Cardiac involvement in Fabry disease: mechanisms beyond storage and forthcoming therapies

Maurizio Pieroni MD, PhD[a], James C. Moon MD[b], Eloisa Arbustini MD[c], Roberto Barriales-Villa MD, PhD[d], Antonia Camporeale MD, PhD,[e] Andreja Cokan Vujkovac, MD,[f] Perry Elliott MBBS, MD,[b] Albert Hagege MD, PhD, FESC, FACC,[g] Johanna Kuusisto MD, PhD,[h] Aleš Linhart MD, PhD,[i] Peter Nordbeck MD[j], Iacopo Olivotto MD,[k] Päivi Pietilä-Effati MD,[l] and Mehdi Namdar MD, PhD[m]

a. Dipartimento Cardiovascolare, Ospedale San Donato, Arezzo, Italy
b. Barts Heart Centre, University College London, London, UK
c. Centre For Inherited Cardiovascular Diseases, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy
d. Unidad de Cardiopatías Familiares, INIBIC, Complejo Hospitalario Universitario de A Coruña, CIBERCV, A Coruña, Spain
e. Multimodality Cardiac Imaging Section, IRCCS Policlinico San Donato, San Donato Milanese, Milan, Italy
f. Department of Internal Medicine, General Hospital Slovenj Gradec, Slovenj Gradec, Slovenia
g. Assistance Publique – Hôpitaux de Paris, Cardiology Department, Hôpital Européen Georges Pompidou, Paris, France
h. Centre for Medicine and Clinical Research, Kuopio University Hospital and University of Eastern Finland, Kuopio, Finland
i. 2nd Department of Internal Cardiovascular Medicine, First Faculty of Medicine, Charles University and General University Hospital, Prague, Czech Republic
j. University Hospital of Würzburg, Würzburg, Germany
k. Cardiomyopathy Unit, Careggi University Hospital, Florence, Italy
l. Cardiac Unit, Vaasa Central Hospital, Vaasa, Finland
m. Hôpitaux Universitaires de Genève, Genève, Switzerland

Brief title Fabry cardiomyopathy

Total word count: 4997/5000 words (text from introduction to the conclusion inclusive of references and figure/table legends but excluding abstract, condensed abstract and highlights)

Funding: Medical writing support funded by Sanofi Genzyme

Disclosures

MP: received advisory board honoraria from Amicus Therapeutics and Sanofi Genzyme; has received speaker honoraria from Amicus Therapeutics, Sanofi Genzyme, and Shire
JCM: received advisory board honoraria and speaker honoraria from Sanofi Genzyme and Shire, and an investigator-led research grant from Sanofi Genzyme
EA: received travel support from Sanofi Genzyme, Shire, and Amicus Therapeutics
RBV: received an unrestricted educational grant Sanofi Genzyme; Board/speaker's fees Amicus Therapeutics, Sanofi Genzyme and Pfizer
AC: received honoraria for presentations and board meetings from Amicus Therapeutics, Sanofi Genzyme and Shire. Research grant from Amicus Therapeutics
ACV: received speaker honoraria and travel support from Sanofi Genzyme and Takeda
PE: received an unrestricted educational grant Pfizer; Board/speaker's fees Myokardia, Cytokinetics, Sanofi Genzyme, Shire, Alnylam, Pfizer
AH: received support from Amicus, Gilead, Myokardia, Novartis, Sanofi Genzyme
J**K:** advisory board attendance Sanofi Genzyme; speaker honoraria and travel support from Sanofi Genzyme, Shire and Amicus
AL: consultant for Amicus Therapeutics, Sanofi Genzyme, and Takeda; has received speaker honoraria and travel support from Sanofi Genzyme and Takeda
PN: received honoraria for lecturing and advisory board participation from Amicus Therapeutics, Genzyme/Sanofi, Greenovation, Idorsia and Shire/Takeda
IO: received grants from Myokardia, Sanofi Genzyme, Shire, Bayer, Amicus, Menarini International. Board/speaker's fees from Myokardia, Cytokinetics, Sanofi Genzyme, Shire
PPE: advisory board attendance and speaker honoraria, and travel support from Sanofi Genzyme; consultancy for Shire
MN: received research support, advisory board attendance and speaker honoraria, and travel support from Sanofi Genzyme; has received research support from Shire HGT

Corresponding author's contact information: Maurizio Pieroni
Maurizio Pieroni

Cardiovascular Department, San Donato Hospital
Via Pietro Nenni 22, 52100 Arezzo Italy
Telephone: +393473732927
Fax: +390575255520
E-mail: mauriziopieroni@yahoo.com

Twitter handle: @mauripieroni72
Tweet: Cardiac involvement in Fabry disease: from pathophysiology to new therapies.

Acknowledgments:
The authors would like to thank Ugo Battaglia, PhD and Alex Goonesinghe, PhD from Team HealthCare21, Lucid, a Lucid Group agency, Macclesfield, Cheshire, SK10 2XA, UK, for providing medical writing support, which has been funded by Sanofi Genzyme.

Abstract

Fabry disease (FD) is a rare, X-linked, inherited lysosomal storage disorder caused by deficiency of α-galactosidase A enzyme activity leading to an accumulation of lysosomal globotriasylceramide (Gb3) in affected tissues, including the heart. Cardiovascular involvement usually manifests as left ventricular hypertrophy, myocardial fibrosis, heart failure, and arrhythmias, limiting quality of life, and representing the most common cause of death. Following the introduction of enzyme replacement therapy, early diagnosis and treatment have become essential to slow disease progression and prevent major cardiac complications. Recent advances in the understanding of FD pathophysiology have indicated that in addition to Gb3 accumulation, other mechanisms may contribute to myocardial damage and the development of Fabry cardiomyopathy. Progress in imaging techniques have improved the diagnostic approach to cardiac variant FD, suggesting a central role for myocardial inflammation in disease progression, and setting the stage for further research. Additionally, with the recent approval of oral chaperone therapy, and new treatment developments, including modified enzymes, substrate reduction therapy, and genetic treatments, the FD-specific treatment landscape is rapidly evolving.

Condensed abstract

Fabry cardiomyopathy is the main cause of impaired quality of life and death in patients with Fabry disease (FD), resulting from the accumulation of lysosomal globotriasylceramide in the heart. Manifesting as a mimic of hypertrophic cardiomyopathy, cardiac involvement in patients with FD progresses silently before significant clinical symptoms occur. Early diagnosis is essential to mitigate disease progression and improve treatment outcomes. Recent advances in cardiovascular imaging and disease-specific therapies have significantly improved the management of patients with FD.

Highlights:

- Fabry cardiomyopathy manifests as left ventricular hypertrophy and is often under-diagnosed
- In addition to glycosphingolipid accumulation, secondary mechanisms of cardiac damage include inflammation and immune activation

- Cardiac imaging, particularly cardiac magnetic resonance imaging, is essential for diagnosis and staging of Fabry disease
- Early treatment with currently available and forthcoming therapies is essential to improve clinical course

Key words: Fabry disease, hypertrophic cardiomyopathy, T1 mapping, lysosome function

Abbreviations list:

ADAs = Anti-drugs antibodies

CMR = cardiac magnetic resonance

ERT = enzyme replacement therapy

FD = Fabry disease

Gb3 = globotriasylceramide

HCM = hypertrophic cardiomyopathy

LGE = late gadolinium enhancement

LVH = left ventricular hypertrophy

Lyso-Gb3 = globotriaosylsphingosine

α-Gal A = α-galactosidase A

INTRODUCTION

Cardiac involvement represents the main cause of impaired quality of life and death in patients with Fabry disease (FD) (1,2) and an under-recognized cause of heart failure with preserved ejection fraction and ventricular arrhythmias in men aged >30 years and women aged >40 years (3). Cardiac damage starts early in life, progresses sub-clinically before significant symptoms occur, and usually manifests as left ventricular hypertrophy (LVH) mimicking hypertrophic cardiomyopathy (HCM) (4,5). A recent reanalysis of 5491 patients with a clinical diagnosis of LVH/HCM screened for FD reported a prevalence of *GLA* pathogenic genetic variant of 0.93% in males and 0.90% in females (5).

Following the introduction of enzyme replacement therapy (ERT), differential diagnosis of FD from other causes of LVH and early recognition have become crucial to limit disease progression (1,6). Recent advances in FD pathophysiology understanding and cardiac imaging have improved diagnostic and therapeutic approaches to FD cardiac manifestations. Additionally, FD-specific treatments are evolving, with the recent approval of oral chaperone therapy, and new treatment developments including modified enzymes, substrate reduction therapy, and gene therapy (7).

This article aims to provide a comprehensive review of current knowledge and ongoing research into the pathophysiology, diagnosis, and treatment of cardiac variant FD.

GENERAL FEATURES OF FABRY DISEASE

Fabry disease is a pan-ethnic rare, X-linked, inherited lysosomal storage disorder caused by pathogenic variants in the *GLA* gene, resulting in reduced α-galactosidase A (α-Gal A) enzyme activity (1). This leads to an accumulation of lysosomal globotriasylceramide (Gb3) and related globotriaosylsphingosine (lyso-Gb3) in affected tissues, including the heart, kidneys, vasculature, and peripheral nervous system (2). The reported incidence between 1:40,000 and 1:117,000 may be underestimated, as screening in newborns suggests a prevalence of up to 1:8800 newborns (8). Over 1000 *GLA* variants have been identified (1,10) and are categorized as pathogenic, benign without clinical relevance, or of unclear significance (9).

Nonsense, missense variants and stop-codons leading to absent or very low α-Gal A enzyme activity are usually associated with “classic” early-onset FD, characterized in males by

childhood onset of symptoms, multi-organ involvement and rapid disease progression, with clinical manifestations often affecting the heart, kidney, and central nervous system (1-3). Extra-cardiac clinical manifestations of FD are summarized according to decade of presentation in **Table 1**. Missense genetic variants allowing for residual α-Gal A activity, cause late-onset FD, which predominantly affects the heart (cardiac variant). Genetic variants associated with the cardiac variant include p.N215S (prevalent in North America and Europe), p.F113L (prevalent in Portugal), and IVS4+919G>A (prevalent in Taiwan) (10-12). In female patients, X-chromosome random inactivation (lyonization) results in mosaicism, with some cells expressing the normal allele and others the mutated one (13). This causes heterogeneous manifestations, from an asymptomatic or mild phenotype manifesting later in life and affecting one or more organs to a severe phenotype resembling classic FD.
In males with classic FD, confirmation of severely reduced or absent α-Gal A activity is often sufficient for a diagnosis. Male patients with late-onset FD have higher residual α-Gal A activity compared with classic FD, although far below normal values. In heterozygous females, α-Gal A activity may be normal or slightly deficient, and diagnosis requires genotype confirmation. Consequently, all FD diagnoses should be confirmed by genetic testing (both enzymatic and genetic testing are easily performed on dry-blood spot cards). Following diagnostic confirmation, cascade family genetic screening according to X-linked inheritance is highly recommended.

PATHOPHYSIOLOGY OF CARDIAC INVOLVEMENT IN FABRY DISEASE

Accumulation of Gb3 affects all cardiac cell types and tissues including myocytes, endothelial and smooth muscle cells of intramyocardial vessels, endocardium, valvular fibroblasts, and conduction tissue (14). Myocardial accumulation leads to progressive LVH and diastolic dysfunction. Involvement of intramural vessels induces structural and functional changes causing myocardial ischemia (15). Fibrosis and involvement of conduction tissue underlie development of ventricular arrhythmias and conduction disturbances (**Figure 1A**). Increasing evidence demonstrates that Gb3 accumulation does not explain the whole spectrum of FD pathophysiology (16). Alongside mechanical effects, Gb3 accumulation causes biochemical and functional impairment in myocytes. *In vitro* studies show that intra-lysosomal Gb3 impairs endocytosis and autophagy, triggers apoptosis, and interferes with mitochondrial energy production (**Figure 1B)** (17). Energy depletion and trophic factors, like

sphingosine, may activate cellular hypertrophy pathways common to other HCMs. Studies on cardiomyocytes isolated from endomyocardial biopsies demonstrated that intracellular glycosphingolipids elicit sarcomeric myofilament dysfunction and myofibrillolysis (18). Similarly, Birket *et al.* demonstrated enhanced sodium and calcium channel function, resulting in higher and shorter spontaneous action potentials, in FD cardiomyocytes derived from induced pluripotent stem cells (19). These findings suggest that stored glycosphingolipids may alter ion channel expression and/or cell membrane trafficking, altering the electrical properties of cardiomyocytes. Indeed, Namdar *et al.* proposed increased conduction velocity in atrial and ventricular myocardium as possible causes of arrhythmias and electrocardiography (ECG) abnormalities in FD, including short PR interval without evidence of an accessory pathway (20).

The model of FD as a simple storage cardiomyopathy has been challenged further by cardiac magnetic resonance imaging (CMR) studies with T1-T2 mapping allowing to assess myocardial lipid content and inflammation at different stages of cardiac FD. Based on their findings, Nordin *et al.* suggested a central role for inflammation in early disease progression (21,22) (**Central illustration**). Clinical and experimental evidence also support the role of inflammation in FD and other lysosomal storage disorders (16, 23-26). Deficiency of α-Gal A limits degradation thus favoring accumulation of lipidic antigens while Gb3 and lyso-Gb3 may also act as antigens themselves activating invariant natural killer T cells, leading to chronic inflammation and potential autoimmunity. (23-25) (**Figure 1B**). Glycosphingolipids-mediated effects are abolished by anti-toll-like receptor-4 antibodies, suggesting a pivotal role of this inflammatory pathway (23,24), also promoting a TGF-β response involved in extracellular matrix remodeling and fibrosis (25). Defective autophagy promotes inflammation through inflammasome activators and release of reactive-oxidative species (23). Yogasundaram *et al*. recently reported elevated inflammatory and cardiac remodeling biomarkers correlating with disease progression in patients with FD (26), while chronic inflammatory activation has been observed in endomyocardial biopsies from FD patients (27). Knott *et al*. have recently linked myocardial inflammation with microvascular dysfunction and perfusion abnormalities in early cardiac involvement (15).

DIAGNOSIS AND STAGING OF CARDIAC INVOLVEMENT

New insights into disease pathophysiology and availability of long-term ERT data have modified the cardiological approach to FD. While early diagnosis remains essential to obtain most benefit from disease-specific therapies (1-3), it is clear that accurate staging of cardiac involvement with imaging and biomarkers has important clinical implications.

In FD registries, LVH is reported in 53% of men and ≥33% of women after the third decade of life, with 60% of patients presenting with symptoms including heart failure with preserved ejection fractions, chest pain, and arrhythmias (1-3). Therefore, FD should be suspected in adult patients with such symptoms of unclear origin. According to a stepwise approach previously proposed for the diagnostic work-up of cardiomyopathies (28), recognition of extra-cardiac red flags should raise the index of suspicion of classic FD (**Table 1**, **Figures 2-3**). In patients with cardiac variant FD, differential diagnosis from other HCMs is more challenging in the absence of systemic manifestations, considering that all patterns of LVH have been reported (**Figure 4**). Cardiological red flags, although with variable sensitivity and specificity (29), may rule out FD in the diagnosis of patients with suspected LVH/HCM (**Table 1**, **Figures 2-3**). Subtle ECG changes, including short PR interval and repolarization abnormalities, precede LVH and may be observed from childhood (20,30). Progressive cardiomyopathy is associated with high voltages, marked strain pattern, and T-wave inversion in the precordial leads. ST-T segment depression and T-wave inversion in the inferolateral leads may develop, reflecting posterolateral fibrosis (**Figures 4 and 5**).

Echocardiography is important for initial diagnosis and monitoring of FD-related cardiomyopathy; typical findings include concentric LVH, disproportionate hypertrophy of papillary muscles, loss of base-to-apex circumferential strain gradient, and right ventricular hypertrophy with normal systolic function, but none of them are pathognomonic (4). In carriers of pathogenic variants, global longitudinal strain and speckle tracking allow early detection of cardiac involvement independently of LVH (4).

CMR has become central to the early differential diagnosis and staging of cardiac FD (**Figure 3**). Typical features include late gadolinium enhancement (LGE), initially in the basal inferolateral wall, and low native T1, likely reflecting glycosphingolipid myocardial storage and occurring before the development of significant LVH. Being FD predominantly an intracellular storage disease, at variance with cardiac amyloidosis, extracellular volume is typically normal except for LGE-positive areas (15,30,33,34).

Application of multi-parametric CMR has provided valuable insights into myocardial biology of FD at different stages and on patients' responses to specific therapies. In a prospective observational study including 182 FD patients, Nordin *et al.* proposed a three-phase model of cardiac FD progression: 1) accumulation, starting in childhood and characterized by progressive lowering of T1 with no LVH or LGE; 2) inflammation/hypertrophy, with low T1, initial LVH (mostly in males), and T2 mapping evidence of inflammation in the basal inferolateral segment associated with LGE (sometimes preceding LVH particularly in females and Taiwanese patients with IVS4 variant); 3) fibrosis/impairment, with increasing T1 values (pseudo-normalization) and LGE with wall thinning in the basal inferolateral segment (21). Increase of myocardial hypertrophy versus storage component, increased interstitial and replacement fibrosis, and myocardial inflammation are all possible mechanisms of progressive T1 "pseudo-normalization" in advanced phases. Other groups reported low native T1 is detectable in up to 59% LVH-negative patients and associated with clinical worsening at 12-month follow-up, while reduction of myocardial blood flow seems to precede T1 lowering in a very initial stage (30,31).

Concerning T2 mapping, Augusto *et al.* showed that when LGE is present, there are significant associations between increased T2 values in the LGE segments, increased troponin/NT-proBNP, ECG changes, and global longitudinal strain impairment. In these patients both LGE-related and global T2 elevation were higher than in other myocardial disorders, like sarcomeric HCM. Persistent T2 and troponin elevation over one year suggested chronic myocardial edema and injury, with associated clinical deterioration (32). If validated by histology or other methods, these findings could demonstrate a pivotal role for inflammation in FD pathogenesis, with potential therapeutic implications.

In association with clinical assessment and imaging, biomarkers like troponin and NT-proBNP are important for cardiac disease staging (**Central illustration**). Preliminary findings also suggest a correlation between inflammation, cardiac-remodeling biomarkers and disease progression (26). Lyso-Gb3 levels are increased since childhood and their assessment may help in evaluating the pathogenicity of *GLA* variants of uncertain significance (33) while its role in disease monitoring is still debated. Endomyocardial biopsy with electron microscopy may be considered for diagnosis of FD in patients with variants of unknown significance and low lyso-Gb3 levels (**Figure 3**).

CLINICAL MANAGEMENT OF CARDIAC FABRY DISEASE

The main goal of FD treatment is to prevent disease progression and irreversible organ damage. Optimal FD management requires a multidisciplinary clinical approach (3). The pharmacological treatment of FD includes disease-specific therapies, and therapies to manage cardiovascular symptoms and prevent major cardiovascular events.

FD-specific therapies

Approved FD-specific treatments include ERTs and the pharmacological chaperone migalastat while new therapeutic approaches are in development (7) (**Table 2** and **Figure 6**).

ERT

ERT is administered intravenously bi-weekly and is indicated in symptomatic patients with an established FD diagnosis. ERT has profoundly changed the natural history of FD and improved patients' quality of life through effective treatment of neuropathic pain, gastrointestinal manifestations, heath and exercise intolerance (1-3). Long-term follow-up studies and registry data show that ERT may delay cardiac disease progression and reduce cardiovascular event rate (1-3,6). Evidence suggests that LVH may be prevented by early treatment (**Figure 7**) and regression of mild LVH has been reported in patients with both classic and cardiac phenotypes, although evidence for late-onset cardiac FD variants is limited. In advanced cardiac FD, response to ERT is poor (**Central illustration**) (1-3,6) with no data suggesting any effect on myocardial fibrosis, and LVH progression.
Several factors influence cardiac response to ERT, including phenotype, gender, timing and dosage of ERT, and anti-drug antibody (ADAs) development against exogenous α-Gal A (1-3,34).

Chaperone therapy

Chaperone molecules are orally administered iminosugars that binding to the catalytic domain of α-Gal A promote its proper folding and transportation to the lysosome. The same molecules at higher doses may act as inhibitors of α-Gal A. The chaperone molecule Migalastat is approved for administration every other day in adult patients with amenable *GLA* variants, defined by the presence of residual α-Gal A activity of at least 3% of normal, and an increase in activity by at least 20% in the presence of 20 μM migalastat in patients' cultured lymphocytes.

Clinical trials and open-label extension studies showed that treatment with Migalastat is associated with a significant decrease in left ventricular mass index (LVMI) (35). However, recent real-world data showed a significant discrepancy between predicted *in vitro* amenability and the effective increase in α-Gal A activity and clinical response in some genetic variants (36). This may be related to intrinsic limitations of the *in vitro* amenability test, and possible dosage-dependent inhibitory effects of Migalastat. These data suggest that biochemical and clinical response to chaperone therapy must be carefully monitored to confirm efficacy.

Management of cardiac complications and monitoring

Further to FD-specific therapies, cardiovascular therapies are necessary to manage cardiovascular manifestations of FD. Updated expert recommendations have been provided in a recent consensus document (3). Clinical monitoring is essential to assess disease progression and optimize treatment. Disease progression may be variable between organs, particularly in patients receiving ERT, due to specific secondary pathways of damage and variable response to therapy in different tissues. Accordingly, a multi-parametric clinical scoring system has been validated (37). The role of lyso-Gb3 in monitoring disease evolution and treatment efficacy is still debated, although new biomarkers including microRNAs and lyso-Gb3 isoforms are under investigation. The use of new CMR techniques in FD monitoring is promising. A recent study showed that in ERT-naïve patients, 1 year of ERT attenuated T1 lowering, with small reductions in maximum wall thickness and stabilized LVMI. In patients with advanced disease and established ERT, however, CMR showed a 1-year increase of T2 in LGE area and worsening global longitudinal strain (22).

New therapies in development

Therapeutic strategies currently in development include second-generation ERTs, substrate reduction therapies, gene and mRNA therapies (8) (**Table 2** and **Figure 6**).

Plant-derived ERTs have been developed to reduce ADA development and improve enzyme biodistribution. Pegunigalsidase alpha is a novel pegylated form of α-Gal A produced in a PlantCell Ex system with a longer circulatory half-life and increased heart and kidney uptake compared to current ERTs (38).

Substrate reduction therapy (SRT) is based on oral administration of iminosugars that inhibit glycosphingolipid synthesis directly, thereby lowering the cellular load of Gb3. These drugs, previously validated in Gaucher disease, may be administered irrespective of FD genotype. Two SRTs, venglustat and lucerastat, are currently in phase II and III clinical trials respectively (39,40).

In a recent phase II trial adopting an *ex vivo* approach, hematopoietic stem cells from a FD patient, transfected with lentiviruses (AVR-RD-01, Avrobio), and re-administered, provided persistent elevation in α-Gal A activity (7).

Pre-clinical *in vivo* approaches using liver-targeted, adenoviral-mediated gene transfer in a-GAL A knockout mouse model demonstrated a dramatic increase of α-Gal A activity and marked lyso-Gb3 reduction (41). However, it remains unclear whether enzyme release by transfected cells will result in adequate uptake by affected tissues. In heterozygous females, cross-correction does not seem sufficient to prevent Gb3 accumulation and disease development. It is also unclear whether males with classic FD and null α-Gal A activity could develop ADAs against the expressed enzyme, although continuous exposure and endogenous synthesis and glycosylation could result in tolerance in most treated patients. Novel cardiotropic vectors, specifically targeting myocardial tissue with increased delivery and reduced immunogenicity (compared with conventional adenoviral vectors), are currently undergoing testing in non-human primates. Finally, gene delivery systems continue to be developed. Encapsulation of human α-Gal mRNA within lipid nanoparticles increased α-Gal levels in liver, heart, and kidney in mice and non-human primates (42).

CURRENT CHALLENGES AND AVENUES FOR FUTURE RESEARCH

Although ERT has significantly changed the natural history of FD, cardiac involvement remains a key prognostic determinant and knowledge gaps prevent optimal management and warrant further research.

Cardiac manifestations benefit from early ERT, but clinical effects are limited in more advanced cases. Several mechanisms that potentially reduce ERT efficacy in myocardial tissue have been proposed. Histologic studies demonstrate that clearance of Gb3 deposits, while significant in endothelial cells, appears limited in cardiomyocytes (43). Indeed, clearance of endothelial cells is facilitated by their higher turnover compared to terminally

differentiated cells such as myocytes and renal podocytes. Additionally, myocardial concentrations of exogenous enzyme can be significantly lower than those reaching endothelial cells. The relevance of ERT dose has been also debated, with evidence suggesting that higher doses provide more effective clearance of podocytes in serial kidney biopsies in children with FD (44). Development of ADAs may also reduce enzyme uptake in target tissues. Lenders *et al.* showed that neutralizing ADAs impaired ERT efficacy, particularly in males with classic FD, suggesting a need for routine ADA titer assessment and dose adjustments to achieve supersaturation and overcome neutralizing activity (36). Other strategies to minimize the detrimental impact of ADAs are being investigated, including immunosuppressive therapy and tolerance induction (36).

Exogenous enzyme instability at tissue level has also been hypothesized, highlighting potential benefits of ERT and chaperone co-administration. A phase II study demonstrated a 1.2- to 5.1-fold increase of enzyme activity in target tissues following ERT/migalastat co-administration compared with ERT alone (45). With the advent of new treatments, different therapeutic combinations may provide opportunities to target different stages of the lysosomal lipid storage pathway, although the increased cost of treatment per patient with the use of two disease-specific therapies would represent a potential limitation of clinical applicability.

Considering the limited accessibility to myocardial tissue from living patients, the use of cardiomyocytes derived from isolated pluripotent stem cells offers an opportunity to assess early changes in FD cardiomyocytes at a genome- and proteome-wide level. Additional studies should also clarify whether pathogenic pathways may become storage-independent, thus representing alternative therapeutic strategies. Recent studies have shown that pentosan polysulfate, a mixture of semisynthetic sulfated polyanions, demonstrated anti-inflammatory activity in mucopolysaccharidosis type II patients, and reduced pro-inflammatory cytokine secretion in cultured peripheral blood mononuclear cells from patients with FD or Gaucher disease (46).

A deeper understanding of mechanisms of cardiac damage in FD may also provide insights for other cardiomyopathies and other non-cardiac conditions. The central role of defective lysosomal/endosomal transport has revealed links between Gaucher and Parkinson disease (18). Additionally, the lysosomal protein NPC1, defects in which result in Niemann Pick disease, is also involved in the Ebola virus infection-replication cycle.

CONCLUSIONS

Recent advances in our understanding of the complexity of cardiac FD have significantly improved diagnostic and therapeutic approaches, particularly with respect to identifying storage-independent mechanisms and detecting early cardiac involvement. A deeper understanding of secondary pathogenic pathways, particularly myocardial, may influence future therapeutic strategies.

Although new disease-specific therapies look promising, diagnostic delay and timely initiation of current treatments remain critical concerns for many patients with FD, particularly those with late-onset cardiac variant disease, who often fail to benefit from disease-specific treatment. Therefore, collaboration between FD specialists and cardiologists remains essential to identify patients before the onset of cardiac involvement, to enable them to gain maximum benefit from current and future therapeutic approaches.

REFERENCES

1. Ortiz A, Germain DP, Desnick RJ, et al. Fabry disease revisited: Management and treatment recommendations for adult patients. Mol Genet Metab 2018;123:416-427.
2. Ortiz A, Abiose A, Bichet DG, et al. Time to treatment benefit for adult patients with Fabry disease receiving agalsidase β: data from the Fabry Registry. J Med Genet 2016;53:495-502.
3. Linhart A, Germain DP, Olivotto I et al. An expert consensus document on the management of cardiovascular manifestations of Fabry disease. Eur J Heart Fail. 2020;22:1076-1096.
4. Perry R, Shah R, Saiedi M, et al. The role of cardiac imaging in the diagnosis and management of Anderson-Fabry disease. JACC Cardiovasc Imaging. 2019;12:1230-1242.
5. Doheny D, Srinivasan R, Pagant S, et al. Fabry disease: prevalence of affected males and heterozygotes with pathogenic GLA mutations identified by screening renal, cardiac and stroke clinics, 1995-2017. J Med Genet 2018;55:261-268.
6. Germain DP, Elliott PM, Falissard B, et al. The effect of enzyme replacement therapy on clinical outcomes in male patients with Fabry disease: A systematic literature review by a European panel of experts. Mol Genet Metab Rep 2019;19:100454.
7. van der Veen SJ, Hollak CEM, van Kuilenburg ABP, Langeveld M. Developments in the treatment of Fabry disease. J Inherit Metab Dis 2020.
8. Burlina AB, Polo G, Salviati L, et al. Newborn screening for lysosomal storage disorders by tandem mass spectrometry in North East Italy. J Inherit Metab Dis. 2018;41:209-219.
9. The Fabry disease mutation database, Available at: http://fabry-database.org/mutants/. Accessed date: 19 September 2020.
10. Germain DP, Brand E, Burlina A, et al. Phenotypic characteristics of the p.Asn215Ser (p.N215S) GLA mutation in male and female patients with Fabry disease: A multicenter Fabry Registry study. Mol Genet Genomic Med 2018;6:492-503.
11. Azevedo O, Gal A, Faria R, et al. Founder effect of Fabry disease due to p.F113L mutation: Clinical profile of a late-onset phenotype. Mol Genet Metab. 2020;129:150-160.
12. Hsu TR, Hung SC, Chang FP, et al. Later onset Fabry disease, cardiac damage progress in silence: experience with a highly prevalent mutation. J Am Coll Cardiol. 2016;68:2554-2563.

13. Echevarria L, Benistan K, Toussaint A, et al. X-chromosome inactivation in female patients with Fabry disease. Clin Genet 2016;89:44-54.
14. Nair V, Belanger EC, Veinot JP. Lysosomal storage disorders affecting the heart: a review. Cardiovasc Pathol 2019;39:12-24.
15. Knott KD, Augusto JB, Nordin, S et al. Quantitative myocardial perfusion in Fabry disease. Circ Cardiovasc Imaging 2019;12:e008872.
16. Platt FM, d'Azzo A, Davidson BL, Neufeld EF, Tifft CJ. Lysosomal storage diseases. Nat Rev Dis Primers. 2018;4:27.doi:10.1038/s41572-018-0025-4.
17. Ivanova M. Altered sphingolipids metabolism damaged mitochondrial functions: lessons learned from Gaucher and Fabry diseases. J Clin Med. 2020;9:1116.
18. Chimenti C, Hamdani N, Boontje NM et al. Myofilament degradation and dysfunction of human cardiomyocytes in Fabry disease. Am J Pathol. 2008;172:1482-1490.
19. Birket MJ, Raibaud S, Lettieri M, et al. A human stem cell model of Fabry disease implicates LIMP-2 accumulation in cardiomyocyte pathology. Stem Cell Reports. 2019;13:380-393.
20. Namdar M. Electrocardiographic changes and arrhythmia in Fabry disease. Front Cardiovasc Med 2016;3:7-7.
21. Nordin S, Kozor R, Medina-Menacho K, et al. Proposed stages of myocardial phenotype development in Fabry disease. JACC Cardiovasc Imaging 2019;12:1673-1683.
22. Nordin S, Kozor R, Vijapurapu R, et al. Myocardial storage, inflammation, and cardiac phenotype in Fabry disease after one year of enzyme replacement therapy. Circ Cardiovasc Imaging. 2019;12:e009430.doi:10.1161/CIRCIMAGING.119.009430.
23. Rozenfeld P, Feriozzi S. Contribution of inflammatory pathways to Fabry disease pathogenesis. Mol Genet Metab 2017;122:19-27.
24. Mauhin W, Lidove O, Masat E, et al. Innate and adaptive immune response in Fabry disease. JIMD Rep 2015;22:1-10.
25. Sanchez-Niño MD, Carpio D, Sanz AB, et al. Lyso-Gb3 activates Notch1 in human podocytes. Hum Mol Genet 2015;24:5720-5732.
26. Yogasundaram H, Nikhanj A, Putko BN, et al. Elevated inflammatory plasma Biomarkers in patients with Fabry disease: a critical link to heart failure with preserved ejection fraction. J Am Heart Assoc 2018;7:e009098.
27. Frustaci A, Verardo R, Grande C, et al. Immune-mediated myocarditis in Fabry disease cardiomyopathy. J Am Heart Assoc 2018;7:e009052.

28. Rapezzi C, Arbustini E, Caforio AL, et al. Diagnostic work-up in cardiomyopathies: bridging the gap between clinical phenotypes and final diagnosis. A position statement from the ESC Working Group on Myocardial and Pericardial Diseases. Eur Heart J. 2013;34:1448-1458.
29. Limongelli G, Monda E, Tramonte S et al. Prevalence and clinical significance of red flags in patients with hypertrophic cardiomyopathy. Int J Cardiol. 2020;299:186-191.
30. Augusto JB, Johner N, Shah D, et al. The myocardial phenotype of Fabry disease pre-hypertrophy and pre-detectable storage. Eur Heart J Cardiovasc Imaging 2020.doi:https://doi.org/10.1093/ehjci/jeaa101.
31. Camporeale A, Pieroni M, Pieruzzi F, et al. Predictors of clinical evolution in prehypertrophic Fabry disease. Circ Cardiovasc Imaging 2019;12:e008424.
32. Augusto JB, Nordin S, Vijapurapu R, et al. Myocardial edema, myocyte injury, and disease severity in Fabry disease. Circ Cardiovasc Imaging 2020;13:e010171.
33. Spada M, Kasper D, Pagliardini V, Biamino E, Giachero S, Porta F. Metabolic progression to clinical phenotype in classic Fabry disease. Ital J Pediatr. 2017;43:1.
34. Lenders M, Neußer LP, Rudnicki M, et al. Dose-dependent effect of enzyme replacement therapy on neutralizing antidrug antibody titers and clinical outcome in patients with Fabry disease. J Am Soc Nephrol. 2018;29:2879-2889.
35. Germain DP, Hughes DA, Nicholls K, et al. Treatment of Fabry's disease with the pharmacologic chaperone Migalastat. N Engl J Med 2016;375:545-555.
36. Lenders M, Nordbeck P, Kurschat C, et al. Treatment of Fabry's disease with migalastat: outcome from a prospective observational multicenter study (FAMOUS). Clin Pharmacol Ther. 2020;108:326-337.
37. Mignani R, Pieroni M, Pisani A, et al. New insights from the application of the FAbry STabilization indEX in a large population of Fabry cases. Clin Kidney J. 2018;12:65-70.
38. Schiffmann R, Goker-Alpan O, Holida M, et al. Pegunigalsidase alfa, a novel PEGylated enzyme replacement therapy for Fabry disease, provides sustained plasma concentrations and favorable pharmacodynamics: A 1-year Phase 1/2 clinical trial. J Inherit Metab Dis. 2019;42:534-544.
39. Peterschmitt MJ, Crawford NPS, Gaemers SJM, et al. Pharmacokinetics, pharmacodynamics, safety, and tolerability of Oral venglustat in healthy volunteers. Clin Pharmacol Drug Dev. 2020. doi: 10.1002/cpdd.865
40. Welford RWD, Mühlemann A, Garzotti M, et al. Glucosylceramide synthase inhibition with lucerastat lowers globotriaosylceramide and lysosome staining in cultured

fibroblasts from Fabry patients with different mutation types. Hum Mol Genet. 2018;27:3392-3403.

41. Yasuda M, Huston MW, Pagant S, et al. AAV2/6 gene therapy in a murine model of Fabry disease results in supraphysiological enzyme activity and effective substrate reduction. Mol Ther Methods Clin Dev. 2020;18:607-619.
42. DeRosa F, Smith L, Shen Y, et al. Improved efficacy in a Fabry disease model using a systemic mRNA liver depot system as compared to enzyme replacement therapy. Mol Ther 2019;27:878–889.
43. Thurberg BL, Fallon JT, Mitchell R, Aretz T, Gordon RE, O'Callaghan MW. Cardiac microvascular pathology in Fabry disease: evaluation of endomyocardial biopsies before and after enzyme replacement therapy. Circulation 2009;119:2561-2567.
44. Skrunes R, Tøndel C, Leh S, et al. Long-term dose-Dependent agalsidase effects on Kidney histology in Fabry disease. Clin J Am Soc Nephrol. 2017;12:1470-1479.
45. Warnock DG, Bichet DG, Holida M, et al. Oral migalastat HCl leads to greater systemic exposure and tissue levels of active α-galactosidase A in Fabry patients when co-administered with infused agalsidase. PLoS One. 2015;10:e0134341.
46. Crivaro AN, Mucci JM, Bondar CM, et al. Efficacy of pentosan polysulfate in in vitro models of lysosomal storage disorders: Fabry and Gaucher disease. PLoS One. 2019;14:e0217780.doi:10.1371/journal.pone.0217780.

Figure legends

Central illustration. Proposed evolution of cardiac involvement in FD

Proposed stages of FD cardiac involvement evolution along with clinical progression, imaging, biomarkers, main means to diagnosis, and in relation to expected treatment efficacy.

ECG = electrocardiography; MBF = Myocardial blood flow; GLS = Global longitudinal strain; LGE = late gadolinium enhancement; lyso-Gb3 = globotriaosylsphingosine; NT-proBNP = NT-pro-brain natriuretic peptide.

Figure 1. Pathophysiology of FD

(A) Classic pathophysiology of FD as a myocardial storage disease and (B) recently reported secondary pathways operating in FD.

Gb3 = globotriaosylceramide; TLR4 = toll-like receptor-4; iNKT = invariant natural killer T; other abbreviations as in Central illustration.

Figure 2. Red flags in FD

Cardiac and systemic red-flags with increasing likelihood of FD diagnosis from outer/brighter to inner/darker circles.

GLS = Global longitudinal strain; HFPEF= heart failure with preserved ejection fraction; LVH = left ventricular hypertrophy; RVH = right ventricular hypertrophy; eGFR = estimated glomerular filtration rate; other abbreviations as in Central illustration and Figure 1.

* T wave ratio = (Tonset–Tpeak)/(Tpeak–Tend) ratio.

Figure 3. Proposed flow chart for diagnosis of FD in patients with idiopathic LVH

Suggested red flag diagnostic approach in classic and cardiac FD. Systematic screening of LVH patients represents an alternative approach.

*Low native T1 values reinforce or generate suspicion of FD. Normal native T1 values do not exclude FD, being rarely observed in untreated patients with mild left ventricular hypertrophy (mostly females), or in advanced disease due to “pseudo-normalization”. With normal native T1 values, genetic analysis remains indicated if other findings suggest FD.

** By lyso-Gb3 levels assessment and endomyocardial biopsy

Abbreviations as in Central illustration and Figures 1 and 2.

Figure 4. Representative case of p.N215S cardiac variant with apical left ventricular hypertrophy

A 54-year-old female referred for chest pain with no systemic red flags suggesting FD.

Electrocardiography (A) showed giant negative T-waves. 2D-echocardiography showed apical HCM (B) with reduced systolic and diastolic velocities at tissue-Doppler (C). Cardiac magnetic resonance confirmed apical HCM (D-E) with low myocardial T1 values (857±20 ms, normal reference value 984±18 ms) suggesting FD. Genetic analysis detected N215S mutation causing cardiac variant of FD.

HCM = hypertrophic cardiomyopathy.

Figure 5. Representative case of a 59-year-old female with classic FD (c.124-125delAT) showing progression of myocardial damage and inflammation

Top: baseline; bottom: changes at 2-year follow-up.

(A) and (E) electrocardiographic progression (particularly deeper, more extensive T-wave inversion); (B) and (F) new basal inferolateral LGE with progression of fibrosis (red arrow); (C) and (G) low T1 (875±22 ms, normal reference value 984±18 ms); (D) and (H) T2 mapping – with new increase in T2 signal (edema) in area of LGE (black arrow).

Abbreviations as in Central illustration and Figure 2.

Figure 6. Schematic representation of currently approved and investigational drugs for FD

Schematic representation of mode of action for approved and investigational therapies for FD.

α-Gal A = α-galactosidase A; ERT = enzyme replacement therapy; other abbreviations as in Central illustration and Figure 1.

Figure 7. Long-term effect of early enzyme replacement therapy

A 42-year-old male with classic FD (c.946delG) after 19 years of ERT.
(A) Angiokeratomas in bathing-trunk region; (B) normal ECG with sinus bradycardia; (C) echocardiography showing mild reduction of longitudinal strain in postero-inferior basal segment; (D-E) CMR with no evidence of LVH nor LGE (D) but with low myocardial T1 values (820 ms, normal reference value 959±20ms) (E).

Table 1. Fabry disease red flags for differential diagnosis of patients with idiopathic left ventricular hypertrophy/hypertrophic cardiomyopathy

	Extra-cardiac red flags		Cardiac red flags		
Presenting decades of age	Any time	Family history of renal failure and/or stroke	Family history of LVH, particularly if no evidence of male to male transmission	History	Diagnostic tool
	1–2	Neuropathic pain	Short PQ interval **	Electrocardiography	
	1–2	Gastrointestinal symptoms	Bradycardia		
	1–2	Angiokeratomas	Chronotropic incompetence		
	1–2	Cornea verticillata*	Atrioventricular blocks **		
	1–2	Hypohidrosis, heat/cold and exercise intolerance	Left ventricular hypertrophy with normal systolic function	2D-echo	
	1–2	Proteinuria	Reduced global longitudinal strain		
	3–4	Juvenile and/or cryptogenic TIA/stroke	Mild-to-moderate aortic root dilation		
	3–4	Hearing loss (either progressive or sudden)	Mitral and aortic valve thickening with mild-to-moderate regurgitation		
	3–4	Renal failure	Hypertrophy of papillary muscles	Cardiac magnetic resonance	
	3–4	Dolichoectasia of the basilar artery, chronic white matter hyperintensities at brain MRI	Mid-layer posterolateral late gadolinium enhancement		
	3–4	Lymphedema	Low T1		

*In the absence of iatrogenic causes (chloroquine/amiodarone); **Short PQ interval in early stages; atrioventricular and bundle branch blocks more common in advanced disease.

LVH = left ventricular hypertrophy; MRI = magnetic resonance imaging; TIA = transient ischemic attack; 2D-echo = 2-dimensional echocardiography.

Table 2. Currently approved and under-development therapies for Fabry disease

	Drug name	Mechanism of action	Route of administration	Dose	Notes
Approved	Agalsidase alfa	ERT	Intravenous	0.2 mg/kg/every other week	• Agalsidase alfa is the human protein α-galactosidase A produced in a human cell line by genetic engineering technology[1] • Agalsidase beta is a recombinant form of human α-galactosidase A and is produced by recombinant DNA technology using a mammalian Chinese Hamster Ovary cell culture. The amino acid sequence of the recombinant form, as well as the nucleotide sequence which encoded it, are identical to the natural form of α-galactosidase A[2] • In patients with late-onset Fabry disease, ERT should be considered and is appropriate in the presence of laboratory, histological, or imaging evidence of injury to the heart, kidney, or central nervous system, even in the absence of typical Fabry symptoms[3] • In the absence of demonstrable Fabry disease-related tissue pathology or clinical symptoms, ERT may not be appropriate, particularly in heterozygous female
	Agalsidase beta	ERT	Intravenous	1.0 mg/kg/every other week	

					patients; however, these patients should be monitored regularly by a multidisciplinary care team • ERT is not recommended in those patients with well-characterized benign alpha-galactosidase variants[3]
	Migalastat	Pharmacological chaperone	Oral	123 mg/every other day	• Indicated only for adult patients with migalastat-amenable alpha-galactosidase variants, i.e., a *GLA* variant translating into α-Gal A proteins that may be stabilized by migalastat, thereby restoring their trafficking to lysosomes and their intralysosomal activity • No food 2 hours prior to and after intake[4] • Not recommended in those patients with well-characterized benign alpha-galactosidase benign variants[3]

Under development (Phase III trials)*	Pegunigalsidase-alfa	ERT	Intravenous	1 mg/kg/every other week	• Produced in tobacco cells and chemically modified with polyethylene glycol • Three ongoing Phase III clinical trials
	Moss-aGal	ERT	Intravenous	– (being tested as 0.2 mg/kg to measure pharmacokinetics and safety)	• Produced in moss • Phase I trial completed. Plans for Phase II and III studies in progress
	Venglustat	SRT	Oral	15 mg/once daily	• Ongoing long-term, Phase II trial • Plans for Phase III trials in progress
	Lucerastat	SRT	Oral	1.0 g/ twice daily (dose adjusted for renal function)	• Ongoing Phase III trial for patients with Fabry disease with neuropathic pain

*Information from therapies under development taken from ClinicalTrials.gov. 1. Shires Pharmaceuticals Limited. Agalsidase alfa. Summary of product characteristics. 2. Sanofi Genzyme. Agalsidase beta. Summary of product characteristics; 3. Ortiz A, Germain DP, Desnick RJ, et al. Fabry disease revisited: Management and treatment recommendations for adult patients. Mol Genet Metab. 2018;123:416-27; 4. Amicus Therapeutics UK Ltd. Migalastat hydrochloride. Summary of product characteristics.

ERT = enzyme replacement therapy; SRT = substrate reduction therapy.