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Case Report

# Fabry disease. A potential pitfall A family with a novel intronic mutation

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ABSTRACT

Fabry disease is a genetic disorder characterized by the accumulation of globotriaosylceramide in cell lysosomes resulting from an X-linked deficiency of α-galactosidase A activity. It presents with multiorgan manifestations, including progressive renal disease, cardiomyopathy and premature demise. Recently, its prevalence has been reported to be higher in hemodialysis (HD) patients than in the general population. We report two cases of homozygous patients with an intronic alpha-galactosidase gene mutation and a classic phenotype of the disease. One of the patients had a kidney transplant and the donor was his brother, before Fabry disease were diagnose.

## 1. Introduction

Fabry disease (FD,MIM#301500) is an inherited metabolic disorder that results from the deficient activity of the lysosomal enzyme α-galactosidase A (α-Gal A, EC 3.2.1.22). [1, 2] This lysosomal hydrolase, encoded by the GLA gene (locus Xq22.1), catalyzes the removal of terminal α-linked galactosyl residues from neutral glycosphingolipids, the most prominent being globotriaosylceramide (Gb3). Patients with FD show progressive accumulation of Gb3 and related glycosphingolipids in neurons, podocytes, cardiomyocytes, endothelial, perithelial and vascular smooth muscle cells. Lyso-Gb3, the deacylated soluble derivative globotriaosylsphingosine (lyso-Gb3) and analogs has recently been proposed as a key pathogenic mediator of the onset and progression of some of FD complications [3]. FD has an estimated birth incidence of 1:40.000 newborns, although recent systematic screenings in different low and high risk cohorts indicate a higher prevalence [4–6]. The phenotypic spectrum of the disease comprises the classic or early onset form and the attenuated forms, diagnosed later in life time, formerly referred as heart or renal variants.

In the classic form, the first disease manifestations may become evident during childhood or adolescence as neuropathic distal pain in limbs, hypohydrosis, angiokeratoma, gastrointestinal symptoms, cornea verticillata, dysautonomia, fatigue and auditive impairment. During adulthood, most males develop left ventricular hypertrophy (LVH) and/or arrhythmia, renal insufficiency and/or stroke.

Recently, there have been amazing advances in molecular diagnostics for FD. > 700 mutations in *GLA* gene have been identified, including missense mutations, small deletions/insertions, splice mutations, and large gene rearrangements. Most of the GLA gene mutations involved are exonic missense mutations. As far as we know there are few descriptions of intronic mutations [7].

## 2. Case 1

A 43-year-old male that at the age of 32 years, was hospitalized for uremic symptoms.

At admission full blood count which showed low amount of red cells (hto 22%). The values of blood biochemical parameters were urea 299 mg/dL, creatinine 6,9 mg/dL.

Twenty-four-hour protein excretion was 1,9 g, and his creatinine clearance was 10 mL/min. On admission, we also performed an abdominal ultrasound, which showed bilateral heterogenic echogenicity, with a decrease in mean cortical thickness and loss of cortico-medullary definition. The echocardiography confirmed the concentric LVH with a normal ejection fraction (63%).

He didn't undergo renal biopsy nor etiology diagnosis.

The patient started RRT (renal replacement therapy) and evolved stable until 5 years later that a kidney transplant was done. The donor was one of his brothers. Patient's outcome after transplantation was satisfactory.

Five years later a nephrologist suspected the presence of Fabry disease (FD) based on the presence of characteristic symptoms of FD, like four limbs neuropathic pain, hypohidrosis and angiokeratomas. Dry blood spot (DBS) assay for lysosomal α-Gal A activity was done finding a very low level enzyme activity (0.1 umol/l/h, normal value ≥4). Genomic DNA was analyzed and the sequencing analysis identified a hemizygous G > C nucleotide change in intron 4 of the GLA gene (C.640-1G > C). This nucleotide change resides 1 base pairs upstream from intrin4/exon5 boundary and it is predicted to cause abnormal mRNA splicing. Therefore, this allele is a disease-causing GLA mutation.

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This mutation was considered a novel mutation by the genetics laboratory from Baylor College in Medicine.

The patient was considered for enzyme replacement therapy (ERT) with recombinant human agalsidase. Unfortunately, the patient suffered a septic arthritis and died few weeks later of generalized sepsis.

His mother, 60 years old, had hypertension and diabetes and a history of renal failure. The patient has four brothers and two sisters.

Genotyping was extended to his relatives. One of his brothers was hemizygous for C.640-1G > C and his mother and one of her sisters was heterozygous for the same mutation.

## 3. Case 2

After diagnosis in the index case, the patient's younger brother a 40-year-old male, came for a clinical examination. The patient had a history of blood hypertension (HTN) and arrhythmia (no details about the kind of arrhythmia) treated with 25 mg/day of atenolol. He was a current smoker and had no history of diabetes mellitus or hypercholesterolemia.

After a good interrogatory and physical examination, we found that the patient had symptoms and signs of a classic FD phenotype that started at childhood (hypohidrosis, heat intolerance, neuropathic pain, and angiokeratomas). Blood pressure was 150/100 mmHg. no heart murmurs, neither vascular disturbances were found.

Fabry diagnosis was also confirmed by demonstration of a very low levels of α-Gal A 0.1 μmol/l/h (normal value ≥4) and the same mutation C.640-1G > C intron 04 that his brother.

Laboratory evaluation showed 1.0 g/24 hs of proteinuria. And a normal estimated glomerular filtration rate (eGFR). Renal ultrasound was normal.

Brain magnetic resonance images (MRI) showed no vertebrobasilar dolichoectasia (VBD), and no evidence of white matter involvement. Echocardiographic studies revealed severe concentric LVH with normal left ventricular ejection fraction.

He started ERT with agalsidase beta immediately after diagnosis was done.

## 4. Discussion

In this paper, we reported the case of two brothers in which clinical history and a very low level of α-Gal A enzyme activity suggested the presence of a classic phenotype of FD. Genomic DNA was analyzed and the sequencing analysis identified a hemizygous G > C nucleotide change in intron 4 of the GLA gene (C.640-1G > C). Intronic mutations have been described in patients with manifestations of FD not severe, affecting a specific organ, and that occur later in life because residual enzyme activity remains high enough to prevent organ damage in childhood and adolescence [7]. In our patients multisystemic involvement and early onset of symptoms seems to suggest a classical form of FD. Our results and those recently reported in the literature support the idea that FD is not only caused by genetic alterations in the exons of GLA, but even by those occurring in regions of gene regulation. Since non-coding regions are not routinely evaluated when sequencing the GLA gene, the occurrence of intronic disease-causing lesions should encourage to analyze them and to perform, in patients with such mutations, further clinical and instrumental evaluations. This can lead to confirm diagnosis of FD in a higher number of patients, thus providing a more realistic estimation of the prevalence of FD in general population.

Patients on hemodialysis therapy are a high-risk group for Fabry disease. Several case-finding studies using currently available technology have revealed a considerably high prevalence of Fabry disease among this population. Four reports described the prevalence and outcomes of Fabry disease among end stage renal disease (ESRD) patients. In Europe and in the United States, the prevalence of Fabry disease among patients on RRT was 0.0188 (83/440,665 patients) and 0.0167 (42/250,352 patients), respectively [8, 9]. Despite this low prevalence new prospective screening among ESRD patients have shown a > 10 times higher prevalence of Fabry disease (0,12–0,3%) [10–12].

In our case the lack of ESRD etiology could be devastating for the donor of the graft. If the donor were his brother with FD (whose diagnosis was done 2 years after de kidney transplant was done) this could accelerate his evolution towards ESRD. Fortunately, the donor was another brother that resulted negative for FD.

## 5. Conclusions

In this paper, we reported the case of two brothers in which clinical history and a very low level of α-Gal A enzyme activity suggested the presence of a classic phenotype of FD. Genomic DNA was analyzed and the sequencing analysis identified a novel hemizygous G > C nucleotide change in intron 4 of the GLA gene (C.640-1G > C). Intronic mutations have been described in patients with attenuated forms of FD but in these cases the phenotype vas classic and severe stressing the importance of studying also intronic regions of the genes involved. We can also see the phenotype differences between brother with same mutation, the first case had more kidney involvement than cardiac and the second case more cardiac involvement than kidney.

These cases highlighted de necessity of screening patients for Fabry disease in high risk population as ESRD patients and that it is extremely important to check the introns when preforming gene analysis on Fabry patients, especially if no definite mutation has been found in the exons.

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