RESEARCH NOTE

Mutational analysis of the *GLA* gene in Mexican families with Fabry disease

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Abstract

Fabry disease (FD) is a lysosomal storage disorder, which develops due to a deficiency in the hydrolytic enzyme, α-galactosidase A (α-Gal A). Alpha-Gal A hydrolyzes glycosphingolipid globotriaosylceramide (Gb3), and an α-Gal A deficiency leads to Gb3 accumulation in tissues and cells in the body. This pathology is likely to involve multiple systems, but it is generally considered to affect primarily vascular endothelium. In this study, we investigated mutations in the *GLA* gene, which encodes α-Gal A, in Mexican families with FD. We included seven probands with FD that carried known mutations. We analysed pedigrees of the probands, and performed molecular screening in 65 relatives with the potential of carrying a GLA mutation. Five mutations (P40S, IVS4^{+4}, G328V, R363H, R404del) were detected in seven unrelated Mexican families with the classic FD phenotype. Of the 65 relatives examined, 42 (64.6%) had a *GLA* gene mutation. In summary, among seven Mexican probands with FD, 65 relatives were at risk of carrying a known *GLA* mutation, and molecular screening identified 42 individuals with the mutation. Thus, our findings showed that it is important to perform molecular analysis in families with FD to detect mutations and to provide accurate diagnoses for individuals that could be affected.

[Gutiérrez-Amavizca B. E., Gal A., Ortíz-Orozco R., Orth U., Prado Montes De Oca E., Gutiérrez-Amavizca J. P. and Figuera L. E. 2017 Mutational analysis of the *GLA* gene in Mexican families with Fabry disease. *J. Genet.* **96**, xx–xx]

Introduction

Fabry disease (FD; MIM 301500) is an X-linked disease caused by a lysosomal storage disorder, due to the deficiency or absence of the hydrolytic enzyme, α-galactosidase A (α-Gal A; EC 3.2.1.22). The α-Gal A enzyme hydrolyses globotriaosylceramide (Gb3), and deficient hydrolyzation leads to Gb3 accumulation in cells and body tissues. This aetiology suggests that this condition may have pleiotropic effects (Desnick and Wasserstein 2001). The phenotypic expression of FD is highly variable. Some individuals display a mild, oligosymptomatic phenotype, with heart or kidney disorders (the cardiac or renal variant). Others display the classic phenotype, which includes acroparesthesia, hypohydrosis, angiokeratoma, cornea verticillata, cardiac abnormalities and renal failure, among other symptoms (Ferri *et al.* 2012). Most male patients with FD have markedly short life span; death occurs between the fourth and fifth decade of life, secondary to renal and cardiovascular complications or stroke. Heterozygous females display a wide spectrum of disease severity, ranging from no symptoms to the classic characteristics observed in men with FD. It is important to diagnose FD carriers, because currently available FD treatment can favourably change the disease course. This

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Keywords. Fabry disease; mutation; *GLA* gene; Mexican.

treatment includes α-Gal A enzyme replacement, which significantly lower the levels of Gb3, and avoids complications (Eng *et al.* 2007). To date, more than 600 mutations in the *GLA* gene (which encodes α-Gal A) have been described. Most of the identified mutations are private (confined to a single family), and they are distributed over seven exons. There are no obvious 'hot spots', which might indicate a region highly prone to mutations. Correlations between genotype and phenotype remain unclear and controversial (Bono *et al.* 2011). In this study, we performed a molecular analysis of *GLA* gene mutations in a Mexican population with FD. We analysed pedigrees of the probands, and performed molecular screening in 65 relatives with the potential of carrying a *GLA* mutation. Five mutations (P40S, IVS4^{+4}, G328V, R363H and R404del) were detected in seven unrelated Mexican families with the classic FD phenotype. Of the 65 relatives examined, 42 (64.6%) had a *GLA* gene mutation.

Material and methods

Patients

We included seven probands who had been diagnosed with FD, based on clinical, enzymatic and molecular analyses. All patients were referred from the 'UMAE Hospital de Especialidades', Centro Médico Nacional de Occidente, Instituto Mexicano del Seguro Social, Guadalajara, México. We analysed the pedigrees of these seven probands, and performed a molecular screening of 65 relatives to determine whether they carried a *GLA* mutation. Written informed consent was obtained from all the recruits before participating in the study. This study was performed according to the tenets of the Declaration of Helsinki and applicable Mexican regulations for health and research. Ethics approval was obtained from the Comisión Nacional de Investigación Científica, Mexico (R-2011-785-009).

Table 1. Primer sequences used for *GLA* exons.

Primer name	Primer sequence (5′–3′)	Amplicon size (pb)
MF1F	TAACTCATCGGTGATTGGTCC	326
MF1R	CACATGGAAAAGCAAAGGGA	
MF2F	ATGGGAGGTACCTAAGTGTTC	288
MF2R	GTGCTTACAGTCCTCTGAATG	
MF3F	ATTGTGCTTCTACAATGGTGAC	277
MF3R	CCATGGCCTCAAAGTTCTTTC	
MF4F	TATAGCCCCAGCTGGAAATTC	230
MF4R	AGGAGACCTTGGTTTCCTTTG	
MF5F	CACAAGGATGTTAGTAGAAAG	270
MF5R	GTCAAAATAGGAAACAAGCCT	
MF6F	CTCCATATGGGTCATCTAGG	351
MF6R	CCAAGACAAAGTTGGTATTGG	
MF7F	GGGCCACTTATCACTAGTTGC	377
MF7R	GGACAGGAAGTAGTAGTTGGC	

Genomic DNA extraction and mutation analysis

Genomic DNA was extracted from 10 mL of peripheral venous blood, collected in EDTA, according to the Miller method (Miller *et al.* 1998). Each of the seven *GLA* exons with flanking intronic sequences was PCR-amplified from genomic DNA. Sequences of the primers for the seven exons and the sizes of the amplified products are shown in table 1. The polymerase chain reaction (PCR) was performed with 20 ng of genomic DNA in a total volume of 20 μL, containing 10× PCR buffer, 1.5 mM $MgCl_2$, 0.1 mM dNTPs, 0.5 pM of each primer and 0.02 U *Taq* polymerase (Invitrogen, Los Angeles, USA). PCR conditions required denaturation at 95°C for 2 min, followed by 29 cycles at 95°C for 40 s (denaturation), 57°C for 30 s (annealing), 72°C for 50 s (elongation); then, a final elongation was completed at 72°C for 3 min. The PCR products were electrophoresed in a 2% agarose gel. Amplicons were removed from the gel and sequenced with an ABI Prism 3700 Capillary Array Sequence Analyzer and the ABI Prism BigDye Terminator Ready Reaction Mix (Perkin–Elmer–Cetus, Norwalk, USA). Each mutation was confirmed by repeating the PCR-amplification and sequencing the opposite strand.

Microsatellite analysis

Two probands with the IVS4^{+4} splice-site mutation were studied further to determine whether the mutation was inherited or occurred independently. Their genomic DNAs were haplotyped with four microsatellite markers close to the *GLA* locus, including DXS990, DXS8020, DXS8096 and DXS1191. The sequences of the primers used in the PCR amplifications were described in the Online Mendelian Inheritance in Online Mendelian inheritance in man database (OMIM). Microsatellites were analysed with an ABI Prism 3100 Genetic Analyzer.

Software tools

Sequence electropherograms were analysed with Genemapper software, ver. 3.5.2 (JSI, Medical Systems, Kippenheim, Germany). Microsatellites were analysed with GeneScan Analysis Software (ver. 3.1.2).

Results

Five *GLA* mutations (P40S, IVS4^{+4}, G328V, R363H and R404del) were detected in seven unrelated Mexican individuals that displayed the classic FD phenotype (table 2). These probands included six males and one female. A pedigree analysis identified 65 relatives at the risk of carrying a *GLA* mutation. A molecular genetic analysis showed that among these 65 family members, 42 (64.6%) individuals carried *GLA* mutations. All mutations found in these families had been previously described (Koide *et al*. 1990; Topaloglu *et al*. 1999; Blaydon *et al*. 2001; Shabbeer *et al*. 2002, 2006).

Table 2. Mutations in *GLA* gene in Mexican families.

Family	Index case	Number of relatives analysed	Presented mutation		Mutation	Type of mutation	Exon	Nucleotide change	Phenotype	Reference
			Yes	No						
1	Male	5	3	2	P40S	Missense	1	c.118C>T	Classic	Koide *et al.* (1990)
2	Male	4	3	1	IVS4^{+4}	Splice site	4		Classic	Topaloglu *et al.* (1999)
3	Male	19	15	4	IVS4^{+4}	Splice site	4		Classic	Topaloglu *et al.* (1999)
4	Female	7	4	3	G328V	Missense	6	c.983G>C	Classic	Shabbeer *et al.* (2006)
5	Male	14	7	7	R363H	Missense	7	c.11066G>A	Classic	Blaydon *et al.* (2001); Shabbeer *et al.* (2002)
6	Male	10	6	4	R363H	Missense	7	c.11066G>A	Classic	Blaydon *et al.* (2001); Shabbeer *et al.* (2002)
7	Male	6	4	2	R404del	Deletion	7	c.1209_1211delAAG	Classic	Shabbeer *et al.* (2006)

Discussion

FD is an X-linked inborn error in glycosphingolipid catabolism. The disease is caused by Gb3 deposits, which is formed due to deficient α-Gal A activity. Once a proband is diagnosed, it is essential to orient relatives about the natural history of the disease, treatment options and its inheritance pattern. Familial screening can detect individuals with mutations that could potentially manifest FD symptoms; early identification allows the individual to obtain timely treatment (Desnick and Brady 2004). Women were previously considered to be genetic carriers, with a low risk of developing FD. However, current evidence has shown that some women also present life-threatening manifestations of FD, including microvascular cardiac ischaemia, cerebrovascular accidents, hypertension, dysrhythmias and renal insufficiency (Wang *et al*. 2007).

No clear hot spot has been identified in the *GLA* gene locus. However, among the seven exons, exons 5 and 6 displayed the highest frequency of mutations (17.2 and 20.8%, respectively). It was also shown that small rearrangements occurred in about one third (30.6%) of all mutations in exon 7. Interestingly, exon 4, which is the smallest exon (92 bp), represents 7.1% of the entire coding sequence, but its mutation frequency is only 5.4%, lower than the expected 7.1% (Gal 2010).

Of the 6448 variants in the *GLA* gene (www.ensembl.org), at least 500 mutations (7.7%) have been associated with FD (Bono *et al.* 2011), and 164 mutations (2.5%) are missense variants. Among the mutations that impact αGal-A enzyme activity, mutation P40S (exon 1) is adjacent to the active site, and mutation G328V (exon 6) causes misfolding of αGal-A, which is predicted to increase the rate of degradation. Both mutations are associated with diminished or undetectable enzyme activity, but detectable levels of enzyme protein. Mutation R363H (exon 7) is localized to a CpG region; CpG regions are generally considered hot spots (Shabbeer *et al.* 2006), due to the deamination of methylcytosine to thymidine (Pastores and Lien 2002).

Other mutations affect alternative splicing; there are seven alternative *GLA* transcripts and 89 splice region variants (1.3% of the total variants). Mutation IVS4^{+4} alters a splice site, which probably affects the correct splicing of one or more overlapping RNA sequences (www.ensembl.org, 2014).

Mutation R404del is localized in exon 7. Interestingly, this mutation has been associated with both the cardiac variant and classic variant in different families (Pastores and Lien 2002). Mexico does not have a national registry of patients with FD. In Mexican population, it has been reported that two clinical studies of patients with FD (Becerra *et al*. 2012; Gutiérrez-Amavizca *et al*. 2014), and a molecular study by Ramos-Kuri *et al.* (2014), who reported *GLA* mutations on three Mexican patients: p.L243F and p.A156V (previously reported), and a new mutation (c.260delA). Although this study analysed a small number of families, it was the second to describe *GLA* gene mutations in Mexican families.

Based on our results, we suggest that in western Mexico, exons 4, 6 and 7 of the *GLA* gene are more prone to mutations than the other exons. This hypothesis was supported by our finding that of seven families with FD, one had a mutation in exon 1 (16.6%), two had mutations in exon 4 (33.3%), one had a mutation in exon 6 (16.6%) and three had mutations in exon 7 (50%). However, we must analyse more families to provide stronger evidence in support of this hypothesis. Future studies should aim to establish genotype–phenotype correlations for mutations in the *GLA* gene to gain a better understanding of the molecular aetiology and to facilitate personalized treatment for FD. In conclusion, this study identified *GLA* gene mutations in 42 of 65 relatives of seven Mexican probands with FD. This identification provided these 42 individuals with the possibility of seeking early treatment. Thus, it is important for each family with FD to undergo molecular screening, because the detection of *GLA* mutations provides an accurate diagnosis of FD risk.

Acknowledgements

We thank the Consejo Nacional de Ciencia y Tecnología (CONACYT) for the scholarship awarded to B. E. Gutiérrez-Amavizca during her doctoral study.

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Received 11 March 2016, in revised form 14 June 2016; accepted 20 June 2016
Unedited version published online: 23 June 2016
Final version published online: 15 February 2017

Corresponding editor: S. GANESH