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ORIGINAL RESEARCH ARTICLE

Cardiomyopathy and kidney function in agalsidase beta-treated female Fabry patients: a pre-treatment vs. post-treatment analysis

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Abstract

Aims Long-term treatment effect studies in large female Fabry patient groups are challenging to design because of phenotype heterogeneity and lack of appropriate comparison groups, and have not been reported. We compared long-term cardiomyopathy and kidney function outcomes after agalsidase beta treatment with preceding treatment-naive outcomes.

Methods and results Self-controlled pretreatment and post-treatment comparison (piecewise mixed linear modelling) included Fabry female patients ≥18 years at treatment initiation who received agalsidase beta (0.9–1.1 mg/kg every other week) for ≥2 years, with ≥2 pretreatment and ≥2 post-treatment outcome measurements during 10-year follow-up. Left ventricular posterior wall thickness (LVPWT)/interventricular septal thickness (IVST) and estimated glomerular filtration rate (eGFR, Chronic Kidney Disease Epidemiology Collaboration creatinine equation) analyses included 42 and 86 patients, respectively, aged 50.0 and 46.3 years at treatment initiation, respectively. LVPWT and IVST increased pretreatment (follow-up 3.5 years) but stabilized during 3.6 years of treatment (LVPWT: n = 38, slope difference [95% confidence interval (CI)] = −0.41 [−0.68, −0.15] mm/year, $P_{\text{pre–post difference}} < 0.01$; IVST: n = 38, slope difference = −0.32 [−0.67, 0.02] mm/year, $P_{\text{pre–post difference}} = 0.07$). These findings were not modified by renal involvement or antiproteinuric agent use. Compared with the treatment-naive period (follow-up 3.6 years), eGFR decline remained modest and stabilized within normal ranges during 4.1 years of treatment (slope difference, 95% CI: −0.13 [−1.15, 0.89] mL/min/1.73m^2/year, $P_{\text{pre–post difference}} = 0.80$).

Conclusions Cardiac hypertrophy, progressing during pretreatment follow-up, appeared to stabilize during sustained agalsidase beta treatment. eGFR decline remained within normal ranges. This suggests that treatment may prevent further Fabry-related progression of cardiomyopathy in female patients and maintain normal kidney function.

Keywords Agalsidase beta; Enzyme replacement therapy; Fabry disease; Cardiomyopathy; Kidney function; Female patients

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Introduction

Fabry disease (OMIM #301500) is an X-linked lysosomal storage disorder caused by a pathogenic variant of the *GLA* gene leading to a deficient activity of the enzyme α-galactosidase (α-Gal).[1,2] This results in progressive, lifelong accumulation of glycosphingolipids, in particular globotriaosylceramide (GL-3) and the deacylated form globotriaosylsphingosine (lyso-GL-3), in plasma, urine and a wide range of cell types, including vascular endothelial cells, all kidney cell types, cardiomyocytes, and neural cells.[1,2] Progressive cell and tissue damage leads to fibrosis and dysfunction of vital organs.[1,2] Currently, more than 900 different variations in the *GLA* gene have been reported, and most Fabry families have unique variants with intra-familial variability in clinical expression.[1,2]

Hemizygous male patients with the severe, 'classic' Fabry phenotype start developing symptoms in early childhood, including neuropathic pain, autonomic dysfunction, gastrointestinal complaints, angiokeratomas, and hypohidrosis.[3] Potentially life-threatening complications involving the kidneys and cardiovascular and cerebrovascular systems may gradually become apparent during adulthood.[1,2,4]

In heterozygous female patients, the spectrum of clinical phenotypes is broad[4,5] and depends on the *GLA* variant and the X-chromosome inactivation pattern in tissues.[6] Both factors influence the level of residual α-Gal activity in female patients, which can range from almost completely absent to normal. Therefore, female patients can be severely affected, present with a more attenuated course, or remain fully asymptomatic.[4,5] Registry studies have shown that left ventricular hypertrophy (LVH), Fabry nephropathy, and ischaemic stroke are prevalent among female Fabry patients,[5,7–11] although cardiovascular complications generally develop at older age compared with male patients.[5,10] Few female patients progress to end-stage kidney disease[1,6,8] and, as in male patients, Fabry-related cardiovascular disease is the leading cause of death in female patients.[12]

Enzyme replacement therapy (ERT) with recombinant agalsidase (intravenous infusions of either agalsidase beta or agalsidase alfa) is available, and clinical experience now exceeds 15 years. Most of the published studies have reported clinical outcomes in populations primarily composed of male Fabry patients[13]; there is relatively limited data on therapeutic outcomes in female patients.[14] In addition, it is challenging to interpret the long-term treatment benefits for organ-specific outcomes without a treatment-naive comparison group or period. Therefore, the objective of our study was to evaluate cardiomyopathy and kidney function in adult female patients enrolled in the Fabry Registry and to compare the outcomes before and after initiation of treatment with agalsidase beta administered at an average dose of 0.9–1.1 mg/kg every other week (EOW).

Methods

Fabry Registry and patient selection

We used data from the Fabry Registry (NCT00196742), which was initiated in 2001 as a multicentre, international, longitudinal, observational programme designed to monitor the natural history and treatment outcomes of patients with Fabry disease. Patient and investigator involvement in the Fabry Registry is voluntary. Recommended schedules of clinical assessments are available, but treating physicians determine assessment frequency according to each patient's individual need for medical care and follow-up. Each site is independent and responsible for obtaining informed written consent from patients to submit their health data to the Fabry Registry and use/disclose their data in analyses. The protocol, informed consent form, and any authorization documents that are locally required for entering patient information into the Fabry Registry are in accordance with the Declaration of Helsinki and are reviewed and approved by the local, fully constituted Institutional Review Board or Independent Ethics Committee unless a specific site provides evidence that approval is not required or that this requirement has been waived by a particular Institutional Review Board/Independent Ethics Committee.

The study included adult female patients enrolled in the Fabry Registry who had initiated treatment with agalsidase beta as first ERT at age ≥18 years and had received an average dose at or near the licensed dose of 1 mg/kg EOW (0.9–1.1 mg/kg EOW) for ≥2 years. The analysis population was restricted to female patients with *GLA* variants categorized in the fabry-database.org database (Fabry Disease Mutation Database, http://fabry-database.org/mutants/) as being associated with classic Fabry disease or variants not entered or classified in the database and to female patients for whom variants were not reported to the Fabry Registry.[15] To be eligible for pretreatment vs. post-treatment analyses, patients had to have ≥2 pretreatment outcome measurements during the 5 years before initiation of treatment (≥2 years apart) and ≥2 post-treatment outcome measurements during the 5 years after treatment initiation (≥2 years apart). Patients with end-stage kidney failure were excluded (i.e. estimated glomerular filtration rate [eGFR] <15 mL/min/1.73m^2 at the first pretreatment or first post-treatment assessment or chronic dialysis or kidney transplant prior to treatment initiation). Furthermore, any record deemed an extreme outlier potentially caused by recording or measurement error was excluded (i.e. serum creatinine level <0.4 or >25 mg/dL; cardiac wall thickness measurement <5 or >35 mm).

Clinical outcomes

The outcomes assessed were end-diastolic left ventricular posterior wall thickness (LVPWT) and intraventricular septal

thickness (IVST), both using standard echocardiography measurements (either 2-dimensional or M-mode), and eGFR using the Chronic Kidney Disease Epidemiology Collaboration equation based on reported serum creatinine.[16]

Statistical analysis

Descriptive statistics were calculated for demographic characteristics and follow-up time. Clinical measurements of LVPWT, IVST, and eGFR by follow-up years before and after treatment initiation were evaluated. Patients were followed from the start of treatment until clinical assessments, cessation of agalsidase beta treatment, or death, whichever came first, using pretreatment records of negative follow-up time and post-treatment records of positive follow-up time. Piecewise linear mixed model was used to examine the LVPWT, IVST, and eGFR change over time during the pretreatment and post-treatment periods and the difference between these periods. The model was initially fitted with follow-up time (random effect) to estimate the slopes for both periods and a spline variable (fixed effect), which compared the slope difference between the two periods ($P_{\text{pre–post difference}}$). The intercept was considered a random effect, and unstructured covariance matrix was applied. Renal involvement was defined as low renal involvement (LRI; urine protein-to-creatinine ratio [UPCR] $\leq$0.5 g/g or urine albumin-to-creatinine ratio [UACR] $\leq$0.3 g/g) or high renal involvement (HRI; UPCR $>$0.5 g/g or UACR $>$0.3 g/g) using available data collected at treatment initiation or during post-treatment follow-up.[17,18] Use of angiotensin-converting enzyme inhibitors (ACEi)/angiotensin receptor blockers (ARBs) was categorized as 'never' vs. 'ever' during the entire follow-up time; the ever group was defined as those who reported using ACEi/ARBs at some time during the entire follow-up, while the never group comprised the rest of the patients. Effect

Table 1 Characteristics of the adult female patients in the Fabry Registry included in the pretreatment vs. post-agalsidase beta treatment analyses

	Cardiac analysis (N = 42)[a]	eGFR analysis (N = 86)
Age at symptom onset (years)	n = 28	n = 56
Mean (SD)	23.6 (15.7)	21.0 (15.8)
Median (25th–75th percentile)	19.1 (11.7–32.3)	15.4 (9.7–30.6)
Min, Max	5.3, 60.6	2.9, 60.6
Age at diagnosis (years)	n = 41	n = 81
Mean (SD)	39.2 (14.5)	36.6 (14.9)
Median (25th–75th percentile)	36.5 (30.2–48.1)	35.1 (28.3–45.1)
Min, Max	10.3, 70.6	3.2, 69.0
Fabry phenotype	n = 42	n = 86
Classic, n (%)	20 (47.6)	46 (53.5)
Unclassified, n (%)	21 (50.0)	31 (36.0)
Missing, n (%)	1 (2.4)	9 (10.5)
Age at first agalsidase beta (years)	n = 42	n = 86
Mean (SD)	51.1 (10.7)	47.2 (11.5)
Median (25th–75th percentile)	50.0 (44.2–59.1)	46.3 (38.8–56.2)
Min, Max	27.7, 72.0	22.1, 69.3
Time from diagnosis to first agalsidase beta (years)	n = 41	n = 81
Mean (SD)	12.1 (11.3)	10.3 (10.9)
Median (25th–75th percentile)	9.0 (3.5–16.8)	5.8 (2.6–13.6)
Min, Max	0.5, 45.3	0.0, 45.3
Pre-agalsidase beta follow-up time (years)	n = 42	n = 86
Mean (SD)	3.6 (0.9)	3.6 (0.8)
Median (25th–75th percentile)	3.5 (2.8–4.4)	3.6 (2.8–4.2)
Min, Max	2.0, 4.9	2.1, 5.0
Post-agalsidase beta follow-up time (years)	n = 42	n = 86
Mean (SD)	3.7 (0.9)	3.9 (0.9)
Median (25th–75th percentile)	3.6 (3.1–4.5)	4.1 (3.2–4.5)
Min, Max	2.0, 4.9	2.0, 5.0
Renal involvement[b]	n = 32	n = 75
LRI, n (%)	22 (68.8)	53 (70.7)
HRI, n (%)	10 (31.3)	22 (29.3)
Use of ACEi/ARBs	n = 42	n = 86
'Ever' reported during follow-up, n (%)	27 (64.3)	52 (60.5)
'Never' reported during follow-up, n (%)	15 (35.7)	34 (39.5)

ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; eGFR, estimated glomerular filtration rate; HRI, high renal involvement; LRI, low renal involvement; SD, standard deviation.

[a]Unique patients with left ventricular posterior wall thickness (LVPWT) or interventricular septal thickness (IVST) were included. Thirty-four patients had both LVPWT and IVST assessments; four patients had LVPWT assessments only, and four patients had IVST assessments only.

[b]Renal involvement was defined as LRI (urine protein-to-creatinine ratio [UPCR] $\leq$0.5 g/g or urine albumin-to-creatinine ratio [UACR] $\leq$0.3 g/g) or HRI (UPCR $>$0.5 g/g or UACR $>$0.3 g/g) using available data collected at treatment initiation or during follow-up.

modification by renal involvement and use of ACEi/ARBs was evaluated by adding cross-product terms to the primary model, which tested the slope difference during the treatment-naive period ($P_{\text{pre difference}}$), the slope difference after treatment ($P_{\text{post difference}}$), and the difference of changing patterns before and after treatment ($P_{\text{interaction}}$) between two subgroups. Of note, additional adjustment of the model for potential confounding by age at first treatment, renal involvement, and use of ACEi/ARBs did not change the results; therefore, these variables were not included in the main analyses (see Supporting information, *Table* S1). All statistical analyses were two sided and performed using SAS statistical software v.9.3 (SAS Institute Inc, Cary, NC, USA). A *P* value <0.05 was considered to represent statistical significance.

Results

Patient demographics

The characteristics of the female Fabry patients with LVPWT/IVST or eGFR assessments included in the final analyses are summarized in *Table 1* and those of patients included in the effect modification analyses in *Table* S2. Fabry Registry data entered up to 1 June 2018 were used in the analyses; the final analysis records were collected from 23 January 1998 to 11 May 2018.

Of 503 agalsidase beta-treated adult females with any LVPWT and/or IVST data available, 42 unique patients (8.3%) were included in the final pre-LVPWT/IVST vs. post-LVPWT/IVST analyses. Of these patients, 34 had both LVPWT and IVST measurements, 4 had LVPWT measures only, and 4 had IVST measurements only. Therefore, the LVPWT and IVST subgroups each had data from 38 patients. The median ages (25th–75th percentile) at diagnosis and first agalsidase beta treatment of the 42 patients were 36.5 (30.2–48.1) and 50.0 (44.2–59.1) years, respectively, and the median time from diagnosis to first treatment was 9.0 (3.5–16.8) years. The median follow-up durations (25th–75th percentile) during the pretreatment and post-treatment periods were 3.5 (2.8–4.4) and 3.6 (3.1–4.5) years, respectively.

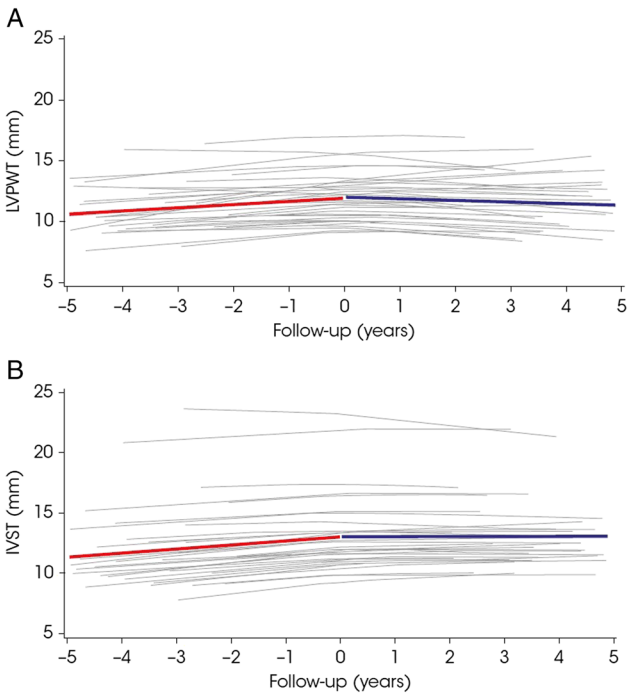
Of 629 agalsidase beta-treated adult female patients who had any eGFR data available in the Fabry Registry, 86 (13.7%) met all criteria for inclusion in the pre-eGFR vs. post-eGFR analysis. The median ages (25th–75th percentile) at diagnosis and first agalsidase beta treatment were 35.1 (28.3–45.1) and 46.3 (38.8–56.2) years, respectively, and the median time from diagnosis to first treatment was 5.8 (2.6–13.6) years. The median follow-up durations (25th–75th percentile) during the pretreatment and post-treatment periods were 3.6 (2.8–4.2) and 4.1 (3.2–4.5) years, respectively.

Of the female patients included in the final analyses, 47.6% (LVPWT/IVST) and 53.5% (eGFR) had *GLA* variants associated with classic Fabry disease and 52.4% and 46.5%, respectively, with unclassified or unreported variants.

Echocardiographic variables of cardiomyopathy

Among the 38 patients with available echocardiographic data, LVPWT and IVST slopes remained stable after agalsidase beta initiation, whereas increasing patterns were observed before treatment. The pretreatment- and post-treatment slopes (95% confidence interval [CI)] for LVPWT were 0.28 (0.10, 0.46) and −0.13 (−0.30, 0.04) mm/year, respectively ($P_{\text{pre–post difference}}<0.01$) (*Figure 1*A), and for IVST 0.33 (0.12, 0.54) and 0.01 (−0.20, 0.21) mm/year ($P_{\text{pre–post difference}}=0.07$)

Figure 1 (A) Estimated LVPWT slopes pre-agalsidase beta and post-agalsidase beta treatment, *N* = 38. Estimated LVPWT group slope (95% confidence interval) pretreatment (red) and post-treatment (blue) of 0.28 (0.10, 0.46) (*P* <0.01) and −0.13 (−0.30, 0.04) mm/year (*P* = 0.13), respectively. Pretreatment vs. post-treatment slope difference = −0.41 (−0.68, −0.15) mm/year (*P* for pretreatment vs. post-treatment slope difference <0.01). Estimated individual slopes for pretreatment and post-treatment were shown as grey lines. LVPWT, left ventricular posterior wall thickness. (B) Estimated IVST slopes pre- and post-agalsidase beta treatment, *N* = 38. Estimated IVST group slope (95% confidence interval) pretreatment (red) and post-treatment (blue) of 0.33 (0.12, 0.54) (*P* <0.01) and 0.01 (−0.20, 0.21) mm/year (*P* = 0.93), respectively. Pretreatment vs. post-treatment slope difference = −0.32 (−0.67, 0.02) mm/year (*P* pretreatment vs. post-treatment slope difference = 0.07). Estimated individual slopes for pretreatment and post-treatment were shown as grey lines. IVST, interventricular septal thickness.



(*Figure 1*B). The change in LVPWT or IVST slopes was not modified by renal involvement or use of ACEi/ARBs (*Table 2*) although the slope difference of IVST appeared to be altered by renal involvement, which might be a chance finding. The trends in each subgroup included in the effect modification analyses were comparable with the observations in the overall analyses, but these results need to be interpreted with caution given the small numbers of patients (*Table 2*). *Table* S3 presents the yearly LVPWT and IVST values assessed during the 10-year follow-up among the overall patients. *Tables* S4A and S4B show the yearly LVPWT and IVST values, respectively, during follow-up by renal involvement and use of ACEi/ARBs.

Kidney function

eGFR slopes were estimated using data from 86 patients. The eGFR slope was −0.83 mL/min/1.73m^2/year (95% CI: −1.52, −0.13) during the pretreatment period and −0.95 mL/min/1.73m^2/year (95% CI: −1.59, −0.32) during the post-treatment period. There was no difference in the modest rates of yearly eGFR decline, which were within the normal range of a healthy population,[19] between the treatment-naive and treatment periods ($P_{\text{pre–post difference}}$ = 0.80) (*Figure 2*). The slope differences were also not statistically different between the 53 female patients with LRI (pretreatment vs. post-treatment slopes [95% CI] −0.68 [−1.56, 0.20] and −0.14 [−0.92, 0.63] mL/min/1.73 m^2/year; $P_{\text{pre–post difference}}$ = 0.43) and the 22 female patients with HRI (−1.95 [−3.23, −0.67] and −2.08 [−3.21, −0.95] mL/min/1.73m^2/year; $P_{\text{pre–post difference}}$ = 0.89) ($P_{\text{interaction}}$ = 0.58) (*Table 2*); nonetheless, patients with HRI reported a higher rate of decline in eGFR compared with those with LRI in both periods. In addition, the changing patterns of decline in eGFR before and after treatment were comparable between 34 patients who had never reported using ACEi/ARBs (0.01 [−1.15, 1.18] and −0.57 [−1.69, 0.56] mL/min/1.73m^2/year; $P_{\text{pre–post difference}}$ = 0.54) and 52 patients who had ever reported using these agents (−1.30 [−2.17, −0.43] and −1.19 [−1.96, −0.43] mL/min/1.73m^2/year; $P_{\text{pre–post difference}}$ = 0.87) ($P_{\text{interaction}}$ = 0.55). Patients who had ever used ACEi/ARBs had a higher rate of yearly eGFR decline than those who had never used these agents in both periods. *Table* S3 presents the overall eGFR values for the patients measured during follow-up; *Table* S4C presents the yearly eGFR values measured during follow-up by renal involvement and use of ACEi/ARBs.

Discussion

This novel analysis among female patients in the Fabry Registry treated with agalsidase beta (0.9–1.1 mg/kg EOW) compared pretreatment and post-treatment outcomes of echocardiographic variables of cardiomyopathy and kidney function as surrogate clinical endpoints of Fabry disease progression. We found a non-progressive cardiac hypertrophy after treatment initiation and a stable, modest decline in eGFR within the normal range. As a result of the heterogeneity of Fabry phenotypes in female patients and the lack of an appropriate comparison group, studies evaluating the long-term effects of agalsidase beta treatment in large groups of female Fabry patients are challenging to design and perform and, therefore, have not been conducted. This novel self-controlled pretreatment vs. post-treatment comparison provided valuable, unique insights into the long-term treatment outcomes of agalsidase beta by investigating end-organ manifestations of Fabry disease in the female patients.

In patients with Fabry disease, accumulation of GL-3 in cardiomyocytes, specific trophic factors, and microcirculatory ischaemia as a result of GL-3 accumulation in vascular endothelial cells is believed to contribute to tissue injury and lead to inflammation, concentric LVH, and cardiac replacement fibrosis.[2,20] Moreover, increased concentrations of lyso-GL-3 presumably trigger promotion of proliferation of smooth muscle cells and development of cardiomyocyte hypertrophy, while GL-3 accumulation appears to marginally contribute to increased ventricular mass.[21,22] Registry studies have shown that approximately one-fifth of untreated female patients had clinical evidence of LVH at a mean age of around 50 years.[5,7]

We observed significant increases in both LVPWT and IVST in female Fabry patients in the pretreatment period. Importantly, cardiac hypertrophy stabilized in this female population after initiation of agalsidase beta treatment at a median age of 50.0 years and during a median follow-up of 3.6 years. Cardiac-replacement fibrosis, which has been reported in female Fabry patients in the absence of LVH or measurable cardiac functional impairments,[4,23] does not typically affect the septal wall initially. Instead, the mid-myocardial layer of the posterolateral left ventricular wall is affected first, which may result in fibrotic wall thinning.[24] Therefore, our observation of stabilization of both LVPWT *and* IVST is encouraging as it supports the agalsidase beta treatment response rather than a fibrotic process influence.

Progressive accumulation of GL-3 in kidney cells (including endothelial, mesangial, and tubular epithelial cells and podocytes) may lead to proteinuria and deterioration of renal function driven by interstitial fibrosis, glomerulosclerosis, and tubular atrophy.[2] There is a marked variability in kidney pathology in female patients who rarely progress to end-stage kidney disease.[1,6,8] We observed a modest rate of decline in eGFR during the pretreatment period, comparable with those documented in other studies among untreated female patients.[25] Our finding of overall stability of the eGFR slope during sustained treatment with agalsidase beta (median 4.1 years) suggests that treatment maintained the normal

Table 2 Pretreatment vs. post-treatment LVPWT, IVST, and eGFR slopes stratified by renal involvement or use of ACEi/ARBs

Subgroup: n[a]	Age at first agalsidase beta[b]	Slope pretreatment[c]	P_{pre}[d]	$P_{\text{pre difference}}$[e]	Slope post-treatment[c]	P_{post}[d]	$P_{\text{post difference}}$[e]	Pretreatment vs. post-treatment slope difference[c]	$P_{\text{pre–post difference}}$[d]	$P_{\text{interaction}}$[e]
LVPWT										
HRI: 9	55.3 (46.5–59.0)	0.54 (0.19, 0.88)	<0.01	0.18	−0.39 (−0.74, −0.05)	<0.05	0.25	−0.92 (−1.36, −0.49)	<0.01	0.09
LRI: 21	51.5 (43.0–57.5)	0.23 (−0.03, 0.49)	0.09		−0.13 (−0.36, 0.11)	0.30		−0.35 (−0.75, 0.04)	0.08	
'Ever' ACEi/ARBs: 26	55.1 (44.7–59.1)	0.37 (0.13, 0.61)	<0.01	0.19	−0.16 (−0.39, 0.07)	0.17	0.66	−0.53 (−0.89, −0.17)	<0.01	0.24
'Never' ACEi/ARBs: 12	44.2 (38.0–53.0)	0.07 (−0.17, 0.31)	0.58		−0.03 (−0.25, 0.19)	0.79		−0.10 (−0.50, 0.30)	0.62	
IVST										
HRI: 10	55.6 (46.5–63.7)	0.56 (0.15, 0.98)	<0.05	0.21	−0.47 (−0.87, −0.06)	<0.05	<0.05	−1.03 (−1.63, −0.42)	<0.01	<0.05
LRI: 20	49.9 (43.7–57.5)	0.24 (−0.08, 0.56)	0.14		0.12 (−0.18, 0.42)	0.43		−0.12 (−0.67, 0.42)	0.66	
'Ever' ACEi/ARBs: 25	55.3 (45.7–59.2)	0.43 (0.16, 0.71)	<0.01	0.20	−0.02 (−0.28, 0.25)	0.91	0.67	−0.45 (−0.89, −0.0)	0.05	0.30
'Never' ACEi/ARBs: 13	46.4 (38.3–48.5)	0.19 (−0.13, 0.51)	0.26		0.02 (−0.29, 0.33)	0.92		−0.17 (−0.72, 0.39)	0.54	
eGFR										
HRI: 22	46.9 (41.8–59.0)	−1.95 (−3.23, −0.67)	<0.01	0.10	−2.08 (−3.21, −0.95)	<0.01	<0.01	−0.13 (−1.99, 1.72)	0.89	0.58
LRI: 53	45.8 (39.1–54.9)	−0.68 (−1.56, 0.20)	0.13		−0.14 (−0.92, 0.63)	0.72		0.54 (−0.81, 1.88)	0.43	
'Ever' ACEi/ARBs: 52	49.4 (42.4–58.5)	−1.30 (−2.17, −0.43)	<0.01	0.08	−1.19 (−1.96, −0.43)	<0.01	0.38	0.10 (−1.13, 1.34)	0.87	0.55
'Never' ACEi/ARBs: 34	40.0 (35.2–48.5)	0.01 (−1.15, 1.18)	0.98		−0.57 (−1.69, 0.56)	0.32		−0.58 (−2.43, 1.27)	0.54	

ACEi, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blocker; eGFR, estimated glomerular filtration rate; HRI, high renal involvement; IVST, interventricular septal thickness; LRI, low renal involvement; LVPWT, left ventricular posterior wall thickness.

[a]Renal involvement was defined as LRI (urine protein-to-creatinine ratio ≤0.5 g/g or urine albumin-to-creatinine ratio ≤0.3 g/g) or HRI (urine protein-to-creatinine ratio >0.5 g/g or urine albumin-to-creatinine ratio >0.3 g/g) using available data collected at treatment initiation or during follow-up.

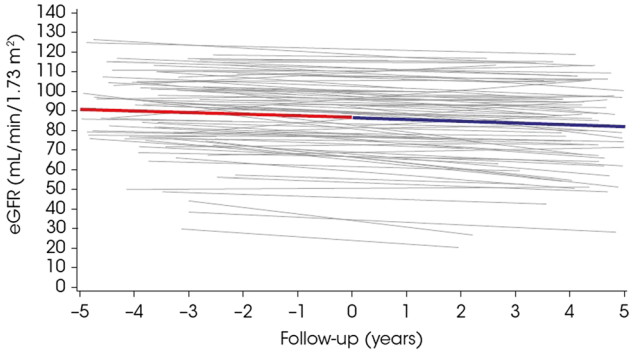
[b]Median in years (25th–75th percentile).

[c]Estimated slopes (95% confidence interval) in mm/year for cardiac parameters and mL/min/1.73 m^2 per year for eGFR.

[d]*P* value calculated by piecewise linear mixed model in each subgroup to test whether the pretreatment slope (P_{pre}) or post-treatment slope (P_{post}) is different from zero and whether the pretreatment slope is different from the post-treatment slope ($P_{\text{pre–post difference}}$) within each subgroup.

[e]*P* value calculated by piecewise linear mixed model with cross-product terms to test the pretreatment slope difference ($P_{\text{pre difference}}$) and the post-treatment slope difference ($P_{\text{post difference}}$) and the difference of changing patterns before and after treatment ($P_{\text{interaction}}$) between subgroups (e.g. HRI vs. LRI).

Figure 2 Estimated eGFR slopes pre-agalsidase beta and post-agalsidase beta treatment, *N* = 86. Estimated eGFR group slope (95% confidence interval) pretreatment (red) and post-treatment (blue) of −0.83 (−1.52, −0.13) (*P* = 0.02) and −0.95 (−1.59, −0.32) mL/min/1.73 m^2/year (*P* < 0.01), respectively. Pretreatment vs. post-treatment slope difference = −0.13 (−1.15, 0.89) mL/min/1.73 m^2/year (*P* for pretreatment vs. post-treatment slope difference = 0.80). Estimated individual slopes for pretreatment and post-treatment were shown as grey lines. eGFR, estimated glomerular filtration rate.



decline in kidney function reported for ageing females in a healthy population.[19] When our data were stratified by renal involvement (defined by the level of UACR or UPCR), the yearly decline in eGFR was greater in female patients with HRI than in those with LRI despite similar changing patterns observed for pretreatment and post-treatment eGFR and similar median ages (46.3 years) at treatment initiation. Two explanations can be given for the difference in yearly GFR decline between HRI and LRI. First, it has been reported that the decline in kidney function in ageing male and female Fabry patients and normal populations is influenced by the patient's stage of chronic kidney disease (CKD) rather than age *per se*.[19,25–29] Albuminuria and proteinuria are strong independent risk factors for CKD progression in patients with and without Fabry disease.[28,29] Thus, treatment-naive patients with more advanced CKD are more likely to show continued decline in eGFR. Second, accumulation of GL-3 within podocytes and other renal cell types has been observed in female Fabry patients, including young patients,[30] and may lead to irreversible kidney damage. Consequently, patients with a more advanced level of tissue damage may not respond to treatment as well as patients who initiate treatment at a younger age and earlier in the disease process.[29] Furthermore, the yearly decline in eGFR pretreatment vs. post-treatment was higher among ever users of ACEi/ARBs than never users, though no pretreatment vs. post-treatment difference within each group or modification of changing patterns between the groups were reported. Patients who had a history of using these antiproteinuric agents had a consistent, modest decline in eGFR before and during treatment, whereas patients who had never used these agents had stable eGFR pretreatment and post-treatment. This may be due to more advanced nephropathy before starting agalsidase beta among patients who had ever used these agents. Because ACEi/ARBs are prescribed as nephroprotective agents, this could be seen as confounding by indication. However, due to the limited number of patients, our analysis was not able to evaluate eGFR in never vs. ever users of ACEi/ARBs matched by baseline level of kidney disease. The lack of apparent benefit of ACEi/ARBs in female patients may reflect the small numbers of patients studied but may also reflect the better renal prognosis of female patients relative to that of male patients, similar to studies with agalsidase alfa.

The novelty of our analysis precludes a direct comparison with results from the limited number of other studies of ERT assessing cardiomyopathy and kidney function outcomes in female patients with Fabry disease that used treatment baseline and follow-up assessments and no self-controlled pretreatment and post-treatment comparison.[14] Female patients with *GLA* variants associated with the classic phenotype of Fabry disease may be as severely affected as classic male patients but, overall, the spectrum of clinical phenotypes in these female patients is broader with varying rates of progression of Fabry cardiomyopathy and nephropathy. Therefore, a thorough comparison of our findings with reported studies of organ-specific outcomes of ERT in male patients (that did not use a treatment-naive comparison group or period)[13] is difficult to perform. Although direct comparison with other studies is not possible, our findings support the general understanding of the beneficial effect of ERT in Fabry patients. It is noteworthy that including a pre-agalsidase beta treatment period provides stronger evidence of long-term effects and avoids uncertainty caused by a lack of appropriate comparison.

Strengths and limitations should be weighed carefully. The current study compared pretreatment and post-treatment clinical assessments and had a relatively long follow-up time and repeated assessments in both periods. Nevertheless, it had a limited number of patients, warranting caution in interpreting the results. In addition, despite employing self-control comparisons among the same populations and adjusting for certain confounders, we cannot rule out residual confounding as an explanation for our findings. Cardiovascular and/or renal risk factors (e.g. hypercholesterolaemia, obesity, tobacco use, and physical activity) as well as comorbid conditions (e.g. diabetes mellitus and arterial hypertension) and co-medication use (other than ACEi/ARBs) were not analysed because of limited available data in the Fabry Registry; this may have influenced the outcomes. However, our findings of cardiomyopathy and kidney function stabilization may result from the interaction between treatment and the co-existing pathologies caused by Fabry disease and the well-known risk factors. We lacked data on X-chromosome inactivation profiles, residual α-Gal activity and the timing of

ACEi/ARBs initiation, and the uniformity in their use, including dose and titration. Incomplete data sets for left ventricular mass (indexed) and systolic function and evidence of cardiac fibrosis (an important prognostic factor) precluded meaningful analysis of these parameters. In addition, we did not capture the evolution of echocardiographic techniques over time (most of the echocardiographic examinations were performed after 2001) and non-standardized evaluations may have resulted in measurement errors. Nonetheless, we did not expect significant inconsistency. Furthermore, female patients enrolled in the Fabry Registry and initiated on treatment with agalsidase beta may have more advanced Fabry disease, which may limit the generalizability of the results to other female Fabry populations. Finally, Fabry disease has a remarkable allelic variability, and many *GLA* variants have unclassified phenotypes. Most of the *GLA* variants considered to be associated with later-onset phenotypes have not yet been well characterized, although cardiac involvement appears significant[31] and treatment outcome data are scarce. To minimize the impact of phenotype heterogeneity on the analyses, we excluded patients with *GLA* variants associated with later-onset phenotypes and variants previously reported to be of uncertain significance, and restricted the main analysis population to female patients with *GLA* variants associated with classic Fabry disease or variants not entered or classified in the fabry-database.org database and to female patients for whom variants were not reported to the Fabry Registry. These inclusion/exclusion criteria were supported by similarity between the demographics and baseline clinical characteristics of the overall female patient population having these variants who started agalsidase beta treatment at age $\geq$18 years and were followed-up in the Fabry Registry (*Table* S5).

Conclusions

Cardiac hypertrophy in female patients with Fabry disease did not progress after initiation of agalsidase beta treatment, in contrast to the increase during the pretreatment period. Additionally, the overall eGFR decline remained stable within the normal range of a healthy population during sustained follow-up in female patients in both pretreatment and post-treatment periods. These findings suggest a beneficial effect of agalsidase beta treatment in female patients on cardiomyopathy and kidney function. Nevertheless, given the scarcity of published studies that used a treatment-naive comparison group or period and the limitations of the current study, the associations between agalsidase beta and long-term organ-specific outcomes merit caution in their interpretations and require further evaluations in female patients with Fabry disease.

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Conflict of interest

C.W. reported receiving Advisory Board honoraria from Sanofi Genzyme, consulting honoraria from Idorsia and Sanofi Genzyme, and speaker honoraria from Sanofi Genzyme and Takeda. U.F-R reported receiving Advisory Board honoraria from Amicus Therapeutics, Freeline, Sanofi Genzyme, and Takeda, and speaker honoraria from Amicus Therapeutics, Sanofi Genzyme, and Takeda. A.J. reported receiving a research grant from Amicus Therapeutics, Advisory Board honoraria from Amicus Therapeutics, Sanofi Genzyme, and Takeda, speaker honoraria from Amicus Therapeutics, BioMarin, and Sanofi Genzyme, and travel support from Amicus Therapeutics and Sanofi Genzyme. A.L. reported receiving consulting honoraria from Amicus Therapeutics, Sanofi Genzyme, and Takeda, and speaker honoraria and travel support from Sanofi Genzyme and Takeda. M.Y. and E.P. are full-time employees of Sanofi Genzyme. E.B. reported receiving Advisory Board honoraria from Amicus Therapeutics, Greenovation, Sanofi Genzyme, and Takeda, speaker honoraria from Amicus Therapeutics, Sanofi Genzyme, and Takeda; and travel support from Amicus Therapeutics. D.P.G. reported receiving consulting honoraria from Sanofi Genzyme and Takeda, and speaker honoraria and travel support from Amicus Therapeutics, Sanofi Genzyme, and Takeda. D.A.H. reported receiving Advisory Board honoraria, speaker honoraria, and travel support from Amicus Therapeutics, Protalix, Sanofi Genzyme, and Takeda. J.L.J. reported receiving Advisory Board honoraria from Sanofi Genzyme. A.M.M. reported receiving Advisory Board honoraria from BioMarin and Sanofi Genzyme, and speaker honoraria and travel support from Alexion, BioMarin, and Sanofi Genzyme. A.N. reported receiving speaker honoraria and travel support from Amicus Therapeutics, Sanofi Genzyme, and Takeda. B.V. reported receiving Advisory Board honoraria from Sanofi Genzyme, and speaker honoraria and travel support from Greenovation, Sanofi Genzyme, and Takeda. F.W. reported receiving speaker honoraria and travel support from Amicus Therapeutics, Sanofi Genzyme, and Takeda. M.L.W. reported receiving speaker honoraria and travel support from Amicus Therapeutics, Sanofi Genzyme, and Takeda. A.O. reported

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Estimated slopes of LVPWT, IVST, and eGFR pre- and post-agalsidase beta after adjusting for potentially confounding effects of age at first treatment, renal involvement, or use of ACEi/ARBs.

Table S2. Characteristics of the adult female patients in the Fabry Registry included the subgroup analyses of renal involvement or use of ACEi/ARBs.

Table S3. Summary statistics of LVPWT, IVST, and eGFR yearly assessments during 5 years pre- and 5 years post-initiation of agalsidase beta treatment.

Table S4A. LVPWT adult female patient population in the Fabry Registry stratified by renal involvement or use of ACEi/ARBs during 5 years pre- and 5 years post-initiation of agalsidase beta treatment.

Table S4B. IVST adult female patient population in the Fabry Registry stratified by renal involvement or use of ACEi/ARBs during 5 years pre- and 5 years post-initiation of agalsidase beta treatment.

Table S4C. eGFR adult female patient population in the Fabry Registry stratified by renal involvement or use of ACEi/ARBs during 5 years pre- and 5 years post-initiation of agalsidase beta treatment.

Table S5. Demographics and clinical characteristics by phenotype: overall adult female patient population in the Fabry Registry starting agalsidase beta 0.9–1.1 mg/kg every other week at age ≥18 years.

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