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Biomarkers associated with clinical manifestations in Fabry disease patients with a late-onset cardiac variant mutation

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ABSTRACT

Background: Fabry disease is a lysosomal storage disorder with an incidence of 1:1600 for the late-onset IVS4 + 919G > A cardiac variant mutation in Taiwan. Signs and symptoms of this cardiac variant include left ventricular hypertrophy, mitral insufficiency and/or arrhythmias. The search for biomarkers that might predict the clinical outcomes and guide treatment options is important. We thus investigated relationships between Fabry disease biomarkers (such as globotriaosylceramide (Gb_3), globotriaosylsphingosine (lyso-Gb_3)/related analogues) and age, gender, enzyme activity, clinical manifestations and severity of the disease in these patients.
Method: Urine and plasma biomarkers were analyzed using tandem mass spectrometry. A large cohort of 191 adult and pediatric Fabry patients carrying the IVS4 + 919G > A mutation was studied. Some patients were members of the same family.
Results: Our results show that the plasma lyso-Gb_3 level, and urinary analogue levels of lyso-Gb_3 at m/z (+16), (+34), and (+50) adjusted for gender and age had a positive association with the left ventricular mass index, and/or the Mainz Severity Score Index.
Conclusions: It might thus be of particular interest to monitor children with high levels of these biomarkers, as part of a longitudinal study in order to determine if the excretion profile at a young age is predictive of the outcomes of disease severity in adulthood.

1. Introduction

Fabry disease (OMIM no. 301500) is an X-linked multisystemic, panethnic, lysosomal storage disorder caused by deficiency of α-galactosidase A (EC 3.2.1.22) activity. This leads to progressive accumulation of globotriaosylceramide (Gb_3) [1–4] and related glycosphingolipids, such as globotriaosylsphingosine (lyso-Gb_3) [5–7] in lysosomes of the heart, kidneys, skin and brain. Clinical features in classically affected patients include acroparesthesia, angiokeratomas, and hypohidrosis in early childhood or adolescence, and progress to renal insufficiency, cardiomyopathy, and cerebrovascular disease in adulthood [8–11]. Patients with late-onset Fabry disease have higher residual enzyme activity than those with the classical type. They lack the classical signs/symptoms of Fabry disease and present relatively fewer or isolated manifestations, such as hypertrophic cardiomyopathy, renal failure, or cryptogenic stroke at later stages in life [12–15]. In fact, the late-onset cardiac phenotype usually presents only with cardiac manifestations, such as hypertrophic cardiomyopathy, mitral insufficiency and/or arrhythmias in the fifth to eighth decade [14,16,17]. The incidence of Fabry disease has been reported as 1:40,000 to 1:117,000 live births in the general population [18–19]. However, recent studies revealed that the prevalence of late-onset Fabry disease is much higher than classical Fabry disease and might be a hidden important health issue in some populations [20–26]. Through newborn screening, our team revealed a high incidence (approximately 1:1600 males) of a Fabry mutation, IVS4 + 919G > A (IVS4) associated with the late-onset cardiac phenotype, in the Taiwanese population [27–29]. Further investigation of the disease onset rate according to ages of male and female adults with this latter mutation revealed that 77% of adult males and 35% of adult females older than 40 years of age had already developed hypertrophic cardiomyopathy [30]. However, the natural course

Abbreviations: ERT, enzyme replacement therapy; Gb_3, Globotriaosylceramide; IVSd, diastolic interventricular septal thickness; LOD, limit of detection; LVIDd, diastolic left ventricular internal diameter; LVIDs, systolic left ventricular internal diameter; LVM, left ventricular mass; LVMI, left ventricular mass index; LVPWd, diastolic left ventricular posterior wall thickness; lyso-Gb_3, globotriaosylsphingosine; MSSI, Mainz Severity Score Index; NBS, newborn screening.

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of Fabry disease in patients with this mutation still remains largely unknown, and important questions are still unanswered: When does the appearance of biomarker accumulation start to signal impairment of cardiomyocytes function in patients bearing the IVS4 mutation? When is the best timing to start enzyme replacement therapy (ERT) to achieve optimal treatment outcomes? Which biomarkers are the most reliable for the identification of IVS4-bearing individuals who are at high-risk to develop clinically significant cardiomyopathy? Which biomarkers are more efficient for monitoring and follow-up of these patients?

Recent studies by our group revealed lyso-Gb_3 analogues in urine and plasma, which are deacylated forms of Gb_3 [31–35]. These analogues were structurally elucidated and each empirical formula was established by exact mass measurements by mass spectrometry [31, 34]. Another study with Fabry patients having a late-onset cardiac variant mutation (*p*.N215S) showed abnormal levels of lyso-Gb_3 or analogues, while the urinary Gb_3 levels were normal [33]. It was also demonstrated that biomarker profiles of Gb_3 and lyso-Gb_3 and related analogues correlated closely with the phenotypic expression in a cohort of Fabry disease patients having classical, late-onset cardiac variant, and atypical mutations of the disease [36]. In this study, we evaluated a cohort of 191 patients carrying the IVS4 late-onset cardiac variant mutation, including both adults and children. Some were members of the same family. The profiles of Gb_3 and lyso-Gb_3 and related analogues were determined, and the relationship between these biomarkers and clinical manifestations of the disease in these patients was investigated.

2. Methods

2.1. Patients

This project was approved by the Research Ethics Board at the Centre hospitalier universitaire de Sherbrooke, and the Institutional Review Board at Taipei Veterans General Hospital. Informed consent was obtained from all Taipei adult subjects and parents of children.

Urine and/or plasma specimens were collected from 191 individuals carrying the Fabry mutation IVS4 + 919G > A. All samples were obtained prior to ERT initiation. Of these 191 individuals, 79 were under 18 years of age (pediatric group) and 112 were adults. Most of the children were detected through a nationwide newborn screening (NBS) program in Taiwan [27,28], while others were found during family studies of newborns identified in the NBS program. All of the individuals had a confirmed Fabry diagnosis by molecular genetic analysis. Demographics are shown in Table 1. There was an imbalance in the representation of males and females in the pediatric cohort, due to the fact that the NBS was based on enzyme activity assessments, thus most females might have been missed by this screening. Samples were stored at −80 °C in Taipei, then shipped to Sherbrooke, Quebec on dry ice and stored at −20 °C until analysis.

2.2. Clinical information and parameters

Mainz Severity Score Index (MSSI) was calculated [37] for each individual in the adult group according to reported signs/symptoms. Plasma α-galactosidase A enzyme activity was measured using a 4-MU fluorescent method according to a previously published methodology [27]. Left ventricular mass (LVM) was calculated according to the American Society of Echocardiography's Guidelines with the following equation [38]:

$$\mathrm{LVM(g)} = 0.8 \times \left(1.04 \times [(\mathrm{LVIDd}) + (\mathrm{IVSd}) + (\mathrm{LVPWd})]^3 - [\mathrm{LVIDs}]^3\right) + 0.6,$$

where LVIDd: diastolic left ventricular internal diameter; IVSd: diastolic interventricular septal thickness; LVPWd: diastolic left ventricular posterior wall thickness; LVIDs: systolic left ventricular internal diameter.

LVM was normalized to height (meter) to 2.7 power (left ventricular mass index (LVMI) = LVM/height$^{2.7}$). Left ventricular hypertrophy (LVH) was defined as LVMI > 51 g/m$^{2.7}$ in men and LVMI > 48 g/m$^{2.7}$ in women [39]. A low glomerular filtration rate (GFR) was defined as <60 mL/min/1.73 m^2. Proteinuria was defined as an albumin/creatinine ratio >30 mg/g creatinine.

2.3. Urinary biomarker measurements

Globotriaosylceramide (Gb_3) was measured in urine collected on filter paper according to a previously published mass spectrometry methodology [4]. Gb_3 concentration was expressed as the total ion count of eight Gb_3 isoform signals (C16:0, C18:0, C20:0, C22:1, C22:0, C24:1, C24:0, C24:OH) normalized to creatinine.

Globotriaosylsphingosine (lyso-Gb_3) and seven analogues were measured in urine by mass spectrometry using a UPLC-Xevo TQ-S instrument (Waters Corp., Milford, MA, USA) as previously described [33]. These analogues are lyso-Gb_3 molecules with different modifications of the sphingosine moieties: lyso-Gb_3 (−28) ($-C_2H_4$), lyso-Gb_3 (−12) ($-C_2H_4 + O$), lyso-Gb_3 (−2) ($-H_2$), lyso-Gb_3 (+14) ($-H_2 + O$), lyso-Gb_3 (+16) (+O), lyso-Gb_3 (+34) ($+H_2O_2$), and lyso-Gb_3 (+50) ($+H_2O_3$). All urinary biomarkers were normalized to creatinine, in order to correct for variations in urine concentrations [31].

2.4. Plasma biomarker measurements

In plasma, lyso-Gb_3 and six related analogues including lyso-Gb_3 (−28), lyso-Gb_3 (−2), lyso-Gb_3 (+16), lyso-Gb_3 (+18), lyso-Gb_3 (+34), and lyso-Gb_3 (+50) were also measured with the UPLC-Xevo TQ-S (Waters) as previously described [35].

2.5. Statistical analyses

Statistical analyses were performed using IBM SPSS Statistics version 22. Correlations between biomarker levels and gender were established using the Mann-Whitney *U* Test. The Spearman rank correlation coefficient was used to evaluate the correlation between age and biomarker excretion levels in adults. Multiple regression analyses were performed to establish the association between biomarker levels and LVMI/MSSI, adjusted for gender and age. As shown in Table 1, the median age of adult Fabry males (57.82 years old) was higher than for adult females (36.75 years old). This was taken into account during the multiple regression statistical analysis. Statistical significance was established at $p < 0.01$ for all comparisons. For each test, statistical significance was evaluated using the Holm-Šídák procedure for multiple comparisons.

3. Results

All data from this manuscript are available in Supplementary file 1.

3.1. Reported signs and symptoms

Registered signs and symptoms were evaluated for all Fabry patients. All children were found to be asymptomatic at the time of specimen collection, while signs/symptoms experienced by adults are shown in Table 2. In the General signs/symptom category, the most prevalent manifestations were NYHA (New York Heart Association)

Table 1
Demographics of patients enrolled in this study.

Fabry patient subgroups	Gender	n	Age range (y)	Median (y)
Pediatric < 18 years old	Females	13	0.10–4.58	0.14
	Males	66	0.05–15.44	0.18
Adult > 18 years old	Females	87	19.62–85.52	36.75
	Males	25	26.33–74.34	57.82