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SHORT COMMUNICATION

Mutation identification and characterization of a Taiwanese patient with fucosidosis

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Abstract Fucosidosis is a rare lysosomal storage disease caused by a defect of the α-L-fucosidase (*FUCA1*) gene. Worldwide 26 mutations underlying the disease have been reported. By direct DNA sequencing of exons and flanking introns, homozygous Y126X mutation and Q281R polymorphism were found in a Taiwanese patient with fucosidosis. Upon expressing in COS-7 cells, 97.4% of α-L-fucosidase activity compared with that of the wild-type construct was observed in the cDNA containing Q281R polymorphism. Western blot analysis revealed a 58-kDa precursor and 56-kDa mature forms for cells transfected with wild-type and Q281R enzymes. Using the fluorogenic substrate, the Michaelis constants and maximal velocities of both enzymes were very similar. While no appreciable enzyme activity (0.0%) was observed with Y126X mutation, no apparent decrease in *FUCA1* mRNA level was seen with Y126X mutation. The expressed truncated Y126X protein was unstable and largely degraded. The delineation of the molecular defect could serve to complement future prenatal diagnosis for this family when necessary.

Keywords α-L-Fucosidase · Fucosidosis · Mutation · Polymorphism · In vitro expression

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Introduction

Fucosidosis is an autosomal recessive lysosomal storage disorder caused by a deficiency of α-L-fucosidase (EC 3.2.1.51) with accumulation of fucose-containing glycolipids and glycoproteins in various tissues (Van Hoof and Hers 1968; Durand et al. 1969; Loeb et al. 1969). The clinical symptoms mainly consist of neurodegeneration with progressive mental and motor deterioration. Additional features include angiokeratoma corporis diffusum, dysostosis multiplex, visceromegaly, ocular abnormalities, hearing loss, seizures, coarse features, recurrent infections, spasticity, contractures, growth retardation, muscle wasting, and dystrophy (Willems et al. 1991; Kanitakis et al. 2005). Although a distinction between the severe type I and less severe type II forms has been described, the wide continuous clinical spectrum of the disease may account for the clinical heterogeneity (Willems et al. 1991).

The human α-L-fucosidase gene (*FUCA1*) spans over 23 kb, contains 8 exons, and has been mapped to chromosome 1p34.1–1p36.1 (Fukushima et al. 1985, 1990; Occhiodoro et al. 1989; Kretz et al. 1992). The 2,053 bp full-length cDNA encodes for a signal peptide of 22 amino acids and a mature protein of 439 amino acids. Worldwide a total of 26 different mutations underlying fucosidosis have been identified (the Human Gene Mutation Database, http://www.hgmd.cf.ac.uk/ac/gene.php?gene=FUCA1). The spectrum of the mutations reported includes 5 missense mutations and 21 null mutations consisting of 9 stop codon mutations, 6 small deletions, 3 large deletions, 1 duplication, 1 small insertion, and 1 splice site mutation. Most mutations led to nearly absent enzymatic activity and severely reduced cross-reacting immunomaterial (Fleming et al. 1998; Akagi et al. 1999; Willems et al. 1999; Ip et al. 2002). Therefore, the observed clinical variability was considered to be due to secondary unknown factors.

In this study, we investigate the molecular lesion of a Taiwanese fucosidosis patient. We used transient expression studies to examine the effects of the identified mutation and polymorphism on α-L-fucosidase catalytic activity, mRNA level, and protein stability.

Materials and methods

Patient

The patient is a 23-year-old Chinese young woman, born to non-consanguineous parents. She has severe mental retardation, progressive loss of psychosocial functions, coarse facial features, short stature, dysostosis multiplex with joint contractures, mild hypertrophic cardiomyopathy, mild aortic stenosis, and diffuse erythematous papules. The clinical presentation started in early childhood with slow progression. A biopsy was taken from a skin lesion. The microscopic examination showed characteristic findings of angiokeratoma composed of dilated, thin-walled, congested capillaries in the papillary dermis. The overlying epidermis showed hyperplastic change. α-Fucosidase activity was conspicuously decreased in peripheral blood leukocytes (0.5 nmol/h/mg protein; normal range 50–200 nmol/h/mg protein).

DNA analysis

Genomic DNA was extracted from whole blood according to standard procedures. Polymerase chain reaction (PCR) of *FUCA1* exons including adjacent intronic regions was done with primers and conditions listed in Supplementary Table 1. PCR products were purified and sequenced directly using the MegaBACE Analyzer (Amersham Biosciences AB, Uppsala, Sweden). The identified Y126X and Q281R were analyzed for population (200 controls) and family studies using *Mse*I and *Sec*I (New England Biolabs, Beverly, MA) restriction analysis, respectively. The *Mse*I site was generated by PCR using mismatch forward primer (5′-GTGGTTTTGCTGTCCACAGTTA-3′) and exon 2 reverse primer.

cDNA constructs

Polyadenylated RNA was isolated from human cultured fibroblasts and reverse transcription-PCR amplification carried out as previously described (Chang et al. 2005). Sense and antisense primers used for amplification of *FUCA1* cDNA are shown in Supplementary Table 1. The amplified full-length 1.4-kb *FUCA1* cDNA were cloned into pGEM-T Easy vector (Promega, Madison, WI) and sequenced. The cDNA was then excised with *Eco*RI and subcloned into pcDNA3 (Invitrogen, Carlsbad, CA) to produce plasmid pcDNA3-FUCA1. The Y126X mutation and Q281R polymorphism in the *FUCA1* cDNA were generated by QuickChange™ XL Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA) (sequences of primers are available on request). The mutated sequence constructs were confirmed by DNA sequencing.

Expression studies

COS-7 cells cultivated in DMEM containing 10% FCS were transfected with the *FUCA1* cDNA constructs by lipofection procedure (Invitrogen). Forty-eight hours after transfection, cells were harvested and extracts prepared by freeze–thawing. The assay for α-L-fucosidase activity was carried out using 0.1 mM 4-methylumbelliferyl fucopyranoside as a substrate (Sigma, St. Louis, MO) for 5 min at 37°C in citrate/phosphate buffer, pH 5.5 (Turner et al. 1975). Kinetic data (K_m and V_{max}) were obtained from Lineweaver–Burk plots with the substrate assayed in a concentration range 0.02–0.1 mM. The total RNA (5 μg) from transfected cells was reversed-transcribed for amplification of the 301-bp *FUCA1* cDNA fragment (primers 5′-AAAAATGGCTTCAAAACACAGCA-3′ and 5′-TGG TGCACATCTCCCACTTGT-3′). A 450-bp neomycin fragment was co-amplified (primers 5′-TTGGGTGGA GAGGCTATTCG-3′ and 5′-CCCTGATGCTCTTCGTCC AG-3′) as internal control for transfection efficiency. Total proteins (10 μg) from transfected cells were separated by SDS/PAGE and blotted on to nitrocellulose filters by transverse electrophoresis. After blocking, the membrane was stained with polyclonal antiserum raised again human placenta enzyme (1:1,000 dilution, Cordero et al. 2001). The immune complexes were detected with alkaline phosphatase–conjugated goat anti-rabbit IgG antibody

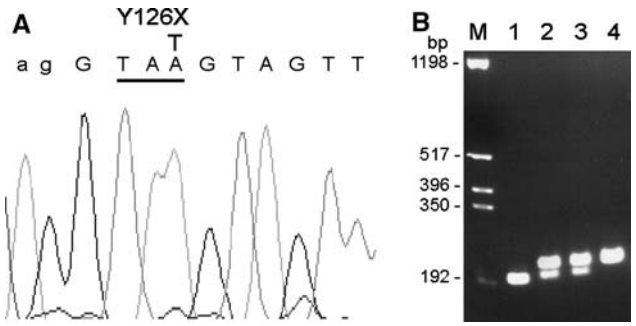

Fig. 1 Mutation identification. **a** DNA sequence analysis of the mutation Y126X. Sequences located in exon 2 are indicated by *uppercase letters* and in intron 1 by *lowercase letters*. **b** Restriction enzyme analysis of the mutant allele. The mismatch primer-amplified exon 2 products from the patient (*lane 1*), the father (*lane 2*), the mother (*lane 3*), and a normal control (*lane 4*) were digested with *Mse*I and resolved on a 2% agarose gel. *Lane M* (*Hin*fI digest of pGEM4 DNA) is the size marker

(1:3,000 dilution, Boehringer Mannheim, Mannheim, Germany).

Results

Mutation and polymorphism analysis

All *FUCA1* exons from the patient were amplified for sequence analysis. In addition to a well-described Q281R (c.842A>G) polymorphism (Seo et al. 1993; Yang et al. 1993; Cragg et al. 1994; Yang and DiCioccio 1994), a recurrent homozygous Y126X (c.378T > A) mutation (Ip et al. 2002) was detected (Fig. 1a). Family analysis verified that the Y126X mutation was inherited from both parents (Fig. 1b). The Y126X mutation was not found on screening 400 control chromosomes. For Q281R change, the R allele frequency was 0.23 and heterozygosity 0.32.

Expression of FUCA1 cDNA variants

The effect of the identified Y126X and Q281R changes was investigated by transient expression in COS-7 cells. As shown in Table 1, no appreciable enzyme activity (0.0%) was observed with Y126X mutation. However, 97.4% of α-L-fucosidase activity compared with that of the wild-type construct was observed in the cDNA containing Q281R polymorphism. Apparent K_m and V_{max} were determined on the wild type and Q281R enzymes and were found to be 0.136 mM and 344.2 nmol/min/mg cell protein and 0.134 mM and 337.3 nmol/min/mg cell protein, respectively. The level of *FUCA1* mRNA expressed was examined by RT-PCR. By gel semi-quantitation, neither Q281R nor Y126X caused an apparent change in mRNA level when compared to that of wild type (Fig. 2a; lanes 2–4).

Table 1 Expression of *FUCA1* in transfected COS-7 cells

Transfected vector	α-L-fucosidase activity (nmole/min/mg protein)	Total (% of wild type)
pcDNA3	5.5	0.0
pcDNA3-FUCA1	120.6	100.0
pcDNA3-FUCA1/Q281R	117.6	97.4
pcDNA3-FUCA1/Y126X	5.5	0.0

The α-L-fucosidase activities in COS-7 cells are the mean of three independent experiments, with a range less than 5%. The endogenous α-L-fucosidase activity in COS-7 cells was subtracted before the percentage of wild type was calculated

The presence of *FUCA1* proteins in transfected COS-7 cell extracts was determined by immunoblot analysis. As shown in Fig. 2b, cells transfected with the parental pcDNA3 vector did not produce cross-reacting protein (lane 1). Cells expressing the wild-type (lane 2) and Q281R (lane 3) cDNA constructs exhibited a 58-kDa precursor and 56-kDa mature forms of the enzyme. Conversely, no *FUCA1* protein was seen with Y126X (lane 4).

Discussion

We have described a patient with late infantile onset fucosidosis with slow progressive symptoms, considered to be type I. We identified a recurrent Y126X mutation and a well-described Q281R polymorphism of the *FUCA1* gene. Upon expressing in COS-7 cells, both wild-type and Q281R enzymes resulted in a 20-fold increase of α-L-fucosidase activity (Table 1). That Q281R does not affect α-L-fucosidase activity may be explained by its location in the disordered region, thus not interfering with proper folding or packing of the enzyme (Sulzenbacher et al. 2004). The Y126X predicts a lack of 314 amino acids at the carboxyl terminus of the protein. No appreciable α-L-fucosidase activity was detected in transfected COS-7 cells (Table 1). This is in agreement with nearly absent α-L-fucosidase activity for other *FUCA1* mutations reported (Fleming et al. 1998; Akagi et al. 1999; Willems et al. 1999; Ip et al. 2002). Neither splicing (http://www.es.embnet.org/~mwang/assp.html) nor the mRNA level (Fig. 2a) was affected by Y126X. Since no *FUCA1* protein was seen on the immunoblot (Fig. 2b), the largely truncated protein was apparently unstable and degraded.

The patient was born to healthy, non-consanguineous parents originated from southern China. The same mutation was reported in a Chinese boy of consanguineous parents also from southern China (Ip et al. 2002). As no mutational hot spots were reported and both the Chinese boy and the patient's parents were natives of China, it is therefore

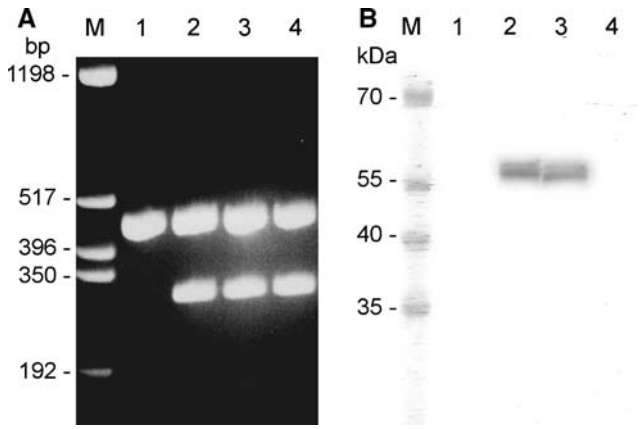

Fig. 2 RT-PCR and Western analyses of *FUCA1* cDNA variants. **a** The amplified *FUCA1* (301 bp) and neomycin (450 bp) fragments were fractionated on 1.8% agarose gels. **b** Total protein was separated on 12% SDS-polyacrylamide gel, transferred onto nitrocellulose membrane, and stained with *FUCA1* polyclonal antibody. Weights of the molecular mass markers in *lanes M* (in bp for *Hin*fI digest of pGEM4 DNA and in kDa for standard proteins from Fermentas) are shown on the left. Lanes *1* pcDNA3; *2* pcDNA3-FUCA1; *3* pcDNA3-FUCA1/Q281R; *4* pcDNA3-FUCA1/Y126X

probable that the two Y126X mutations arose in a common ancestor of Chinese origin many years ago.

In conclusion, we identified and characterized the Y126X and Q281R in a Taiwanese fucosidosis patient. While expression studies are useful in determining whether a variation is deleterious or benign, it would be more informative to investigate cultured fibroblasts to determine the level of mRNA and protein and their kinetics with respect to α-L-fucosidase substrate.

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