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REVIEW

# Spectrum of mutations in fucosidosis

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**Fucosidosis is a lysosomal storage disorder characterised by progressive psychomotor deterioration, angiokeratoma and growth retardation. It is due to deficient α-l-fucosidase activity leading to accumulation of fucose-containing glycolipids and glycoproteins in various tissues. Fucosidosis is extremely rare with less than 100 patients reported worldwide, although the disease occurs at a higher rate in Italy, in the Hispanic-American population of New Mexico and Colorado, and in Cuba. We present here a review study of the mutational spectrum of fucosidosis. Exon by exon mutation analysis of *FUCA1*, the structural gene of α-l-fucosidase, has identified the mutation(s) in nearly all fucosidosis patients investigated. The spectrum of the 22 mutations detected to date includes four missense mutations, 17 nonsense mutations consisting of seven stop codon mutations, six small deletions, two large deletions, one duplication, one small insertion and one splice site mutation. All these mutations lead to nearly absent enzymatic activity and severely reduced cross-reacting immunomaterial. The observed clinical variability is, therefore, not due to the nature of the fucosidosis mutation, but to secondary unknown factors.**

**Keywords: fucosidosis; fucosidase; lysosomal storage disorder; mutations; polymorphisms**

## Introduction

Fucosidosis is due to deficient activity of a lysosomal enzyme, α-l-fucosidase (E.C. 3.2.1.51).[1] When fucosidase activity is deficient, fucose is not hydrolysed from fucose-containing glycoconjugates leading to accumulation of fucosyl-glycolipids, glycopeptids and oligosaccharides in various tissues. The clinical picture mainly consists of neurodegeneration with progressive mental and motor deterioration. Additional features are angiokeratoma corporis diffusum, dysostosis multiplex, visceromegaly, ocular abnormalities, hearing loss, seizures, coarse features, recurrent infections, spasticity, contractures, growth retardation, muscle wasting and dystrophy.[2–4] All these clinical abnormalities are progressive, leading to cachexia and early death. Although clinical heterogeneity with a very rapidly progressive course and death in infancy (type I), and a slightly milder variant with death in adulthood (type II) has been described, further studies have shown that fucosidosis has a wide continuous clinical spectrum without any evidence for real clinical heterogeneity.[4]

In a clinical review study compiling information from the literature and from an international questionnaire survey in North America, Western Europe, Japan and Australia, 77 patients affected with fucosidosis from 54 sibships have been reviewed.[4] A few additional patients

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have been reported after 1990, but in total less than 100 fucosidosis patients have been identified, indicating that the incidence of fucosidosis must be very low. The ethnic origin of fucosidosis has a worldwide distribution and fucosidosis patients have been reported in more than 20 different countries from Europe, North and South America, Asia and Africa. All fucosidosis patients have nearly absent α-l-fucosidase enzyme activity.[5,6] In this molecular review study we present the spectrum of mutations in *FUCA1* responsible for polymorphisms and fucosidosis.

## *FUCA1* Gene

### *cDNA Sequence*

The compiled cDNA sequence of *FUCA1* consists of 2053 base pairs.[7,8] This includes 5' untranslated sequences, an open reading frame of 1383 bp, a consensus polyadenylation signal AATAAA and a poly(A)$^+$ tail. The open reading frame encodes for a signal peptide of 22 amino acids and a mature protein of 439 amino acids, with a calculated molecular weight of 51338.

The canine α-l-fucosidase cDNA encodes for a mature protein of 465 amino acids, and has an overall similarity with the human fucosidase of 84%.[9]

### *Genomic Structure*

*FUCA1* is composed of eight exons spanning approximately 23 kb. The sizes of the different exons are > 374 bp (exon 1), 135 bp (exon 2), 138 bp (exon 3), 106 bp (exon 4), 201 bp (exon 5), 191 bp (exon 6), 100 bp (exon 7) and > 772 bp (exon 8), respectively.[10] The canine *FUCA1* gene consists of 8 exons spanning approximately 12 kb, with a genomic structure which is similar to that of the human gene.[9]

## *FUCA1P* Gene

A sequence on chromosome 2 with homology to the structural fucosidase gene *FUCA1* has been identified by Southern blot analysis of somatic cell hybrids with *FUCA1* cDNA. It is 80% identical to the *FUCA1* cDNA, but does not have an open reading frame and does not encode fucosidase enzyme activity, indicating that it is a processed pseudogene of *FUCA1*.[10] It was therefore designated *FUCA1P*. The *FUCA1P* gene has been sublocalised to chromosome 2q31–q32 by FISH, in close proximity to the *COL3A1* gene.[11]

## Fucosidosis Mutations

The characterisation of the genomic structure of *FUCA1* with all its exon–intron boundary sequences[10] facilitated the mutational analysis in fucosidosis patients. PCR amplification of the eight different *FUCA1* exons followed by direct sequencing or SSCP/sequencing resulted in the identification of the majority of fucosidosis mutations including point mutations, deletions of 1 to 10 bp, and insertions of 1 to 66 bp (Table 1). Only four (18%) of the 22 mutations are missense mutations, whereas the remaining 18 (72%) are inactivating mutations consisting of seven nonsense mutations, six small deletions, two large deletions, one small insertion, one duplication of 66 bp, and one splice site mutation (Table 1). The mutations are spread throughout the open reading frame of *FUCA1* without hot spots (Figure 1). Only one fucosidosis mutation in a total of 40 patients has not yet been identified, indicating that fucosidosis is genetically homogeneous and due to mutations in only one gene. Furthermore, fucosidosis mutations nearly always disrupt the open reading frame and not the regulatory region of *FUCA1*. All these mutations including the missense mutations result in nearly absent α-l-fucosidase enzymatic activity and severely deficient cross-reacting immunomaterial (CRIM), indicating that the mutant fucosidase proteins are unstable and degrade rapidly.[5,6] All but two mutations were found in homozygous form, confirming the very high rate of consanguinity found in fucosidosis families.[4] The different mutations are discussed below. The patients' initials refer to the appendix table in the review study by Willems *et al*.[4]

## Missense Mutations

Only four missense mutations have been identified. These include the G60D, the S63L, the N329Y and the P5R mutations described below.

### *G60D Mutation*

The substitution of the neutral glycine residue by the acidic aspartic acid at amino acid position 60 in exon 1 ($^{179}$G A) is present in homozygous forms in a large French-American (Cajun) family with three affected patients, SB/CB/RL, and in three Italian families, AM, MZ/GZ and SD. The ancestors of SD and MZ/GZ originate from the same village of Formia (Lazio), whereas AM originates from the nearby Forio (Campania), suggesting a common ancestor. However, an

**Table 1** Spectrum of FUCA1 mutations in fucosidosis

| *Mutation* | *Nucleotide change* | *Type* | *Exon/ Intron* | *Patients* | *Ethnic origin* | *References* |
|---|---|---|---|---|---|---|
| P5R | $^{14}$C→G | missense | exon 1 | MoA(47)[a] | Sudanese[b] | 6 |
| G60D | $^{179}$G→A | missense | exon 1 | SB/CB/RL(19) | French-American | 16 |
| | | | | AM(10) | Italian (Campania) | |
| | | | | MZ/GZ(3) | Italian (Lazio) | |
| | | | | SD(2) | Italian (Lazio) | |
| S63L | $^{188}$C→T | missense | exon 1 | FX(13) | Italian (Veneto) | 32 |
| Q77X | $^{229}$C→T | stop codon | exon 1 | GM/RM(1) | Italian (Campania) | 17,33 |
| | | | | AgC(14) | Italian (Campania) | 17 |
| E113fs | 340del10 | deletion | exon 1 | MB | Austrian | 6 |
| P141fs | 421delC | deletion C | exon 2 | MS/SS/C(4) | Italian (Calabria) | 33 |
| | | | | SI | Italian (Calabria) | |
| | | | | RZ/AZ(8) | Italian (Calabria) | |
| | | | | RobF/RosF | Italian (Calabria) | |
| K151fs | 451delAA | deletion AA | exon 2 | CS(12) | Italian (Puglia) | 16 |
| W183X | $^{549}$G→A | stop codon | exon 3 | MM | Austrian | 6 |
| Y211X | $^{633}$C→A | stop codon | exon 3 | MB(25) | Belgian | 33 |
| S216fs | 646delA | deletion A | exon 3 | EN(40) | Canadian-Indian | 6,33 |
| | | | | GH | British | |
| E253fs | 758delA | deletion A | exon 5 | C(49) | Turkish | 34 |
| S265fs | 794delC | deletion C | exon 5 | GD(20) | Portuguese | 33 |
| | $^{954+1}$G→A | splice site | intron 5 | SB | East Indian-Zambian | 35 |
| N329Y | $^{985}$A→T | missense | exon 6 | GS | Austrian | 6 |
| Y330fs | 1988insT | insertion T | exon 6 | MoA(47) | Sudanese[b] | 6 |
| | 1030ins66 | duplication | exon 6 | JH(44) | | 36 |
| E375X | $^{1123}$G→T | stop codon | exon 6 | JT(31) | Hispanic-American | 16,37 |
| | | | | LA(32) | | |
| | | | | JC/FC(38) | | |
| | | | | BL(39) | | |
| | | | | FV(34) | | |
| | | | | JG/GG(33) | | |
| W382X | $^{1145}$G→A | stop codon | exon 6 | CS(12) | Italian (Puglia) | 33 |
| | | | | TB | Italian (Puglia) | |
| G401X | $^{1201}$G→T | stop codon | exon 7 | AB | German (Volga) | 38,39 |
| | | | | SW | German | |
| Gross deletion | | deletion | exons 7-8 | FZ/SZ(48) | Algerian | 4,16 |
| Q422X | $^{1264}$C→T | stop codon | exon 8 | LS(9) | Italian (Umbria) | 12,39,40 |
| | | | | DeG/DaG(45) | Cuban | |
| | | | | RP/LP(46) | Cuban | |
| | | | | DM/SM(18) | French | |
| | | | | GM/RM(1) | Italian (Sicilia) | |
| | | | | CM | French/Scottish | |
| Gross deletion | | deletion | exon 4 | JDH(26) | Dutch | Coucke and Willems, unpubl, result. (1996) |
| | | | | CN(27) | | Seo and O'Brien, unpubl, result. (1996) |

[a]The numbers after the patients' initials represent the numbering system in the clinical review study by Willems *et al*[5];
[b]The P5R and Y330fs mutations are present in the same patient.

obvious common ancestor of the Cajun family, SB/CB/RL, has not been identified.

## *S63L Mutation*

The substitution of serine by leucine at amino acid position 63 in exon 1 ($^{188}$C→T) has only been identified in a single patient from the Veneto region in Northern Italy. Diminished *in vitro* expression of mutant α-l-fucosidase proved that the S63L mutation was the disease-causing mutation.

## *N329Y Mutation*

This missense mutation in exon 6 ($^{985}$A→T) replacing the conserved asparagine residue at amino acid position 329 by a larger and hydrophobic amino acid,

**Table 2** Polymorphisms in *FUCA* 1 and fucosidase-related genes

| *Gene* | | *Chromosomal localisation* | *Polymorphism* |
|---|---|---|---|
| *FUCA*1 | structural gene | 1 | PvuII RFLP |
| *FUCA*1 | structural gene | 1 | BglI RFLP |
| *FUCA*1 | structural gene | 1 | electrophoretic protein polymorphism |
| *FUCA*1P | pseudogene | 2 | low or high intracellular activity |
| *FUCT* | regulating gene[a] | 1 | BclI RFLP |
| *FUCA*2 | regulating gene[a] | 6 | low or high plasma activity |

[a]Genes for which there is no formal proof of existence

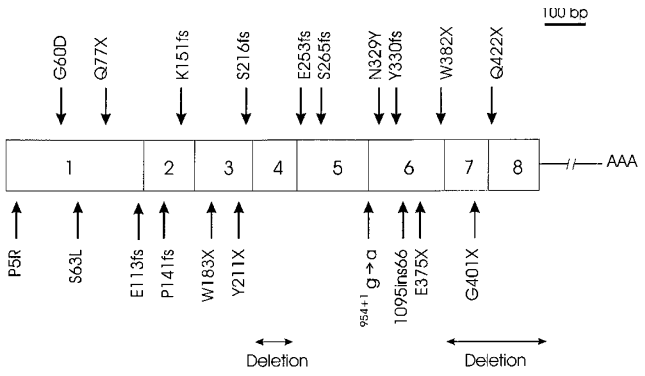


**Figure 1** *Schematic representation of* FUCA1 *mutations in fucosidosis. The cDNA structure representing the different exons is shown with the different* FUCA1 *mutations.*

tyrosine, was found in homozygous form in an Austrian patient.

### *P5R Mutation*

This mutation was found in a single Sudanese patient. The substitution of proline by arginine at amino acid position 5 in exon 1 might cause a conformational change in the secondary structure of the α-l-fucosidase leader peptide. Although the P5R mutation was found in homozygous form in one patient it is not certain that the P5R mutation causes the disease, as the patient also has a Y330fs mutation in homozygous form. As the Y330fs mutation results in a truncated fucosidase protein which is probably unstable, this mutation alone probably leads to fucosidosis. Therefore, the significance of the P5R mutation is not clear, and it rather represents a polymorphism than a disease-causing mutation.

## Inactivating Mutations

Eighteen inactivating mutations described below have been identified in fucosidosis.

### *Q77X Mutation*

This stop codon mutation in exon 1 ($^{229}$C→T) is present in homozygous form in an Italian patient AgC and in heterozygous form in the siblings GM/RM. The parents of AgC and the mother of GM/RM are from Napoli (Campania), which suggests they are related.

### *E113fs Mutation*

A deletion of 10 bp in exon 1 (340del10) causing a frameshift and a premature stop codon has been found in an Austrian patient.

### *P141fs Mutation*

A deletion of a single C at nucleotide position in 421 (421delC) in exon 2 causing a frameshift and a premature stop codon has been identified in three Italian families, AZ/RZ, SS/MS/patient C, and SI from Calabria. The siblings AZ and RZ belong to the same pedigree as Rob F and Ros F, the initial fucosidosis patients described by Durand *et al*.[2] Patient SS, who had similar clinical and pathological abnormalities as Durand's original family, was the first patient in whom an α-l-fucosidase deficiency was demonstrated.[1] Also MS and patient C belong to this fucosidosis family. Large pedigrees of both Calabrian families have been published.[4] The two families and patient SI have not yet been linked by pedigree analysis, but the fact that they are from nearby villages in southern Calabria, suggests that they are all related and descendants from a common ancestor carrying the P141fs mutation. The relatively high consanguinity of Calabrian families is, therefore, the most likely explanation for the high frequency of fucosidosis in Calabria.

### *K151fs Mutation*

A deletion of 2 bp AA in exon 2 (451delAA) giving rise to a frameshift has been found in heterozygous form in an Italian patient, CS, from the Puglia region, who is also heterozygous for the W382X mutation.

### *W183X Mutation*

This stop codon mutation in exon 3 ($^{549}$G→A) was found in homozygous form in an Austrian patient.

### *Y211X*

This stop codon mutation in exon 3 ($^{633}$C→A) was identified in homozygous form in a Belgian patient, MB.

### *S216fs*

A deletion of a single A in exon 3 (646delA) was found in EN, in which the second mutation has not yet been found. EN has a native Indian mother, whereas his father is Irish. As the S216fs is also present in homozygous form in a British patient, GH, EN and GH might be related.

### *E253fs Mutation*

A deletion of a single A in exon 5 (758delA) was found in homozygous state in a Turkish patient, Ce.

### *S265fs Mutation*

A deletion of a single C in exon 5 (794delC) was found in homozygous form in a Portugese patient, GD.

### *Splice Site Mutation*

A homozygous G→A point mutation of the first nucleotide of the 5' splice site of intron 5 ($^{954+1}$G→A) was found in an East Indian-Zambian patient, SB. The consequence of this splice site mutation on the protein is unknown.

### *Y330fs Mutation*

A homozygous insertion of T in exon 6 (988insT) was found in a patient that was also homozygous for the P5R mutation (see above).

### *A 66 bp Duplication*

An in-frame 66 bp duplication of nucleotides 1030 to 1095 (1030ins66) of exon 6 was identified in homozygous form in patient JH.

### *E375X Mutation*

The stop codon mutation E375X ($^{1123}$G→T) in exon 6 has been found in homozygous form in six Hispanic-American families from New Mexico and Colorado. As the families have the same fucosidosis mutation and the same Hispanic background, and the population is known to be highly consanguineous, it is likely that they are all descendants from a common ancestor carrying the E375X mutation.

### *W382X Mutation*

This stop codon mutation in exon 6 ($^{1145}$G→A) is present in heterozygous form in CS and in homozygous form in TB, two Italian patients originating from the Puglia region, suggesting that they are related.

### *G401X Mutation*

The G401X mutation creates a premature stop codon in exon 7 ($^{1201}$G→T) and was found in two patients, AB and SW. Patient AB originates from the former German Volga Republic in Russia, whereas patient SW lives near Würzberg in Germany. Both patients have consanguineous parents but pedigree analysis could not indicate a relation between the two families. Nevertheless, it is likely that they have a common ancestor carrying the G401X mutation as this has never been found in other patients.

### *Q422X Mutation*

This stop codon mutation in exon 8 ($^{1264}$C→T) was the first mutation found in fucosidosis patients. The Q422X mutation has now been identified in six different families, including LS from the Umbria region (Italy), GM/RM from Sicily (Italy), DeG/DaG and RP/LP from Cuba, DM/SM from France, and CM, a Canadian patient with mixed Scottish/French origin. More than 10 fucosidosis patients from the same province in Cuba (Holguin), have been found to carry the Q422X mutation in homozygous state, which makes it very likely that they have a common ancestor (Coucke and Willems, unpublished observations). It is unclear whether all patients with the Q422X mutation have a common ancestor in view of their different ethnic origin, although the Q422X mutation is present on the same haplotype background for 2 RFLPs (PvuII and BglI).

### *Deletion of Exon 4*

In two Dutch patients, JdH and CN, a deletion of exon 4 and its flanking intronic sequences was found in homozygous form suggesting that they are probably related.

### *Deletion of Exons 7 and 8*

A homozygous deletion of exons 7 and 8 was detected in Algerian siblings, FZ/SZ.

## Polymorphisms

### *Restriction Fragment Length Polymorphisms (RFLPs) in* FUCA1

Both a PvuII and a BglI RFLP have been identified in *FUCA1*. PvuII identifies a two-allele RFLP[13] with bands at either 7.0 kb (70%) or 6.0 kb (30%). BglI identifies a two-allele polymorphism with either one band at 12 kb or two bands at 6.5 kb (63%) and 5.5 kb (37%).

An additional BclI RFLP can be detected by *FUCA1* cDNA probes. This RFLP, however, it not located in

*FUCA1*, but in the fucosidase pseudogene *FUCA1P* on chromosome 2.[14]

### *Electrophoretic Polymorphism in* FUCA1

A common fucosidase enzyme polymorphism can be detected in blood and tissues by starch gel electrophoresis and isoelectric focusing.[15] Isoenzymes with the Fu1 phenotype have a more acidic pI than Fu2 isoenzymes. Allele frequencies of Fu1 and Fu2 are 0.72 and 0.28, respectively, with only minor differences in allele frequency between different ethnic populations. A $^{842}$A → G missense mutation, responsible for the substitution of glutamine by arginine at amino acid position 281, has been identified in exon 5 of *FUCA1*,[16–18] and $^{281}$Arg is associated with the Fu2 phenotype of more basic isoenzymes.[19,20]

### *BclI RFLP in the Fucosidase Pseudogene* FUCA1P

A two-allele BclI polymorphism is present in *FUCA1P*.[14] The BclI RFLP is best revealed by BclI/PstI double digestion, which reveals polymorphic bands of 12.0 kb (absence of BclI site) and 8.1/3.9 kb (presence of BclI site). Allele frequencies are 0.58 (12 kb) and 0.42 (8.1 kb). The BclI RFLP can also be identified by *FUCA1* cDNA probes because of the high degree of homology between *FUCA1P* and *FUCA1*.

### *Polymorphism in a Putative Regulatory Gene* FUCT

Variation in the pattern of intracellular α-l-fucosidase synthesis has been reported by Tümmler *et al*[21] who proposed to call this polymorphic regulatory gene *FUCT*, and suggested that *FUCT* mapped in close proximity to the structural fucosidase gene *FUCA1*, in view of its linkage disequilibrium with *FUCA1*.

DiCioccio and Brown,[22] however, found no evidence for such a regulatory gene, and therefore the existence of *FUCT* is uncertain.

### *Low Plasma Activity Polymorphism in* FUCA2

Some normal individuals have low plasma α-l-fucosidase activity. This variant trait is not associated with any kind of disease and represents an autosomal recessive enzyme polymorphism.[23,24] The mean allele frequencies are 0.72 and 0.28 for high and low enzyme activity, respectively. Analogous plasma low activity polymorphisms have been described for other lysosomal enzymes such as α-L-iduronidase, arylsulfatase A, hexosaminidase A, galactocerebrosidase and α-galactosidase A. (For a review on low activity polymorphisms, see Thomas.[25])

The enzymatic fucosidase activity in plasma of variants is between 10 and 30% of the control mean.[23,24,26] Variants have also a lower fucosidase activity in cultured fibroblasts.[27] In leucocytes and lymphoblastoid cell lines, however, the fucosidase activity of variants and normals is not significantly different.[23,24] All eight *FUCA1* exons and exon–intron boundaries from individuals who were homozygous for the low activity trait, were sequenced but no mutation was found (Seo and O'Brien, unpublished results). On the other hand, weak evidence for linkage between a putative gene controlling plasma fucosidase activity, *FUCA2*, and the plasminogen gene on chromosome 6 has been provided by Eiberg *et al*,[28] but this has never been confirmed nor refuted. Therefore, the existence of *FUCA2* is uncertain.

## Animal Model for Fucosidosis

Severe α-L-fucosidase deficiency with residual enzyme activity below 5% of controls can be found in English and Australian springer spaniels derived from a few original English stud dogs. Especially in Australia the incidence of fucosidosis in these dogs is exceedingly high (4%) causing breeding problems. Fucosidosis in springer spaniels manifests itself as a mainly neurodegenerative condition with progressive tremor, ataxia, proprioceptive defects, deafness, blindness, inability to swallow, loss of learned behaviour and wasting. This results in a lethargic state and death at the age of 3–5 years. The pathologic abnormalities are similar to those of the human condition.[29] A 14 bp deletion at the 3' end of exon 1 of Fuca1 was found, resulting in a frame shift with a premature stopcodon.[9,30] The affected springer spaniel dogs have been used as an animal model to treat fucosidosis *in vivo* with bone marrow transplants and *in vitro* by retrovirus mediated gene transfer.[31]

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