



## Novel mutations in the *FUCA1* gene that cause fucosidosis

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**ABSTRACT.** Fucosidosis is a rare lysosomal storage disorder inherited in an autosomal recessive manner. Its estimated frequency is below 1 in 200,000 live births. Its clinical phenotypes include progressive neurological and mental deterioration, coarse facial features, growth retardation, visceromegaly, angiokeratomas, and seizures. The disease is caused by mutations in the *FUCA1* gene that lead to deficiency of  $\alpha$ -L-fucosidase. Here, we describe the clinical and molecular features of a Thai boy with fucosidosis. Whole exome sequencing and array-based

comparative genomic hybridization analysis revealed that the patient was compound heterozygous for a single base-pair deletion (c.670delC; p.P224LfsX2) inherited from his father, and a 3281-base-pair deletion covering exon 3 inherited from his mother. Neither mutation has been reported before so the *FUCA1* mutational spectrum is herein expanded.

**Key words:** Fucosidosis; *FUCA1*; Novel mutations; Deletion

## INTRODUCTION

Fucosidosis (MIM No. 230000) is an autosomal recessive lysosomal storage disorder that was first described in 1966 by Durand et al. (1966). The disease is rare; only approximately 100 cases have been reported worldwide (Muthusamy et al., 2014). Its occurrence is higher in Southern Italy, the Hispanic populations of New Mexico and Colorado, and Cuba. The occurrence of fucosidosis in Cuba is about 0.63 per 100,000 live births (Menéndez-Sainz et al., 2012). The disease is caused by a deficiency of  $\alpha$ -L-fucosidase (EC 3.2.1.51) activity leading to the accumulation of fucose-containing glycolipids and glycoproteins in multiple tissues (Van Hoof and Hers, 1968; Durand et al., 1969). Its clinical phenotypes include progressive neurological deterioration, coarse facial features, hearing loss, growth retardation, visceromegaly, dysostosis multiplex, angiokeratoma corporis diffusum, recurrent infections, and seizures (Willems et al., 1991).

$\alpha$ -L-fucosidase is an enzyme that is encoded by the *FUCA1* gene; it is involved in one of the early steps of glycoprotein and glycolipid breakdown in lysosomes. So far, 30 mutations in the *FUCA1* gene associated with the disease have been reported. The spectrum of mutations includes 17 missense/nonsense mutations, six small deletions, three large deletions, two splice site mutations, one small insertion, and one duplication. The mutations are distributed along the coding region of *FUCA1*. They are located in the glycoside hydrolase domain (catalytic domain, amino acid residues 35-370) and the C-terminal domain (amino acid residues 372-463) of  $\alpha$ -L-fucosidase, and result in nearly absent enzymatic activity (Willems et al., 1999) (Human Gene Mutation Database, <http://www.hgmd.cf.ac.uk>, accessed on June, 2016).

In this study, we identified two novel mutations in the *FUCA1* gene including a single base deletion, c.670delC (p.P224LfsX2), and a whole exon 3 deletion in a Thai case affected with fucosidosis.

## MATERIAL AND METHODS

The study was approved by the institutional review board of the Faculty of Medicine, Chulalongkorn University. Written informed consent was obtained from the parents.

### Mutation analysis

Genomic DNA was extracted from peripheral blood leukocytes obtained from the patient and his parents using a QIAamp<sup>®</sup> DNA blood mini kit according to the manufacturer instructions (Qiagen, Valencia, CA, USA), and sent to Macrogen Inc. (Seoul, Korea) for whole exome sequencing (WES). The sample was captured and enriched using an Agilent SureSelect Human All Exon V4 Capture kit (Agilent Technologies, Santa Clara, CA, USA). The enriched

DNA library was subsequently sequenced using a pair-end 100-bp configuration on a HiSeq 2000 platform (Illumina, San Diego, CA, USA). Sequence reads were aligned to the reference genome UCSC hg19 (<http://genome.ucsc.edu/>) using the BWA software (<http://bio-bwa.sourceforge.net/>). The single nucleotide polymorphisms (SNPs) and indels were detected using SAMTOOLS software (<http://samtools.sourceforge.net/>). The Single Nucleotide Polymorphism Database (dbSNP) and 1000G were used as variant databases.

To identify a causative variant, we focused on variants located in the *FUCAI* gene. The variants found in the dbSNP, the ExAC database, and our in-house exome database were excluded.

To confirm the identified mutation, we performed polymerase chain reaction (PCR)-Sanger sequencing of the entire exon 4 and flanking sequences using the forward primer F2 (5'-GGAGGCTTAGGCAAGAGGAT-3') and the reverse primer R1 (5'-AAGGGAGCCAGGGAAGATTA-3'). The PCR was performed in a total reaction volume of 20  $\mu$ L. The mixture consisted of 0.2  $\mu$ M dNTPs, 1X Taq buffer with  $(\text{NH}_4)_2\text{SO}_4$ , 0.75 mM  $\text{MgCl}_2$ , 0.5 U Taq DNA polymerase (Thermo Fisher Scientific Inc., USA), 0.2  $\mu$ M each primer, and 50 ng genomic DNA. PCR amplification was performed using the touchdown PCR program described as follows: initial denaturation at 94°C for 5 min, followed by 19 cycles of denaturation at 94°C for 45 s, annealing at 63°C for 45 s (during which time the temperature was reduced by 0.5°C every cycle until the calculated  $T_m$  range was reached), extension at 72°C for 45 s, followed by 14 cycles of denaturation at 94°C for 45 s, annealing at 53°C for 45 s, extension at 72°C for 45 s, and a final extension at 72°C for 2 min. The PCR products were then treated with ExoSAP-IT (USP Corporation, Cleveland, OH, USA) at 37°C for 20 min followed by inactivation at 80°C for 15 min, then sent to Macrogen Inc. (Seoul, Korea) for Sanger sequencing.

### Array comparative genomic hybridization (aCGH) analysis

Genomic DNA of the proband was sent to Macrogen Inc. (Seoul, Korea) for aCGH analysis on chromosome 1 using a custom SurePrint G3 Human CGH Microarray, 1 x 1 M (Agilent®), according to the manufacturer instructions. Briefly, 1000 ng genomic DNA was digested with *AluI* and *RsaI*, and then random primers were added. Test and reference DNA samples were labeled with Cy5-dUTP and Cy3-dUTP, respectively. The labeled test and reference DNA samples were paired and co-hybridized to the SurePrint G3 Human CGH Microarrays, 1 x 1 M (Agilent®). The hybridized array was immediately scanned using an Agilent Microarray Scanner (Agilent Technologies, Inc.). CGH data were then extracted from the scanned images (TIFF files). Genomic positions refer to the Human Genome February 2009 assembly (GRCh37/hg19).

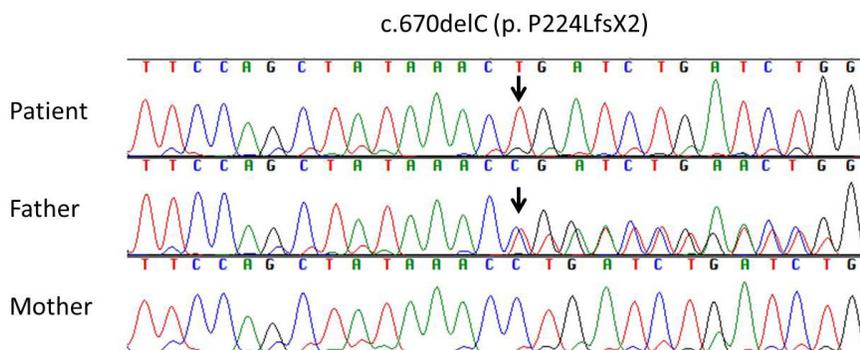
To identify the precise deletion breakpoints, duplex PCR-amplification was performed using the forward primer F1 (5'-GCGCCCAGCAGGACTATAC-3') and the reverse primer R1 (5'-AAGGGAGCCAGGGAAGATTA-3'). The PCR was performed in a total volume of 20  $\mu$ L. The mixture consisted of 0.2  $\mu$ M dNTPs, 1X Immo™ Buffer, 0.75 mM  $\text{MgCl}_2$ , 0.5 units IMMOLASE™ DNA Polymerase (Bioline USA Inc.), 0.2  $\mu$ M each primer, and 50 ng genomic DNA. PCR amplification was performed using the Touchdown PCR program with initial denaturation at 94°C for 10 min, followed by 19 cycles of denaturation at 94°C for 45 s, annealing at 63°C for 30 s (during which time the temperature was reduced by 0.5°C every cycle until the calculated  $T_m$  range was reached), extension at 72°C for 40 s, followed by 14 cycles of denaturation at 94°C for 45 s, annealing at 53°C for 30 s, extension at 72°C for 40 s,

and a final extension at 72°C for 2 min. The PCR products were then treated with ExoSAP-IT (USP Corporation) at 37°C for 20 min followed by inactivation at 80°C for 15 min, and were sent to Macrogen Inc. (Seoul, Korea) for Sanger sequencing.

## RESULTS

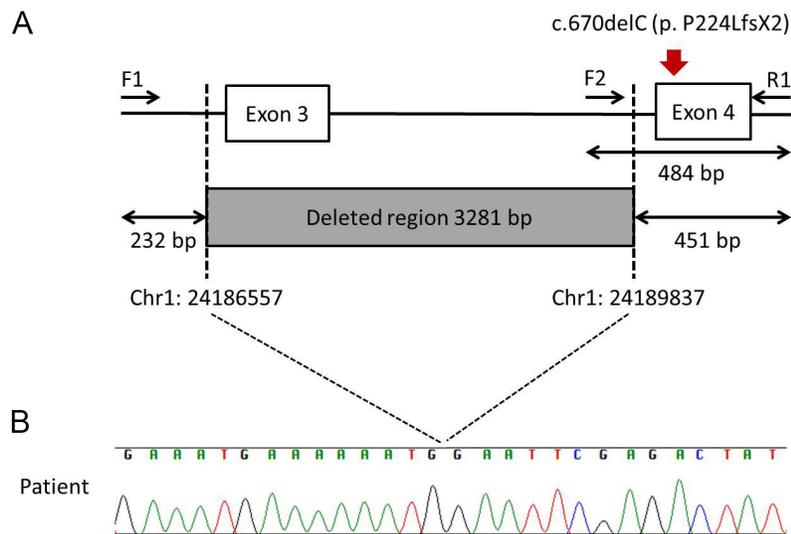
The patient is a Thai boy who was born to non-consanguineous parents with an uneventful perinatal history. He was the first child of the family. His milestones were reportedly attained within normal limits until two years of age. He started to toe walk with an unsteady gait. He knew approximately 10 one-syllable words and followed simple commands. At age 3 years and 6 months, he presented to our hospital with psychomotor regression. He had lost his ability to communicate verbally. Physical examination revealed no distinct dysmorphism. He had spasticity in which the lower extremities were more affected than the upper. Brisk tendon reflexes were also observed. An ophthalmologic evaluation and auditory brainstem response were normal. Magnetic resonance imaging (MRI) of the brain showed symmetrical hypersignal intensity of subcortical white matter involving the periventricular area and U fibers and hypointensity of the bilateral globi pallidi with curvilinear hypersignaling within both lentiform nuclei on T2WI. His growth parameters and development continued to deteriorate. He was lost to follow-up until he presented again at 7 years old. He was bed-ridden and suffered multiple episodes of aspiration pneumonia along with seizures. Coarse facial features with macroglossia became obvious with no corneal opacities or hepatosplenomegaly. Oar-like ribs, bullet-shaped vertebrae, and widening of both clavicular heads were noted on chest radiographs. Punctate non-blanchable erythematous papules pathologically confirmed to be angiokeratomas were present at age 9. Follow-up MRI of the brain showed increasing degrees of cerebral atrophy with significant signal changes in the thalamus.

Exome sequencing identified a novel single base-pair deletion in exon 4, c.670delC (p.P224LfsX2). We further confirmed its presence by PCR-Sanger sequencing of the genomic DNA of the patient and his parents using the forward primer F2 located at the 3' end of intron 3 and the reverse primer R1 located at the 5' end of intron 4. We found that the patient harbored only the mutant allele, inherited from his father. His mother unexpectedly showed only the wild-type allele (Figure 1).



**Figure 1.** Electropherograms of the patient and both parents. The c.670delC (p.P224LfsX2) mutation is indicated by the arrows. It was present in the patient and his father but absent in his mother.

This finding prompted us to hypothesize that the maternal allele was a large deletion that interfered with the PCR amplification using primers F2 and R1. Custom array CGH analysis of chromosome 1 revealed a gross deletion in the *FUCA1* gene. The precise deletion breakpoints were examined by direct sequencing. The deletion size of 3281 base pairs was identified, which encompassed the 3' portion of intron 2, all of exon 3, and most of intron 3 (Figure 2). Duplex PCR-amplification confirmed that the deletion was inherited from the patient's mother.



**Figure 2.** A. Schematic representation of the two novel mutations, c.670delC (p.P224LfsX2) and the 3.3-kb deletion. The deletion encompasses the 3' end of intron 2, all of exon 3, and most of intron 3 of the *FUCA1* gene. The location of the primers and the expected size are shown. B. Electropherogram of the patient showing the precise breakpoints of the 3.3-kb deletion.

## DISCUSSION

We described the first Thai patient with fucosidosis from a non-consanguineous family. Using WES together with aCGH, two different novel pathogenic mutations were identified in the *FUCA1* gene. The patient was compound heterozygous for a single base-pair deletion in exon 4, c.670delC (p.P224LfsX2) and a complete deletion of exon 3.

The deletion of a cytosine at cDNA nucleotide position 670 in exon 4 (c.670delC) was predicted to cause a frameshift at codon 224 leading to the generation of a termination codon at two base pairs downstream (p.P224LfsX2). The premature stop codon resulted in a truncated protein of 226-461 amino acids long. Only six small deletions have been reported worldwide (Seo et al., 1993a,b, 1994; Cragg et al., 1997).

The newly identified 3.3-kb deletion (g.24186557\_24189838del) encompassing the entire exon 3 of the *FUCA1* gene was also found in this patient. There have been three reports describing large deletions including a deletion of exon 4, a deletion of exons 7 and 8, and a whole gene deletion (Willems et al., 1991; Akagi et al., 1999; Willems et al., 1999). The patient with compound heterozygosity for a whole gene deletion and a nonsense mutation

(p.W148X) in the *FUCAI* gene was reported to have late infantile onset fucosidosis with slow progressive symptoms (Akagi et al., 1999). Because a limited number of patients with molecularly confirmed fucosidosis have been reported, a genotype-phenotype correlation cannot be determined (Willems et al., 1991; Akagi et al., 1999; Willems et al., 1999). Further studies of more patients with fucosidosis are needed to establish the correlation.

In conclusion, we reported the first Thai family with fucosidosis and successfully identified two pathogenic mutations in the *FUCAI* gene. The c.670delC (p.P224LfsX2) and the whole exon 3 deletion have not been previously described, so the *FUCAI* mutation spectrum has been expanded.

### Conflicts of interest

The authors declare no conflict of interest.

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