Correction: Assessment of plasma lyso-Gb3 for clinical monitoring of treatment response in migalastat-treated patients with Fabry disease

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Genetics inMedicine | ARTICLE

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Assessment of plasma lyso-Gb_3 for clinical monitoring of treatment response in migalastat-treated patients with Fabry disease

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Purpose: To assess the utility of globotriaosylsphingosine (lyso-Gb_3) for clinical monitoring of treatment response in patients with Fabry disease receiving migalastat.

Methods: A post hoc analysis evaluated data from 97 treatment-naive and enzyme replacement therapy (ERT)–experienced patients with migalastat-*amenable GLA* variants from FACETS (NCT00925301) and ATTRACT (NCT01218659) and subsequent open-label extension studies. The relationship between plasma lyso-Gb_3 and measures of Fabry disease progression (left ventricular mass index [LVMi], estimated glomerular filtration rate [eGFR], and pain) and the relationship between lyso-Gb_3 and incidence of Fabry-associated clinical events (FACEs) were assessed in both groups. The relationship between changes in lyso-Gb_3 and kidney interstitial capillary (KIC) globotriaosylceramide (Gb_3) inclusions was assessed in treatment-naive patients.

Results: No significant correlations were identified between changes in lyso-Gb_3 and changes in LVMi, eGFR, or pain. Neither baseline lyso-Gb_3 levels nor the rate of change in lyso-Gb_3 levels during treatment predicted FACE occurrences in all patients or those receiving migalastat for ≥24 months. Changes in lyso-Gb_3 correlated with changes in KIC Gb_3 inclusions in treatment-naive patients.

Conclusions: Although used as a pharmacodynamic biomarker in research and clinical studies, plasma lyso-Gb_3 may not be a suitable biomarker for monitoring treatment response in migalastat-treated patients.

Genetics in Medicine (2021) 23:192–201; https://doi.org/10.1038/s41436-020-00968-z

Key words: biomarker; clinical monitoring; Fabry disease; lyso-Gb_3; migalastat

INTRODUCTION

Fabry disease (OMIM 301500) is a rare, progressive X-linked lysosomal disorder caused by pathogenic variants in the α-galactosidase A gene (*GLA*), resulting in functional deficiency of α-galactosidase A (α-Gal A) and accumulation of glycosphingolipids within lysosomes, including globotriaosylceramide (Gb_3) and globotriaosylsphingosine (lyso-Gb_3).[1,2] Glycosphingolipids accrue in many cell types, such as capillary endothelial, renal, cardiac, and nerve cells[1,2] and several studies implicate activation of Toll-like receptors as a trigger of inflammatory and fibrotic cascades,[3] which ultimately lead to multisystem dysfunction and death from cardiac disease, renal failure, or cerebrovascular disease.[4] Lyso-Gb_3 is the hydrophilic deacylated form of Gb_3 and is detected at high levels in plasma in patients with classic Fabry disease.[2,5] Various analogs of lyso-Gb_3 with modifications to the sphingosine chain were also detected in plasma of patients with Fabry disease.[6] It has been reported in mouse models that lyso-Gb_3 accumulates most in the liver and spleen, organs that are not affected in Fabry disease.[2] Although elevated intracellular Gb_3 and lyso-Gb_3 are considered to trigger inflammation,[3] the persistence of altered cellular signaling subsequent to Gb_3 clearance indicates that inflammation, when activated, could be uncoupled from substrate accumulation,[7] and that at some point, the pathologic consequences are irreversible.

Approved treatments for Fabry disease include intravenous enzyme replacement therapy (ERT) and the oral pharmacological chaperone migalastat.[8–10] ERT compensates for α-Gal A deficiency in patients with Fabry disease and is delivered through intravenous infusion.[8,9] Migalastat is an orally administered small molecule that binds to and stabilizes endogenous α-Gal A in patients with migalastat-*amenable GLA* variants, facilitating lysosomal trafficking and restoration of native enzyme activity.[10,11] As a small molecule, migalastat has broad tissue distribution and

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Submitted 8 July 2020; revised 3 September 2020; accepted: 4 September 2020
Published online: 30 September 2020

penetration.[10,12] In phase 3 clinical studies, migalastat treatment effectively decreased disease substrates, stabilized renal function, reduced left ventricular mass index (LVMi), and improved gastrointestinal symptoms in patients with Fabry disease and *amenable* variants.[13,14]

Given that treatment options with different mechanisms of action exist and our understanding of Fabry pathophysiology and genetics is evolving, there is an increasing need for prognostic biomarkers to monitor and evaluate therapeutic efficacy and disease progression. A prognostic biomarker is one that is validated to identify the likelihood of a clinical event or progression of disease.[15] Although some biomarkers have been identified that show diagnostic and pharmacodynamic value, no prognostic biomarkers have been validated for any Fabry disease therapy.[15] Lyso-Gb_3 is frequently and appropriately used for primary screening and diagnosing patients with Fabry disease. Studies have demonstrated that lyso-Gb_3 effectively identifies unrecognized Fabry disease probands in patients referred from multispecialty clinics[16] and detects clinically relevant Fabry disease phenotypes (classic vs. late onset).[17] In cross-sectional studies, plasma lyso-Gb_3 levels were found to associate with disease severity in patients with Fabry disease.[18,19] Relationships between lyso-Gb_3 and clinical manifestations of Fabry disease were also examined based on cross-sectional data.[19–21] For example, plasma lyso-Gb_3 adjusted for sex and age correlated with LVMi in a cross-sectional study of untreated patients with Fabry disease and the late-onset variant IVS4+919G>A.[19] Similarly, plasma lyso-Gb_3 correlated with left ventricular hypertrophy and myocardial fibrosis in patients with Fabry disease in recent prospective multicenter studies.[20,21] However, these studies did not address how changes in lyso-Gb_3 may relate to treatment outcomes (e.g., LVMi and estimated glomerular filtration rate [eGFR]) over time.

Although lyso-Gb_3 has commonly been used in treatment monitoring, it has not been validated for this purpose, and few longitudinal studies have evaluated the association of lyso-Gb_3 with treatment outcomes.[22,23] One study showed that neither the lyso-Gb_3 concentration at baseline, lyso-Gb_3 concentration during treatment, absolute decrease of lyso-Gb_3, nor the relative decrease of lyso-Gb_3 predicted the risk of clinical events in patients receiving ERT.[22] In addition, the absence or presence of end-organ damage was not predicted by absolute lyso-Gb_3 levels, and undetectable or low lyso-Gb_3 levels in patients with the late-onset presentation of Fabry disease did not protect patients from end-organ clinical events.[24] It is increasingly recognized that the mechanism of Fabry disease is more complex than previously thought and that substrate accumulation alone does not explain disease severity and therapeutic response to a given treatment.[3] However, the downstream effects of substrate accumulation are not well understood.

Given the desire for validated biomarkers of clinical disease progression in treated patients with Fabry disease, the value of lyso-Gb_3 as a prognostic biomarker needs to be evaluated in migalastat-treated patients. Here, we examined plasma lyso-Gb_3 profiles in treatment-naive and ERT-experienced patients with migalastat-*amenable GLA* variants in the phase 3 clinical studies FACETS (NCT00925301)[13] and ATTRACT (NCT01218659)[14] and subsequent long-term open-label extension studies to assess the relationship between plasma lyso-Gb_3 and measures of clinical disease progression of Fabry disease (LVMi, eGFR, and pain), and Fabry-associated clinical events (FACEs) over time. We also aimed to confirm the utility of lyso-Gb_3 as a pharmacodynamic biomarker by assessing its relationship with kidney interstitial capillary (KIC) Gb_3, a commonly used, "reasonably likely surrogate endpoint" for accelerated approval of treatments for Fabry disease in the United States.[25]

MATERIALS AND METHODS

Ethics statement

FACETS, ATTRACT, AT1001-041, and AT1001-042 were all designed and monitored in accordance with the ethical principles of Good Clinical Practice guidelines and the Declaration of Helsinki.[13,14] The clinical study protocols were reviewed and approved by the appropriate Independent Ethics Committee/Institutional Review Board at each study site. All participants provided written informed consent prior to initiation of any studies.

Study design and patients

This post hoc analysis includes data from treatment-naive and ERT-experienced adult patients with Fabry disease who enrolled in the phase 3 clinical studies FACETS[13] (NCT00925301) and ATTRACT[14] (NCT01218659), respectively. Briefly, FACETS comprised a 6-month randomized, double-blind, placebo-controlled phase, followed by a 6-month open-label extension (OLE) phase with crossover of patients in the placebo arm to receive migalastat 150 mg every other day (QOD), and a 12-month migalastat treatment extension phase. ATTRACT was an open-label, randomized study comprising an 18-month active-controlled (ERT), randomized phase and a 12-month optional OLE phase with crossover of patients in the ERT arm to receive migalastat 150 mg QOD. Data were collected during the phase 3 trials and the long-term OLE safety and efficacy studies AT1001-041 (NCT01458119) and AT1001-042 (NCT02194985) (Fig. S1). Eligibility criteria and study designs for FACETS and ATTRACT were published previously.[13,14] This analysis included all patients who had an *amenable GLA* variant based on the Good Laboratory Practice–validated migalastat amenability assay in human embryonic kidney cells and had received at least one dose of migalastat.

Assessments

Plasma lyso-Gb_3 and measures of Fabry disease progression including LVMi, eGFR, and pain were assessed in both ERT-naive and ERT-experienced patients. Plasma lyso-Gb_3 levels were analyzed on a research basis at Amicus Therapeutics, Inc. by liquid chromatography–tandem mass spectrometry using plasma samples collected at study enrollment and every

6 months thereafter; LVMi was calculated based on echocardiography measures assessed through blinded, centralized evaluation;[13,14] eGFR was determined using the Chronic Kidney Disease Epidemiology Collaboration equation ($eGFR_{CKD\text{-}EPI}$); and worst pain in 24 hours was collected using the Brief Pain Inventory Short Form in patients with Fabry disease in the FACETS and ATTRACT studies,[13,14] analyzing only responses to the question on the worst pain over a 24-hour period.

The relationship between lyso-Gb_3 and FACEs was also analyzed. FACEs occurring during the trial were defined previously in the ATTRACT study and included cardiac, renal, and cerebrovascular events, and death.[14] Cardiac, renal, and cerebrovascular events were defined as follows:

Cardiac events

- Myocardial infarction
- Unstable cardiac angina, as defined by the American College of Cardiology/American Heart Association national practice guidelines
- New symptomatic arrhythmia requiring antiarrhythmic medication, direct current cardioversion, pacemaker, or defibrillator implantation, or
- Congestive heart failure, New York Heart Association class III or IV

Renal events

- A decrease in $eGFR_{CKD\text{-}EPI} \geq 15$ mL/min/1.73 m^2, with the decreased eGFR <90 mL/min/1.73 m^2 relative to baseline, or
- An increase in 24-hour urine protein ≥33%, with elevated protein ≥300 mg relative to baseline

Cerebrovascular events

- Stroke, or
- Transient ischemic attack

KIC Gb_3 inclusions were assessed quantitatively using the Barisoni Lipid Inclusion Scoring System in biopsy samples from ERT-naive patients as described previously.[13,26]

Statistical analyses

For this post hoc analysis, baseline (month 0) was defined as the beginning of migalastat treatment. The data cutoff date for the ongoing AT1001-042 study was 25 May 2019.

Relationships between changes in lyso-Gb_3 and changes in measures of disease progression (i.e., LVMi, eGFR, and pain) were assessed for all patients with *amenable* variants and migalastat exposure. In addition, the relationship between baseline values of lyso-Gb_3 and LVMi, eGFR, and pain was evaluated. Three subgroup analyses were performed in which data were stratified by prior ERT treatment status (naive or ERT-experienced), sex, or age (≤40 and >40 years). Spearman rank correlation coefficients and P values were calculated to assess correlations between changes in lyso-Gb_3 and changes in LVMi, eGFR, or pain at months 12, 18, 24, 30, and 36 for ERT-naive and ERT-experienced patients during migalastat treatment, specifically. Correlations between changes in lyso-Gb_3 and changes in KIC Gb_3 were assessed at months 6 and 12 only in ERT-naive patients during migalastat treatment.

Relationships between variables over time were assessed in longitudinal analyses via random coefficient mixed models. A separate set of regression coefficients was fitted for each response variable (LVMi, eGFR, and pain) and the correlations among these random coefficients were examined in a pairwise manner with regression coefficients for lyso-Gb_3. The model was implemented via PROC MIXED using SAS Enterprise Guide version 8.1 (SAS Institute; Cary, NC, USA). The method assesses if the slopes for the two variables tested (e.g., lyso-Gb_3 and LVMi) are independent.[27]

Cox proportional hazard models were used to assess any relationships between lyso-Gb_3 and the incidence of FACEs. This analysis was performed for all patients and patients who had continued migalastat therapy for ≥24 months. For patients with recurrent events, only the first events were analyzed. The data were right censored if a patient dropped out of the study before an event occurred (at the time of dropout) or had experienced no event at the end of the follow-up (May 25, 2019). Modeling was conducted using SAS Enterprise Guide version 8.1, and the following covariates were included in this analysis: age, time from diagnosis, presence of FACEs prior to the start of migalastat therapy, urine protein values at baseline, LVMi at baseline, and eGFR at baseline.

Previous cardiac and cerebrovascular events were identified based on Medical Dictionary for Regulatory Activities codes for the preferred terms from medical history with the exception of arrhythmias, which were evaluated by a physician. Previous renal events were defined as baseline $eGFR_{CKD\text{-}EPI} < 60$ mL/min/1.73 m^2 or baseline urine protein ≥300 mg, or any renal events as identified by a physician in the medical history. All variables were introduced into the Cox model tested, P values were calculated using Wald chi-square statistics, and $P < 0.05$ was considered significant.

The statistical analyses were not adjusted for multiplicity.

RESULTS

Patient demographics and baseline characteristics

Patient demographics and disease characteristics at baseline (start of migalastat) are shown for all 97 patients included in the analysis, and by prior treatment status and sex in Table 1. Overall, mean (standard deviation) age was 46.2 (13.1) years, 60 (61.9%) patients were female, and 86 (88.7%) patients had ≥1 prior FACE. At baseline, LVMi, eGFR, and history of previous clinical events were generally comparable between patient subgroups. The upper limit of the normal reference range for plasma lyso-Gb_3 was 1.19 nmol/L.[14] The median

Table 1 Baseline[a] characteristics.

	Overall $N = 97$	ERT-naive[b] $n = 48$	ERT-experienced $n = 49$	Males $n = 37$	Females $n = 60$	Age ≤ 40 years $n = 31$	Age > 40 years $n = 66$
Age, years, mean (SD)	46.2 (13.1)	43.0 (11.3)	49.4 (14.1)	45.8 (14.1)	46.4 (12.6)	30.6 (6.8)	53.5 (7.9)
Time since Fabry diagnosis, years, mean (SD)	9.0 (10.5)	6.3 (7.8)	11.6 (12.0)	7.1 (8.9)	10.1 (11.2)	8.4 (9.4)	9.2 (11.0)
Duration of ERT exposure,[c] years, mean (SD)	3.4 (2.4)	NA	3.4 (2.4)	3.5 (2.3)	3.4 (2.5)	3.7 (3.3)	3.4 (2.1)
Duration of migalastat exposure, years, median (range)	5.1 (0.1–8.5)	6.5 (0.1–8.5)	5.0 (0.1–7.2)	5.6 (0.5–8.4)	5.1 (0.1–8.5)	2.5 (0.1–8.2)	5.6 (0.1–8.5)
KIC Gb_3 inclusions, median (range)	0.2 (0.01–5.69)	0.2 (0.01–5.69)	NA	0.6 (0.01–5.69)[d]	0.2 (0.02–0.60)[d]	0.2 (0.05–5.69)[d]	0.1 (0.01–3.05)[d]
LVMi, g/m^2, mean (SD)	93.9 (29.6)	96.5 (32.9)	91.5 (26.3)	113.3 (34.5)	82.0 (17.9)	78.1 (17.6)	101.1 (31.2)
$eGFR_{CKD\text{-}EPI}$, mL/min/1.73 m^2, mean (SD)	91.2 (22.5)	93.1 (24.4)	89.3 (20.5)	88.5 (26.3)	92.9 (19.8)	109.8 (18.9)	82.5 (18.4)
24-hour urine protein, mg/24 hours, median (range)	198.0 (0.0–5566.0)	245.0 (44.0–2663.0)	116.0 (0.0–5566.0)	242.0 (0.0–5566.0)	152.0 (0.0–2663.0)	151.0 (0.0–2663.0)	215.0 (0.0–5566.0)
History of prior FACEs,[e] *n* (%)	86 (88.7)	45 (93.8)	41 (83.7)	33 (89.2)	53 (88.3)	24 (77.4)	62 (93.9)
Plasma lyso-Gb_3,[f,g] nmol/L							
Median (range)	9.9 (0.8–218.3)	16.8 (1.2–218.3)	6.4 (0.8–59.1)	21.8 (0.8–218.3)	8.2 (0.8–31.7)	13.0 (0.8–135.3)	9.0 (0.8–218.3)
Mean (SD)	24.1 (39.1)	45.2 (54.2)	10.1 (11.7)	47.7 (55.1)	9.3 (6.7)	30.6 (42.2)	21.2 (37.6)

$eGFR_{CKD\text{-}EPI}$ estimated glomerular filtration rate using the Chronic Kidney Disease Epidemiology Collaboration equation, *ERT* enzyme replacement therapy, *FACE* Fabry-associated clinical events, Gb_3 globotriaosylceramide, *KIC* kidney interstitial capillary, *LVMi* left ventricular mass index, *lyso-Gb_3* globotriaosylsphingosine, *NA* not applicable, *NC* not calculated, *SD* standard deviation.

[a]Baseline was defined as the beginning of migalastat treatment. For patients in the ERT-migalastat group, this was month 18 of ATTRACT, prior to starting migalastat treatment in the 12-month open-label extension. Age, time since diagnosis, and duration of ERT exposure were recorded at study (FACETS or ATTRACT) enrollment.

[b]Patients were ERT-naive or had not received ERT for ≥6 months prior to FACETS enrollment.[13]

[c]Prior ERT treatment duration was not reported for 4 males and 2 females.

[d]ERT-naive patients only.

[e]FACEs included renal, cardiac, and cerebrovascular events and death.

[f]In ERT-experienced patients, lyso-Gb_3 values were measured 2–17 days after the last dose of ERT.

[g]The upper limit of the normal reference range for plasma lyso-Gb_3 is 1.19 nmol/L.[14]

(range) duration of migalastat exposure was 5.1 (0.1–8.5) years in the overall patient group, and the median (range) duration of migalastat exposure was 6.5 (0.1–8.5) years in treatment-naive patients and 5.0 (0.1–7.2) years in ERT-experienced patients (Table 1).

Relationship between plasma lyso-Gb3 and measures of Fabry disease progression

At baseline, plasma lyso-Gb_3 levels were not correlated with eGFR or worst pain in 24 hours in treatment-naive and ERT-experienced patients (Table S1). When analyzed by sex or age, no correlations were identified between lyso-Gb_3 and eGFR; however, a significant correlation between baseline lyso-Gb_3 and baseline pain was identified in male patients (Spearman correlation unadjusted $P = 0.0038$). Baseline lyso-Gb_3 was shown to correlate with baseline LVMi in both ERT-naive and ERT-experienced patients (unadjusted $P = 0.0002$ and unadjusted $P = 0.0016$, respectively) and patients aged ≤40 and >40 years (unadjusted $P = 0.0007$ and unadjusted $P = 0.0003$, respectively). In contrast, no correlation was identified between baseline lyso-Gb_3 and baseline LVMi when patients were analyzed by sex.

During migalastat treatment, no correlation was identified between changes from baseline in lyso-Gb_3 and changes from baseline in LVMi, eGFR, or worst pain in 24 hours at any timepoint analyzed in treatment-naive and ERT-experienced patients (Table 2). When analyzed by sex, no correlations between changes in lyso-Gb_3 and changes in LVMi, eGFR, or worst pain in 24 hours were identified in male or female patients for any timepoint with one exception. A correlation was identified between lyso-Gb_3 and eGFR at month 18 in male patients (unadjusted $P = 0.03$). Similarly, when analyzed by age, no correlations were identified between lyso-Gb_3 and LVMi, eGFR, and pain at any timepoint assessed except for a correlation between lyso-Gb_3 and eGFR at month 12 in patients aged >40 years (unadjusted $P = 0.02$). The individual changes from baseline in lyso-Gb_3 were plotted against changes from baseline in LVMi, eGFR, or worst pain in 24 hours at selected timepoints by treatment status (Fig. 1), and no trends between lyso-Gb_3 and LVMi, eGFR, or pain were observed.

When assessing the rate of change in lyso-Gb_3 and LVMi or eGFR during follow-up, no longitudinal correlation was identified in the overall patient group or subgroups stratified by prior ERT treatment status and sex (Table 3). A longitudinal correlation was identified between lyso-Gb_3 and worst pain in 24 hours in the overall patient group ($r = 0.82$; unadjusted $P < 0.01$), ERT-experienced patients ($r = 0.69$; unadjusted $P = 0.04$), and male patients ($r = 0.99$; unadjusted $P = 0.02$), but not in treatment-naive or female patients (Table 3). When analyzed by age, no longitudinal correlation was identified between lyso-Gb_3 and LVMi, eGFR, or pain in patients aged ≤40 years. However, a longitudinal correlation was identified between lyso-Gb_3 and LVMi in patients aged >40 years ($r = 0.63$; unadjusted $P = 0.02$).

Table 2 Spearman correlation coefficients between changes in plasma lyso-Gb_3 and changes in LVMi, eGFR, pain, and KIC Gb_3 at specific timepoints during migalastat treatment.

		Overall ($N = 97$)		ERT-naive ($n = 48$)		ERT-experienced ($n = 49$)		Males ($n = 37$)		Females ($n = 60$)		Age ≤ 40 years ($n = 31$)		Age > 40 years ($n = 66$)	
Parameter	Visit	n[a]	P value[b]	n[a]	P value[b]	n[a]	P value[b]	n[a]	P value[b]	n[a]	P value[b]	n[a]	P value[b]	n[a]	P value[b]
LVMi	Month 12	55	0.9283	17	0.5164	38	0.5311	21	0.4506	34	0.2742	16	0.4782	39	0.9254
	Month 18	44	0.5210	10	0.6515	34	0.9133	19	0.0663	25	0.4538	8	0.2604	36	0.1185
	Month 24	13	0.8164	13	0.8164	0	NA	3	0.6667	10	0.1921	6	0.1108	7	0.2939
	Month 30	27	0.2404	0	NA	27	0.2404	11	0.4669	16	0.1682	4	0.2000	23	0.3144
	Month 36	3	0.6667	0	NA	3	0.6667	1	NA	2	NA	0	NA	3	0.6667
eGFR	Month 12	61	0.1294	18	0.9773	43	0.1338	23	0.6183	38	0.1072	18	0.8357	43	0.0189
	Month 18	52	0.1052	13	0.8166	39	0.3793	21	0.0320	31	0.6475	11	0.7092	41	0.0660
	Month 24	27	0.4925	16	0.8201	11	0.2981	8	0.2894	19	0.9943	10	0.2763	17	0.1743
	Month 30	37	0.5586	0	NA	37	0.5586	15	0.8298	22	0.4373	6	0.8717	31	0.4231
	Month 36	29	0.3506	0	NA	29	0.3506	11	0.7092	18	0.2795	4	0.6000	25	0.2165
Pain	Month 12	61	0.8523	18	0.6612	43	0.8123	23	0.2968	38	0.1402	18	0.9224	43	0.6802
	Month 18	48	0.1935	13	0.3920	35	0.4525	20	0.8846	28	0.1310	10	0.1004	38	0.5628
	Month 24	18	0.8782	16	0.7804	2	NA	6	0.3123	12	0.8353	8	0.9081	10	0.8633
	Month 30	29	0.3309	0	NA	29	0.3309	13	0.3157	16	0.5872	4	0.4000	25	0.4522
	Month 36	5	0.4925	0	NA	5	0.4925	2	NA	3	1.0000	1	NA	4	0.2000
KIC Gb_3[c]	Month 6	18	0.0003	18	0.0003	NA	NA	5	0.0374	13	0.0707	10	0.0022	7	0.0208
	Month 12	17	0.0470	17	0.0470	NA	NA	4	0.6000	13	0.7208	10	0.1076	8	0.4821

eGFR estimated glomerular filtration rate, *ERT* enzyme replacement therapy, Gb_3 globotriaosylceramide, *KIC* kidney interstitial capillary, *LVMi* left ventricular mass index, *lyso-Gb_3* globotriaosylsphingosine, *NA* not applicable.
[a]*n* indicates number of patients with values for both lyso-Gb_3 and the other assessment at the specified timepoint.
[b]*P* values represent Spearman correlations.
[c]KIC Gb_3 data were derived from FACETS only.

Relationship between plasma lyso-Gb3 and incidence of FACEs

Overall, FACEs occurred in 47 (48.5%) patients receiving migalastat treatment. When analyzed by ERT treatment status, FACEs occurred in 22 (45.8%) treatment-naive patients and 25 (51.0%) ERT-experienced patients while on migalastat treatment. Among male and female patients, 22 (59.5%) and 25 (41.7%) patients experienced FACEs, respectively.

When we assessed the ability of various baseline variables to predict the incidence of FACEs, only baseline eGFR was associated with the occurrence of FACEs during migalastat treatment (hazard ratio [HR]: 0.74 for every 10 mL/min/1.73 m^2; unadjusted $P = 0.02$) (Table 4). Similarly, higher baseline eGFR was associated with a decreased incidence of FACEs when analyses were controlled for prior ERT treatment status (HR: 0.72 for every 10 mL/min/1.73 m^2; unadjusted $P = 0.02$) or sex (HR: 0.74 for every 10 mL/min/1.73 m^2; unadjusted $P = 0.02$). Neither lyso-Gb_3 levels at baseline nor the rate of change in lyso-Gb_3 levels during treatment predicted the occurrence of FACEs in the overall patient group (HR: 1.05 for every 10 nmol/L in baseline lyso-Gb_3 levels; unadjusted $P = 0.60$ and HR: 1.02 for every 0.05 nmol/L/month in the rate of change in lyso-Gb_3 level; unadjusted $P = 0.62$). Similarly, neither variable predicted FACEs when analyses were controlled for prior ERT treatment status (HR: 1.06; unadjusted $P = 0.54$ and HR: 1.00; unadjusted $P = 0.96$, respectively) or sex (HR: 1.05; unadjusted $P = 0.56$ and HR: 1.02; unadjusted $P = 0.54$, respectively).

Similar results were observed when the analysis was restricted to all patients who had received ≥24 months of migalastat treatment (Table 4). Higher baseline eGFR was associated with decreased incidence of FACEs (HR: 0.72 for every 10 mL/min/1.73 m^2; unadjusted $P = 0.02$). Neither lyso-Gb_3 baseline levels nor the rate of change in lyso-Gb_3 levels was associated with the occurrence of FACEs (HR: 1.07; unadjusted $P = 0.47$ and HR: 1.02; unadjusted $P = 0.54$, respectively). In addition, neither was associated with the occurrence of FACEs when analyses were controlled for prior ERT treatment status (HR: 1.08; unadjusted $P = 0.43$ and HR: 1.01; unadjusted $P = 0.78$) or sex (HR: 1.08; unadjusted $P = 0.40$ and HR: 1.02; unadjusted $P = 0.47$) in this long-term treatment subgroup.

Relationship between plasma lyso-Gb_3 and KIC Gb_3

KIC Gb_3 was only assessed in the FACETS study up to 12 months after which biopsies were not obtained. The relationship between plasma lyso-Gb_3 and KIC Gb_3 was evaluated in ERT-naive patients only. A correlation was identified between lyso-Gb_3 and KIC Gb_3 in ERT-naive patients at months 6 and 12 (unadjusted $P < 0.01$ and unadjusted $P = 0.05$, respectively) (Table 2). When patients were analyzed by sex, a correlation was identified in male patients at month 6 (unadjusted $P = 0.04$). However, no

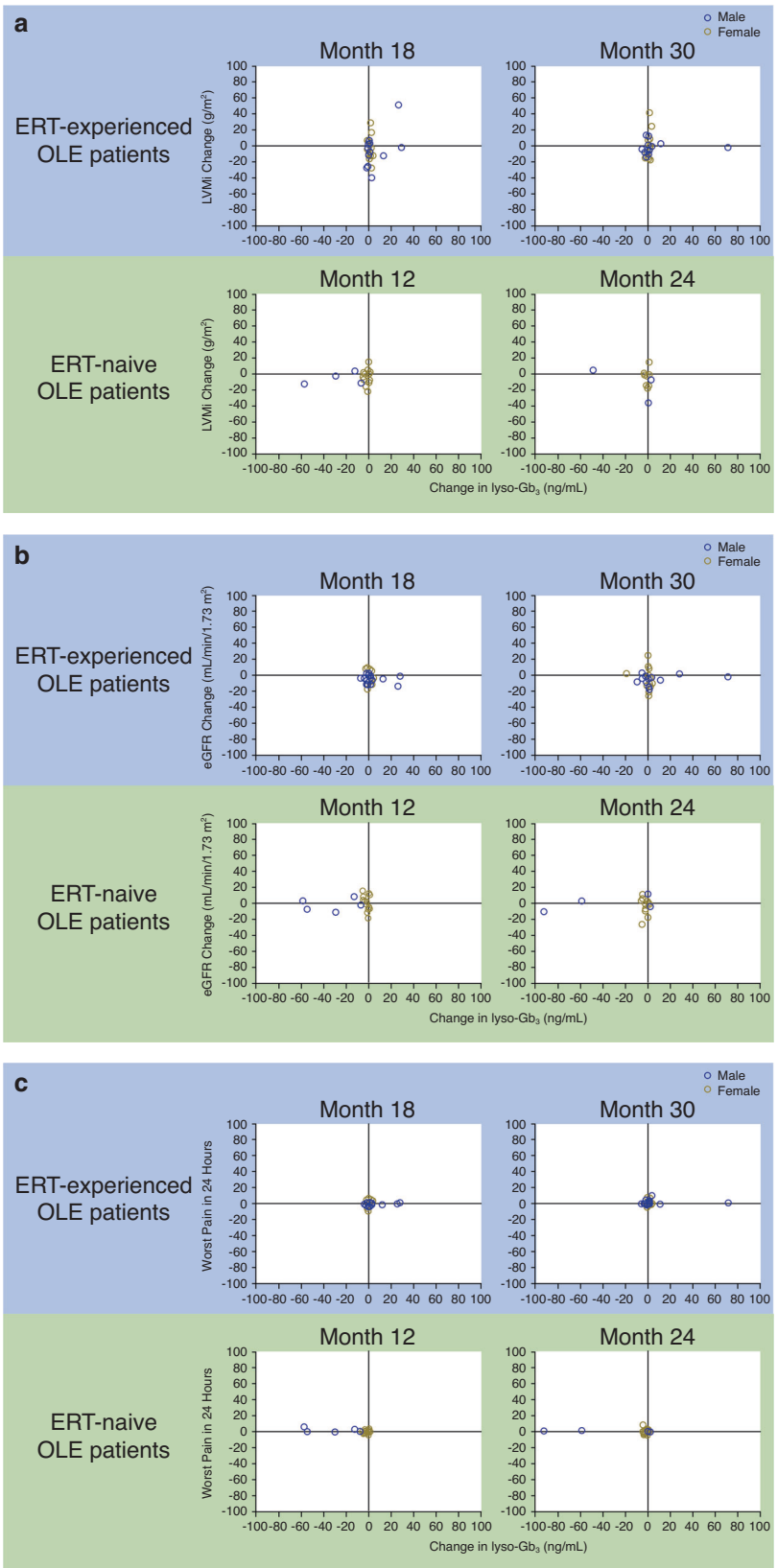

Fig. 1 Lack of correlation between changes in plasma lyso-Gb_3 and changes in LVMi, eGFR, and pain at selected timepoints of migalastat treatment. (**a**) Relationship between changes in lyso-Gb_3 versus changes in LVMi. (**b**) Relationship between changes in lyso-Gb_3 vs. changes in eGFR. (**c**) Relationship between changes in lyso-Gb_3 versus changes in worst pain in 24 hours. *eGFR* estimated glomerular filtration rate, *ERT* enzyme replacement therapy, *LVMi* left ventricular mass index, *lyso-Gb_3* globotriaosylsphingosine, *OLE* open-label extension.

Table 3 Random coefficient mixed models results for longitudinal correlation between plasma lyso-Gb_3 and LVMi, eGFR, and pain during migalastat treatment.

Parameter	Overall ($N = 97$)	ERT-naive ($n = 48$)	ERT-experienced ($n = 49$)	Males ($n = 37$)	Females ($n = 60$)	Age ≤ 40 years ($n = 31$)	Age > 40 years ($n = 66$)
LVMi[a]							
n	66	25	41	27	39	14	52
Correlation coefficient	0.2056	0.2075	0.3389	−0.0412	0.1723	0.6263	0.6329
P value	0.2675	0.4407	0.2460	0.8824	0.4536	0.1391	0.0185
eGFR[b]							
n	97	48	49	37	60	31	66
Correlation coefficient	0.0851	0.5198	0.0511	−0.0342	0.1984	0.7603	−0.0954
P value	0.7342	0.4341	0.8484	0.9319	0.6393	0.1157	0.6913
Pain[c]							
n	96	47	49	37	59	31	65
Correlation coefficient	0.8230	0.4473	0.6868	0.9872	NE[d]	0.3146	NE
P value	0.0032	0.3834	0.0380	0.0165	NE[d]	0.4543	NE

eGFR estimated glomerular filtration rate, *ERT* enzyme replacement therapy, *LVMi* left ventricular mass index, *lyso-Gb_3* globotriaosylsphingosine, *NE* not estimable.
[a]LVMi data were derived from FACETS, ATTRACT, and the open-label extension study AT1001-042.
[b]eGFR data were derived from FACETS, ATTRACT, and the open-label extension study AT1001-042.
[c]Pain data were derived from FACETS and ATTRACT.
[d]These values were not estimated due to insufficient data.

correlation was identified in female patients at either time-point (unadjusted $P = 0.07$ and unadjusted $P = 0.72$, respectively). When patients were analyzed by age, a correlation was identified between lyso-Gb_3 and KIC Gb_3 in patients aged ≤40 and >40 years at month 6 only (unadjusted $P = 0.002$ and unadjusted $P = 0.02$, respectively).

DISCUSSION

There is skepticism about the usefulness of monitoring plasma lyso-Gb_3 to evaluate treatment response in Fabry disease.[22,24] Despite its utility for diagnosis, disease severity assessment, and as a pharmacodynamic biomarker,[16,17,28] lyso-Gb_3 values showed little association with treatment outcomes in ERT-treated patients (agalsidase alfa or agalsidase beta).[22] In the current study, changes in plasma lyso-Gb_3 levels did not correlate with changes in measures of clinical disease progression (LVMi, eGFR, or pain) in treatment-naive or ERT-experienced patients during migalastat treatment. However, without adjusting for multiplicity, lyso-Gb_3 correlated with LVMi at baseline, and a longitudinal correlation was identified between lyso-Gb_3 and LVMi in patients aged >40 years. Plasma lyso-Gb_3 measurements, including levels at baseline or rate of change in lyso-Gb_3 levels during treatment, did not predict FACEs in treatment-naive or ERT-experienced patients, suggesting that changes in plasma lyso-Gb_3 may not predict therapeutic outcome and may have limited utility in clinical monitoring and decision making for migalastat-treated patients from this cohort.

These observations are in line with the evolving understanding of Fabry pathophysiology, which likely includes multiple disease mechanisms beyond substrate storage. In support of this, glycosphingolipid substrate clearance by ERT did not reverse several Fabry disease–associated pathophysiological processes in a recent study in cultured podocytes.[7] Furthermore, the origin of plasma lyso-Gb_3 is not well understood. Nevertheless, a possible biochemical relationship between Gb_3 and lyso-Gb_3 was revealed in a urine metabolomic study where the mass spectrometry fragmentation approach showed that methylated Gb_3-related analogs might be intermediate compounds leading to Gb_3 deacylation and lyso-Gb_3 generation.[29] Furthermore, studies in mouse models suggest that lyso-Gb_3 is either actively formed or preferentially stored in the liver and spleen, organs not affected by Fabry disease.[2,30,31] Elevated plasma lyso-Gb_3 could be a "spillover" from these organs and therefore may not reflect the substrate levels in clinically relevant organs such as the heart, kidney, or peripheral nerves.[1] In addition, as migalastat and lyso-Gb_3 primarily occupy distinct compartments (lysosomes versus plasma),[2,10] lyso-Gb_3 may not be subject to catalysis by migalastat-stabilized α-Gal A.

Given that lyso-Gb_3 generally did not correlate with measures of Fabry disease progression or predict the incidence of FACEs during migalastat treatment, our results confirm for migalastat what was already demonstrated for ERT regarding the poor value of lyso-Gb_3 for monitoring treatment.[22]

Table 4 Influence of plasma lyso-Gb_3 on the occurrence of Fabry-associated clinical events during migalastat treatment.

	Overall ($N = 72$)			Patients with ≥ 24 months migalastat treatment ($N = 66$)		
	HR	95% CI	*P* value	HR	95% CI	*P* value
Age, years (per 5 years)	0.99	(0.79–1.24)	0.90	0.99	(0.78–1.26)	0.93
Sex	1.23	(0.50–3.04)	0.65	1.05	(0.42–2.62)	0.92
Time from Fabry diagnosis (per 5 years)	0.90	(0.75–1.08)	0.24	0.89	(0.74–1.08)	0.23
Lyso-Gb_3 concentration at baseline (per 10 nmol/L)	1.05	(0.88–1.25)	0.60	1.07	(0.90–1.27)	0.47
Rate of change in lyso-Gb_3 levels during treatment (per 0.05 nmol/L/month)	1.02	(0.96–1.08)	0.62	1.02	(0.96–1.09)	0.54
LVMi at baseline (per 5 g/m^2)	1.03	(0.97–1.10)	0.39	1.02	(0.96–1.09)	0.47
eGFR at baseline (per 10 mL/min/1.73 m^2)	0.74	(0.57–0.95)	0.02	0.72	(0.55–0.94)	0.02
24-hour urine protein at baseline (per 1000 mg/24 hours)	1.30	(0.95–1.77)	0.10	1.22	(0.87–1.72)	0.24
Prior FACE	3.74	(0.48–29.33)	0.21	3.46	(0.44–27.54)	0.24

CI confidence interval, *eGFR* estimated glomerular filtration rate, *ERT* enzyme replacement therapy, *FACE* Fabry-associated clinical event, *HR* hazard ratio, *LVMi* left ventricular mass index, *lyso-Gb_3* globotriaosylsphingosine.

Although these data suggest that lyso-Gb_3 is not a suitable prognostic biomarker for Fabry disease, lyso-Gb_3 and Gb_3 are robust pharmacodynamic biomarkers commonly used in research of new treatments for Fabry disease.[13,14,32] ERT and migalastat have effectively reduced plasma lyso-Gb_3 levels in patients with Fabry disease.[5,14,28] It was also observed in a retrospective study that among ERT-treated male patients with Fabry disease, those who developed antibodies against ERT had significantly higher plasma lyso-Gb_3 levels than those without ERT antibodies ($P = 0.02$).[33] In addition, ERT substantially reduced Gb_3 inclusions in KIC and glomerular cells after 6 months of treatment in patients with Fabry disease,[8,9] and migalastat decreased podocyte volume and partially cleared Gb_3 inclusions in podocytes after 6 months of treatment in male patients with Fabry disease.[34] These observations demonstrate a clear biological response in individuals treated with migalastat and ERT, which is the purpose of a pharmacodynamic biomarker.[35] Consequently, KIC Gb_3 has served as a "reasonably likely surrogate endpoint" and is the basis of regulatory approval for ERT and migalastat in the United States and continues to be used in clinical development programs.[13,25,32] In this analysis, changes in lyso-Gb_3 correlated with changes in KIC Gb_3 in ERT-naive patients at months 6 and 12, supporting its utility as a pharmacodynamic biomarker in the clinical development of treatments for Fabry disease.

Several studies have suggested associations between lyso-Gb_3 and manifestations of Fabry disease, including LVMi and myocardial fibrosis.[19–21,23] However, it should be noted that these associations were found using cross-sectional data in either untreated patients[19,21] or in heterogeneous patient populations in which a subset of patients received ERT.[20,23] Indeed, our finding that baseline lyso-Gb_3 levels correlated with baseline LVMi is consistent with previous report,[19] but to our knowledge, no publication had explored the relationship between changes in lyso-Gb_3 and LVMi during treatment. One study evaluated longitudinal changes in myocardial fibrosis in untreated patients and found baseline lyso-Gb_3 was not a predictor of fibrosis during follow-up.[21] Another study identified a trend toward a correlation between lyso-Gb_3 and decline in pulmonary function with age as assessed by spirometry in a mixed population of ERT-treated and untreated patients with Fabry disease.[23] Therefore, the value of lyso-Gb_3 for treatment monitoring remains uncertain as these studies did not address the association between changes in lyso-Gb_3 levels and treatment outcomes. However, the current analysis using longitudinal data and FACEs fills an important gap in research by evaluating the clinical utility of lyso-Gb_3 for treatment monitoring of patients with Fabry disease receiving migalastat.

Study limitations include the fact that this is a post hoc analysis of data from trials not specifically designed to explore the research question and lack of adjustment for multiplicity in statistical analyses, which could be a source of bias considering the relatively small patient number and may account for significant correlations that were identified between lyso-Gb_3 and KIC Gb_3 and measures of Fabry disease progression in a subset of patients at certain timepoints. In addition, few patients had LVMi and pain data beyond 36 months of migalastat treatment, and longitudinal correlations between slopes of lyso-Gb_3 and pain could not be calculated for female patients due to the small sample size. Pain measurements included the worst pain in 24 hours only, suggesting that these analyses may not have fully explored the relationship between lyso-Gb_3 and general pain levels.

Although this is a post hoc analysis, patients were stratified by baseline lyso-Gb_3 level to calculate Spearman correlation coefficients or longitudinal correlations. Furthermore, adjustment for baseline lyso-Gb_3 and change over time was included in Cox proportional hazard models to assess FACEs. However, a prospective study that includes formal biomarker validation methodology would be informative.

Few studies have investigated potential prognostic biomarkers for Fabry disease progression and clinical response to treatment for the guidance of treatment decisions.[22,23] Therefore, new biomarkers should be explored for monitoring

treatment effect in Fabry disease. More studies are needed to confirm the findings of this study and explore the value of existing biomarkers including proteinuria/albuminuria,[36] podocyturia,[36] inflammatory markers such as tumor necrosis factor,[20] potential biomarkers of renal disease including podocalyxin[36] and fibroblast growth factor 23,[37,38] and markers of cardiac disease.[21,39] Moreover, future studies are warranted to assess any relationships between lyso-Gb_3 analogs and Fabry disease severity given that lyso-Gb_3 analogs constitute a substantial proportion of total lyso-Gb_3 in plasma and urine of patients with Fabry disease.[19,28] Proteomic and metabolomic profiling of plasma and/or urine samples of patients with Fabry disease compared with healthy controls may also identify potential biomarkers of Fabry disease progression.[38]

In conclusion, these post hoc analyses show that plasma lyso-Gb_3 levels generally do not correlate with measures of disease progression (LVMi, eGFR, and pain) or predict FACEs in migalastat-treated patients regardless of their previous treatment history or sex. Our results confirm that lyso-Gb_3 is not a prognostic biomarker of migalastat treatment response in patients with Fabry disease, a finding similar to what has been published for ERT.[22] For patients receiving migalastat, the ongoing effectiveness of treatment should be determined based on the totality of biochemical and clinical evidence as well as patient-reported outcomes for consistency with current treatment monitoring guidelines.[40] Clinical decision making must consider effectiveness, safety and tolerability, and patient preference.

SUPPLEMENTARY INFORMATION

The online version of this article (https://doi.org/10.1038/s41436-020-00968-z) contains supplementary material, which is available to authorized users.

ACKNOWLEDGEMENTS

We thank Simon Heales, David Kasper, and Sarah Young for their contributions to the development of this study. Third-party medical writing assistance was provided by Lei Bai and Stephanie Agbu (ApotheCom, Yardley, PA)

AUTHOR CONTRIBUTIONS

DGB, ABM, and NS participated in study design; DGB, ABM, NS, and EK analyzed the data; and DGB, CAB, HM, ABM, NS, and EK interpreted the data. JMA drafted the article. All authors critically revised the manuscript, gave final approval of the submitted version, and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

FUNDING

This study was funded by Amicus Therapeutics, Inc.

DISCLOSURE

D.G.B. has served as a consultant and speaker for, and received research funding and honoraria from, Amicus Therapeutics, Inc. and Sanofi Genzyme. J.M.A. has served as a consultant for Azafaros and as a speaker for Amicus Therapeutics, Inc. and Sanofi Genzyme. C.A.-B. has served as a consultant and speaker for Amicus Therapeutics, Inc. and Sanofi Genzyme; has served as a collaborator for 4D Molecular Therapeutics, Avrobio, and Protalix; has received research grants and honoraria as an investigator for BioMarin Pharmaceutical Inc., Shire/Takeda, and University Health Network; and has received research equipment and supplies from Waters Corporation. H.M. has received research support from Amicus Therapeutics, Inc. and Idorsia; and has received speaker fees from Amicus Therapeutics, Inc. and Sanofi K.K. A.T.B. has received honoraria from and served as a consultant and investigator for Amicus Therapeutics, Inc., Protalix/Pfizer, Sanofi Genzyme, and Shire/Takeda. N.S. is an employee of and holds stock in Amicus Therapeutics, Inc. E.K. is a paid consultant for Amicus Therapeutics, Inc. R.S. has served as a consultant for Chiesi Farmaceutici and Amicus Therapeutics, Inc., and has served as an investigator for Idorsia, Protalix, and Sanofi Genzyme.

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