MUTATION IN BRIEF

Fabry Disease: 20 Novel GLA Mutations in 35 Families

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Thirty two mutations have been found in 35 unrelated patients of European origin with Fabry disease, including 8 females. Twenty of the mutations are novel and comprise of 13 missense: H46Y, W47G, R49P, C94S, F113S, G258R, P259R, Q279H, Q280H, R363H, A377D, P409A, P409T; 1 nonsense: L294X; 5 small deletions: 154delT, 520delT, 909-918del10, 1152-1153delCA, 1235-1236delCT and 1 splice site mutation: IVS5+2t® c. The remaining 12 mutations have all been reported previously. All patients with deletions had the classic form of the disease but it was not possible to predict the phenotype from the missense mutations. © 2001 Wiley-Liss, Inc.

KEY WORDS: Fabry Disease; α-Galactosidase A; GLA; SSCP; European

INTRODUCTION

Fabry disease (MIM# 301500) is an X-linked, recessive, lysosomal storage disease resulting from a deficiency of the lysosomal enzyme, α-galactosidase A (GLA; EC3.2.1.22)(Desnick et al. 1989), which leads to a build up of neutral glycosphingolipids with terminal α-galactosyl moieties in the lysosomes of a variety of tissues. Affected hemizygous males develop various symptoms in childhood or early adolescence, including severe pain and paresthesias in the extremities, characteristic skin lesions, or angiokeratoma and corneal opacities. Later there is a progression to renal and cardiovascular disease and death usually occurs in the fifth decade (Desnick et al. 1989). Heterozygous females either suffer from a milder form of the disease, or they maybe asymptomatic, but occasionally they may be as severely affected as the hemizygous males. Demonstration of α-galactosidase deficiency in leukocytes is diagnostic in affected males. However, enzymatic detection of female carriers is often inconclusive, due to random X-chromosomal inactivation, underlining the need for molecular investigations for accurate genetic counselling.

The gene for α-galactosidase A is localized at Xq22.1. It is 12 kb in length and consists of 7 exons (Kornreich et al. 1989, accession number NM 000169). All of the exons are small enough to allow amplification by the polymerase chain reaction (PCR) and investigation of sequence changes by single strand conformation polymorphism (SSCP) analysis and sequencing. This strategy was employed to identify mutations in 35 families from the United Kingdom and other European countries in male and female patients with both classic and variant forms of Fabry disease (Table 1). Tests for the detection of heterozygotes and for prenatal diagnosis are also presented. Genotype/phenotype correlations are considered.

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MATERIALS AND METHODS

Patients

Blood samples were obtained from 35 patients who had been diagnosed clinically and enzymatically to be suffering from either classic or a variant form of Fabry disease (Table 1).

Genomic DNA Extraction and Amplification

Genomic DNA was extracted from the blood samples by following an ammonium acetate salting out method described previously (Davies et al. 1993a). The seven exons of the α-galactosidase A gene, along with the flanking intronic sequences, were amplified from genomic DNA using pairs of oligonucleotide primers reported previously (Davies et al. 1993b).

SSCP Analysis and DNA Sequencing

The amplified DNA fragments were then subjected to single-strand conformation polymorphism (SSCP) analysis by electrophoresis in MDETM gels (FMC Bioproducts). Two μl of a loading dye mixture (95% (v/v) formamide, 10mM NaOH, 0.25% (w/v) bromophenol blue, 0.25% (w/v) xylene cyanol) was added to 2 μl of PCR product. The samples were denatured at 94°C for 5 minutes prior to loading onto a 0.5 x MDETM gel. Electrophoresis was carried out in 0.5 x TBE at 15W overnight at 4°C, or at 45W for 3-4 hours at room temperature to find optimal conditions for detecting sequence changes. DNA bands were detected using a silver staining procedure (Davies et al. 1993b).

Fragments that showed band shifts on SSCP gels, were purified from excess primers and dNTPs by ultrafiltration through Microspin™ S-400 HR Columns (Pharmacia). Products were then sequenced in both forward and reverse directions, either manually or by using the ABI Prism™ dRhodamine Terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer Applied Biosystems) and analysed on the ABI Prism 377 Automated DNA Sequencer (Perkin Elmer Applied Biosystems).

For patients where no obvious band shifts were detected upon SSCP analysis, all 7 PCR fragments of the α-galactosidase A gene were sequenced.

Restriction Digest Tests

Sequence changes were confirmed by reamplification of the DNA fragment and digestion with an appropriate restriction enzyme (Table 2). If the sequence change did not alter an existing restriction site then a restriction site was created by amplifying the DNA fragment with a primer which contained an altered base, ACRS (amplification created restriction site). These digest or ACRS tests were then used to detect heterozygous females in the families.

RESULTS

Thirty two mutations were detected in 35 unrelated patients of European origin with Fabry disease (Table 1). Eight of the patients were females. Twenty of the mutations are novel and comprise of 13 missense (H46Y, W47G, R49P, C94S, F113S, G258R, P259R, Q279H, Q280H, R363H, A377D, P409A, P409T), one nonsense (L294X), five small deletions (154delT, 520delT, 909-918del10, 1152-1153delCA, 1235-1236delCT) and one splice site mutation (IVS5+2T→C). The remaining 12 mutations (W95S, R112C, A143T, N215S, M267I, N298H, D313Y, W162X, R301X, R342X, 359-364del6 and 1176-1179del4) have all been reported previously as indicated in Table 1.

DISCUSSION

We have detected 32 mutations, including 20 novel ones, in 35 unrelated European pedigrees. The novel mutations constist of 14 single base substitutions (13 missense and 1 nonsense), 5 small deletions and one splice site alteration.

All of the missense mutations, except for D313Y and R363H, occur in highly conserved residues of the α-galactosidase A amino acid sequence. In most cases the change in amino acid is predicted to be disease causing because of its expected effect on the conformation, stability or catalytic function of the α-galactosidase A enzyme. These changes include: a change in charge: H46Y, G258R, Q279H, Q280H, N298H, D313Y, A377D; a change

Table 1. Mutations and phenotypes of 35 Fabry patients.

Mutation*	Exon	Nucleotide Change	cDNA Base	Protein Change	Phenotype
Missense					
H46Y	1	CAC→TAC	136	His→Tyr	Female
H46Y					Classic
W47G	1	TGG→GGG	139	Trp→Gly	Female with renal problems
R49P	1	CGC→CCC	146	Arg→Pro	Classic
C94S	2	TGT→TCT	281	Cys→Ser	Classic
W95S (Ashton-Prolla et al. 2000)	2	TGG→TCG	284	Trp→Ser	Gut problems
R112C (Ishii et al. 1992, Ashton-Prolla et al. 2000)	2	CGC→TGC	334	Arg→Cys	Classic
F113S	2	TTT→TCT	338	Phe→Ser	Female
A143T (Eng et al. 1997)	3	GCA→ACA	427	Ala→Thr	Classic
N215S (Davies et al. 1993b, Eng et al. 1993)	5	AAT→AGT	644	Asn→Ser	Mild, cardiac variant
N215S					Mild
G258R	5	GGA→CGA	772	Gly→Arg	Female
P259R	5	CCA→CGA	776	Pro→Arg	Classic
M267I (Topaloglu et al. 1999)	5	ATG→ATA	801	Met→Ile	Classic
Q279H	6	CAG→CAC	837	Gln→His	Pain in hands and feet
Q280H	6	CAA→CAT	840	Gln→His	Female
N298H (Davies et al. 1996)	6	AAT→CAT	894	Asn→His	Classic
D313Y (Eng et al. 1993)	6	GAT→TAT	937	Asp→Tyr	Female with acroparaethesias
R363H	7	CGC→CAC	1088	Arg→His	Renal presentation
A377D	7	GCC→GAC	1130	Ala→Asp	Classic
P409A	7	CCC→GCC	1225	Pro→Ala	Classic
P409T	7	CCC→ACC	1225	Pro→Thr	Female
Nonsense					
W162X (Rosenberg et al. 1999)	3	TGG→TGA	486	Trp→Stop	Classic
L294X	6	TTA→TGA	881	Leu→Stop	Classic
R301X (Eng et al. 1994b)	6	CGA→TGA	901	Arg→Stop	Joint pains
R301X					Kidney involvement
R342X (Davies et al. 1993b)	7	CGA→TGA	1024	Arg→Stop	Classic
Deletions					
154delT	1	1 bp deletion	154	68 altered aa, stop	Female with classic symptoms
359-364del6 (Eng et al. 1994b)	2	6 bp deletion	359	1 altered aa, in-frame	Classic
520delT	3	1 bp deletion	520	17 altered aa, stop	Classic
909-918del10	6	10 bp deletion	908	10 altered aa, stop	Classic
1152-1153delCA	7	2 bp deletion	1152	13 altered aa, stop	Classic
1176-1179del4 (Topaloglu et al. 1999)	7	4 bp deletion	1176	1 altered aa, stop	Classic
1235-1236delCT	7	2 bp deletion	1235	37 altered aa, stop	Classic

Splice site					
IVS5+2T® C	intron 5	Ggt→Ggc			Classic

*Mutations found are novel unless a reference is given in which they have been previously described. Patients were males with classic forms of Fabry presentation unless otherwise indicated.

Table 2. Detection of band shift on SSCP gel and restriction enzyme digest test or ACRS test used to confirm each mutation found.

Mutation*	SSCP band shift	Restriction enzyme test	ACRS test
H46Y	Yes	+RsaI	
W47G	Yes	-BsrI	
R49P	Yes	+Bsp1286I	
C94S	Yes	+HinfI	
W95S	Yes	+TaqI	
R112C	Yes	-HhaI	
F113S	Yes	+BseRI	
A143T	Yes	-HhaI	
N215S	Yes	-TspEI	
G258R	No	+TaqI	
P259R	No	-AvaII	
M267I	Yes	-BslI	
Q279H	Yes	+HphI	
Q280H	No	+NlaIII	
N298H	Yes	+BspHI	
D313Y	Yes		+RsaI
R363H	Yes		-BfaI
A377D	Yes	-HaeIII	
P409A	Yes		+NlaIII
P409T	No		+RsaI
W162X	Yes	+MnlI	
L294X	Yes	+HinfI	
R301X	Yes		+DdeI
R342X	Yes		+RsaI
154delT	Yes	-MwoI	
359-364del6	Yes	-AluI	
520delT	Yes	+MaeII	
908-917del10	Yes	+NlaIII	
1152-1153delCA	Yes	Sequence	
1176-1179del4	Yes		+MboII
1235-1236delCT	Yes	-AlwNI	
IVS5+2t® c	No		+MwoI

*Novel mutations are indicated by boldface type.

in hydrophobicity: W95S, A143T; introduction or loss of aromatic group: W47G, F113S, R363H; replacement or insertion of a proline: R49P, P259R, P409A, P409T; which disrupts the folding of the polypeptide chain, or a cysteine: C94S, R112C; which is highly reactive and can be involved in formation of disulphide bridges. All novel missense and splicing mutations were confirmed as causative by checking 100 normal control individuals on SSCP gels or, for those mutations that did not show an SSCP band shift, 50 normal control individuals were digested.

The structural consequences of two of the previously reported mutations N215S and M267I are not predictable. However, the N215S change has been commonly found in patients with a cardiac variant of Fabry disease. Although it abolishes a functional N-glycosylation consensus site the expressed mutant enzyme retains up to 25% of normal activity accounting for the observed phenotype (Eng et al. 1993). M267I does not alter the

hydrophobicity or charge of the side chain but it is conserved in both mouse and human α-galactosidase and results in a classic Fabry phenotype.

Only one of the nonsense mutations reported here, L294X, is novel. It could be causing premature termination of protein translation in exon 6, a decrease in mRNA (Zhang et al. 1994) or exon skipping (Dietz et al. 1993). It is also possible that a mutated protein could be produced by reading through the nonsense codon (Peltola et al. 1994). Two of the other three nonsense mutations occur at CpG dinucleotides, which are known mutational hotspots due to the deamination of methylcytosine to thymidine.

All of the small deletions found result in a shift in the codon reading frame and termination, except for 359-364del6 which results in the loss of 3 original amino acids and gain of a new one, histidine, which remains in-frame. This deletion has been seen previously and is thought to be due to inverted repeats allowing self-complementarity within the DNA strand and deletion during replication by read-through errors of the polymerase (Eng et al. 1994b). Another deletion, 1176-1179del4, has also been seen previously (Topaloglu et al. 1999), and a 2-base pair deletion at the same nucleotide position (Eng et al 1994a). The deletion 1152-1153delCA is part of a CA repeat sequence and is probably the result of slipped mispairing due to the presence of short direct repeats at the breakpoint.

We also observed a novel splice-site mutation, IVS5+2T→C, in which the 5' donor consensus splice site of intron 5 has been changed from Ggt to Ggc, which would be predicted to result in aberrant mRNA processing.

SSCP analysis and DNA sequencing have been efficient and reliable methods for detecting mutations in our cohort of Fabry patients. There are, however, other effective methods for detecting mutations in the α-galactosidase A gene, including fluorescence-assisted mismatch analysis (Germain et al. 1996) and fluorescent chemical cleavage of mismatches (Germain et al. 1999).

This report further increases the number of private mutations found in the α-galactosidase A gene associated with Fabry's disease. As all types of mutations were found in patients with the classic phenotype, it was not possible to correlate the phenotype with the genotype.

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