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Three novel homozygous mutations in the *GNPTG* gene that cause mucolipidosis type III gamma

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ABSTRACT

Background: Mucolipidosis type III gamma (MLIII gamma) is an autosomal recessive disease caused by a mutation in the *GNPTG* gene, which encodes the γ subunit of the N-acetylglucosamine-1-phosphotransferase (GlcNAc-1-phosphotransferase). This protein plays a key role in the transport of lysosomal hydrolases to the lysosome.
Methods: Three Chinese children with typical skeletal abnormalities of MLIII were identified, who were from unrelated consanguineous families. After obtaining informed consent, genomic DNA was isolated from the patients and their parents. Direct sequencing of the *GNPTG* and *GNPTAB* genes was performed using standard PCR reactions.
Results: The three probands showed clinical features typical of MLIII gamma, such as joint stiffness and vertebral scoliosis without coarsened facial features. Mutation analysis of the *GNPTG* gene showed that three novel mutations were identified, two in exon seven [c.425G>A (p.Cys142Val)] and [c.515dupC (p.His172Profs27X)], and one in exon eight [c.609+1G>C]. Their parents were determined to be heterozygous carriers when compared to the reference sequence in GenBank on NCBI.
Conclusions: Mutation of the *GNPTG* gene is the cause of MLIII gamma in our patients. Our findings expand the mutation spectrum of the *GNPTG* gene and extend the knowledge of the phenotype–genotype correlation of the disease.

1. Introduction

Mucolipidosis type III, originally called a variant of pseudo-Hurler polydystrophy, is an autosomal recessive disease that results from a deficiency of the membrane-bound enzyme UDP-GlcNAc-1-phosphotransferase (Tappino et al., 2009). This enzyme is responsible for the initial step in the synthesis of the mannose 6-phosphate (M6P) recognition markers on high mannose-type oligosaccharides in the Golgi. A lack of GlcNAc-1-phosphotransferase leads to the M6P marker losing its function. Without M6P, the trafficking process that moves the lysosomal hydrolases to lysosomes is impaired (Tappino et al., 2009).

The GlcNAc-1-phosphotransferase is composed of a 540-kDa α 2-β 2-γ 2 hexameric complex. Two genes, *GNPTAB* (Raas-Rothschild et al., 2000) and *GNPTG* (Kudo et al., 2005; Tiede et al., 2005), encode α/β- and γ-subunits of the GlcNAc-1-phosphotransferase, respectively. The *GNPTAB* is located on chromosome 12q23.3, contains 21 exons spanning 85 kb and encodes a protein of 1256 amino acids with a predicted molecular mass of 144 kDa (a/b precursor) (Kudo et al., 2005; Raas-Rothschild et al., 2000; Tiede et al., 2006, 2005). A mutation of the *GNPTAB* gene will cause MLIII alpha/beta (α/β) (MIM #252600). The *GNPTG* gene (*GNPTG*) is located on chromosome 16 and was discovered in 2000. *GNPTG* contains 11 exons and encodes a soluble protein made up of 305 amino acids with a predicted molecular mass of 34 kDa. Mutation of the *GNPTG* gene is responsible for MLIII gamma (γ) (MIM #252605). It is believed that the γ-subunit is capable of forming disulfide-linked dimers, and the α/β-precursor appears to be a prerequisite for the catalytic activity of the enzyme (Pohl et al., 2009).

Clinically, MLIII α/β and MLIII γ are both rare diseases and are indistinguishable. The typical clinical symptoms include progressive joint stiffness, short stature, scoliosis and mild mental retardation. Most patients also exhibit cardiac valve involvement and experience bone pain and disability because of destruction of the hip joints. Moderate

Abbreviations: A, adenosine; α, alpha; bp, base pair(s); β, beta; C, cytidine; cDNA, DNA complementary to RNA; dNTP, deoxyribonucleoside triphosphate; G, guanosine; γ, gamma; GlcNAc-1-phosphotransferase, N-acetylglucosamine-1-phosphotransferase; *GNPTAB*, gene encoding the alpha/beta subunit of the N-acetylglucosamine-1-phosphotransferase; *GNPTG*, gene encoding the gamma subunit of the N-acetylglucosamine-1-phosphotransferase; kb, kilobase(s) or 1000 bp; kDa, kilodalton(s); Km, kanamycin; MLIII, mucolipidosis type III; M6P, mannose-6-phosphate; MW, molecular weight; NMD, nonsense-mediated mRNA decay; NN, Neural Network; ORF, open reading frame; T, thymidine; Wt, wild type.

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to severe dysostosis multiplex with vertebral changes is evident upon radiological examination (Pohl et al., 2009; Zarghooni and Dittakavi, 2009).

Here we report two unique Chinese children with MLIII gamma caused by three novel homozygous mutations in the *GNPTG* gene.

2. Methods and materials

2.1. Patients

The study was approved by the Peking Union Medical College Hospital Institutional Review Board, and peripheral blood samples were collected from the patients and their parents with informed consent.

Three affected children were included in the study, two girls and one boy that were 13, 12 and 17 years of age. The participants are from unrelated intermarried families. All of the children were born normally. They were admitted to our clinic for progressive joint stiffness a minimum of nine years ago.

2.2. Lysosomal enzyme assay

The plasma was separated from 2 ml of peripheral blood from the three patients and their parents. 4-Mu-β-D-gluronide (MW: 352 g/mol) and 4-Mu-α-D-mannopyronoside (MW: 338 g/mol) (Sigma-Aldrich) were used as the fluorogenic substrates to determine the activity of the lysosomal enzymes. The activity was expressed as the amount of substrate (nmol) cleaved per h per mg of protein in the cell lysates. The normal range in Chinese controls was found to be 10.7–33.7 nmol h^{-1} per mg protein for β-D-glucuronidase and to be 13.7–66.7 nmol h^{-1} per mg protein for α-D-mannosidase.

2.3. Isolation of the DNA and mutational analysis

Genomic DNA was isolated from the whole blood obtained from the three patients and their parents. DNA isolation was performed using the Qiagen DNA isolation kit (D-5000) (Gentra Systems, Inc., Minneapolis, MN, USA) according to the manufacturer's protocol.

The full coding of the exons, the exon–intron boundaries and the 5′- and 3′-flanking regions of the *GNPTAB* gene (GenBank: NM_024312) and the *GNPTG* gene (GenBank: NM_032520.4) were amplified using primers designed according to a published sequence. Primers and conditions are available upon request. PCR was performed in a 25 μl reaction mixture containing 20 ng of genomic DNA, 2.5 μl of 10× PCR buffer I, 10 mmol of dNTPs, 10 pmol each of the forward and reverse primers and 0.5 U of AmpliTaq (Takara Biotechnology, Dalian, China). Samples were incubated in a thermocycler for 5 min at 95 °C followed by 35 cycles of 94 °C for 30 s, annealing at 58.5 °C for 30 s and 72 °C for 40 s and a final extension at 72 °C for 10 min.

The PCR products were sequenced in both directions using an ABI 3730XL sequencer (Biomed Corporation, Beijing, China).

The results were compared with normal controls (GenBank: NM_024312 and NM_032520.4). The mutations found were confirmed by sequencing the second PCR amplicons. As a reference, the A of the ATG translation initiation codon of the coding sequence of *GNPTAB* and *GNPTG* is referred to as nucleotide +1.

3. Results

The plasma activity of the two lysosomal hydrolases (β-D-glucuronidase and α-D-mannosidase) in all affected patients was significantly increased over its activity in the controls (18.8–22.4-fold for β-D-glucuronidase and 12.4–13.7-fold for α-D-mannosidase) (Table 1).

Three novel homozygous mutations in the *GNPTG* gene were found in the affected consanguineous children. In the 13-year-old girl, an adenine was substituted for the guanine at nucleotide 425 in exon seven (c.425G>A; p.Cys142Val); in the 12-year-old girl, a duplication of cytosine was displayed in exon seven at nucleotide 515 (c.515dupC (p.His172Profs27X)); and in the 17-year-old boy, a transition was present in the necessary AG-dinucleotide of the intron eight donor splice site (c.609+1G>C). All of the parents were heterozygous carriers of these mutations (Fig. 3). We did not find any mutation in the *GNPTAB* gene.

The boy, who had the latest onset of skeletal symptoms, began complaining of joint stiffness and loss of flexibility at six years old. The two girls first presented contracture of the hand and progressive joint stiffness at the age of three years, especially in the fingers, wrists, hips and knees. Patient three showed serious hip pain and scoliosis of the spine. All of the patients complained of difficulty with walking after nine years of age.

Physical examination showed that the height of all three children was below the 3rd percentile. They did not show any coarse facial features or corneal clouding, and their intelligence was normal. The skeletal symptoms present in all patients included short necks, scoliosis of the spine and contracture of the knees, spine, wrists, elbows and fingers. Patient two had genu valgum with an intermalleolar distance of 7 cm.

Radiographic evaluations showed signs of spondyloepiphyseal dysplasia (Figs. 1 and 2). Echocardiography showed a thickening of the cardiac valve involving the mitral valve for the 13-year-old girl and in the aortic valve for the boy. Lung function testing and an abdominal ultrasound did not show any abnormalities. The pattern and quantity of urinary oligosaccharides and glycosaminoglycans were normal. (Table 1).

4. Discussion

Prior to June 2013, a total of 25 mutations in the *GNPTG* gene from 44 patients had been reported, including 10 deletions, five insertions, four missense mutations, two nonsense mutations and four splicing mutations. All of the mutations are randomly distributed in each exon, without the presence of a distinct hot spot (Encarnacao et al., 2009; Gao et al., 2011; Kang et al., 2010; Persichetti et al., 2009; Pohl et al., 2010; Raas-Rothschild et al., 2000, 2004; Zarghooni and Dittakavi, 2009). The *GNPTG* mutation was predicted to lead to the dysfunction of the γ-subunits, which is expected to result in malfunction of the proper folding of the α/β-subunits or the balance of the phosphotransferase subunits, leading to MLIII gamma. In individuals with MLIII gamma, homozygous or compound heterozygous mutations are detected. To date, no correlation between the severity of the disease and the type of mutation has been reported. In 2010, Yong Gao et al. reported the first and only *GNPTG* mutation in the Chinese population. They identified two novel mutations, c.471delC and IVS4-1G>C (Gao et al., 2011). In this study, we have found three novel homozygous mutations that cause MLIII gamma in three unrelated Chinese consanguineous families. This phenomenon also highlights the impact of consanguineous marriage on the development of autosomal recessive diseases.

In the 13-year-old girl, we found the c.425G>A (p.Cys142Val) substitution, which is located in a conserved domain, by comparing the sequences of eight orthologous vertebrate *GNPTG* proteins from human to zebrafish (known as protein kinase C substrate 80K-H; PRKCSH). Consistent with their pathological authenticity, no nucleotide change was found in a screen of 150 healthy Chinese control subjects, nor is substitution listed as a polymorphism in dbSNP (www.ncbi.nlm.nih.gov/projects/SNP). This missense mutation was submitted to the predictive methods implemented in Polyphen (http://genetics.bwh.harvard.edu/pph2/index.shtml) and SIFT (http://blocks.fhcrc.org/sift/SIFT.html) bioinformatic tools, and both were expected to be potentially damaging to the protein function with a high probability of functional impairment. Therefore, we predicted that the patient with elevated lysosome enzyme activities belongs to the ML gamma subtype caused by the p.Cys142Val mutation in the *GNPTG* gene.