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ORIGINAL RESEARCH

# Long-Term Clinical-Pathologic Results of Enzyme Replacement Therapy in Prehypertrophic Fabry Disease Cardiomyopathy

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**BACKGROUND:** The limited ability of enzyme replacement therapy (ERT) in removing globotriaosylceramide from cardiomyocytes is recognized for advanced Fabry disease cardiomyopathy (FDCM). Prehypertrophic FDCM is believed to be cured or stabilized by ERT. However, no pathologic confirmation is available. We report here on the long-term clinical–pathologic impact of ERT on prehypertrophic FDCM.

**METHODS AND RESULTS:** Fifteen patients with Fabry disease with left ventricular maximal wall thickness ≤10.5 mm at cardiac magnetic resonance required endomyocardial biopsy because of angina and ventricular arrhythmias. Endomyocardial biopsy showed coronary small-vessel disease in the angina cohort, and vacuoles in smooth muscle cells and cardiomyocytes ≈20% of the cell surface containing myelin bodies at electron microscopy. Patients received α-agalsidase in 8 cases, and β-agalsidase in 7 cases. Both groups experienced symptom improvement except 1 patients treated with α-agalsidase and 1 treated with β-agalsidase. After ERT administration ranging from 4 to 20 years, all patients had control cardiac magnetic resonance and left ventricular endomyocardial biopsy because of persistence of symptoms or patient inquiry on disease resolution. In 13 asymptomatic patients with FDCM, left ventricular maximal wall thickness and left ventricular mass, cardiomyocyte diameter, vacuole surface/cell surface ratio, and vessels remained unchanged or minimally increased (left ventricular mass increased by <2%) even after 20 years of observation, and storage material was still present at electron microscopy. In 2 symptomatic patients, FDCM progressed, with larger and more engulfed by globotriaosylceramide myocytes being associated with myocardial virus-negative lymphocytic inflammation.

**CONCLUSIONS:** ERT stabilizes storage deposits and myocyte dimensions in 87% of patients with prehypertrophic FDCM. Globotriaosylceramide is never completely removed even after long-term treatment. Immune-mediated myocardial inflammation can overlap, limiting ERT activity.

**Key Words:** enzyme replacement therapy ■ Fabry disease cardiomyopathy ■ globotrioasylceramide ■ mannose-6-phosphate receptors



Fabry disease (FD) is an X-linked inborn error of glycosphingolipid catabolism caused by pathogenic mutations in the α-galactosidase A gene encoding the lysosomal hydrolase, α-galactosidase A.[1] The marked deficiency or absence of α-galactosidase A activity results in the systemic accumulation of globotriaosylceramide and related glycosphingolipids within the lysosomes, particularly in microvascular

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## CLINICAL PERSPECTIVE

### What Is New?

- Through a long-term pathologic follow-up (ranging from 4 to 20 years) obtained by sequential left ventricular endomyocardial biopsy to investigate the impact of enzyme replacement therapy (ERT) on Fabry disease cardiomyopathy treated at the early stage of the disease (prehypertrophic phase), a general stabilization of the disease with substantially unchanged cardiomyocyte dimensions as well as extent of storage material (cell surface/vacuolar surface ratio) is documented for the first time.

### What Are the Clinical Implications?

- Persistence of glycolipid accumulation in cardiomyocytes that correlates with a marked reduction (>85%) of mannose 6 phosphate receptors which mediates ERT internalization into cardiac cells.
- This study promotes the association of ERT with inductors of receptor synthesis like growth hormone to enhance ERT efficacy.

### Nonstandard Abbreviations and Acronyms

| | |
|---|---|
| **EMB** | endomyocardial biopsy |
| **ERT** | enzyme replacement therapy |
| **FDCM** | Fabry disease cardiomyopathy |
| **M6Pr** | mannose-6-phosphate receptor |

endothelial cells, vascular smooth muscle cells, renal tubular cells, podocytes, and cardiomyocytes.[2–7] Their progressive accumulation, especially in endothelial cells, podocytes, and cardiomyocytes, leads to renal failure, cardiac and cerebrovascular disease, and premature demise.

Currently available treatments for FD include enzyme replacement therapy (ERT, α- agalsidase, and β-agalsidase since 2001,[8,9]) and chaperone therapy (galactose since 2001[10] and migalastat[11] since 2016).

The efficacy of ERT on globotriaosylceramide clearance is cell type–specific and dose-dependent.[12–16]

In particular, advanced Fabry disease cardiomyopathy (FDCM), particularly those patients with left ventricular (LV) maximal wall thickness >15 mm, appears poorly responsive to ERT.[17–19] The reasons seem related mainly to the long life span[20] of cardiomyocytes (cell turnover ≤1% per year) and the remarkable downregulation in Fabry myocytes (up to 90% versus normal myocardium) of the mannose-6-phosphate receptor (M6Pr) that mediates cell uptake and internalization of both α- and β-agalsidase.[21] Otherwise, pre-hypertrophic and early hypertrophic FDCM are believed to be cured or stabilized by ERT.[22–24] However, no pathologic confirmations are available so far.

In the present study, a case series of patients with prehypertrophic FDCM, the clinical and pathologic results of long-term treatment with α- and β-agalsidase are reported.

## METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

All patients with prehypertrophic FDCM (LV maximal wall thickness ≤10.5 mm) were included in our study. In all patients, cardiac investigations included noninvasive (ECG, Holter monitoring, 2-dimensional echocardiography, and cardiac magnetic resonance [CMR]) and invasive (coronary, LV angiography, and endomyocardial biopsy [EMB]) studies. The study complies with the Declaration of Helsinki, the locally appointed ethics committee (Opinion No. 6/2019) approved the research protocol, and informed consent was obtained from all subjects. Our institution is a tertiary referring center for the diagnosis and treatment of cardiomyopathies, myocarditis, and heart failure and has obtained permission from local ethical committee (Sapienza University, Rome, Italy; Opinion No. 2016-003014-28 [FARM12JCXN]). The patients underwent endomyocardial biopsy because of the presence of disabling symptoms like chest pain and cardiac arrhythmias in the context of normal or nearly normal cardiac parameters at echocardiogram and nondiagnostic findings at CMR. Although the patients had a genetic pattern consistent with FD, we were unable to explain the importance of symptoms with FD and preserved cardiac structure and function. Other explanation could have been responsible for symptoms like myocarditis that sometimes overlap FD.[25] The patients were strongly motivated to clarify the origin of symptoms and gave their written consent to the procedure. All patients were reevaluated with ECG, echocardiogram (atrioventricular chambers dimension, LV end-diastolic diameter and ejection fraction, valve structure and function, presence of pericardial abnormalities), 24-hour Holter, CMR and endomyocardial biopsy after a time range between 4 and 20 years of ERT administration. Control biopsy after a treatment period of 4 to 20 years was often required by patients because of worsening of symptoms or the patient's inquiry on disease resolution. Control EMB was carried out after obtaining the patient's informed consent.

## Cardiac Magnetic Resonance

CMR exams were performed on a 1.5 Tesla scanner (Avanto, Siemens). Standard CMR protocol included (1) cine-CMR sequence acquired during breath holds in the short-axis, 2-chamber, and 4-chamber; (2) black blood T2-weighted short tau inversion recovery images on short-axis planes covering the entire left ventricle during 6 to 8 consecutive breath holds for myocardial edema detection; (3) late gadolinium-enhanced imaging performed 15 minutes after injection of 0.2 mmol/kg of gadoterate meglumine and signal intensity value 2 SDs above the mean signal intensity of the remote normal myocardium, which were considered suggestive for myocardial fibrosis; (4) performance of native T1 mapping (nT1) imaging, when available, using the Modified Look-Locker inversion recovery sequence on 3 short-axis views (1 basal and 2 midventricular); and (5) T2 map obtained using a T2-prepared True-FISP prototype sequence producing 3 single-shot images with 3 different T2 pulse preparation. A nonrigid registration algorithm and the 2-parametric automatic curve fitting were automatically applied to generate the map. CMR image analysis was performed as previously described,[25] extending the analysis method for T1 maps also to T2 maps. In particular, global myocardial nT1 and T2 values were measured by semiautomatically contouring subepicardial and subendocardial layers on respective maps, carefully avoiding late gadolinium enhancement areas. A 1-cm$^2$ region of interest with 20% offset were drawn for nT1 in the midseptum for the assessment of septal values. The values of T1 and T2 global were defined as normal, reduced, or increased compared with a reference range developed on a multiage sample of 100 healthy subjects of both sexes (normal value nT1, 970–1027 milliseconds, T2<49.7 milliseconds). CMR was performed with the same protocol during the follow-up.

## Endomyocardial Biopsy

EMB in our institution is regularly performed whenever a symptomatic heart muscle disease remains undiagnosed by noninvasive procedures including echocardiography and CMR.[26,27] Cardiac catheterization with LV and coronary angiography was obtained in all patients. EMB was performed in the septal–apical region of the left ventricle. Biopsy samples, 5 to 8 per patient, were cut and stored at −80 °C. For the histologic analysis, the endomyocardial samples were fixed in 10% buffered formalin and paraffin embedded. Five-micron-thick sections were stained with hematoxylin and eosin and Masson trichrome. For electron microscopy studies, additional samples were fixed in 2% glutaraldehyde in a 0.1 M phosphate buffer at pH 7.3, postfixed in osmium tetroxide, and processed following a standard protocol for embedding in Epon resin. Ultrathin sections were stained with uranyl acetate substitute and lead hydroxide. Two frozen samples from each patient were processed for real-time polymerase chain reaction for the most common cardiotropic viruses in case of observation of overlapping myocarditis at histology. The molecular study was performed in the patients with real-time polymerase chain reaction for the most common cardiotropic viruses (adenovirus, enterovirus, influenza A and B virus, Epstein–Barr virus, parvovirus B19, hepatitis C virus, cytomegalovirus, human herpes virus 6, herpes simplex types 1 and 2) for the possible identification of viral genomes.

In the remaining frozen myocardial tissue from the control biopsies of 6 patients (3 men: patient 6, patient 10 and patient 14; and 3 women: patient 2, patient 5, and patient 7) M6Pr was evaluated by western blot analysis.[21] Results were compared with values from surgical control unloaded myocardium (papillary muscle of patients with mitral stenosis undergoing valve replacement).

The cardiomyocyte cross-sectional area was computed measuring the cardiomyocyte diameter across the nucleus in 50 to 100 cells in transverse sections. At that level, the diameter of the perinuclear vacuoles was also measured, and the percent cardiomyocyte area occupied by vacuoles was calculated. These measurements were analyzed using Nis-Elements BR software. The normal value for cardiomyocyte diameter was derived from our long-term published experience.[26]

## CMR Was Performed With the Same Protocol During Follow-Up

### *Enzyme Replacement Therapy*

After the histologic diagnosis of prehypertrophic FDCM was confirmed by endomyocardial biopsy, 8 patients received intravenous α-agalsidase at a dosage of 0.2 mg/kg and 7 β-agalsidase at a dosage of 1 mg/kg every other week. To date, there are no specific indications from current literature regarding which to choose beween α-agalsidase and β-agalsidase, particularly for the prehypertrophic form of FDCM, where the interstitium is still reasonably preserved with limited or absent myocardial fibrosis. The 2 formulations were alternatively administered to test possible different impact.

We deliberately decided to treat all patients with pathologic α-galactosidase A gene mutations and globotriaosylceramide deposition into cardiomyocytes, as previous reports[28,29] have demonstrated in such untreated subjects an average increase in LV mass index of 4.07±1.03 g/m$^2$ per year in men and 2.31±0.81 g/m$^2$ per year in women.[30,31]

**Table. Clinical, Genetic, Electrocardiographic, Echocardiographic, Cardiac Magnetic Resonance and Histological Characteristics of Patients Treated With ERT (N=15) and Patients Untreated With ERT at Baseline and Follow-Up**

| | ERT group | No ERT group | *P* value |
|---|---|---|---|
| Age, y | 36 (25.5–49.5) | 31.5 (30.25–39.75) | NS |
| Male sex, n (%) | 6 (40) | 6 (60) | NS |
| Type of ERT | | | |
| α-Agalsidase, n (%) | 8 (53.3) | … | … |
| β-Agalsidase, n (%) | 7 (46.7) | … | … |
| α-Galactosidase A gene mutations | | | |
| *c.666delC* | 4 (26.7) | 2 (20) | NS |
| *c.335G>A* | 2 (13.3) | 3 (30) | NS |
| *Deletion exons 3 and 4* | 2 (13.3) | 1 (10) | NS |
| *c.215A>G* | 2 (13.3) | … | … |
| *c.946delG* | 2 (13.3) | … | … |
| *c.668G>A* | 1 (6.7) | … | … |
| *c.548>G* | 1 (6.7) | … | … |
| *c.680A>T* | 1 (6.7) | … | … |
| *N215S* | … | 4 (40) | … |

| | ERT group (n=15) | | | No ERT group (n=10) | | |
|---|---|---|---|---|---|---|
| **Variables** | **Baseline** | **Follow-up** | ***P* value** | **Baseline** | **Follow-up** | ***P* value** |
| Cardiac manifestations | | | | | | |
| Palpitations, n (%) | 12 (80) | 6 (40) | NS | 6 (60) | 6 (60) | NS |
| Chest pain, n (%) | 4 (26.7) | 0 | NS | 4 (40) | 3 (30) | NS |
| None, n (%) | 0 | 9 (60) | 0.001* | 1 (10) | 2 (20) | NS |
| Extracardiac manifestations | | | | | | |
| Cornea verticillata, n (%) | 10 (76.9) | 10 (76.9) | NS | 3 (30) | 3 (30) | NS |
| Acroparesthesias, n (%) | 6 (46.2) | 1 (7.7) | NS | 3 (30) | 2 (20) | NS |
| Gastrointestinal disorders, n (%) | 5 (38.5) | 4 (30.8) | NS | 2 (20) | 2 (20) | NS |
| Angiokeratomas, n (%) | 5 (38.5) | 0 | 0.04* | 1 (10) | 1 (10) | NS |
| Weight loss, n (%) | 4 (30.8) | 0 | NS | … | … | … |
| Hypoanhidrosis, n (%) | 3 (23.1) | 0 | NS | … | … | … |
| Proteinuria, n (%) | 3 (23.1) | 2 (15.4) | NS | 5 (50) | 5 (50) | NS |
| Hypoacusia, n (%) | 2 (15.4) | 0 | NS | … | … | … |
| None, n (%) | … | … | … | 2 (20) | 3 (30) | NS |
| Electrocardiographic findings | | | | | | |
| Normal | 1 (6.7) | 7 (46.7) | 0.04* | 1 (10) | 1 (10) | NS |
| Atrioventricular conduction abnormalities, n (%) | 5 (33.3) | 4 (26.7) | NS | 4 (40) | 4 (40) | NS |
| Intraventricular conduction abnormalities, n (%) | 2 (13.3) | 2 (13.3) | NS | 0 | 0 | NS |
| PVC/VT, n (%) | 12 (80) | 2 (13.3) | 0.001* | 1 (10) | 1 (10) | NS |
| Echocardiographic | | | | | | |
| MWT, mm | 10 (9.5–10) | 10 (9.5–10.5) | NS | 10 (9.5–10) | 12 (11.9–12.5) | 0.001* |
| LV mass, g/m$^2$ | 45.3 (44.2–46.1) | 47.1 (46.2–49.3) | NS | 44.1 (43.6–44.9) | 63 (58.5–68.8) | 0.001* |
| LVEDD, mm | 48.1 (45.8–49.6) | 47.7 (47.2–48.1) | NS | 46.4 (43.3–48.4) | 46.4 (46.5–48.6) | NS |
| LVEF, % | 59.3 (58.7–61.3) | 58.1 (57.7–58.9) | NS | 58.5 (58.8–62.2) | 58.4 (58.2–58.4) | NS |
| Cardiac magnetic resonance findings | | | | | | |
| LV mass, g/m$^2$ | 45 (43.9–50.9) | 45.7 (43.8–50.5) | NS | … | … | … |
| LVEF, % | 63.1 (59.6–65.9) | 62.2 (58.8–66.5) | NS | … | … | … |
| Global nT1, ms | 975 (952–1003) | 990 (952.5–1006) | NS | … | … | … |

(*Continued*)

**Table. Continued**

| Variables | ERT group (n=15) | | | No ERT group (n=10) | | |
|---|---|---|---|---|---|---|
| | Baseline | Follow-up | *P* value | Baseline | Follow-up | *P* value |
| Septal nT1, ms | 971 (943–1001) | 974 (942–1002) | NS | … | … | … |
| Global T2, ms | 47 (46–49) | 47 (46–48.5) | NS | … | … | … |
| Histologic findings | | | | | | |
| Myocyte diameter, μm | 25.2±5.3 | 26.1±6.3 | NS | … | … | … |
| Vacuolar area, % | 21±0.5 | 23±0.7 | NS | … | … | … |
| Fibrosis, % | 1±0.05 | 1±0.1 | NS | … | … | … |
| Inflammation, n (%) | 3 (20) | 2 (13.3) | NS | … | … | … |

Data are presented as n (%), mean±SD, or median (interquartile range). ERT indicates enzyme replacement therapy; LV, left ventricular; LVEDD, left ventricular end diastolic diameter; LVEF, left ventricular ejection fraction; MWT, maximal wall thickness; NS, not significant; PVC, premature ventricular complex; and VT, ventricular tachycardia.

*$P<0.001$.

### *Control Group*

Patients who were recruited between 1990 and 2001, in the pre-ERT period, served as the control group. Follow-up was obtained by cardiac 2-dimensional echocardiography as CMR was not yet diffusely available. Analyzed echocardiographic parameters included atrioventricular chamber dimension, LV end-diastolic diameter and ejection fraction, valve structure and function, and presence of pericardial abnormalities. Analysis of echocardiographic data was blinded. Holter monitoring was obtained to analyze the possible presence of arrhythmias.

### *Statistical Analysis*

Descriptive statistics were used to summarize the data; results are reported as medians and interquartile ranges or means and SDs, as appropriate. Categorical variables were summarized as counts and percentages. Baseline demographic and clinical characteristics are presented in table format. Changes observed before 2 EMBs were examined by paired *t* test and McNemar test. All statistical analyses were performed using R statistical analysis software (R Foundation for Statistical Computing, Vienna, Austria).

## RESULTS

### Clinical Manifestations

Baseline, clinical, and instrumental features of patients with prehypertrophic FDCM treated with ERT are reported in Table and Figures 1 through 3. In brief, patients were members of 8 different families; 8 (53.3%) patients were treated with α-agalsidase, and 7 (46.7%) patients with β-agalsidase.

Cornea verticillata, present in 67% of patients, persisted at follow-up in all, while gastrointestinal disorders, present in 4 patients (patients 5, 6, 8, and 15) of the same family, disappeared during ERT treatment (Table; Table S1). Globally, cardiac and extracardiac symptoms improved or remained stable on ERT (Table; Table S1).

### CMR Parameters

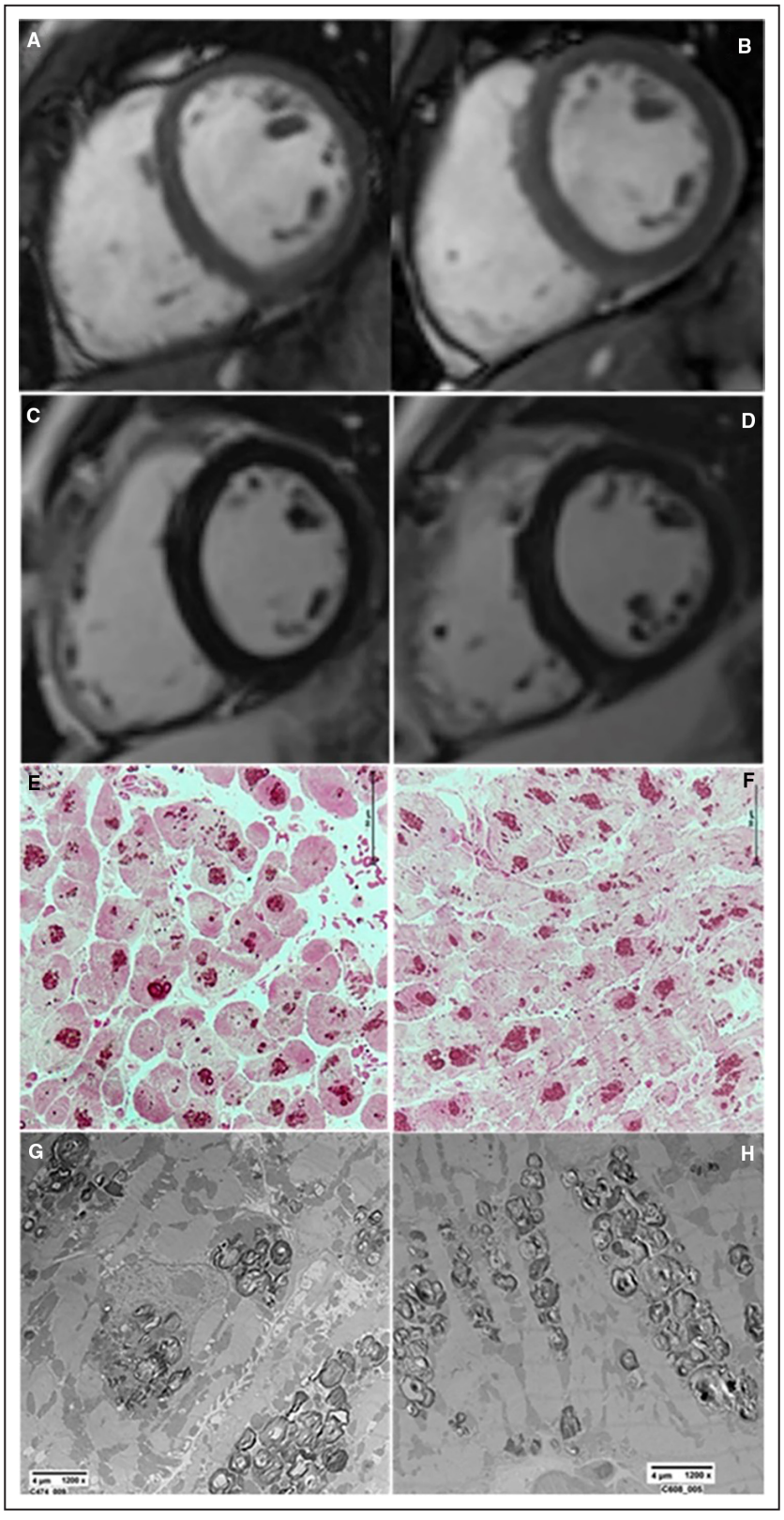
LV mass index at CMR did not significantly (<2%) increase in most patients (87%) during follow-up (Figure 4 and Table S2). The overall median rate of change (interquartile range) in LV mass index was 0.28 g/m$^2$ (0.02–0.94 g/m$^2$) per year; in 11 women, the median rate of increase was 0.50 g/m$^2$ (0.15–0.94 g/m$^2$) per year, while in the remaining 4 men the median rate of increase was 0.04 g/m$^2$ (0–0.96 g/m$^2$) per year (Table; Table S2).

In patient 7, the LV mass index increased from 47.7 to 68.5 g/m$^2$ and in patient 14 from 53.3 to 69 g/m$^2$. In these 2 patients, the increased LV mass was associated with progression of the cardiomyocytes globotriaosylceramide deposition, as documented by percent area occupied by vacuoles (from 24% to 36% in patient 7 and from 24% to 35% in patient 14).

T1 mapping imaging showed abnormal values (nT1 <970 milliseconds) in 6 patients at baseline, remaining below the normal range at follow-up (Figure 2A and 2B), and T2 mapping showed edema in patients 7 and 14 presenting overlapping myocarditis at histology. No late gadolinium enhancement was evident at baseline and follow-up CMR (Figures 1C and 1D and 2C and 2D).

### Histologic Findings

At baseline, cardiomyocyte diameter was mildly increased compared with normal values[26,27] (21.9±8.1 μm versus normal value 12.0±2.8 μm; $P<0.001$) because of the presence of intramyocyte vacuoles occupying an average of 21±5% of the cell area (Figures 1E and 2E). At electron microscopy, the vacuoles consisted of concentric lamellar figures in single membrane–bound vesicles (myelin bodies), diagnostic for FD (Figures 1G and 2G). Intramural arteries showed endothelial and




smooth muscle cell glycosphingolipid infiltration with lumen narrowing only in the 4 patients presenting with angina. Myocardial fibrosis was mild and not significantly different compared with normal controls (3.3±1.2% versus normal value 3.1%±2%) and did not increase significantly during follow-up (3.4%±1.9%) (Figure 1F). Myocardial inflammation was detected in 2 patients at baseline, persisting at follow-up despite

**Figure 1. Patient 11 (24-year-old man) with genetic and biopsy-proven diagnosis of FDCM before and after 20 years of ERT.** CineMR (**A** and **B**) and LGE (**C** and **D**) images, performed at baseline (**A** and **C**) and 20 years after ERT (**B** and **D**) show normal LV wall thickness (8 and 10 mm) and absence of fibrotic areas. Semithin section of Epon-embedded EMB samples, stained with basic fuchsin, showing mildly hypertrophied cardiomyocytes containing storage material that result unmodified after long-term ERT administration at baseline (**E**) and 20 years after ERT (**F**). Ultrastructural evidence of storage material consisting of myelin bodies at baseline (**G**) that remained unchanged after ERT (**H**). CineMR indicates cine magnetic resonance; EMB, endomyocardial biopsy; ERT, enzyme replacement therapy; FDCM, Fabry disease cardiomyopathy; and LGE, late gadolinium enhancement.

ERT (Figure 3G, Table S3). In these patients, polymerase chain reaction for the most common cardiotropic viruses was negative in both baseline and control biopsy. Importantly, myocardial vacuoles did not disappear in follow-up biopsy even after up to 20 years but remained stable in 87% of cases (Figures 1H and 2F and 2H) and increased in 2 patients (patients 7 and 14) (Figure 3H through 3K).

Protein analysis, obtained in only 6 patients (3 men and 3 women) because of tissue shortage, showed M6Pr in FDCM to be 8.74-fold lower than in a normal heart (24 046±2912 versus 2751±2912, respectively; $P<0.001$): specifically, 8.72-fold lower in men ($P<0.001$) and 8.75-fold lower in women ($P<0.001$).

## Control Group

Baseline, clinical, and instrumental features of untreated patients with prehypertrophic FDCM were reported in Table and Table S4. Palpitations were the most common cardiac symptom (60%), while proteinuria was the most common extracardiac manifestation (40%). No significant difference in term of clinical manifestations were observed during follow-up.

After a median (interquartile range) follow-up of 96 months (96–108 months), the overall mean LV mass increased by 2.91 g/m$^2$ (2.22–3.60 g/m$^2$) per year. In particular, LV mass increased by 3.26 g/m$^2$ (2.24–4.28 g/m$^2$) per year in men and 2.39 g/m$^2$ (1.68–3.10 g/m$^2$) per year in women (see Table and Table S4).

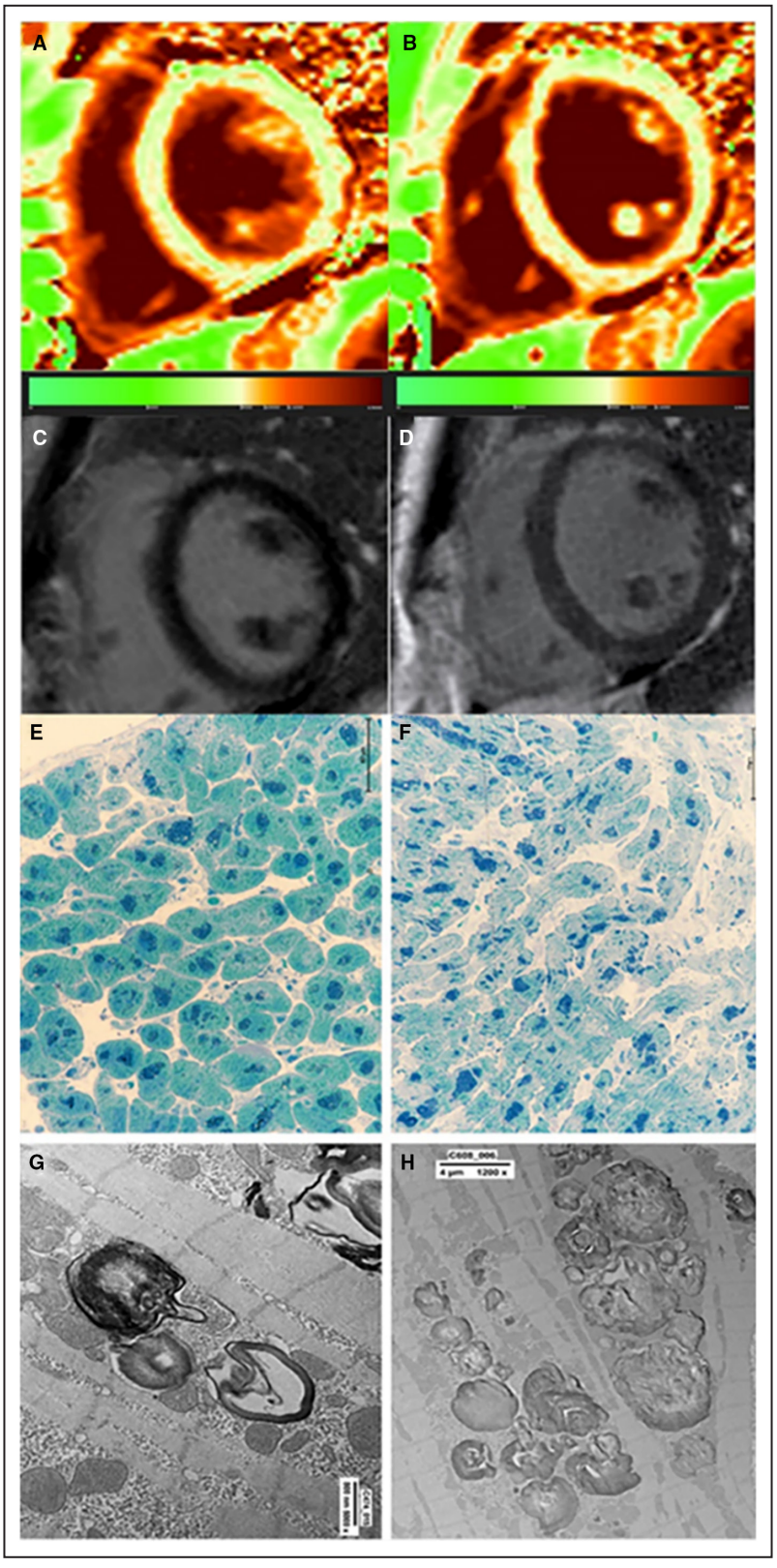
# DISCUSSION

This is the first study to explore the pathologic impact of long-term ERT administration in the prehypertrophic FDCM. Its importance relies on the clinical observation that while the advanced form of the disease (LV maximal wall thickness >15 mm) appears to be resistant to ERT, the prehypertrophic variant (LV maximal wall thickness ≤10.5 mm) suggests that it can be stabilized or even cured by the treatment. This consideration is also supported by the histologic observation of a normal interstitium in prehypertrophic FDCM and the presence of progressive myocardial fibrosis in the advanced form of the disease that represent an obstacle to ERT delivery. Nevertheless, cardiomyocytes along with podocytes are less susceptible to globotriaosylceramide clearance as postmitotic cells provided a prolonged life span and limited turnover (for cardiomyocytes estimated to be ≈1% per year). In this regard, Thurberg et al[4] reported globotriaosylceramide clearance in cardiac endothelial cells after 5 months of β-agalsidase (1 mg/kg every other week) but no clearance of cardiomyocytes, and in our experience, 2-year ERT was able to remove globotriaosylceramide from Fabry enterocytes (which are completely replaced in 48–72 hours), while myocardial storage remained unaffected.[32]

The present study suggests that in prehypertrophic FDCM, ERT is able to stabilize globotriaosylceramide deposit and cardiomyocyte dimensions in 87% of treated patients with improvement of cardiac symptoms including chest pain and palpitations. These findings are documented at a control follow-up including cardiac magnetic resonance, Holter monitoring, cardiac catheterization, coronary angiography, and LV endomyocardial biopsy ranging from 4 to 20 years. Invasive cardiac studies documented normal intracavitary pressures and coronary arteries but failed to report procedural complications. On the other hand, untreated patients in prehypertrophic FDCM, as reported by Kampamann, manifest an average increase in LV mass index of 4.07±1.03 g/m$^2$ per year in men and 2.31±0.81 g/m$^2$ per year in women. Similar disease progression has been observed in our center in untreated prehypertrophic FDCM in patients enrolled and followed in a time range between 1990 and 2001, in the pre-ERT era (see Table S1).

It may be argued that amelioration of symptoms was not supported by appreciable histologic and ultrastructural variations. Nevertheless, a previous report[33] has observed that globotriaosylceramide deposits in cardiac cells are accompanied by oxidative damage that particularly at the vascular endothelial level can be improved by ERT increasing nitric oxide availability and then justify the disappearance of angina and the reduction of electrical instability in the absence of visible structural changes.

Persistence of storage material in cardiomyocytes despite very long (median, 4 years [interquartile range, 4–5.5]) ERT administration raises the observation that metabolic derangement characterizing FDCM is not completely corrected by actual treatment even in its prehypertrophic expression. Possible explanations include a downregulation of myocardial M6Pr[21] that is the major molecular pathway involved in the myocyte




uptake and internalization of α- and β-agalsidase as well as along with Lyset protein for the lysosomal targeting of the enzyme.[34,35] Determination of M6Pr in 6 of 15 of our patients with prehypertrophic FDCM show a reduced receptor expression by >85%, confirming a major role in the process of ERT resistance.

Molecular derangement of FDCM has been reported to be associated with marked elevation of myocardial

**Figure 2. Clinical–pathologic results of 48 months ERT in a 27-year-old man (patient 6) affected by prehypertrophic FDCM.**
CMR acquired on midventricular short axis view at baseline (**A** and **C**) and after treatment (**B** and **D**). The analysis of native T1 maps (**A** and **B**) show a diffuse reduction of the myocardial T1 value and a slight increase at follow-up (nT1: 920 vs 949.5 milliseconds). Myocardial ECV and T2 values (maps not shown in the figure) were within normal range in both exams. LGE images at baseline (**C**) and after treatment (**D**) show that neither ventricular hypertrophy nor areas of fibrosis occurred over the observation interval. Semithin sections showing diffuse intracytoplasmic accumulation of dark blue deposits at baseline (**E**) that mildly reduce after treatment (**F**). Ultrastructural detection of myelin bodies at baseline (**G**) that are still visible after ERT (**H**). CMR indicates cardiac magnetic resonance; ECV, extracellular volume; FDCM, Fabry disease cardiomyopathy; and LGE, late gadolinium enhancement.

ubiquitin, suggesting a potential cytoplasmic degradation of M6Pr, being normal M6Pr mRNA.[21]

If this mechanism is definitely confirmed, addition to ERT of inhibitors of proteasomal proteolysis or inductors of protein synthesis like growth hormone and estradiol[36] could enhance M6Pr expression and improve disease outcome.

In 2 cases treated with α-agalsidase and β-agalsidase respectively, prehypertrophic FDCM progressed despite ERT. Control biopsy at 5-year follow-up documented overlapping of virus-negative myocardial inflammation. This finding has been observed in as many as 56% of patients with FDCM and its incidence correlated with the disease severity.[25] Its high frequency is linked to the immunogenic property of globotriaosylceramide and the constitutional secretory pathway that externalizes globotriaosylceramide from cardiac cells, exposing the myocardium to an adverse immune reaction. This event may be considered as an human leukocyte antigen–specific immune response to the constitutional secretion of globotriaosylceramide from cardiomyocytes. This response may concur to ERT resistance as well as to progression of the disease. It would represent a potential target for use in the most pronounced cases of immunosuppression associated with ERT. On the other hand, globotriaosylceramide secretion by myocytes explains survival up to the fifth to sixth decade of patients with untreated FDCM even in the total absence of enzyme α-galactosidase A.

Myocardial inflammation interferes with enzyme internalization in cardiac cells through expansion of interstitial space by over-imposition of inflammatory cells and the advent of edema and fibrosis.



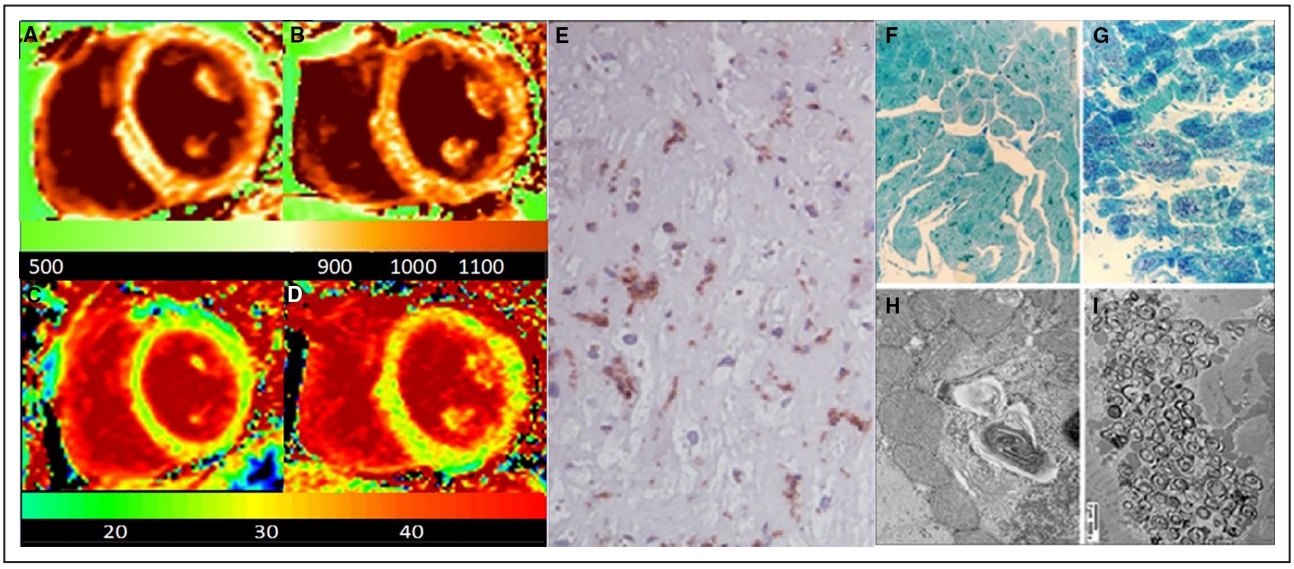



**Figure 3. CMR images (A through D) with histologic (E through G) and ultrastructural (H and I) documentation of ERT results in a 52-year-old (patient 7) woman with prehypertrophic FDCM.**
CMR images are acquired at baseline (**A** and **C**) and after 60 months ERT (**B** and **D**). Native T1 (**A** and **B**) and ECV (**C** and **D**) maps show progressive increase of myocardial mass (LV mass/BSA from 37.7 to 68.5 g/m$^2$), myocardial nT1 and ECV values (nT1 from 946 to 968 milliseconds; ECV from 30% to 34% at follow-up), reflecting occurrence of interstitial fibrosis. **E**, Section of left ventricular endomyocardial biopsy stained with immunohistochemistry for CD45Ro show hypertrophied cardiomyocytes containing large vacuoles with T lymphocytes associated with necrosis of adjacent cells, denoting over overlapping myocarditis. **F** and **G**, Diffuse intracytoplasmic accumulation of small dark blue deposits that at TEM can be identified as typical glycolipid bodies at baseline (**F**). After 5 years of ERT the amount of dark blue deposit is drastically increased in number and size (**G**). Ultrastructural details of glycolipid bodies at baseline (**H**) and after 5 years of ERT (**I**) denoting increase of storage material. BSA indicates body surface area; CMR, cardiac magnetic resonance; ERT, enzyme replacement therapy; FDCM, Fabry disease cardiomyopathy; LV, left ventricular; and TEM, transmission electronic microscope.

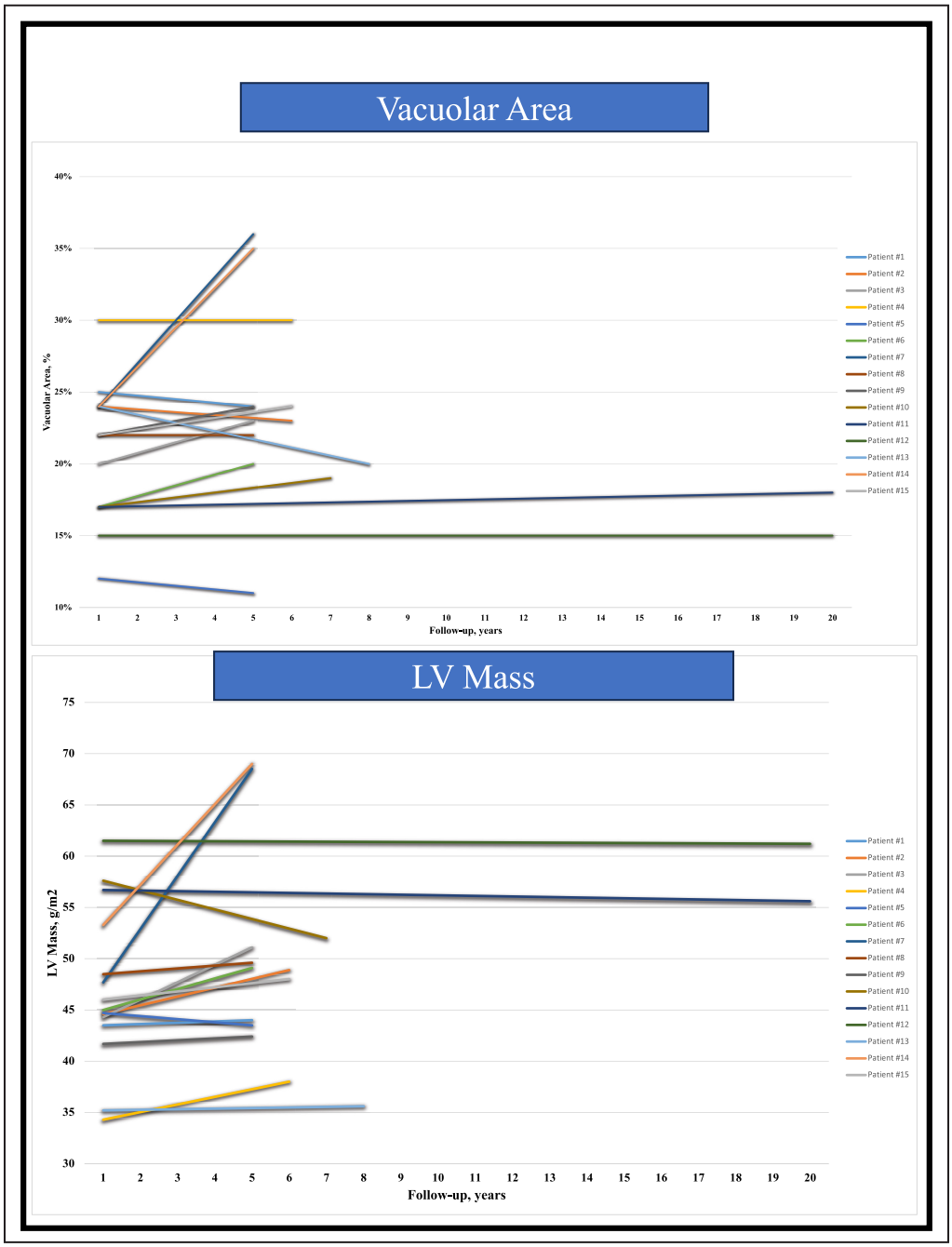



**Figure 4. Vacuolar area (%) and LV mass changes during follow-up of 15 patients with prehypertrophic FDCM treated with long-term ERT.**
Data show stabilization of vacuolar area and LV mass in 13 patients, whereas overlapping myocarditis indicates disease progression in 2 patients. ERT indicates enzyme replacement therapy; FDCM, Fabry disease cardiomyopathy; and LV, left ventricular.

## Limitations of the Study

Investigation of the molecular pathway involved in the resistance to ERT is limited to M6Pr determination. Other abnormal mechanisms as Lyset protein, responsible for lysosomal transport of the infused enzyme may concur. Moreover, the mechanism responsible for reduced M6Pr expression remains unclarified. Elevation of ubiquitin would suggest an its enhanced degradation; however, the involved molecular pathway is still undefined. The control endomyocardial samples used for M6Pr determination, represented by surgical biopsies from papillary muscle obtained from patients with pure mitral stenosis and normal LV dimension and function, are not perfectly normal but the closest to normal myocardium.

Furthermore, we are well aware of the cell mosaic of women with FD due to X-chromosome inactivation and also to *sampling limitation* of endomyocardial biopsy. However, every biopsy study included the extraction of at least 3 endomyocardial samples that always included affected and unaffected cardiomyocytes, allowing systematically a pathologic description of severity of the disease and of its evolution in the control samples. In addition, the inclusion in the study of different categories of patients with FD bring the advantage to recognize possible differences among men and women and patients with classic and late-onset disease.

Reference samples of tissues from surgically operated patients with mitral valve stenosis may represent a limitation of the study because the left ventricle is suffering from low volume load, and cardiomyocytes may be smaller than normal. Nevertheless, these represent the best possible controls close to normal.

## CONCLUSIONS

In conclusion, our study suggests that ERT stabilizes storage deposits and myocyte dimensions in 87% of patients with prehypertrophic FDCM.

Globotriaosylceramide is never completely removed even after long-term treatment. Immune-mediated myocardial inflammation can overlap, limiting ERT activity.

## ARTICLE INFORMATION

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### Disclosures

None.

### Supplemental Material

Tables S1–S4.

## REFERENCES

1. Desnick RJ, Brady R, Barranger J, Collins AJ, Germain DP, Goldman M, Grabowski G, Packman S, Wilcox WR. Fabry disease, an under-recognized multisystemic disorder: expert recommendations for diagnosis, management, and enzyme replacement therapy. *Ann Intern Med*. 2003;138:338–346.
2. Desnick RJ. Fabry disease (α-galactosidase A deficiency). *Genetic Diseases of the Kidney*. Elsevier; 2009:597–616.
3. Germain DP. Fabry disease. *Orphanet J Rare Dis*. 2010;5:30. doi: 10.1186/1750-1172-5-30
4. 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. doi: 10.1161/CIRCULATIONAHA.108.841494
5. Najafian B, Svarstad E, Bostad L, Gubler MC, Tøndel C, Whitley C, Mauer M. Progressive podocyte injury and glo-botriaosylceramide (GB-3) accumulation in young patients with Fabry disease. *Kidney Int*. 2011;79:663–670. doi: 10.1038/ki.2010.484
6. Chimenti C, Morgante E, Tanzilli G, Mangieri E, Critelli G, Gaudio C, Russo MA, Frustaci A. Angina in Fabry disease reflects coronary small vessel disease. *Circ Heart Fail*. 2008;1:161–169. doi: 10.1161/CIRCHEARTFAILURE.108.769729
7. Von Scheidt W, Eng CM, Fitzmaurice TF, Erdmann E, Hübner G, Olsen EG, Christomanou H, Kandolf R, Bishop DF, Desnick RJ. An atypical variant of Fabry's disease with manifestations confined to the myocardium. *N Engl J Med*. 2010;324:395–399.
8. Eng CM, Guffon N, Wilcox WR, Germain DP, Lee P, Waldek S, Caplan L, Linthorst GE, Desnick RJ; International Collaborative Fabry Disease Study Group. Safety and efficacy of recombinant human alpha-galactosidase A replacement therapy in Fabry's disease. *N Engl J Med*. 2001;345:9–16. doi: 10.1056/NEJM200107053450102
9. Germain DP, Waldek S, Banikazemi M, Bushinsky DA, Charrow J, Desnick RJ, Lee P, Loew T, Vedder AC, Abichandani R, et al. Sustained, long-term renal stabilization after 54 months of Agalsidase therapy in patients with Fabry disease. *J Am Soc Nephrol*. 2007;18:1547–1557. doi: 10.1681/ASN.2006080816
10. Frustaci A, Chimenti C, Ricci R, Natale L, Russo MA, Pieroni M, Eng CM, Desnick RJ. Improvement in cardiac function in the cardiac variant of Fabry's disease with galactose-infusion therapy. *N Engl J Med*. 2001;345:25–32. doi: 10.1056/NEJM200107053450104
11. Migalastat MA. First global approval. *Drugs*. 2016;76:1147–1152. doi: 10.1007/s40265-016-0607-y
12. Ramaswami U, Bichet DG, Clarke LA, Dostalova G, Fainboim A, Fellgiebel A, Forcelini CM, An Haack K, Hopkin RJ, Mauer M, et al. Low-dose agalsidase beta treatment in male pediatric patients with Fabry disease: a 5-year randomized controlled trial. *Mol Genet Metab*. 2019;127:86–94. doi: 10.1016/j.ymgme.2019.03.010
13. Najafian B, Tøndel C, Svarstad E, Sokolovkiy A, Smith K, Mauer M. One year of enzyme replacement therapy reduces globotriaosylceramide inclusions in podocytes in male adult patients with Fabry disease. *PLoS One*. 2016;11:e0152812. doi: 10.1371/journal.pone.0152812
14. Thurberg BL, Byers HR, Granter SR, Phelps RG, Gordon RE, O'Callaghan M. Monitoring the 3-year efficacy of enzyme replacement therapy in Fabry disease by repeated skin biopsies. *J Investig Dermatol*. 2004;122:900–908. doi: 10.1111/j.0022-202X.2004.22425.x
15. Thurberg BL, Randolph Byers H, Granter SR, Phelps RG, Gordon RE, O'Callaghan M. Globotriaosylceramide accumulation in the Fabry kidney is cleared from multiple cell types after enzyme replacement therapy. *Kidney Int*. 2002;62:1933–1946. doi: 10.1046/j.1523-1755.2002.00675.x
16. Skrunes R, Tøndel C, Leh S, Larsen KK, Houge G, Davidsen ES, Hollak C, van Kuilenburg ABP, Vaz FM, Svarstad E. Long-term dose-dependent agalsidase effects on kidney histology in Fabry disease. *Clin J Am Soc Nephrol*. 2017;12:1470–1479. doi: 10.2215/CJN.01820217
17. Ortiz A, Germain DP, Desnick RJ, Politei J, Mauer M, Burlina A, Eng C, Hopkin RJ, Laney D, Linhart A, et al. Fabry disease revisited: management and treatment recommendations for adult patients. *Mol Genet Metab*. 2018;123:416–427. doi: 10.1016/j.ymgme.2018.02.014
18. Pieroni M, Moon JC, Arbustini E, Barriales-Villa R, Camporeale A, Vujkovac AC, Elliott PM, Hagege A, Kuusisto J, Linhart A, et al. Cardiac involvement in Fabry disease: JACC review topic of the week. *J Am Coll Cardiol*. 2021;77:922–936. doi: 10.1016/j.jacc.2020.12.024

19. Germain DP, Elliott PM, Falissard B, Fomin VV, Hilz MJ, Jovanovic A, Kantola I, Linhart A, Mignani R, Namdar M, 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. doi: 10.1016/j.ymgmr.2019.100454
20. Bergmann O, Bhardwaj RD, Bernard S, Zdunek S, Barnabé-Heider F, Walsh S, Zupicich J, Alkass K, Buchholz BA, Druid H, et al. Evidence of cardiomyocyte renew in humans. *Science*. 2009;324:98–102. doi: 10.1126/science.1164680
21. Frustaci A, Verardo R, Scialla R, Bagnato G, Verardo M, Alfarano M, Russo MA. Downregulation of mannose-6-phosphate receptors in Fabry disease cardiomyopathy: a potential target for enzyme therapy enhancement. *J Clin Med*. 2022;11:5440. doi: 10.3390/jcm11185440
22. Ortiz A, Abiose A, Bichet DG, Cabrera G, Charrow J, Germain DP, Hopkin RJ, Jovanovic A, Linhart A, Maruti SS, 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. doi: 10.1136/jmedgenet-2015-103486
23. Weidemann F, Niemann M, Breunig F, Herrmann S, Beer M, Störk S, Voelker W, Ertl G, Wanner C, Strotmann J. Long-term effects of enzyme replacement therapy on fabry cardiomyopathy: evidence for a better outcome with early treatment. *Circulation*. 2009;119:524–529. doi: 10.1161/CIRCULATIONAHA.108.794529
24. Hopkin RJ, Cabrera G, Charrow J, Lemay R, Martins AM, Mauer M, Ortiz A, Patel MR, Sims K, Waldek S, et al. Risk factors for severe clinical events in male and female patients with Fabry disease treated with agalsidase beta enzyme replacement therapy: data from the Fabry registry. *Mol Genet Metab*. 2016;119:151–159. doi: 10.1016/j.ymgme.2016.06.007
25. Frustaci A, Verardo R, Grande C, Galea N, Piselli P, Carbone I, Alfarano M, Russo MA, Chimenti C. Immune-mediated myocarditis in Fabry disease cardiomyopathy. *J Am Heart Assoc*. 2018;7:e009052. doi: 10.1161/JAHA.118.009052
26. Chimenti C, Frustaci A. Contribution and risks of left ventricular endomyocardial biopsy in patients with cardiomyopathies: a retrospective study over a 28-year period. *Circulation*. 2013;128:1531–1541. doi: 10.1161/CIRCULATIONAHA.13.001414
27. Cooper LT, Baughman KL, Feldman AM, Frustaci A, Jessup M, Kuhl U, Levine GN, Narula J, Starling RC, Towbin J, et al. The role of endomyocardial biopsy in the management of cardiovascular disease: a scientific statement from the American Heart Association, the American College of Cardiology, and the European Society of Cardiology. Endorsed by the Heart Failure Society of America and the Heart Failure Association of the European Society of Cardiology. *J Am Coll Cardiol*. 2007;50:1914–1931.
28. Kampmann C, Linhart A, Baehner F, Palecek T, Wiethoff CM, Miebach E, Whybra C, Gal A, Bultas J, Beck M. Onset and progression of the Anderson-Fabry disease related cardiomyopathy. *Int J Cardiol*. 2008;130:367–373. doi: 10.1016/j.ijcard.2008.03.007
29. Chimenti C, Padua L, Pazzaglia C, Morgante E, Centurion C, Antuzzi D, Russo MA, Frustaci A. Cardiac and skeletal myopathy in Fabry disease: a clinicopathologic correlative study. *Hum Pathol*. 2012;43:1444–1452. doi: 10.1016/j.humpath.2011.09.020
30. Linhart A, Germain DP, Olivotto I, Akhtar MM, Anastasakis A, Hughes D, Namdar M, Pieroni M, Hagège A, Cecchi F, et al. An expert consensus document on the management of cardiovascular manifestations of Fabry disease. *Eur J Heart Fail*. 2020;22:1076–1096. doi: 10.1002/ejhf.1960
31. Pieroni M, Chimenti C, De Cobelli F, Morgante E, Del Maschio A, Gaudio C, Russo MA, Frustaci A. Fabry's disease cardiomyopathy: echocardiographic detection of endomyocardial glycosphingolipid compartmentalization. *J Am Coll Cardiol*. 2006;47:1663–1671. doi: 10.1016/j.jacc.2005.11.070
32. Frustaci A, Najafian B, Donato G, Verardo R, Chimenti C, Sansone L, Belli M, Vernucci E, Russo MA. Divergent impact of enzyme replacement therapy on human cardiomyocytes and enterocytes affected by Fabry disease: correlation with mannose-6-phosphate receptor expression. *J Clin Med*. 2022;11:1344. doi: 10.3390/jcm11051344
33. Chimenti C, Scopelliti F, Vulpis E, Tafani M, Villanova L, Verardo R, De Paulis R, Russo MA, Frustaci A. Increased oxidative stress contributes to cardiomyocyte dysfunction and death in patients with Fabry disease cardiomyopathy. *Hum Pathol*. 2015;46:1760–1768. doi: 10.1016/j.humpath.2015.07.017
34. Coutinho MF, Prata MJ, Alves S. Mannose-6-phosphate pathway: a review on its role in lysosomal function and dysfunction. *Mol Genet Metab*. 2012;105:542–550. doi: 10.1016/j.ymgme.2011.12.012
35. Pechincha C, Groessl S, Kalis R, de Almeida M, Zanotti A, Wittmann M, Schneider M, de Campos RP, Rieser S, Brandstetter M, et al. Lysosomal enzyme trafficking factor LYSET enables nutritional usage of extracellular proteins. *Science*. 2022;378:eabn5637. doi: 10.1126/science.abn5637
36. Bannoud N, Carvelli FL, Troncoso M, Sartor T, Vargas-Roig LM, Sosa M. Cation-dependent mannose-6-phosphate receptor expression and distribution are influenced by estradiol in MCF-7 breast cancer cells. *PLoS One*. 2018;13:e0201844. doi: 10.1371/journal.pone.0201844