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BRIEF COMMUNICATION

Maternal germline mosaicism in Fabry disease

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Abstract

Fabry disease (FD) is an X-linked monogenic disorder caused by mutations in the *GLA* gene which leads to a deficiency of the functionally active lysosomal α-galactosidase A enzyme. Here, we report on a family of five members: unaffected parents, one unaffected son, and another son and daughter both carrying the same mutation (p.G138E) in the *GLA* gene. Genotype analysis using intragenic *GLA* markers confirmed the maternal origin of the mutation. The affected son and daughter carried the same mutation; however, it was not detected in the peripheral blood, buccal cells, and urinary sediment cells of their mother. Moreover, the unaffected son without the alteration in the *GLA* gene carried the same maternal chromosome X (disease-associated) haplotype. To the best of our knowledge, this study represents the first case of maternal germline mosaicism in FD.

Keywords Fabry disease · Mutation · Germline mosaicism · GLA

Introduction

Fabry disease (FD [MIM: 301500]) is a rare X-linked lysosomal storage disease caused by the deficiency of α-galactosidase A (α-Gal A) activity [1]. The enzyme deficiency causes the intracellular accumulation of complex sphingolipids, especially globotriaosylceramide (Gb3) and its deacylated derivative globotriaosylshingosine (lyso-Gb3) in various tissues and organs and their progressive dysfunction [1].

The clinical manifestations include early signs like acroparesthesias, hypohidrosis, angiokeratoma, gastrointestinal symptoms, and the characteristic corneal opacities (cornea verticillata) [2]. Other crucial organs involved in FD are the heart, kidney, and CNS, with severe clinical consequences due to hypertrophic cardiomyopathy, renal failure, and stroke. Enzymatic replacement therapy (ERT) is available from more than 15 years, and more recently, chaperone therapy became available only in case of amenable mutations [2].

Affected hemizygous males usually develop a classic severe phenotype with early onset of symptoms (very low or null α-Gal A activity and high levels of lyso-Gb3). Clinical variants of FD in male patients with high residual activity of α-Gal A and normal levels of plasma lyso-Gb3 have been described [2]. Heterozygous female patients may present with a mild-to-severe phenotype, with a normal or reduced α-Gal A activity and a normal or elevated plasmatic lyso-Gb3 [3]. These latter findings are the result of random and uneven X-chromosomal inactivation [4]. Over 600 different mutations are known in the GLA gene coding for α-Gal A [5]. Both familial and sporadic forms of the disease are known [1]. A case of germline mosaicism in a father of two female Fabry patients with a severe form of the disease has been described [6].

In this report, we describe a family (father, mother, two sons, and one daughter) without a familial history of FD. Both the oldest son and the daughter were affected, carrying the pathogenic mutation p.G138E. This mutation was not detected in their mother and in the younger son. We

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Table 1 Genetic and clinical details of family members

Patient	Age/ sex	GLA mutation	α-Gal A activity[a]	Lyso-Gb3[b]	Cardiac evaluation[c]	Neurological evaluation[d]	Skin biopsy[e]	Ocular abnormalities	Renal abnormalities[f]	ERT
I-1	70/M	None	ND	ND	ND	ND	ND	ND	ND	
I-2	66/F	None	37	0.28	Normal	Normal	Normal	None	None	
II-1	40/M	p.G138E	3.4	ND	Mild left ventricular hypertrophy	Neuropathic pain and hypohidrosis	ND	Cornea verticillata	Chronic kidney disease stage 5D	Agalsidase alfa
II-2	38/M	None	35	ND	ND	ND	ND	ND	ND	
II-3	37/F	p.G138E	25	8.03	Normal	Normal	ND	Cornea verticillata	Chronic kidney disease stage 1	Refused

ND, not done; *ERT*, enzyme replacement therapy

[a] Normal value, 17–64 nmol/mg/h

[b] Normal value, ≤ 0.43 nmol/L

[c] Cardiac evaluation includes physical examination, medical history, ECG, echocardiography, and cardiac magnetic resonance imaging (MRI)

[d] Neurological evaluation includes physical examination, medical history, psychometric tests, brain MRI, MR angiography, electromyography (EMG), and electroneurography (ENG)

[e] Skin biopsy for nerve fibers evaluation

[f] Renal abnormalities according to the Kidney Disease Outcomes Quality Initiative-Chronic Kidney Disease (KDOQI CKD) classification

demonstrated that a maternal germline mosaicism is the most likely the explanation.

Materials and methods

Subjects

All subjects gave their informed consent to participate in the present study according to the principles of the Declaration of Helsinki. The genetic and clinical data are detailed in Table 1 and Fig. 1.

Plasma lyso-Gb3 measurement

Plasma lyso-Gb3 was measured by reverse-phase liquid chromatography tandem mass spectrometry (LC-MS/MS), according to Polo et al. [7].

Analysis of genomic DNA

Genomic DNA was isolated from peripheral blood leukocytes with the MagNA Pure Compact Nucleic Acid isolation kit by an automated nucleic acid extractor (MagNA Pure Compact, Roche). The search for mutations was started by amplification

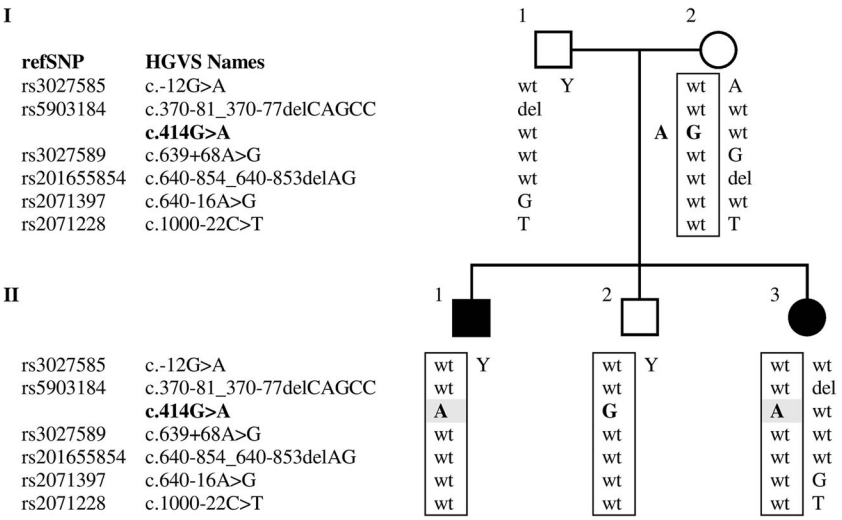

Fig. 1 Family pedigree with haplotype. The haplotype of the family members listed according to the intragenic *GLA* polymorphisms indicated on the left (RefSeq gene transcript sequences, NM_000169.2). The mutation c.414G>A (p.G138E) is also listed. All three offspring (individuals II-1, II-2, and II-3) carry the same maternal (individual I-2) chromosome X haplotypes. The mutation c.414G>A (p.G138E) is present only in individuals II-1 and II-3 (gray shading), suggesting that it is only contained in a percentage of cells in the germline of the mother. wt, wild type; Y, chromosome Y

of all exons of *GLA* gene including the cryptic exon in intron 4, using previously described primers [8, 9]. The PCR products were directly sequenced for both sense and antisense strands using an automated fluorescent sequencing method (Big Dye Terminator Kit v3.1) on an ABI Prism 3130 Genetic Analyzer (Applied Biosystems, Foster City, USA) after treatment with exonuclease I and shrimp alkaline phosphatase (illustra ExoProStar 1-step, GE Healthcare, UK). The sequences of the PCR products were aligned with the published human *GLA* DNA sequence and analyzed with the SeqScape software (Applied Biosystems). Mutations were confirmed using a second independent amplification of the affected genomic region and re-sequencing on another day. For the individual I-2, genomic DNA was also isolated from buccal cells and urinary sediment cells according to the instructions of the manufacturer.

Results and discussion

The family pedigree is shown in Fig. 1. DNA sequencing of the *GLA* gene of patient II-1 revealed a missense hemizygous mutation, a G to A transition at exon 3 (c.414G>A), which led to a substitution of the neutral, polar, highly conserved glycine with a glutamic acid at codon 138 (p.G138E). This mutation had previously been described to be associated with a classic form of FD, supported by the fact that lyso-Gb3 was elevated in the female patient [10]. The presence of this mutation was then tested in the genomic DNA isolated from leukocytes of the sister (II-3) and of the asymptomatic brother (II-2) and parents (I-1; I-2). The sister was found heterozygous for the same mutation, while neither the other brother nor the parents carried this mutation. As one of the two affected subjects is a male, the mother should have been an obligate carrier (FD is an X-linked disease), but the mother was unaffected. The possibility of a de novo mutation was ruled out because all two siblings carried the same mutation. Therefore, we decided to test the hypothesis of a mutation transmitted via maternal germline mosaicism. In order to determine the maternal allele carrying the mutation, haplotype analysis was performed using intragenic *GLA* polymorphisms previously described by Ferri et al. with no clinical relevance [11]. The results, shown in Fig. 1, defined a disease-associated haplotype shared by the three siblings (two affected and one healthy) and the asymptomatic mother. In addition, analyses of different maternal cells (blood, buccal, and urinary sediment) did not reveal the c.414G>A mutation, suggesting that the mosaicism is more likely restricted to the germline cells. In conclusion, our findings strongly support the existence of germinal mosaicism for c.414G>A mutation in the mother.

In 2005, Dobrovolny et al. reported the first case of paternal germinal mosaicism in FD [6]. To the best of our knowledge, this study represents the first documented case of maternal germline mosaicism in Fabry disease.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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