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

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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.

1. 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

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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.

2. 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.

2.1. 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 °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].

2.2. 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 $g/m^{2.7}$) [17].

2.3. 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.

3. 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),

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/1.73 m^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 ($g/m^{2.7}$)	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. ERT: enzyme replacement therapy, aGAL: alpha galactosidase, LysoGb3: globotriaosylsphingosine, eGFR: estimated glomerular filtration rate (CKD-EPI), CKDA: chronic kidney disease albuminuria categories, CKD category A2 is defined as AER: 30–300 mg/day or equivalent, CKD category A3 is defined as AER > 300 g/day or equivalent. LVMI: left ventricular mass index measured by echocardiography (upper reference limit for adult men is 48 $g/m^{2.7}$) [17], WML: white matter lesions. 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.