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Plasma mutant α-galactosidase A protein and globotriaosylsphingosine level in Fabry disease

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ABSTRACT

Fabry disease is an X-linked genetic disorder characterized by deficient activity of α-galactosidase A (GLA) and accumulation of glycolipids, and various GLA gene mutations lead to a wide range of clinical phenotypes from the classic form to the later-onset one. To investigate the biochemical heterogeneity and elucidate the basis of the disease using available clinical samples, we measured GLA activity, GLA protein and accumulated globotriaosylsphingosine (Lyso-Gb3), a biomarker of this disease, in plasma samples from Fabry patients. The analysis revealed that both the enzyme activity and the protein level were apparently decreased, and the enzyme activity was well correlated with the protein level in many Fabry patients. In these cases, a defect of biosynthesis or excessive degradation of mutant GLAs should be involved in the pathogenesis, and the residual protein level would determine the accumulation of Lyso-Gb3 and the severity of the disease. However, there are some exceptional cases, i.e., ones harboring p.C142Y, p.R112H and p.M296I, who exhibit a considerable amount of GLA protein. Especially, a subset of Fabry patients with p.R112H or p.M296I has been attracted interest because the patients exhibit almost normal plasma Lyso-Gb3 concentration. Structural analysis revealed that C142Y causes a structural change at the entrance of the active site. It will lead to a complete enzyme activity deficiency, resulting in a high level of plasma Lyso-Gb3 and the classic Fabry disease. On the other hand, it is thought that R112H causes a relatively large structural change on the molecular

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surface, and M296I a small one in a restricted region from the core to the surface, both the structural changes being far from the active site. These changes will cause not only partial degradation but also degeneration of the mutant GLA proteins, and the degenerated enzymes exhibiting small and residual activity remain and probably facilitate degradation of Lyso-Gb3 in plasma, leading to the later-onset phenotype. The results of this comprehensive analysis will be useful for elucidation of the basis of Fabry disease.

1. Introduction

Fabry disease (MIM 301500) is an X-linked genetic disease caused by deficient activity of α-galactosidase A (GLA) [1,2]. The lack of enzyme activity results in progressive accumulation of neutral glycolipids including globotriaosylceramide (Gb3) and globatriaosylsphingosine (Lyso-Gb3) in lysosomes of cells and body fluids. The disease is basically divided into two clinical phenotypes, the classic type and the later-onset one [3]. Affected males with the classic form usually exhibit little or no GLA activity, and acroparesthesias, angiokeratomas, hypohidrosis, and corneal opacities in childhood or adolescence. With advancing age, the occurrence of renal disorders, cardiac disease, and stroke leads to premature death in adulthood. On the other hand, affected males with the later-onset type, who have residual GLA activity, develop renal and/or cardiac disorders in adulthood without the childhood symptoms. The clinical manifestations in heterozygous Fabry females range from asymptomatic to severe due to random X-chromosomal inactivation [4]. Up to now, over 600 gene mutations on the *GLA* gene have been identified (http://fabry-database.org/) and it is thought that such genetic heterogeneity reflects the clinical and biochemical diversity of Fabry disease.

Since enzyme replacement therapy (ERT) is now available, many Fabry patients have been successfully treated with recombinant GLAs, if the treatment is started early [5,6]. However, recent outcome survey analysis revealed that ERT is less efficacious when started after the disease has progressed to such as the occurrence of tissue fibrosis [7]. Considering the requirement of early therapy, neonatal and high-risk screening has been widely performed for an early diagnosis [8–11], and it has become more and more important to elucidate the basis of Fabry disease and to identify parameters influencing the disease progression.

In this study, we measured GLA activity, GLA protein and Lyso-Gb3 in plasma samples from Fabry patients with various phenotypes and gene mutations, and examined the relation between them for an insight into the basis of the disease. Furthermore, we focussed on a subset of Fabry patients harboring p.R112H or p.M296I, because it has been reported that the plasma Lyso-Gb3 level in this group does not increase, the biochemical findings being different from other Fabry cases [12–14]. We tried to elucidate the pathogenesis of the specific cases.

2. Materials and methods

2.1. Patients and samples

Plasma samples for measurement of the enzyme activity and protein concentration of GLA, and the Lyso-Gb3 concentration were obtained from 8 classic Fabry males, 12 later-onset Fabry males, 18 heterozygous Fabry females, and 30 healthy volunteers. The ages of the patients and their genotypes are summarized in Table 1. This study involving human samples was approved by the Ethics Committees of Meiji Pharmaceutical University. All participants and/or their parents provided written informed consent to participation in this study.

2.2. Measurement of plasma GLA activity

GLA activity in plasma was fluorometrically measured using 4-methylumbelliferyl-α-D-galactopyranoside (Calbiochem, La Jolla, CA) as a substrate and N-acetyl-D-galactosamine (Sigma, St. Louis, MO) as an inhibitor of