증 례

18개월 남아에서 간비장비대, 성장 부진을 동반한 3형 고셔병 증례: 효소 대체 요법 후 임상 경과

성균관대학교 의과대학 삼성서울병원 소아청소년과[1], 진단검사의학과[2], 영상의학과[3]

임영신[1] · 황정윤[1] · 김진섭[1] · 양아람[1] · 박형두[2] · 전태연[3] · 조성윤[1] · 진동규[1]

A Case of an 18-month-old Boy with Type 3 Gaucher Disease Presenting with Hepatosplenomegaly and Growth Retardation: The Clinical Course after Enzyme Replacement Therapy

Young Shin Lim[1], Jeongyun Hwang[1], Jinsup Kim[1], Aram Yang[1]
Hyung Doo Park[2], Tae Yeon Jeon[3], Sung Yoon Cho[1], Dong-Kyu Jin[1]

Department of Pediatrics[1], Samsung Medical Center,
Sungkyunkwan University School of Medicine, Seoul, Korea,
Department of Laboratory Medicine and Genetics[2],
Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea,
Department of Radiology and Center for Imaging Science[3],
Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea

Gaucher disease (GD) is an autosomal recessive lysosomal storage disorder caused by beta-glucosidase deficiency. An 18 month-old male with hepatosplenomegaly, anemia, thrombocytopenia, and growth retardation referred to our hospital. The patient showed neurological symptoms, such as supranuclear gaze palsy and developmental delay. Bone marrow biopsy performed to rule out malignancy and the results revealed no malignant cell; however, abnormal histiocytes suggesting storage disease was noted. Based on hepatosplenomegaly, bicytopenia and unexplained neurologic manifestations, enzyme activity and genetic analysis were conducted emergently with a strong suspicion of GD. Beta-glucosidase activity in leukocyte was decreased. GBA sequencing to confirm the diagnosis revealed compound heterozygous pathogenic variants (i.e., c.754T>A, c.887G>A), both previously reported as the cause of neuronopathic GD. Under the diagnosis of type 3 GD, the patient immediately received enzyme replacement therapy (ERT). After 17 months of ERT, the size of spleen decreased, and hemoglobin and platelet count returned to normal. In addition, the activity of chitotriosidase and angiotensin converting enzyme decreased. However, myoclonic movement and generalized seizure occurred at the age of 19 months and antiepileptic drug was started. Other neurological deterioration including supranuclear gaze palsy and developmental delay also persisted. A new therapy to overcome neurologic problems should be developed for patients with type 3 GD.

Key words: Gaucher disease, Glucocerebrosidase, Hepatosplenomegaly, Growth retardation, Enzyme replacement therapy

Introduction

Gaucher disease (GD, MIM #606463), autosomal recessive inherited lysosomal storage dis-

책임저자: 조성윤, 서울시 강남구 일원로 81
성균관대학교 의과대학 삼성서울병원 소아청소년과
Tel: 02)6190-5227, Fax: 02)3410-0830
E-mail: nadri1217@naver.com

order, is caused by a deficiency of the beta-glucocerebrosidase enzyme (*GBA*), leading to the accumulation of glucocerebroside within tissue macrophages in multiple organs. GD is most prevalent in people of Ashkenazi Jewish ancestry with the prevalence rate of 1 in 450 as compared to 1 in 40,000-60,000 in non-Jewish populations [1]. There are three subtypes of GD by the Knudsen and Kaplan classification, classified as types 1, 2, and 3, according to the presence of neurological deterioration, age at identification, and progression rate[2].

Type 1 GD is the most common subtype and differentiated from type 2 and 3 GD by the absence of central nervous system (CNS) involvement[3]. The manifestations of type 1 GD include splenomegaly, hepatomegaly, anemia, thrombocytopenia, bone disease (i.e., bone marrow infiltration, Erlenmeyer flask deformity of the distal femur, osteopenia, osteoporosis, infarction, avascular necrosis, and pathologic fracture) and growth retardation[3]. The patients with type 2 GD typically exhibit neurodegeneration and hepatosplenomegaly before one year of age. Visceral symptoms are extensive and severe, and cranial nerve involvement deteriorates rapidly. Most type 2 patients die at birth or within 2-3 years of life[4]. Type 3 GD is a chronically progressive form with the onset of any neurological symptom at the age of 1 and older. It is a highly heterogeneous subtype encompassing a variable degree of visceral involvement combined with diverse neurological signs. Three forms of type 3 GD are recognized, although there is a marked overlap supporting that this is a spectrum of disease manifestation[5]. The patients with type 3a GD have mild hepatosplenomegaly and earlier development of neurologic symptoms, including seizures, strabismus, and supranuclear gaze palsy. Type 3b has extensive visceral and bone involvement with more slowly progressive neurodegenerative symptoms. Type 3c is rare and characterized by supranuclear gaze palsy, corneal opacity, and cardiovascular calcification with little visceral and bone disease. Neurologic involvement can begin late and progression is variable[5]. In Jewish and non-Jewish Caucasian, over 95% of patients are type 1 GD, while in East Asia such as Japan, China and Korea, the percentage of type 2 and 3 GD account for about 60%[6].

GD is the first lysosomal storage disorder which is treated with macrophage-targeted enzyme replacement therapy (ERT) and purified placental glucocerebrosidase was approved by the FDA in 1991. The modified form of the acid beta-glucosidase enzyme, imiglucerase, has been used commercially as a standard treatment of GD since 1994[7]. With this background, early diagnosis and prompt initiation of treatment when indicated is crucial, but the patients with GD frequently experience a significant diagnostic delay that leads to complications (i.e., avascular necrosis, severe bleeding, chronic bone pain, life-threatening sepsis, pathologic fractures, and growth failure)[1]. The rarity of the disease and heterogeneous nature of symptoms may impede the consideration of GD as in the differential diagnosis of the patients with hepatosplenomegaly and cytopenia. Increased awareness of GD should reduce the rate of inaccurate or delayed diagnosis, and enable an earlier initiation of treatment.

Here, we describe an 18 month-old Korean patient diagnosed with type 3 GD by genetic analysis of *GBA*. Even though typical Gaucher cell was not found in bone marrow examination, a strong clinical suspicion enabled reaching early diagnosis and starting ERT immediately.

Case Report

The patient was the first boy of non-consanguineous Korean parents and was born by a Cesarean section at 39th week of gestation. His birth height was 49 cm (75^{th}-90^{th} percentile), birth weight was 3.2 kg (25^{th}-50^{th} percentile), and head circumference was 35 cm (75^{th}-90^{th} percentile). At the age of 18 months, he was admitted to a hospital because of high fever and cough. Vital sign were blood pressure of 108/56 mmHg, pulse of 120 bpm, respiratory rate of 28 per minute, body temperature of 36.5℃. On physical examination, the spleen was palpated to 4 cm and the liver was extending to 7 cm under the right costal margin. Coarse breath sounds with bilateral crackles were checked. He did not show remarkable dysmorphic facial features besides low-set ears and flat nasal bridge. His height was 75.6 cm (-1.96 standard deviation score [SDS], his weight was 9.4 kg (-2.01 SDS), and head circumference was 47.2 cm (-0.55 SDS). On neurologic examination, horizontal supranuclear gaze palsy was noted. Deep tendon reflex was normal; bilateral muscle tone was decreased (grade 4/4) and slightly rigid. He was able to hold his head while sitting at 5 months, sat without support at 10 months, and walked with support at 15 months. However, at the age of 18 months, he could not sit, walk, or speak at all and a global developmental delay was noted on the Denver developmental screening test. The laboratory studies revealed hemoglobin level of 8.8 g/dL, leukocytes count of 11,880/μL with normal differential count, platelet count of 76,000/μL, Prothrombin time (PT) INR of 1.53 (reference range, 0.9-1.2), and ferritin of 2,360.6 ng/mL (reference range, 20-150 ng/mL). Chest X-ray showed pneumonic infiltration on the both perihilar regions. As the patient continuously showed high fever and respiratory symptoms over 1 month despite antibiotic therapy for pneumonia, he was transferred to Samsung Medical Center. Based on hepatosplenomegaly, bicytopenia, persistent fever, and severe growth retardation, bone marrow biopsy was performed to rule out malignancy. Bone marrow examination showed no evidence of malignancy, but abnormal histiocytes suggesting storage disease were noted (Fig. 1A, B). We considered lysosomal storage disease including types 1 and 2 Niemann-Pick disease as differential diagnosis, but GD was most strongly suspected considering the patient's

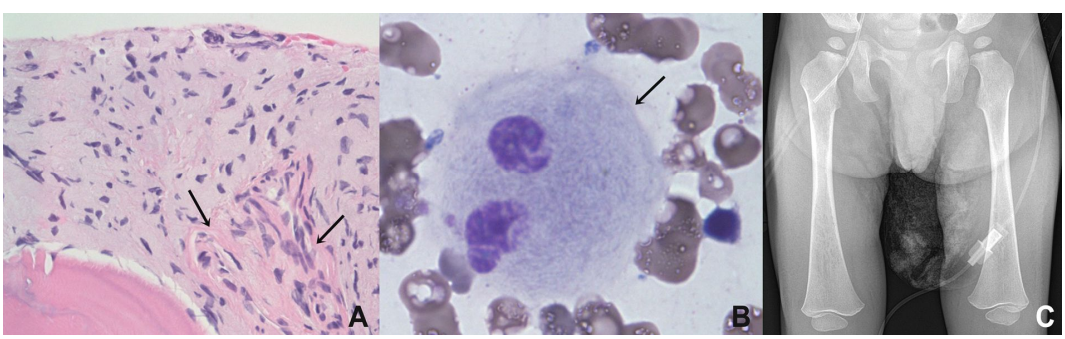

Fig. 1. (A) Bone marrow biopsy showed nodular aggregates of large storage histiocytes (arrow) among hematopoietic cells (hematoxylin-eosin, original magnification ×400). (B) Bone marrow abnormal histiocytes (arrow) with a very low N/C ratio abundant pale blue cytoplasm were observed, but subtle striations, characteristic of Gaucher cells, were not definite (hematoxylin-eosin, original magnification ×1,000). (C) The mottled bone lesions with cortical thinning were observed in the metadiaphysis of the both femur and generalized osteopenia.

clinical features, specifically, supranuclear gaze palsy. Although Gaucher cell was not detected in bone marrow examination, we decided to carry out beta-glucosidase activity assay emergently and direct sequencing of *GBA* gene with approval of the Institutional Review Board (IRB) of Samsung Medical Center (IRB number, PHO017001) under prompt clinical impression of GD. The beta-glucosidase activity of peripheral blood leukocytes deceased to 3.5 nmol/hr/mg protein (reference range, 5.1-11.3 nmol/hr/mg protein). Compound heterozygous pathogenic variants, c.754T>A (F213I), c.887G>A (R257Q), in the *GBA* gene were found and the parents were heterozygous carriers of each variant (Fig. 2). These two mutations have been reported previously in neuronopathic GD. The skeletal survey revealed generalized osteopenia with cortical thinning and mottled bone lesions suggesting impaired remodeling process in the metadiaphysis of the both femurs (Fig. 1C). Brainstem-evoked response audiometry test revealed bilateral abnormal auditory thresholds (90 dBnHL/100 dBnHL), suggesting profound sensorineural hearing loss. Echocardiogram was normal. As confirmed by GD, the patient immediately started ERT with imiglucerase, recombinant human *GBA* with the dose of 60 U/kg every 2 weeks. Since then, the size of spleen was decreased (Fig. 3) and blood transfusion was not necessary with improved anemia and thrombocytopenia after a month of treatment. Irritability possibly caused by bone pain was alleviated. However, myoclonic movement and generalized seizure occurred at the age of 19 months. Brain magnetic resonance imaging (MRI) revealed non-specific multiple hyperintense lesions in both frontal white matter and an electroencephalography (EEG) showed right or left central regional sharp wave and spike.

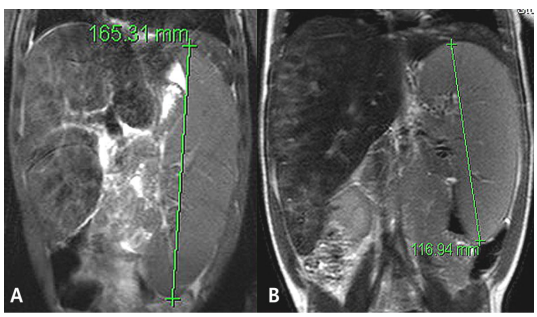

Fig. 3. (A) Hepatosplenomegaly showed in coronal T1 MRI of the abdomen at diagnosis. (B) The spleen length at the hilum decreased from 16 cm to 12 cm after four months of enzyme replacement therapy.

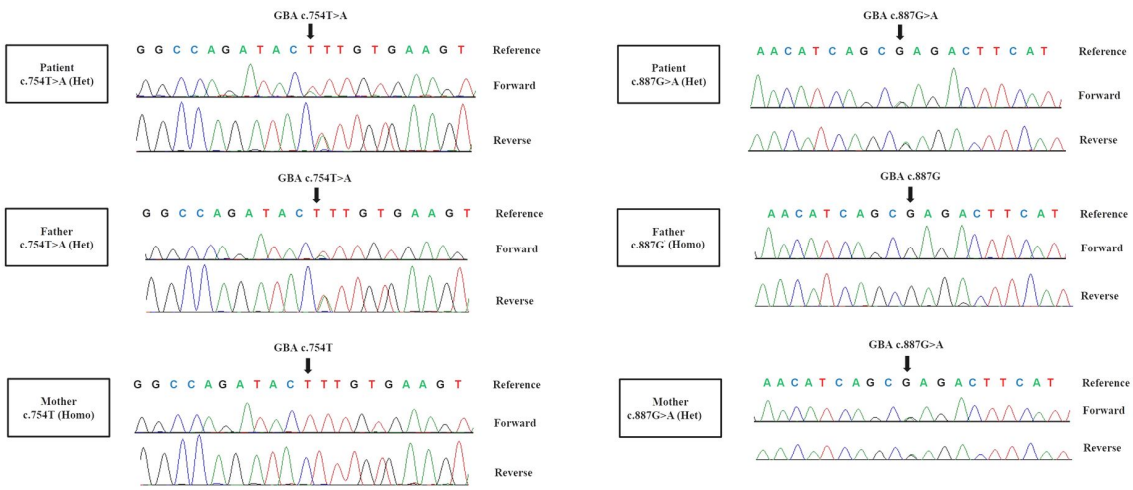

Fig. 2. Genotype analysis revealed compound heterozygous pathogenic variants (c.754T>A, c.887G>A) in the *GBA* gene.

This EEG is suggestive of focal seizure disorder arising from right temporal or left fronto-central area. He started taking antiepileptic drug (levetiracetam). The patient underwent gastrostomy and fundoplication because of frequent vomiting and swallowing difficulty at the age of 22 months. Tracheostomy was done because of severe oral secretion and respiratory difficulty at the age of 24 months. At the last follow-up, 39 month-old, the patient was slowly growing except for head circumference. Body measurements at the last follow-up were as follows: height 94 cm (-0.386 SDS), weight 13.8 kg (-0.674 SDS), head circumference 48.3 cm (-1.11 SDS). After 17 months of ERT, chitotriosidase activity decreased from 9,810 nmol/h/mL to 3,813 nmol/h/mL (reference range, 55.21±27.81 nmol/hr/mL), and angiotensin-converting enzyme decreased from 150 U/L to 74 U/L (reference range, 12-78 U/L) (Table 1, Fig 4). A rhythmic sharp wave was still observed on the EEG and intermittent background suppression was also noted. This is suggestive of a severe diffuse cerebral dysfunction. Even after 17 months of ERT, despite taking multiple antiepileptic drugs, the frequency of seizures has increased. He had been treated with ambroxol (25 mg/kg/day) for 3

Table 1. Changes of the Gaucher Disease Biomarkers During ERT

Biomarker	ERT (months)				
	0	1	2	8	17
ACE (U/L)	150	150	114.9	117.2	74
Chitotriosidase (nmol/h/mL)	9,810	8,618	6,692	4,897	3,813
Ferritin (ng/mL)	2,682	1,274	683.1	80.4	28.4
Hemoglobin (g/dL)	7.2	11.5	13.6	11.5	11.1
Platelet (10^3/μL)	27	85	149	187	159

Abbreviations: ERT: enzyme replacement therapy; ACE: angiotensin-converting enzyme.

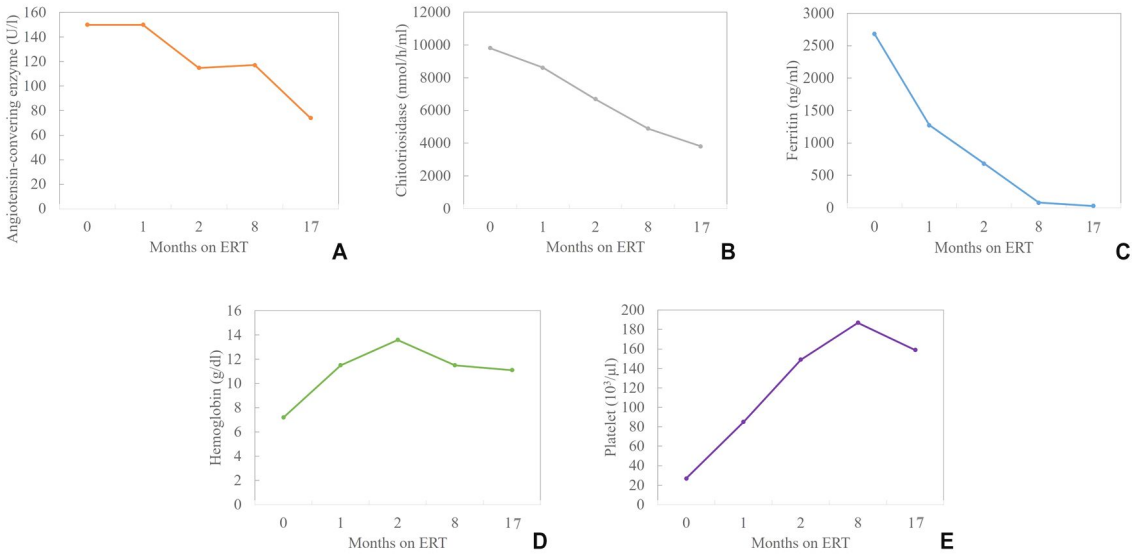

Fig. 4. Treatment with imiglucerase resulted in a gradual decrease of Gaucher disease biomarkers. (A) Angiotension-converting enzyme; (B) Chitotriosidase; (C) Ferritin; (D) Hemoglobin; (E) Platelet.

months at the age of 37 months, as one of the *GBA* mutation (c.754T>A) is effective to chaperone therapy. However, the frequency of seizures did not decrease and muscle rigidity persisted.

Discussion

GD is the first lysosomal storage disorder available for macrophage-targeted enzyme replacement therapy, and early initiation of ERT may contribute to a better treatment outcome. We analyzed type 3 GD patient with a genetic background of neuronopathic GD (compound heterozygous c.754T>A, c.887G>A mutations) and treated him from an early age. Our results revealed that ERT rapidly reverted the patient's visceral symptoms and his quality of life improved, even though neurological outcomes had a limited effect.

To date, over 200 different *GBA* mutations have been reported in the Human Gene Mutation Database at the Institute of Medical Genetics in Cardiff (http://www.hgmd.cf.ac.uk/; August 2017). The mutation varies according to ethnic groups[8]. In the Korean patients, the prevalent *GBA* mutant alleles are very different from those in the Jewish and non-Jewish Caucasian, but similar to those of the Japanese and Chinese groups[9]. Particularly, two mutant alleles found in our patient, c.754T>A and c.887G>A, are included in the common *GBA* mutant alleles in the Korean individual with GD[9].

According to previous reports on the Korean GD patients, c.754T>A and c.887G>A alleles have been known to be found in types 2 and 3 GD patients, respectively. The patient with c.754T>A mutation showed chronic progressive neurologic symptoms including seizure, lateral gaze impairment, cognitive deficit, and tremor, and most of them were diagnosed with type 3 GD[6,9]. On the other hand, c.887G>A allele can lead to a more severe and acute form of neuronopathic GD. For instance, Jeong et al. described homozygous c.887G>A 5-month-old infant with severe clinical phenotypes diagnosed with type 2 GD[9]. This supports the relationship between genotypes and clinical phenotype of GD. In general, making a clear distinction between types 2 and 3 is difficult due to the genetic heterogeneity and overlap of the age at onset[5]. In this study, we classified our patient with type 3 GD rather than type 2, because the onset of neurological symptoms was the age of 1 and older (mean age at onset of the type 2 patient was 7 months[10]). Moreover, when considering early manifestation of neurological symptoms including supranuclear gaze palsy and generalized seizure, the patient was finally diagnosed with type 3a GD.

ERT, hematopoietic stem cell transplantation, substrate reduction therapy (SRT), and pharmacological chaperone therapy (PCT) are currently being used to treat the patients with GD and the potential of gene and stem cell therapies have been investigated[11-13]. ERT reduces the size of the liver and spleen, as well as increases hemoglobin levels, platelet count, and bone mineral density in the patients with GD[11]. However, the efficacy of ERT to improve neurologic symptoms was not consistent[11]. In our case, ERT rapidly reverted patient's visceral symptoms and decreased activity of biomarkers that reflected the burden of disease, such as angiotensin-converting enzyme (ACE), chitotriosidase, and ferritin. However, neurological symptoms, including horizontal gaze palsy and generalized seizure, persisted. Because tight junctions of the blood-brain barrier (BBB) block the passage of the ERT drugs, improvement in the CNS involvement is limited. PCT is hypothesized to offer a new strategy for treating the neurological manifestations. Ambroxol, a com-

monly used mucolytic agent, has been proposed as a candidate pharmacological chaperone[13]. It can increase lymphocyte glucocerebrosidase activity, permeate the BBB, and decrease glucosylsphingosine levels in the cerebrospinal fluid. The combination of ERT and PCT should enhance the effect of ERT, since PCT assists in trafficking of the endogenous mutant beta-glucosidase out of the endoplasmic reticulum to lysosomes where they may have some residual activity[13]. As our patient's genotype (c.754T>A) is a known *GBA* mutation in which the chaperone effects of ambroxol were detected[13], he had been treated ambroxol (25 mg/kg/day) for 3 months at the age of 37 months. In a study of five patients with neuronopathic GD, Narita et al. reported that PCT with high-dose oral ambroxol from 6 to 48 months was well tolerated and effective in the treatment of neurological manifestations, particularly in myoclonus and pupillary light reflex dysfunction[13]. However, in this study, there was no change in neurological symptoms after the treatment of ambroxol, and further analysis of the treatment effect is needed.

The large variability in the treatment outcomes of type 3 GD might arise from the differences in age to start the treatment. In the literature, Tajima et al. described 24 Japanese patients with type 3 GD starting ERT from median age of 1.8 years old[10]. The authors emphasized that the interval between onset of the disease and the initiation of the treatment might have affected disease prognosis. More recently, Ni-Chung et al. reported 7 early-treated type 3 GD patients, and their median age at treatment initiation was 2.1 years[14]. The results revealed that early initiation of ERT decreased somatic burden of the patients, such as hematological impairment and hepatosplenomegaly. This fact suggests that prompt initiation of ERT of type 3 GD patients is effective for the life-threatening hematologic, visceral, and skeletal symptoms of the disease and improves mortality and modality. In our study, although neurological outcomes showed a limited effect, the frequency of transfusion significantly decreased, and irritability caused by bone pain was alleviated after early initiation of ERT. Therefore, early diagnosis and ERT should be recommended for type 3 GD considering patient's quality of life.

In conclusion, this study describes the clinical course of type 3 GD patients who underwent prompt initiation of ERT and emphasizes awareness of GD for early diagnosis. Moreover, considering the high proportion of the neuronopathic GD in the Korean population, investigations into new therapeutic strategies targeting the nervous system are required.

Acknowledgments

This study was supported by a grant from Samsung Medical Center (#GFO217006).

요 약

고셔병은 리소좀축적병으로 lysosomal hydrolase glucocerebrosidase 결여로 간비장비대, 골격계 증상, 빈혈, 혈소판 감소증의 증상을 나타내는 드문 상염색체 유전 질환이다. 본 증례에서는 18개월 남아에서 간비장비대, 성장 부진이 관찰되었으며 안구 운동 장애 및 발달 지연이 동반되어 제 3형 고셔병을 의심하였고 효소 분석 및 유전자 검사를 통해 확진하였다. 환아에서 한국인 신경형 고셔병에서 흔하게 관찰되는 c.754T>A (F213I)와 c.887G>A (R257Q)가 이형 접합체 돌연변이로 확인되었고 17개월 간의 효소 대체 요법을 통해 성장, 혈액학적 지표, 간비장비대 및 골증상은 호전되었지만 신경학적 증상의 호전은 없었고, 샤프론 중 암브록솔에 유의한 반응이 있다고 알려져 있는 c.754T>

A이 확인됨에 따라 환아에서 3개월간 암브록솔 치료를 시도하였지만 뚜렷한 임상적 치료 효과를 확인할 수 없었기에 본 증례를 보고하는 바이다.

참고문헌

1) Mistry PK, et al. Consequences of diagnostic delays in type 1 Gaucher disease: the need for greater awareness among hematologists-oncologists and an opportunity for early diagnosis and intervention. Am J Hematol 2007;82:697-701.
2) Barneveld RA, et al. Assignment of the gene coding for human beta-glucocerebrosidase to the region q21-q31 of chromosome 1 using monoclonal antibodies. Hum Genet 1983;64:227-31.
3) Kaplan P, et al. The clinical and demographic characteristics of nonneuronopathic Gaucher disease in 887 children at diagnosis. Arch Pediatr Adolesc Med 2006; 160:603-8.
4) Gupta N, et al. Type 2 Gaucher disease: phenotypic variation and genotypic heterogeneity. Blood Cells Mol Dis 2011;46:75-84.
5) Park JK, et al. Myoclonic epilepsy in Gaucher disease: genotype-phenotype insights from a rare patient subgroup. Pediatr Res 2003;53:387-95.
6) Lee JY, et al. Clinical and genetic characteristics of Gaucher disease according to phenotypic subgroups. Korean J Pediatr 2012;55:48-53.
7) Weinreb NJ, et al. Effectiveness of enzyme replacement therapy in 1028 patients with type 1 Gaucher disease after 2 to 5 years of treatment: a report from the Gaucher Registry. Am J Med 2002;113:112-9.
8) Beutler E, Gelbart T, Scott CR. Hematologically important mutations: Gaucher disease. Blood Cells Mol Dis 2005;35:355-64.
9) Jeong SY, Park SJ, Kim HJ. Clinical and genetic characteristics of Korean patients with Gaucher disease. Blood Cells Mol Dis 2011;46:11-4.
10) Tajima A, et al. Clinical and genetic study of Japanese patients with type 3 Gaucher disease. Mol Genet Metab 2009;97:272-7.
11) Vellodi A, et al. Management of neuronopathic Gaucher disease: revised recommendations. J Inherit Metab Dis 2009;32:660-4.
12) Cox-Brinkman J, et al. Potential efficacy of enzyme replacement and substrate reduction therapy in three siblings with Gaucher disease type III. J Inherit Metab Dis 2008;31:745-52.
13) Narita A, et al. Ambroxol chaperone therapy for neuronopathic Gaucher disease: A pilot study. Ann Clin Transl Neurol 2016;3:200-15.
14) Lee NC, et al. Outcome of early-treated type III Gaucher disease patients. Blood Cells Mol Dis 2014;53: 105-9.