Enzyme replacement therapy in Fabry disease, towards individualized treatment

Arends, M.

Publication date
2017
Document Version
Other version
License
Other

7

Favourable effect of early versus late start of enzyme replacement therapy on plasma globotriaosylsphingosine levels in men with classical Fabry disease

Maarten Arends, Frits A. Wijburg, Christoph Wanner, Frédéric M. Vaz, André B.P. van Kuilenburg, Derralynn A. Hughes, Marieke Biegstraaten, Atul Mehta, Carla E.M. Hollak, Mirjam Langeveld

Molecular Genetics and Metabolism (2017), in press

Abstract

Background: The level of plasma globotriaosylsphingosine (lysoGb3) is an indication of disease severity in Fabry disease (FD) and its decrease during enzyme replacement therapy could be a reflection of treatment efficacy. Early treatment of FD may improve clinical outcome, but data to support this hypothesis are scarce. In this study we compared lysoGb3 decrease after ERT initiation in men with classical FD who started ERT before the age of 25 (early-treatment) with those who started later in life (late-treatment).

Methods: Treatment naïve men with classical FD from three centers of excellence in Europe were included. Measurements of lysoGb3 levels by tandem mass spectroscopy and antibodies by an inhibitory assay were performed in a single laboratory. Results were adjusted for lysoGb3 at baseline, first ERT (*i.e.* agalsidase alfa or beta) and the average ERT dose.

Results: 85 patients were included, 21 in the early-treatment and 64 in the late-treatment group. LysoGb3 level at baseline was not different between the two groups (112 vs 114 nmol/l, p=0.92). The adjusted odds ratio for reaching a lysoGb3 level <20 nmol/L was 7.38 for the early-treatment versus late-treatment group (95% CI: 1.91 – 34.04, p=0.006). The adjusted lysoGb3 levels one year after ERT initiation was 12.9 nmol/l lower in the early-treatment (95% CI: -20.1 – -5.8, p<0.001) compared to the late-treatment group.

Conclusion: The current retrospective cohort study shows that initiation of ERT at younger age in men with classical Fabry disease results in a better biochemical response.

Introduction

The optimal timing for of initiating enzyme replacement therapy (ERT) in Fabry disease (FD) patients is currently unclear.[1] When treatment is started in patients with considerable organ damage (impaired renal function and/or cardiac fibrosis), the disease tends to progress despite start of ERT.[2-4] Thus, it is frequently suggested that treatment before the occurrence of irreversible manifestations may provide better outcome[3,5]. This is of particular importance for classically affected men, who exhibit the most severe and progressive symptomatology. Classical FD is characterized by the presence of specific symptoms, most importantly cornea verticillata, angiokeratoma and neuropathic pain.[6] In men with classical FD alpha galactosidase (aGAL, enzyme commission number: 3.2.1.22) activity is very low or absent and plasma globotriaosylsphingosine (lysoGb3) levels are high.[7] Women and men with non-classical FD usually have a more variable and milder disease course, biochemically characterized by the presence of residual enzyme activity and only slight to modest elevations in lysoGb3.[7,8] The most prominent decrease in plasma lysoGb3 levels is observed in men with a classical FD phenotype.[9] We hypothesize that the magnitude of the decrease in plasma lysoGb3 levels depends on the timing of start of treatment and that the decrease would be greater in those patients who start ERT early versus those in whom treatment was initiated later in life. To test this hypothesis, the lysoGb3 response was investigated in two groups of men with a classical FD phenotype: those who started ERT before the age of 25 and those who started treatment later in life.

Methods

This investigation is part of the multicenter retrospective cohort study on FD supported by the Dutch Government to establish appropriate use of ERT.[10] Data from three European centers of excellence for the treatment of FD (Academic Medical Center, the Netherlands; Royal Free London NHS Foundation Trust, United Kingdom and the University Hospital Wuerzburg, Germany) were entered in a single database. This database contains historical medical data retrieved from patient records and clinical letters, as well as prospectively collected data from the first visit to the center onwards.

From this database a selection was made for this retrospective cohort study using the following criteria: treatment naïve men, definite diagnosis of FD according to previously developed criteria, and classical FD phenotype.[11] A classical phenotype in men was defined as: 1) low aGAL activity (<5% of reference mean) and the presence of one or more characteristic FD symptoms (Fabry neuropathic pain, the presence of clustered angiokeratoma and/or cornea verticillata). A detailed description of this classification method has been published before.[6,8]

Patients had to be treated for at least two months at the time of data collection, with either agalsidase alfa or agalsidase beta. Two sets of patients were identified: 1) patients who started treatment before the age of 25 (early-treatment) and 2) patients who started treatment at any point after the age of 25 (late-treatment). The cut-off of 25 years was chosen because previous studies have shown that before this age severe clinical disease manifestations are scarcely present.[8,12]

Baseline was defined as start of ERT, last included time point was at discontinuation of ERT, the last recorded visit or death. If the patients were switched from one to the other ERT type (agalsidase alfa to agalsidase beta or vice versa), data were still included in the analysis.

Biochemistry

Plasma lysoGb3 levels were measured at the laboratory Genetic Metabolic Diseases in the Academic Medical Center using an (adjusted) tandem mass spectrometry method with glycine labeled (all samples from the Royal Free Hospital and the University Clinic Würzburg, as well as all samples after August 2015 from the Academic Medical Center) or isotope labeled lysoGb3 (samples from before August 2015 from the Academic Medical Center) as an internal standard.[13] There was a good correlation between results using the different internal standards (intraclass correlation coefficient 0.98, 95% CI: 0.97-0.99; p<0.001).[8] Samples were stored at -80 ^{0}C in all centers, exploratory analysis showed that lysoGb3 is very stable over time (unpublished data) . The presence of neutralizing antibodies against ERT was determined as previously described.[14] To assess neutralizing activity different dilutions of serum were incubated with a standard amount of recombinant agalsidase A. The serum dilution that resulted in 50% reduction of the enzyme activity was determined. A titer of ≥6 was considered as antibody positive.[14]

Clinical parameters

Clinical event rate, cardiac mass and renal function were assessed. Clinical events were defined as follows: end stage renal disease, stroke, TIA, cardiac events (atrial fibrillation, admission for any rhythm disturbance or congestive heart failure, implantation of an implantable cardiac defibrillator (ICD) or pacemaker (PM), myocardial infarction, coronary artery bypass graft surgery or a percutaneous transluminal angioplasty intervention) and death.

Renal function was evaluated by the estimated glomerular filtration rate (eGFR) and the amount of protein excretion in urine. The eGFR was calculated using the CKD-EPI in adults [15] and the Schwartz formula in children up to 18 years of age [16]Albuminuria and proteinuria excretion was categorized following Kidney Disease Improving Global Outcomes guidelines [15] The left ventricular mass index (LVMI) was calculated by the Devereux formula on the basis of echocardiography measurements and adjusted for height ($m^{2.7}$). The upper reference limit for adult men is 48 gram/$m^{2.7}$).[17]

Statistical analysis

R (version 3.3.1) was used for statistical analyses. Data are presented as median and range. Logistic regression was used to compare the proportion of patients who reached a lysoGb3 level <20 nmol/l at the measurement closest to the time point 1 year after ERT initiation. A linear mixed effect model (package: nlme) was used to analyze lysoGb3 levels after initiation over time. The average received ERT dose was categorized into <0.4 mg/kg every other week (EOW), 0.4 – 0.8 mg/kg EOW and >0.8 mg/kg EOW. Mixed effect model assumptions were visually tested by diagnostic plots. Variance inflation factor (VIF) was used to explore potential multicollinearity. Models were selected in a combined expert judgement and stepwise manner, and the Akaike Information Criterion (AIC) was used to evaluate the goodness of fit. P-values <0.05 were considered statistically significant. Where appropriate 95% confidence intervals (95% CI) are given.

Results

At the three treatment centers a total of 147 men with classical FD had started ERT since 1999. Sixty-two were excluded from the analysis because of missing baseline and/or follow-up lysoGb3 measurements (supplemental material A). Data of 85 men with classical FD were included, baseline characteristics of the patients are given in table 1. In 21 patients ERT was started before the age of 25 (early-treatment group), at a median age of 18.0 years. In the 64 patients who started treatment after the age of 25 (late-treatment group) the median age of ERT start was 41.7. There were no differences between both groups in first treatment modality (p=1.0). The median follow-up time in the early-treatment group was 6.7 years and 5.4 years in the late-treatment group (p=0.29). During the follow up the average received ERT dose in the early-treatment group was 0.5 (0.2 – 1.0) mg/kg/EOW compared with 0.7 (0.2 – 1.0) mg/kg/EOW in the late-treatment group (p=0.17).

At treatment initiation, four early-treated patients received a 0.5 mg/kg/EOW or equivalent dose of agalsidase beta in the context of the FIELD study,[18] and six patients in the late-treatment group received a 0.2 mg/kg/EOW dose of agalsidase beta during an ERT comparison trial.[19] ERT type or dose was changed in five early-treated patients and 34 late-treated patients (p=0.02).

Table 1 Patient characteristics at start of ERT

	Initiation of ERT <25	Initiation of ERT ≥25	p-value
Patients	21	64	
Age at start ERT (years)	18.0 (9.5-24.6)	41.7 (25.0-64.9)	<0.001
Agalsidase alfa as first ERT	6 (29%)	17 (27%)	0.99
Agalsidase beta as first ERT	15 (71%)	47 (73%)	0.99
Missense mutation	10/21 (48%)	32/64 (50%)	0.99
Nonsense mutation	11/21 (52%)	27/64 (42%)	0.46
Splice site mutation	0/21 (0%)	5/64 (8%)	0.33
aGAL activity (% of mean reference)	1.1 (0.0-8.6%)	2.9 (0.0-8.6%)	0.02
LysoGb3 before ERT (nmol/l)	114 (84-124)	112 (32-175)	0.92
Acroparesthesia	21/21 (100%)	59/62 (95%)	0.57
Angiokeratoma	12/20 (60%)	53/64 (83%)	0.06
Cornea verticillata	14/19 (74%)	39/61 (64%)	0.58
eGFR (ml/min/1.73m^2)	125 (89-139)	87 (10-136	<0.001
CKDA category A2 or higher	9/19 (47%)	31/37 (84%)	0.02
CKDA category A3	0/19 (0%)	24/37 (65%)	<0.001
LVMI (gram /m$^{2.7}$)	34 (21-64)	52 (24-150)	<0.001
WML	5/16 (31%)	15/18 (83%)	0.004
Clinical event(s) before ERT	1/21 (5%)	23/64 (36%)	<0.001

Data are presented as median and range, CKD category A2 is defined as AER: 30-300 mg/day or equivalent, CKD category A3 is defined as AER >300 gram/day or equivalent. LVMI: left ventricular mass index measured by echocardiography (upper reference limit for adult men is 48 gram/m$^{2.7}$),[17] Clinical events were defined as follows: end stage renal disease, stroke, TIA and cardiac events (atrial fibrillation, admission for any rhythm disturbance or congestive heart failure, implantation of an implantable cardiac defibrillator (ICD) or pacemaker (PM), myocardial infarction, coronary artery bypass graft surgery or a percutaneous transluminal angioplasty intervention) and death. Missing values (percentage): angiokeratomen (1%), acroparesthesia (2%), cornea verticillata (6%), eGFR (1%), CKDA category (60%), LVMI (9%), WML (60%). Mutations, enzyme activity and lysoGb3 per patient can be found in supplemental material B.

LysoGb3

Plasma lysoGb3 values at baseline did not differ between the early-treatment (114 nmol/l) and late-treatment (112 nmol/l) groups (p=0.92). There was a decrease in plasma lysoGb3 in all patients after ERT start (figure 1). The median lysoGb3 determined at the time point closest to 1 year after ERT initiation (figure 2) was lower for the early-treatment compared with the late-treatment group (24.4 vs 27.7 nmol/l, p=0.04). In addition, 10/21 (48%) patients in the early-treatment group reached a lysoGb3 concentration <20 nmol/l compared to 15/64 (22%) in the late-treatment group. Thus, the odds ratio for a lysoGb3 <20 nmol/L in early versus late-treatment group was 2.93 (95% CI: 0.92 – 9.39, p=0.052). When adjusted for lysoGb3 at baseline, first ERT preparation and the average ERT dose, the adjusted OR was 7.38 (95% CI: 1.91 – 34.04, p=0.006). Inclusion of ERT type change did not result in a better fit of the model nor was it associated with lysoGb3 values during treatment. ERT dose, as categorical variable was included in the model as covariate, since it did have a significant influence on lysoGb3 values.

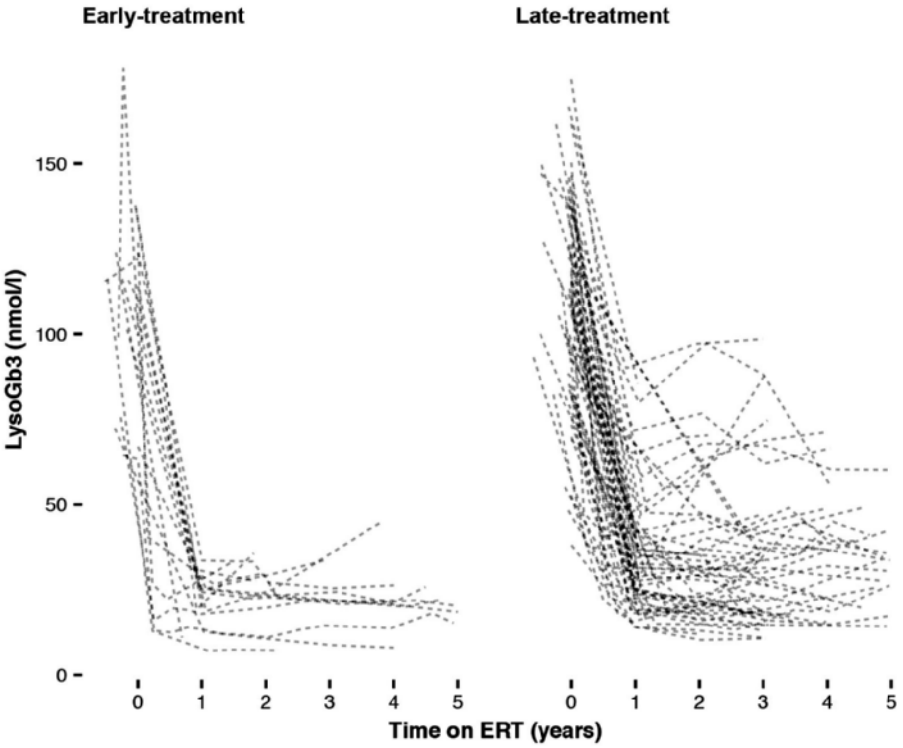


Figure 1 LysoGb3 over time for early-treatment and late-treatment patients

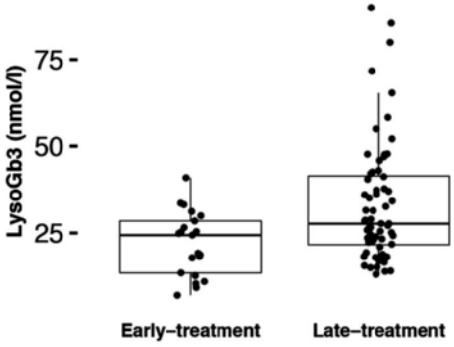


Figure 2 LysoGb3 one year after ERT initiation or closest time point

In order to adjust for differences in lysoGb3 at baseline, first ERT preparation and the average ERT dose longitudinal lysoGb3 data was analyzed in a linear mixed model. Which showed that adjusted lysoGb3 values of the early-treatment group were 12.9 nmol/l lower (95% CI: -20.1 – -5.8, p<0.001) compared to the late-treatment group.

Antibodies

Data of antibody levels were missing in one early-treated and two late-treated patients. Thirteen out of the 20 (65%) early-treated patients never developed antibodies, compared to 24 out of 64 (39%) of the late-treated patients. A persistent positive antibody (only positive antibody measurements during follow-up) response was observed in 4 out of 20 (20%) and 23 out of 64 (37%) of the early-treated versus late-treated patients. In addition, 3 out of 20 (15%) early-treated and 15 out of the 64 (24%) late-treated patients had a mixed antibody response over time (negative as well as positive antibody measurements). There was a trend towards a higher proportion of patients with a persistent or mixed antibody response in the late-treatment group (38 out of 62, 61%) compared with the early-treatment group (7 out of 20, 35%) (OR: 2.9, 95% CI: 0.92 – 9.91, p=0.07). When adjusted for lysoGb3 at baseline, average dose and first ERT type similar results were found (OR: 3.7, 95% CI: 1.14 – 13.27, p=0.03). The difference in lysoGb3 response between the early and late treated patients was not explained by the presence of antibodies since inclusion of the presence of antibodies as covariate in the mixed model of the lysoGb3 response did not change the results substantially (β: 9.8 nmol/l, 95% CI:-16.8 – 2.6, p=0.008).

Clinical outcomes

One patient in the early-treatment group (5%) had an ischemic stroke 12 years after initiation of ERT at age 30 (7 events per 1000 patient years). In the late-treatment group 36 out of 63 patients (57%) developed one or more clinical events (114 events per 1000 patient years). The first events were cardiac in 15, cerebral in 11, renal in 8 and death in 2 patients. No one in the early-treatment group died, but 10 patients in the late-treatment cohort died during follow-up. The median time from start ERT to first event in the later-treated group was 3.5 years (range 0.1 – 13.0), and the median age at the time of first event was 48 years (range 28-66).

Comparison of eGFR (supplemental material C) and LVMI (supplemental material D) did not yield meaningful results. This is due to the following factors: 1. eGFR is calculated using a different formula for pediatric and adult patients; 2. the apparent decline in eGFR during adolescence can be caused reduction in hyperfiltration and alteration in body composition; 3. cardiac mass may also be influenced by changes in growth and maturation during adolescence; and 4. at baseline, there was only one patient with LVH in the early-treatment group, thus reduction of LVH could not be studied in this group.

Discussion

In the current study in men with classical FD, start of ERT before the age of 25 results in a greater reduction of plasma lysoGb3 compared to those patients that start treatment later in life. Furthermore, the risk of developing antibodies may be lower when ERT is initiated at an earlier age.

LysoGb3 is most likely formed by deacetylation of Gb3 by the lysosomal enzyme acid ceramidase.[20] This is supported by results from a mouse study which suggest that the intracellular accumulated lysoGb3 has an endogenous origin.[21] In untreated pediatric patients, podocyte Gb3 inclusions increase progressively with age. Although plasma lysoGb3 levels were similar in both age groups in the current study, one could speculate, based on the pediatric podocyte findings, that intracellular lysoGb3 storage increases with age. This may explain why later treatment initiation results in less effective lysoGb3 clearance. An alternative explanation for the observed difference in lysoGb3 response might be the lower proportion of patients which developed antibodies against the recombinant enzyme in the early versus the late-treatment group. However, our multivariate model did not support this hypothesis.

The higher lysoGb3 levels during ERT in the late-treated group may reflect a greater residual disease burden, putting these patients at greater risk of clinical events. Disease progression despite ERT initiation is indeed observed in FD patients in whom organ damage (*e.g.* myocardial fibrosis, reduced kidney function) is already present at start of ERT.[4,22,23]
In Gaucher disease, plasma biomarker levels (chitotriosidase activity) correlate well with the amount of residual disease (Gaucher cell burden) and the incidence rate of clinical events.[24] We speculate that this may also be the case for FD. However, although the current study evaluates the biochemical and not the clinical response per se, previous studies show a relationship between plasma (lyso)Gb3, storage in tissue and clearance from affected organs.[18,25] In older patients with irreversible disease, no relation can be expected between clearance of plasma lysoGb3, but for young patients, we hypothesize that a decline in lysoGb3 is a predictor of preservation of organ function.

All data and samples analyzed in this study were collected retrospectively and results reflect real life treatment settings. Consequently, patients used different ERT formulations and different doses, potentially influencing the reduction of lysoGb3 and the risk of antibody development. However, the effect of age at start of ERT on plasma lysoGb3 levels and on the risk to develop antibodies did not change when adjusting for these factors.

To confirm an improved clinical outcome in patients who start ERT earlier in life, large studies with a long follow-up are needed. Not surprisingly, events were almost absent in the early-treated patients and common in the late-treated patients. Comparison of the effect on ERT on renal function, cardiac mass or risk of events between the early and late-treatment

group in the current study would not generate meaningful data, since the occurrence of these disease complications are highly age dependent.

Even in the patients who started treatment under the age of 25, a significant proportion already showed signs of organ involvement. In one third of these patients, one or more WML were found on cerebral MRI. The presence of early signs of cerebrovascular disease in children has been described before.[18,26] In almost half of the early-treated patients albuminuria was present, those patients with albuminuria were all older than 16 year of age. This may mean that for some patients, there may be a need to start ERT even before age 25 to prevent organ damage. Identifying these patients remains a challenge, as lifelong treatment of patients who will not benefit significantly needs to be avoided.

Recently the results of a 6.5 year open-label study in 11 children treated with agalsidase alfa (mean age at start treatment ±12 years) were published, showing that agalsidase alfa was well tolerated and in these patients cardiac disease remained stable. However, without an untreated control group no solid conclusions can be drawn on the effectiveness of ERT in these patients. The results of the FIELD study investigating the effect of agalsidase beta in pediatric patients are not yet published, but this study also lacks an untreated control group.[18]

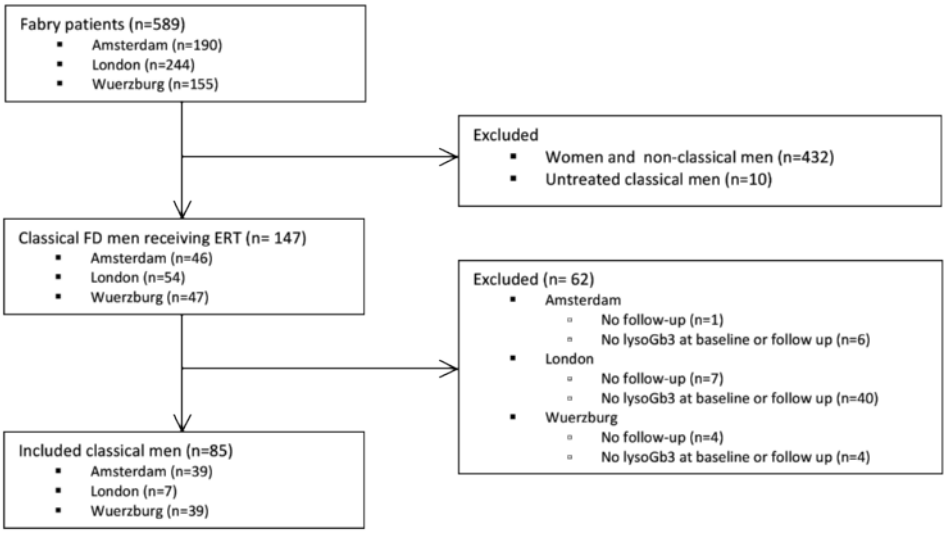
In conclusion, results from the current retrospective cohort study suggest that initiation of ERT at younger age in men with classical FD results in a better biochemical response.

References

1. Schiffmann, R. *et al.* Screening, diagnosis, and management of patients with Fabry disease: conclusions from a "Kidney Disease: Improving Global Outcomes" (KDIGO) Controversies Conference. *Kidney international* **91**, 284-293, doi:10.1016/j.kint.2016.10.004 (2017).
2. Lenders, M. *et al.* Renal function predicts long-term outcome on enzyme replacement therapy in patients with Fabry disease. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association*, doi:10.1093/ndt/gfw334 (2016).
3. Weidemann, F. *et al.* Long-term outcome of enzyme-replacement therapy in advanced Fabry disease: evidence for disease progression towards serious complications. *Journal of internal medicine* **274**, 331-341, doi:10.1111/joim.12077 (2013).
4. Banikazemi, M. *et al.* Agalsidase-beta therapy for advanced Fabry disease: a randomized trial. *Annals of internal medicine* **146**, 77-86 (2007).
5. Rombach, S. M. *et al.* Long term enzyme replacement therapy for Fabry disease: effectiveness on kidney, heart and brain. *Orphanet journal of rare diseases* **8**, 47, doi:10.1186/1750-1172-8-47 (2013).
6. van der Tol, L. *et al.* Uncertain diagnosis of fabry disease in patients with neuropathic pain, angiokeratoma or cornea verticillata: consensus on the approach to diagnosis and follow-up. *JIMD reports* **17**, 83-90, doi:10.1007/8904_2014_342 (2014).
7. Smid, B. E. *et al.* Plasma globotriaosylsphingosine in relation to phenotypes of Fabry disease. *Journal of medical genetics* **52**, 262-268, doi:10.1136/jmedgenet-2014-102872 (2015).
8. Arends, M. *et al.* Characterization of Classical and Nonclassical Fabry Disease: A Multicenter Study. *Journal of the American Society of Nephrology : JASN* **in press**, doi:10.1681/ASN.2016090964 (2017).
9. van Breemen, M. J. *et al.* Reduction of elevated plasma globotriaosylsphingosine in patients with classic Fabry disease following enzyme replacement therapy. *Biochimica et biophysica acta* **1812**, 70-76, doi:10.1016/j.bbadis.2010.09.007 (2011).
10. ZonMW. *Treatment of patients with Fabry disease with agalsidase alfa and agalsidase beta: phenotypic diversity necessitates the development of individualized treatment guidelines.* https://www.zonmw.nl/nl/onderzoek-resultaten/doelmatigheidsonderzoek/programmas/project-detail/goed-gebruik-geneesmiddelen/treatment-of-patients-with-fabry-disease-with-agalsidase-alfa-and-agalsidase-beta-phenotypic-divers/> (2013).
11. Smid, B. E. *et al.* Uncertain diagnosis of Fabry disease: Consensus recommendation on diagnosis in adults with left ventricular hypertrophy and genetic variants of unknown significance. *International journal of cardiology* **177**, 400-408, doi:10.1016/j.ijcard.2014.09.001 (2014).
12. Germain, D. P. *et al.* Analysis of left ventricular mass in untreated men and in men treated with agalsidase-beta: data from the Fabry Registry. *Genetics in medicine : official journal of the American College of Medical Genetics* **15**, 958-965, doi:10.1038/gim.2013.53 (2013).
13. Gold, H. *et al.* Quantification of globotriaosylsphingosine in plasma and urine of fabry patients by stable isotope ultraperformance liquid chromatography-tandem mass spectrometry. *Clin Chem* **59**, 547-556, doi:10.1373/clinchem.2012.192138 (2013).
14. Linthorst, G. E., Hollak, C. E., Donker-Koopman, W. E., Strijland, A. & Aerts, J. M. Enzyme therapy for Fabry disease: neutralizing antibodies toward agalsidase alpha and beta. *Kidney international* **66**, 1589-1595, doi:10.1111/j.1523-1755.2004.00924.x (2004).
15. Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group. KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. *Kidney International Supplements* **3**, 1-150 (2013).
16. Schwartz, G. J. *et al.* New equations to estimate GFR in children with CKD. *Journal of the American Society of Nephrology : JASN* **20**, 629-637, doi:10.1681/ASN.2008030287 (2009).
17. Lang, R. M. *et al.* Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the

European Society of Cardiology. *Journal of the American Society of Echocardiography : official publication of the American Society of Echocardiography* **18**, 1440-1463, doi:10.1016/j.echo.2005.10.005 (2005).
18. Wijburg, F. A. *et al.* Characterization of early disease status in treatment-naive male paediatric patients with Fabry disease enrolled in a randomized clinical trial. *PloS one* **10**, e0124987, doi:10.1371/journal.pone.0124987 (2015).
19. Vedder, A. C. *et al.* Treatment of Fabry disease: outcome of a comparative trial with agalsidase alfa or beta at a dose of 0.2 mg/kg. *PloS one* **2**, e598, doi:10.1371/journal.pone.0000598 (2007).
20. Ferraz, M. J. *et al.* Lysosomal glycosphingolipid catabolism by acid ceramidase: formation of glycosphingoid bases during deficiency of glycosidases. *FEBS letters* **590**, 716-725, doi:10.1002/1873-3468.12104 (2016).
21. Ferraz, M. J. *et al.* Lyso-glycosphingolipid abnormalities in different murine models of lysosomal storage disorders. *Molecular genetics and metabolism* **117**, 186-193, doi:10.1016/j.ymgme.2015.12.006 (2016).
22. Weidemann, F. *et al.* Long-term effects of enzyme replacement therapy on fabry cardiomyopathy: evidence for a better outcome with early treatment. *Circulation* **119**, 524-529, doi:10.1161/CIRCULATIONAHA.108.794529 (2009).
23. Warnock, D. G. *et al.* Renal outcomes of agalsidase beta treatment for Fabry disease: role of proteinuria and timing of treatment initiation. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association* **27**, 1042-1049, doi:10.1093/ndt/gfr420 (2012).
24. van Dussen, L. *et al.* Value of plasma chitotriosidase to assess non-neuronopathic Gaucher disease severity and progression in the era of enzyme replacement therapy. *Journal of inherited metabolic disease* **37**, 991-1001, doi:10.1007/s10545-014-9711-x (2014).
25. Eng, C. M. *et al.* A phase 1/2 clinical trial of enzyme replacement in fabry disease: pharmacokinetic, substrate clearance, and safety studies. *American Journal of Human Genetics* **68**, 711-722 (2001).
26. Cabrera-Salazar, M. A., O'Rourke, E., Charria-Ortiz, G. & Barranger, J. A. Radiological evidence of early cerebral microvascular disease in young children with Fabry disease. *The Journal of pediatrics* **147**, 102-105, doi:10.1016/j.jpeds.2005.03.004 (2005).

Supplemental material A



Supplemental figure A1 CONSORT Diagram

Supplemental material B

Supplemental table B1 Early-treated patients

Patient	Mutation		Enzyme activity		Reference range enzyme activity	LysoGb3
1.01	D136Y	c.406G>T	0	nmol/mg protein/hour	17 - 55	123.9
1.02	D136Y	c.406G>T	0	nmol/mg protein/hour	17 - 55	67
1.03	D136Y	c.406G>T	0	nmol/mg protein/hour	17 - 55	94.4
1.04	D136Y	c.406G>T	0	nmol/mg protein/hour	17 - 55	126.8
1.05	D170N	c.508G>A	0.02	nmol/min/mg	0.4 - 1	107.8
1.06	G183D	c.548G>A	1.2	nmol/mg protein/hour	50 - 100	72.4
1.07	G260E	c.779G>A	0.5	nmol/mg protein/hour	15 - 45	116
1.08	G373D	c.1118G>A	0.2	nmol/mg protein/hour	32 - 70	124
1.09	I268fs	c.757delA	0.04	nmol/min/mg	0.4 - 1	137.8
1.10	L268S	c.803T>C	0.3	nmol/mg protein/hour	32 - 60	148.6
1.11	N53fs*57	c.157_160delAACC	0.2	nmol/mg protein/hour	20 - 65	136.8
1.12	N53fs*57	c.157_160delAACC		absent - very low		122.1
1.13	Q312*	c.943C>T	0.06	nmol/min/mg	0.4 - 1	84.2
1.14	R227*	c.679C>T	0.6	nmol/mg protein/hour	33 - 77	123.5
1.15	R301*	c.901C>T	0.8	nmol/mg protein/hour	32 - 60	75.6
1.16	R301*	c.901C>T	0.5	nmol/mg protein/hour	32 - 60	63.3
1.17	R301*	c.901C>T	2.1	nmol/mg protein/hour	32 - 60	110
1.18	R332fs*7	c.994dupA	0.03	nmol/min/mg	0.4 - 1	101.4
1.19	R342Q	c.1025G>A	0.3	nmol/mg protein/hour	32 - 60	83.9
1.20	W226*	c.677G>A	0.5	nmol/mg protein/hour	20 - 60	177.8
1.21		duplication exon 3+4	0.2	nmol/mg protein/hour	32 - 70	114

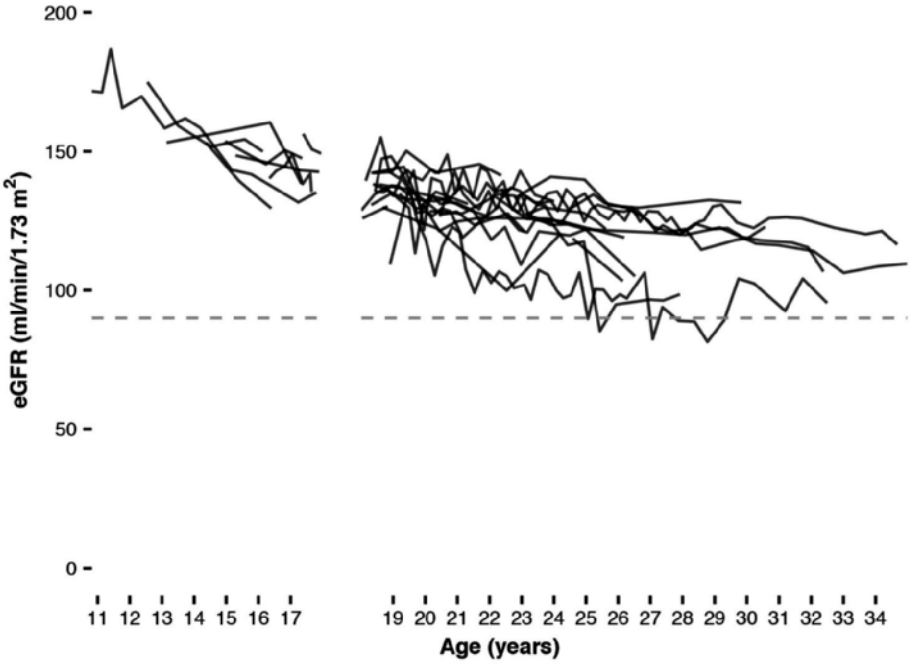
Supplemental table B2 Late-treated patients

Patient	Mutation		Enzyme activity		Reference range enzyme activity	LysoGb3
2.01	A135V	c.404C>T	0.02	nmol/min/mg	0.4 - 1	146
2.02	A13P	c.37G>C	0.2	nmol/h/mg	4 - 21.9	31.8
2.03	C63Y	c.188G>A	0.02	nmol/min/mg	0.4 - 1	126.3
2.04	D136E	c.408T>A	0.02	nmol/min/mg	0.4 - 1	83.7
2.05	D165V	c.494A>T	0.02	nmol/min/mg	0.4 - 1	75.9
2.06	E341K	c.1021G>A	0.02	nmol/min/mg	0.4 - 1	117.5
2.07	E341K	c.1021G>A	0.03	nmol/min/mg	0.4 - 1	107.1
2.08	F18S	c.53T>C	1.1	nmol/mg protein/hour		61
2.09	G132E	c.395G>A	0.03	nmol/min/mg	0.4 - 1	136.9
2.10	G325fs*23	c.972delG	0.06	nmol/min/mg	0.4 - 1	96.1
2.11	G35R	c.103G>A	0.02	nmol/min/mg	0.4 – 1	85.4
2.12	I319fs*10	c.955_969delinsTTGC	0.1	nmol/mg protein/hour	15 - 45	100
2.13	I319fs*10	c.955_969delinsTTGC	0	nmol/mg protein/hour	15 - 45	69.1
2.14	K248fs	c.717-718delAA	0.02	nmol/min/mg	0.4 - 1	174.8
2.15	K248fs	c.717-718delAA	0.03	nmol/min/mg	0.4 - 1	146.5
2.16	L129P	c.368T>C	0.014	nmol/min/mg	0.4 - 1	138
2.17	L129P	c.368T>C	2.1	nmol MU/mg/h	33.2 - 109	81.9
2.18	L268S	c.803T>C	0	nmol/mg protein/hour	32 - 70	93.1
2.19	L311V	c.931C>G	0.06	nmol/min/mg	0.4 - 1	82.6
2.20	M187fs	c.560delT	0.02	nmol/min/mg	0.4 - 1	137.7
2.21	M72R	c.215T>G	0.1	nmol/mg protein/hour	32 - 60	52.7
2.22	P205T	c.614C>T	2.9	nmol/hr/mg ptn	33 - 134	62
2.23	Q357*	c.1069C>T	0.02	nmol/min/mg	0.4 - 1	133.6
2.24	Q386*	c.1156C>T	1	nmol/mg protein/hour	32 - 60	115.1
2.25	Q386*	c.1156C>T	0.1	nmol/mg protein/hour		88.4
2.26	Q416*	c.1246C>T	0.5	nmol/mg protein/hour	15 - 45	118.7
2.27	R112C	c.334C>T	0.02	nmol/mg protein/hour	23 - 58	82.3
2.28	R112C	c.334C>T	0.1	nmol/hr/nl	4 - 21.9	109.8
2.29	R220*	c.658C>T	0	nmol/mg protein/hour		102.3
2.30	R220*	c.658C>T	0.4	nmol/mg protein/hour		119.4
2.31	R220*	c.658C>T	0	nmol/mg protein/hour	17 - 55	115.3
2.32	R220*	c.658C>T	1	nmol/mg protein/hour	17 - 55	115.6

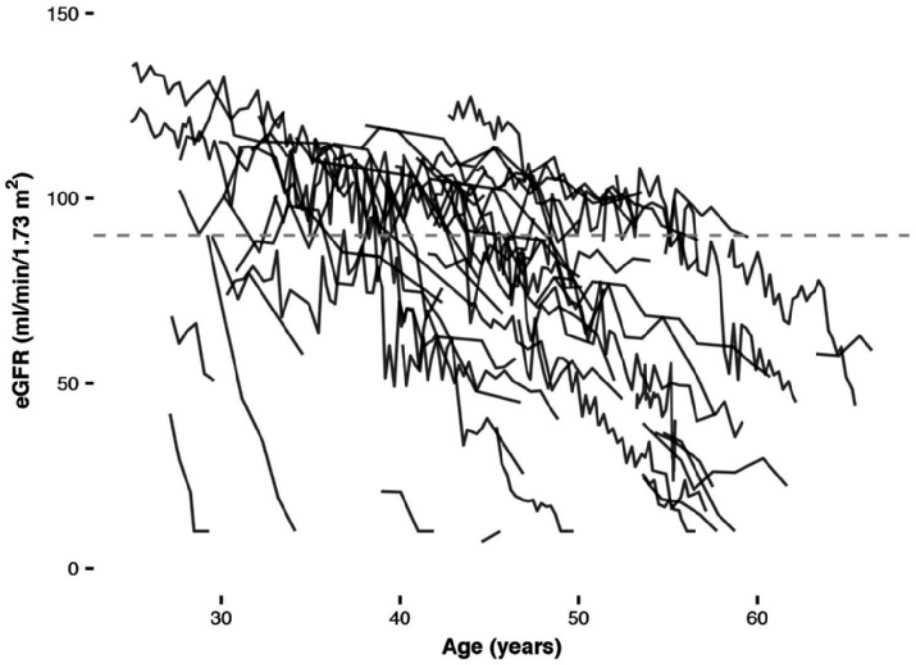
Supplemental table B2 Late-treated patients (Continued)

Patient	Mutation		Enzyme activity		Reference range enzyme activity	LysoGb3
2.33	R227*	c.679C>T	0.02	nmol/min/mg	0.4 - 1	161.6
2.34	R227*	c.679C>T	1.6	nmol/hr/mg ptn	33 - 134	126.9
2.35	R227*	c.679C>T	1.1	nmol/hr/mg ptn	33 - 134	122.3
2.36	R227*	c.679C>T	1.5	nmol/hr/mg ptn	33 - 124	105.8
2.37	R227Q	c.681G>A	0.1	nmol/mg protein/hour	32 - 60	96.2
2.38	R332fs*7	c.994dupA	0.02	nmol/min/mg	0.4 - 1	166.7
2.39	R342*	c.1027C>T	0.01	nmol/min/mg	0.4 - 1	173.1
2.40	R342L	c.1025G>T	0.03	nmol/min/mg	0.4 - 1	84.9
2.41	R342L	c.1025G>T	0.02	nmol/min/mg	0.4 - 1	82.2
2.42	R342Q	c.1025G>A	1	nmol/mg protein/hour	32 - 70	151
2.43	R342Q	c.1025G>A	2	nmol/mg protein/hour	50 - 130	124.3
2.44	R342Q	c.1025G>A	0.9	nmol/mg protein/hour	33 - 77	69.8
2.45	R342Q	c.1025G>A	0.3	nmol/mg protein/hour	17 - 55	111.6
2.46	R342Q	c.1025G>A	1.5	nmol/mg protein/hour	33 - 77	105.5
2.47	R342Q	c.1025G>A	0.4	nmol/mg protein/hour	15 - 45	150.3
2.48	R403*	c.1208-1211delTAA	0.03	nmol/min/mg	0.4 - 1	126.3
2.49	R49C	c.145C>T	0.013	nmol/min/mg	0.4 - 1	127.3
2.50	S374fs*29	c.1029-1030delTC	0.02	nmol/min/mg	0.4 - 1	98.8
2.51	T282I	c.845C>T	0.03	nmol/min/mg	0.4 - 1	48.3
2.52	T282I	c.845C>T	0.02	nmol/min/mg	0.4 - 1	38.1
2.53	V269A	c.806T>C				73.4
2.54	W204*	c.611G>A	0.03	nmol/min/mg	0.4 - 1	145.7
2.55	W226*	c.677G>A	1.2	nmol/mg protein/hour	18 - 50	92.6
2.56	W236C	c.708G>C	0.001	nmol/min/mg	0.4 - 1	161.7
2.57	W349*	c.1047G>A	0.02	nmol/min/mg	0.4 - 1	114.8
2.58	W399*	c.1196G>A	0.03	nmol/min/mg	0.4 - 1	142.1
2.59	W399*	c.1196G>A	0.025	nmol/min/mg	0.4 - 1	140.5
2.60		IVS6-2A>T (c.1000-2A>T)	0	nmol/mg protein/hour		100
2.61		IVS3+1G>A	5.8	nmol MU/mg/h	33.2 - 109	122.5
2.62		IVS3+1G>A	0.02	nmol/min/mg	0.4 - 1	111.7
2.63		IVS2+1G>A	0.02	nmol/min/mg	0.4 - 1	113.3
2.64		IVS6-10G>A	0.04	nmol/min/mg	0.4 - 1	55.1

Supplemental material C

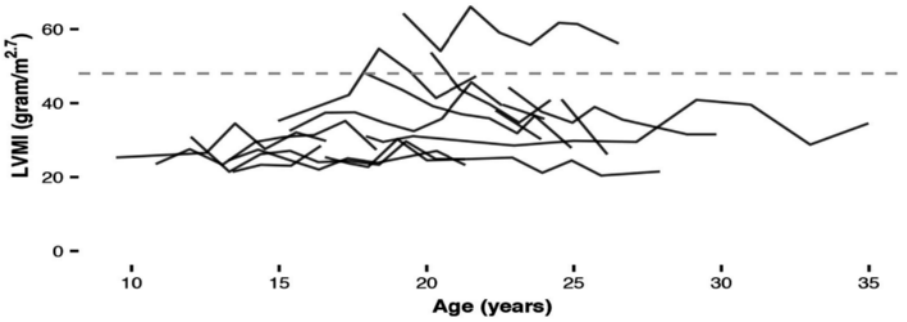


Supplemental figure C1 eGFR over time in early-treated patients. The dashed grey line represents an eGFR of 90 ml/min/1.73m^2

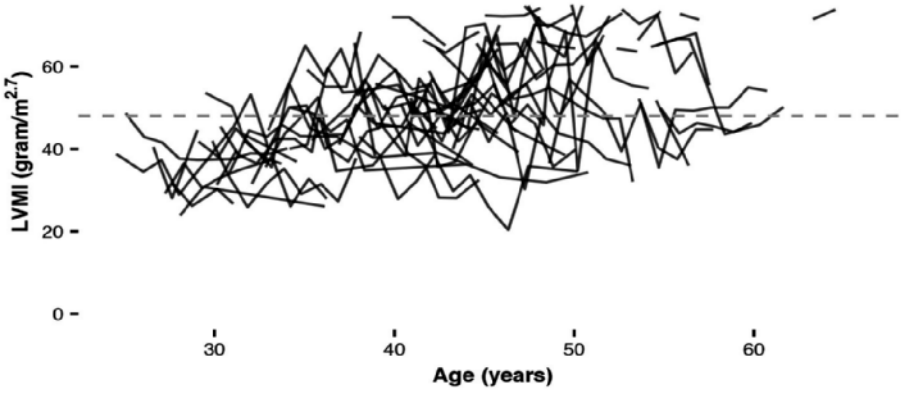


Supplemental figure C2 eGFR over time in late-treated patients. The dashed grey line represents an eGFR of 90 ml/min/1.73m^2

Supplemental material D



Supplemental figure D1 LVMI over time in early-treated patients. The dashed grey line represents the upper reference limit of normal in adult men (48 gram/$m^{2.7}$)



Supplemental figure D2 LVMI over time in late-treated patients. The dashed grey line represents the upper reference limit of normal in adult men (48 gram/$m^{2.7}$)