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SHORT COMMUNICATION

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Mutation analysis of a Japanese patient with fucosidosis

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Abstract Fucosidosis is a rare autosomal recessive disorder resulting from a deficiency of α-L-fucosidase. Recently, various mutations have been reported in this disease, but it is difficult to elucidate the phenotype from the genetic mutations. We report a patient with chronic infantile type fucosidosis, with a compound heterozygote of a nonsense mutation (W148X, Trp at codon 148 to stop codon) and a large deletion, including all exons. This is the first report of a large deletion demonstrated in fucosidosis. It is interesting that this patient has a relatively mild clinical course despite the absence of the mRNA. This case also indicates the difficulty in determining the phenotype from the genotype in fucosidosis.

Key words Fucosidosis · Nonsense mutation · Large deletion · Japanese patient

Introduction

Fucosidosis, first described by Durand et al., in 1966, is an autosomal recessive lysosomal storage disease resulting from a deficiency of α-L-fucosidase (EC 3.2.1.51). Most fucosidosis patients have slow neurological deterioration, surviving to only their second or third decade (Willems et al. 1991; Thomas and Beaudet 1995), but several patients, including the original ones, had rapid progressive neurological deterioration, leading to decerebration and death before the age of 5 years. Historically, severe forms of fucosidosis were referred to as type I and less severe forms as type II. As more patients affected with fucosidosis were described, the clinical gap between type I and type II fucosidosis was gradually filled in with a continuum of patients with intermediate form. In Japan, several patients of different types have been reported (Matsuda et al. 1973; Ikeda et al.1984; Honjoh et al. 1985), but there have been no reports of mutations.

The structural gene encoding α-L-fucosidase (*FUCA1*) has been cloned (Fukushima et al. 1985; Fukushima et al. 1990; Kretz et al. 1992). *FUCA1* contains 8 exons over 23 kb of genomic *FUCA1*, and the gene is mapped on the short arm of chromosome 1 at position 1p34.1–1p36.1. To date, more than 20 mutations in the α-L-fucosidase structural gene (*FUCA1*) have been reported. It was speculated that clinical differences, such as types I and II, were genetically determined by different mutations. However, after several mutations were identified, it was found to be difficult to explain the observed phenotypic differences from the genotypic differences. From this point, it is important to clarify molecular defects in many patients. In this report, we describe a chronic, slow progressive type of fucosidosis in a patient with a novel nonsense mutation and a large deletion. This is the first patient in whom *FUCA1* gene mutation was identified in Japan.

Case report

The patient was a 15-year-old Japanese girl. She was born by normal delivery from nonconsanguineous parents and developed normally until approximately 1 year of age. She was able to walk at age 19 months. At 23 months, she had delayed speech and hearing difficulty. At 3 years and 7 months, she was referred to our hospital. She had a coarse face, small stature, and kyphoscoliosis. She did not have organomegaly and mucopolysaccharideuria. She was diagnosed with fucosidosis because of deficiency of α-L-fucosidase in leukocytes (0 nmol/h per mg protein compared

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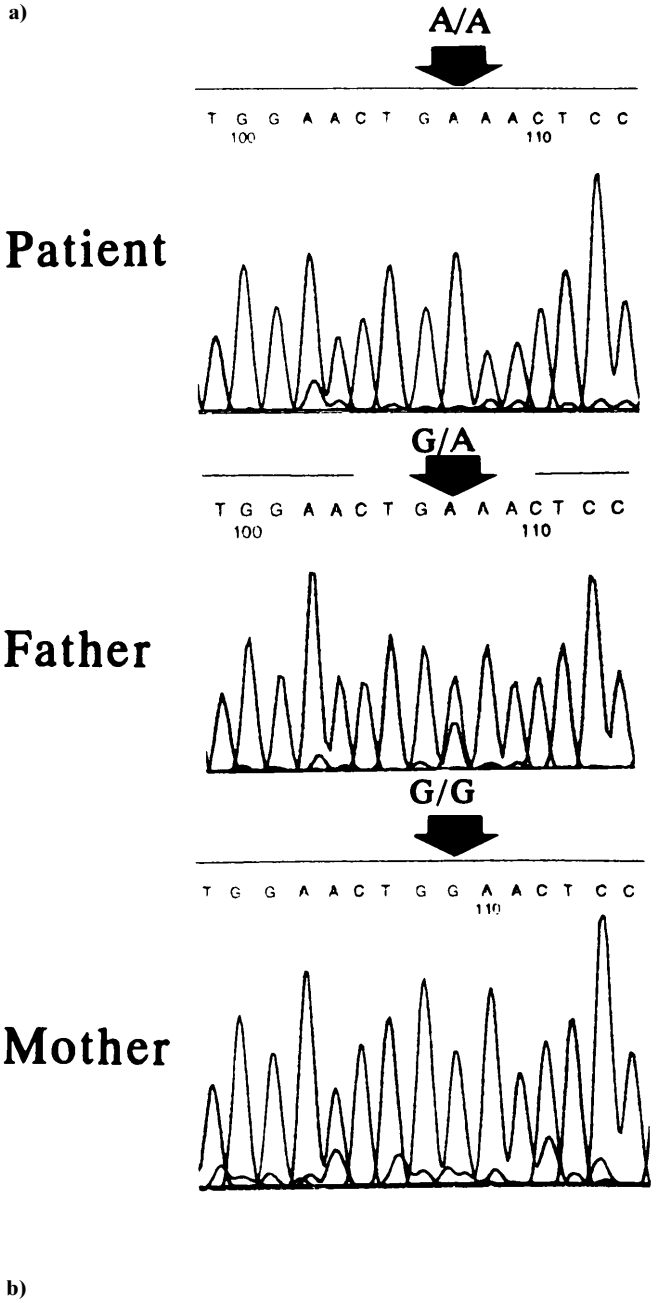

with 29.1 ± 4.7 nmol/h per mg protein in control). At 4 age years, angiokeratoma appeared on her palms. Her bone abnormalities and motor dysfunction gradually progressed, until, at age 6 years, she was unable to walk. She also developed myoclonic seizures. At age 13 years, she showed spasticity and dystonia of all extremities with involuntary movements. She developed generalized angiokeratoma corporis diffusum, but showed no hepatosplenomegaly or pubertal development.

Subjects and methods

Mutation analysis. Genomic DNA was isolated from peripheral blood leukocytes of the patient and her parents by standard methods. All eight exons of *FUCA1* were amplified from genomic DNA by PCR methods, using primer sets (Seo et al. 1993a). The amplified products were purified and directly sequenced by Dye Terminator Cycle Sequencing Ready Reaction (ABI 310 Genetic Analyzer, Perkin-Elmer, Foster City, CA, USA). To confirm the mutation identified in the patient, the PCR products of exon 2 were digested with *Bsr*I and subjected to 3% NuSieve (FMC Bio Products, Rockland, ME, USA) agarose gel electrophoresis.

Southern and Northern blot analysis. Fifteen micrograms of genomic DNA from the patient, the parents and normal individuals were digested with the restriction enzymes *EcoR*I, *Bam*HI, or *Hind*III. Southern blot analysis was performed according to standard procedures. Hybridization probes were PCR-amplified from genomic DNA. Total RNA from cultured skin fibroblasts of the patient and normal individuals were isolated by a guanidium thiocyanate-phenol-chloroform method, and 20 μg of RNA was used for Northern blot analyses. α-L-Fucosidase and β-actin cDNA were radiolabelled, and hybridized using standard methods.

Fluorescence in situ hybridization (FISH) analysis. Metaphase chromosomes of the patient and her mother were prepared. Cosmid probes containing exons 1–8 of *FUCA1* were cloned from a genomic library of normal lymphocytes and labeled with biotin-16-dUTP. A chromosome

Fig. 1a, b. Direct sequence analyses of *FUCA1* genomic DNA and restriction enzyme analyses in the exon 2 region in genomic DNA. **a** Direct sequence analysis of the exon 2 region showed homozygous amino acids of codon 148 (TGA) in the patient, but heterozygous amino acids (TGG and TGA) in her father, and homozygous amino acids of the codon (TGG) in her mother. **b** In the normal sequence the genomic amplified PCR products containing two sites of *Bsr*I were digested to 117-bp, 105-bp, and 24-bp products with Bsr I, and two bands, of 117- and 105 bp, were visible on 3% NuSieve agarose gel electrophoresis (*upper panel* and *lane 3* in *lower panel*) (FMC Bio Products, Rockland, ME, USA). When there was the transition of G to A at codon 148, one site of *Bsr*I was lost resulting in 141-bp and 105-bp products produced after *Bsr*I digestion (*upper panel* and *lane 2* in *lower panel*). In the patient, only the 141-bp and 105-bp products were detected, which showed homozygous mutations at the codon 148 (*lane 2* in *lower panel*). The mother showed a normal pattern and the father showed a heterozygous pattern (*lanes 3 and 4* in the *lower panel*, respectively). These results are compatible with the direct sequence analysis shown in **a**

1 probe (cC11-163; Ariyama et al. 1995) served as a control and was labeled with digoxigenin. The control and FUCA1 probe were detected simultaneously with avidin-fluorescein isothiocyanate (FITC) and antidigoxigenin-rhodamine (Boehringer Mannheim, Penzberg, Germany), after which the chromosomes were counterstained with DAPI (Sigma, St. Louis, MO, USA) in an antifade solution. Signals were examined on a Nikon Optiphoto fluorescent microscope (Nikon, Tokyo, Japan).

Results

Mutation analysis. DNA sequence analyses revealed that the patient had a G-to-A transition at cDNA nucleotide number 444 in exon 2, resulting in Trp to stop codon at codon 148 (TGG to TGA, designated W148X). Her father was found to be heterozygous (G/A) for the W148X mutation. However, the mother had a normal sequence (Fig. 1a). To confirm this evidence, restriction site analyses of genomic DNA were performed. The W148X mutation obliterates a *Bsr*I site. In the presence of *Bsr*I sites, the 246-bp PCR product of exon 2 was digested into 117-, 105-, and 24-bp, but fragments of 141-bp and 105-bp were obtained with the mutation. In the father, 141-, 117-, and 105-bp fragments were obtained. However, 141-bp and 105-bp fragments were obtained in the patient, and 117-bp and 105-bp fragments were obtained in the mother (Fig. 1b). These data show that one allele has the W148X mutation and the other has a deletion at that position in the patient. Therefore, her father is a carrier for the W148X mutation and her mother probably has a deletion.

Southern and Northern blot analysis. Southern blot analyses of genomic DNA of the patient and her parents, digested with *Eco*RI, *Bam*HI, or *Hind*III, showed no differences among them, suggesting a large deletion in the patient and her mother (data not shown). Northern blot analyses from lymphoid cells of a healthy individual showed a band of 2.3 kb, but no band could be detected in the patient (data not shown).

Fluorescence in situ hybridization analysis. Fluorescence in situ hybridization analyses of lymphocytes from the patient and her mother showed only one signal of *FUCA1* (Fig. 2, red color).

Discussion

We have described a patient with late infantile onset fucosidosis with slow progressive symptoms, considered to be an intermediate form between types I and II. We identified a new mutation, W148X, and a large deletion, including all exons, of the *FUCA1* gene. From DNA sequence analyses of amplified PCR products of exon 2 in the patient, homozygosity for the W148X mutation was considered. However, genomic DNA analyses of her parents at that position (nucleotide number 444 of cDNA) showed that her father was heterozygous for the mutation and that her mother had no mutation. To explain this result, a deletion in the patient's and mother's genome at that poisition was considered. However, Southern blot analyses of the patient, her parents, and the control showed the same patterns as those in the restriction enzymatic digestion studies using a FUCA1 cDNA probe. From this evidence, a large deletion, including the whole *FUCA1* gene, was suspected. To confirm this evidence, FISH analysis was performed, using a cosmid genomic clone containing the whole *FUCA1* gene. The patient and her mother showed only one signal in chromosome 1 from the FISH study. It was concluded that the patient has the nonsense mutation from a paternal allele and the large deletion from a maternal allele.

To date, 25 mutations, including our patient's mutations, have been reported; 8 types of nonsense mutations, 5 types of missense mutations, 8 types of frame-shift mutations, 2 types of insertion, and 2 types of deletions (Yang et al. 1992; Seo et al. 1993a, b; Williamson et al. 1993; Yang et al. 1993; Seo et al. 1994a, b; Yang and DiCioccio 1994; Seo et al. 1995, Seo et al. 1996, Cragg et al. 1997, Fleming et al. 1998). Most mutations are unique, yet one mutation (Q77X) was repeatedly observed (Yang et al. 1993; Seo et al. 1993b). Of the 25 known mutations, only 5 yield amino acid substitutions, whereas the other 20 are presumed to result in unstable or defective mRNA, similar to findings in our patient. This evidence is unique compared with findings in other lysosomal storage diseases. The clinical spectrum of this disease is very wide, and the coexistence of types I and II within the same family has been reported, while identical mutations have been identified in the homozygous state in patients (Willems et al. 1988). At the protein biochemical level, no heterogeneity was observed (Willems et al. 1991). Our patient had no residual activity of α-L-fucosidase, as reported in most patients (Willems et al. 1991). From the mutation and biochemical analysis, it seems that fucosidosis is homogeneous, despite the clinical variations. Most likely, modifying genes or environmental effects, which are currently unknown, are operative in fucosidosis.

References

Ariyama T, Inazawa J, Ezaki T, Nakamura Y, Hori A, Abe T (1995) High-resolution cytogenetic mapping of the short arm of chromosome 1 with newly isolated 411 cosmid markers by fluorescence *in situ* hybridization: the precise order of 18 markers on 1p36.1 on prophase chromosomes and "stretched" DNAs. Genomics 25:114–123

Cragg H, Williamson M, Young E, O'Brien J, Alhadeff J, Fang-Kircher S, Paschke E, Winchester B (1997) Fucosidosis: genetic and biochemical analysis of eight cases. J Med Genet 34:105–110

Durand P, Borrone C, Della Cella G (1966) A new mucopolysaccharide lipid-sotrage disease? Lancet II:1313–1314

Fleming CJ, Sinclair DU, White EJ, Winchester B, Whiteford ML, Connor JM (1998) A fucosidosis patient with relative longevity and a missense mutation in exon 7 of the α-fucosidase gene. J Inher Metab Dis 21:688–689

Fukushima H, de Wet JR, O'Brien JS (1985) Molecular cloning of a cDNA for human α-L-Fucosidase. Proc Natl Acad Sci USA 82:1262–1265

Fukushima H, Nishimoto J, Okada S (1990) Sequencing and expression of full-length cDNA for human α-L-Fucosidase. J Inher Metab Dis 13:761–765

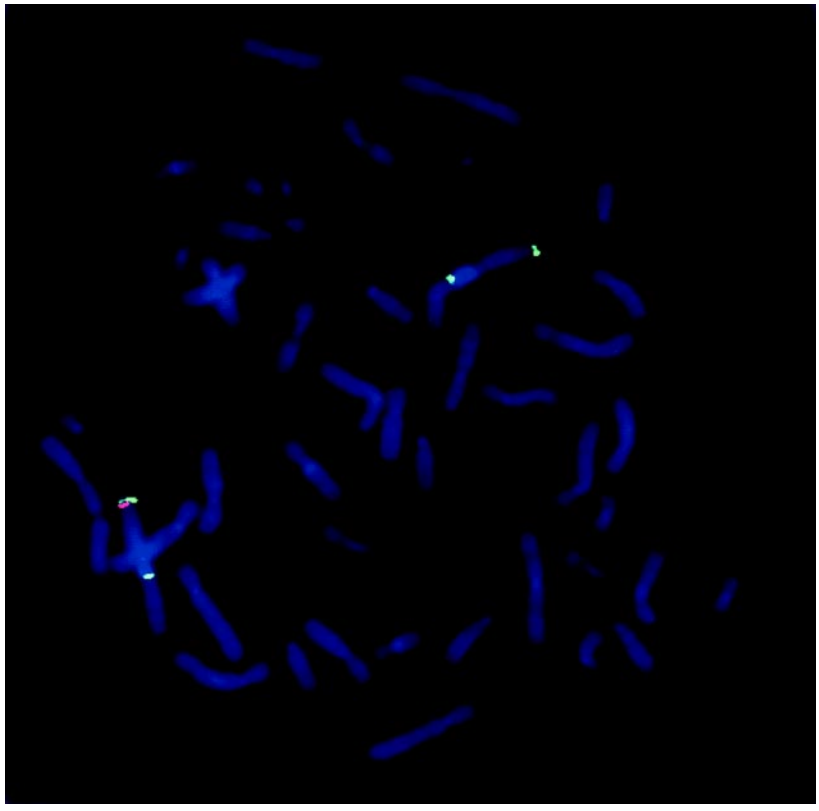

Fig. 2. Fluorescence in situ hybridization (FISH) analysis of the patient's lymphocytes. The control probe yielded FISH signals fluorescein isothiocyanate (FITC) on 1p36.1 and 1q21. Only one signal of the *FUCA1* probe (rhodamine) was detected, close to the control marker of 1p36.1

Honjoh M, Yamaguchi S, Kohda N, Mori Y, Nishimura H (1985) Fucosidosis type 3 with angiokeratoma corporis diffusum. J Dermatol 12:174–182

Ikeda S-I, Kondo K, Oguchi K, Yanagisawa N, Horigome R, Murata F (1984) Adult fucosidosis: histochemical and ultrastructural studies of rectal mucosa biopsy. Neurology 34:451–456

Kretz KA, Cripe D, Carson GS, Fukushima H, O'Brien JS (1992) Structure and sequence of the human α-L-Fucosidase gene and pseudogene. Genomics 12:276–280

Matsuda I, Arashima S, Anakura M, Ege A, Hayata I (1973) Fucosidosis. Tohoku J Exp Med 109:41–48

Seo H-C, Willems PJ, Kretz KA, Martin BM, O'Brien JS (1993a) Fucosidosis: four new mutations and a polymorphism. Hum Mol Genet 2:423–429

Seo H-C, Williems PJ, O'Brien JS (1993b) Six additional mutations in fucosidosis: three nonsense mutations and three frameshift mutations. Hum Mol Genet 2:1205–1208

Seo H-C, Kunze J, Williems PJ, Kim AH, Hanefeld, O'Brien JS (1994a) A single-base with fucosidosis deletion mutation in a Turkish patient with fucosidosis. Hum Mutation 3:407–408

Seo H-C, Yang M, Tonlorenzi R, Williems PJ, Kim AH, Filocamo M, Gatti R, DiCioccio RA, O'Brien JS (1994b) A missense mutation (S63L) in α-L-fucosidase is responsible for fucosidosis in an Italian patient. Hum Mol Genet 3:2065–2066

Seo H-C, Heidemann PH, Lutz E, O'Brien JS (1995) A nonsense mutation in two German patients with fucosidosis. Hum Mutation 6:184–185

Seo H-C, Yang M, Kim AH, O'Brien JS, DiCioccio RA, Gordon BA (1996) A 66-basepair insertion in exon 6 of the α-L-fucosidase gene of a fucosidosis patient. Hum Mutation 7:183

Thomas GH, Beaudet AL (1995) Fucosidosis. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The metabolic and molecular basis of inherited disease, 7th edn. McGraw Hill, pp 2539–2542

Willems PJ, Garcia CA, de Smedt MCH, Martin-Jimenez R, Darby JK, Duenas DA, Granado-Villar D, O'Brien JS (1988) Intrafamilial variability in fucosidosis. Clin Genet 34:7–14

Willems PJ, Gatti R, Darby JK, Romeo G, Durand P, Dumon JE, O'Brien JS (1991) Fucosidosis revisited: a review of 77 patients. Am J Med Genet 38:111–131

Williamson M, Cragg H, Grant J, Kretz K, O'Brien J, Willems PJ, Young E, Winchester B (1993) A 5′ splice site mutation in fucosidosis. J Med Genet 30:218–223

Yang M, Allen H, DiCioccio RA (1992) A mutation generating a stop codon in the gene of a fucosidosis patient. Biochem Biophys Res Commun 189:1063–1068

Yang M, Allen H, DiCioccio RA (1993) Pedigree analysis of α-L-fucosidase gene mutations in a fucosidosis family. Biochim Biophys Acta 1182:245–249

Yang M, DiCioccio RA (1994) A Gln-281 to Arg substitution in a-L-fucosidase is responble for a common polymorphism detected by isoelectric focusing. Hum Genet 93:597-599