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Clinical and molecular characteristics of 20 Chinese probands with Mucolipidosis type II and III alpha/beta

Yuyu Feng[1], Yonglan Huang[1], Xiaoyuan Zhao[1], Huiying Sheng[1], Xueying Su[1], Xi Yin[1], Liu Li[1] and Wen Zhang[1*]

Abstract

Background Mucolipidosis (ML) II and III alpha/beta are lysosomal disorders caused by mutations in the *GNPTAB* gene which encodes the alpha and beta subunits of the heterohexameric enzyme, N-acetylglucosamine-1-phosphotransferase.

Method To explore the clinical and molecular characteristics of the 20 ML II and III alpha/beta patients, clinical data was collected and *GNPTAB* gene was analyzed by nest PCR and direct Sanger-sequencing. The activity of several lysosomal enzymes was measured in the plasma.

Results Among the 20 ML II and III alpha/beta patients, 6 patients were classified as ML II and 14 as ML III alpha/beta. The main clinical manifestations were joint stiffness, skeletal deformity, mental retardation and short stature. Bone X-ray examination showed radiological changes. The plasma arylsulfatase A and hexosaminidase A enzyme activities increased significantly. Urinary glycosaminoglycan values were normal. We detected mutations in *GNPTAB* in 35 of 40 alleles (87.5%). Mutation c.2715 + 1G > A and c.2404 C > T (p.Gln802Ter) were the most prevalent variants, accounting for 14.3% and 11.4%, respectively. Five novel mutations c.3335 + 5G > A, c.1284 + 1G > A, c.571 + 4 A > G, c.1634_1635delAA (p.Lys545Serfs*16) and c.1582T > C(p.Cys528Arg) were identified.

Conclusion Our study expands the spectrum of *GNPTAB* gene in China. Mutation c.2715 + 1G > A was the most prevalent mutation in our study. The novel mutation c.1284 + 1G > A might be a severe mutation associated with ML II.

Keywords Mucolipidosis, *GNPTAB* gene, Clinical and molecular characteristics

Background

Mucolipidosis (ML) is an autosomal recessive lysosomal storage disease caused by reduced enzyme activity of N-acetylglucosamine-1-phosphotransferase (GlcNAc-1-PT) (EC 2.7.8.17). The term "mucolipidosis" was first described by Wiedmann et al. in 1970, because its clinical manifestations were similar to mucopolysaccharidosis and sphingolipidosis [1, 2]. GlcNAc-1-PT catalyzes the first step in the synthesis of the mannose 6-phosphate (M6P) recognition marker required for targeting of newly synthesized lysosomal hydrolases to the lysosome. Without the M6P recognition markers, the overflowed lysosomal enzymes will leak out of the cells and elevated in the serum and body fluids [3, 4]. Among live births, the incidence is 1/123,500 in Portugal [5], 1/252,500 in Japan [6], 1/625,500 in the Netherlands [7], and 1/2,229,516 in Malaysia [8].

*Correspondence:
Wen Zhang
zhw2001zhw@163.com
[1]Department of Genetics and Endocrinology, Guangzhou Women and Children's Medical Center, Guangzhou Medical University, 9 Jinsui Road, Guangzhou 510623, China

GlcNAc-1-PT is a 540-kDa hexameric complex composed of three subunits: 2α, 2β and 2γ. The α/β subunit is encoded by the *GNPTAB* gene and the γ subunit is encoded by the *GNPTG* gene. The alpha/beta subunit contains the catalytic domain of GlcNAc-1-PT enzyme and the gamma subunit plays an auxiliary role in the GlcNAc-1-PT enzyme. *GNPTAB* gene (MIM# 607840, ENSG00000111670) spans up to 85Kb of chromosome 12q23.3 and contains 21 exons encoding 1256 amino acid residues. *GNPTG* (MIM#252605, ENSG00000090581) gene spans 11.13 kb of chromosome 16p13.3 and contains 11 exons encoding 305 amino acid residues. Mutations in *GNPTAB* are known to be responsible for ML II and ML III alpha/beta. Mutations in the *GNPTG* gene cause ML III gamma [9–12].

The classic ML is divided into 4 types, namely ML I-IV. Currently, ML I and ML IV have been classified into other diseases. ML I, named sialidosis, is due to deficiency of neuraminidase, while ML IV is a neurodevelopmental disorder with retinal degeneration and normal lysosomal hydrolase activities [2]. Recently, mucolipidosis type V was reported in the literature. It found that TMEM251 was a regulator of M6P, which was crucial for the cleavage and activity of GlcNAc-PT enzyme. Individuals carrying pathogenic TMEM251 mutations might show severe symptoms similar to ML II, so they were classified as ML V [13]. The etiology of ML II and ML III are both caused by the deficiency of GlcNAc-1-PT enzyme activity. ML II patients are completely deficient in GlcNAc-1-PT enzyme activity and characterized by stiffness of the joints, coarse facies, serious progressive bone diseases and intellectual disability [14, 15]. Most of them developed the disease between the age of 6 to 12 months and died within 10 years of age [6]. A certain number of patients may be evident at birth or prenatally [16, 17]. ML III alpha/beta is the moderate form of ML II. Patients with ML III are partial deficient in GlcNAc-1-PT enzyme activity and have a later onset at the age of 2 to 4 years. The ML III progress slowly and could survival into adulthood [18].

At present, there is no effective treatment for ML disease, except for symptomatic treatment. It was reported that intravenous treatment with pamidronate could improve reduced bone density [19]. But there weren't many cases, so it wasn't widely used in clinical practice. Surgical orthopedic treatment for bones and joints, such as bilateral hip and knee replacements, spinal fusion procedures and carpal tunnel release, could help improve movement limitations. The heart valve damage can affect the function of the heart and patients should require surgical intervention [20].

In this study, we described clinical, biochemical and molecular characteristics of 20 Chinese patients with ML II and III alpha/beta to further analyze the relationship between genotype and phenotype, which helps us to predict the prognosis of patients and to offer genetic counseling.

Methods

Patients

Twenty probands from 17 unrelated families with ML II and III alpha/beta from South China (Guangdong, Hunan, Hubei, Guangxi, Sichuan, Yunnan province) were enrolled in this study. Eleven were male and nine were female. The diagnosis of ML II and III alpha/beta was based on clinical findings and confirmed by lysosomal enzyme activities in plasma measured at the Guangzhou Women and Children's Medical Center from August 2011 to January 2023. Informed consent was obtained from parents of patients. This study was approved by the Ethics Committee of Guangzhou Women and Children's Medical Center (2015-92).

Lysosomal enzyme activity

The activities of several lysosomal hydrolases in plasma, including arylsulfatase A (ASA) and hexosaminase A (HexA), were tested with reference to previous methods and modified slightly [21, 22]. The fluorogenic substrate 4-nitrocatechol sulfate (Sigma-Aldrich, St. Louis, MO, USA) and 4-methylumbelliferyl-6-sulfo-2-acetamido-2-deoxy-β-Dglucopyranoside (Glycosynth, Warrington, UK) were used in the detection, respectively. If the enzyme activity values were increased 10–20 times than the normal range, then the diagnosis of ML II or III alpha/beta was supported. The normal reference range of ASA enzyme activity was 50–140 nmol/mg.17 h, and the normal reference range of HexA enzyme activity was 29.8–63.8 nmol/mg.h. Enzyme activities measurements of our lab participate in the quality assurance of IEM laboratory tests (www.eqa.erndim.org).

Urinary glycosaminoglycan (GAG) determination

Urinary GAG was measured using dimethylmethylene blue/Tris by spectrophotometry and corrected for urinary creatinine (Cr) content. The ratio of GAG/Cr (mg/mmol) was compared to age-matched normal controls. Normal reference values of urine GAG/Cr were GAG/Cr normal reference range: <64.0 mg/mmol(<28days), <49.9 mg/mmol(28days-6months), <37.8 mg/mmol(6months-1year), <30.0 mg/mmol(1-3years), <19.9 mg/mmol(3-5years), <16.0 mg/mmol(5-7years), <12.6 mg/mmol(7-18years), <7.1 mg/mmol(>18years) [23].

Molecular analysis

Genomic DNA was extracted from peripheral blood samples. *GNPTAB* gene was analyzed by direct Sanger-sequencing. Primers (Table 1) were designed using

Table 1 Primers used for PCR amplification and sequencing of *GNPTAB*

Exons	Forward primers(5′-3′)	Reverse primers(5′-3′)	Product size(bp)
1	CGTCCGTCGCCGGAGCTGCAATG	GGCAAAACCCCGTCTCTAATAATG	386
2	GATCTAACACGATGTATGTGGTAGGCAG	GGCCACACTAATCTTCTCTGTATCGTTC	458
3–4	TACAGTTTGAAAACATGCAGTTCTGTGA	AGATAACAATGCACCAAGAGGGAACTAA	1951
5	GCTCTATCTTTGGAGTTGGGTTTAACAA	TGTTTTGCTTCTCTTTGTGCATTTTTAG	552
6–7	TCAACGTACACTGATTTACATTGTTCCC	CAGGAAACAAGCAAACCAAATAAGACTC	863
8–10	AGGAAAGATTAAAGAGCAGAGTGGGAAT	CGGGCCCAGATAATTATTTTACTTTTTG	1430
11	AAACTTAAAGCACTTGTGAGTGTGAACG	CCTCCCAAGTAGCTAGGACTACAGGTTT	576
12	CACCCAGTCCAGAACTGTCTTTC	GCTAAGGTAAATCTGCTTGGTCC	522
13	TGCCTACTTCAGCAGCACATATAC/ TCCAAGTCAGCCTTG CTGAG	CCTGAGCATGAGAAAGAATGAGG/ CTCAGCAAGGCTGA CTTGGA	1345
14–16	TGAGTCCTGCTAAGTCCAGTTTAGATCC	ATGAATGATTCGGATTACCTGTGCTACT	2066
17–18	GGTGTTTTTCTTACCTCCAGAGAGCATA	CTGGGTCTCTCTGAACAGCTTGTTATTA	812
19	CCACATCCTTGTTTTGTTAGGTCAG	AGAAGAATCATTGTACCCAGGAGG	450
20	TGAACTCGTGTTGGAAATGTATATTGTG	TATTTGCTGCCTGAATATTGTGAAACAT	428
21	AGGCATACTGTCCCTACAAAGCT	GGCTATATTCATGCCACAAAACAG	433

Primer 5 software (Biosoft International, Palo Alto, USA). The coding and splicing regions of the *GNPTAB* gene were amplified with Ex Taq DNA polymerase (TaKaRa Bio Inc., Otsu, Japan). The PCR products were sequenced by an ABI 3730 sequencer (Applied Biosystems, Foster City, CA, USA) and the sequencing chromatograms were analyzed by comparing with the corresponding reference sequences (NCBI: NM_024312.5) using Sequencer software DNAMAN (Lynnon Biosoft, Inc., Ouebec, Canada). Once variants were identified, the PCR and sequencing on the corresponding exons were repeated at least twice to verify reliability of the results. Pathogenic assessment of novel missense variants were analyzed by the Mutation Taster2021 [24] (https://www.mutationtaster.org/) and Polymorphism Phenotyping v2 (PolyPhen-2) (http://genetics.bwh.harvard.edu/pph2/index/shtml). To predict the pathogenicity of novel splicing mutation c.3335+5G>A, c.1284+1G>A and c.571+4 A>G, we performed in silico analysis using the Mutation Taster2021 (https://www.mutationtaster.org/), FATHMM (http://fathmm.biocompute.org.uk/) and NetGene2 (https://services.healthtech.dtu.dk/services/NetGene2-2.42/).

Results

Clinical features

Patients with ML II

As shown in Table 2, the ML II group contains 6 patients, including 4 females and 2 males. Patient 1a and 1b were fraternal twins. The onset age of the 6 patients ranged from 3 to 6 months, and the diagnosis age ranged from 6 months to 2 years and 6 months. Two patients died when they were about 3 years of age (patient no.4 and no.5). All 6 patients had joint stiffness, short stature and developmental delay or intellectual disability. Other main clinical features included coarse facies (5 cases) and skeletal deformity (4 cases). Some patients had gingival hyperplasia (2 cases), Mongolian spots (1 case) and left ventricular hypertrophy (1 case).

Patients with ML III alpha/beta

The ML III alpha/beta group contained 14 patients, including 5 females and 9 males. Patient 7c and 7d, 17e and 17f were affected siblings. The onset age of the 14 patients ranged from 7 months to 9 years of age, and the diagnosis age ranged from 7 months to 14 years of age. The clinical symptoms were similar to ML II and progressed with age. The 14 ML III alpha/beta patients all had joint stiffness. Eight of them had skeletal deformity, six had coarse facies, four had developmental delay and Mongolian spots and three had valvular disease or left ventricular hypertrophy. Notably, patient 7c and 7d were finally classified into ML III alpha/beta, though the onset age was within 1 year of age. Though the siblings began to develop joint stiffness and coarse facies in infancy, the disease progressed slowly. By their mid-teens, they were still alive and relied on assistive wheelchair for longer distances.

Radiographic findings

The bone X-ray examination of most patients was characterised by "dysostosis multiplex". The combination of radiographic features included "J" shaped sella turcica, oar shaped ribs, anterior inferior beaking of lower thoracic to upper lumbar vertebral bodies, flared iliac wings, constricted iliac bodies, dysplastic femoral heads, "bullet-shaped" proximal phalanges and central pointing of proximal metacarpals (Fig. 1).

Biochemical analysis

As shown in Table 2, plasma activities of ASA and HexA were significantly higher than the reference values. The average of ASA activities was 5712nmol/

Table 2 Clinical features and genotypes of 20 Chinese probands with ML II and III alpha/beta

Patient no.	Sex	Age of onset	Age of diagnosis	Type	HS	SD	CF	DD	SS	MS	HD	GH	ASA	HexA	GAG/Cr	GNPTAB mutation	GNPTAB mutation	Mutation type
1a	M	3 m	2y6m	II	+	+	+	+	+	-	-	-	4323	724.9	41.9	c.1523delG (p.Gly508Aspfs*39)	**c.3335 + 5G > A**	NS/Spl
1b	F	3 m	2y6m	II	+	+	+	+	+	-	-	-	4607	759.7	34.6	c.1523delG (p.Gly508Aspfs*39)	**c.3335 + 5G > A**	NS/Spl
2	F	3 m	6 m	II	+	-	+	+	+	-	-	-	3230	420.6	46.6	c.2404 C > T (p.Gln802Ter)	c.2404 C > T (p.Gln802Ter)	NS/NS
3	F	3 m	7 m	II	+	+	+	+	+	-	-	+	7776	594.2	ND	c.88_89delAC (p.Thr30Hisfs*24)	c.2550-2554del (p.Lys850Asnfs*10)	FSH/FSH
4	F	5 m	11 m	II	+	+	-	+	+	+	-	+	4516	232.9	ND	c.1090 C > T (p.Arg364Ter)	c.2404 C > T (p.Gln802Ter)	NS/NS
5	M	6 m	2y6m	II	+	-	+	+	+	-	+	-	8930	510.1	ND	c.1090 C > T (p.Arg364Ter)	**c.1284 + 1G > A**	NS/Spl
6	F	1y	3y	III	+	+	+	-	+	-	+	-	8505	417	ND	c.1760G > A (p.Arg587Pro)	c.1760G > A (p.Arg587Pro)	MS/MS
7c	M	7 m	7 m	III	+	+	+	-	+	-	-	-	5730	828.9	55.9	c.118-1G > A	ND	Spl
7d	F	10 m	3y10m	III	+	+	+	-	+	-	-	-	5346	655	9.2	c.118-1G > A	ND	Spl
8	M	1y9m	4y9m	III	+	+	+	-	+	-	-	-	6581	695.5	10.4	c.1760G > A (p.Arg587Pro)	c.2693delA (p.Lys898Serfs*13)	MS/FSH
9	M	2y	4y	III	+	-	+	+	+	+	-	-	6298	622.2	17.6	c.3094delA (p.Thr1032Hisfs*11)	**c.571 + 4 A > G**	FSH/Spl
10	F	2y	5y	III	+	+	-	-	-	+	-	-	2309	650.9	9.7	c.2455G > T (p.Glu819Ter)	ND	NS
11	F	3y	12y	III	+	+	-	+	+	-	+	-	7107	279.4	ND	c.673 C > T (p.Gln225Ter)	ND	NS
12	M	3y	11y	III	+	+	+	+	+	+	-	-	ND	939.3	8.9	c.2404 C > T (p.Gln802Ter)	c.2715 + 1G > A	NS/Spl
13	M	3y	14y	III	+	+	-	+	+	-	-	-	5903	721.1	6.6	c.196 C > T (p.Gln66Ter)	c.2715 + 1G > A	NS/Spl
14	M	3y	12y	III	+	-	-	-	+	-	-	-	3604	172	6.6	c.1090 C > T (p.Arg364Ter)	c.3571 C > T (p.Arg1191Cys)	NS/MS
15	M	4y	10y	III	+	-	-	-	+	-	-	-	1897	303.6	6.6	**c.1582T > C (p.Cys528Arg)**	c.2715 + 1G > A	MS/Spl
16	M	6y	7y	III	+	-	-	-	-	+	-	-	6085	597.1	7.7	c.77G > A (p.Gly26 > Asp)	ND	MS
17e	M	5y	10y	III	+	-	-	-	-	-	+	-	7685	304.2	4.5	**c.1634_1635delAA (p.Lys545Serfs*16)**	c.2715 + 1G > A	FSH/Spl
17f	F	9y	13y	III	+	-	-	-	-	-	-	-	8100	348.7	5.6	**c.1634_1635delAA (p.Lys545Serfs*16)**	c.2715 + 1G > A	FSH/Spl

Novel mutations are indicated in bold letters.

a, b: fraternal twins; c,d: affected siblings; e, f: affected siblings; F: female; M: male; m: month; y: year; ASA: arylsulphatase A; HexA: β-hexosaminidase A; CF: coarse facies; Cr: urine creatinine; DD: developmental delay; GAG: urine glycosaminoglycan; GH: gingival hyperplasia; HS: hand stiffness; HD: heart disease; MS: Mongolian spots; SD: skeletal deformity; SS: short statue; ND, not detected; FSH: frameshift; MS: missense; NS: nonsense; Spl: splice site.

ASA normal reference range: (50–140)nmol/mg.17 h.

HexA normal reference range: (29.8–63.8)nmol/mg.h.

GAG/Cr normal reference range: <64.0 mg/mmol(< 28days), < 49.9 mg/mmol(28days-6months), < 37.8 mg/mmol(6months-1year), < 30.0 mg/mmol(1-3years), < 19.9 mg/mmol(3-5years), < 16.0 mg/mmol(5-7years), < 12.6 mg/mmol(7-18years), < 7.1 mg/mmol(> 18years). The detection time of GAG/Cr is the diagnosed time.

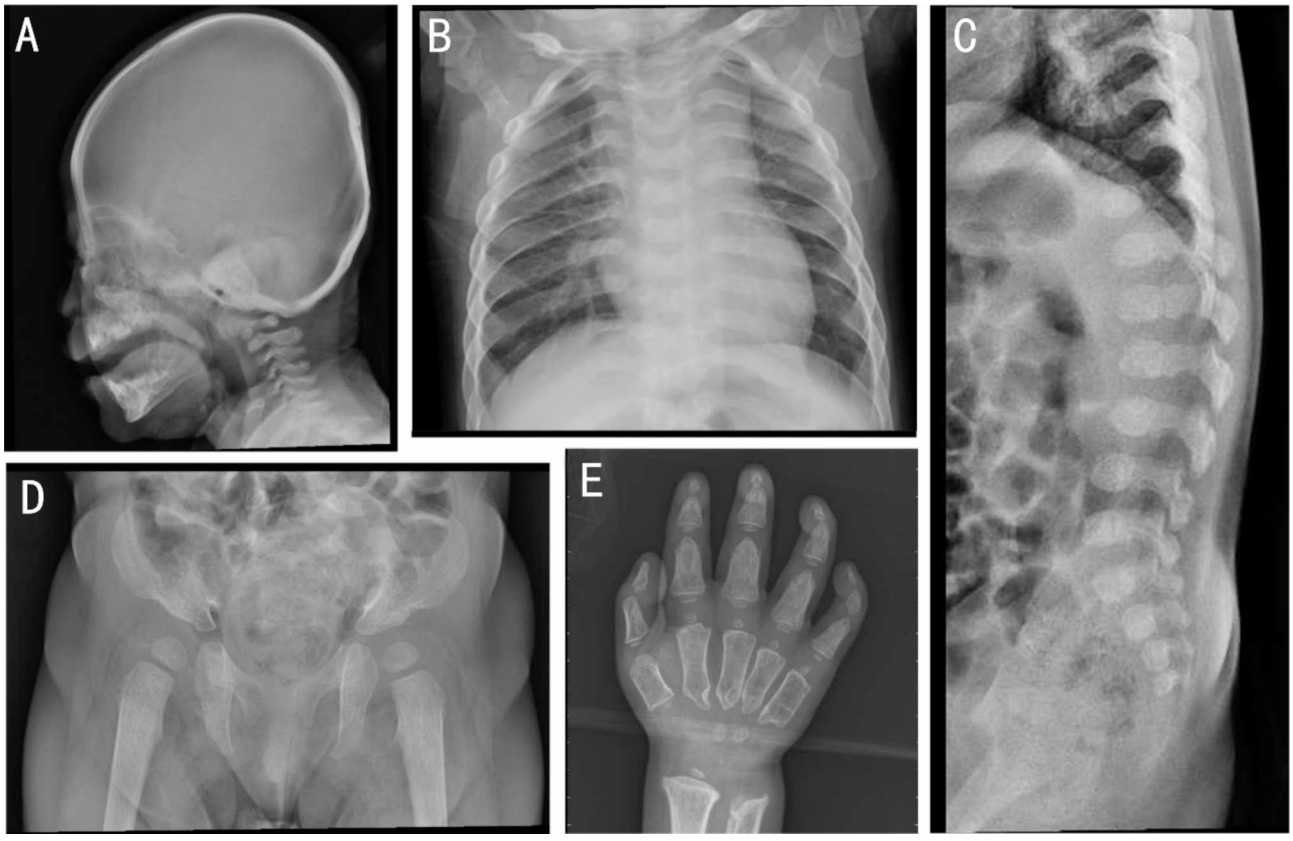

Fig. 1 Radiographic findings of patient no.2 (**A, B, C, D**) and patient no.9 (**E**). (**A**) 'J' shaped sella turcica. (**B**) Oar shaped ribs. (**C**) Anterior inferior beaking of lower thoracic to upper lumbar vertebral bodies. (**D**) Flared iliac wings, constricted iliac bodies, dysplastic femoral heads. (**E**) 'bullet-shaped' proximal phalanges and central pointing of proximal metacarpals

mg.17 h. The ASA activities of patients increased 13 to 63 times of reference value. The average of HexA activities was 538.9nmol/mg.h. The HexA activities of patients increased 3 to 4 times of reference value. Most GAG/Cr ratios of patients were normal. The GAG/Cr ratios of patient 1a, 1b and 7c were slightly elevated. The GAG/Cr ratios in patients with mucopolysaccharidosis (MPS) generally increase more than 2 times of the reference value. Combined with the results of enzyme activities, we could exclude MPS.

GNPTAB mutations

As shown in Table 3, out of 40 mutant alleles in 20 children with mucolipidosis we collected, only 35 mutant alleles (87.5% identification)were detected. The remaining five mutations were not detected. It may be related to large fragments are missing. This is just speculation. And we need to perform cDNA or CNVs studies to detect the variants we didn't find in the affected cases. There were 20 different variants, including 15 reported mutations (c.2715+1G>A, c.3571 C>T, c.2455G>T, c.2404 C>T, c.1760G>C, c.1090 C>T, c.673 C>T, c.196 C>T, c.77G>A, c.3094delA, c.2693delA, c.2550_2554del, c.1523delG, c.88_89delAC, c.118-1G>A) and five novel mutations (c.3335+5G>A, c.1284+1G>A, c.571+4 A>G, c.1634_1635delAA, c.1582T>C). None of the novel mutations were found in the 1000 Genomes database (www.1000genomes.org) and HGMD database. In addition, novel missense mutation c.1582T>C (p.Cys528Arg) was predicted to be damaging by Mutation Taster and Polyphen-2 web software. The Cys528 is located in the immunoglobulin domain of GlcNAc-1-PT. This domain participates in specific recognition and binding to lysosomal hydrolases, allowing the catalytic domain to tag their glycans. Mutation p.Cys528Arg may affect the recognition and binding of lysosomal hydrolases [3]. Novel splicing mutation c.1284+1G>A was predicted to be deleterious by Mutation Taster, FATHMM and NetGene2. Mutation c.3335+5G>A and c.571+4 A>G were predicted to be influential by FATHMM, while to be benign by Mutation Taster. It is necessary to perform cDNA analysis of c.3335+5G>A and c.571+4 A>G mutations to be sure the effect they cause. The missense mutation was the most common form, accounting for 45.7% (16/35) in this study, followed by splicing mutation and deletion mutation, accounting for 31.4% (11/35) and 22.9% (8/35), respectively. Mutation c.2715+1G>A and c.2404 C>T accounted

Table 3 *GNPTAB* gene mutation summary in our 20 patients

	Alteration	Location	effect	alleles	frequency	Phenotype
1	c.2715 + 1G > A	I13	Splicing	5	14.3%	III
2	c.2404 C > T	E13	p.Gln802Ter	4	11.4%	II/III
3	c.1760G > C	E13	p.Arg587Pro	3	8.6%	III
4	c.1090 C > T	E9	p.Arg364Ter	3	8.6%	II/III
5	c.118-1G > A	I1	Splicing	2	5.7%	III
6	c.3335 + 5G > A	I17	Splicing	2	5.7%	II
7	c.1523delG	E12	p.Gly508Aspfs*39	2	5.7%	II
8	c.1634_1635delAA	E13	p.Lys545Serfs*16	2	5.7%	III
9	c.77G > A	E1	p.Gly26 > Asp	1	2.9%	III
10	c.196 C > T	E2	p.Gln66Ter	1	2.9%	III
11	c.673 C > T	E7	p.Gln225Ter	1	2.9%	III
12	c.1582T > C	E12	p.Cys528Arg	1	2.9%	III
13	c.2455G > T	E13	p.Glu819Ter	1	2.9%	III
14	c.3571 C > T	E19	p.Arg1191Cys	1	2.9%	III
15	c.571 + 4 A > G	I5	Splicing	1	2.9%	III
16	c.1284 + 1G > A	I10	Splicing	1	2.9%	II
17	c.88_89delAC	E1	p.Thr30Hisfs*24	1	2.9%	II
18	c.2550_2554del	E13	p.Lys850Asnfs*10	1	2.9%	II
19	c.2693delA	E13	p.Lys898Serfs*13	1	2.9%	III
20	c.3094delA	E15	p.Thr1032Hisfs*11	1	2.9%	III

E: exon, I: intron

for 14.3% (5/35) and 11.4% (4/35), respectively. About 34.2% (12/35) detected mutations were localized in exon 13, suggesting that this exon was hotspot region for *GNPTAB* mutations.

Discussion

In this study, joint stiffness and skeletal deformities were the earliest and the most important manifestations of patients. Plasma lysosomal hydrolase activities (ASA and HexA) increased significantly. Bone X-ray examinations showed radiological changes. The earliest age of onset of our ML II patients was 3 months of age, and no cases of neonatal onset have been found in our study. However, it was reported that severe cases of ML II could present earlier as prenatal skeletal dysplasia and neonatal severe secondary hyperparathyroidism [16, 17]. Most ML II patients presented with symptoms immediately after birth. The median age of symptom onset was 0 years [18]. In America, 14 ML II patients were born with the abnormalities and half of them died between the ages of 3 days and 8 years [2]. In Australia, 6 out of 15 ML II cases had neonatal onset and died at around 27 months [25]. In Beijing, 1 out of 8 ML II patient had neonatal onset, the remaining 7 patients had onset range from 2 months to 6 months [26]. In Eastern China, 4 out of 15 ML II patients had neonatal onset, the remaining 11 patients had onset range from 3 months to 18 months [27]. Our research observed that the proportion of Chinese patients who got sick in infancy was relatively fewer compared to those from abroad. It may be related to the difference of mutation spectrum of *GNPTAB* gene.

Previous research has found that a 45-year-old patient with no obvious abnormalities in the pre-birth period was diagnosed with atypical juvenile rheumatoid arthritis when he was 3 years old. The skeletal deformity progressed with age and by the age of 10 years, mucolipidosis was confirmed by enzyme examination [28]. Similarly, patient no.10 of our study had costal valgus and mild finger joints inflexibility at the age of 2 years. She was diagnosed with the sequelae stage of rickets at that time. As the stiffness of the finger joints worsened, she was diagnosed with mucolipidosis by enzyme activities around the age of 5 years. Although there are some clinical and imaging overlaps between ML and mucopolysaccharidosis diseases, urinary GAG/Cr and lysosomal hydrolase activities can be distinguished. Through the above biochemical indicators and imaging examination, mucolipidosis can be distinguished from rheumatoid arthritis and rickets as well. *GNPTAB* gene analysis can further confirm the diagnosis.

There were regional and ethnic differences in the variation spectrum of *GNPTAB* gene. According to reports, c.3503delTC variant was common in Portugal, Brazil, Italy, Turkey, Argentina and America. The c.3503delTC variant accounted for 45% and 22.7% in Portuguese and the United States, respectively. And it was speculated to be associated with ML II [2, 29]. In Japan and South Korea, p.Arg1189Ter was the most common variant, accounting for 41% and 30%, respectively [30, 31]. In eastern China, p.Arg364Ter was the most frequent mutation, accounting for 18% [32]. In northern China, c.2715 + 1G > A was the most common

variant, accounting for 28%, followed by p.Arg364Ter and c.2345 C>T, accounting for 13% and 9%, respectively [26]. Similarly, c.2715+1G>A was the most common variant in our research, accounting for 14.3%. The *GNPTAB* mutations reported in ML patients of other countries were concentrated in exon 19, while it was exon 13 in Chinese.

Cathey, et al. found homozygous or compound heterozygous nonsense mutation and frameshift mutation caused more serious phenotypes. The ML II phenotype correlates directly with those *GNPTAB* genotypes that produce no or nearly no gene products [2]. In our study, there were one patient homozygous for p.Gln802Ter variant (patient no.2), one compound heterozygous for two different nonsense mutations (patient no.4), and one compound heterozygous for two different frameshift mutations (patient no.3). They developed the disease within one year of age and progressed rapidly. All of them were associated with ML II phenotype. And the p.Arg587Pro homozygote was detected in a patient with ML III alpha/beta. She had finger joint stiffness and scoliosis at the age of 1 year. By the age of 20 years, she could barely walk due to the worsening bone deformities. Our study similar to the previous reports that mutation p.Gln802Ter and p.Arg587Pro was associated with ML II and ML III α/β, respectively [27, 33].

Among our 5 ML III α/β patients with c.2715+1G>A heterozygote, they carried another mutation p.Gln802Ter, p.Gln66Ter, p.Arg364Ter and p.Lys545Serfs*16, respectively. They were characterized by a later onset form and showed mild phenotype, which speculated that the c.2715+1G>A mutation might be a mild mutation associated with ML III α/β. Similarly, in eastern China, c.2715+1G>A was identified in four patients with ML III α/β [27]. In northern China, c.2715+1G>A was identified in both ML II α/β (3 patients) and ML III α/β (6 patients) [26]. In Japan, c.2715+1G>A has been reported to be a mild form of gene mutation involved in ML III [30]. Patient no.5 was p.Arg364Ter/c.1284+1G>A genotype and classified as ML II. He developed the disease when he was 6 months of age, presenting with developmental delay and stiff fingers. The disease progressed rapidly and he died at about 3 years of age. The nonsense mutation p.Arg364Ter was reported to be a severe mutation associated with ML II [2]. Yang Ke, et al. also found very similar mutation p.Arg364Ter/c.1284+1G>T in a ML II patient [34]. We speculated that the novel mutation of c.1284+1G>A we found might be a severe mutation associated with ML II. Under the influence of two highly pathogenic mutations, our patient presented severe symptoms as ML II.

Conclusions

The mutation spectrum of *GNPTAB* gene exhibits ethnic and regional disparity in ML patients. Mutation c.2715+1G>A was the most prevalent mutation in our study. The novel mutation c.1284+1G>A might be a severe mutation associated with ML II.

Abbreviations

ML	Mucolipidosis
GlcNAc-1-PT	N-acetylglucosamine-1-phosphotransferase
M6P	Mannose 6-phosphate
ASA	Arylsulfatase A
HexA	Hexosaminidase A
GAG	Glycosaminoglycan
Cr	Creatinine

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Author contributions

YYF, YLH acquisition of data, analysis and interpretation of data, drafting the article. YLH, LL and WZ analysis and interpretation of data, revising the manuscript critically for important intellectual content. XYZ and XYS Performed biochemical analysis. HYS and XY performed genetic analysis. All authors reviewed and approved the manuscript.

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Data availability

The datasets generated and/or analysed during the current study are available in the ClinVar repository. The ClinVar accession number are SCV004041869, SCV004041870, SCV004041871, SCV004041872, SCV004041873, SCV004041874, SCV004041875, SCV004041876, SCV004041877, SCV004041878, SCV004041879, SCV004041880, SCV004041881, SCV004041882, SCV004041883, SCV004041884, SCV004041885, SCV004041886, SCV004041887, SCV004041888.

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Guangzhou Women and Children's Medical Center (2015-92). The patients' parents provided written informed consent for all genetic studies performed in relation to this case.

Consent for publication

The written informed consents for publication of identifying images and clinical details were obtained from all parents.

Competing interests

The authors declare no competing interests.

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