

Original Article

Compound heterozygous *GNPTAB* mutations cause mucopolidosis II or III alpha/beta in two Chinese families

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Abstract: Objective: Mucopolidosis II and III alpha/beta (ML II & ML III alpha/beta) are rare autosomal recessive lysosomal storage disorders. ML II is clinically evident from birth with a progressive course and fatal outcome in childhood. The typical phenotypes of ML II include limited statural growth, craniofacial abnormality, skeletal malformation, intelligence developmental deficiency and visceral organ abnormality. ML III is milder than ML II. Mutations in *GNPTAB* cause the ML II/III. Methods: Two families with ML II/III (initially undiagnosed) were recruited. We applied whole-exome sequencing (WES) and filtered mutations by genes causing lysosomal storage diseases with skeletal involvement. Mutational analysis and co-segregation confirmation were then performed. Results: We presented two families with ML II or ML III alpha/beta. By WES, the compound heterozygosity of *GNPTAB* (c.2404C>T, p.Q802* and c.2590dup, p.E864Gfs*4) is identified in a family with ML II, and c.1364C>T, p.A455V and c.2715+1G>A are detected in a family with ML III alpha/beta. Conclusion: We detected the causative mutations in two ML II/III families by WES and confirmed their diagnosis of the diseases. The present identification of mutations expands the spectrum of known *GNPTAB* mutations and it may contribute to novel approaches to genetic diagnosis and counseling for patients with ML II/III.

Keywords: *GNPTAB*, ML II, ML III, GlcNAc-1-PT, whole-exome sequencing, lysosomal storage disorders

Introduction

Mucopolidosis II alpha/beta (ML II; OMIM 252-500) and Mucopolidosis III alpha/beta (ML III alpha/beta; OMIM 252600) are very rare autosomal recessive lysosomal storage disorders, originally called Inclusion Cell Disease (I-cell disease) [1, 2]. Both of them are severe multi-organ diseases and result from a deficiency of N-acetylglucosamine-1-phosphotransferase (GlcNAc-1-PT or GNPT) [3]. Symptoms of ML II are observed at birth and cause progressive deterioration, and may result in death in childhood (most often during the first decade of life) [4]. The typical phenotypes of ML II include limited statural growth, craniofacial abnormality (including facial coarseness, prominent forehead, shallow orbits, depressed nasal bridge and hyperrophic gingiva), skeletal malformation

(including scoliosis, hip dislocation, long bone deformities, hand contracture and clubfeet), intelligence deficiency and visceral organ abnormality [5-7]. ML III is milder than ML II, with an onset in early childhood, slowly progressive course, and fatal outcome from early adulthood [8, 9].

GlcNAc-1-PT is a Golgi-resident enzyme complex and catalyzes the initial step in the production of the mannose 6-phosphate (M6P) recognition marker, which is essential for lysosomal hydrolase transport into lysosomes [10, 11]. GlcNAc-1-PT contains two α , two β and two γ polypeptides [12]. *GNPTAB* gene encodes the α - and β -subunits, and *GNPTG* encodes the γ -subunit [13, 14]. Human *GNPTAB* is located on chromosome 12q23.3 (13), it spans approximately 80 kb of cDNA consisting of 21 exons,

and encodes a 1256 amino acid (AAs) precursor protein (16). This precursor protein needs to be processed into mature α - and β -polypeptides by proteolytic cleavage at the Lys928-Asp929 bond [10, 15]. Mutations in the *GNPTAB* lead to ML II and ML III alpha/beta, and *GNPTG* mutations cause ML III gamma (OMIM 252605) [16].

In this study, we present two families from Central-South region of China, one with ML II and the other with ML III alpha/beta. In the first family, a compound heterozygosity of *GNPTAB* (c.2404C>T, p.Q802* and c.2590dup, p.E86-4Gfs*4) is identified, and we found c.1364C>T, p.A455V and c.2715+1G>A in the second family.

Materials and methods

Patients and subjects

The Review Board of Xiangya Hospital of the Central South University has approved this research. All subjects have consented to this study. Blood was collected from the proband and related family members. Segregation analysis was applied to all family members according to the WES results.

Whole-exome sequencing

Genomic DNA was extracted with a DNeasy blood and tissue kit (Qiagen, Valencia, Calif., USA). The Novogene Bioinformatics Institute (Beijing, China) provided the exome capture, high throughput sequencing and common filtering. All the exomes were captured by means of Agilent SureSelect Human All Exon V5 kits and were sequenced with an Illumina HiSeq2000 platform. Filtering the common variants (frequency >0.05) from the 1000 Genomes Project (<https://www.genome.gov/27528684/1000-genomes-project/>), YH (<http://yh.genomics.org.cn/>), dbSNP (<https://www.ncbi.nlm.nih.gov/SNP/>) and ESP (<http://evs.gs.washington.edu/EVS/>) databases, unique single-nucleotide polymorphisms (SNPs) were detected in subjects. Potential causative variants were screened by the list of genes about lysosomal storage diseases with skeletal involvement (Table S1) and then predicted by bioinformatics programs including MutationTaster (<http://www.mutation-taster.org/>), Polyphen-2 (<http://genetics.bwh.harvard.edu/pph2/>) and SIFT (<http://provean.jcvi.org/index.php>). The analyses of gene func-

tion, inheritance pattern, and clinical phenotype were conducted by Online Mendelian Inheritance in Man (OMIM) (<https://www.omim.org>).

Sanger sequencing

Primer pairs were designed by DNASTAR and the sequences of primers will be provided upon request. The target fragments were amplified with polymerase chain reaction (PCR) and determined by the ABI 3100 Genetic Analyzer (ABI, Foster City, CA).

Result

Clinical features

We first identified a family with ML II (Family 1) (**Figure 1A**). The proband (II:1) is a 3 year and 8 months old boy. He was born at term and diagnosed with inguinal hernia. One year after his birth, he had coarse facial features and no ability to speak and walk. Now, the proband has a short stature (75.3 cm, 10.4 kg), facial coarseness with depressed nasal bridge and shallow orbits, metopic prominence, thickened alveolar ridges, cup-ear and sparse hair in a part of the head, scoliosis, hand contracture, broad thumbs and big toes with abnormal nails, bowed lower limbs, intelligence developmental deficiency (his verbal expression was still limited to a few words) and inguinal hernia (**Figure 1B-G**). The family went to our department because the proband failed in unaided walking. We primarily diagnosed him with lysosomal storage disorder, most likely Mucopolysaccharidosis type 1H (MPS-1H, also named Hurler syndrome; OMIM 607015), and confirmed he had ML II after genetic diagnosis. His parents (I:1 and I:2) were not affected.

In the second family (Family 2) (**Figure 2A**), the proband (II:1) is a 14-year-old girl (150 cm, 42 kg) diagnosed with ML III alpha/beta, without intelligence developmental deficiency. No abnormalities were present at her birth and she walked independently at 2 and half years old. She was observed to have bilateral contracture of 2-5 fingers and talipes cavus before age 3 years (**Figure 2B, 2C**). The proband presented to our department for the pain of hip joints and was diagnosed with congenital dislocation of the hip (CDH) presenting unusual symptoms (shallow acetabulum and caput femoris, rug-

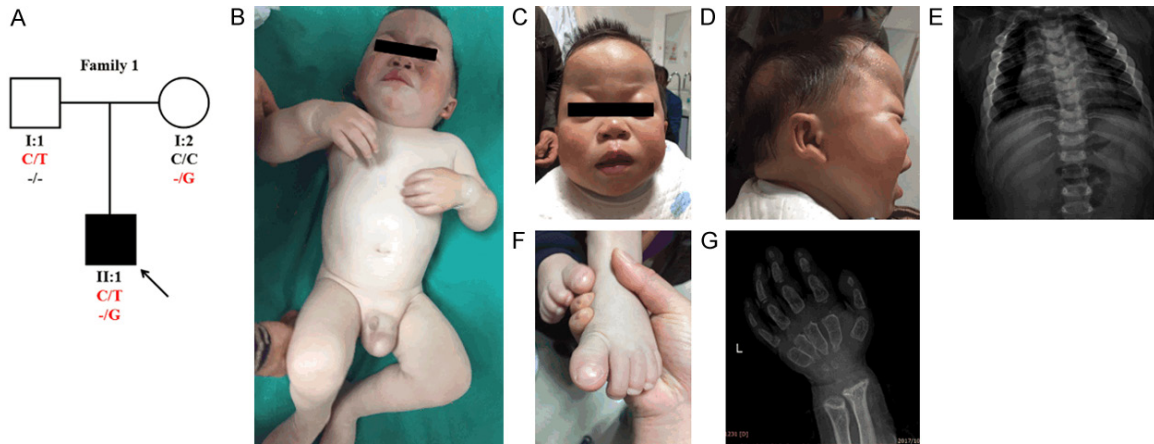


Figure 1. (A) Pedigree of the Family 1 with segregation analysis. Family members are identified by their generation (indicated by Roman numerals) and a number. Squares represent male family members and circles, female members. The black symbols represent a member affected and the white symbols represent unaffected members. The arrow indicates the proband. Genotype is identified by letters and slash, red represents mutations. (B-G) Phenotypes of the proband of Family 1 (B) is a full body shot of the proband of Family 1, who has coarse facial features (C), cup-ear (D), mild scoliosis (E), broad thumbs and big toes with abnormal nails (F, G) and inguinal hernia (B).

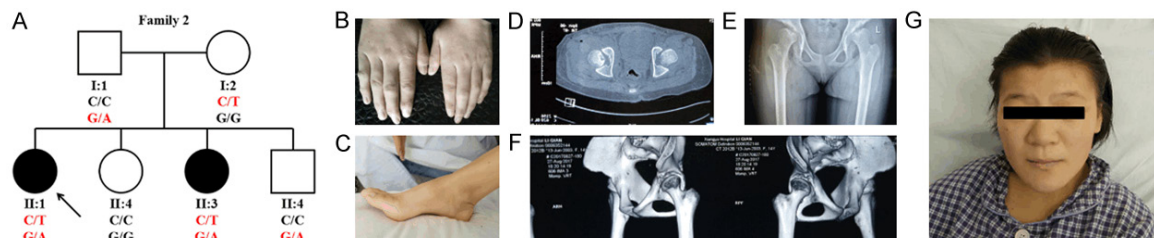


Figure 2. (A) Pedigree of the Family 2 with segregation analysis. The meanings of all symbols are same as Figure 1A. (B-G) Phenotypes of the proband of Family 2. The proband of Family 2 with ML III has bilateral contracture of 2-5 fingers (B), talipes cavus (C), congenital dislocation of the hip (D-F), and minor facial changes (G).

ged articulation coxae with low density focus) by the X-ray, CT and 3-D image (Figure 2D-F). Also, the proband had minor facial changes (Figure 2G). We initially diagnosed her with MPS or other lysosomal storage disorder. We thought her sister (II:3), a 4-year-old girl with bilateral contracture of digital joints and having the ability to walk independently at 3 years, might suffer from the same disease. Other members (I:1, I:2, II:2 and II:4) were not affected.

Genetic analysis

By WES, we detected 1156 unique SNPs in the proband of Family 1 and 1064 SNPs in the proband of Family 2. Variants were filtered by genes for lysosomal storage diseases with skeletal involvement. 2 *GNPTAB* variants were identified in each of two families, without other variants of genes of lysosomal storage diseases

(Table 1). The bioinformatic prediction about pathogenicity and the analysis of inheritance pattern, OMIM clinical phenotypes and American College of Medical Genetics classification all suggested these mutations causing these two families' diseases.

In Family 1, Sanger sequencing showed that a nonsense mutation (c.2404C>T, p.Q802*) of *GNPTAB* in the proband was inherited from his father and a frameshift mutation (c.2590dup, p.E864Gfs*4) from his mother (Figure 3A). Similarly, in Family 2, the mutations (c.1364C>T, p.A455V and c.2715+1G>A) of the proband were inherited from parents respectively, and co-segregated with the affected family members (Figure 3B). Neither of the mutations was identified in the 200 control cohorts that our group studied previously. The final diagnosis

GNPTAB mutations cause mucopolidosis II/III alpha/beta

Table 1. Variants identified by WES in combination with lysosomal storage diseases (skeletal involvement)-related gene-filtering in the present families

Family	Gene	Variant	MutationTaster	Polyphen-2	SIFT	OMIM clinical phenotype	American College of Medical Genetics classification
1	GNPTAB	NM_024312: c. 2404C>T: p.Q802*	D (1.000)	-	-	AR; Mucopolidosis II alpha/beta/AR; Mucopolidosis III alpha/beta	PVS1
1	GNPTAB	NM_024312: c.2590dupG: p.E864Gfs*	D (1.000)	-	-		PVS1
2	GNPTAB	NM_024312: c.2715+1G>A	D (1.000)	-	-		PVS1
2	GNPTAB	NM_024312: c. 1364C>T: p.A455V	D (1.000)	D (0.973)	D (0.000)		PM5

D, disease causing; AR, autosomal recessive; PVS, pathogenic very strong; PM, pathogenic moderate.

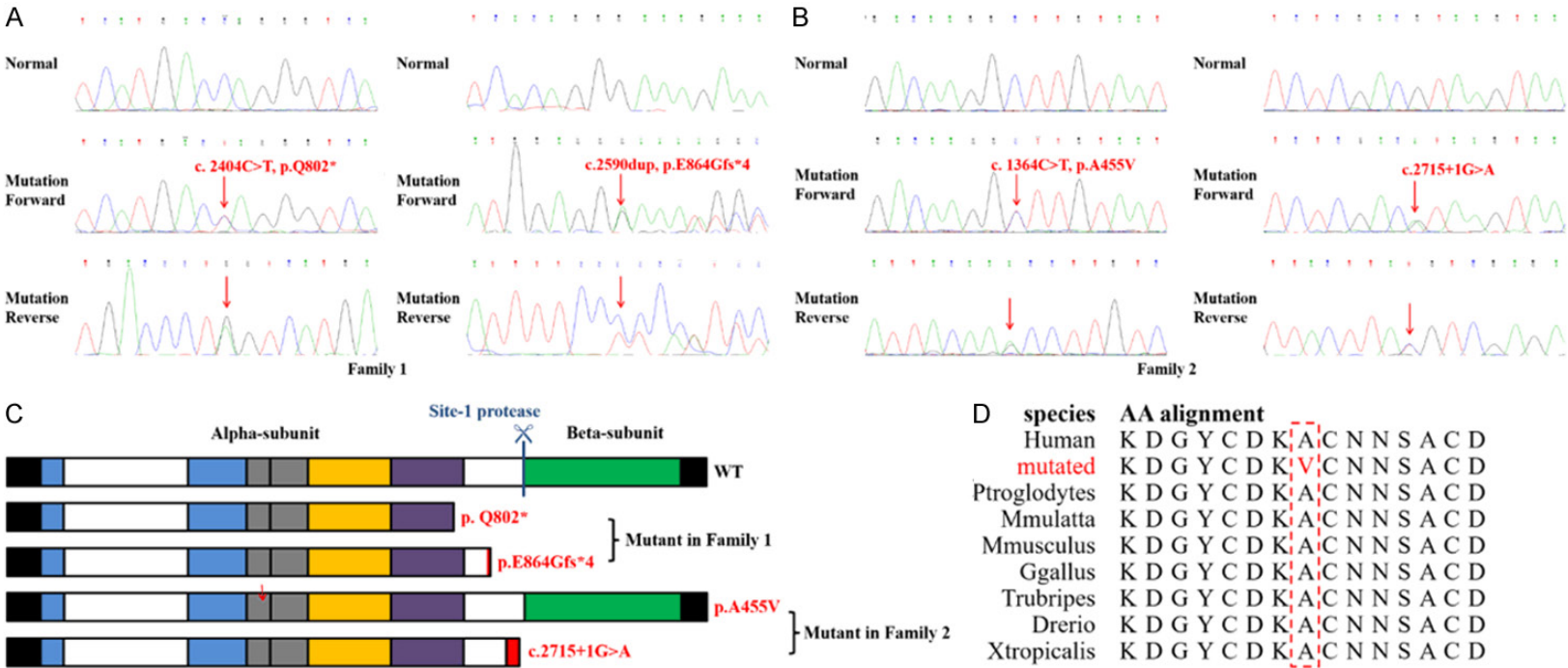


Figure 3. (A, B) Sequencing results of the *GNPTAB* mutations. Sequence chromatograms indicate compound heterozygosity (c.2404C>T, p.Q802* and c.2590dup, p.E864Gfs*4) in the proband of Family 1 (A) and indicate compound heterozygosity (c.1364C>T, p.A455V and IVS13+1G>A) in the proband of Family 2 (B). (C) A schematic presentation of the domain of the wild-type (indicated by “WT”) *GNPTAB* precursor and mutant *GNPTAB* precursor proteins in the present study (indicated by red words). The rectangular box represents the precursor protein with the N-terminus on the left and C-terminus on the right. The black box represents the trans-membrane domain, blue represents the Stealth domain, gray represents the Notch-repeat-like domain, yellow represents the γ-binding domain, purple represents the DMAP domain and green represents a conserved region. The position of the site-1 protease cleavage site is indicated by a blue vertical bar. Incorrect amino acids sequence in frameshift mutants are indicated by the red box, and the red arrows represent a mutated AA site. (D) This site of the α subunit with A455V is highly evolutionarily conserved across species. Red grapheme represents mutated amino acid, red box emphasizes these sites cross species to compare.

was that proband of Family 1 (II:1) has ML II and patients in Family 2 (II:1 and II:3) have ML III.

Discussion

In this study, we identified 4 mutations of *GNPTAB* in two families with ML II/III. *GNPTAB* precursor includes two transmembrane domains, two Stealth domains, Notch-repeat-like domains, a γ -binding domain, a DMAP domain and a conserved region (**Figure 3C**) [1, 17]. In the patient of Family 1, the nonsense mutation (c.2404C>T, p.Q802*) is expected to produce truncated protein and the frameshift mutation (c.2590dup, p.E864Gfs*4) transcribe into an incorrect transcript with a premature stop codon, which results in the GlcNAc-1-PT with deficiency or lack of biological activity and causes ML II (**Figure 3C**). Both have been described by Wang et al. (2018) and Liu et al. (2015) respectively in Chinese families with ML II/III [18, 19]. The splice-site mutation (c.2715+1G>A) has described already in patients from Japan, Korea and China [19-22]. In fact, it is the second most common mutation and appears to be quite frequent in Chinese patients. The mutation produces a transcript without the 13rd exon (1103 bases) and formations a new stop codon. The missense mutation (c.1364C>T, p.A455V) happens at Notch-repeat-like domains and the site is predicted to be evolutionarily conserved (**Figure 3C, 3D**).

At least 207 variants/mutations of *GNPTAB* has been reported (**Figure 4**). Summarizing these variants/mutations and referencing the previous predictions, it suggests that 1) most *GNPTAB* genotypes in the patients of ML II are homozygous or compound heterozygous either for frameshift mutations or for nonsense mutations; 2) the patients of ML III alpha/beta carry homozygous or compound heterozygous mutations at least including one missense or splice-site mutation [8]. Nonsense mutations produce a truncated protein, and frameshift mutations result in a premature stop codon or an entirely different following sequence. These “null” or “amorph” alleles would lead to endoplasmic reticulum (ER) retention of the mutant proteins [1, 17]. Therefore these mutants are always inactive and cause the severe ML II phenotypes. ML III patients mostly carry mutations that induce only partial loss of activity. Given II:3 in Family 2 harboring a missense mutation and a splice-site mutation of *GNPTAB* and

beginning to present slight symptoms, we diagnosed her with ML III alpha/beta in the early stage and advised her to receive treatment, for example physical therapy, to slow down the progression. This showed the superiority of genetic diagnosis in the early diagnosis of inherited progressive disease. Prediction is helpful to genetic diagnosis and also needs more cases to demonstrate it.

The symptoms of ML II and ML III are similar with MPS-diseases, including onset in the early period of life, facial coarseness, skeletal dysplasia, dysgnosia and visceral organ abnormality [23, 24]. But their disease-causing genes are different; for example, MPS-IH is caused by homozygous or compound heterozygous mutations of *IDUA* (encoding alpha-L-iduronidase) [25]. Genetic diagnosis is one of the best ways to distinguish between ML II/III and MPS-diseases, which can be widely used to distinguish various lysosomal storage disorders. Urine screens support the diagnosis. In patients with MPS-diseases, urinary excretion of glycosaminoglycans (GAG) increases. But GAG excretion of ML II/III is normal, while oligosaccharide may be excessive [4]. As to our patient of Family 1, urinary screening by toluidine blue test for GAG was negative. ML II and ML III are pathologically similar, but ML III is milder than ML II [2]. In the present study, the symptoms of the patient of Family 1 were more severe than patients of Family 2.

In conclusion, the present study identified two families from Central-South region of China, one with ML II and the other with ML III alpha/beta. In the first family, a compound heterozygosity of *GNPTAB* (c.2404C>T, p.Q802* and c.2590dup, p.E864Gfs*4) was identified, and we found c.1364C>T, p.A455V and c.2715+1G>A in the second family. The mutation c.1364C>T, p.A455V is first reported here. The present identification of mutations expands the spectrum of known *GNPTAB* mutations and it may contribute to novel approaches to genetic diagnosis and counseling for patients with ML II/III.

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V41Ffs
 1-2A>G W44* Q104*
 S15Y
 A34P
 S33Sfs
 Y56*
 V78del
 V77del
 D76G
 N148Tfs
 A147Afs
 T205P
 S187T
 S252*
 L251Tfs
 N250Kfs
 Y158Sfs
 E217Sfs
 A194T
 R209Lfs
 T284Lfs
 Y331C
 F374L
 C442Y
 R334P
 R334L
 R334Q
 R375*
 S445Ffs*4
 S385L
 S399F
 S399Afs
 A455S
 A455V
 G508Dfs
 Q507*
 C505Y
 E542Afs
 G575R
 H588Qfs
 R587*
 R587P
 G648D
 E667*
 I601M
 P625L
 S685Kfs
 K732N
 L730Cfs
 L730Kfs
 Q802*
 E864Gfs
 N865Kfs
 Q882*
 R879A
 L889*
 Y888*
 S887Kfs
 Y899Vfs
 K898Sfs
 H956Y
 A955V
 Y937_M972del
 I941Nfs
 Q967R
 M972Ifs
 L1033P
 T1032Hfs
 R1031*
 V1130Afs
 Y1111Ifs
 Y1111Hfs
 N1153S
 T1223del
 K1236R
 K1236M
 3335+6T>G
 3335+1G>A
 I1154Qfs
 E1248Lfs
 G1049Rfs
 V1130Afs
 L1054V
 T1163*
 L1137*
 H1158Qfs
 3136-2A>G
 S1058*
 L1054V
 L1137*
 H1158Qfs
 E1248Lfs

1-2A>G W44* Q104*
 S15Y
 A34P
 S33Sfs
 Y56*
 V78del
 V77del
 D76G
 N148Tfs
 A147Afs
 T205P
 S187T
 S252*
 L251Tfs
 N250Kfs
 Y158Sfs
 E217Sfs
 A194T
 R209Lfs
 T284Lfs
 Y331C
 F374L
 C442Y
 R334P
 R334L
 R334Q
 R375*
 S445Ffs*4
 S385L
 S399F
 S399Afs
 A455S
 A455V
 G508Dfs
 Q507*
 C505Y
 E542Afs
 G575R
 H588Qfs
 R587*
 R587P
 G648D
 E667*
 I601M
 P625L
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 L730Cfs
 L730Kfs
 Q802*
 E864Gfs
 N865Kfs
 Q882*
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 L889*
 Y888*
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 L1137*
 H1158Qfs
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 S1058*
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 L1137*
 H1158Qfs
 E1248Lfs

K4Q
 R46Q
 W81L
 I167N
 D190V
 Q278*
 D305Efs
 W357*
 I403Yfs
 I403T
 Y404*
 D407A
 C461G
 C528Vfs
 F566Lfs
 E637Dfs
 K694T
 L697Pfs
 E757Kfs*1
 E757Kfs*2
 D760Efs
 Q786*
 V781M
 S782F
 S654Pfs
 I656*
 N642Lfs
 T644M
 W894*
 N859Qfs
 R861K
 E849Kfs
 K850Nfs
 R925A
 Q926P
 K928R
 D1018G
 D1018V
 Q1021*
 L1025S
 L1001P
 A1104Hfs
 L1168Qfs
 3249+1G>C
 3250-1G>C
 3250-2A>G
 P1085Rfs
 R986C
 Y1078Tfs
 Y1079Lfs
 A994Sfs
 Y997C
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 2715+2T>G
 2715+93A>G
 R986Tfs
 3435+1G>A
 V1148Afs
 M1175Pfs
 3434+1G>A
 3435-1G>A
 R1189Qfs
 R1189*
 R1191C
 R1191H

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Disclosure of conflict of interest

None.

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GNPTAB mutations cause mucopolipidosis II/III alpha/beta

Table S1. Genes causing lysosomal storage diseases with skeletal involvement and disorders caused by them

Gene	Protein	Disorder	Inheritance
<i>IDUA</i>	Alpha-1-Iduronidase	Mucopolysaccharidosis type 1H/1S	AR
		Mucopolysaccharidosis type 5	AR
<i>IDUS</i>	Iduronate-2-sulfatase	Mucopolysaccharidosis type 2	XLR
<i>HSS</i>	Heparan sulfate sulfatase	Mucopolysaccharidosis type 3A	AR
<i>NAGLU</i>	N-Ac-beta-D-glucosaminidase	Mucopolysaccharidosis type 3B	AR
<i>HSGNAT</i>	Ac-CoA: alpha-glucosaminide Nacetyltransferase	Mucopolysaccharidosis type 3C	AR
<i>GNS</i>	N-Acetylglucosamine 6-sulfatase	Mucopolysaccharidosis type 3D	AR
<i>GALNS</i>	Galactosamine-6-sulfate sulfatase	Mucopolysaccharidosis type 4A	AR
<i>GLBI</i>	Beta-Galactosidase	Mucopolysaccharidosis type 4B	AR
<i>ARSB</i>	Arylsulfatase B	Mucopolysaccharidosis type 6	AR
<i>GUSB</i>	Beta-Glucuronidase	Mucopolysaccharidosis type 7	AR
<i>FUCA</i>	Alpha-Fucosidase	Fucosidosis	AR
<i>MANA</i>	Alpha-Mannosidase	Alpha-Mannosidosis	AR
<i>MANB</i>	Beta-Mannosidase	Beta-Mannosidosis	AR
<i>AGA</i>	Aspartyl-glucosaminidase	Aspartylglucosaminuria	AR
<i>GLB1</i>	beta-Galactosidase	GMI Gangliosidosis, several forms	AR
<i>NEU1</i>	Neuraminidase	Sialidosis, several forms	AR
<i>SLC17A5</i>	Sialic acid transporter	Sialic acid storage disease	AR
<i>PPGB</i>	Beta-Galactosidase protective protein	Galactosialidosis, several forms	AR
<i>SUMF1</i>	Sulfatase-modifying factor-1	Multiple sulfatase deficiency	AR
<i>GNPTAB</i>	GlcNAc-1-PT, alpha/beta subunits	Mucopolipidosis II alpha/beta	AR
		Mucopolipidosis III alpha/beta	AR
<i>GNPTG</i>	GlcNAc-1-PT, gamma subunits	Mucopolipidosis III gamma	AR

GlcNAc-1-PT = N-acetylglucosamine-1-phosphotransferase.