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Urinary biomarker investigation in children with Fabry disease using tandem mass spectrometry

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ABSTRACT

Background: Fabry disease is an X-linked lysosomal storage disorder affecting both males and females with tremendous genotypic/phenotypic variability. Concentrations of globotriaosylceramide (Gb_3), globotriaosylsphingosine (lyso-Gb_3)/related analogues were investigated in pediatric and adult Fabry cohorts. The aims of this study were to transfer and validate an HPLC–MS/MS methodology on a UPLC–MS/MS new generation platform, using an HPLC column, for urine analysis of treated and untreated pediatric and adult Fabry patients, to establish correlations between the excretion of Fabry biomarkers with gender, treatment, types of mutations, and to evaluate the biomarker reliability for early detection of pediatric Fabry patients.
Method: A UPLC–MS/MS was used for biomarker analysis.
Results: Reference values are presented for all biomarkers. Results show that gender strongly influences the excretion of each biomarker in the pediatric Fabry cohort, with females having lower urinary levels of all biomarkers. Urinary distribution of lyso-Gb_3/related analogues in treated Fabry males was similar to the untreated and treated Fabry female groups in both children and adult cohorts. Children with the late-onset p.N215S mutation had normal urinary levels of Gb_3, and lyso-Gb_3 but abnormal levels of related analogues.
Conclusions: In this study, Fabry males and most Fabry females would have been diagnosed using the urinary lyso-Gb_3/related analogue profile.

1. Introduction

Fabry disease (OMIM #301500) is a multisystemic, panethnic X-linked lysosomal storage disorder. It is caused by the defect in the activity of the alpha-galactosidase A (α-GAL or GLA; EC 3.2.1.22) enzyme, which results in the accumulation of glycosphingolipids, particularly globotriaosylceramide (Gb_3) and globotriaosylsphingosine (lyso-Gb_3, a deacylated Gb_3 molecule) in the heart, eyes, kidneys, vascular endothelium, and biological fluids. Clinical manifestations occur in both male and female patients; however, females generally have less severe symptoms at a later onset than their male counterparts. The symptomatology is quite diverse and may present with neurological, dermatologic, gastrointestinal, ocular, renal, and cardiac manifestations [1].

The signs and symptoms of Fabry disease are less severe in childhood and have been well described [2]. Children commonly present with acroparesthesias, intolerance to heat and cold, and hypohidrosis, and less commonly with renal involvements such as mild proteinuria, microalbuminuria, and changes in glomerular filtration rate (GFR) [2,3]. Najafian et al. found a relation between age and clinical progression and the accumulation of podocyte Gb_3 deposits in Fabry renal

Abbreviations: Gb_3, globotriaosylceramide; lyso-Gb_3, globotriaosylsphingosine; α-GAL, alpha-galactosidase A; LVH, left ventricular hypertrophy; ERT, enzyme replacement therapy; CFDI, Canadian Fabry Disease Initiative; REB, Research Ethics Board; CHUS, Centre hospitalier universitaire de Sherbrooke; PLNO, partial loop with needle overfill; PASW, Predictive Analytics Software; CFM, children Fabry males; CFF, children Fabry females.

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disease [4]. This study also reports a relationship between early renal structural changes and proteinuria in young patients. Cardiac manifestations may also occur in adolescence with left ventricular hypertrophy (LVH) and mild valvular insufficiency [2]. Ophthalmologic manifestations are present even in young children with cornea verticillata and retinal vascular tortuosities, especially in those with severe loss-of-function *GLA* mutations [5].

As expected for an X-linked disease, major differences in the symptomatology of pediatric Fabry disease have been reported between genders. In pediatric Fabry patients, it was reported that boys expressed more pain related to acroparesthesia than girls. However, proteinuria was reported to be more common in female children with Fabry disease than in male children [2,3]. Moreover, the occurrence of cardiac complications in the pediatric Fabry cohort appear as subtle variations progressing slowly over time, with boys being more affected than girls [6].

Although treating Fabry patients with enzyme replacement therapy (ERT) has been found to reduce Gb_3 storage in different type of cells, as well as to reduce the clinical symptoms of the disease, the reversal of existing tissue damage is generally incomplete [7]. Overall, ERT is generally well tolerated by pediatric Fabry patients and reduces pain [8]; however, long term evaluation in the pediatric population is needed to assess the impact of early initiation of ERT on the progression of major organ dysfunction associated with the disease [9]. Reliable tools are now available to evaluate pain in children in order to better investigate the effect of ERT [10]. Identification and evaluation of biomarkers might prove to be useful for the assessment of other aspects of the progression of the disease, as well as the response to treatment, and are reliable for screening purposes. Biomarkers might also be useful to establish genotype/phenotype correlations [3,11].

Previous research studies revealed that lyso-Gb_3 [12–15] and its analogues [16–20] might be useful biomarkers reflecting Fabry disease severity, and of significant value in the screening of Fabry disease as well as for the follow-up of treatment. Fig. 1 presents the chemical structures of Gb_3, lyso-Gb_3 and 7 analogues of the latter. Lyso-Gb_3 and its analogues have already been characterized using a mass spectrometry approach [16]. Considering the tremendous genotypic variability (more than 600 mutations have been reported) [21] and phenotypic heterogeneity among Fabry patients, a need exists to evaluate the relationship between these complex biomarkers and different types of *GLA* mutations.

This study has 4 objectives: 1) to transfer and validate the current methodology for the analysis of urinary lyso-Gb_3 and its 7 related analogues performed on an Alliance-Quattro Micro system (high performance liquid chromatography/tandem mass spectrometry, HPLC-MS/MS) (Waters Corp., Milford, MA) to a new generation Acquity-Xevo TQ-S system (ultra performance liquid chromatography/tandem mass spectrometry, UPLC–MS/MS) (Waters); 2) to analyze urine samples from treated and untreated pediatric and adult Fabry patients; 3) to establish correlations between the excretion of Gb_3, lyso-Gb_3 and its 7 related analogues with the gender, ERT treatment, and types of *GLA* mutations in children; and 4) to evaluate the reliability of these biomarkers for early detection of pediatric Fabry patients, as well as the potential therapeutic response. Indications for ERT according to the Canadian Fabry Disease Initiative (CFDI) guidelines are also presented.

2. Material and methods

2.1. Ethics approval

This project was approved by the Research Ethics Board (REB) at the Faculty of Medicine and Health Sciences of the Centre hospitalier universitaire de Sherbrooke (CHUS), as well as by the institutional REBs of all the collaborators. Informed consent was obtained from all subjects.

2.2. Collection of urine specimens from Fabry patients and controls

Random urine specimens were collected from children and adults with Fabry disease in whom the diagnosis was confirmed by enzyme analysis and identification of a pathogenic mutation in the *GLA* gene. Urine samples were stored at −20 °C after being collected as part of routine clinical care. No degradation of lyso-Gb_3 nor its 7 analogues was observed while storing urine samples in these conditions for at least 9 weeks. For the purpose of this study, individuals <18 years were classified in the pediatric group. Urine specimens from 55 pediatric Fabry patients were thus analyzed: 29 males (age range: 1 to 17 years, median age: 8 years), and 26 females (age range: 0.5 to 18 years, median age: 11 years). In the pediatric cohort, 6 males and 2 females were receiving ERT. Data are presented in Supplementary Information Table 1. In the adult group, 108 urine specimens were analyzed: 46 males (age range: 19 to 72 years, median age: 42 years), and 62 females (age range: 19 to 79 years, median age: 48 years). In the adult cohort, 38 males and 31 females were treated with ERT. Data are presented in Supplementary Information Table 2. Two urine specimens were taken from a Fabry patient before (at 10 years) and after (at 14 years) the initiation of ERT. This patient experienced acroparesthesia and fatigue attributed to Fabry disease. Indications for ERT according to the Canadian Fabry Disease Treatment Guidelines (2012) are the same for all genders and age groups and are provided in Supplementary Information Table 3. For reference purposes, we include indications for ERT for CFDI Cohort 1a

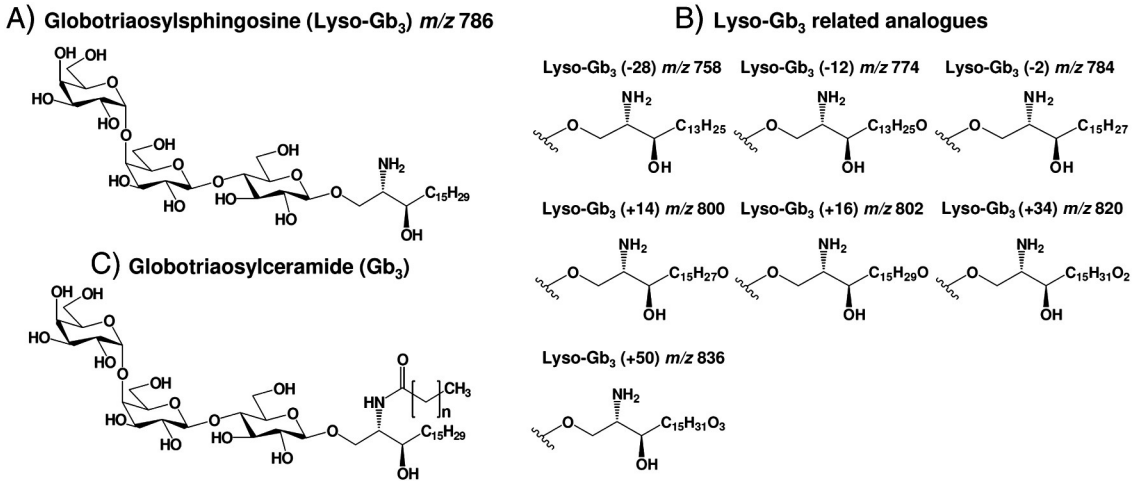

Fig. 1. Chemical structures of A) globotriaosylsphingosine (lyso-Gb_3); B) its 7 related analogues; and C) globotriaosylceramide (Gb_3). The analogues of lyso-Gb_3 have modifications on the sphingosine moiety of the lyso-Gb_3 molecule, as displayed in B).