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Review

Two homozygous nonsense mutations of *GNPTAB* gene in two Chinese families with mucolipidosis II alpha/beta using targeted next-generation sequencing

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ABSTRACT

Mucolipidosis II alpha/beta (ML II alpha/beta; I-cell disease) is a rare, inherited, metabolic disease and has often been clinically misdiagnosed. ML II alpha/beta results from a deficiency of the enzyme N-acetylglucosamine-1-phosphotransferase (GlcNAc-PT), which causes the lysosomal enzymes to accumulate in plasma. We identified two new Chinese patients with ML II alpha/beta by lysosomal enzyme assay. Using targeted next-generation sequencing genetic analysis, we located two homozygous nonsense mutations in the *GNPTAB* gene, c.1071G>A (p.W357X) and c.1090C>T (p.R364X). These results were confirmed by Sanger sequencing. To our knowledge, the c.1071G>A mutation has not been previously reported. Our findings add to the number of reported cases of this rare illness and to the *GNPTAB* pathogenic mutation database. This work also demonstrates the application of lysosomal enzyme assay and targeted next-generation sequencing for the genetic screening analysis and diagnosis of ML II alpha/beta.

Contents

1. Introduction

Mucolipidosis II alpha/beta (ML II alpha/beta; I-cell disease; OMIM #252500) is a rare, autosomal recessive disorder caused by impaired trafficking of lysosomal hydrolases to lysosomes [1,2]. Mutations of the *GNPTAB* gene cause functional deficits in the N-acetylglucosamine-1-phosphotransferase enzyme (EC 2.7.8.17), which is responsible for the phosphorylation of mannose residues to mannose-6-phosphate (M6P) on newly synthesized lysosomal enzymes in the Golgi apparatus. Without the M6P to recruit synthesized lysosomal enzymes to the

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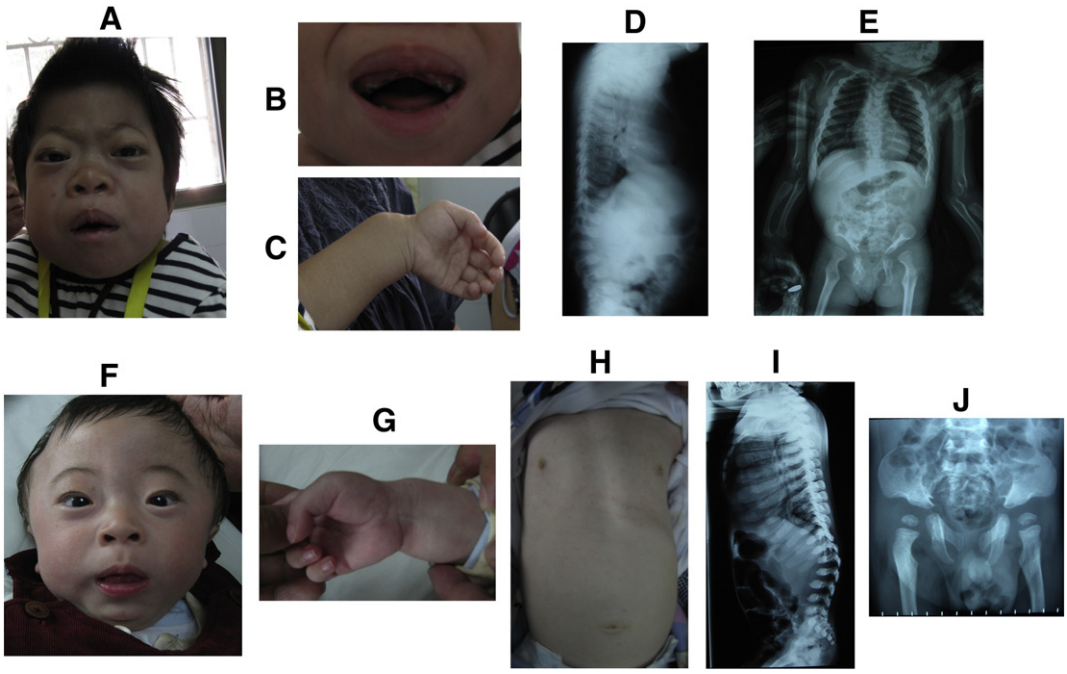

Fig. 1. Clinical features of the two Chinese patients with ML II alpha/beta. For Patient 1: (A–B) A "coarse" face was noted with a low nasal bridge, inner epicanthic folds, gum hypertrophy, and partially erupted teeth. (C) Patient 1 had broad wrists bilaterally. (D–E) Radiographs of Patient 1 showed multiple dysostosis including generalized osteopenia, oar-shaped ribs, and scoliosis. For Patient 2: (F) He had noted "coarsening" of face with narrow forehead, thin eyebrows, low nasal bridge, long philtrum, open mouth. (G–H) Patient 2 also had broadening of wrists and fingers, a deformed chest. (I–J) Dysotosis multiplex (short, thick clavicles, thick elevated scapula with poorly formed glenoid fossa, oar-shaped ribs, and scoliosis) in Patient 2.

lysosomes, the enzymes are secreted from the Golgi to the extracellular space [3,4]. As a result, these substances accumulate and cause the symptoms of the disease, ML II alpha/beta is characterized by failure to thrive and developmental delays and manifests as thickened skin, coarse facial features, hypertrophic gingiva, thoracic deformity, kyphosis, clubfeet, and restricted joint movement [5]. Patients with ML II alpha/beta usually die before seventh year of age from cardiorespiratory failure or recurrent respiratory tract infections [6].

N-acetyglucosamine-1-phosphotransferase is a heterohexamer ($\alpha2\beta2\gamma2$) composed of six subunits. The subunits α and β are encoded by the *GNPTAB* gene and the γ subunit is encoded by the *GNPTG* gene [7,8]. Mucolipidosis II alpha/beta and III alpha/beta are caused by mutations in the *GNPTAB* gene [9,10], whereas mucolipidosis III gamma results from the mutations in the *GNPTG* gene [11]. While more than 60 different mutations in the *GNPTAB* gene have been reported [10,12–18], there is only one reported ML II alpha/beta patient from China. This patient had two compound heterozygous mutations: c.2422delC in exon 13, which was a novel mutation, and c.3565C>T in exon 19, which had been previously reported [17,19].

Sanger sequencing is the traditional method for identifying these mutations and has been used as gold standard of sequencing for more than three decades. Nevertheless, Sanger sequencing for this screening purposes has relatively high cost and low throughput, which greatly limits its use. Since ML II alpha/beta is often misdiagnosed because of its complicated clinical and radiographic features, often many different gene tests need to be conducted before the final diagnosis can be made.

Here we describe the clinical and biochemical findings of two new cases of ML II alpha/beta from China. Genetic analysis of *GNPTAB* was carried out by targeted next-generation sequencing in the two patients and confirmed.

Table 1
Lysosomal enzymes activity in plasma.

Enzyme	Enzyme activity (nmol/1 h/ml)		
	Patient 1	Patient 2	Normal range
β-Mannosidase	2410	3142	65–360
β-galactosidase	148	115	0–18.7
β-glucuronidase	288.6	325	3.7–11.2
α-L-fucosidase	119.2	132	5–23
Hexosaminidase	3284	3626	60–500

2. Materials and methods

This study was approved by the Medical Ethics Committee of General Hospital of Beijing Military Region and informed consents were signed by the participants' legal guardian. The activity of several lysosomal enzymes in serum was assayed using methylumbelliferone (MU)-labeled fluorescent substrate. Genomic DNA from peripheral blood leukocytes derived from the affected individuals and the available family members was extracted using the QIAamp DNA Blood Midi Kit (Qiagen, Hilden, Germany). The list of 505 inheritable genetic disease related genes in the panel for captured and targeted next-generation sequencing can be seen in Supplementary Table 1. The exon regions of 505 genes including *GNPTAB* were specifically enriched using biotinylated capture probe (MyGenostics, Baltimore, MD, USA) as described previously [20,21]. In brief, 1 μg DNA library was mixed with Buffer BL and GenCap probe (MyGenostics, MD, USA), heated at 95 °C for 7 min and 65 °C for 2 min on a PCR machine; 23 μL of the 65 °C pre-warmed Buffer HY (MyGenostics, MD, USA) was then added to the mix. The mixture was held at 65 °C for 22 h with PCR lid heat on for hybridization. Fifty microliters of MyOne beads (Life Technologies Corporation, USA) were washed in 500 μL 1 × binding buffer 3 times and resuspended in 80 μL 1 × binding buffer. Sixty-four μL 2 × binding buffer was added to the hybrid mix and transferred to the tube with 80 μL MyOne