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Identification of a missense mutation (G261D) in the alpha-galactosidase A gene in a Libyan family with Fabry disease

Ahlam Masaud Ellafi[1,2], Abdulhafid Shebani[3], Refaat Tabagh[4]*, Ahmed Zaid[5]. Almin Mohamed[5], Mohamed A. Al-Griw[1], Mohamed Marwan[4]

[1]*Histology and Genetics Department, Faculty of Medicine, University of Tripoli, Libya*
[2]*National Authority for DNA fingerprinting Research and Analysis, Libya*
[3]*Libyan National Organ Transplantation Program, Tripoli Central Hospital, Libya.*
[4]*Division Developmental Biology, Zoology Department, Faculty of Sciences, University of Tripoli, Libya.*
[5]*Biochemistry and Molecular Biology Department, Faculty of Medicine, University of Tripoli, Libya.*
Corresponding author: Refaat M. Tabagh refaat_tb@yahoo.com

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ABSTRACT

Background: Fabry disease (FD) is a rare X-linked genetic lysosomal storage disease with a defect in the activity of the alpha-galactosidase A (α-GLA) lysosomal hydrolase enzyme, which breaks down globotriaosylceramide in lysosomes. Fabry disease is characterized by many unique mutations. Molecular diagnosis is essential for identifying heterozygosity in patients with Fabry disease. Case-series: This case series includes two Libyan brothers with classic Fabry disease predominantly with nephropathy and on hemodialysis, and their sister, who had mainly proteinuria. They were studied clinically and genetically. Only patient 1 had renal and cardiac involvement. None of the patients had enzyme replacement therapy. This study aimed to identify the mutation in the alpha-galactosidase A gene in these patients. Methods. DNA samples from patients with FD at age 30 and 45 years were screened by DNA-PCR and direct DNA sequence analysis to search for genetic sequence variations in the α-GLA gene. Results: Missense point mutation c.782G>A, p. G261D in exon five of the alpha-galactosidase A gene was detected in the three patients. Conclusion: This work highlights that there is a genotype-phenotype association between FD clinical findings and mutations in the α-GLA gene, which may be useful in the genetic counseling of FD patients.

Keywords: Alpha-galactosidase A gene; Fabry disease; Hemodialysis; Proteinuria.

1. Introduction

Fabry disease (FD) is a rare X-linked genetic disease caused by a defect in the activity of the alpha-galactosidase A (α-GLA) enzyme, which results in the accumulation of globotriaosylceramide (Gb3) in lysosomes, leading to damage to different organs and systems, as reviewed by by [1]. FD is treated with enzyme replacement therapy (ERT) [2],

which is safe and effective in reducing Gb3 accumulation [3].

The disease is first manifested in childhood or adolescence and can lead to death in the forties or fifties of life if it is untreated. Because of the variety of manifestations of FD, clinical diagnosis is sometimes difficult and misdiagnosis may occur [4].

Screening can detect affected neonates and the cardiac variant of FD [5]. Screening of family members enables early diagnosis and counseling of all affected members in the family [4], as well as early administration of ERT to protect the patients from irreversible complications to increase their life expectancy and improve the quality of their lives [6]. Mutations causing FD in Libya had not been described, which motivated this study.

2. Case series

This study was carried out between Sep/2017 and Sep/2018 in the department of Biochemistry and Molecular Biology, Faculty of Medicine, the University of Tripoli and the Centre Hospitalier Universitaire de Tours, Hospital Bretonneau, France.

The study was approved by the University of Tripoli, and written consent was obtained from the patients before initiation of work related to this case report and for publication. Three symptomatic Libyan siblings with FD were included in this case series: two are males who had been diagnosed with classic Fabry nephropathy in 2001 based on α-GLA enzymatic activity and renal biopsy, and one is female.

3. Material and Methods

3.1 Subjects

The pedigree of the three individuals across three generations of Libyan family was enrolled in this study Figure 1.

Patient 1: is a male aged 46 year who was diagnosed with FD when he was 29 years old. He was divorced and had one daughter. His marriage was not consanguineous. He was an ex-smoker and worked as a driver. He had coarse features, periorbital fullness, prominent lobules of the ears, a generous nose with bulbous nasal tip, prominent supraorbital ridges, full lips, prominent veins, and clubbed fingers. He had been experiencing hypohidrosis, acroparasthesia in feet and hands, vertigo, delayed wound healing, and maculopapular rash more noticeable in the lower abdomen since childhood. The maculopapular rash was confirmed by skin biopsy in 2007 as angiokeratoma Figure 2 (1). The disease manifestations have had a major impact on the quality of his life. These were hypohidrosis, nervous and pain that was aggravated by stress, long walks, and physical activity, especially while playing and walking at school or with his friends. He left school early.

During adolescence, the patient had progressive heavy proteinuria, arthralgia, chest pain, and decreased vision. Three years ago, he was diagnosed by electrocardiography and electrocardiophonography with left ventricular hypertrophy, mild mitral regurgitation, sinus bradycardia, and incomplete right bundle-branch block. Moreover, lymphedema developed mainly in the left leg. The last biochemical measurements show high creatinine and urea levels, low ferritin, normocytic hypochromic anemia, high uric acid level, and thrombocytopenia. In mid-2018, the patient developed end-stage renal disease then he has been on hemodialysis.

Patient 2: This thirty-three years old single male had coarse features and broad fingers. During childhood, he experienced acroparesthesia, which was aggravated by hot weather, changes in the weather, long walks, and physical activity. He also had angiokeratoma mainly around the umbilicus Figure.2 (2), which proved to be hypohidrosis. He also had a disturbance of vision. After ten years, he began undergoing hemodialysis. The patient had been suffering from weakness, fatigue, dizziness, tinnitus, and dyspnea on mild exertion. Furthermore, a decrease in hearing moreover, anxiety, depression, and heat intolerance affected the quality of his life. He had microcytic hypochromic anemia, thrombocytopenia, and low ferritin. An echocardiogram showed mild mitral regurgitation and his electrocardiogram was normal, and consequently, both brothers left school early.

Patient 3: This 44-year-old female was a widow with a son and a daughter. She has been

experiencing maculopapular rash around the umbilicus, back, and breasts since 2000. After nine years, a skin biopsy was done to confirm angiokeratoma, and she was also diagnosed with heavy proteinuria at that time. Renal biopsy and biochemical tests were not done at that time. In 2017, she experienced acroparesthesia, microcytic hypochromic anemia, weakness, and unilateral lower limb edema.

3.2 Gene analysis

Genomic DNA was obtained from peripheral blood leukocytes using (nucleoSpin kit). The concentration of extracted DNA samples was measured by Nanodrop spectrophotometerand ranged from 34.1 ng/µl to 63.2 ng/µl. The α-GLA gene was amplified. Upstream and downstream primers (Table 1) were designed for the seven exons in the α-GLA gene [7] to perform amplification PCR reaction in a total volume 25µl using GoTaq® qPCR Master Mix. The PCR was initiated with a 3 min hold at 95°C, followed by 35 cycles of 95°C for 45 s, 58°C for 40 s, 72°C for 1 min, and a final extension step at 72°C for 5 min. The concentration of PCR products was verified on an agarose gel. 9 µL of PCR products were treated by 0.8 U shrimp alkaline phosphatase (SAP, Fermentas) and 8 U exonuclease I functional prediction tools such as Polymorphism Phenotyping version 2 (PolyPhen-2, http://genetics.bwh.harvard.edu/pph2/) , SIFT (http://sift.jcvi.org/ scores less than 0.05 are deleterious), and MutationTaster (http://www.mutationtaster.org/) were applied to evaluate the possible effects of amino acid alteration on protein structure and function [9] (Fermentas), and analyzed directionally using Applied Biosystems 3500 Series Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) at the department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Tripoli and the Centre Hospitalier Universitaire de Tours, Hospital Bretonneau, France.

Table (1): primers of α-GLA gene

Exon No	Forward	Reversed
1	5' TGACAATGCAGCTGAGGAAC 3'	5' TCTTCCTGGCAGTCAAGGT 3'
2	5' CTCAGAAGGCTGGAAGGATG 3'	5' GCCATGAGGGCTGTTTCTAA 3'
3	5' GACATTGATGCCCAGACCTT 3'	5' AGCTCTGGCACATGGAGAAT 3'
4	5' CACCCTGGATGACAGACTGA 3'	5' CCTTTTGAAAGGGCCACATA 3'
5	5' GGCGAAATTTTGCTGACATT 3'	5' ATAAAGCCTCCTCCCAGGAA 3'
6	5' AACCTGTTTAATTTTCTTCTCAGAGC 3'	5' CCCTGCCCTCATGAAACTT 3'
7	5' AGCCTGGGCTGTAGCTATGA 3'	5' TGCCTGTGGGATTTATGTGA 3'

3.3 Bioinformatics analysis of the mutation

The Basic Local Alignment Search Tool was used to perform multiple sequence alignments and conservation analysis http://blast.st-va.ncbi.nlm.nih.gov/Blast.cgi) [8] .To assess the potential effects of amino acid alteration on protein structure and function,

4. Results

4.1 Clinical findings and genetic analysis

The clinicopathological features of the patients are summarized in (Table 2). All three patients had blood group A. None of them had ever taken ERT. The clinical and biochemical findings were collected from the medical records and the patients' information sheets. The coding region of the α-GLA gene of all patients was sequenced. The point mutation c.782G>A in the α-GLA gene, which leads to the substitution of the glycine at the position by aspartic acid, was identified in all three patients Figure 3.

Table 2. Clinicopathological features of the Libyan family members with Fabry nephropathy

Patient	Age sex	Blood group	α-GLA	c.782G>A mutation	Childhood	Adolescence	Adulthood
1	46 male	A+	Decreased	Present	Hypohidrosis, Acroparasthesia in both foot and hands, Vertigo, Delay wound healing Angiokeratoma.	Heavy proteinuria, Arthralgia, Chest pain, Decreased vision**.**	Left ventricular hypertrophy. Mild mitral regurgitation, Sinus bradycardia Incomplete right bundle branch block Lymphedema, End stage renal disease. On hemodialysis microcytic hypochromic anemia
2	33 male	A+	Decreased	Present	Proteinuria acroparesthesia, anginokeratoma, hypohidrosis and alternation in vision.	End-stage renal disease. On hemodialysis	weakness, fatigue, dizziness, tinnitus, decrease of hearing, dyspnea on mild exertion, anxiety, depression, heat intolerance. mitral regurgitation. microcytic hypochromic anemia
3	44 female	A+	___	present	___	Angiokeratoma	heavy proteinuria weakness , unilateral lower limb edema**,** acroparesthesia, microcytic hypochromic anemia

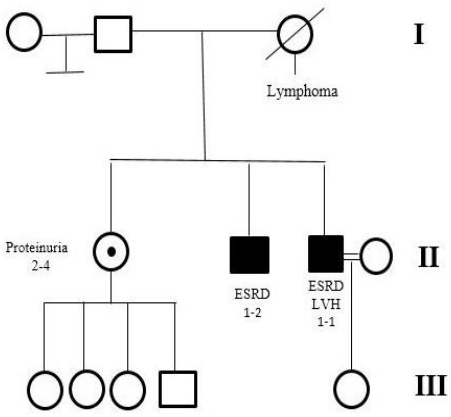

Fig.1. Pedigree chart of Libyan family. Two brothers had Fabry nephropathy (1, 2) and one carrier sister (3) had proteinuria. ESRD: end-stage renal disease; LVH- left ventricular hypertrophy

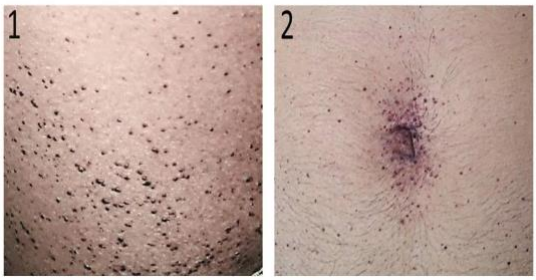

Fig.2. (1) Asymptomatic maculopapular rash over the lower abdomen. (2) Multiple and concentric asymptomatic maculopapular rash around the umbilicus. Both were confirmed histopathologically as angiokeratoma. With permission from both patients

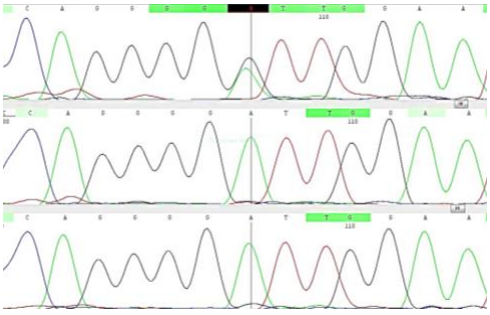

Fig.3. Electropherogram of exon five of α- GLA in three Libyan patients with Fabry disease. Two affected males (1 & 2) and one female carrier 3. The missense point mutation c.782G>A results in amino acid substitution G261D.

5. Discussion

This is the first molecular study carried out in Libya to detect the missense point mutation c.782G>A, p.G261D in exon five of the α-GLA gene in FD patients. The c.782G>A, p.G261D missense mutation has been previously identified in patients with classic FD in six studies listed in the Fabry mutations database from 1997[10] to 2008. However, three different genetic mutations at the same position as our study, c.782G>T (p.Gly261Val) [11,12] and c.782dupG and c.782delG were reported in 2002 and 2020 respectively[13].

The three patients remained undiagnosed for many years. After their diagnosis, the health authorities provided ERT from abroad specifically for them free of charge, but they refused to take it because they were worried about its potential side effects. Since then, the medicine is still not available locally, and after they agreed to take it, many steps were taken to provide it. Although there is no correlation between blood groups and the severity of FD [14], in this study, all three patients had blood group A, which might be associated with renal changes. Although women with FB are considered carriers, mild to severe manifestations can affect the quality of their lives [15-17].

Left ventricular hypertrophy was present only in patient 1. However, other main manifestations (acroparesthesia, angiokeratoma, and hypohidrosis) had been present in both brothers when they were children. Late-onset heavy proteinuria, acroparesthesia, and angiokeratoma developed in patient 3. Their father was alive and healthy, but during the study, he was diagnosed with DM. Their mother had died in middle age after being diagnosed with lymphoma, but she did not have Fabry features, according to her husband. However, a detailed medical history of the mother was unavailable. Tisi et al. (2013) reported a coincidence of FD with lymphoma in an old woman [18].

In the third generation, all children are phenotypically healthy in this study, but inherited FD could be hidden in these children; therefore, genotypic screening is needed. Although the environmental factors were not investigated, the family had moved to different areas of Tripoli, and there is no history of direct chemical exposure to a mutagen. Due to limited resources, neither Gb3 nor LysoGb3 was analyzed before or during this study. Consequently, the relevance of these biomarkers to diagnosis was not demonstrated. LysoGb3 analysis may detect the important α-GLA mutations that lead to FD [19], and is a valuable indicator to improve the specificity of FD diagnosis in neonatal screening due to its high and persistent deposition during the newborn period [20].

In conclusion, the point mutation c.782G>A p. G261D in exon five of the -GLA gene caused FD in our patients. The delay in its diagnosis and treatment was caused by a combination of the inadequacy of thehe healthcare system and a lack of awareness. Delay in FD diagnosis has been reported elsewhere [21]. The similarity of the clinical manifestations of FD during its early stages to some other diseases and the unavailability of a specialized center for rare diseases impede the timely diagnosis and treatment of FD. This disease should be considered along with the other differential diagnoses to achieve early diagnosis and treatment. In the absence of a specialized center for genetic diseases in resource-limited settings, healthcare providers should not fail to provide appropriate diagnosis and treatment. Importantly, patients should not be penalized for having refused to take treatment out of fear or lack of awareness.

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