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An *Alu* insertion in compound heterozygosity with a microduplication in *GNPTAB* gene underlies Mucolipidosis II

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Abstract

Mucolipidosis type II (ML II) is a fatal, autosomal recessive, lysosomal storage disorder characterized by severe clinical and radiologic features. ML II results from mutations in alpha and beta subunits, encoded by the GlcNAc-1-phosphotransferase gene (*GNPTAB*). Most of the 40 different *GNPTAB* mutations reported so far are insertions and deletions predicting diverse types of aberrant proteins. *Alu* mobile elements have however never been involved in these events up to now. The Italian ML II patient of our study showed an *Alu* retrotrasposition in *GNPTAB* exon 5. The *Alu* insertion mutation (NM_024312.3:c.555_556insHSU14569) generated a transcript with a skipping of the target exon 5 and a frameshift p.S122fs, causing a premature translation termination codon at position 123. This insertion mutation was found in compound heterozygosity with the frameshift p.S887KfsX33, resulting from a new mono-nucleotide duplication (c.2659dupA) that occurred in *GNPTAB* exon 13. A possible involvement of *cis*-splicing elements having an exonic location, such as exon enhancers (ESEs), is discussed as mechanism that led to the production of the aberrant mRNA splicing.

Keywords: Mucolipidosis type II; I-cell disease; GlcNAc-1-phosphotransferase; *GNPTAB*; Exon skipping; Exonic *Alu* insertion; Microduplication; Exon enhancers

Introduction

Mucolipidosis type II (ML II; MIM: 252500) is a fatal, autosomal recessive, lysosomal storage disorder characterized by severe clinical and radiologic features. Congenital dislocation of the hip, thoracic deformities, hernia and hyperplastic gums are evident soon after birth. ML II results from defects in the multimeric GlcNAc-1-phosphotransferase (EC 2.7.8.17). GlcNAc-1-phosphotransferase is responsible for the initial step in the generation of the mannose 6-phosphate marker (M6P) required to bind to the M6P receptors which mediate the intracellular targeting of newly synthesized lysosomal hydrolases to the endosomal/prelysosomal compartment. Failure of acquisition of this marker, therefore, leads to mistargeting of lysosomal enzymes requiring the M6P markers to enter the lysosome. Consequently, they are nearly completely absent in many cell types. The mis-sorting of multiple lysosomal hydrolases results in accumulation of the respective substrates which appear as phase-dense intracytoplasmic inclusion bodies that are the hallmarks of Mucolipidosis, also called "inclusion (I)-cell disease" [1].

GlcNAc-1-phosphotransferase is a heterohexamer composed of two alpha, two beta and two gamma subunits [2]. Mutations in alpha and beta subunits, encoded by the *GNPTAB* gene (GenBank-EMBL No. NM_024312.3) cause Mucolipidosis type II [3]. Mutations in gamma subunit, encoded by the *GNPTG* gene (GenBank-EMBL No. NM_032520.3), are responsible for Mucolipidosis type III, another clinically distinct disorder [4].

GNPTAB gene contains 21 exons and spans a genomic region of approximately 80 kb on chromosome 12q23.2. The cDNA encodes a protein of 1256 amino acids (GenBank-EMBL No. NP_077288.2) [5].

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Forty different *GNPTAB* mutations have been reported to date [3,6–12]. Although the majority are insertions and deletions that predict diverse types of aberrant proteins, *Alu* mobile elements have so far never been involved in these events.

Alu elements are the predominant type of short interspersed elements (SINEs) in the human genome with over one million copies comprising ~10% of the total genome. Based on a hierarchical series of sequence mutations, *Alu* elements are classified into three major families designated as J, S, and Y; each of these families is further divided into one or more levels of subfamilies [13]. The amplification of *Alu* elements is thought to occur by retrotransposition, a process involving the insertion of reverse transcribed DNAs of *Alu*-derived transcripts back into the genome. De novo *Alu* insertions into the coding region of a gene can cause genetic diseases by disrupting the open reading frame which generates nonsense or frameshift mutations as well as affecting the splicing of a gene [14–23].

The Italian ML II patient of our study showed an *Alu* retrotrasposition event in *GNPTAB* exon 5. The *Alu* insertion mutation (NM_024312.3:c.555_556insHSU14569) generated a transcript with a skipping of the target exon 5 and a frameshift p.S122fs, causing a premature translation termination codon at position 123. This insertion mutation was found in compound heterozygosity with the frameshift p.S887KfsX33, resulting from a new mono-nucleotide duplication (c.2659dupA) that occurred in *GNPTAB* exon 13.

A possible mobile-element-involving mechanism that led to the production of the aberrant mRNA splicing is also discussed.

Materials and methods

Patient

The male patient was born to non-consanguineous parents of Italian origin. The diagnosis of ML II was suspected early at birth when the clinical features of the patient included thoracic deformities, coarse face, thick skin over trunk and extremities, asymmetrical skull (parieto-occipital platycephalus in the right region), lumbar gibbus, puffy eyelids, thick earlobes anteverted nostrils, low nasal bridge and hyperplastic gums.

Lysosomal enzyme activities were significantly increased in the serum of the patient. A skin biopsy was made and a fibroblast culture was established according to standard procedures. Aliquots of the patient's cell line were also cryopreserved in the "Cell Line and DNA Biobank from Patients affected by Genetic Diseases" (http://www.gaslini.org/labdppm.htm), at our laboratory. The enzymatic fluorimetric assays performed on cellular extracts showed low intracellular levels of the following lysosomal enzymes: β-galattosidase, α-fucosidase, β-esosaminidase total, and α-mannosidase.

Molecular analysis

DNA was extracted from cultured fibroblasts of the patient using standard protocols. Total RNA was extracted from cultured fibroblasts using RNeasy mini Kit (Qiagen) and reverse transcribed using Advantage RT-for-PCR Kit (BD Biosciences Clontech). Set of PCR primers and temperature profiles to produce the appropriate cDNA and DNA amplimers with the two mutations, have been reported in Table 1. PCR products were cloned into the TOPO TA Cloning KIT (with pCR2.1-TOPO vector) (Invitrogen, San Diego, CA). Sequence analysis was performed by ABI 377 DNA automated sequencer with dye terminator cycle sequencing kit (Applied Biosystems). Putative mutations were confirmed by sequencing in both directions of the duplicate PCR/RT-PCR products. To confirm by PCR-RFLP analysis, at DNA level, the mutation c.2659dupA, which neither created nor destroyed a restriction site, the amplification was carried out by PCR-mediated site direct mutagenesis introducing the new DdeI cleavage site of restriction [24]. The hypothesis of new possible genetic polymorphisms was also excluded ascertaining that none of the 50 control individuals had these alterations.

Nucleotide numbers of mutations are derived from cDNA *GNPTAB* sequences (GenBank-EMBL Accession No. NM_024312) considering nucleotide +1 the A of the first ATG translation initiation codon.

The two mutations are described according to the recommended nomenclature at http://www.hgvs.org/mutnomen/ [25].

Results

The two mutant *GNPTAB* transcripts were identified by RT-PCR analysis on RNA and both mutations, an *Alu* insertion (NM_024312.3:c.555_556insHSU14569) and a microduplication (c.2659dupA) were confirmed on DNA. Total RNA and DNA were extracted from a cryopreserved cell line of the ML II patient. The primers used to obtain specific cDNA and DNA amplimers are listed in Table 1.

Alu insertion c.555_556

RT-PCR analysis, encompassing exons 2–7, revealed the presence of an abnormally smaller product (359 bp) besides the expected 565 bp, as shown in Fig. 1a. Both products were cloned and sequenced. The shorter abnormal product

Table 1
List of primers used for PCR-amplification of *GNPTAB* gene

Mutation	Exons	Forward (5′ > 3′)	Reverse (5′ > 3′)	Annealing *T* (°C)	Product size (bp)
cDNA amplification					
Alu insertion	2–7	ggagccgagatcaataccat	ccactcaggaaagccaaatc	60	565
Microduplication	13–15	gaggtgaaaattcccctggt	ggcagttcgtgaattctggt	58	1048
	Exons (ex)/introns (int)				
DNA amplification					
Alu insertion	4 int–5 ex–5 int[a]	gtagtatgacaagcttctcatt	gaatcatttctattccactcag	60	364
Microduplication	13 ex–14 int	agctgcagcattacacagct[b]	gaagtgctgaataaatggtagc	58	170

GenBank Accession Nos. NM_024312, NC_000012.

[a] 74 bp of intron 4 (upstream) and 84 bp of intron 5 (downstream) flanking exon 5.

[b] Primer containing a mismatch for PCR-mediated site direct mutagenesis.