

Case Report

Elevated Bone Turnover in an Infantile Patient with Mucopolysaccharidosis II; No Association with Hyperparathyroidism

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Abstract. This present report concerns an infantile patient with mucopolysaccharidosis II, who showed transient cortical bone hyperostosis followed by severe osteopenia. The diagnosis of mucopolysaccharidosis II was made based on the leakage of lysosomal enzymes in serum and conditioned media of the patient's skin fibroblasts, low activity of lysosomal enzymes of the fibroblasts and mutation of c.2086_2089insC (p.L697fs) and c.3565C>T (p.R1189X) in the *GNPTAB* gene. Bone X-ray analysis demonstrated a periosteal reaction and elevated bone resorption at the age of 2 mo. Bone markers, including alkaline phosphatase, osteocalcin and urine deoxypyridinoline, also indicated a high turnover of bone metabolism; however, no apparent rickets-like changes and no increased levels of PTH were observed. Elevated bone resorption is possibly associated with the leakage of lysosomal enzyme from osteoclasts into bone matrices. Bone formation gradually reduced, and increased bone resorption persisted. This led to severe osteopenia at the age of 6 mo. Characteristic bone findings may contribute to early diagnosis of mucopolysaccharidosis II, but their pathogenesis remains to be clarified.

Key words: lysosome, bone formation, bone resorption, parathyroid hormone, osteoclast

Introduction

Lysosomes are organelles involved in protein recycling using various proteolytic enzymes under acidic conditions (1). In addition, lysosomes play significant roles in autophagy and apoptosis (2, 3). The dysfunction of lysosomes leads to many diseases, including

mucopolysaccharidosis type II alpha/beta (I-cell disease, MIM #252500) and III alpha/beta (pseudo-Hurler polydystrophy, MIM #252600), a genetic lysosomal storage disorder (4, 5). Mucopolysaccharidosis II is a rare congenital metabolic disorder caused by deficiency of the uridine diphosphate (UDP)-N-acetylglucosamine:lysosomal enzyme N-acetylglucosamine-1-phosphotransferase (abbreviated to GlcNAc-phosphotransferase). GlcNAc-phosphotransferase consists of an alpha-2/beta-2/gamma-2 hexameric complex. Defect of the enzyme causes failure of the biosynthesis of mannose 6-phosphate, which serves as a recognition signal for targeting acid

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proteolytic enzymes to lysosomes (1). Thus, lysosomal enzymes fail to be localized in lysosomes, and their substrates accumulate in the lysosomes of patients. The human *GNPTAB* gene contains 21 exons and spans 85 kb, encoding the 1256-amino acid peptide consisting of the 928-amino acid N-terminal alpha subunit and the 328-amino acid C-terminal beta subunit (6). The gamma subunit is encoded by a separate gene (*GNPTG*) (7).

Mucopolidosis II is characterized by severe clinical and radiological features, including a coarse face, retarded psychomotor development and restricted joint mobility (8). Neonates with mucopolidosis II often show small birth weight/length, inguinal hernia, gingival hypertrophy and hip dislocation. Mucopolidosis II is usually more severe than Hurler disease, which has similar phenotypes and a poor prognosis. The most unusual feature of mucopolidosis II is vacuoles in fibroblasts or lymphocytes and inclusion bodies in fibroblasts, causing markedly enlarged lysosomes filled with undigested substrates. High levels of lysosomal enzymes, such as β -hexosaminidase, β -galactosidase, α -mannosidase and arylsulfatase A, are detected in serum or cultured media due to leakage of enzymes from the cell. In addition, these lysosomal enzymes have low activity in the skin fibroblasts of patients.

A characteristic finding on X-ray films of the long bones is the transient increase of periosteal bone formation followed by severe osteopenia (9); however, the causes of the transient increase in periosteal bone formation and osteopenia remain unknown. At an early stage, subperiosteal resorption, rickets-like changes and cloaking may look similar to neonatal hyperparathyroidism. Some cases of mucopolidosis II are associated with neonatal hyperparathyroidism (10). A recent report consisting of 25 individuals with mucopolidosis types II and III indicated normal levels of PTH and PTH-related peptide (PTHrP) associated with normal calcium and phosphorus levels despite the resemblance of the bone

phenotype to that of neonatal hyperparathyroidism (11). In that report, the progressive bone and mineral disorder in mucopolidosis II and III is called osteodystrophy of mucopolidosis, and the radiographic features might be caused by the increased sensitivity of skeletal tissue to normal circulating levels of PTH; however, no evidence, such as an elevated level of urinary cAMP, was obtained to support the hypersensitivity to PTH. A few documents on metabolic bone markers in mucopolidosis II have described rapid changes from high bone turnover to low bone formation in neonatal mucopolidosis II and bone histology with findings of osteoclast activation, although they did not clarify the causes of these rapid changes of bone metabolism or the activation of osteoclasts (12).

PTH stimulates bone resorption, increases serum calcium levels and decreases serum phosphate levels, while intermittent administration of PTH promotes bone formation. Severe hyperparathyroidism in neonates or infants is caused genetically by a calcium-sensing receptor defect and is life-threatening due to severe hypercalcemia and multiple rib fractures (13). Maternal hypoparathyroidism or vitamin D deficiency also causes transient neonatal hyperparathyroidism (14). Exposure of the fetus to hypocalcemia is the basis of neonatal hyperparathyroidism. As described above, mucopolidosis II may be an additional cause of neonatal hyperparathyroidism, with impaired transport of calcium through the placenta being a possible reason (10).

In the present paper, we investigated serum and urinary bone markers and PTH levels to elucidate the mechanism of bone changes of neonatal mucopolidosis II.

Case Report

The patient was a boy born small for his gestational age at 40 wk and 2 d of gestation, with Apgar scores of 6 (1 min)/4 (5 min). His birth weight was 2,076 g, and his height was

unclear. There was no parental consanguinity. At birth, he showed micrognathia, overlapping fingers and severe skeletal changes, including thoracic deformity and limited flexion of the joints. Bone X-ray at birth revealed increased periosteal bone formation (periosteal reaction) in the long bones (arrow; Fig. 1A). He also showed severe hemolytic jaundice and needed repeated intubation for deterioration of respiratory status, although these symptoms improved a month after birth. High levels of serum alkaline phosphatase (ALP; over 5,000 U/l) and thinning of total bones continued from birth. At 2 and 6 mo of age, the blood and urine levels of bone markers and serum levels of cytokines were determined (Table 1). At 2 mo of age, he had elevated levels of serum ALP, osteocalcin (BGP) and procollagen type I C-propeptide (PICP), as well as increased urinary excretion of deoxypyridinoline (u-DPD), indicating the presence of high turnover bone disease; however, his renal and liver functions were normal. C-terminal PTH was 0.25 ng/ml (reference: <0.5) at one month old, and the intact PTH level was 59 pg/ml (reference range: 10–60) at 3 mo old, indicating no hyperparathyroidism. At 6 mo of age, the serum level of ALP was decreased by 45% (still above the normal level), and serum BGP and PICP were slightly decreased. On the other hand, u-DPD was almost unchanged. Serum calcium remained in the normal range, and the phosphate levels also returned to normal. The intact PTH level was 25 pg/ml. At 6 mo of age, X-ray films showed severe osteopenia (Fig. 1B). The bone mineral density was low at 1 yr old (L_2-L_4 BMD; 0.226 g/cm²: 39% of that in age-matched normal controls, ref. 15). His lymphocytes showed vacuolation at 2 mo of age. The clinical findings suggested that bone abnormalities were caused by a congenital metabolic disease. At 4 mo old, we diagnosed him with mucopolidosis II by lysosomal enzymatic analysis. He showed approximately half the normal level of β -galactosidase and normal levels of β -hexosaminidase, α -mannosidase and

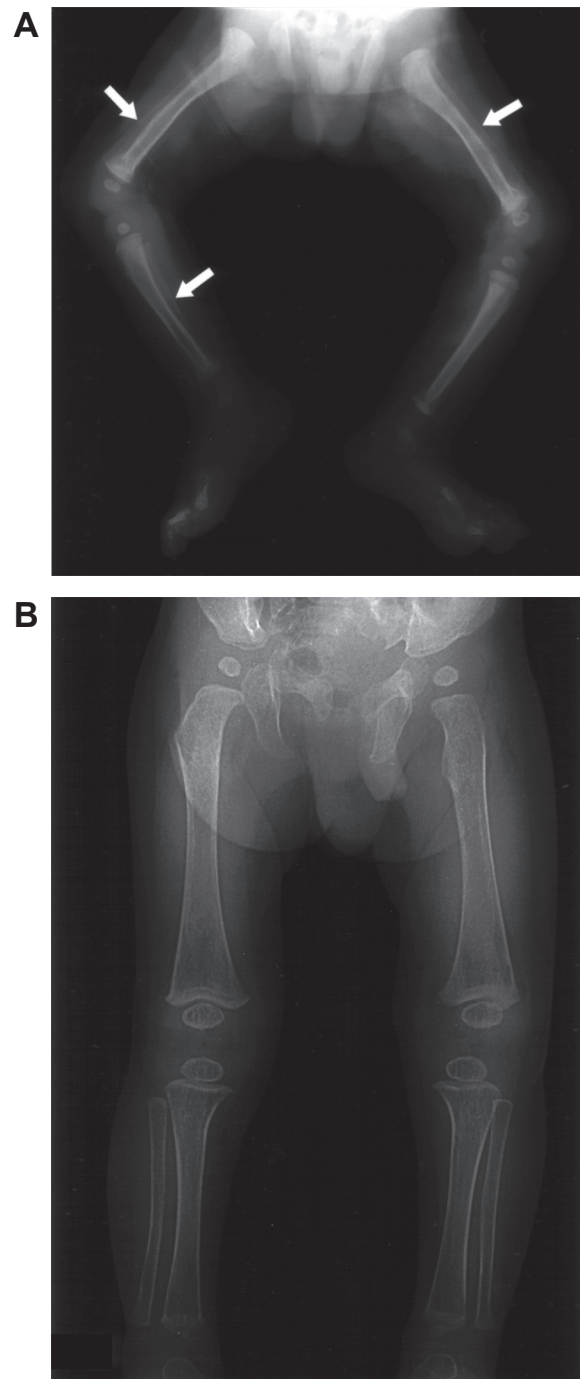


Fig. 1 Bone X-ray analysis. (A) At birth, increased periosteal bone formation in the long bones was shown (arrow). (B) At 6 mo of age, X-ray films showed severe osteopenia.

Table 1 Laboratory data

	2 mo	6 mo	Normal range for infants
Blood parameters			
Ca (mg/dl)	8.4	10.4	8.4~10.2
P (mg/dl)	4.4	5.9	5.5~7.4
ALP (IU/l)	6,605	2,948	430~800
BGP (ng/ml)	71	58.2	10.5~19.9
PICP (ng/ml)	2,420	1,800	540~580
1,25(OH) ₂ D (pg/ml)	160	121.4	33~128
Intact PTH (pg/ml)	59	25	10~60
Urine parameters			
Ca/Cr	0.03	0.047	~0.21
%TRP (%)	89	98.8	85~
u-DPD (μ mol/molCr)	167	150	30~40

Blood and urine levels of bone markers and serum levels of calcium, phosphate and hormones at 2 and 6 mo of age. BGP and PICP are measured by the IRMA method. DPD (deoxypyridinoline) is measured by the EIA method.

Table 2 Lysosomal enzyme activities

	β -galactosidase	β -hexosaminidase	α -mannosidase	α -fucosidase	Arylsulfatase A
Lymphocyte					
Patient	56	763	76	40	ND
Control	132 \pm 30	1,088 \pm 246	74 \pm 32	46 \pm 14	
Plasma					
Patient	133	7,140	1,320	2,440	ND
Control	8 \pm 8	782 \pm 518	59 \pm 36	442 \pm 194	
Skin fibroblast					
Patient	1.4	687	11.1	ND	59
Control	290 \pm 77	2,379 \pm 948	158 \pm 48		322 \pm 150

Lysosomal enzyme activities in lymphocytes, plasma and skin fibroblasts. The units are nmol/mg protein/h (lymphocytes and skin fibroblasts) or nmol/ml / h (plasma). Control: mean \pm SD. ND: not done.

α -fucosidase in lymphocytes. The plasma levels of these enzymes were highly elevated. These results were confirmed by the lysosomal enzyme activities of skin fibroblasts. He showed extremely low or low levels of β -galactosidase, β -hexosaminidase, α -mannosidase and arylsulfatase A (Table 2). A genetic test was performed and showed mutations of c.2086_2089insC (p.L697fs) and c.3565C>T (p.R1189X) in the *GNPTAB* gene compound

heterozygously (case 2 in ref. 16). He was not given therapy for osteopenia, but did not suffer from any bone fractures. He died at three years old from sepsis following pneumonia. The parents did not consent to an autopsy.

Discussion

The X-ray changes of neonatal mucopolidosis II at 2 mo of age are characteristic. The

subperiosteal resorption and new bone formation resembled bone abnormalities in neonatal patients with treatment of prostaglandin E1 to maintain the patency of the ductus arteriosus or with neonatal hyperparathyroidism (17). Consistent with the bone X-ray findings, bone markers show increased turnover of bone. In our patient, the serum levels of ALP and BGP, bone formation markers, were elevated, and an increased level of u-DPD, a bone resorption marker, was also observed. Subsequently, the serum ALP levels decreased markedly from 2 to 6 mo of age; however, the level of u-DPD showed only a slight decrease. We also found that increased periosteal bone formation in the long bones disappeared on X-ray films at 6 mo of age, and the bone mineral density was low at 1 yr old. These findings imply that high bone turnover occurs in patients with mucopolipidosis II at a few months of age followed by imbalanced high bone resorption, which may lead to low bone density at the age of 6 mo. In other expressions, this metabolic bone disorder shows a transition from the coupling state to uncoupling.

The association of neonatal hyperparathyroidism and mucopolipidosis II has been reported. In the reported cases, the radiological features resembled those of intrauterine hyperparathyroidism, and PTH and ALP were elevated (10). Secondary hyperparathyroidism usually resolves spontaneously by 3 or 4 mo of age. Our case seemed to be different from these cases because there was no apparent metaphyseal irregularity and no elevation of PTH, at least after one month old. Indeed, a recent report suggested two radiological patterns in mucopolipidosis types II and III, transient neonatal hyperparathyroidism and progressive osteodystrophy (11). The authors suggested the hypothesis that the progress of osteodystrophy is due to the hypersensitivity of bone to PTH because it is similar to chronic osteitis fibrosa cystica without an increase in PTH levels. More neonatal cases of mucopolipidosis are necessary to reach a conclusion on the pathogenesis of the

bone phenotype; however, it is unlikely that elevated levels of PTH are always associated with bone changes of infantile patients with mucopolipidosis II.

Osteoclasts are terminally differentiated multinucleated giant cells specific for bone resorption. Decalcification of bone and degradation of the bone matrix are undertaken at the site of a resorption pit sealed by a sealing zone and encircled by a ruffled border of osteoclasts and bone matrix because it contains acid and proteolytic enzymes, such as cathepsin K, the defect of which causes pycnodysostosis (18). The membrane of the ruffle border has characteristics identical to those of lysosome in osteoclasts, and thus it is called hemilyosome (19). Therefore, it is reasonable to hypothesize that the function of osteoclasts is impaired in mucopolipidosis II, as suggested by Robinson *et al.*, while bone resorption is enhanced in mucopolipidosis II (12). The increased bone resorption in mucopolipidosis may imply that the targeting of enzymes involved in bone resorption is not disturbed in this disorder. Further examination of the targeting of lysosomal enzymes is necessary to elucidate the mechanism by which bone resorption is enhanced in mucopolipidosis II. Another possible mechanism by which bone resorption is enhanced in mucopolipidosis II is that the excessive release of lysosomal enzymes by osteoclasts leads to increased bone resorption.

In conclusion, a neonatal patient with mucopolipidosis II that showed characteristic bone X-ray findings and elevated bone turnover without elevation of the PTH levels was reported in this paper. Characteristic X-ray findings of the bone may contribute to early diagnosis and treatment of mucopolipidosis II. Analysis of bone metabolism in mucopolipidosis will lead to better understanding of the relationship between bone formation and resorption.

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