

jrpe-2018-0217.R1

## Case Report

### Perinatal form hypophosphatasia caused by a novel large duplication of *ALPL* gene and one year follow-up under enzyme replacement therapy; a case report

**Short title:** A novel duplication on ALPL gene

Bulent Hacıhamdioğlu\*, Gamze Ozgurhan\*\*, Catarina Pereira\*\*\*, Emre Tepeli\*\*, Gülşah Acar\*\*, Serdar Cömert\*\*

\* Istinye University, Istanbul, Turkey, Istanbul, Turkey

\*\* University of Health Sciences, Suleymaniye Women Maternity and Child Diseases Training and Research Hospital, Istanbul

\*\*\* Centogene AG, Rostock, Germany

**Correspondence author:** Bülent Hacıhamdioğlu, MD, Istinye University, Istanbul, Turkey, Istanbul, Turkey

bhacihamdioglu@gmail.com.tr,

+90 212 481 36 55

orcid.org/0000-0001-7070-6429

### What is already known on this topic?

Hypophosphatasia is caused by mutations in the gene encoding tissue-nonspecific isoenzyme of alkaline phosphatase. Missense mutations are the most common type of mutations described for this gene, while duplications are rarely described. It has been shown that enzyme replacement therapy mineralizes the skeleton and improves respiratory function and survival in the life-threatening perinatal form of HPP.

## What this study adds?

We report a novel large homozygous duplication encompassing exons 2 to 6 of the *ALPL* gene. Early diagnosis and rapid intervention with enzyme replacement therapy is life-saving in the severe form of hypophosphatasia.

## Abstract

Hypophosphatasia is a rare disease caused by mutations in the gene encoding tissue-nonspecific isoenzyme of alkaline phosphatase. Duplications of the *ALPL* gene account for fewer than 1% of the mutations causing HPP. It has been shown that asfotase alfa treatment mineralizes the skeleton and improves respiratory function and survival in severe forms of hypophosphatasia. The newborn was evaluated for respiratory failure and generalized hypotonia after birth. Diagnosis of HPP was based on low-serum ALP activity, high levels of substrates of tissue-nonspecific isoenzyme of alkaline phosphatase and radiologic findings. On day 21 after birth, enzyme replacement therapy using asfotase alfa (2 mg/kg three times per week, subcutaneous injection) was started. We were able to discharge our patient when he was 7 months old. His respiratory support was gradually reduced and skeletal mineralization improved during treatment. No mutation was detected in the *ALPL* gene by all exon sequencing, and additional analysis was done by quantitative polymerase chain reaction. As a result, a novel homozygote duplication encompassing exons 2 to 6 was detected. Early diagnosis and rapid intervention with enzyme replacement therapy is life-saving in the severe form of hypophosphatasia. Quantitative polymerase chain reaction can detect duplications if a mutation cannot be detected by sequence analysis in patients with hypophosphatasia.

**Key words:** Hypophosphatasia, perinatal form, *ALPL* gene, duplication, enzyme replacement therapy

## Introduction

Hypophosphatasia (HPP) is a rare disease caused by mutations in the gene encoding tissue-nonspecific isoenzyme of alkaline phosphatase (TNSALP) (1). It is estimated that the incidence of severe forms of the disease is approximately 1 in 300,000 in Europe and approximately 1 in 435,517 in Turkey (2,3). Patients have been classified traditionally as having perinatal, infantile, childhood, or adult HPP based on symptom severity and presentation age. Currently, specific bone-targeted recombinant enzyme replacement therapy (ERT) (asfotase alfa; STRENSIQ®, Alexion Pharmaceuticals, Inc.) is available for HPP patients, and it is suggested for patients with pediatric-onset HPP (1,4).

Alkaline phosphatases (ALPs) are membrane-bound ectoenzymes that hydrolyze monophosphate esters. Human ALP is classified into four types: TNSALP, intestinal, placental (PLAP), and germ cell. TNSALP's expression is widespread, especially in the liver, bone, kidney, neuronal cells, and neutrophils. It is expressed on the cell membrane of hypertrophic chondrocytes, osteoblasts, and odontoblasts and is also concentrated on the membranes of budding matrix vesicles on these cells. TNSALP is essential for tissue biomineralization (5-7).

TNSALP is encoded by an *Alkaline Phosphatase-Liver (ALPL)* gene on the chromosome 1p36.12. To date, over 300 different mutations in *ALPL* gene have been identified. Missense mutations are the most common type of mutation. Duplications on this gene have been reported very rarely. Herein, we report a novel duplication in the *ALPL* gene in a patient with perinatal form of HPP, as well as the patient's clinical characteristics and briefly the results of 12-months follow-up under ERT.

## Case report

The patient was evaluated for respiratory failure and generalized hypotonia after birth. He was born from second cousin consanguineous parents at full-term weighing 3,440 g. The birth length was 50 cm, and the head circumference was 35 cm. Diagnosis of HPP was based on low-serum ALP activity, high levels of substrates of TNSALP (Table 1) and radiologic findings (Figure 1). The parents were of Turkish origin and healthy. At the time of the assessment (age father 37 years, age mother 32 years), the parents had no clinical symptoms of HPP.

No mutation was detected in the *ALPL* gene by full gene sequencing, and thus, we decided to do additional analysis by quantitative polymerase chain reaction (qPCR). Blood samples were collected from the patient and parents. DNA isolation was done by salt precipitation method. The qPCR analysis is done by LightCycler 480 Software (Roche), and a relative quantification analysis is performed that compares the target DNA sequence (that of the patient) with a reference DNA sequence (used for normalization of the ratio). Primers were designed for the coding exons 2 to 12 of the gene of interest; 0,5  $\mu$ L primer forward, 0,5  $\mu$ L primer reverse, 10  $\mu$ L SYBR Green I Master, and 2  $\mu$ L DNA were used for reaction mix in a total volume of 20  $\mu$ L. As a result, a novel homozygous duplication encompassing exons 2 to 6 was detected (Figure 2). This mutation was classified as likely pathogenic (class 2) according to the American College of Medical Genetics (ACMG) and Centogene's guidelines. Genetic analysis of the parents demonstrated that both were carriers of the same mutation (Figure 3).

Asfotase alfa was kindly provided by Alexion Pharmaceuticals as part of the compassionate use program. On day 21 after birth, ERT using asfotase alfa (2 mg/kg three times per week, subcutaneous injection) was started.

After birth, he was intubated and ventilated by SIMV mode. The inspiratory requirement was gradually reduced during treatment. Due to an ongoing requirement for

mechanical ventilation, tracheostomy was performed at the age of 6 months. The patient was discharged from the hospital at 7 months of age. At 12 months he needed ventilation via tracheostomy only during sleep (8 hours a day).

Improved mineralization during the treatment was observed (Figure 1). There was no significant hypercalcemia before the treatment, and hypocalcemia was not observed during the same period. Actually we have not performed and an objective test, for example Bayley Scales of Infant and Toddler Development, for evaluate neurocognitive functions during first 12 months old. However we noticed that that relational acts emerge (for example placing spoon in cup. We could not evaluated speech function due to tracheostomy. At the age of one, the patient was able to sit up with support, with full head control, but he was not yet able to stand. Seizures were not observed before or during treatment, and there was no sign of craniosynostosis at 12 months old. At 12 months old his weight was 8.0 kg (SDS: -1.87), his height was 75.0 cm (SDS: -0.59), and his head circumference was 46.0 cm (SDS: -0.75 ). He continued to display normal oral intake. Kidney function and renal ultrasound were normal. No side effects were observed during the first 12 months of treatment. The patient is still in treatment with asfotase alfa, and we hope to share long-term follow-up results in the future.

Written informed consent was obtained from the parents of the patients.

## **Discussion**

To our knowledge large duplications on the ALPL gene have not been reported to date, but minor duplications is not rare (8,9). Herein, we report a novel large homozygote duplication encompassing exons 2 to 6 of the ALPL gene. It is well known that missense mutations are the most common type of mutation of this gene (9). This case highlights that duplication or deletion analysis should be performed if a mutation cannot be detected by s6equencing in a patient clinically diagnosed with HPP.

Recently, it has been shown that ERT (asfotase alfa) mineralizes the skeleton and improves respiratory function and survival in the life-threatening perinatal form of HPP (9). Survival is the main goal, but not the only goal, for patients with a perinatal form of HPP who receive ERT. Other goals of the treatment are the improvement of respiratory status, skeletal mineralization, growth and physical development, promotion of normal developmental milestones, treatment of craniosynostosis, seizure control, and shortening of hospitalization (11). We were able to discharge our patient when he was 7 months old. His inspiratory support was gradually reduced and skeletal mineralization improved during treatment. Asfotase alfa treatment has a good safety profile for children. Common adverse reactions are hypersensitivity reactions, localized lipodystrophy, ectopic calcification of the eye, and nephrocalcinosis. Severe hypocalcemia has also been reported (11,12). We monitored our patient according to the current guidelines, and we did not observe any adverse reactions during the first 12 months (11).

In this report, we describe a child diagnosed with the perinatal form of HPP with a novel large duplication in the *ALPL* gene. Although we were unable to perform cDNA studies/mRNA, this large duplication is very likely to be pathogenic based on ACMG guidelines. Early diagnosis and rapid intervention with ERT is life-saving in the severe form HPP. Duplications can be detected by qPCR if a mutation cannot be detected by sequence analysis in patients with HPP.

#### **Editorship Contribution**

Follow-up of patient and medical practice: Bulent Hacıhamdioğlu, Gamze Özgürhan, Gülşah Acar, Serdar Cömert, Genetic Analyses and Comment Catarina Pereira, Emre Tepeli, Literature Search: Bulent Hacıhamdioğlu, Gamze Özgürhan, Writing: Bulent Hacıhamdioğlu.

Conflict of Interest

Alexion Pharmaceuticals Inc. provided a scientific/medical review and English grammar editing of the case report as a courtesy upon authors' request; authors had final authority over all content in this publication.

Dr. BH received payment from Alexion Pharmaceuticals Company for being part of the HPP advisory board. Other authors have no financial relationships relevant to this article to disclose. The authors declare that they have no conflict of interest.

#### References

1. Mornet E. Hypophosphatasia. *Metabolism*. 2018;82:142-155.
2. Mornet E, Yvard A, Taillandier A, Fauvert D, Simon-Bouy B. A molecular-based estimation of the prevalence of hypophosphatasia in the European population. *Ann Hum Genet* 2011;75:439-445.
3. Sağlam H, Erdöl Ş, Dorum S. Clinical and Genetic Findings of Turkish Hypophosphatasia Cases. *J Clin Res Pediatr Endocrinol*. 2017;9:229-236.
4. Whyte MP, Greenberg CR, Salman NJ, Bober MB, McAlister WH, Wenkert D, Van Sickle BJ, Simmons JH, Edgar TS, Bauer ML, Hamdan MA, Bishop N, Lutz RE, McGinn M, Craig S, Moore JN, Taylor JW, Cleveland RH, Cranley WR, Lim R, Thacher TD, Mayhew JE, Downs M, Millán JL, Skrinar AM, Crine P, Landy H. Enzyme-replacement therapy in life threatening hypophosphatasia. *N Engl J Med* 2012;366:904–913.
5. Orimo H. The mechanism of mineralization and the role of alkaline phosphatase in health and disease. *J Nippon Med Sch*. 2010;77:4-12.
6. Orimo H. Pathophysiology of hypophosphatasia and the potential role of asfotase alfa. *Ther Clin Risk Manag*. 2016;12:777-786.
7. Rader BA. Alkaline Phosphatase, an Unconventional Immune Protein. *Front Immunol*. 2017;8:897.

8. Mornet E, Hofmann C, Bloch-Zupan A, Girschick H, Le Merrer M. Clinical utility gene card for: hypophosphatasia - update 2013. *Eur J Hum Genet.* 2014;22(4).
9. Tenorio J, Álvarez I, Riancho-Zarrabeitia L, Martos-Moreno GÁ, Mandrile G, de la Flor Crespo M, Sukchev M, Sherif M, Kramer I, Darnaude-Ortiz MT, Arias P, Gordo G, Dapía I, Martínez-Villanueva J, Gómez R, Iturzaeta JM, Otaify G, García-Unzueta M, Rubinacci A, Riancho JA, Aglan M, Temtamy S, Hamid MA, Argente J, Ruiz-Pérez VL, Heath KE, Lapunzina P. Molecular and clinical analysis of ALPL in a cohort of patients with suspicion of Hypophosphatasia. *Am J Med Genet A.* 2017;173:601-610.
10. Whyte MP, Rockman-Greenberg C, Ozono K, Riese R, Moseley S, Melian A, Thompson DD, Bishop N, Hofmann C. Asfotase Alfa Treatment Improves Survival for Perinatal and Infantile Hypophosphatasia. *J Clin Endocrinol Metab.* 2016;101:334-342.
11. Kishnani PS, Rush ET, Arundel P, Bishop N, Dahir K, Fraser W, Harmatz P, Linglart A, Munns CF, Nunes ME, Saal HM, Seefried L, Ozono K. Monitoring guidance for patients with hypophosphatasia treated with asfotase alfa. *Mol Genet Metab.* 2017;122:4-17.
12. Scott LJ. Asfotase Alfa: A Review in Paediatric-Onset Hypophosphatasia. *Drugs.* 2016;76:255-262.



## Tables and legends

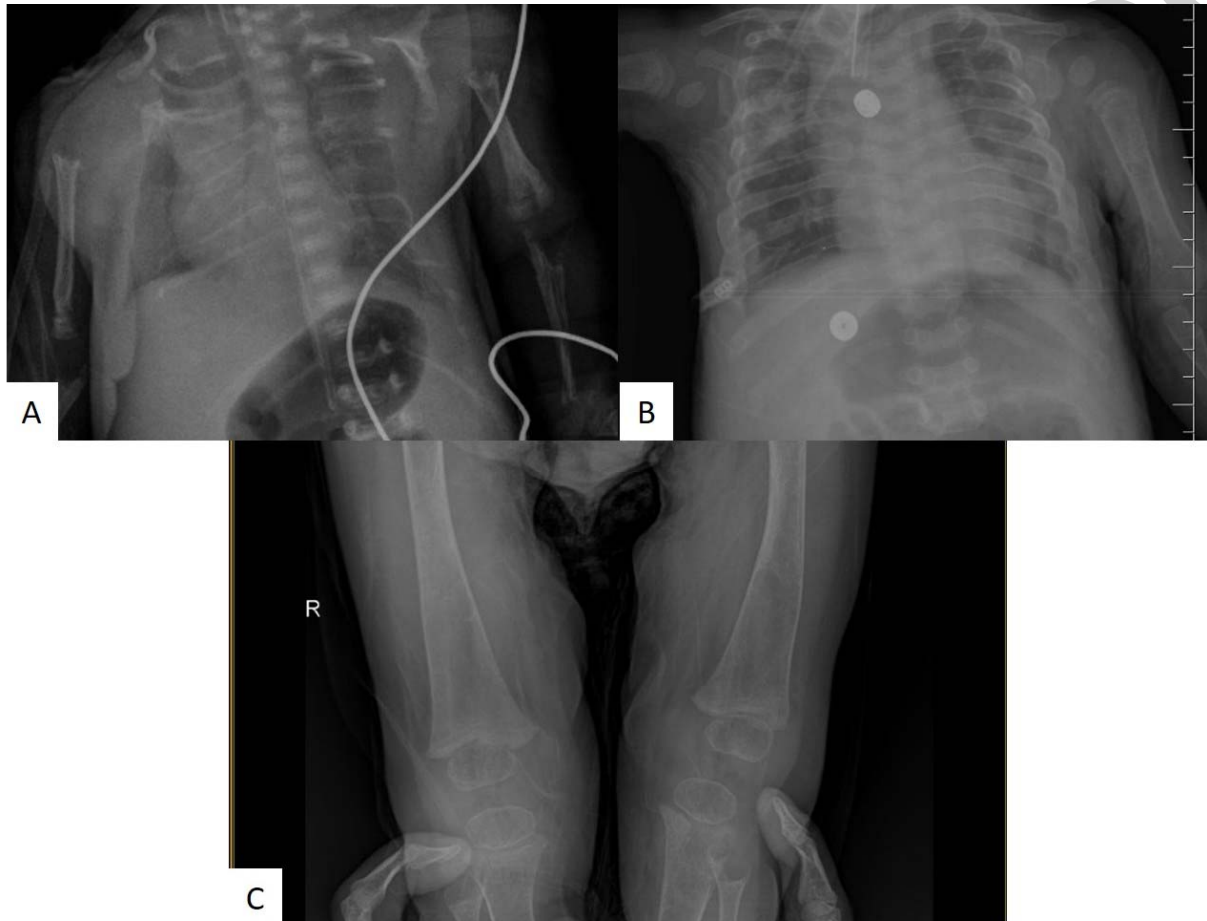
**Table 1; Patient and parents' laboratory results**

	<b>Patient</b>	<b>Mother</b>	<b>Father</b>
Alkaline phosphatases (ALP) (IU/l)	8 (100-380)	52.00 (25-94)	27.0 (33-107)
Pyridoxal 5'-phosphate (PLP) ( $\mu\text{g/l}$ )	14104.0 (5-30)	7.8 (5-50)	20.2 (5-50)
Phosphoethanolamine (PEA) ( $\mu\text{mol/g creatinine}$ )	2767.5 (15-341)	41.6 (0-48)	21.8 (0-48)
Inorganic pyrophosphate (PPi)	NA	NA	NA

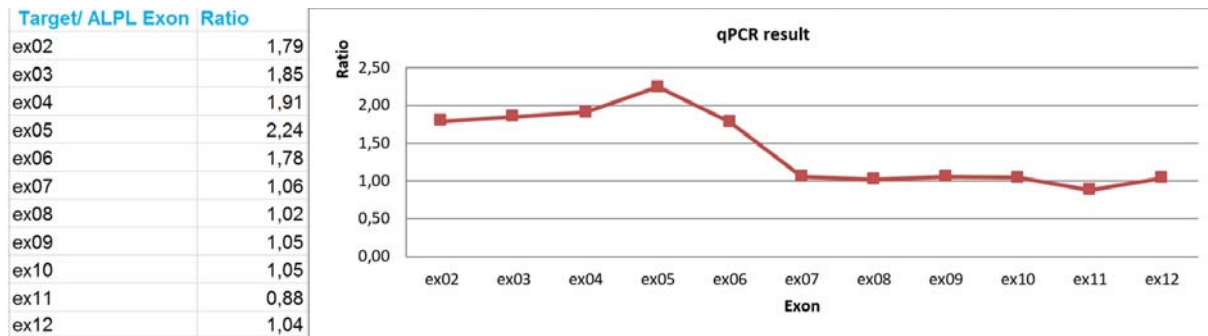
Uncorrected proof

## Figure legends

**Figure 1:** X-ray of the patient; (A) before treatment, (B and C) at 12 months of treatment. Note the general improvement of mineralization and rachitic changes under asfotase alfa enzyme replacement therapy.



**Figure 2:** qPCR assay by using 11 gene-specific amplicons encompassing the coding exons 2 to 12 ALPL gene. Normalized qPCR ratios are WT (0.70 -1.35) and homozygous duplication (4n) (1.75 -2.35).



**Figure 3:** Mother and father heterozygous carriers of the same mutation.

MOTHER		FATHER	
Target/ ALPL Exon	Ratio	Target/ ALPL Exon	Ratio
ex02	1,598	ex02	1,627
ex03	1,489	ex03	1,589
ex04	1,624	ex04	1,495
ex05	1,711	ex05	1,661
ex06	1,544	ex06	1,523
ex07	1,061	ex07	1,077
ex08	1,095	ex08	1,015
ex09	0,986	ex09	1,121
ex10	1,034	ex10	1,069
ex11	1,094	ex11	1,112
ex12	0,984	ex12	0,994

