J Korean Med Sci 2003; 18: 722-6
ISSN 1011-8934

Biochemical Characteristics of a Korean Patient with Mucolipidosis III (Pseudo-Hurler Polydystrophy)

We performed a biochemical study on the patient with mucolipidosis III (ML-III, pseudo-Hurler polydystrophy) in Korea. Confluent fibroblasts from the patient and from normal controls were cultured for 4, 12, 24, 48, and 72 hr, respectively. Lysosomal enzyme activities in culture media after different incubation times and in plasma, leukocytes, and fibroblasts were determined. Most of the leukocyte lysosomal enzymes were within normal limits or slightly lowered; however, plasma lysosomal enzyme activities such as those of hexosaminidase and arylsulfatase A were markedly increased. Numerous phase-dense inclusions were present in the cytoplasm of cultured fibroblasts. Lysosomal enzyme activities of fibroblasts were markedly decreased except for β-glucosidase. The rates of increase of the lysosomal enzyme activities with incubation time were greater in the culture medium of the patient than in normal control, whereas no difference in the β-glucosidase activity of the culture media of the patient and the control was found. This study describes the first case of ML-III in Korea, with its typical biochemical characteristics, i.e., a problem with targeting and transporting of lysosomal enzymes which results in a marked increase in plasma lysosomal enzyme activities and a high ratio of extracellular to intracellular lysosomal enzyme activities in cultured fibroblasts.

Key Words : *Mucolipidoses; Pseudo hurler polydystrophy; Fibroblasts*

Junghan Song, Dong Soon Lee, Han Ik Cho, Jin Q Kim, Tae-Joon Cho*

Department of Laboratory Medicine, Department of Orthopaedic Surgery*, Seoul National University College of Medicine, Seoul, Korea

Received : 12 February 2003
Accepted : 26 May 2003

Address for correspondence
Junghan Song, M.D.
Department of Laboratory Medicine, Seoul National University College of Medicine, 28 Yongon-dong, Chongno-gu, Seoul 110-744, Korea
Tel : +82.2-760-2805, Fax : +82.2-764-6542
E-mail : songjhcp@plaza.snu.ac.kr

*This work was supported by grant no. 01-1999-051-0 from the Seoul National University Hospital Research Fund.

INTRODUCTION

Mucolipidosis was first described by Leroy and Demars (1) in 1967 as a new disorder that clinically resembled Hurler syndrome but lacked mucopolysacchariduria. Another distinguishing feature of mucolipidois is the presence of large, phase-dense inclusions in fibroblasts. Because these cells are called inclusion cells (I-cells), the disorder came to be called I-cell disease (mucolipidosis II, or ML-II). Similar inclusions are seen in pseudo-Hurler polydystrophy (mucolipidosis III, or ML-III), which is clinically milder than I-cell disease and is presented later (2). ML-II and III are biochemically related genetic diseases that are rare and recessively inherited in autosomes. One of the most striking biochemical features of these disorders is the finding of markedly elevated levels of many lysosomal enzymes in serum (2, 3). Cultured fibroblasts from these patients show lower intracellular activities of the same lysosomal enzymes (2, 4-6) and contain the characteristic inclusion bodies. In both diseases, lysosomal enzyme transport is abnormal in cells of mesenchymal origin. In normal cells, the targeting of lysosomal enzymes to lysosomes is mediated by receptors that bind mannose 6-phosphate recognition markers to the enzymes (7). The recognition marker is synthesized in a two-step reaction in the Golgi complex, and is the enzyme that catalyzes the first step in the process. This enzyme was identified as N-acetylglucosamine-1-phosphotransferase, and found to be defective in ML-II and ML-III (8, 9). As a consequence of a defective recognition marker, newly synthesized lysosomal enzymes are not targeted to lysosomes, but are secreted into the extracellular space.

Ahn and Chung (10) reported upon a sibling of ML-III with carpal tunnel syndrome, but the patients were not Korean. To the best of our knowledge, this is the first report on a Korean patient with ML-III. Biochemical studies were performed upon the cultured fibroblast cells from this patient.

MATERIALS AND METHODS

Patient

A 1.5-yr-old girl first was taken into our out-patient clinic for the evaluation of motor developmental delay and spine deformity. She was delivered through normal, full-term, and spontaneous process with 2.3 kg of the weight at birth. Chromosomal study was normal. Characteristics of facial appearance included frontal bossing, exophthalmos, hypertelorism, and depressed midface. Simple radiographs revealed thoracolumbar kyphosis and acetabular dysplasia, but no epiphyseal dysplasia was noted. At age of 3.5 yr, she was measured

at 87 cm in height (<1%). She had 30 degree flexion contracture of both hip and knee joints (Fig. 1). She could walk alone only after she became 4 yr-old. At that time, hepatosplenomegaly and frequent constipation were noted. Cardiac echocardiography showed a thick left ventricular wall. At age of 5 yr, papilledema due to increased intracranial pressure and craniosynostosis was noted and confirmed. Conductive loss of hearing due to chronic otitis media, mild mental retardation, and language developmental delay were found. Urinary mucopolysaccharide spot test was negative. Under the impression of mucolipidosis, laboratory tests including enzyme assay from circulating leukocyte, plasma, and cultured dermal fibroblast were performed. No specific therapeutic measurement was carried out.

Chemicals

4-Methylumbelliferone, 4-methylumbelliferyl (MU)-β-D-glucuronide, 4-MU-β-D-glucopyranoside, 4-MU-β-D-galactopyranoside, 4-MU-α-D-galactopyranoside, 4-MU-2-acetamido-2-deoxy-α-D-glucopyranoside, 4-MU-2-acetamido-2-deoxy-β-D-glucopyranoside, and p-nitrocatechol sulfate were purchased from the Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Samples

Leukocytes and plasma were prepared from heparinized peripheral blood specimens. Skin fibroblasts were obtained from skin punch biopsies of the patient and from normal controls. Cultured skin fibroblasts were propagated and maintained in Medium 199 supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin. Confluent cells were trypsinized for lysosomal enzyme assay.

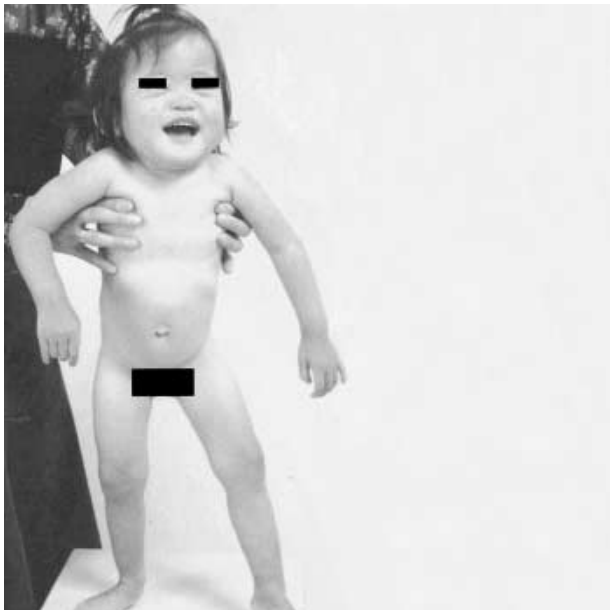

Fig. 1. A patient with pseudo-Hurler polydystrophy at the age of 3.5 yr; note the coarse face and 30 degree flexion contracture of both hip and knee joints.

Lysosomal enzyme activity changes in culture medium with time

After seeding the equal number of cells into five culture flasks, the old medium was replaced with the fresh medium. Each flask was cultured for 4, 12, 24, 48, and 72 hr, respectively, and lysosomal enzyme activities in culture medium were determined.

Lysosomal enzyme assays

Lysosomal enzyme activities including α-N-acetylglucosaminidase, hexosaminidase, α-galactosidase, β-galactosidase, β-glucosidase and β-glucuronidase in samples of plasma, leukocytes, cultured fibroblasts and culture media were determined by using appropriate methylumbelliferyl fluorogenic substrates and by commonly used methods (11). The fluorescence was compared with a standard solution of 4-methylumbelliferone using a Jasco FP-55A spectrofluorometer (Easton, MD, U.S.A.) with the excitation wavelength at 360 nm and the emission wavelength at 448 nm. Arylsulfatase A and B were also measured, using p-nitrocatechol sulfate as substrate (11). The normal ranges of lysosomal enzymes were adopted from those of the laboratory manuals (11) and were verified by measuring several control samples in the present study. Protein was measured by using the method of Lowry et al. (12). Enzyme activities of leukocytes and cultured fibroblasts were expressed as nmoles of substrate cleaved per mg of cell protein per hour at 37°C. Enzyme activities in plasma and in culture medium were expressed as nmoles of substrate cleaved per mL per hour at 37°C.

Table 1. Lysosomal enzyme activities in leukocyte, plasma and cultured fibroblasts from the patient with pseudo-Hurler polydystrophy

	Lysosomal enzyme activities (normal range)		
	Leukocytes*	Plasma†	Cultured Fibroblasts*
α-N-acetylglucosaminidase	1.2 (0.7-1.7)	nd	3.9 (11-21)
β-galactosidase	52 (80-140)	nd	26 (80-140)
Arylsulfatase B	354 (115-226)	nd	67 (530-950)
β-glucuronidase	202 (350-500)	nd	47 (75-155)
Hexosaminidase	nd	11,687 (800-1,600)	911 (3,000-5,000)
Arylsulfatase A	nd	62.2 (1.3-2.4)	16 (190-405)
β-glucosidase	nd	nd	329 (320-550)
α-galactosidase	nd	nd	11 (40-110)

*Enzyme activity is expressed as nanomoles of substrate liberated per mg of cell per hour. †Enzyme activity is expressed as nanomoles of substrate liberated per ml of plasma per hour. nd: not determined.

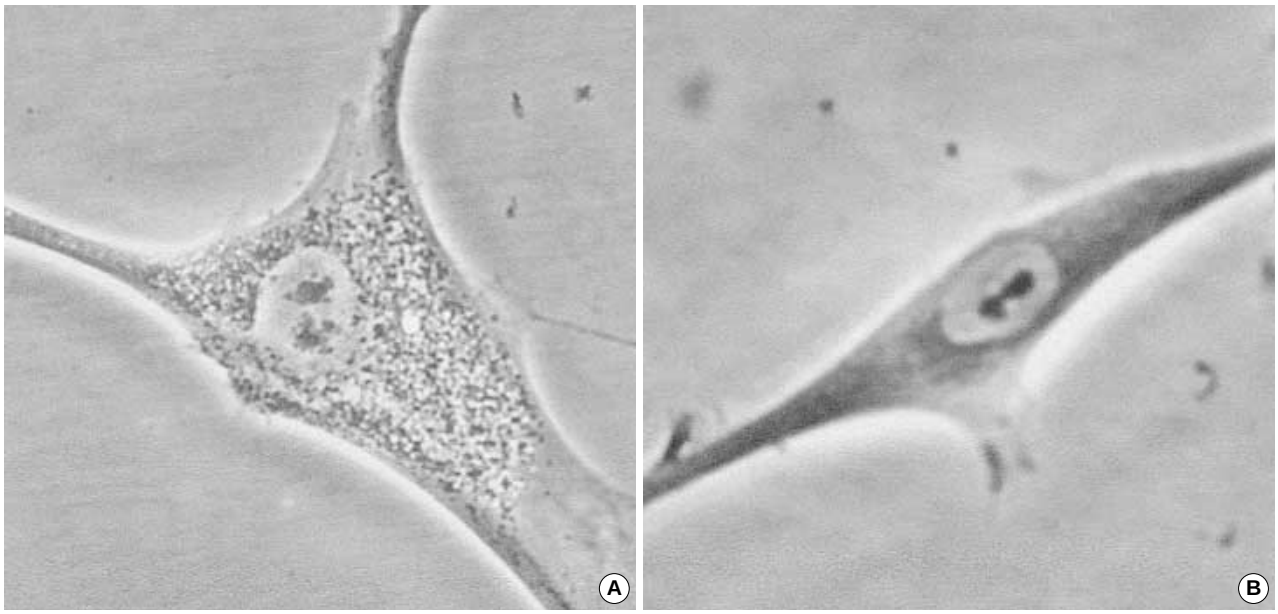

Fig. 2. Phase contrast microscopy of cultured fibroblasts; (A) from a patient with pseudo-Hurler polydystrophy and (B) from a control. Dense inclusions are present throughout the cytoplasm of a patient.

RESULTS

Leukocyte and plasma lysosomal enzyme activities are shown in Table 1. Most of the leukocyte lysosomal enzyme levels were normal or slightly reduced; however, plasma lysosomal enzymes, such as hexosaminidase and arylsulfatase A, were markedly elevated. A complete assay for all lysosomal enzymes was not performed because of the loss of the patient to conduct a follow-up.

In the cytoplasm of cultured fibroblasts, numerous phase-dense inclusions was observed, although not prominent (Fig. 2). Lysosomal enzyme levels in cultured fibroblasts were markedly reduced except for β-glucosidase (Table 1).

The changes in the lysosomal enzyme activities in culture medium with incubation time are shown in Fig. 3. The rates of increase of lysosomal enzyme activities in the culture medium of the patient were greater than in the culture medium of normal control, whereas no difference in β-glucosidase activity was found in the culture media of the patient and of the control.

DISCUSSION

ML-II and III reflect multiple deficiencies of many lysosomal hydrolases, and require posttranslational processing to form a recognition site that permits their cellular uptake. The fundamental defect is found in N-acetylglucosaminyl phosphotransferase, which results in abnormal lysosomal enzyme transport in cells (8, 9, 13). In such cells, newly synthesized lysosomal enzymes are secreted into the extracellular medium instead of being targeted correctly to lysosomes. Patients with ML-II have a complete deficiency of this enzyme, while patients with ML-III have varying amounts of residual enzymic activities. Both ML-II and III show many of clinical and radiographic features of the classic mucopolysaccharidoses: such as dysostosis multiplex and coarse facial features. However, unlike patients with mucopolysaccharidoses, patients with ML have no mucopolysaccharidoses. ML-II shows as a severe progressive psychomotor retardation, and death usually occurs in the first decade. ML-III is milder and presents later, and survival into adulthood is possible (2). In the case described, until biochemical confirmatory diagnosis for ML-III had been obtained, the patient was clinically considered as mucopolysaccharidosis with unusual laboratory findings, such as no mucopolysaccharidoses and normal or slightly reduced leukocyte enzyme activities for mucopolysacchridoses. Even though the assay for N-acetylglucosaminyl phosphotransferase enzyme activity was not performed in this patient, the patient was diagnosed as ML-III because she had relatively mild clinical manifestations and typical biochemical findings.

The biochemical diagnosis of ML-II and ML-III can be made by measuring the activities of lysosomal enzymes in serum or in cultured fibroblasts. In general, a ten- to twenty-fold increase in serum lysosomal enzymes is diagnostic of these disorders (2, 3). If cultured fibroblasts are available, the characteristic pattern of lysosomal enzyme deficiencies may be used, as well as the ratio of extracellular to intracellular enzyme activities for diagnosis (5, 6). In this presentation, the patient showed a marked increase in plasma lysosomal enzymes, such as hexosaminidase and arylsulfatase A. Lysosomal enzyme levels in cultured fibroblasts were markedly decreased, and also

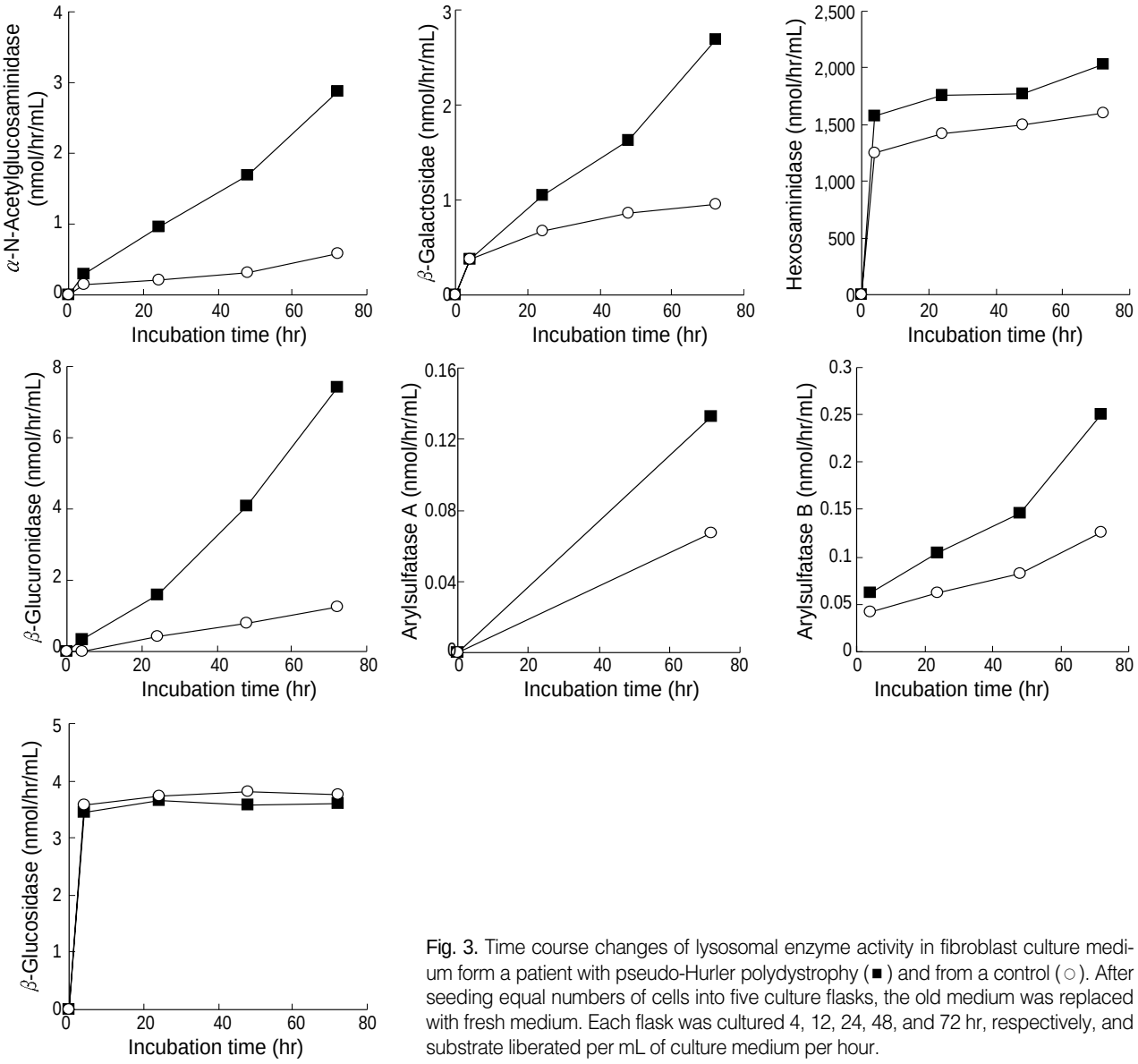

Fig. 3. Time course changes of lysosomal enzyme activity in fibroblast culture medium form a patient with pseudo-Hurler polydystrophy (■) and from a control (○). After seeding equal numbers of cells into five culture flasks, the old medium was replaced with fresh medium. Each flask was cultured 4, 12, 24, 48, and 72 hr, respectively, and substrate liberated per mL of culture medium per hour.

those in cultured fibroblast media were markedly reduced. This was caused by the secretion of newly synthesized intracellular lysosomal enzyme into extracellular media instead of being targeted correctly to lysosomes. Moreover, the fibroblasts were deficient in a large number of lysosomal enzymes due to the hypersecretion of these proteins.

To elucidate this secretory phenomenon into the extracellular media more precisely, we investigated the time course of enzymatic activities in the fibroblast culture medium from a patient with pseudo-Hurler polydystrophy and from a control (Fig. 3). After seeding equal numbers of cells into five culture flasks, the old medium was replaced with fresh medium. Each flask was cultured for 4, 12, 24, 48, and 72 hr, respectively, and lysosomal enzyme activities in culture medium were determined. The rates of increase of the lysosomal enzyme activities with incubation time were greater in the media of pseudo-Hurler polydystrophy (ML-III) fibroblast culture than in normal fibroblast culture; which means that the normal transport of lysosomal enzymes to lysosomes is impaired and that extracellular secretion is increased as a consequence.

Interestingly, no difference was found in β-glucosidase activity of the culture media of the patient and of the control. Furthermore, the activity of β-glucosidase in the fibroblasts of the patient was normal. This enzyme is known to be targeted to lysosomes by a phosphorylation-independent mechanism (14). However, the exact targeting mechanism of β-glucosidase remained to be clarified. Acid phosphatase is another enzyme that does not use the mannose 6-phosphate targeting pathway. Acid phosphatase is made as a transmembrane protein,

and its lysosomal targeting signal is contained in its cytoplasmic tail (15).

ML-III is genetically and clinically heterogenous (16-18). It can be divided into three distinct complementation groups: Complementation group A, the classic and the more frequent, is characterized by decreased phosphotransferase when determined using artificial and natural substrates: Complementation group C is characterized by a deficient activity when determined using natural substrate but normal activity using artificial substrate; and Complementation group B is very rare and its phosphotransferase properties are similar to those group A. Recently, there was a report on mutation analysis in a group C patient (19, 20). However, because the phosphotransferase assay was not conducted in our patient, it is not possible to place it in a complementation group. More studies on phosphotransferase activity and mutation analysis are needed.

In conclusion, we report the first Korean case of ML-III, pseudo-Hurler polydystrophy, with typical biochemical findings that show the problem of lysosomal enzyme targeting and transport, which results in a marked increase in plasma lysosomal enzyme activity and an increased ratio of extracellular to intracellular lysosomal enzyme activity in cultured fibroblasts.

REFERENCES

1. Leroy JG, Demars RI. *Mutant enzymatic and cytological phenotypes in cultured human fibroblasts. Science 1967; 157: 804-6.*
2. Kelly TE, Thomas GH, Taylor HA Jr, McKusick VA, Sly WS, Glaser JH, Robinow M, Luzzatti L, Espiritu C, Feingold M, Bull MJ, Ashenhurst EM, Ives EJ. *Mucolipidosis III (pseudo-Hurler polydystrophy): clinical and laboratory studies in a series of 12 patients. Johns Hopkins Med J 1976; 137: 156-75.*
3. Thomas GH, Taylor HA, Reynolds LW, Miller CS. *Mucolipidosis 3 (pseudo-Hurler polydystrophy): multiple lysosomal enzyme abnormalities in serum and cultured fibroblast cells. Pediatr Res 1973; 7: 751-6.*
4. Leroy JG, Ho MW, MacBrinn MC, Zielke K, Jacob J, O'Brien JS. *I-cell disease: biochemical studies. Pediatr Res 1972; 6: 752-7.*
5. Wiesmann UN, Lightbody J, Vasella F, Herschkowitz NN. *Multiple lysosmal deficiency due to enzyme leakage. N Engl J Med 1971; 284: 109-10.*
6. Wiesmann UN, Herschkowitz NN. *Studies on the pathogenetic mechanism of I-cell disease in cultured fibroblasts. Pediatr Res 1974; 8: 865-9.*
7. Kornfeld S. *Trafficking of lysosomal enzymes. FASEB J 1987; 1: 462-8.*
8. Reitman ML, Varki A, Kornfeld S. *Fibroblasts from patients with I-cell disease and pseudo-Hurler polydystrophy are deficient in uridine 5´-disphosphate-N-acetylglucosamine:glycoprotein N-acetylglucosaminyl phosphotransferase activity. J Clin Invest 1981; 67: 1574-9.*
9. Varki AP, Reitman ML, Kornfeld S. *Identification of a variant of mucolipidosis III (pseudo Hurler polydystrophy): a catalytically active N-acetylglucosaminyl phosphotransferase that fails to phosphorylate lysosomal enzymes. Proc Natl Acad Sci USA 1981; 78: 7773-7.*
10. Ahn DS, Chung C. *Congenital carpal tunnel syndrome in mucolipidosis III. J Korean Soc Plast Reconstr Surg 1990; 17: 73-6.*
11. Shapira E, Blitzer MG, Miller JB, Africk DK. *Biochemical Genetics. A laboratory manual. 1st ed. New York: Oxford University Press 1989: 1-46.*
12. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. *Protein measurements with the folin phenol reagent, J Biol Chem 1951; 193: 265-75.*
13. Hasilik A, Waheed A, von Figura K. *Enzymatic phosphorylation of lysosomal enzymes in the presence of UDP-N-acetylglucosamine. Absence of the activity in I-cell fibroblasts. Biochem Biophys Res Commun 1981; 98: 761-7.*
14. Aerts JM, Schram AW, Strijland A, van Weely S, Jonsson LM, Tager JM, Sorrell SH, Ginns EI, Barranger JA, Murray GJ. *Glucocerebrosidase, a lysosomal enzyme that does not undergo oligosaccharide phosphorylation. Biochim Biophys Acta 1988; 964: 303-8.*
15. Waheed A, Gottschalk S, Hille A, Krentler C, Pohlmann R, Braulke T, Hauser H, Geuze H, von Figura K. *Human lysosomal acid phosphatase is transported as a transmembrane protein to lysosomes in transfected baby hamster kidney cells. EMBO J 1988; 7: 2351-8.*
16. Honey NK, Mueller OT, Little LE, Miller AL, Shows TB. *Mucolipidosis III is genetically heterogeneous. Proc Natl Acad Sci USA 1982; 79: 7420-4.*
17. Little LE, Mueller OT, Honey NK, Shows TB, Miller AL. *Heterogeneity of N-acetylglucosamine 1-phosphotransferase within mucolipidosis III. J Biol Chem 1986; 261: 733-8.*
18. Tylki-Szymanska A, Czartoryska B, Groener JE, Lugowska A. *Clinical variability in mucolipidosis III (pseudo-Hurler polydystrophy). Am J Med Genet 2002; 108: 214-8.*
19. Raas-Rothschild A, Cormier-Daire V, Bao M, Genin E, Salomon R, Brewer K, Zeigler M, Mandel H, Toth S, Roe B, Munnich A, Canfield WM. *Molecular basis of variant pseudo-Hurler polydystrophy (mucolipidosis IIIC). J Clin Invest 2000; 105: 673-81.*
20. Sly WS. *The missing link in lysosomal enzyme targeting. J Clin Invest 2000; 105: 563-4.*