



## Case Report

## Two children with hypophosphatasia with a heterozygous c.1559delT variant in the *ALPL* gene, the most common variant in Japanese populations

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## ABSTRACT

Hypophosphatasia (HPP), a genetic disorder characterized by decreased tissue-nonspecific alkaline phosphatase (TNSALP) activity, is caused by loss-of-function mutations in the *ALPL* gene, which encodes TNSALP. The most frequent pathogenic variant in Japanese patients with HPP is a frameshift mutation in the *ALPL* gene, c.1559delT, and its carrier frequency is reported to be one in 480 in the Japanese population. We report the cases of two Japanese children with HPP who had a heterozygous c.1559delT variant in the *ALPL* gene. One case (involving a neonate) exhibited respiratory insufficiency associated with vitamin B6 dependent convulsions, significant defective mineralization similar to the severe form of HPP, and extremely low ALP activity. Enzyme replacement therapy (ERT) using asfotase alfa promptly improved her respiratory insufficiency, bone mineralization, and maintained her motor development during infancy. The second case involved a 10-year-old boy who demonstrated diffuse musculoskeletal pain and weakness that progressively disturbed mobility. Although he showed no bony lesions, the clinical symptoms and biochemical abnormalities were compatible with childhood HPP. ERT successfully relieved the severe generalized pain and significantly improved motor function.

## 1. Introduction

Hypophosphatasia (HPP) is a rare skeletal dysplasia characterized by defective bone and tooth mineralization, which are associated with impaired activity of tissue-nonspecific alkaline phosphatase (TNSALP). HPP is caused by loss-of-function mutations in the *ALPL* gene which encodes TNSALP. It has been classified as perinatal lethal, benign prenatal, infantile, childhood, adult, and odontohypophosphatasia, according to the age of onset and severity (Ozono and Michigami, 2011; Wenkert et al., 2011). Phenotypes of patients with HPP are closely related to the residual enzyme activity of *ALPL* variants (Whyte et al., 2018). The first and second most frequent pathologic variants in the *ALPL* gene in Japanese patients with HPP were c.1559delT and p.F327L, respectively (Taketani et al., 2014). Michigami et al. (2005) measured enzymatic activities of some TNSALP protein variants using reconstruction experiments and demonstrated that the p.F327L variant retained approximately 70 % of its enzymatic activity, whereas the c.1559delT variant developed a complete loss of activity. Watanabe

et al. (2011) reported the frequency of c.1559delT carriers to be one in 480 among Japanese individuals. Additionally, most of these individuals had normal levels of HPP biochemical markers, such as serum ALP and urinary phosphoethanolamine (PEA). Here, we report two children with symptomatic HPP who had a heterozygous c.1559delT variant in the *ALPL* gene. Enzyme replacement therapy (ERT) successfully reduced the pathological symptoms in both children. Written informed consent was obtained from the patient's parents who viewed this report and approved its publication.

## 2. Case report

## 2.1. Case 1

This patient was delivered via vaginal delivery at 37 weeks of gestation. Her birth weight and height were 2050 g (−2.2 SD) and 42.5 cm (−2.8 SD), respectively. Her 1- and 5-min Apgar scores were 8 and 9, respectively. She was the second child of healthy non-consanguineous

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parents. Her parents and a sibling were unremarkable for any genetic conditions, and they did not experience symptoms compatible with HPP, such as premature loss of deciduous teeth. She was managed in the neonatal intensive care unit of a tertiary care hospital because of low birth weight and respiratory distress. On day 13, convulsions were observed, which were poorly managed with intravenous phenobarbital. On day 19, she was intubated and ventilated due to uncontrolled convulsions. A HPP diagnosis was subsequently confirmed based on extremely low serum ALP activity (1 IU/L, measured using the Japan Society Clinical Chemistry: JSCC method, reference interval: 530–1610 IU/L) and whole-body radiographic findings, including pulmonary hypoplasia with thin ribs and evidence of rickets at the metaphysis of the long tubular bones (Fig. 1A, E). Serum calcium and phosphorus were within normal range; however, pyridoxal-5'-phosphate (PLP) was significantly elevated (2892.3 nmol/L; reference interval, 20.5–151 nmol/L) (Tables 1, 2). ERT with recombinant human bone-targeted TNSALP asfotase alfa (2 mg/kg  $\times$  3/week) was initiated on day 22. Prolonged convulsions were successfully treated using intravenous pyridoxine hydrochloride (30 mg/kg/day). She was fully weaned from mechanical ventilation on day 39 and discharged at the age of 5 months. The serum PLP level was significantly decreased after 1 month of asfotase alfa treatment (Table 2). To confirm a diagnosis, the whole coding region with exon/intron boundaries of the *ALPL* gene was sequenced by the Sanger method, using a genomic DNA sample extracted from the patient's peripheral blood leukocytes. A heterozygous c.1559delT variant was identified at the age of 6 months. The patient continued to receive ERT as outpatient treatment. Sanger sequencing of the *ALPL* gene using DNA samples extracted from her parents' saliva demonstrated that a heterozygous c.1559delT variant was detected in her asymptomatic mother, who never reported low levels of serum ALP.

Mineralization of the long bones in the upper and lower limbs recovered, and the thoracic size was enlarged within 4 months of

**Table 1**

Serum bone markers in Cases 1 and 2.

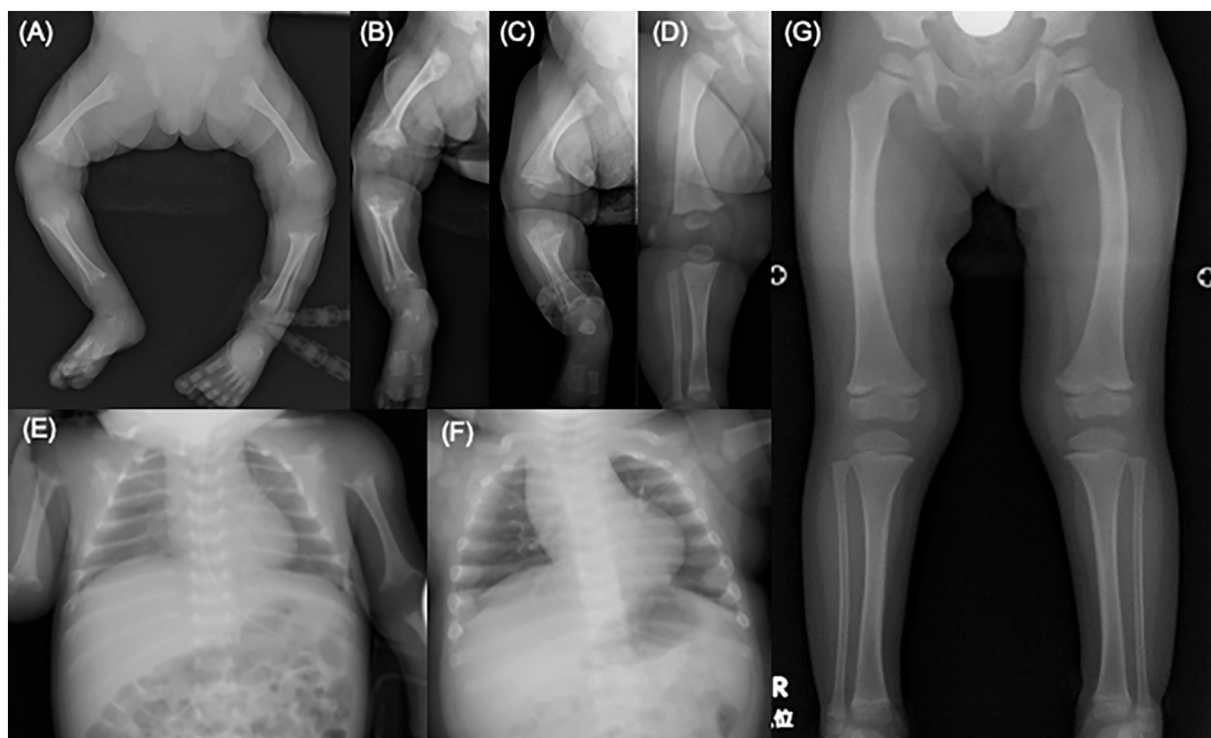
	Case 1 (reference interval)	Case 2 (reference interval)
Ca (mg/dL)	10.4 (9.0–11.0)	9.7 (8.7–10.2)
P (mg/dL)	6.5 (5.0–7.7)	5.1 (3.9–5.8)
ALP (IU/L)	1 (530–1610)	97 (161–508)

Ca: calcium, P: phosphorus, ALP: alkaline phosphatase.

asfotase alfa treatment (Fig. 1B–D, F). Motor development was initially delayed, but gradually caught up, and she could walk independently at the age of 13 months. No serious adverse events were observed during ERT. At the latest follow-up, at 2.8 years old, she exhibited no physical or mental restrictions on daily life, except for significant short stature (height: 81.3 cm,  $-2.8$  SD) (Fig. 2). An anteroposterior standing radiograph of the bilateral lower limbs demonstrated mild bowing of the long bones, without evidence of rickets-like hypomineralization (Fig. 1G).

## 2.2. Case 2

A 10-year-old boy visited a local orthopaedic clinic due to atraumatic right hip pain (day 0), which was initially diagnosed as transient coxitis. He was healthy, of normal stature, exhibited no musculoskeletal impairment, and had no history of premature tooth loss. His parents and younger brother had no suspicious findings for HPP or other genetic diseases. In addition to right hip pain, he gradually suffered from right upper arm, bilateral knee, ankle, and lower back pain; the location of which varied daily. He visited a local pediatrician on day 13 because of persistent, diffuse musculoskeletal pain requiring school absences. Laboratory findings were normal, except for a low serum ALP level (112 IU/L, measured using the International Federation Clinical Chemistry and Laboratory Medicine: IFCC method; reference interval, 161–508 IU/

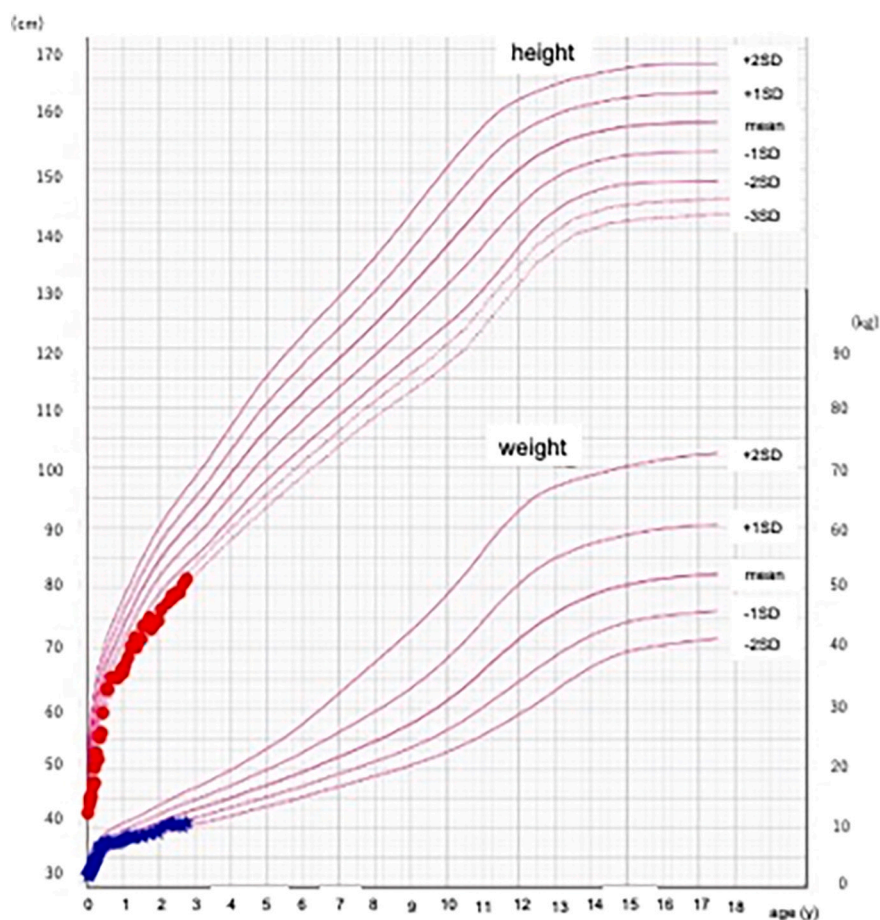


**Fig. 1.** Anteroposterior radiographs of the lower limbs in Case 1 before asfotase alfa treatment (A) showing remarkable rickets-like hypomineralization at the metaphysis of the long bones. Anteroposterior radiographs of the right lower limb in Case 1 at 1 month (B), 2 months (C), and 4 months (D) after asfotase alfa treatment, demonstrating acceleration of mineralization and rapid disappearance of rickets-like changes. Anteroposterior radiographs of the chest in Case 1 before treatment (E) and at 4 months after asfotase alfa treatment (F) revealing enlargement of the thoracic size with increased rib thickness after treatment. An anteroposterior radiograph of the bilateral lower limbs at 2.8 years old in Case 1 (G) demonstrating mild bowing of the long bones without evidence of rickets-like hypomineralization.

**Table 2**  
Change in serum PLP and PL levels before and after the administration of asfotase alfa.

	Case 1		Case 2		Reference interval
	Pre-treatment	1 month after ERT	Pre-treatment	1 month after ERT	
PLP (nmol/L)	2892.3	96.2	139.8	25.5	20.5–151.0
PL (nmol/L)	82.8	25,485.2	23.0	21.1	8.8–58.7
PLP/PL	34.9	0	6.1	1.2	1.0–4.2

PLP: pyridoxal 5'-phosphate, PL: pyridoxal, ERT: enzyme replacement therapy with asfotase alfa.



**Fig. 2.** Growth curves of Case 1. Short stature persists despite enzyme replacement therapy.

L), which was overlooked by the pediatrician. In addition to diffuse, progressive musculoskeletal pain, the patient experienced anxiety and depression. He was referred to our orthopaedic clinic on day 22.

At the first examination, he reported right hip pain; however, he was able to walk independently at a slow speed. There were no remarkable findings in his hips, knees, and ankles, nor neurological abnormalities in his lower limbs; however, the second serum ALP measurement remained low (97 IU/L) and the serum phosphorus level was normal (Table 1). HPP was considered as a differential diagnosis, and we measured urinary PEA and performed *ALPL* gene analysis on day 28, although no bony deformity or rickets were observed on radiographs. His prepubertal form corresponded with Tanner stage 1. Bone mineral density (BMD) of the lumbar vertebrae was measured using dual-energy X-ray absorptiometry (DXA) and was within the normal range (L1-L4 average, 0.528 g/cm<sup>2</sup>; -1.9 SD of normal values). He was referred to a general pediatrician on day 28, considering the possibility of psychogenic disorders. The general pediatrician suspected psychosomatic disorders and prescribed Chinese medicine. The bilateral lower limb pain and weakness deteriorated, and he became unable to walk on day 35. He was

referred to a pediatric rheumatologist on day 42 who excluded inflammatory diseases such as juvenile idiopathic arthritis. During this time, his mobility significantly decreased due to progressive skeletal pain that had spread to the lower and upper limbs. Urinary PEA, from the samples extracted on day 28, was elevated (184.8 μmol/gcr; reference interval for adults, 7.0–70.0 μmol/gcr), and a heterozygous c.1559delT variant in the *ALPL* gene was identified by the Sanger sequencing. Based on low serum ALP activity, increased urinary PEA, and the genetic variant in the *ALPL* gene, we diagnosed him with childhood HPP with diffuse musculoskeletal pain, and asfotase alfa treatment 1 mg/kg was administered subcutaneously 6 times per week on day 62. Samples extracted just before the first administration of asfotase alfa demonstrated significantly decreased serum ALP (66 IU/L) and increased urinary PEA (517.0 μmol/gcr) (Table 3). His serum PLP level was within the normal limits (139.8 nmol/L; reference interval, 20.5–151 nmol/L); however, the PLP/PL ratio was elevated (6.1; reference interval, 1–4.2), which is a consistent finding in HPP (Table 2). A heterozygous c.1559delT variant in the *ALPL* gene was also identified in his asymptomatic mother, who reported that she had been pointed out to have a low ALP level (24 IU/L;

**Table 3**

Change in biomarkers related to HPP before treatment with asfotase alfa in Case 2.

	Day 14	Day 22	Day 41	Day 62 <sup>a</sup>	Reference interval
Serum ALP (IU/L)	112	97	90	66	161–508
Urinary PEA (μmol/gcr)	–	184.8	–	517.0	7.0–70.0 <sup>b</sup>

HPP: hypophosphatasia, ALP: alkaline phosphatase, PEA: phosphoethanolamine.

<sup>a</sup> Samples on day 62 were extracted just before the first administration of asfotase alfa.

<sup>b</sup> Reference interval for adults.

reference interval, 38–113) and decreased BMD in a past medical examination.

Asfotase alfa administration remarkably improved his physical function, as assessed by the 6-minute walk test. His 6-minute walk distance was 26 m before treatment and significantly increased to 234 m after 1-month of ERT (Table 4). Pain was evaluated using the visual analog scale (VAS), which is used to determine the pain intensity experienced by an individual (a score of 0 indicating no pain and a score of 10 indicating maximum pain). During treatment, his VAS-score gradually declined, and he exhibited marked improvement in his diffuse skeletal pain, with an average VAS-score of 5.1 before treatment and 1.9 after 2 months of treatment. Serum PLP levels significantly decreased and the PLP/PL ratio normalized after ERT (Table 2). Despite the rapid improvement in physical function, his mental state slightly improved but had not completely recovered after 3 months of asfotase alfa treatment.

### 3. Discussion

Michigami et al. (2020) analyzed *ALPL* variants in 98 unrelated patients with HPP in Japan and demonstrated that c.1559delT was the most frequently detected variant at a rate of 45.4 %. A heterozygous c.1559delT variant in the *ALPL* gene, whose carrier frequency is estimated to be 1/480, may be a founder mutation in Japanese individuals. Although Watanabe et al. (2011) demonstrated that most heterozygous c.1559delT carriers had normal levels of ALP activity and urinary PEA, significantly low serum ALP levels were observed in our patients. One patient developed vitamin B6 dependent convulsions and significant defective mineralization on radiographs, and the other developed childhood HPP with marked generalized musculoskeletal pain and weakness. Koyama et al. (2020) reported on an adult patient with HPP with a heterozygous c.1559delT variant in the *ALPL* gene, who showed progressive general fatigue and muscle weakness with low serum ALP activity. Herein, we reported the second cases of symptomatic HPP caused by a heterozygous c.1559delT variant.

Respiratory distress associated with vitamin B6 dependent convulsions, hypomineralization with rickets-like changes, and significantly reduced ALP activity in Case 1 suggested a severe form of HPP. However, the subsequent clinical course with a favorable response to asfotase alfa, differed from that of perinatal lethal or infantile HPP (Whyte et al., 2012). Rickets-like metaphyseal abnormalities promptly disappeared, the respiratory insufficiency with convulsion recovered within a short period, and motor development was maintained during continuous ERT.

Establishing a diagnosis of HPP in heterozygous carriers who present with atypical symptoms, such as in Case 2, remains a challenge (Rush, 2018). Using magnetic resonance imaging, Beck et al. (2011) demonstrated that patients with childhood HPP can experience lower limb pain with significant metaphyseal bone edema. Although there was no defective bone mineralization in Case 2, the deterioration of diffuse musculoskeletal pain was associated with a decline in serum ALP, increased PLP/PL ratio, and elevated urinary PEA levels, despite being a heterozygous carrier (Tables 1, 2). Similar to our case, Strandbech et al.

**Table 4**

Change in 6MWD (m) before and 1 month after treatment using asfotase alfa in Case 2.

	1 min	2 min	3 min	4 min	5 min	6 min
Pre-treatment	4.5	11	16	20	13.5	26
Post-treatment	42	79	115	157	195	234

6MWD: 6-minute walking distance.

(2021) demonstrated that asfotase alfa significantly contributed to pain reduction and improvement in quality of life in a patient with childhood HPP who showed no radiographic abnormalities and had a debilitating pain phenotype. Chronic pain is linked to depression (Sheng et al., 2017); however, our patient's mental health did not recover despite the disappearance of pain on ERT. He could not overcome his depression even after 3 months of asfotase alfa treatment.

According to the *ALPL* variant database (<https://alplmutationdatabase.jku.at/>), patients with HPP with a c.1559delT variant were homozygous or compound heterozygotes. It was unlikely that a heterozygous c.1559delT variant alone produced overt disease. However, sequencing throughout the coding region and exon-intron boundaries of the *ALPL* gene could not identify any other pathologic variants in both patients. From our current limited data, factors that may have contributed to disease development in our patients cannot be elucidated. As we did not perform sequencing on other genes or a genome-wide analysis, the possibility of pathological variants in a gene other than *ALPL* cannot be excluded. Moreover, genetic defects on the modifier genes or a large deletion in the *ALPL* gene should be considered. Furthermore, environmental or epigenetic factors may have contributed to the clinical manifestations in our patients.

In conclusion, we demonstrated that carriers of the heterozygous c.1559delT variant, the most common variant among Japanese individuals, can develop symptoms of HPP associated with decreased ALP activity. Physicians should determine the targeted therapy based on clinical judgment indicating evidence of overt disease supported by compatible laboratory findings, not only by the presence of the *ALPL* variant.

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### CRedit authorship contribution statement

**Hiroshi Kitoh:** conceptualization, data collection, funding acquisition, investigation, writing-original draft. **Masako Izawa:** conceptualization, data collection. **Hiroshi Kaneko:** data collection, writing-review & editing. **Akiko Kitamura:** data collection, writing-review & editing. **Saori Matsuyama:** data collection, writing-review & editing. **Kohji Kato:** formal analysis, resources. **Tomoo Ogi:** formal analysis, resources.

### Conflict of interest

None.

### Data availability

Data will be made available on request.

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