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Plasma globotriaosylsphingosine as a biomarker of Fabry disease

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ABSTRACT

Fabry disease is an X-linked genetic disorder caused by a deficiency of α-galactosidase A (GLA) activity. As enzyme replacement therapy (ERT) involving recombinant GLAs has been introduced for this disease, a useful biomarker for diagnosis and monitoring of therapy has been strongly required. We measured globotriaosylsphingosine (lyso-Gb3) and globotriaosylceramide (Gb3) in plasma samples from ten hemizygous males (six classic and four variant cases) and eight heterozygous females with Fabry disease, and investigated the responses of plasma lyso-Gb3 and Gb3 in a male Fabry patient who had undergone ERT for 4 years to determine whether plasma lyso-Gb3 and Gb3 could be biomarkers of Fabry disease. The results revealed that plasma lyso-Gb3 was apparently increased in male patients and was higher in cases of the classic form than those of the variant one. In Fabry females, plasma lyso-Gb3 was moderately increased in both symptomatic and asymptomatic cases, and there was a correlation between the increase in lyso-Gb3 and the decrease in GLA activity. As to plasma Gb3, the levels in the variant Fabry hemizygotes and Fabry heterozygotes could not be distinguished from those in the controls, although those in the classic Fabry hemizygotes were increased. The plasma lyso-Gb3 level in the Fabry patient who had received ERT was elevated at the baseline and fell more dramatically on ERT than that of Gb3. Plasma lyso-Gb3 could thus be a potential biomarker of Fabry disease.

Introduction

Fabry disease (MIM 301500) is an X-linked genetic disorder due to a deficiency of α-galactosidase A (GLA, EC3.2.1.22) activity [1]. This deficiency causes the lysosomal accumulation of glycolipids, predominantly globotriaosylceramide (Gb3). Male patients with the classic form of this disease having no GLA activity experience pain in the peripheral extremities, cutaneous angiokeratomas, hypohidrosis, corneal opacities, cerebrovascular disorders, and renal and cardiac involvement. On the other hand, male patients with the variant form having residual GLA activity develop milder clinical manifestations, sometimes limited to heart disorders. Heterozygous Fabry females exhibit a quite wide range of clinical phenotypes. They either suffer from a milder form of the disease or are asymptomatic, but sometimes they may exhibit severe clinical features as well as the classic form seen in male patients [1]. A recent clinical study revealed that the vast majority of Fabry heterozygotes are symptomatic and develop cardiac involvement with increasing age [2]. The diagnosis of Fabry hemizygotes is essentially based on the demonstration of decreased GLA activity. However, the GLA assay is inconclusive in heterozygous Fabry females, the activity ranging from relatively low to normal levels.

Recombinant GLAs have been produced in Chinese hamster ovary cells [3,4] and genetically modified human fibroblasts [5], and they are clinically available for enzyme replacement therapy (ERT) for Fabry disease. Although many patients have been successfully treated with these recombinant GLAs, little or no improvement in cardiac and renal functions can be obtained in some patients, especially in ones in whom the stage of the disease is advanced [6,7]. This suggests that early diagnosis and early treatment are important, and thus the determination of a useful biomarker for the diagnosis of and monitoring of the response to ERT in Fabry disease has been strongly required.

The levels of Gb3 in plasma and/or urine have been measured for the above purposes [8–11], but recent systemic analysis revealed that Gb3 was not an ideal marker for a diagnosis or the response to treatment in patients with this disease [12,13]. Recently, Aerts et al. reported that globotriaosylsphingosine (lyso-Gb3) was increased in the plasma of classically affected male Fabry patients,

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Table 1
Plasma lyso-Gb3, Gb3, GLA activity, and clinical manifestations in the patients with Fabry disease.

	Case No.	Age	Clinical manifestations				Plasma		
			Pain	Renal involvement	Cardiac involvement	Cerebrovascular involvement	Lyso-Gb3 (nmol/L)	Gb3 (μg/mL)	GLA activity (nmol/h/mL)
Hemizygotes	1	18	+	–	–	–	193	21.0	<1
	2	21	+	–	–	–	58	6.8	<1
	3	32	+	+	+	+	132	15.2	<1
	4*	33	+	+	+	–	134	23.7	<1
	5*	40	+	+	+	–	139	23.7	<1
	6	50	+	+	+	–	150	23.1	<1
	7	60	–	–	+	–	38	9.2	<1
	8	65	–	–	+	–	74	5.2	<1
	9	66	–	–	+	–	35	7.4	1.4
	10	69	–	–	+	–	19	4.3	<1
Heterozygotes	11	17	–	–	–	–	11	6.8	6.6
	12	21	–	+	–	–	9	7.0	7.9
	13	35	+	–	–	–	17	7.1	4.4
	14	40	+	–	–	–	35	5.5	3.3
	15	44	–	–	–	–	14	7.2	6.9
	16	46	+	–	–	–	13	14.0	5.2
	17	63	–	+	+	–	29	6.9	4.8
	18	70	+	–	–	–	13	3.3	6.4
Control subjects (n = 6)		22–60					<2	5.3 ± 1.3**	8.0 ± 3.0**

Case No. 1–6: classic Fabry hemizygotes; Case No. 7–10: variant Fabry hemizygotes.
* Brothers in the same family.
** Expressed as means ± standard deviation.

and in the plasma and tissues of Fabry mice, and they suggested that circulating lyso-Gb3 could be a candidate biomarker for monitoring Fabry disease [13].

In this study, we measured the plasma lyso-Gb3 and Gb3 concentrations in male and female patients with both the classic and variant forms of Fabry disease, and compared them, and examined the response to ERT to determine whether lyso-Gb3 is a useful biomarker of Fabry disease.

Materials and methods

Materials

Lyso-Gb3, Gb3 and *N*-acetyl-D-galactosamine were obtained from Sigma Chemical Co. (St. Louis, MO). *o*-Phthalaldehyde (OPA) was purchased from Nacalai Tesque (Kyoto, Japan). 4-Methylumbelliferyl-α-D-galactopyranoside was obtained from Calbiochem (LaJolla, CA). All other chemicals used were of analytical grade.

Plasma samples and patients

Plasma samples for measurement of the lyso-Gb3 and Gb3 concentrations, and GLA activity were obtained from ten hemizygous Fabry males (six classic and four variant cases), eight heterozygous Fabry females (6 symptomatic and 2 asymptomatic cases), and six healthy volunteers. The clinical information is summarized in Table 1. A clinical diagnosis was established from the clinical manifestations, family history, GLA activity in white blood cells (WBCs) and/or cultured fibroblasts, pathological findings and the results of gene analysis.

To monitor ERT, plasma samples were obtained from a male patient with Fabry disease who had been followed for a long time after ERT with recombinant GLA, agalsidase beta (Fabrazyme®; Genzyme Co., Cambridge, MA), before treatment and then at regular intervals during the treatment. When the ERT began, the patient was 40-years-old, and exhibited angiokeratomas, diarrhea, tinnitus, and palpitations. An ST-T change was observed on electrocardiography. A two-dimensional echocardiogram revealed a mitral valve prolapse, and the thicknesses of the intraventricular septum and the left ventricule posterior wall at end distol were calculated to be 9 and 10 mm, respectively. He had no proteinuria, and his serum creatinine and urea nitrogen levels were 0.8 and 11 mg/dl, respectively. The GLA activity in his white blood cells was <1 nmol/h/mg protein (normal range, 101–141), and gene analysis revealed a missense mutation, p.Q250P. He had received ERT with the enzyme at the dose of 1.0 mg/kg body weight for four years. No antibodies to the enzyme had been detected throughout the therapy. During the ERT, no progression of the clinical manifestations or findings had been observed.

This study involving human plasma samples was approved by the Ethical Committee of our institute.

Measurement of lyso-Gb3 in human plasma

Extraction of lyso-Gb3 from the plasma samples was performed according to Aerts's method [13]. To 50 μl of plasma, 25 μl of water was added, and then the lipids were extracted with 450 μl of chloroform/methanol (1/2, v/v). Then the extract was centrifuged for 10 min at 14,000g, and the supernatant was removed. To the supernatant, 150 μl of chloroform and 225 μl of water were added, followed by mixing and then brief centrifugation of the sample. Then the upper phase was removed, and the lower chloroform phase was extracted once more with 600 μl of methanol/water (1/1, v/v). Then the combined upper phases were dried and taken up in 500 μl of water, and the water phase was extracted twice with 500 μl of water-saturated 1-butanol. The butanol phase was dried and dissolved in 60 μl of methanol. Then, 50 μl of the solution was taken to dryness, and the lyso-Gb3 was derivatized with 25 μl of OPA reagent (pH 11). The OPA-derivatized lyso-Gb3 was separated by high performance liquid chromatography (HPLC), and then quantitated by fluorescence detection. Chromatographic separation was carried out on a Unison UK-C18 column (150 × 4.6 mm I.D., 3 μm; Imtakt, Kyoto, Japan) with a mobile phase of methanol/water (85/15, v/v). A calibration curve for