Mutation and Polymorphism Report

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Title : Identification of a novel *de novo* mutation (G373D) in the α-galactosidase A gene (*GLA*) in a patient affected with Fabry disease

Keywords: Fabry disease; α-galactosidase A; *GLA;* enzyme replacement therapy.

Species: Homo sapiens

Change is: Mutation

Gene/Locus

Name:	α-galactosidase A
Symbol:	*GLA*
Genbank accession number:	X14448
OMIM accession number:	301500
Locus specific database:	
Chromosomal location:	Xq22
Inheritance:	X-linked recessive

Mutation / polymorphism name

Nucleotide change–Systematic name:	g11096G>A ; c1178G>A
Amino acid change–Trivial name:	G373D
Mutation / polymorphism type:	Missense
Polymorphism frequency:	
Detection method:	RT-PCR and direct sequencing
Detection conditions:	Sequence of primers cDNA : 757-f5' : ATT GTT GAT GTT GCT GGA CCA G-3' 1281-r3' : GTC TTT TAA TGA CAT CTG CAT T-3' Genomic DNA : Exon 7-F : 5'-GGG CCA CTT ATC ACT AGT TG-3' Exon 7-R : 5'-TAG CCT TGA GCT TTT AAA GT-3'

PCR conditions: The PCR reactions were performed on cDNA of the hemizygous male proband, affected with classical Fabry disease, in 20 µL final volume, using 5 pmol of each primer, 1.5 mM MgCl2, 250 µM dNTPs, Taq DNA Polymerase, 1 unit (ATGC Biotechnologie) : 5 min at 95°C ; 35 cycles of 1 min at 95°C, 1 min at 58°C, 90 sec at 72°C ; 10 min at 72°C. Direct Sequencing : the direct sequencing was performed with BigDye Terminators Ready Reaction Kit (PE Biosystems) on both DNA strands. ABI PRISM 310 Genetic Analyzer (PE Biosystems) was used to obtain DNA sequences which were handled with Navigator 2.0 software. The mutation was subsequently confirmed by direct sequencing of genomic DNA for the proband, whereas his mother was found to be a normal homozygote.

Diagnosis method developed: Direct sequencing (genomic DNA)

Evidence for existence and effect of mutation:

	Yes	No	Don't know
1. Base change found on repeat PCR sample	X		
2. Base change segregates or appears with trait	X		
3. Base change affects conserved residue	X		
4. Expression analysis supports hypothesis for causation			X
5. Normals tested (50 required)	X		

Ancillary data

1. **Haplotype association :**
2. **Ethnic background/Population association :** Caucasian
3. **Geographic association :** France
4. **Frequency** (of mutation) **in population:**
5. **Clinical phenotype of proband :** Classical Fabry disease
6. **Homologous allele** (if recessive trait)**:**
7. **PIC:** (if microsatellite)
8. **Other:** *de novo* mutation
9. **Present in HGMD listing:** Yes: No: X
   (http://www.cf.ac.uk/uwcm/mg/hgmd0.html)

Comments

Fabry disease (FD; MIM# 301500) is an X-linked lysosomal storage disorder caused by the deficient activity of the lysosomal enzyme α-galactosidase A (GLA, EC 3.2.1.22). Progressive glycosphingolipid deposition in the microvasculature of hemizygous males, affected with classical FD, leads to failure of target organs, and death usually occurs during the fifth decade of life from renal, cardiac or cerebrovascular complications. However, recent studies have demonstrated that genetic engineering has removed many of the obstacles to the clinical use of enzyme replacement, and that the infusions of purified recombinant α-galactosidase A are safe and biochemically active (Eng et al., 2000; Schiffmann et al., 2000), emphasizing the need for timely diagnosis and accurate identification of affected family members (Germain and Poenaru, 1999). In the present study, the full-length cDNA of the *GLA* gene was screened by direct automated sequencing in a 22-year-old hemizygous male, affected with symptoms of classical Fabry disease. The patient was shown to carry a G to A transition at position 1178 in the cDNA sequence. This nucleotide substitution alters the codon (GGT) for glycine to the codon (GAT) for aspartic acid at position 373 of the α-galactosidase A protein (G373D). Despite scanning of the rest of the gene, no other sequence abnormality was

found. These data were confirmed by direct sequencing of exon 7 in the proband. It is interesting to note that, apart from C378Y (Topaloglu et al., 1999), G373D is the most carboxy-terminal missense mutation identified to date in the α-galactosidase A gene. Targeted genomic DNA sequencing, limited to exon 7 of the *GLA* gene, subsequently demonstrated that the proband's mother did not carry the mutation. Similarly, no mutation was found in the proband's siblings (three brothers and one sister). These results are at odds with the extremely low occurrence of *de novo* mutations in Fabry disease, which have been described only rarely (Madsen et al., 1995 ; Redonnet-Vernhet et al., 1996).

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