Clinica Chimica Acta 426 (2013) 114–120

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

Plasma globotriaosylsphingosine (lysoGb3) could be a biomarker for Fabry disease with a Chinese hotspot late-onset mutation (IVS4 + 919G>A)☆

Hsuan-Chieh Liao [a,b,n], Yu-Hsiu Huang [a,c,n], Yann-Jang Chen [a,d], Shu-Min Kao [b], Hsiang-Yu Lin [a,e,f], Chun-Kai Huang [c], Hao-Chuan Liu [c], Ting-Rong Hsu [c], Shuan-Pei Lin [e,f], Chia-Feng Yang [c], Cathy S.J. Fann [g], Pao-Chin Chiu [h], Kai-Sheng Hsieh [h], Yun-Ching Fu [a,i], Yu-Yuan Ke [i], Ching-Yuang Lin [j], Fuu-Jen Tsai [k], Chung-Hsing Wang [k], Mei-Chyn Chao [l], Wen-Chung Yu [m], Chuan-Chi Chiang [b,n,*], Dau-Ming Niu [a,c,n,**]

[a] Institute of Clinical Medicine, National Yang-Ming University, Taipei, Taiwan
[b] Chinese Foundation of Health, Section of Newborn Screening Center, Taipei, Taiwan
[c] Department of Pediatrics, Taipei Veterans General Hospital, Taipei, Taiwan
[d] Department of Life Sciences and Institute of Genome Sciences, National Yang-Ming University, Taipei, Taiwan
[e] Department of Pediatrics, Mackay Memorial Hospital, Taipei, Taiwan
[f] Department of Medicine, Mackay Medical College, Taipei, Taiwan
[g] Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan
[h] Department of Pediatrics, Kaohsiung Veterans General Hospital, Kaohsiung, Taiwan
[i] Department of Pediatrics, Taichung Veterans General Hospital, Taichung, Taiwan
[j] College of Medicine, China Medical University, Taichung, Taiwan
[k] Department of Pediatrics, China Medical University Hospital, Taichung, Taiwan
[l] Department of Pediatrics, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan
[m] Division of Cardiology, Taipei Veterans General Hospital, Taipei, Taiwan
[n] Taiwan Clinical Trial Consortium in Fabry Disease, Taiwan

ARTICLE INFO

Article history:
Received 7 August 2013
Accepted 10 September 2013
Available online 19 September 2013

Keywords:
Fabry disease
IVS4 + 919G>A
LysoGb3
Newborn screening
Hypertrophic cardiomyopathy

ABSTRACT

Background: Previous studies revealed a high incidence of late-onset Fabry disease mutation, IVS4 + 919G>A, in Taiwan. However, the natural course is largely unclear and suitable biomarkers for monitoring disease progress are unavailable.

Methods and results: Patients carrying IVS4 + 919G>A or classical Fabry mutations were enrolled in this study. The subjects ranged from newborn to eighty year old adults. Plasma globotriaosylceramide (Gb3) and globotriaosylsphingosine (lysoGb3) were measured by LC-MS/MS in subjects to evaluate the sensitivity of these two biomarkers. All adult males and symptomatic females could be distinguished from healthy controls by an elevated plasma lysoGb3 level. The lysoGb3 level was also related to the left ventricular mass considering gender and age ($p < 0.01$). Moreover, approximately 70% of male and 45% of female newborns already had an elevated plasma lysoGb3 level which increased gradually as the subjects got older ($p < 0.01$).

Conclusions: Plasma lysoGb3 is a more sensitive and reliable biomarker than plasma Gb3. LysoGb3 also correlated with age and left ventricular mass index in Fabry patients with IVS4 + 919G>A mutation. Because lots of infants with the IVS4 + 919G>A mutation already had elevated lysoGb3 levels at birth, that indicates that the development of hypertrophic cardiomyopathy may require a long and insidious course after lysoGb3 accumulation.

Abbreviations: Gb3, globotriaosylceramide; LysoGb3, globotriaosylsphingosine; LVMI, left ventricular mass index; LC-MS/MS, high performance liquid chromatography mass spectrometry; ERT, enzyme replacement therapy.

* Correspondence to: C.-C. Chiang, Chinese Foundation of Health, Taipei, Section of Newborn Screening, 11070, 5F., No. 55, Dongxing Rd., Xinyi Dist., Taipei City 110, Taiwan. Tel.: +886 2 8768 1020; fax: +886 2 8768 1021.
** Correspondence to: D.-M. Niu, Department of Pediatrics, Taipei Veterans General Hospital, 201, Section 2, Shih-Pai Road, Taipei 112, Taiwan. Tel.: +886 2 2875 7128; fax: +886 2 2876 7181.
E-mail addresses: ccchiang@cfoh.org.tw (C.-C. Chiang), dmniu1111@yahoo.com.tw (D.-M. Niu).

0009-8981/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.cca.2013.09.008

1. Introduction

Fabry disease (MIM 301500) is a panethnic, X-linked, inborn error of glycosphingolipid metabolism resulting from mutations in the α-galactosidase A gene leading to deficient activity of a lysosomal enzyme, α-galactosidase A (GLA, EC 3.2.1.22) [1,2]. Deficiency of this enzyme, α-galactosidase A, which is involved in the metabolic breakdown of globotriaosylceramide (Gb3) results in progressive accumulation of Gb3 and related glycosphingolipids in lysosomes of all cells in the body. Affected male patients who have little or no GLA activity exhibit the classic phenotype of Fabry disease, presenting acroparesthesias, angiokeratomas, hypohidrosis in early childhood or adolescence [3], progressing to renal insufficiency, cardiomyopathy, and cerebrovascular disease in adulthood. A wide spectrum of phenotypic expression found in heterozygous females is considered generally as effects of random inactivation of X-chromosome during embryogenesis. Despite the wide range of disease severity, several heterozygous females still develop vital organ damages and lead to severe morbidity and mortality [4–6]. Over the past decades, different atypical types of Fabry disease have been identified [7,8], which have drawn the attention of more physicians. Patients with atypical Fabry disease have higher residual enzyme activities than those of the classical type. They lack the classic symptoms of Fabry disease and present relatively fewer or isolated symptoms such as hypertrophic cardiomyopathy, renal failure, or cryptogenic stroke at later stages in life [7–10]. The estimated incidence of classic Fabry disease is 1 in 40,000–60,000 males in the general population [11]. Previous studies of Fabry newborn screening, however, revealed a remarkably higher prevalence of about 1:900 to 1:4000 in males and 1:400 to 1:2000 in females for atypical Fabry disease [12–15]. These findings indicated that the atypical Fabry disease might represent a major neglected public health problem in at least some ethnic groups. However, the natural course of atypical Fabry diseases is still unclear, and suitable biomarkers for monitoring the disease progression have not been established.

Our team first discovered a surprisingly high incidence (≈1 in 1600 males) of a cardiac variant *GLA* mutation, IVS4 + 919G>A, in Taiwanese through newborn screening [16] and further identified this mutation in a number of adult patients with idiopathic hypertrophic cardiomyopathy [13]. Thereafter, another newborn screening center in Taiwan also found a similar incidence (1 in 1460 males) of this mutation in their study [14]. Higher prevalence rate (72% of males and 35% of females) of this IVS4 + 919G>A mutation was also detected in over 40 year-old adults with cardiomyopathy [6]. This mutation not only leads to hypertrophic cardiomyopathy, but can also potentially cause mild impairment of the kidneys and the eyes [17]. Recently, a DNA-based newborn screening for this mutation revealed a higher incidence of this mutation (1/875 in males and 1/399 in females) than our previous enzyme-based Fabry newborn screening in Taiwan [18]. In addition to Taiwan, this mutation has also been found in Japan [19], Mainland China, Singapore (Han population), and Malaysia (Han population) by our team (unpublished data). Therefore, we believe that IVS4 + 919G>A Fabry mutation may be one of the most common pathogenic mutations of Fabry disease. It is important to understand the natural course of the disease and determine the best time to start enzyme replacement therapy (ERT).

Gb3 in plasma or urine has been used as a biomarker to monitor the clinical course and the response to ERT in Fabry disease. However, these biomarkers cannot truly reflect Fabry disease manifestations and therapeutic outcomes [20–22]. Furthermore, these biomarkers, especially plasma Gb3, were not sensitive enough to detect a large proportion of the heterozygous females and atypical variants [23,24]. Recently, Aerts et al. introduced a new marker for Fabry disease, namely globotriaosylsphingosine (lysoGb3), which is dramatically increased in the plasma of classical Fabry patients [25]. Thereafter, plasma lysoGb3 was proven to have a significantly higher diagnostic sensitivity compared to plasma Gb3 [24,26,27], especially in heterozygous females [24,28]. Because a reduction of lysoGb3 levels both in plasma and tissue was clearly observed upon ERT, plasma lysoGb3 might be a reliable marker for monitoring the therapeutic outcomes of ERT [26,27]. A diagnostic method for measuring urine lysoGb3 was recently developed and showed the close correlation of the urinary excretion of lysoGb3 with the genotype. That suggested a possible value of lysoGb3 for predicting clinical severity [29].

More recently, a fast and reliable LC-MS/MS assay was also developed to analyze plasma lysoGb3 levels [29,30]. Low residual enzyme activity is usually related to high plasma lysoGb3 levels [31]. However, most of these studies were limited to classical Fabry disease; atypical variants have rarely been analyzed. In our previous study, we found that almost all adult males (including symptomatic and asymptomatic) and symptomatic females with the IVS4 + 919G>A mutation can be distinguished from the normal controls by an elevated plasma lysoGb3 level, but not Gb3, and some of the newborns with this mutation already had elevated lyso-Gb3 levels [32,33]. A similar finding was also observed by another newborn screening center in Taiwan [34]. In this study, we first enrolled subjects with classical or IVS4 + 919G>A mutation to evaluate the sensitivity of plasma Gb3 and lyso-Gb3 levels in these individuals. We then further analyzed plasma lysoGb3 levels in symptomatic and asymptomatic subjects with the IVS4 + 919G>A mutation, and evaluated the relationship between the values of plasma lysoGb3 and left ventricular mass index (LVMI) in subjects with the IVS4 + 919G>A mutation. Finally, we evaluated the nature course of the manifestation of plasma lysoGb3 levels in the subjects carrying the IVS4 + 919G>A mutation.

2. Materials and methods

2.1. Ethics approval

The study was approved by the institutional review board of Taipei Veterans General Hospital (No. 2012-02-065B). Written informed consents were obtained from parents of children and from adult subjects (over 20 years old) before beginning individual examinations.

2.2. Subjects

2.2.1. Evaluating the sensitivity of plasma Gb3 and lysoGb3 levels in the subjects with a classical or IVS4 + 919G>A mutation

A total of 75 adults (>20 years old, including 32 males and 43 females) carrying the IVS4 + 919G>A mutation and 20 Fabry patients (including 3 newborns and 17 adults) carrying classical mutations were enrolled in the first part of this study. Plasma Gb3 and lysoGb3 levels were measured to evaluate the sensitivity of these two biomarkers in these individuals. Except for three infants, all the individuals with the classical Fabry mutations were symptomatic patients. All blood samples in this study were obtained before the initiation of ERT. Control samples from 31 healthy adults (16 males and 15 females) were enrolled to establish normal reference values of plasma lysoGb3. All individuals in the normal control group were confirmed to have normal enzyme activity and without carrying any known mutation in *GLA* gene.

2.2.2. Evaluation of plasma lysoGb3 levels and the relationship of plasma lysoGb3 and left ventricular mass index (LVMI) in subjects with a IVS4 + 919G>A mutation

The objective of the second part of this study was to investigate the relationship between lysoGb3 and clinical manifestation in the adults (>20 years old) carrying the IVS4 + 919G>A mutation. In this part of the study, our sample size was extended to 146 individuals and divided into symptomatic and asymptomatic subjects. Another 72 healthy adults (35 males and 37 females) were enrolled as normal controls. The adults carrying the IVS4 + 919G>A mutation without any cardiac symptoms and signs (such as left ventricular hypertrophy [35], left ventricular diastolic dysfunction, etc.) were defined as asymptomatic group, while individuals who have already developed cardiac symptoms (left ventricular mass index (LVMI) >51 g/m$^{2.7}$ in males and