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Mutations in Fucosidosis Gene: a Review

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Abstract. Fucosidosis is an autosomal recessive disorder caused by a deficiency of alpha-L-fucosidase. Up to now 79 cases have been described and several others identified but not yet published. The higher incidence of the disease is in Italy, where nearly 20 patients have been identified. Fourteen disease-causing mutations have been detected and four of them, Q422X, G60D, E375X, P141fs are present in more than 70% of the forty patients studied. In Italian patients, only seven mutations have been described and P141fs and G60D mutations are present in more than 50% of the cases. The P141fs mutation is absent in other ethnic groups. It has been impossible to establish genotype-phenotype correlation so far and the clinical variability of the disease cannot be explained only by genetic heterogeneity.

Key words: Inborn metabolic diseases, Fucosidosis, Mutations

INTRODUCTION

Fucosidosis is an autosomal recessive lysosomal storage disorder caused by a deficiency of alpha-L-fucosidase, leading to the accumulation of fucoglycoconiugates. Its main clinical features are progressive mental and neurological deterioration, coarse facies, growth retardation, recurrent infections, dysostosis multiplex, angiokeratoma.

Since the first description, nearly thirty years ago, [3-5], 60 cases have been reported in literature [1, 9, 22], 19 cases were personal communications [22] and several other cases have been identified but not published. The higher incidence of the disease is in Italy, where nearly 20 patients have been described.

Another ethnic group with a relatively high frequency is the Hispanic community of New Mexico and Colorado where 8 patients have been diagnosed.

A cDNA encoding alpha-L-fucosidase was first cloned in 1984, and in 1986 the structural fucosidase gene was assigned to 1p34-1p36.

At present, the structure and sequence of the gene is well known [11], and fourteen

mutations have been described [12, 14, 15, 16, 17, 20, 22, 23]. A mutation responsible for a common polymorphism has also been identified [14, 24].

In this paper we summarize the most important clinical, biochemical, pathological and genetic features of the disorder, wich have been recently reviewed [22], and report all the mutations of alpha-L-fucosidase gene up to now identified.

CLINICAL FEATURES

The most frequent symptoms are mental retardation, which ranges from profound to moderate, a progressive neurologic deterioration which generally begins after the first year of normal development and can lead till decerebrate rigidity, more or less coarse facies, growth retardation, recurrent infections, kyphoscoliosis, dysostosis multiplex, angiokeratoma.

Angiokeratoma is present in most patients, especially in those surviving after ten years and, although it is not patognomonic for fucosidosis, it offers an easy clue to the diagnosis. Less constant features are joint contractures, seizures, visceromegaly. Hearing loss, hernia and loss of visual acuity are occasional findings.

However, the course of the disease is highly variable. Most patients have a slow neurologic deterioration and survive into the second or third decade of life. A minority have a very severe form with death in the first decade.

A wide range of severity also exists in the same family. This is an evidence against the former distinction between two forms of the disease, type I (more severe) and type II (milder), caused by different mutations. Instead type I and type II represent two extremes of a continuous clinical spectrum.

BIOCHEMICAL AND PATHOLOGICAL ABNORMALITIES

Nearly 90% of storage material in fucosidosis patients consists of glycoproteins containing L-fucose.

The release of fucose residues from the carbohydrate chain, promoted by alpha-L-fucosidase, is the initial step in glycoprotein degradation. Oligosaccarides, mucopolysaccarides and glycolipids containing L-fucose have been demostrated in limited amount.

An abnormal expression of Lewis blood groups has also been reported. Most patients are Lewis a+b+ even when their parents are a- or b-. Alpha-L-fucosidase enzyme activity in fibroblast, leucocytes and liver tissue of patients is nearly absent, while the range of enzyme activity of heterozygotes largely overlaps with that of controls. In patients, the residual fucosidase protein amount determined by radioimmunoassay in fibroblast and lymphoblastoid cells is below 6% of the normal mean; in heterozygotes it is 29% of the normal mean in fibroblasts and 53% in lymphoblastoid cells.

About 6-10% of phenotypically normal individuals have an alpha-L-fucosidase activity reduced to less than 10% of the mean control in serum and plasma, whereas in leukocytes the activity is only slightly reduced. An autosomal recessive inheritance has been suggested for this polymorphism [8, 13].

Another polymorphism has been detected by electrophoretic focusing. Two forms

have been described: Fu-1, the most frequent and Fu-2, present in about 25% of subjects in a Caucasian population sample. There is no correlation between this electrophoretic polymorphism and the low-activity polymorphism [19].

Autopsy studies in fucosidosis patients have shown abnormalities in almost every organ and tissue. Brain, heart, lung, liver and kidney are the most involved. Clear vacuoles containing a fine reticular component wich stains faintly with usual techniques have been demonstrated by ultrastructural studies in almost every tissue. Dark inclusions with granular material were also shown especially in brain. Such combination of clear and dark vacuoles is typical of fucosidosis. Moreover, lamellar inclusion bodies, probably consisting of glycolipids, have been described.

GENETICS

Autosomal recessive inheritance of fucosidosis has been demonstrated by family-based studies. However, a significant excess of male patients remains unexplained.

The high incidence in Italian and Hispanic-American patients could be due to a founder effect, a high imbreeding coefficient or a high frequency of fucosidosis mutation in such populations. However, it could be due also to the deeper knowledge of the clinical features of the disease and to the intensive screening of fucosidosis performed by our group in Italy and by another group in Colorado.

At Gaslini Institute of pediatrics in Italy, 15 patients have been diagnosed and six affected fetuses have been identified by prenatal diagnosis. Genetic heterogeneity (see after) has been demonstrated, but it cannot explain the wide clinical variability of the disease.

ALPHA-L-FUCOSIDASE GENE

In 1991, a study reported the structure and sequence of alpha-L-fucosidase gene, located in the short arm of chromosome 1 [11]. The gene is composed of eight exons dispersed over 23 Kb of genomic DNA. The size of exons ranges from 100 bp (exon 7) to 772 bp (exon 8). A pseudogene on chromosome 2 is 80% identical to alpha-L-fucosidase cDNA. It does not contain introns and has no protein coding potential.

ALPHA-L-FUCOSIDASE MUTATIONS

Up to now, fourteen different disease-causing mutations have been identified [12, 14, 15, 16, 17, 20, 22, 23] (Table 1): five of them (Q422X, E375X, Q77, W382X, Y211X) are non-sense due to a single base change, five are frameshift due to single-base (P141fs, S265fs, S216fs, E253fs) or double-base (K151fs) deletions, two (G60D, S63L) are missense, one is a 5exon/5intron splice site mutation and one is a deletion of exons 7-8.

Forty patients in twenty-eight families have been studied (Table 2). Three of them (one patient with Q77 mutation, one with P141fs and one with W382X) were not published previously but were recently observed, at our Institute.

Table 1 - Mutations in alpha-L-fucosidade gene: econ, base change, aminoacid change

Mutation	Exon	Base change	Aminoacid change	Reference
Q422X	8	C → T	Gln → Stop	[12]
E375X	6	G → T	Glu → Stop	[14]
G60D	1	G → A	Gly → Asp	[14]
P141fs	2	C del.	Frameshift	[15]
Q77	1	C → T	Gln → Stop	[15]
W382X	6	C → A	Trp → Stio	[15]
Exon 7-8 deletion	7-8	/	/	[14]
K151fs	2	AA del.	Frameshift	[14]
Exon5/Intron5 junction	+1 ex5/ intr5	G → A	Alteration of splicing	[23]
Y211X	3	C → A	Tyr → Stop	[15]
S265fs	5	C del.	Frameshift	[15]
S216fs	3	A del.	Frameshift	[15]
Z253fs	5	A del.	Frameshift	[16]
S63L	1	C → T	Ser → Leu	[17]

Three patients resulted to be compound heterozygous: two sibs were Q77/Q422X and one patient was K151fs/W382X. The other thirty-seven patients were all homozigous.

Surely additional mutations exist, since a study analyzed several other fucosidosis patients but did not report any of the foregoing mutations [15].

However, the most frequent mutations identified so far are: Q422X (nine patients in five families) E375X (eight patients in six families), G60D (seven patients in four families) and P141fs (five patients in three families).

Seventy per cent of patients studied carry one of these four mutations.

The ethnic origin of patients carrying the Q422X mutation is Italian (three patients in two families), Cuban (four patients in two families) and French (two patients in one family). All the patients with the E375X mutation are Hispanic American. Of the patients carrying the G60D mutation, four belong to three Italian families and three to one French-American family. All the patients with the P141fs mutations are Italian. All the Q422X patients, except the two compound heterozygous sibs, have the PvuII–BgII aplotype (strictly linked with the fucosidosis gene) 2-2,2-2, all the E375X patients have the PvuII–BgII aplotype 1-1,1-1 and all the G60D patients have the 2-2,2-2 haplotype. This could suggest a founder effect.

Seven mutations (Q422X, G60D, K151fs, 5exon/5intron splice site, W382X, Y211X and P141fs) obliterate or generate a restriction enzyme site (Table 3). The Q281R mutation in exon 5 is responsible for the common alpha-L-fucosidase polymorphism, without clinical consequences, detected by isoelectric focusing [14, 24] (Table 4).

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Table 2 - Mutations in alpha-L-fucosidase gene: number of patients, number of families and ethnic origin of familes

Mutation	N. patients	N. families	Ethnic Origin
Q422x	9	5	Italian (2), Cuban (2), French (1)
E375X	8	6	Hispanic-American
G60D	7	4	Italian (3), French-American (1)
P141fsd	5	3	Italian
Q77	3	2	Italian
W382X	2	2	Italian
Exon 7-8 deletion	2	1	Algerian
K151fs	1	1	Italian
Exon5/Intron5 junction	1	1	Asian
Y211X	1	1	Belgian
S265fs	1	1	Portuguese
S216fs	1	1	Canadian-Indian
E2353fs	1	1	Turkish
S63L	1	1	Italian
Total *	40	28	

* Two sibs are compound heterozygous Q422X-Q77 and one patients is compound heterozygous W382X-K151fs. Thus total number of patients is not 43 but 40 and the total number of famiies is not 30 but 28.

Table 3 - Mutations in alpha-L-fucosidase gene: obliteration or generation of restriction enzyme sites

Mutation	Enzyme	Effect	Fragments in patients (bp)	Fragments in controls
Q422X	EcoRi	Obliter.	6000	4400 and 1600
G60D	AflIII	Generat.	609 and 474	1083
K151fs	BstXI	Obliter.	246	131 and 115
	BpmI	Generat.	129 and 117	246
Exon5/Intron5 Junction	TaqI	Generat.	1800 and 3200	5000
W382X	HphI	Obliter.	298	245 and 53
Y211X	RSAI	Obliter.	237	166 and 71
P141fs	EaeI	Obliter.	246	146 and 100

All the patients studied with the PvuII–BgII aplotype 2-2,2-2 are homozygous for the Q281R mutation, and none of them with 1-1,1-1 aplotype have the Q281R mutation.

This suggests that the Q281R mutation is co-inherited with the 2-2 aplotype.

Table 4 - Polymorphism in alpha-L-fucosidase gene

Mutation	Exon	Base change	Aminoacid. change	N. pat.	N. fam.	Ref.
Q281R	5	A → G	Gln → Arg	19	12	[14, 17, 24]

Table 5 - Mutations in alpha-L-fucosidase gene an italian patients: number of patients, number of families, region and town of origin

Mutation	N. patients	N. families	Origin
P141fs	5	3	Calabria (Mammola, Grotteria, Giffoni)
G60	4	3	Campania (Montecorvino Forio); Lazio (Formia)
Q422X	3	2	Umbria (Magione Passignano); Sicilia (Modica)
Q77	3	2	Campania
K151fs	1	1	Puglia (Trani)
W382X	2	2	Puglia (Giovinazzo, Trani)
S63L	1	1	Veneto (Arzignano)
Total *	16	12	

* Two sibs are compound heterozygous Q422X-Q77 and one patients is a compound heterozygous W382X-K151fs. Thus the total number of patients is not 19 but 16 and the total number of families is not 14 but 12. The father of the Q422X-Q77 sibs is from Sicilia and carries the Q77X mutation. Both the parents of the W382X-K151fs patient are from Puglia.

In Italian patients only seven mutations have been detected (Table 5). All the patients, excluding the case with the S63L mutation, come from center-south regions (Figure 1). The most frequent mutation in Italy is P141fs which has been identified in five patients belonging to three unrelated families in Calabria and has not been found in other ethnic groups. The P141fs and the G60D mutation togheter are present in more than 50% of Italian patients.

GENOTYPE-PHENOTYPE CORRELATION

In order to attempt a genotype-phenotype correlation we rewieved the clinical and biochemical features of the patients carrying the most frequent mutations: Q422X, G60D, E375X and P141fs.

Using the same initials as those used in a recent review [22], the patients with the Q422X mutations are RP, LS, DeG, SM, DM, RM, GM. Mutation analysis was not performed in DaG and LP, but they presumably carry the Q422X mutation since they are

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Figure 1. Alpha-L-Fucosidase Gene Mutations in Italy. In brackets is indicated the number of patients in which they have been detected. The two Q422X-Q77 compound heterozygous sibs inherited the Q422X mutation from the father who is from Sicilia and the Q77X mutation from the mother who is from Campania. Thus the two mutations are shown in their respective regions of origin. The parents of the W382X-K151fs compound heterozygous patient are both from Puglia.

sibs of DeG and RP respectively. The G60D patients are MZ, GZ, RL, CB, SB, AM, SD. The E375X patients are FV, JT, BL, JC, FC, LA, JG, GG. The P141fs patients are MS, SS, AZ RZ and a new patient who was not reported previously (SI).

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Of the children with the Q422X mutation, we found in literature a description of the clinical features of patients RP, DeG DM and SM [6, 10, 20, 22].

In those children with the G60D mutation, we found patients AM, MZ, GZ, RL, CB, SB [7, 13, 20]. Of the children with the E375X mutation we found patients FV, JC, FC [18]. Children with the P141fs mutation were MS, SS, AZ and RZ [7].

According to our experience an individual clinical description of patients LS (Q422X), SD (G60D), JT, BL, LA, JG, GG (E375X), SI (P141fs) does not exist in literature, but most of them are personal communications [22].

We excluded from our analysis patients GM and RM (Q422X), since they are compound heterozygous. We did not find any particular clinical or biochemical feature peculiar of a specific mutation.

At the biochemical level all the patients have a very low alpha-L-fucosidase activity and, when examined, a severely reduced CRIM (cross reactive immunologic protein). Of the Q422X patients, RP showed a slow progression and died after 20 years, his sister (LP) had a fast neurologic decline and died at 4 years, DM, SM and DeG had an intermediate course and died after 10 years. Of the G60D patients, SB and CB showed a fast progression and died at 4 years, RL a slow progression and died at 19 years, GZ and MZ an intermediate course and were still alive after 10 years, AM had a severe decerebrate rigidity.

Those patients with the E375X mutation (FV, JC, FC) had a slow progression, although we do not know the age of death. They have ocular abnormalities, such as dilated and tortuous conjunctival and retinal vessels. However, we do not think that ocular abnormalities are peculiar of E375X patients since probably an ophtalmologic evaluation was not performed in most cases. Of the patients JG and GG (E375X), we only know that they died at 9.5 and 16 years respectively [22]. Thus, we have no knowledge of E375X patients who died before five years. Also the patients with the P141fs mutation are clinically heterogeneous since MS and SS were described as type I while AZ and RZ as type II [7, 22].

CONCLUSIONS

The number of mutations identified in alpha-L-fucosidase gene is rapidly increasing.

Up to now fourteen disease-causing mutations have been identified but several other surely exist.

At the moment four mutations (Q422X, G60D, E375X and P141fs) are present in seventy per cent of patients. Only seven mutations have been identified in Italian patients and two of them, P141fs and G60D, are present in more than 50% of Italian patients. The P141fs mutation is the most frequent mutation in Italy. It is present in all the cases studied from Calabria and is absent in all the other ethnic groups.

However, the wide clinical variability of the disease cannot be explained only by genetic heterogeneity.

Indeed, patients with very fast neurologic deterioration and death before 5 years, such as SB and CB, were found to have the same mutation as patients with slow progression and death at 19 years, such as RL.

It has been suggested that clinical variability could be due to different combina-

tions of fucosidosis mutations [2]. In this case the patients would be compound heterozygotes for different mutations. However, only three compound heterozygotes have been found. The cause of the wide clinical variability of fucosidosis is still unexplained.

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