Clinical, Histological and Molecular Characteristics of Mexican Patients with Fabry Disease and Significant Renal Involvement

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Background and Aims. Fabry’s disease (FD) is an X-linked lysosomal disorder caused by a deficiency of the enzyme α-galactosidase A that produces accumulation of glycosphingolipids with clinical abnormalities of skin, eye, kidney, heart, brain, and peripheral nervous system. We undertook this study to describe the molecular characteristics of the first four Mexican patients with diagnosis of FD with significant renal involvement, correlating these molecular characteristics with clinical, pathological and biochemical findings.

Methods. Genomic DNA from Mexican nonrelated patients with presumptive diagnosis of FD was sequenced by polymerase chain reaction (PCR). DNA sequences were compared against sequences in world data bank gene for alpha-galactosidase A (α-GLA, ENSG00000102393) using the BLAST database.

Results. Three patients were confirmed as having FD by displaying mutations in the α-GLA gene. The mutations found are a substitution (p.L243 F) in patient 1, and a substitution (p.A156 V) in patient 3. These two mutations had been previously reported. The new mutation was in patient 2 who displayed a deletion (c.260delA) changing the open reading frame from codon 86 and a stop codon at the 105th residue of the protein, (instead of 429 AA). The fourth patient had lack of mutations in any of the seven exons of α-GLA or 25 base-pair flanking regions; had mild manifestations with kidney histopathological diagnosis of FD that gave us a final diagnosis of atypical phenotype of FD.

Conclusions. Although the sample is small, it gives a first idea of the molecular and clinical heterogeneity of FD in a Mexican population. © 2014 IMSS. Published by Elsevier Inc.

Key Words: Fabry disease, α-galactosidase A, α-GLA-A enzyme, Fabry nephropathy.

Introduction

Fabry’s Disease (FD) is an inherited lysosomal storage disorder characterized by accumulation of neutral glycosphingolipids in multiple organ systems due to a decrease in the enzymatic activity of α-galactosidase A (α-GLA-A) (1). The gene that encodes this enzyme is located in the Xq22.1 region. Mutations generate low level of activity of the α-GLA-A enzyme, leading to the progressive accumulation of globo-triaosyl-ceramide (Gb3) and α-galactosyl break-down products in male hemizygotes and, to a lesser extent, in female heterozygotes (2–4).
FD is pan-ethnic, with a frequency estimated at 1:40,000 to 1:60,000 males. Recent recognition of late-onset variants suggests that this phenotype is underdiagnosed and the incidence may be 1/4600 individuals with a ratio of 7:1 between late-onset and classical phenotypes (3,4).

Progressive renal insufficiency is one of the main causes of morbidity and mortality in FD. Accumulation of Gb3 in glomerular and tubular epithelial cells results in proteinuria and progressive deterioration of renal function. In the third to fifth decades of life, end-stage renal disease ensues, often requiring dialysis and/or kidney transplant (5,6).

The advent of effective enzyme replacement therapy (ERT), which has been evaluated in randomized trials of patients with advanced FD, has raised considerable interest, particularly regarding its effect on preventing renal complications. FD now represents one of the treatable causes of hereditary chronic kidney disease (6,7). Because of the high cost of treatment and difficulties for genetic counseling, an accurate clinical, biochemical and molecular characterization of every patient is warranted (8).

The aim of this study was to describe the molecular characteristics of the first four Mexican patients with diagnosis of FD and significant nephropathy, as well as clinical, pathological and biochemical correlation.

Subjects and Methods

Subjects

Four unrelated propositi and their respective family members who reside in states of central Mexico were seen at the Nephrology Department of the National Institute of Medical Sciences and Nutrition in Mexico City and diagnosed as patients with FD. Clinical, pathological, and biochemical characteristics of these patients were obtained by chart review and are summarized in Table 1. Levels of α-GLA-A enzyme activity assessment were obtained from plasma of the patients and several of their relatives (Figure 1).

Treatment

Patients 1, 2 and 3 received ERT (enzyme replacement treatment) with a dose of 0.2 mg/kg/week of α-galactosidase as well as kidney transplant. Patients had a good general response, although therapy is not analyzed in the present paper.

Molecular Analysis

Blood samples and DNA extraction. Genomic DNA was obtained by using standard methods. After informed consent, a 5-mL peripheral blood sample was collected from the four patients with FD and from some of their relatives. Genomic DNA was extracted from whole blood using the Puregene DNA Isolation Kit (Gentra Cambridge, MA). DNA concentration was measured by spectrophotometry and the quality of the molecules was assessed by DNA agarose-gel electrophoresis.

DNA amplification. Polymerase chain reaction (PCR) of genomic DNA was used to amplify and sequence the seven exons that encode the α-Gal-A gene. Seven pairs of oligonucleotides were designed and constructed, one for each exon of the gene. In order to amplify the complete exon and part of their introns, primers were placed in the introns flanking each exon, including the base pairs for splicing and ~25 base pairs of nucleotides of the intron. All have a Tm close to 60°C. Before the PCR experiments, we verified that

Table 1. Clinical and laboratory characteristics of the four Mexican cases with FD

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at diagnosis</td>
<td>19</td>
<td>22</td>
<td>24</td>
<td>9</td>
</tr>
<tr>
<td>Follow-up (months)</td>
<td>189</td>
<td>208</td>
<td>5</td>
<td>136</td>
</tr>
<tr>
<td>Kidney manifestations:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum creatinine (mg/dL)</td>
<td>1.43</td>
<td>0.94</td>
<td>0.90</td>
<td>0.54</td>
</tr>
<tr>
<td>eGFR (MDRD) (mL/min/1.73 m²)</td>
<td>23</td>
<td>45</td>
<td>104</td>
<td>108</td>
</tr>
<tr>
<td>Proteinuria (mg/dL)</td>
<td>0.006</td>
<td>0.005</td>
<td>0</td>
<td>0.006</td>
</tr>
<tr>
<td>Hematuria</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Lipiduria</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Renal biopsy</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Systemic manifestations:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neurology</td>
<td>Acroparesthesia</td>
<td>Acroparesthesia</td>
<td>Acroparesthesia, stroke</td>
<td>Normal</td>
</tr>
<tr>
<td>Eye</td>
<td>Normal</td>
<td>Normal</td>
<td>Cornea verticillata</td>
<td>Normal</td>
</tr>
<tr>
<td>Heart</td>
<td>Normal</td>
<td>LVH</td>
<td>Normal</td>
<td>Conduction abnormality</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>Normal</td>
<td>Abdominal pain</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Skin</td>
<td>Hypohydrosis, Angiokeratoma</td>
<td>Hypohydrosis, Angiokeratoma</td>
<td>Hypohydrosis, Angiokeratoma</td>
<td>Normal</td>
</tr>
<tr>
<td>Plasma GLA-A activity (2.0–14.6 μmol/L/h)</td>
<td>0.00</td>
<td>0.18</td>
<td>0.01</td>
<td>2.1</td>
</tr>
</tbody>
</table>

FD, Fabry disease; eGFR (MDRD), Glomerular Filtration Rate estimated by the Modification of Diet in Renal Disease (MDRD-GFR); LVH, left ventricular hypertrophy.
the primers were not compatible with other homologous sequences of the human genome (http://www.ncbi.nlm.nih.gov/BLAST). Finally, the optimal conditions of temperature and MgCl₂ concentration for PCR amplification for each amplicon were standardized by ordinary methods.

DNA sequencing. The seven exons of the four index patients were sequenced. In order to amplify the DNA of these exons by PCR, we mixed 100 ng of genomic DNA with 1 IU of Taq DNA polymerase (Golden Taq Polymerase, Applied Biosystems, Foster City, CA) and 20 pmol of each primer. For PCR sequencing, 90 ng of the previous ampli- con were purified from agarose gel by Qiaquick columns (QIAGEN Cambridge, MA) and subjected to sequencing reaction (Big Dye, Applied Biosystems). For this reaction we used the same PCR amplification conditions but with only the 5′ primer. In exons where a mutation was found, the DNA sequence was corroborated with the 3′ primer. An ABI PRISM 310 Genetic Analyzer (Applied Biosys- tems) was used in order to obtain the DNA sequence or electropherogram of the reaction. Finally, these sequences were compared against the consensus sequence reported in world data bank gene for α-GLA (ENSG00000102393) by the BLAST database as well as against the reported muta- tions associated with FD in the Ensembl database (www. ensembl.org).

Ethical Issues

We performed α-GLA-A gene analyses on seven subjects from four unrelated families (four male patients with clinical diagnosis of FD and three related females) (Table 1).

The study was approved by the Ethics Committee of the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán and all participants gave their informed consent for the study.

Results

Clinical and Molecular Description

The present work is the first molecular characterization of FD in Mexican individuals. Table 1 summarizes clinical, pathological and biochemical characteristics of the four patients. Three had classical FD phenotype and one displayed basically only data of Fabry’s nephropathy.

Patient 1

Patient 1 was a 19-year-old male with nephrotic syndrome secondary to FD characterized by acroparesthesias, generalized pain crisis, multiples angiokeratoma, hypohydrosis and heat intolerance since childhood. A nerve conduction velocity (NCV) test showed combined polyneuropathy. Ophthalmological and cardiac evaluations were normal. Activity of the α-GLA-A enzyme was 0.0 μmol/L/h. Renal manifestations were initially proteinuria of 0.30 g/day and estimated glomerular filtration rate (GFR) of 133 ml/min as estimated using the Modification of Diet in Renal Disease (MDRD-GFR) formula (9). Due to progressive in- crease in proteinuria and development of nephrotic syndrome, a renal biopsy was performed (10–12), showing lamellar inclusions of zebra bodies in endothelial cells. The patient quickly progressed to end-stage renal disease.
DNA sequencing of a Molecular Diagnosis (Case 2)

The patient showed a change at DNA level of G > C at the second position in codon 243 or cDNA 728 G > C, which resulted in a mistranslation that substitutes amino acid L (lysine, Lys) for an F (phenylalanine, Phe) at the 243 residue of the protein (p.L243 F) at exon 5. Activity of α-GAL-A gene in this case showed a molecular genotyping of α-Gal-A in his two female children confirmed their carrier status of the mutation.

Patient 2

Patient 2 was a male who began experiencing recurrent generalized pain and acroparesthesias at the age of 7. At age 12, a physical examination confirmed the presence of angiokeratomas and hypohydrosis. Ophthalmologic evaluation was reported as normal. Electrocardiography and echocardiography demonstrated left ventricular hypertrophy and NCV test compatible with motor neuropathy. At age 22 he was referred to the Nephrology Department due to proteinuria with estimated GFR of 45 ml/min. Urinary sediment revealed oval fat bodies and Maltese cross pattern. Renal biopsy showed Gb3 deposits in podocytes, mesangial expansion, significant glomerulosclerosis and arterial remodeling. The levels of α-GAL-A enzyme showed decreased activity with a value of 0.18 μmol/L/h (range: 2.0–14.6). The patient had accelerated progression to ESRD and required hemodialysis and a kidney transplant.

Molecular Diagnosis (Case 2)

DNA sequencing of α-Gal-A gene in this case showed a punctual deletion of an adenine (A) at the 260 position of the cDNA (c.260delA) in exon 2, which had not been previously described. This deletion changes the open reading frame from codon 87. Relative II-6, his sister (see Figure 1), is a carrier of the mutation and is asymptomatic so far, but with subnephrotic proteinuria. The 260delA deletion has not been previously reported as a cause of FD (13,14).

Patient 3

This male patient has a history of heat intolerance, hypohydrosis, and angiokeratomas since age 10. At age 24 he presented with an ischemic stroke of the right thalamic area. Ophthalmologic evaluation demonstrated the presence of cornea verticillata characteristic of FD (15). Electrocardiography and echocardiography were normal. Urinalysis displayed microscopic hematuria, non-nephritic proteinuria and lipiduria. At that time point his estimated GFR was 104 ml/min. A classic Maltese cross pattern was present in urine sediment. The level of α-GLA-A enzyme activity reported a value of 0.01 μmol/L/h. The patient began ERT and currently continues with preserved renal function.

Molecular Diagnosis (Case 3)

The patient showed a point mutation at position 467 C > T, resulting in a change of the amino acid sequence of the protein at the 156th codon from alanine to valine (p.A156 V). This mutation in the first exon of the gene has been previously reported.

Patient 4

This 20-year-old male patient was clinically diagnosed as an atypical phenotype of FD with hematuria of glomerular origin since 9 years of age as the only clinical manifestation. Due to persistent glomerular hematuria, a renal biopsy was performed and demonstrated the presence of renal lipoidosis with affection of glomerular, tubule-interstitial and endothelial cells, all compatible with Fabry’s nephropathy (Figure 2). His urinalysis demonstrated lipiduria with Maltese cross patterns and normal renal function. Enzyme activity determination was 2.1 μmol/L/h at the lower limit of normal range.

Molecular Diagnosis (Case 4)

Molecular analysis showed integrity in all seven exons sequenced and in the 25 bp of intron flanking exons studied, including the splicing places as described above. The patient had a low-normal level of α-GAL-A activity (2.1 μmol/L/h) and certain characteristics suggestive of FD: significant hematuria, Maltese cross pattern in urinary sediment, and a characteristic renal biopsy consistent with FD (Figure 2).

Discussion

The present paper reports and analyzes the genetic mutations of four cases of FD in a Mexican population and is the first genetic molecular analysis of Mexican patients with Fabry’s disease. Although the number of patients is small, our report gives a first idea of the molecular and clinical heterogeneity of FD in Mexican mestizo families. Previous to our work, there were only clinical reports: two remote reports of a single Mexican patient each (16,17) and a recent report of 13 patients with ophthalmologic clinical data only (18). None of the previous Mexican reports provided biochemical (α-GAL-A activity) or molecular analysis.

The molecular analysis of the seven exons of the gene in these Mexican patients with FD demonstrated a punctual mutation in three of them. The three mutations we found
are different, consistent with previous reports indicating that most FD patients have individual mutations (19).

Our study allows a first characterization of FD in Mexico where the prevalence of this disease is unknown and we believe it may be underestimated. In addition, we confirmed the usefulness of the molecular analysis to characterize the genetic mutations and their relation to the functional activity of the α-GAL-A enzyme.

**Clinical-Molecular Correlation**

There was a good correlation between the clinical and the molecular diagnosis. We made this correlation for each patient as follows:

**Patient 1.** This patient had a mutation on the residue 243 of the protein (p.L243 F) at exon 5. The patient displayed the most classic phenotypic manifestations of this series of patients, and the three phases of the disease can be identified. In this case the molecular diagnosis provided one more piece of useful data: the molecular genotyping in his two female children confirmed that both of his daughters were carriers of the mutation (Figure 1) and with low level of α-GAL-A enzyme. Because a high percentage of women carriers who eventually develop the disease (20,21), the recommendation was that they would not be kidney donors.

**Patient 2.** This patient was the one with a novel mutation represented by a deletion of an adenine (A), at the 260 position of the cDNA (c.260delA) in exon 2. Clinically, the patient showed a classic phenotype of FD. Theoretical analysis of this mutation shows a significant change in the open reading frame from the 86th codon that finally ends with a stop codon at the 105th residue of the protein, producing a truncated small protein with only 105 amino acids (AA) instead of the normal one of 429 AA. However, his plasma enzyme level was low (0.18 nmol/h/mL), which correlated well with his sister (relative II-6), a heterozygous carrier (Figure 1) who is asymptomatic so far but has a low level of enzyme (2.65 nmol/h/mL) and subnephrotic proteinuria.

**Patient 3.** This patient has a mutation at the AA 156 (A156 V). This mutation has been already described and is predicted to be clinically significant based on reports of one individual with FD (22) and a predicted change in protein structure (23). As reported, the level of enzyme activity in patients with this mutation is low but not absent (22) as in our patient who has a level of 0.01 nmol/h/mL (Table 1).

**Patient 4.** This was a difficult case of FD in which, although we did not find the mutation, the molecular analysis was helpful for his final diagnosis. He did not have the classical phenotype of the disease, but he displayed mild clinical and biochemical manifestations of FD including two important signs of Fabry nephropathy: Maltese crosses in urine and a positive biopsy. As has been reported, 7% of FD patients have a genotype associated with non-classic FD (19) in which it lacks the more classic clinical phenotype: acropaesthesias, angiookeratoma, hypohidrosis and corneal opacities (17) as seen in this patient.

**Low Enzyme Level**

Although the enzyme level of this patient was 2.1 nmol/h/mL and most reports consider a normal range level for α-
GAL-A between 2 and 15 nmol/h/mL, some authors consider as abnormal a level <2.5 nmol/h/mL (24, 25).

Lack of Mutations in Exonic Regions

The molecular diagnosis that did not show any mutation in the seven codifying exons of α-GAL-A gene, including the two bases for splicing and 25 bp flanking regions, ruled out the patient as a classical phenotype of FD. The favorable clinical course of the patient was consistent with the diagnosis of atypical FD: he never needed substitutive enzyme therapy or kidney transplant or even renal dialysis. Finally, he remained with only micro-albuminuria.

Final Diagnosis of the 4th Patient

All previous data strongly suggest that the most likely final diagnosis was an atypical phenotype of FD with relative low renal damage, secondary to mutations of the α-GAL-A gene in the promoter or intronic regions, as has been previously reported (17). In order to find other possible alterations such as low expression levels of the α-GAL-A gene or mutations in the final transcription cDNA, one could perform other molecular assays like cDNA analysis and MLPA (multiplex ligation-dependent probe amplification) (17). However, this patient was lost to follow-up.

Final Considerations

FD in Mexico is clinically and genetically heterogeneous. The present work gave a first characterization of FD variants in our country and provide a first clue of FD in the Nephrology Department of our institute. At the molecular level, variants were mainly (three out of four) of single nucleotide polymorphisms, giving as a result structural change in exons, with low or total absence of α-GAL-A enzyme biochemical activity. In addition, we show that mutations in two cases were identical to others reported, yet a new variant was described. We also describe the presence of genetic modifications in some members of their families.

Our study portrays the usefulness of a detailed clinico-pathological study of the patient and the critical role of the molecular diagnosis, which was useful in order to identify patients and related asymptomatic carriers.

References