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Short Communication

Frequency of *de novo* mutations in Japanese patients with Fabry disease

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ABSTRACT

We examined alpha-galactosidase A (GLA) gene mutations in 74 Japanese families with Fabry disease (FD) to determine the frequency of *de novo* mutations. In 5 of 74 families (6.8%), the probands had no positive family histories and were diagnosed as *de novo* because their parents had no mutations in GLA gene. The parents of Fabry patients do not necessarily have mutations in GLA gene which is an important consideration in genetic counseling for FD.

1. Introduction

Fabry disease (OMIN 301500, FD) is an X-linked lysosomal storage disorder resulting from a deficiency of alpha-galactosidase A (EC 3.2.1.22; GLA) activity [1]. The estimated incidence of the disease is 1 per 1250–117,000 live male birth [2–5]. The deficiency of GLA activity leads to the accumulation of the principal substrate globotriaosylceramide (GL3) in various tissues including vascular endothelium, renal glomeruli and tubules, dorsal root ganglia, cardiac myocytes and valves, cornea and skin.

Classically affected male patients with FD have a markedly shortened lifespan with death occurring in the fourth or fifth decade of life. Although the clinical severity of female patients is heterogeneous, most of them present with life-threatening complications in their fifth or sixth decade of life [6–10]. Some reports

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have indicated that enzyme replacement therapy (ERT) is also efficacious for female patients with FD [11,12].

Based on Mendelian inheritance, while mothers of male patients with X-linked disorders are expected to be obligate heterozygotes, sometimes the mothers of the male patients with FD may not be heterozygotes. It is therefore important to diagnose them accurately because they may not require ERT. To determine the frequency of *de novo* mutations in Fabry families, we examined the GLA gene mutations in patients and families with FD.

2. Material and methods

2.1. Study patients

Study patients were 126 Japanese patients (61 male patients and 65 female patients) from 74 families with FD. They were referred to us for diagnosis of FD between 1999 and 2012 and diagnosed with FD based on gene analysis.

2.2. Gene analysis

Genomic DNA was extracted from leukocytes using blood and cell culture DNA Midi Kit (Qiagen, Hilden, Germany). Each exon and flanking intron sequence of the GLA gene was amplified by PCR using AmpliTaq gold 360 master mix (Applied Biosystems, Foster city, CA, USA), and directly sequenced using the BigDye Terminator Kit, version 3.1 (Applied Biosystems, Foster city, CA, USA).

2.3. In vitro mutagenesis and expression study in Cos-1 cells

Mutation was introduced to normal GLA cDNA using Quick Change Lighting Site-Directed Mutagenesis Kit (Agilent Technologies, La Jolla, CA, USA) following the manufacture's protocol and ligated to mammalian expression vector pcDNA 3.1 (Invitrogen, Carlsbad, CA, USA). This plasmid was transfected to Cos-1 cells using Lipofectamine 2000 Reagent (Invitrogen, Carlsbad, CA, USA) following the manufacture's protocol. Forty eight hours after transfection, GLA activities were assayed using a fluorogenic substrate, 4-methylumbelliferyl-α-D-galactopyranoside, as described previously [13].

This study was performed under the approval of the ethical committee of The Jikei University School of Medicine. Written informed consent was obtained from all study subjects or legal guardian.

3. Results

In 74 study families, 50 different disease causing mutations were identified in the exons and intron/exon boundaries. Five patients (from 5 families) had no positive family histories (Fig. 1A). The probands of families 1, 2, 3 and 4 were classically affected male patients and that of family 5 was a female heterozygote patient (Fig. 1B). The mothers of the male probands and the parents of the female proband had no characteristic symptoms of FD. All of the probands in families 1, 2, 3, 4 and 5 had disease-causing mutations. Two mutations (IVS5 − 2 and IVS3 + 1) of the 5 probands were splicing defect and previously reported as disease causing mutations [14,15]. The 2 missense mutations (c.3G>A and c.605G>A) were previously reported as disease causing mutations [16,17]. The remaining one missense mutation (c.1019G>C, p.W340S) was a novel mutation. We confirmed the deficiency of GLA activity (2.3% of wild type) by expression study using Cos-1 cells transfected with c.1019G>C mutation and wild type GLA cDNA. These 3 missense mutations (c.3G>A, c.605G>A and c.1019G>C) were confirmed not to be polymorphism based on SNP analysis using NCBI dbSNP and Human Genetic Variation Browser [18,19], and the amino acid substitutions of these 3 missense mutations changed the GLA structure in the result of PolyPhen-2 test [20]. They were diagnosed as *de novo* because their mutations in the GLA gene were not detected in their parents and siblings. In the other 69 families, *de novo* mutations were excluded by family history of affected parents and/or siblings. In this study, the frequency of *de novo* mutations was 5/74 (6.8%). In this study, the affected family members with positive family histories were not confirmed to have the same disease causing mutations as study patients.