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# Identification of Five Novel Mutations Causing Rare Lysosomal Storage Diseases

Authors' Contribution:

Study Design A  
Data Collection B  
Statistical Analysis C  
Data Interpretation D  
Manuscript Preparation E  
Literature Search F  
Funds Collection G

ABCDEF **Chenxi Yang**  
CE **Jiyan Pan**  
C **Siyuan Linpeng**  
E **Zhuo Li**  
BE **Hu Tan**  
EG **Lingqian Wu**

Center for Medical Genetics, School of Life Sciences, Central South University, Changsha, Hunan, P.R. China

**Corresponding Author:** Lingqian Wu, e-mail: [wulingqian@sklmg.edu.cn](mailto:wulingqian@sklmg.edu.cn)

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**Background:** Lysosomal storage diseases (LSDs), a group of rare inherited metabolic disorders, result from specific lysosomal proteins deficiencies in the degradation of biomacromolecule, including over 70 different diseases, most of which are autosomal recessive. LSDs are multisystem disorders, and the clinical manifestations are usually broad and severe, involving the skeletal system, central nervous system (CNS), cardiovascular system, etc. Besides, patients with some subtypes of LSD have distinctive facial features.

**Material/Methods:** We performed next generation sequencing on 4 suspected mucopolysaccharidosis (MPS) cases to determine the genetic causes of the disease. By *in vitro* molecular cell assay, such as real-time polymerase chain reaction (RT-PCR) and western blot, we tested the pathogenicity of candidate variants.

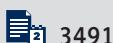
**Results:** We detected 5 novel mutations in 4 patients. The mutations were: c.211\_214del and c.1270C>T in *GUSB*; c.1284+1C>A and c.2404C>T in *GNPTAB*; and c.717C>A in *FUCA1*. We identified a rare mucopolysaccharidosis VII patient, a rare fucosidosis patient, and 2 rare mucopolipidosis II patients, one of which was an atypical patient. We also present a new pathogenic conjecture about a small deletion in *GUSB*.

**Conclusions:** Our study described rare diseases in Chinese patients and our results enrich the phenotype spectrum of related diseases, as well as mutation spectrum of related genes, which might be significant for clinical disease diagnosis and prenatal diagnosis.

**MeSH Keywords:** **Fucosidosis • Lysosomal Storage Diseases • Mucopolipidoses • Mucopolysaccharidosis VII**

**Abbreviations:** **LSD** – lysosomal storage disease; **CNS** – central nervous system; **GAGs** – glycosaminoglycans; **CS** – chondroitin; **DS** – dermatan; **KS** – keratin; **HS** – heparan sulfate; **M6P** – Mannose 6-phosphate; **NMD** – nonsense-mediated mRNA decay; **PVDF** – polyvinylidene difluoride; **MPS VII** – mucopolysaccharidosis; **HGMD** – Human Gene Mutation Database; **LNR** – Lin/Notch repeats; **ESS** – exon splice silence; **ML II** – Mucopolipidosis II; **CNV** – copy number variation

**Full-text PDF:** <https://www.medscimonit.com/abstract/index/idArt/915876>



## Background

Lysosomes as digestive organelles play an important role in life events. It is now acknowledged that lysosomes host more than 60 soluble lysosomal hydrolases and accessory proteins as well as over 120 lysosomal membrane proteins and transitory residents [1], which participate in multiple processes such as immune response, nutrient sensing, gene regulation, secretion, plasma membrane repair, metal ion homeostasis, and cholesterol transport. Mutations in genes which encode lysosomal hydrolase, enzyme activators, or membrane proteins can cause accumulation of macromolecules in cells or tissues, forming pathological changes in the body and leading to lysosomal storage diseases (LSDs). LSDs are a spectrum of rare genetic metabolic disorders comprising over 70 monogenic disorders of lysosomal catabolism [2], including mucopolysaccharidoses (MPS), mucopolipidoses (ML), sphingolipidoses, partial glycogen storage diseases, oligosaccharidosis, and so on, most of which are inherited as autosomal recessive traits. The clinical presentations of LSDs are broad, involving the skeletal system, central nervous system (CNS), cardiovascular system, digestive system, and immune system. Besides, some LSD patients have special facial features like arched eyebrow, ocular hypertelorism, low nasal bridge, or anteverted nostrils. The clinical manifestations generally are severe for most LSD patients, who usually died before adulthood.

Mucopolysaccharidoses (MPS) are a group of genetic disorders caused by defective lysosomal enzymes which hydrolyze different glycosaminoglycan (GAGs). MPS are divided into 7 types: MPS type I, II, III, IV, VI, VII, IX. MPS type II and IV, which are caused by mutations in gene *GALNS* and *IDS* respectively, have higher incidence than other types, in China. MPS type VII (MIM#253220) has extremely low incidence in China, characterized by the inability to degrade glucuronic acid-containing GAGs. Gene *GUSB* (NM\_000181) encodes for beta-glucuronidase, which plays an important role in the degradation of dermatan and keratan sulfates [3,4]. MLs are caused by the loss of UDP-GlcNAc-1-phosphotransferase (GNPT) activity, which prevents the formation of mannose 6-phosphate (M6P) recognition marker. M6P cannot bind to the M6P receptors (MPR), which subsequently leads to miss sorting and hypersecretion of multiple lysosomal enzymes [5]. MLs are divided into 3 types: ML II alpha/beta (MIM#252500), ML III alpha/beta, and ML III gamma. ML II alpha/beta and ML III alpha/beta are caused by mutations in the *GNPTAB* gene (NM\_024312); the *GNPTAB* gene codes for the alpha/beta subunit of GNPT. ML III gamma can cause mutations in the *GNPTG* gene, and *GNPTG* codes for the gamma subunit of GNPT. The clinical manifestation of ML is similar to that of some types of MPS, and are difficult to identified without molecular genetic analysis. Oligosaccharidoses are a group of rare LSDs caused by defective oligosaccharide hydrolyzes with accumulation of related

oligosaccharide in tissues, including fucosidosis (MIM#230000) and  $\alpha$ -mannosidosis. The incidence of these disorders is extremely low. Fucosidosis is caused by abnormal alpha-L-fucosidase which hydrolyze the alpha-1,6-linked fucose joined to the reducing-end N-acetylglucosamine of the carbohydrate moieties of glycoproteins [6]. Gene *FUCA1* (NM\_000147) encodes for the alpha-L-fucosidase.

LSDs are a group of diseases with variety of forms, and some individual diseases have similar clinical manifestations. The diagnosis of LSDs is based on clinical findings combined with genetic analyses (the classic strategy), and in many cases, the enzyme/substrate abnormal assay is also crucial for diagnosis. Because of the phenotype heterogeneity, classification of LSD has to depend on genetic analyses using technology. In this study, by using next generation sequencing combined with clinical manifestations, we were able to identify 5 novel mutations causing LSD in 4 patients: 1 patient had MPS type VII mutation, 1 patient had fucosidosis mutation, and 2 patients had ML II mutation. We confirmed the pathogenicity of each mutation.

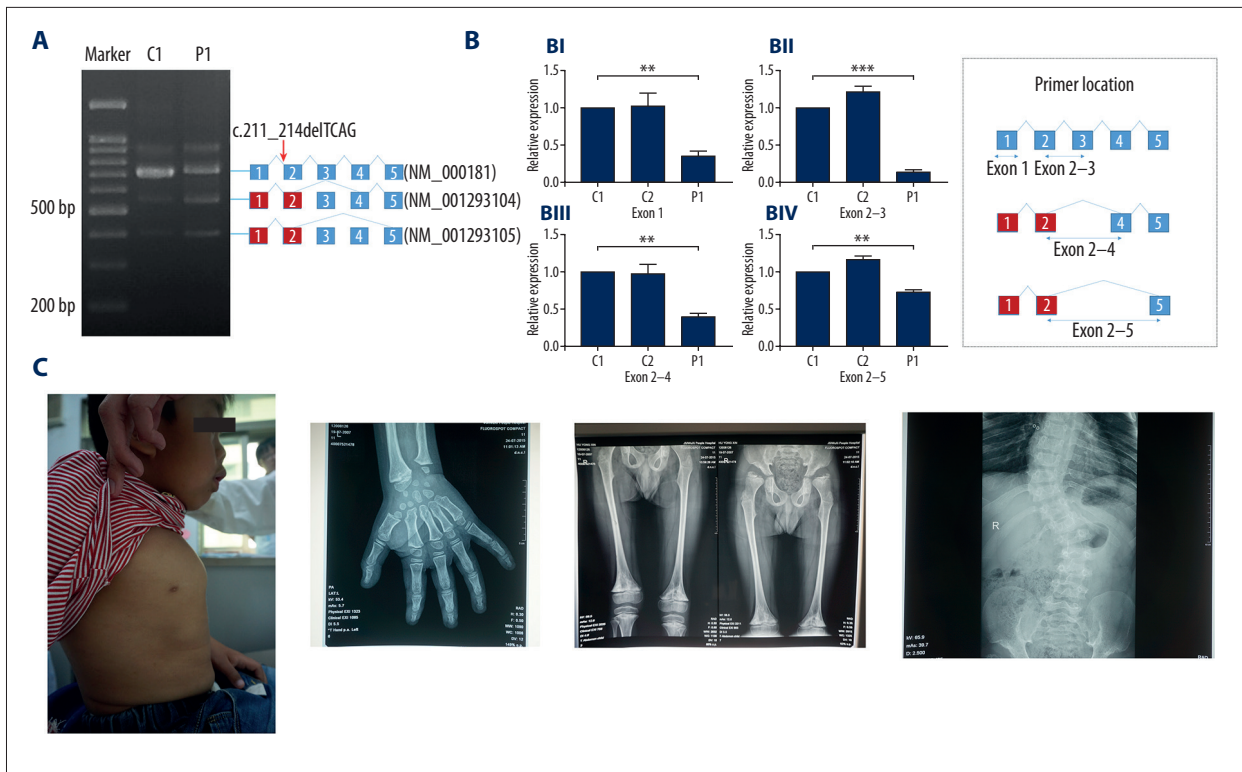
## Material and Methods

### Patients and controls

This study included 4 affected children, from 4 unrelated families, who were recruited at Hunan Jiahui Genetic hospital of China from 2015 to 2017. One of these patients was from a consanguineous marriage family. All of the patients had dysostosis multiplex with multisystem disorder, and were diagnosed with suspected mucopolysaccharidosis, while we recruited 2 normal individuals as controls. Informed consent was obtained from the parents of all patients. Research complied with the Declaration of Helsinki and its later amendments.

### Patient 1

Patient 1 was a male whose first visit to the hospital was at 8 years old. He was 110 cm tall ( $<P_{97}$ ), which was shorter than other children of the same age. He weighed 22 kg ( $P_3 \sim P_{50}$ ), and his head circumference was 54 cm (normal). The patient appeared normal at birth and developed normally before 1-year of age. He was found to have a thoracic deformity when he was 1 year of age, presented as bulging, but he wasn't treated subsequently. Until he was 6 years old, the condition of skeletal dysplasia worsened, and he was found to have limbs length discrepancy, and lumbar scoliosis. X-ray of his chest and hands at the age of 8 years was suggestive of thoracolumbar gibbus, left pointed proximal metacarpals, and increased inhomogeneities of density of the distal radius, sharpened bilateral iliac bone, and shallowed acetabulum (Figure 1C). His left



**Figure 1.** The pathogenic of mutation c.211\_214delTCAG in *GUSB*. **(A)** Amplification results show that Patient 1 and the control both had 3 transcripts (NM\_000181, NM\_001293104, NM\_001293105), transcript variant 3 skipped exon 3, and transcript variant 4 skipped exon 3 and 4, but Patient 1's 739 bp bands became lighter, as well as 554 bp and 411 bp bands were always brighter than the control's. Schematic diagram shows the pattern of each transcript, the red block represents 5'-UTR. **(B)** The results of quantitative real-time polymerase chain reaction results of Patient 1 with small deletion mutation and the 2 normal controls. **(BI)** *GUSB* total mRNA levels were nearly 70% lower in Patient 1 than in normal controls (\*\*  $P=0.0024$ ). **(BII)** The mRNA level of product of transcript variant 1 (\*\*\*)  $P=0.0003$ ) was nearly 90% lower in Patient 1 than in normal controls, but the **(BIII, BIV)** mRNA levels of products of transcript variant 3 (\*\*  $P=0.0016$ ) and transcript variant 4 (\*\*  $P=0.0012$ ) remained over 40% and even 70% compared with controls. **(C)** Patient 1 presented typical MPS skeletal malformation: lumbar scoliosis, limbs length discrepancy, and carpal dysplasia.

leg length was 67.5 cm, and his right leg length was 65 cm. We learned after follow-up that he died of severe pneumonia at the age of 11 years.

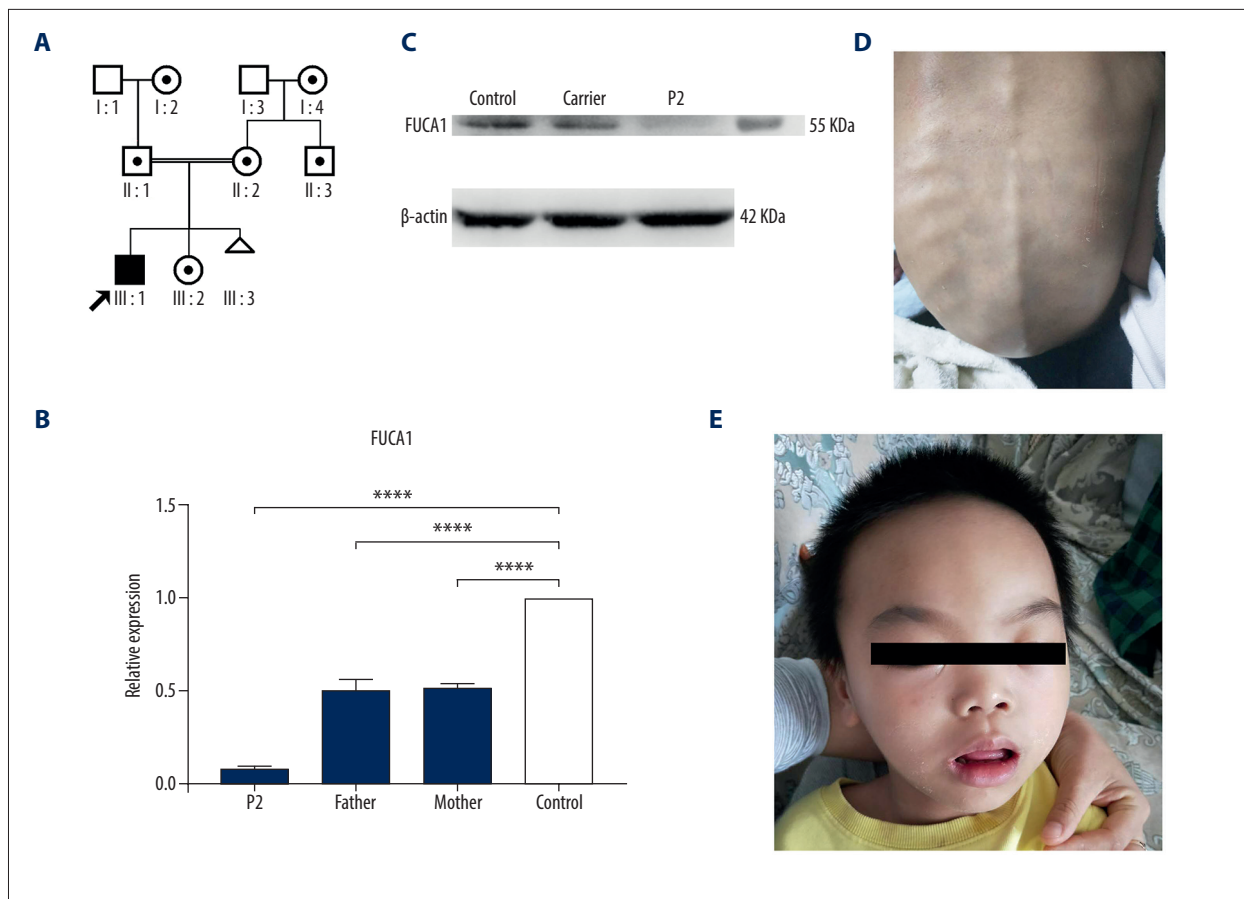
**Patient 2**

Patient 2 was a male whose first visit to the hospital was at 4 years old. He was from a consanguineous marriage family (Figure 2A). He was 90 cm tall ( $<P_{97}$ ) and weighed 17 kg ( $P_3 \sim P_{50}$ ). He was shorter than most children of the same age. He was admitted to the hospital for seizures and psychomotor retardation at the age of 3 years. Biochemical analysis suggested alkaline phosphatase was 325.8 U/L (45.0–125.0 U/L) and fucosidase was 0.5 IU/L (5.0–40.0 IU/L). The patient could not sit up by himself and did not respond when his name was called. His skeletal symptoms are atypical and mainly presented as mild scoliosis (Figure 2D). X-ray of his femoral suggested no abnormality. Besides, there was no observable abnormal sign in his dermal system, while his special facial features

presented as frontal bossing, epicanthus, low nasal bridge, long philtrum, and thick lips (Figure 2E). He died 2 years after his visit to the hospital.

**Patient 3**

Patient 3 was a female whose first visit to our hospital was at 3 years of age. She was admitted to the hospital previously for recurrent pneumonia at the age of 4 months. The patient showed multiple malformations: aortic insufficiency, patent foramen ovale, arched eyebrow, low nasal bridge, broad nasal tip, long philtrum, clyllum, and talipes valgus. She had a heart operation at the age of 2 years, and died of heart failure at the age of 3 years. Besides, she also had some uncommon presentations for ML II patients, like long fingers and toes, and auricle anomaly presented as auricle notch (Figure 3A–3D).



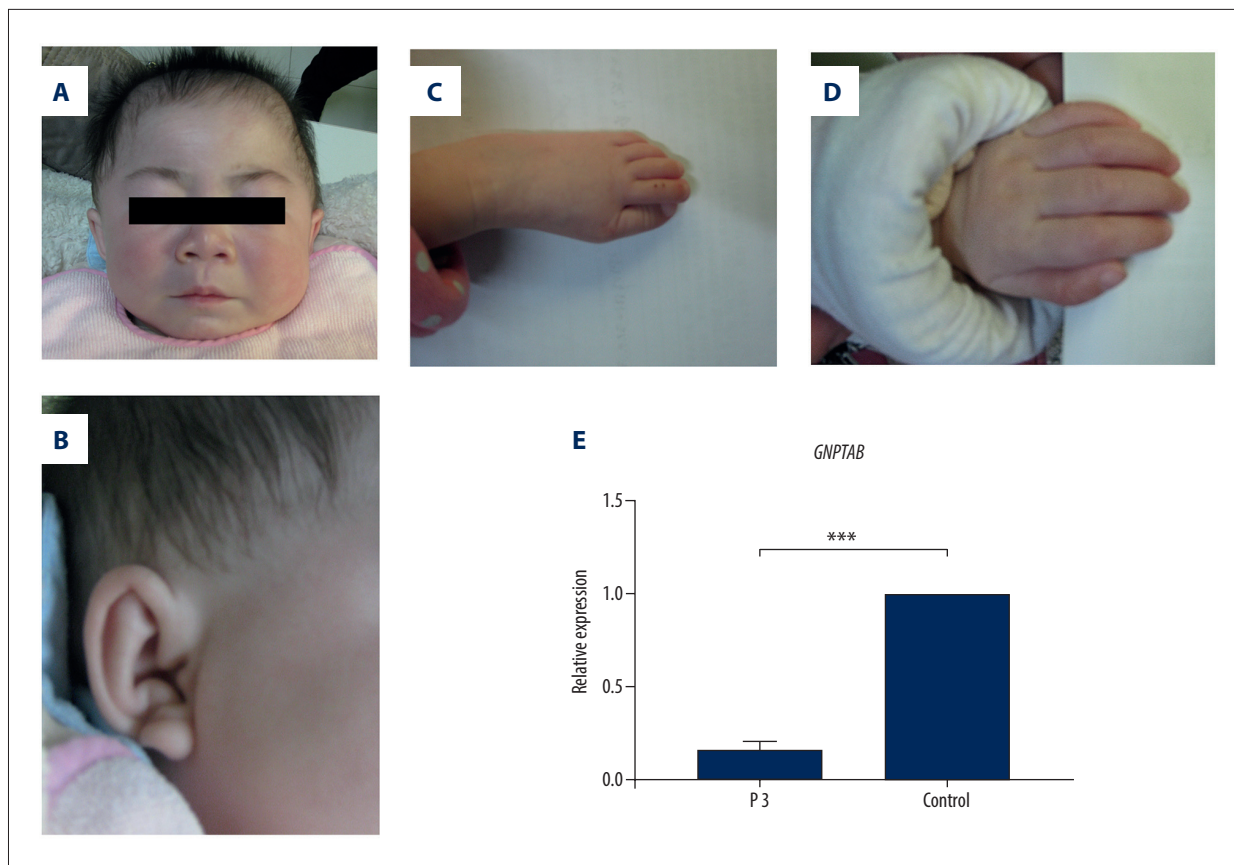
**Figure 2.** The pathogenic of mutation *c.717C>A* in *FUCA1*. **(A)** The pedigree of Patient 2, the arrow points out proband. **(B)** Quantitative real-time polymerase chain reaction results of Patient 2, his parents, and normal control: *FUCA1* mRNA levels were nearly 90% and 50% lower in Patient 2 and in his parents, respectively, than in normal controls (Patient 2: \*\*\*\*  $P<0.0001$ ; Father and Mother: \*\*\*\*  $P<0.0001$ ). **(C)** The western blot result of patient, mutation carrier, and normal control show that mutation *c.717C>A* affected protein expression. **(D, E)** The atypical phenotype of Patient 2 with fucosidosis. **(D)** Patient 2 has mild scoliosis. There was no observable abnormal presentation in dermal system. **(E)** Facial features mainly present as thick lips.

#### Patient 4

Patient 4 was a female whose visit to the hospital was at 3 years old. She was 79 cm tall ( $<P_{97}$ ), weighed 9 kg ( $<P_{97}$ ), and her head circumference was 44 cm ( $<P_{97}$ ). She was shorter than most children of the same age. At the age of 2 months, she had been admitted to hospital for recurrent pneumonia, and then found to have patent ductus arteriosus and thus underwent surgery for therapy. She presented as psychomotor retardation at the age of 4 months. When she was 5 months old, she was reported to have scoliosis, left ribs bulging, and talipes valgus. General examination found obvious facial features: ocular hypertelorism, low nasal bridge, short neck, and gingival edema. She died of heart failure at 4 years old.

#### Mutation analysis by next generation sequencing and Sanger sequencing

Genomic DNA was extracted from the peripheral blood lymphocytes of all patients and their parents according to standard phenol-chloroform extraction methods. We prepared a minimum of 3  $\mu$ g patient's DNA sample to establish libraries and enriched the target using GenCap custom enrichment kit (MyGenostics Inc., Beijing, China). The target library hybridization in solution utilized the biotinylated single-strand DNA capture probes, and exome libraries were sequenced on an Illumina HiSeq 2500 sequencer (Illumina, San Diego, CA, USA). After filtering and analyzing the data, we selected the suspected variants and used Sanger bidirectional and automated sequencing to validate the variants and parent source.



**Figure 3.** The atypical phenotype of Patient 3 with mucopolipidosis II. (A, B) Patient 3 had low nasal bridge, broad nasal tip, long philtrum, arched eyebrow and abnormal auricle, besides skin was white and smooth. (C, D) Patient 3's skeletal abnormalities included long fingers and long toes, which is rare in mucopolipidosis II patients. (E) Quantitative real-time polymerase chain reaction results of Patient 3 with mutation c.2404C>T and normal control. GNPTAB mRNA levels were nearly 80% lower in Patient 3 than in normal controls (Patient 3: \*\*\*  $P=0.0008$ )

#### Identification of splice transcripts by real-time polymerase chain reaction (RT-PCR)

Total RNA was extracted from the lymphocyte cell lines of all patients and controls using a standard TRIzol method. The cDNA was reverse transcribed from 1000 ng mRNA using RevertAid First Strand cDNA Synthesis Kit (Thermo, USA). Gene *GUSB* and *GNPTAB* related exons of Patient 1 and Patient 4 were amplified with primers (see Supplementary Table 1). The products were verified by agarose gel electrophoresis and polyacrylamide gel electrophoresis (PAGE), and were sequenced by Sanger Sequencing (Biosune, China).

#### Quantitative mRNA analysis by RT-PCR

To explore the mRNA expression patterns of Patient 1, Patient 2, and Patient 3 with the mutations in *GUSB*, *FUCA1*, and *GNPTAB* genes, we performed real-time polymerase chain reaction (RT-PCR) using cDNA of Patient 1, Patient 2, and Patient 3, in a total volume of 10  $\mu$ L consisting of 5  $\mu$ L Maxima SYBR Green

qPCR Master Mix (Thermo Fisher, Carlsbad, CA, USA), 0.25  $\mu$ L of 10  $\mu$ M each primer, 2.5  $\mu$ L cDNAs diluted to one-third total concentration, and water. The amplification reaction was performed using ASA-9600 qRT-PCR System (BAIYUAN GENE-TECH, Suzhou, China) in triplicate and run according to the manufacturer's 2-step cycling protocol. Specific primers were designed for target gene based on cDNA sequence using Primer 5 software (Supplementary Table 2). Amplification levels of gene *GUSB*, *FUCA1*, and *GNPTAB* were calculated according to the  $2^{-\Delta\Delta CT}$  method, while mRNA level of housekeeping gene *ACTB* was normalized as internal control.

#### Protein expression level analysis by western blot analysis

Lymphocyte cells from Patient 2 were lysed in RIPA lysis buffer (Beyotime) containing protease inhibitor PMSF (Beyotime). Protein concentrated from the lysed cells was quantitated using the BCA Protein Assay Kit (Thermo Scientific). Then, 50  $\mu$ g of total protein was subjected to 10% SDS-PAGE and transferred onto a polyvinylidene difluoride (PVDF) membrane.

**Table 1.** Summary of the phenotype and genotype characteristics of each patient.

	1	2	3	4
<b>Sex</b>	Male	Male	Female	Female
<b>Age</b>	8 years	4 years	3 years	3 years
<b>Height</b>	<97%	<97%	–	<97%
<b>Weight</b>	Normal	Normal	Birth weight normal	<97%
<b>Head circumference</b>	Normal	–	Normal	<97%
<b>Gene</b>	GUSB	FUCA1	GNPTAB	GNPTAB
<b>Genotype</b>	c.211_214delTCAG* c.1270C>T*	c.717C>A*	c.2404C>T* c.1090C>T	c.1284+1C>A* c.1090C>T
<b>Age of onset</b>	1 year	3 years	neonate period	2 months
<b>Skeletal malformations</b>	Thoracic deformity, lumbar scoliosis, limbs length discrepancy	Scoliosis	Cyllum, long fingers and toes	Scoliosis, left ribs bulging, clubfeet, uneven bone density at distal femur
<b>Cognitive and psychiatric manifestations</b>	Psychomotor retardation	Psychomotor retardation	–	Psychomotor retardation
<b>Facial characteristic</b>	Short neck	Frontal bossing, epicanthus, low nasal bridge, long philtrum, thick lips	Low nasal bridge, broad nasal tip, long philtrum, arched eyebrow, auricle anomaly	Ocular hypertelorism, low nasal bridge, short neck
<b>Others</b>	–	Seizures, poor response, amyosthenia, hypoimmunity, alkaline phosphatase 325.8	Aortic insufficiency, patent foramen ovale, pneumonia	Gingival edema, patent ductus arteriosus
<b>Outcome</b>	Died at 11 years old	Died at 6 years old	Died at 3 years old	Died at 4 years old

Height, weight and head circumference refer to standardized growth curve of height weight and head circumference for children and adolescents aged at 0 to 18 years in China.

The membrane was treated with blocking solution (5% skim milk powder solution) for 1 hour at room temperature and then incubated with rabbit anti-FUCA1 antibody (1: 100 dilution; Sangon Biotech, Shanghai, China) overnight at 4°C. After being washed with TBST, goat anti-rabbit antibodies (1: 10 000 dilution; Sigma) were incubated for 1 hour at room temperature. Specific protein bands were visualized after incubation with ECL™ Prime Western Blotting Detection Reagent (GE Healthcare Life Sciences). We used β-actin as the internal control.

## Results

We detected 6 mutations including 5 novel mutations for 4 affected individuals. Compound heterozygous mutations c.211\_214del and c.1270C>T in the *GUSB* gene were detected in Patient 1, which caused MPS VII. Homozygous mutation c.717C>A was detected in the *FUCA1* gene of Patient 2, which

caused a rare fucosidosis. Compound heterozygous mutations c.2404C>T and c.1090C>T were found in the *GNPTAB* gene of Patient 3, which caused ML II. We also detected compound heterozygous mutations c.1284+1C>A and c.1090G>A in the *GNPTAB* gene of Patient 4. We identified patients' characteristics (genotype and phenotype); information of identified novel mutations are summarized in Tables 1 and 2, respectively. Verification results of novel mutations pathogenicity were performed.

### Compound heterozygosity for 2 mutations in the *GUSB* gene cause mucopolysaccharidosis VII

We detected compound heterozygous mutations c.211\_214delTCAG and c.1270C>T in the *GUSB* gene, both reported first in this paper. We confirmed that the detected mutations were on different alleles by sequencing samples from the patients of Patient 1. Mutation c.211\_214del TCAG was

**Table 2.** Detailed information of identified novel mutations in all patients.

Gene	Genotype	Mutation at protein level	Mutation type	Mutation Taster prediction and score	PolyPhen-2.0 prediction and score	The sorting intolerant from tolerant SIFT and score	Human Splicing Finder3.1	Conservation analysis GERP++	Population frequency ExAC 1000G	
<i>GUSB</i>	c.211_214del	p.S72Afs*34	Frame-shift	N/A	N/A	N/A	Site broken/ESS site creation	Conserved (2.57)	0	0
	c.1270C>T	p.H424Y	Missense	Polymorphism	Possibly_damaging (0.785)	Damaging (0.019)	N/A	Conserved (2.92)	0	0
<i>FUCA1</i>	c.717C>A	p.Y239*	Nonsense	Disease causing	N/A	N/A	N/A	Conserved (4.3)	0	0
<i>GNPTAB</i>	c.2404C>T	p.Q802*	Nonsense	Disease causing	N/A	N/A	N/A	Conserved (3.8)	0	0
	c.1284+1G>T	N/A	splicing	N/A	N/A	N/A	Site broken	Conserved (4.78)	0	0

predicted to lead to the premature stop codon (p. S72Afs\*34). Besides, the missing TCAG in the former 4 bases of exon 2 of gene *GUSB*, this deletion might affect the splicing of mRNA. According to the predicted result of Human Splicing Finder, the small deletion would lead to the splicing site being broken and ESS (exon splice silence) site being activated.

To explore if the splicing pattern was affected by mutation c.211\_214delTCAG in the *GUSB* gene, we performed RT-PCR with the mRNA of Patient 1. According to the result of PAGE, Patient a and the normal control had the same transcript pattern, but there was a difference in brightness (Figure 1A). Based on UCSC Genome Browser, direct sequencing of all amplification products verified that products 739 bp, 554 bp, and 411 bp bands were from different transcripts (NM\_000181, NM\_001293104 and NM\_001293105, respectively). Transcript variant 3 (NM\_001293104) skipped exon 3, and transcript variant 4 (NM\_001293105) skipped exon 3 and 4. We also performed RT-qPCR to compare the *GUSB* gene expression level of Patient 1 with that of normal control. *GUSB* total mRNA levels were nearly 70% lower in Patient 1 than in the normal control. We designed primers to detected expression level of each transcript. The expression level of transcript variant 1 (NM\_000181) was nearly 90% lower in Patient 1 than in the normal control, while the expression levels of transcript variant 3 and variant 4 still remain 40% or even 70% in Patient 1 compared with the control (Figure 1B).

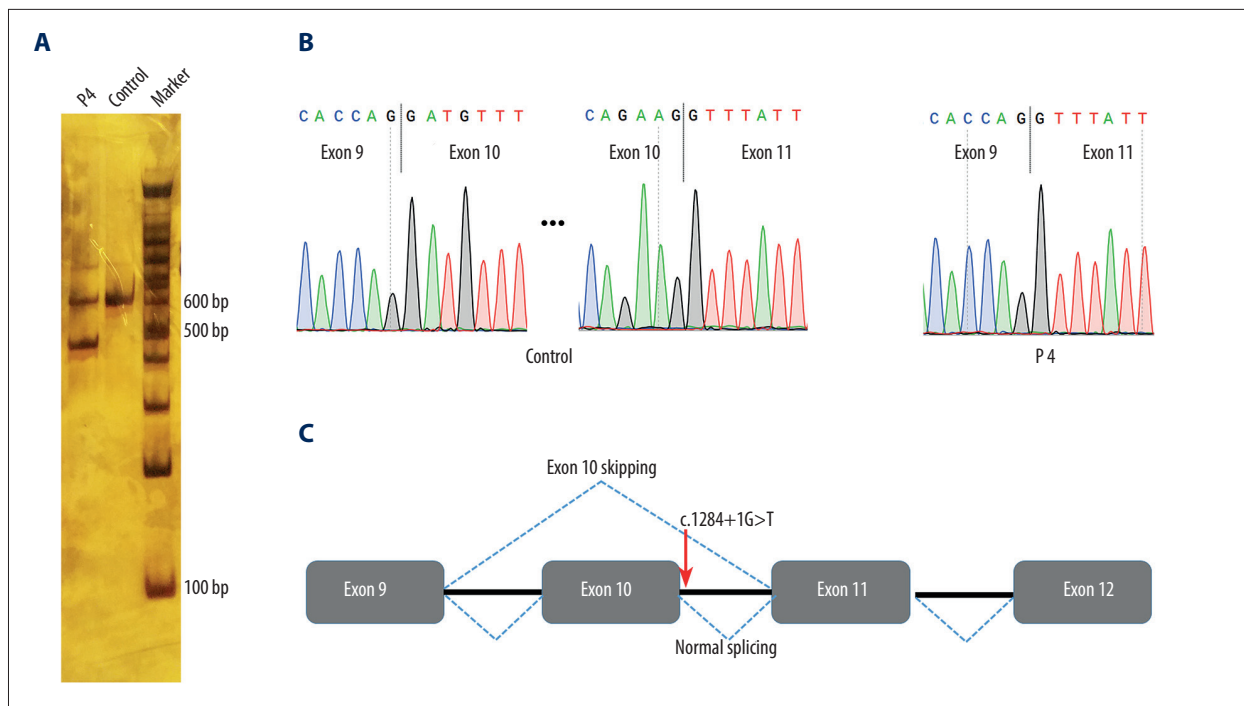
#### Novel homozygous mutation in *FUCA1* caused a rare fucosidosis case

We found a novel homozygous mutation c.717C>A in the *FUCA1* of Patient 2, which was inherited from his parents.

We performed RT-qPCR to compare the gene *FUCA1* expression levels of Patient 2, his parents, and normal control. There was almost no expression of *FUCA1* mRNA in Patient 2, and mRNA expression levels were nearly 50% lower in mutation carriers than in the normal control ( $P<0.0001$  for all) (Figure 2B). The western blot showed that mutation caused the decrease of protein *FUCA1* expression level (Figure 2C), and mutation carriers still had partial protein expression, but lower than the normal control.

#### Compound heterozygous mutations in gene *GNPTAB* caused 2 mucopolipidosis II cases

We detected 3 mutations in *GNPTAB* in Patient 3 and Patient 4, including 2 novel mutations c.2404C>T and c.1284+1C>A. The patients shared a same known nonsense mutation c.1090G>A, but had different phenotypes. We performed RT-qPCR to detect mRNA expression levels in heterozygous mutation c.2404C>T carrier. Primers we designed were located before the mutation c.1090G>A. The mRNA expression level of Patient 3 decreased to 30% compared to the normal control ( $P=0.0008$ ) after assay verification (Figure 3E). We used PAGE to explore the splicing pattern and found that heterozygous mutation c.1284+1G>T caused the generation of an additional transcript product (Figure 4A). Sanger sequencing results of the PCR amplification products confirmed that the alternative splicing from exon 9 directly to exon 11 generated a shorter isoform in which exon 10 was skipped (Figure 4B, 4C).



**Figure 4.** Real-time polymerase chain reaction results of mutation c.1284+1G>T in *GNPTAB*. (A) Amplification results shows that Patient 4 had additional amplification product compared with control, the 630 bp band represents the normal products, the 459 bp band represents the deletion of 1 exon. (B) Sanger sequencing results of cDNA product in Patient 4 compared with control shows the additional amplification product skip the exon 10. (C) Schematic diagram showing the pattern of exon skipping in Patient 4.

## Discussion

We reported molecular genetic study of an MPS VII male patient in China with compound heterozygous mutations c.211\_214delTCAG and c.1270C>T in gene *GUSB*. Incidence of MPS VII in China is extremely low, with almost no reports. There are 66 mutations associated with MPS VII reported in the Human Gene Mutation Database (HGMD professional 2018.4), most of which are missense mutations, and only 5 small deletion mutations. Because missing TCAGs are located at the beginning of exon 2, and the HSF predicted that the mutation might break the splicing site, we speculate that the small deletion mutation c.211\_214delTCAG not only caused frame-shift, but might also affect the splicing of mRNA. Transcript variant 1 (NM\_000181) is the main product in healthy individuals. There is merely a small amount of transcript variant 3 (NM\_001293104) and variant 4 (NM\_001293105) products in healthy individual lymphocytes. The results of our experiment suggested the mRNA, which was derived from the patient exhibited an increased ratio of transcript variant 3 and 4 to transcript variant 1 compared with controls. We speculate that mutation c.211\_214delTCAG might lead to transcript variant 1 substituted by transcript variant 3 and 4 due to potential ESS site activated, as seen in the similar research [7]. Mutation c.211\_214delTCAG might have a dual effect. As for

why the expression level of total mRNA remained less than 50%, we speculate that another missense mutation c.1270C>T might destroy the stability of mRNA, and the specific mechanism needs further experiments to verify. The mutation c.1270C>T has not been reported in 1000G or ExAC. To judge the pathogenicity of this novel mutation, we used the online predicted software. The predictions from PolyPhen-2\_HDIV was "POSSIBLY DAMAGING" and the Sorting Intolerant from Tolerant (SIFT) was "Damaging". Combining with the results of our clinical analysis, our conclusion was that the compound heterozygous mutations c.211\_214delTCAG and c.1270C>T were likely to be pathogenic for this patient.

Patient 2 have fucosidosis, which was caused by homozygous mutation c.717C>A in gene *FUCA1*. There are only 34 mutations associated with fucosidosis reported in HGMD, so far. Most of them are missense/nonsense mutations, in which there are 7 missense mutations and 10 nonsense mutations. Fucosidosis has been rarely reported in China, especially in the mainland. There was 1 case reported in Hongkong and Taiwan [8] respectively. In this paper, we reported and studied the molecular genetic basis of a male fucosidosis child who was from a consanguineous marriage family. Fucosidosis has been classified into 2 subtypes according to the severity of clinical signs and symptoms. Type I has a severe infantile presentation (neurological



functions impairment starting before 1 year of age) and severe skeletal malformations; type II has progressive features (dermal system impairing obviously). In our study, Patient 2 had no dermal system signs for fucosidosis, just mild skeletal symptoms and psychomotor retardation, and his presentations look like a German case [9], which was more inclined to belong to type I. Nonsense mutation c.717C>A in *FUCA1* can lead to atypical fucosidosis Type I.

We detected 3 mutations of *GNPTAB* in 2 ML II patients, including 2 novel mutations. Two patients shared the same known nonsense mutation c.1090C>T, but their phenotypes were different. The  $\alpha/\beta$  subunit of GNPT has a complex modular structure composed of at least 3 domains including an N-terminal domain with a putative nucleotide binding site, 2 Notch repeat-like domains, and a DMAP1 binding-like domain [10,11]. The function of these 3 domains is unknown, but they could play an important role in the interaction with other protein partners or maintaining stability of multi-subunits assembly of GNPT. In our study, Patient 4 had the splicing mutation c.1284+1G>T and had typical phenotype features of MPS, including scoliosis, left ribs bulging and talipes valgus. Patient 3 showed some uncommon phenotypes like long fingers and toes, and auricle anomaly. Splicing mutation c.1284+1G>T leads to exon 10 skipping; exon 10 maps to N-terminal domain, and mutation c.2404C>T (p. Q802X) maps to the DMAP1 binding-like domain. We suspected the splicing mutation led to the broken of N-terminal domain in Patient 4, so she presented without atypical phenotype. Because of significant reducing of mRNA, the GNPT of Patient 3 almost lost the function totally, so she presented with more diverse dysmorphism.

Most MLII patients usually show short stature, skeletal abnormalities, cardiomegaly, and developmental delay, while brachyphalangia is the main hands and feet abnormality. But Patient 3 in our study showed long fingers and toes, and auricle notch, which are uncommon in MLII patients. Bone development and remodeling requires a balance between bone-forming osteoblasts and bone-resorbing osteoclasts [12–18]. The increased osteoclastogenesis is a major skeletal pathomechanism in MLII [14]. Because of the uncommon phenotype, we re-analyzed the WES whole genome sequencing) data, and excluded other skeletal abnormality related genes. We also excluded

the possible copy number variation by single nucleotide polymorphism (SNP)-array. We think that gene *GNPTAB* mutations could lead to such rare phenotypes as long fingers and toes, and auricle notch.

In our study, we detected 6 mutations in 4 patients, of which 5 were novel mutations, including 1 missense mutation, 1 frameshift mutation, 1 splicing site mutation, and 2 nonsense mutations. According to these results, 3 mutations (c.211\_214delTCAG in *GUSB*, c.717C>A in *FUCA1*, and c. c.2404C>T in *GNPTAB*) caused significant reduction of the expression level of mRNA. We speculate nonsense-mediated mRNA decay (NMD) might be the cause of these results. NMD is identified as a widespread mRNA surveillance machinery in degrading “aberrant” mRNA species with premature termination codons (PTCs) rapidly, which protects the cells from the accumulation of truncated proteins [19–21]. Our comparison of height, weight and head circumference used the standardized growth curve of height weight and head circumference for children and adolescents aged at 0 to 18 years in China [22].

In order to verify this speculation, further research is needed, for example cycloheximide (CHX, an inhibitor of NMD) treatment assay.

## Conclusions

Mucopolysaccharidosis VII, fucosidosis and mucopolipidosis II are all rare LSDs in China or even in the world, characterized by low incidence and sporadic cases reported. In our study, we identified 4 rare LSD cases, and described the phenotypes of all patients in detail, including a rare mucopolipidosis II patient with uncommon phenotypes. These results expand the phenotype spectrums of related diseases. We also detected 5 novel mutations, and confirmed the pathogenicity of each mutation by molecular assays. These results will help us to understand the genotype-phenotype correlations in the disease, promote the clinical and molecular diagnosis, and even prenatal diagnosis.

## Conflicts of interest

None.

## Supplementary Data

**Supplementary Table 1.** Primers used in the RT-PCR.

Genes	Exon	Name	Sequence (5'-3')	Tm (°C)	Product length (bp)
<i>GUSB*</i>	1-5	C1F	ATGCTGTACCCCAAGGAGAG	59	739
		C1R	CATTCGCCACGACTTTGTTTT		
<i>GNPTAB**</i>	8-12	C1F	GCCAGTCTAAGCAGGATGAAG	57	630
		C1R	CACAGAACTTATCAGCGAGCC		

\* Based on reference sequence NM\_000181; \*\* based on reference sequence NM\_024312.

**Supplementary Table 2.** Primers used in the RT-qPCR.

Genes	Exon	Name	Sequence (5'-3')	Tm (°C)	Product length (bp)
<i>GUSB*</i>	1	1F	TGTACCCCAAGGAGAGCCCG	60	115
		1R	CGCCGGTACCACTGCTCCTC		
	2-3	2-3F	TGTGGTACGAACGGGAGGTG	60	177
		2-3R	GGACCAGGTTGCTGATGTCCG		
	2-4	2-4F	CATTCCTATGCCATCGTGGTATC	60	206
		2-4R	TGAACAGGTTACTGCCCTTGACA		
2-5	2-5F	TCCTATGCCATCGTGGGCTG	60	190	
	2-5R	AGATAGGCAGGGCGTTCGTG			
<i>FUCA1**</i>	2-3	Q1F	GTCCTGTGTCTTGGAACTGGAAGCTC	60	174
		Q1R	GCTGTGTTTTGAAGCCATTTTTCTT		
<i>GNPTAB***</i>	8-9	Q1F	ATAACCCCAAGGATTTTCAAG	54	194
		Q1R	GATAGATCGCAATGAGTACCTC		
<i>ACTB</i>	4-5	ACTB-F	GCACTCTTCCAGCCTTCCTT	60	106
		ACTB-F	CGTACAGGCTTTGCGGATG		

\* Based on reference sequence NM\_000181; \*\* based on reference sequence NM\_000147; \*\*\* based on reference sequence NM\_024312.

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