RESEARCH NOTE

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

Short running title: Mutations in the *GLA* gene in Mexican families

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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 Aenzymehydrolyzesglobotriaosylceramide (Gb3), and deficient hydrolyzation leads to Gb3 accumulation in cells and body tissues. This etiology suggests that this condition may have pleiotropic effects (Desnick and Wasserstein, 2001). The phenotypic expression ofFDis highly variable. Some individuals display a mild, oligosymptomatic phenotype,withheart or kidneydisorders (the cardiac or renal variant). Others display the classic phenotype, which includes acroparesthesia, hypohydrosis, angiokeratoma, cornea verticillata, cardiac abnormalities, and renalfailure, among other symptoms (Ferrietal., 2012). Most male patients with FD have markedly shortened life spans; 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 FDtreatment can favorably change the disease course. This treatment includes α-Gal Aenzyme replacement, which significantly lowers 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 analyzed pedigrees of the probands, and performed molecular screening in 65 relatives with the potential of carrying a *GLA* mutation.Five mutations (P40S, 639+4A>T, G328V, R363H, R404del)were detected in seven unrelated Mexican families with the classic Fabry disease phenotype. Of the 65 relatives examined, 42 (64.6%) had a *GLA* gene mutation.

Material and methods

Patients

We included 7 probands that had been diagnosed with FD, based on clinical, enzymatic, and molecular analyses. Allpatientswerereferredfromthe “UMAE Hospital de Especialidades”, Centro Médico Nacional de Occidente, Instituto Mexicano del Seguro Social, Guadalajara, México. We analyzed the pedigrees of these seven probands, and we performed a molecular screening of 65 relatives to determine whether they carried a *GLA* mutation. Written informed consent from all the recruits was obtained before they participated in this 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 ComisiónNacional de InvestigaciónCientífica, Mexico (R-2011-785-009).

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 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 mMdNTPs, 0.5 pM of each primer, and 0.02 U Taq polymerase (Invitrogen). 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, CT). Each mutation was confirmed by repeating the PCR-amplification and sequencing the opposite strand.

Microsatellite analysis

Two probands with the 639+4A>T 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 primersused in the PCR amplificationswere described in the Online Mendelian Inheritance in Man database (OMIM). Microsatellites were analyzed with an ABI Prism 3100 Genetic Analyzer.

Software tools

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

Results

Five *GLA* mutations (P40S, IVSA4^{+4}, G328V, R363H, 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 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; Shabbeer et al., 2006).

Discussion

FD is an X-linked inborn error in glycosphingolipid catabolism. The disease is caused by Gb3 deposits, which form, 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 ischemia, cerebrovascular accidents, hypertension, dysrhythmias, and renal insufficiency (Wang et al., 2007).

No clearhot spot has been identified in the *GLA*gene locus. However, among the seven exons,exons5 and 6 displayedthe highestfrequencyofmutations(17.2and 20.8%, respectively).It was alsoshownthat small rearrangements occurredin about one third(30.6%) of allmutationsinexon 7.Interestingly, exon4, which isthe smallest exon (92bp),represents 7.1% of the entirecoding 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 (Bonoet 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 hotspots (Shabbeer et al., 2006), due to the deamination of methylcytosine to thymidine (Pastores and Lien, 2002).

Other mutations affect alternative splicing; there are 7 alternative *GLA* transcripts and 89 splice region variants (1.3% of the total variants). Mutation 639 + 4A >T alters a splice site, which probably affects the correct splicing of one or more overlapping RNA sequences (www.ensembl.org).

Mutation R404del is localized in exon 7. Interestingly, this mutation has been associated with both the cardiac variant and the classic variant in different families (Pastores and Lien, 2002).

Mexico does not have a national registry of patients with FD.In Mexican population, have been reported two clinical studies of patients with Fabry disease (Becerra et al., 2012, and 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 analyzed 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 mutation 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 analyze 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 etiology and to facilitate personalized treatment for FD. In conclusion, this study identified *GLA* gene mutations in 42 out 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.

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TABLES

Table 1. Primers sequences used for *GLA* exons

Primer name	Primer Sequence (5'- 3')	Ampliconsize (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	

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

Family	Index case	Number of Relatives analyzed	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}	Splicesite	4		Classic	Topaloglu et al., 1999
3	Male	19	15	4	IVS4^{+4}	Splicesite	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	Shabbbeer et al., 2002; Blaydon et al., 2001
6	Male	10	6	4	R363H	Missense	7	c.11066G>A	Classic	Shabbbeer et al., 2002; Blaydon et al., 2001.
7	Male	6	4	2	R404del	Deletion	7	c.1209_1211delAAG	Classic	Shabbbeer et al., 2006