Case Report

A case of perinatal hypophosphatasia with a novel mutation in the ALPL gene: clinical course and review of the literature

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Abstract. Hypophosphatasia (HPP) is a metabolic bone disease characterized by failure of bone calcification and vitamin B6 dependent seizures. It is caused by loss-of-function mutations in the ALPL gene. A newborn girl required respiratory support by nasal-directional positive airway pressure at birth, and pyridoxine hydrochloride administration for vitamin B6-dependent seizures observed from day two. Umbilical cord blood showed low alkaline phosphatase (ALP) activity and high pyridoxal phosphate levels. Radiographs showed severe rickets-like appearance of the bones. Genetic analysis of the ALPL gene revealed compound heterozygous mutations, c.1559delT/p.Ser188Pro. We diagnosed her with perinatal severe HPP, and started the patient on asfotase alfa from day six. Following enzyme replacement therapy (ERT), skeletal mineralization and respiratory insufficiency improved with no remarkable side-effects. Crying vital capacity (CVC) was used to evaluate respiratory status, which continuously improved from 13.3 mL/kg (day 22) to 20.6 mL/kg (day 113). Since no seizures occurred, pyridoxine hydrochloride was tapered off at one year of age. Strategies to manage perinatal severe HPP cases following ERT have not been established till date. A review of the literature shows that CVC may be a good indicator for weaning from ventilatory support. In addition, ERT will most likely enable withdrawal of pyridoxine treatment.

Key words: asfotase alfa, crying vital capacity, ALPL, pyridoxal phosphate, pyridoxine hydrochloride

Introduction

Hypophosphatasia (HPP, OMIM #241500) is a congenital metabolic bone disease characterized by failure of bone calcification and vitamin B6-dependent seizures. HPP is caused by mutations in the ALPL gene (OMIM *171760) leading to impaired activity of tissue-nonspecific alkaline
phosphatase (TNSALP). Patients with severe hypophosphatasia often die at or soon after birth from respiratory insufficiency due to pulmonary hypoplasia that occurs as a sequel to poorly mineralized ribs. Respiratory infection often complicates the clinical picture (1, 2). Additionally, vitamin B6-dependent seizures may also occur because pyridoxal cannot traverse the blood brain barrier in the phosphorylated form.

Although there were no effective treatments for HPP, asfotase alfa, a recombinant TNSALP, has been shown to be effective in infants and young children with this disease (1). While the prognosis of patients with severe HPP has remarkably improved with enzyme replacement therapy (ERT) (3), management strategies for the chronic phase following ERT have not been established.

Here, we report the clinical course of a perinatal patient with severe HPP and a novel ALPL mutation who was subjected to ERT, and review the literature focusing on sequelae and management strategies following ERT.

Materials and Methods

Measurement of crying vital capacity (CVC)

The reservoir bag was removed from a Jackson Rees circuit (Inspiron©) and connected to a spirometer (CHESTAC-8900: Chest Inc., Tokyo, Japan). The mask was firmly fitted onto the patient’s face and she was stimulated to cry. The tidal volume was measured when she cried and exhaled to the maximum.

Genetic analysis

After obtaining written informed consent from the parents, the ALPL gene was sequenced using genomic DNA extracted from peripheral mononuclear cells of the patient and both parents. The primers and PCR conditions have been described previously (4). Both strands were directly sequenced. The ethical committee of the Research Institute of Osaka Women’s and Children’s Hospital approved the protocol of this study.

Biochemical examination

Serum alkaline phosphatase (ALP) concentration was measured by the enzyme reaction-rate method using a Quick Auto Neo ALP JS kit (Shino-Test Science Laboratories Inc., Tokyo, Japan). To evaluate serum phosphorus (P), Sysmex IP reagents L “Kokusai” (Sysmex corporation, Kobe, Japan) was used. Serum pyridoxal phosphate and pyridoxal levels were measured using high-performance liquid chromatography with fluorescence detection, using pre-column derivatization by semicarbazide (5).

Case Report

A six-day-old girl, conceived by in vitro fertilization, was born as the first child of healthy and non-consanguineous Japanese parents. Shortening of her femurs was observed by fetal ultrasonography at 17 wk of gestation. She was delivered at a gestational age of 41 wk and 5 d by emergency cesarean section due to non-reassuring fetal heart rate. At birth, she had a weight of 1,940 g (–4.2 SD), height of 40.0 cm (–5.6 SD), head circumference of 30.0 cm (–2.1 SD), and chest circumference of 26.5 cm (–4.2 SD). Apgar’s score at 1 and 5 min were 2 and 8, respectively. There were no obvious deformities except for an open posterior fontanel. The mother was asymptomatic, but her ALP activity was low (62 IU/L, normal range: 100–325 IU/L). Data on the father’s ALP activity was not available, although he had fractured his lower limb during exercise.

ALP activity was low (11 IU/L, normal range: 530–1,610 IU/L) in the umbilical cord blood (Table 1), but the levels of substrates of ALP were elevated. Umbilical cord blood pyridoxal phosphate level was 3,095.6 nmol/L (normal range: 20.5–151 nmol/L). Urine phosphoethanolamine level was 2,790 nmol/mgCre (normal range: 39.4–93.5 nmol/mgCre).
The patient had seizures on days 2–4. While phenobarbital was ineffective, pyridoxine hydrochloride (30 mg/kg/d iv) initiated on day 4 terminated the seizures. Serum Ca and P concentrations were within the normal range. Radiographs of the long bones and ribs showed under-mineralization and severe rickets-like appearance (Fig. 1).

Due to the clinical symptoms and low ALP activity, she was clinically diagnosed with HPP, and ERT with asfotase alfa was initiated on day 6 (2 mg/kg subcutaneously, thrice per wk). Subsequent genetic analysis of the \textit{ALPL} gene revealed compound heterozygous mutations (c.1559delT/p.Ser188Pro) (Fig. 2), which confirmed the diagnosis. Following ERT, ALP activity rose to 13,423 IU/L on day 7. On day 12, serum Ca and ionized Ca levels decreased to 7.8 mg/dL and 1.15 mmol/L, respectively (Table 1). Hypocalcemia improved without treatment, and no clinical symptoms were observed. In the first two months, the peak values of ALP were mostly between 30,000–40,000 IU/L, and the trough values were 16,000–27,000 IU/L. The rickets-like appearance rapidly improved after initiating ERT, and almost disappeared by day 63 (Fig. 1). Her weight and chest circumference reached the normal range by 5 months of age. However, her height was still below the third percentile (Fig. 4).

After birth, the patient required nasal-directional positive airway pressure (nDPAP) to support respiration. Without nDPAP, she showed retractive breathing and cyanosis during the first few weeks. Since a previous case had been successfully weaned using CVC to assess respiratory function (2), we decided to use this measurement. She was gradually weaned off nDPAP by intermittently disconnecting the device. On day 21, her CVC was 13.3 mL/kg. She was completely weaned on day 36 and her CVC had increased to 14.5 mL/kg by day 52, which was near the criterion of 15 mL/kg for extubation (6). Her CVC gradually increased to 20.6 mL/kg on day 114, but did not increase thereafter (Fig. 3). After confirming a CVC over 20 mL/kg, we prepared her for discharge, and she was discharged on day 184.

Pyridoxine hydrochloride treatment was initiated at 30 mg/kg/d and was continued following asfotase alfa administration, since there was no evidence that the drug could ameliorate the vitamin B6-dependent seizures. The dose of pyridoxine hydrochloride was gradually tapered to 8 mg/kg/d. At 12 mo of age, pyridoxine hydrochloride was discontinued.
Fig. 1. Radiographs before and after enzyme replacement therapy (ERT). Radiographs on day six. (a) Metaphyses of the long bones in the lower limbs showed fraying, cupping, and tongue-like projections. (b) Metaphyses of the long bones in the upper limbs also showed fraying and cupping. (c) Narrow thorax and poorly mineralized ribs. (d) Following ERT, mineralization of the bones began to show signs of improvement on day 21, and the rickets-like appearance in the metaphyses disappeared by day 63.

Fig. 2. Genetic analysis of the ALPL gene. (a) Genetic analysis revealed that the patient had a previously reported c.1559delT mutation on the maternal allele and a novel p.Ser188Pro mutation on the paternal allele. (b) Serine 188 is highly conserved across species.
because seizures were not observed after starting ERT. Subsequently, she has had no seizures.

**Discussion**

We diagnosed the present case as perinatal severe HPP, due to low ALP activity, vitamin B6-dependent seizures, and respiratory insufficiency. The narrow thorax necessitated nDPAP, but the patient did not develop respiratory failure. The modest clinical course may be due to relatively well-mineralized bones at birth and early introduction of ERT. Some cases manifest hypocalcemia following initiation of ERT, which results most probably from rapid calcium uptake into the bone (2). Although our case also developed mild hypocalcemia (Table 1), it resolved spontaneously and no treatment was needed.

Gene analysis revealed compound heterozygous ALPL gene mutations: c.1559delT on the maternal allele and p.Ser188Pro on the paternal allele (Fig. 2). The c.1559delT mutation has no residual activity and the prevalence of its homozygosity has been estimated to be more than 1/900,000 (7, 8). It has also been shown to account for 40.9% of severe alleles in the Japanese population (4). In contrast, the p.Ser188Pro substitution has not been reported or registered in any database. However, the serine residue

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**Fig. 3.** Crying vital capacity (CVC).

**Fig. 4.** Growth curves of the patient.
at position 188 is preserved across species, and \textit{in silico} analysis by PolyPhen-2, SIFT, and Mutation Taster predicted the p.Ser188Pro mutation as ‘probably damaging’ with a score of 0.998, sensitivity of 0.27, and specificity of 0.99, ‘damaging’ with a score of 0, and ‘disease causing,’ respectively (9–11). We, therefore, reasoned that the compound heterozygous mutations (c.1559delT/p.Ser188Pro) in the \textit{ALPL} gene were responsible for the phenotype observed in the patient.

Cases of perinatal HPP treated with asfotase alfa before 1 yr of age were searched for on PubMed. Clinical information of seven patients was found as of September 2017. We analyzed the data focusing on two points: management of respiratory function and the effect of asfotase alfa on vitamin B6-dependent seizures.

Assessment and management of the respiratory function is critical in the early phases of severe perinatal HPP. However, there have been few reports describing how respiratory function was monitored during weaning (Table 2). The respiratory status of patients with HPP is widely diverse (1, 3, 12, 13). Moreover, it is not well known whether respiratory function will recover to levels comparable to those in normal healthy infants following ERT. Thus, it is necessary to establish indicators to objectively evaluate respiratory function. To this end, we monitored both CVC and chest circumference. We reasoned that CVC would reflect respiratory function more precisely, since chest circumference can be influenced by subcutaneous tissue. A previous report recommended extubating when CVC reaches 15 mL/kg (6). We discharged the patient when her CVC was 20 mL/kg, with the 5 mL/kg buffer as a safety margin (2), such that she would not succumb to respiratory infections. Following discharge, our patient has undergone several episodes of respiratory infections, but has not required respiratory support. Therefore, CVC over 20 mL/kg may be a good indicator when discharging patients with severe HPP.

In some cases of vitamin B6-dependent seizures, the seizures had ceased after ERT, and in one of the cases, vitamin B6 administration had been terminated (Table 3) (14). Since asfotase alfa is designed to target bones, it is not clear whether the drug can dephosphorylate pyridoxal phosphate sufficiently enough to supply the brain with pyridoxal. In our case, the pyridoxal phosphate/pyridoxal ratio decreased after administration of asfotase alfa (Table 1). Similar to case 6 in Table 3 (14), although we stopped pyridoxine administration, no seizures

\begin{table}[h]
\centering
\caption{Literature review of respiratory status/management in perinatal hypophosphatasia patients who were subjected to ERT during infancy}
\begin{tabular}{llllll}
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Respiratory status & Serum ALP (IU/ml) & Age at start of ERT & Indicators of respiratory management \\
\hline
Baseline & Recent & & & & \\
1 Whyte MP et al.\textsuperscript{1}\textsuperscript{1} & CPAP & Fully weaned & 20 & 7.5 months & ND \\
2 Whyte MP et al.\textsuperscript{1}\textsuperscript{1} & Intubation and Ventilation & Dead & 21 & 20 days & ND \\
3 Whyte MP et al.\textsuperscript{1}\textsuperscript{1} & Progressive respiratory deterioration & Fully weaned & 20 & 5 weeks & ND \\
4 Whyte MP et al.\textsuperscript{1}\textsuperscript{1} & Progressive respiratory deterioration & Nasal canula / O\textsubscript{2} & 6 & 2 months & ND \\
5 Okazaki Y et al.\textsuperscript{2}\textsuperscript{2} & HFO / iNO & Tracheostomy / O\textsubscript{2} & 2 & 1 day & CVC \\
6 Kitaoka T et al.\textsuperscript{3}\textsuperscript{9} & CPAP & Fully weaned & 23 & ND & ND \\
7 Kitaoka T et al.\textsuperscript{3}\textsuperscript{9} & CPAP & Fully weaned & 0 & ND & ND \\
8 Kitaoka T et al.\textsuperscript{3}\textsuperscript{9} & HFO / iNO & O\textsubscript{2} & 15 & ND & ND \\
9 Kitaoka T et al.\textsuperscript{3}\textsuperscript{9} & Nasal canula & Fully weaned & 39 & ND & ND \\
10 Kitaoka T et al.\textsuperscript{3}\textsuperscript{9} & SIMV & SIMV & 23 & ND & ND \\
11 Our case & nasal DPAP & Fully weaned & 11 & 6 days & CVC \\
\hline
\end{tabular}
\end{table}

\textbf{AS: Apgar score, CPAP: continuous positive airway pressure, CVC: crying vital capacity, DPAP: directional positive airway pressure, ERT: enzyme replacement therapy, HFO: high-frequency oscillation, ND: not determined, SIMV: synchronized intermittent mandatory ventilation.}
have been observed so far. This implies that asfotase alfa treatment can generate sufficient amounts of dephosphorylated vitamin B6 that diffuse across the blood brain barrier.

In conclusion, we reported a case of perinatal severe HPP with a novel compound heterozygous mutation. Previous studies have indicated that CVC may be a useful indicator of respiratory function in the early management of these patients. In addition, ERT may allow for the termination of pyridoxine hydrochloride administration.

Conflict of Interest: N.N. consults for and has received honoraria as a speaker from Alexion. All other authors have no conflict of interests.

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