receiving treatment, despite clear signs of disease burden, as this contrasted with available literature showing that therapy is effective in females (7). The low rate of treatment in females with FD reflects the fact there is often a delay in starting treatment either because they are asymptomatic and believed to be at low risk of progression or because patients themselves refuse the proposed therapy, especially if they are asymptomatic or paucisymptomatic. However, several reports have documented a high risk of events in untreated females (68) or better cardiac and renal outcomes in females after the beginning of treatment than in the pre-treatment period (69).

The effects of therapy depend on the disease stage at treatment initiation (70); if treatment is initiated at a later stage when irreversible organ damage has already occurred (e.g., the patient has renal failure and is already on dialysis, or the patient has advanced hypertrophic cardiomyopathy), therapy may not be able to stabilize disease progression in the associated organ systems. The experts recommended that treatment in females should be started based on the age of the patient, *GLA* mutations, pathogenicity, and signs and symptoms of FD. It was previously recommended that treatment in females should only be initiated if there are significant symptoms or there is evidence of progression of organ damage, such as chronic peripheral neuropathic pain resistant to conventional therapies, persistent proteinuria (300 mg/24 h), eGFR <80 ml/min/1.73 m^2, clinically significant cardiac involvement, history of brain stroke, or TIA or ischemic changes on brain MRI (71). However, survival data showed a lower life expectancy in untreated females with FD compared with the general female population (2), leading to a change in treatment approach.

According to the revised recommendations, ERT can also be initiated in asymptomatic females on the basis of laboratory parameters (e.g., eGFR <90 ml/min/1.73 m^2; albuminuria >30 mg/dl), histologic findings (e.g., renal Gb3 inclusions), imaging (e.g., silent strokes or cerebral white matter lesions on brain MRI; cardiac fibrosis on contrast cardiac MRI), or molecular analysis (e.g., skewed XCI pattern with predominant expression of the mutant *GLA* allele) (17). In the authors' opinion, increasing levels of lyso-Gb3 should be considered when deciding whether to initiate treatment in asymptomatic females. Moreover, in patients with late-onset FD, ERT should be considered if there is evidence of injury to the kidney, heart, or the CNS (as detailed above), even if there are no typical FD symptoms. Such abnormalities should be attributable to FD and require histologic assessment or biochemical evidence of Gb3 accumulation (17).

There are several data that support the efficacy of ERT (agalsidase-β and agalsidase-α) and chaperone therapy (migalastat) in female patients (69, 72–74). Agalsidase-β was particularly effective in stabilizing left ventricular posterior wall thickness, interventricular septal thickness, and eGFR decline in females (69). Moreover, females who were treated with agalsidase-β for up to 2 years experienced significant improvements in various aspects of health-related quality of life, including body pain, vitality, physical function, general health perception, mental health, and social function (73). Similarly, the long-term effectiveness of agalsidase-α has been demonstrated in females with FD, with reductions in the severity of disease (as measured by the Mainz Severity Score Index) and left ventricular mass (LVM), an improvement in New York Heart Association heart failure classification, and stabilization of kidney function over 4 years of treatment (72). In a phase 3 study of migalastat vs. ERT, in which 56% of the study cohort were female, after 18 months, the treatments had similar effects on renal function, renal/cardiac/cerebrovascular events and patient-reported

outcomes (including assessment of pain), but migalastat induced a greater decrease of LVM (74).

There are limited data comparing ERT tolerability between males and females, but females may tolerate ERT better because they are less likely to develop anti-drug antibodies than male patients (75).

4 Expert Opinion

4.1 Recommendations for current practice

Diagnosis, monitoring, and management of FD in females has been an area of concern for many years (76). The recommendations from this expert group are summarized in Table 1.

TABLE 1 Expert panel recommendations on the diagnosis, monitoring, and initiation of treatment for female patients with Fabry disease.

Diagnosis and screening
• Screen all at-risk female relatives (identified by pedigree creation) of an index case to minimize the number of patients going undiagnosed
• Genetic analysis is the only reliable method for FD diagnosis in female patients
• Do not use α-Gal A activity and plasma lyso-Gb3 levels for diagnosis of FD in females as these parameters have low diagnostic sensitivity in females
• XCI analysis can be a useful adjunct to guide monitoring and treatment decisions
• Consider all organ systems that may be affected (e.g., heart, kidney, PNS, CNS and GI tract) and evaluate the global clinical picture
• In older patients with late-onset diagnosis, evaluate whether clinical manifestations are related to FD or aging
• Organ biopsy (renal or cardiac), and/or cutaneous biopsy, should be considered in selected asymptomatic patients and in those with *GLA* VUS, to assess histological organ involvement
• Biopsy can be avoided for FD diagnosis in patients with classical FD because the results are predictable, but they can be useful during follow up to assess disease progression despite treatment
• Cutaneous biopsy may be advisable in patients with suspected FD and those with VUS
Monitoring
The monitoring schedule for asymptomatic females must balance the need to detect organ damage with the need to maintain adherence (asymptomatic females are often lost to follow-up)
• Assess renal function annually using daily proteinuria and eGFR
• Begin peripheral neurologic testing every 2 or 5 years at 20–30 years of age to detect early changes
• One-off assessment of anhidrosis is an easy-to-measure parameter of autonomic dysfunction
• Annual CNS monitoring using MRI and cognitive function tests is useful
• Begin annual echocardiographic monitoring from 35 years of age because signs of damage appear after age 45 years
• The role of α-Gal A activity and plasma lyso-Gb3 levels on disease progression has not been confirmed
Treatment initiation
• The decision on when to initiate therapy in females should be based primarily on specific signs and symptoms of FD (see Diagnosis and screening)
• Treatment can also be initiated in asymptomatic patients taking into account their age, results of family screening, *GLA* mutations, Gb3/lyso-Gb3 accumulation, and histologic or other evidence of organ damage (in the absence of overt symptoms)

α-Gal A, α-galactosidase A; CNS, central nervous system; eGFR, estimated glomerular filtration rate; FD, Fabry disease; Gb3, globotriaosylsphingosine; *GLA*, alpha-galactosidase A gene; GI, gastrointestinal; lyso-Gb3, lyso-globotriaosylsphingosine; MRI, magnetic resonance imaging; PNS, peripheral nervous system; VUS, variant of unknown significance; XCI, X chromosome inactivation.

Regarding the diagnosis of females with FD, the experts noted that FD indeed causes less obvious symptoms in females, but also that the clinical manifestations of FD in females are less explored. It is important to ensure that all at-risk female relatives of an index case are screened for FD, to minimize the number of cases remaining undiagnosed. At-risk females may be identified by building a genealogical tree of mutations (i.e., pedigree creation). As FD is a multisystemic disease, to accurately diagnose FD, it is important to consider all of the organ systems that may be affected (e.g., heart, kidney, nervous system, and GI tract), rather than limiting the evaluation to one particular organ. It is also important to remember that symptoms in the different systems, particularly in the nervous system, may be blurred, and that diagnosis can only be achieved by carefully examining the global clinical picture. In the case of a late-onset diagnosis, it is critical to evaluate whether the clinical manifestations are related to FD or aging.

Although genetic analysis is the only reliable method for FD diagnosis in females, many laboratories outside of Italy still rely on the less sensitive measurements of α-Gal A activity and plasma lyso-Gb3 levels. The experts strongly suggest that laboratories should discontinue the use of these less sensitive methods for FD diagnosis. Although no epigenetic evaluation was reported in this survey, the XCI profile (when assessed) may guide treatment decisions in female patients with FD (17).

The experts suggested that it is important to pay attention to the earliest signs of organ damage to avoid disease progression. Females tend to ignore their symptoms, while at the same time their symptoms are often misunderstood or not given the attention they deserve by clinicians. Because organ damage is common, even in the absence of symptoms, a biopsy may be extremely useful for diagnostic and therapeutic decision making and monitoring purposes, even outside the research setting. Thus, the experts recommend that organ biopsy (either renal or cardiac) and/or cutaneous biopsy (a less invasive diagnostic tool) should be considered in female patients, in particular in those who are young, asymptomatic, and with *GLA* VUS. Biopsy may be avoided, however, in patients with classical FD as the outcome is predictable.

A follow-up approach based on age should be implemented, considering the increased risk of certain manifestations at different times of life. For example, echocardiographic parameters are normal until the ages of 35–44 years in female patients with N215S *GLA* mutation, but signs of damage appear after the age of 45 years (77). It is important to take care when monitoring young patients, as they may tire of repeated negative outcomes and may not be as willing to participate in ongoing follow-up.

The experts also suggest that it may be helpful to consider neurologic/psychologic symptoms (e.g., depression), which tend to be neglected in females, or neurosensitive symptoms, which are often misinterpreted and lead patients to consult different specialists and may sometimes result in an incorrect diagnosis. Females with neurosensitive symptoms or pain without cause are often diagnosed as having fibromyalgia. In addition, more FD centers should adopt brain MRI for CNS involvement.

The experts agreed that the Italian approach to treatment initiation has been conservative, but they believe that while symptomatic females

should be immediately started on therapy, asymptomatic females should be carefully monitored, and treatment initiated at the first signal of change. However, in the absence of symptoms, plasma Gb3/lyso-Gb3 levels, presence of missense or nonsense *GLA* mutation, and family history should be considered when initiating treatment, especially in younger patients. In female patients with VUS, the decision to start treatment may be considered only if there is evidence of pathogenicity.

4.2 Recommendations for future research

Despite recent progress, many unanswered questions remain about the assessment and management of female patients with FD. It will be important to specifically design studies to identify new sensitive and reliable biomarkers for the diagnosis and monitoring of FD in female patients. Knowledge gaps exist in the following areas: the diagnostic role and impact of XCI; the role of XCI in the decision to start therapy early; the use of biomarkers for diagnosis; the mechanisms underlying organ damage in FD; understanding the pathogenesis of Gb3/lyso-Gb3 accumulation and basic cellular pathology; identification of prognostic markers to establish the most appropriate time for treatment initiation, especially in asymptomatic or paucisymptomatic patients; the natural course of the disease in females with classical and non-classical FD; and the effect of therapy on non-classical female cases.

XCI evaluation depends on the following: extensive assessment of DNA methylation patterns of CpG islands of X chromosomes; the choice of tissue, as the mosaicism is tissue-dependent and may result in different test outcomes; and the use of uniform methodologies so that studies can be compared. However, in patients with large *GLA* mutation variabilities, including VUS, it would be difficult to find correlations between XCI and phenotype because of upstream bias.

XCI may affect the phenotype but the severity of the *GLA* mutation prevails over XCI; therefore, it is essential to study patients with homogeneous *GLA* mutations to identify a reliable biomarker for genotype-phenotype correlation. Table 2 lists recommendations for the steps needed to identify a predictive marker.

The experts noted that it would be interesting to study organ-specific lyso-Gb3 isoforms as this may explain the variability of disease phenotype within the same family carrying the same mutation. Currently, some data may be available in animal models, but not in humans or organ biopsy samples.

The data on untreated females must be reviewed to understand whether the failure to receive treatment depends on the actual absence of symptoms, patients' resistance to treatment, clinicians' therapeutic inertia, or underestimation of symptoms, or on a difference in attitude towards the treatment of male and female patients with FD. There is also a need to dissipate the perception that heterozygous female carriers have less severe disease and are not eligible for treatment unless they are symptomatic. ERT should be proposed with equal conviction in male and female patients and should also be based on molecular testing/genetic diagnosis and not only on phenotype.

TABLE 2 Expert panel recommendations for the assessment of predictive epigenetic markers.

• Consider testing for predictive epigenetic markers in large families
• Consider genes close to *GLA*, as their interaction can define the target organ and also influences disease expression in the family setting
• After establishing the haplotypic structure of individual genes within the family, study each epiallele using integrated whole-exome and mRNA sequencing
• Verify the marker in four reference tissues that can be easily collected, such as blood, saliva, urinary exosomes, and skin biopsy, and in all identified families to assess the tissue-specific variability
• Statistically validate the marker

GLA, alpha-galactosidase A gene.

Additional therapeutic agents are being studied, including substrate reduction therapies such as lucerastat and venglustat. Gene therapy has also been assessed in both animal models and early-phase clinical trials, with initial results indicating favorable tolerability and efficacy. Given the activity in this area, it is likely that more therapeutic options will become available in the near future, which should provide clinicians with the ability to offer patients a more individualized approach to the management of FD (78).

There is a need to have more data on FD treatment in females to address uncertainties about disease evolution in treated vs. untreated females. Such knowledge may help to maximize adherence to treatment in female patients, especially if they are uncertain of the benefit.

It is important to aim for personalized therapy in females. To do so, a genealogical tree of GLA mutations should be created to trace patterns of disease transmission and predict the possible clinical implications for the patient, as this information influences the choice of treatment. Notably, the phenotype-genotype correlation is not constant within a family. Decision-making algorithms based on the patient's genetic profile and polymorphisms will allow clinicians to decide who to treat, irrespective of symptoms or organ damage.

Lastly, key messages about clinical management of female patients with FD need to be widely and effectively disseminated, with more conventions, investigator meetings, and educational meetings on FD in females to increase awareness among clinicians.

5 Conclusions

Many challenges and unmet needs exist regarding the management of FD in females, with evidence indicating that females are at a significant disadvantage compared to males with FD in terms of timeliness of diagnosis and initiation of treatment. More research into FD in females is needed and greater physician awareness is required to improve outcomes in this underserved patient population.

Author contributions

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Investigation, Writing – original draft, Writing – review & editing. VC: Conceptualization, Writing – original draft, Writing – review & editing. MG: Conceptualization, Investigation, Writing – original draft, Writing – review & editing. CL: Conceptualization, Investigation, Writing – original draft, Writing – review & editing. AL: Conceptualization, Investigation, Writing – original draft, Writing – review & editing. GL: Conceptualization, Investigation, Writing – original draft, Writing – review & editing. RM: Conceptualization, Investigation, Writing – original draft, Writing – review & editing. IO: Conceptualization, Investigation, Writing – original draft, Writing – review & editing. FP: Conceptualization, Investigation, Writing – original draft, Writing – review & editing. AP: Conceptualization, Investigation, Writing – original draft, Writing – review & editing.

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Conflict of interest

AT received remuneration for participation in advisory boards on Fabry disease from Sanofi. CC received remuneration for participation in advisory boards on Fabry disease from Sanofi and Takeda. VC received remuneration for participation in advisory boards on Fabry disease from Sanofi Genzyme, Amicus, and Takeda. MG received remuneration for participation in advisory boards on Fabry disease and presentations in Fabry disease symposia from, and was sponsored for participating in Fabry disease meetings by, Sanofi Genzyme, Amicus, and Takeda; acted as a consultant for Becton Dickinson in clinical studies; was a member of a steering committee for Vifor in clinical studies; and received sponsorship for meeting participation and fees for presentations in symposia from Amicus, AstraZeneca, Baxter, Medtronic, Sanofi, and Vifor. CL received remuneration for participation in advisory boards on Fabry disease from Sanofi. ALR received remuneration for participation in advisory boards on Fabry disease from Sanofi. GL received remuneration for participation in advisory boards on Fabry disease from Sanofi. RM received sponsorship for meeting participation, fees for presentations in symposia, and remuneration for participation in advisory boards on Fabry disease from Sanofi Genzyme, Amicus, Chiesi, and Takeda. IO received remuneration for participation in advisory boards on Fabry disease from Sanofi, Shire Takeda, Amicus, and Chiesi; and received research support from BMS, Cytokinetics, Amicus, Genzyme, Shire Takeda, Menarini International, Chiesi, and Boston Scientific. FP received sponsorship for meeting participation for presentations in symposia and fees as a consultant in advisory boards on Fabry disease from Sanofi, Amicus, Chiesi, and Takeda. AP received sponsorship for meeting participation, fees for presentations in symposia, and remuneration for participation in advisory boards on Fabry disease from Sanofi Genzyme, Amicus, Chiesi, and Takeda.

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Supplementary material

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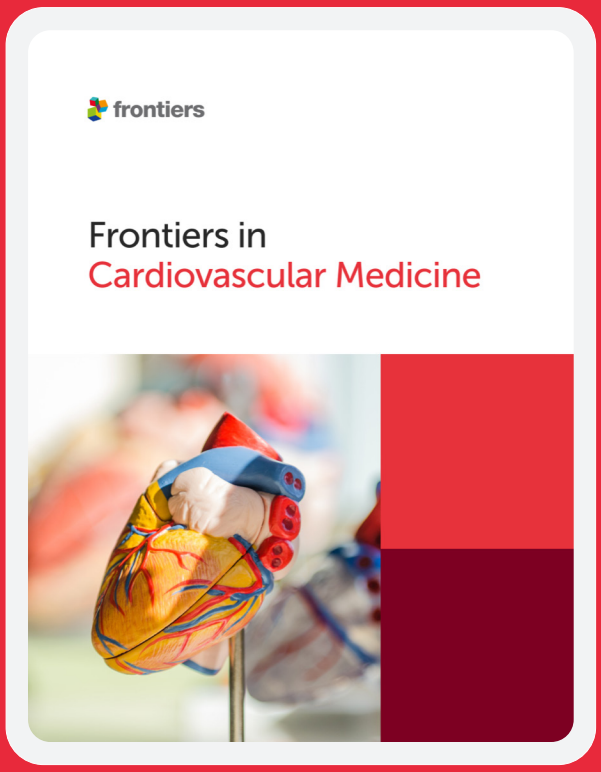

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