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A Case of Hypophosphatasia that was Overlooked for a Long Time in a Patient with a Novel Duplication Mutation in *ALPL*

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Abstract

We report a case of hypophosphatasia that was overlooked for a long time in a patient with a novel mutation in the *ALPL* gene, coding for tissue-nonspecific alkaline phosphatase. A 49-year-old man who had been experiencing frequent fractures since childhood showed a low serum alkaline phosphatase level in a routine health checkup. His bone mineral density was also low. Analysis of his *ALPL* gene showed that he was heterozygous for c.1171dup (p.Arg391ProfsTer14), which was a frameshift variant. From these results, he was diagnosed as having hypophosphatasia caused by a novel mutation.

Keywords: Alkaline phosphatase; Fracture; Bone mineral density; Checkup; Heterozygous

Abbreviations: ALP: Alkaline phosphatase; BMD: Bone mineral density; HPP: Hypophosphatasia; NMD: nonsence-mediated mRNA dacay

Introduction

The main features associated with hypophosphatasia (HPP) are frequent or intractable fractures owing to bone weakness accompanied with muscle pain and growth retardation. A wide range of symptoms involving various organs and functions, including neurological symptoms, may occur. HPP is caused by homozygous, compound heterozygous, or heterozygous mutations in the *ALPL* gene coding for tissue-nonspecific alkaline phosphatase, on chromosome 1p36. Fraser classified HPP into the following forms according to the age of onset: perinatal, infantile, childhood, and adult [1]. Whyte indicated a fifth form of HPP with primarily only dental manifestations, referred to as odontohypophosphatasia (OMIM:146300). More than 400 *ALPL* gene mutations (mostly missense) have been reported to date (https://alplmutationdatabase.jku.at/portal/; accessed on November 27, 2023) [2], inherited in either an autosomal dominant or recessive manner. In this report, we present a case of a patient with a novel *ALPL* gene mutation who had a history of frequent bone fractures, but was overlooked for a long time.

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The patient was a 49-years-old man who was found to have an abnormally low level of ALP at 37 U/L (normal range: 38–113) at a routine health checkup. His past medical history included 10 bone fractures without any particular diagnosis, since a young age. His teeth were normal. His family history showed neither bone fractures, arthralgia, kidney diseases nor neurological diseases. He had neither arthralgia nor muscle pain. He complained of sleeping problems. Values of common laboratory tests were unremarkable except for his serum ALP level. His ionized Ca (iCa) level was high, at 2.77

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mmol/L (normal range: 2.41–2.72) compared with normal serum Ca. Serum Zn and 25-hydroxy vitamin D levels were low at 55 µg/dL and 12.6 ng/mL, respectively. His TRACP (Tartrate-Resistant ACid Phosphatase)-5b level was normal. Other laboratory findings were normal, as shown in Table 1. Calcification was not visible on X-ray. Abdominal ultrasound displayed no calcification of the kidney. Bone mineral density (BMD) of the lumbar spine and left femoral neck were 0.995 g/cm^2 and 0.759 g/cm^2, respectively. T-scores of the lumbar spine and left femoral neck were low, at −0.9 (87%) and −2.1 (73%), respectively. His serum Zn level was low, and he began treatment with Zn medication (NOBELZIN○RR) and a RNKL inhibitor. He has not had any fractures since starting these treatments.

Table 1: Laboratory data of the patient.

Item	Value	Unit	Normal range
WBC	5690	/µL	3,500–9,000
Neutrophils	69.3	%	40–60
Eosinophils	1.2	%	1–5
Basophils	1.1	%	0–5
Lymphocytes	23.7	%	18–50
Monocytes	4.7	%	2–10
Hb	13.3	g/dL	13.5–17.6
Ht	40	%	39.8-–51.8
Plt	22.8	×10^4/µL	15–40
TP	6.6	g/dL	6.7–8.3
T-Bil	1.3	mg/dL	0.3–1.2
AST	14	IU/L	8–33
ALT	16	IU/L	4–45
γ-GTP	18	IU/L	10–47
LDH	154	IU/L	119–229
ALP	28	IU/L	38–113
Ca	9.3	mg/dL	8.8–10.1
iCa	**2.77**	mEq/L	2.41–2.72
IP	3.5	mg/dL	2.7–4.6
Mg	2.1	mg/d	1.8–2.4
Zn	**59**	µg /d	65–110
25-Vitamin D	**16.4**	ng/mL	20–
BUN	14.4	mg/dL	8.0–22.0
Cr	0.84	mg/dL	0.6–1.1
Amylase	139	IU/L	44–132
T-chol	225	mg/dL	128–219
HDL-chol	79	mg/dL	48–103
LDL-chol	129	mg/dL	65–163
TG	71	mg/dL	30–149
UA	4.7	mg/dL	3.6–7.0
Glucose	90	mg/dL	70–109
Na	141	mEq/L	138–146
K	3.8	mEq/L	3.6–4.9
FT3	2.99	pg/mL	2.3–4
FT4	1.11	ng/dL	0.9–1.7
TSH	0.78	µIU/mL	0.5–5
P1NP	27.1		
TRACP-5b	127	mU/dL	120–420
Urinalysis			
Protein	**(+)**		(–)
Blood	(-)		(–)
*Values in bold indicate abnormal values			

Analysis of the *ALPL* gene demonstrated that he had a heterozygous mutation of c.1171dup (p.Arg391ProfsTer14), which is likely to be pathogenic. ClinVar functional analysis indicated that this mutation was pathogenic/likely pathogenic. The gene mutation was also demonstrated to be a protein change that was also "Likely pathogenic" (Pathogenicity: 0.971) by AlphaMissense [3]. A 3-dimensional (3D) model of the mutant protein is shown in Figure 1. The effect of this mutation was determined by MutationTaster (https://www.mutationtaster.org [accessed on February 7, 2024]), and it was determined that there was no protein synthesis from the mutant allele owing to a nonsense-mediated mRNA decay (NMD) mechanism.

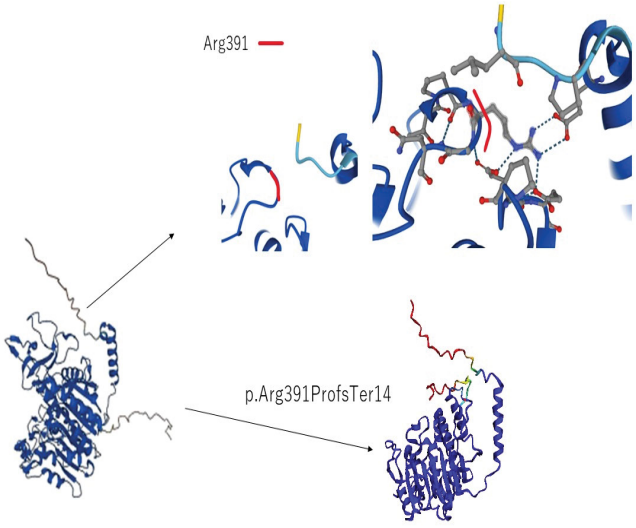

Figure 1: Three-dimensional structures of the normal and mutant ALP proteins of this patient, predicted from their amino acid sequences using AlfaFold 2.1 and AlfaFold DB. The mutant protein with the novel duplication mutation showed interruption of the structure.

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Discussion

HPP was first described by Rathbun in 1948 [4]. He described a new fatal disease entity in a 3-week old boy who had a previously unreported type of bone development abnormality. The low to absent ALP appeared to be the primary defect responsible for the disease. His long bones showed grossly irregular cartilage maturation and lack of calcification. His cranial vault was almost devoid of calcium.

HPP is a rare metabolic disease in which an enzyme called ALP, which is involved in the normal formation of bones and teeth, becomes inactive owing to genetic causes [5,6]. ALP levels are low in patients with HPP. Approximately 1 in 600 people are mutant gene carriers in Japan [7], and in some countries the rate is as high as 1 in 100 [8,9]. As patients with mild types of HPP have minimal symptoms, mild cases are often overlooked for a long time, until the patient is an adult. Approximately 50% of such patients have previously experienced fractures [10]. Clinically, HPP has a wide spectrum of presentations, ranging from neonatal death to an apparent lack of symptoms. The clinical manifestations of the disease include rickets-like bone changes, bone demineralization, fragility fractures, reduced muscular strength, chest deformities, pulmonary hypoplasia, nephrolithiasis, nephrocalcinosis, and chondrocalcinosis. The development of asfotase alfa (enzyme replacement therapy) has changed the prognosis of HPP by reducing bone deformities and improving bone mineralization, lung function, and muscle weakness, among other benefits. In adult patients, teriparatide and the anti-sclerostin antibody have been used off-label in selected cases, demonstrating benefits in accelerating fracture healing and in the concomitant treatment of osteoporosis [11].

HPP in most patients is caused by heterozygous or compound heterozygous (mostly missense; about 74%) mutations in the *ALPL* gene [12]. There are very few reports of associations between the phenotype and genotype. The disease type with a severe phenotype is transmitted in an autosomal recessive manner. Mild types are transmitted in either a dominant or recessive manner [13]. There is no clear report to date about the symptoms of *ALPL* mutation carriers. Calmarza et al. [14] reported 10 patients with mild HPP who had heterozygous mutations, with symptoms of only musculoskeletal pain and weakness [14]. In the present study, we were unable to perform genetic analyses of the patient's family members. However, his family history, including a son, did not show any bone problems. In cases of mild or moderate forms off HPP with an autosomal dominant inheritance, the underlying disease mechanism involves either a dominant-negative effect (DNE) of a single heterozygous mutation, intronic mutations, or mutations in the regulatory sequence of *ALP* [12,15]. In a recently published cohort of 424 HPP patients consisting of 166 heterozygotes and 258 homozygotes or compound heterozygotes, the disease could be subdivided into 3 subtypes (severe, moderate, and mild adult subtypes). Severe forms of HPP are mostly caused by homozygosity or compound heterozygosity; moderate forms of HPP are caused by a DNE of missense variants; whereas mild adult HPP, which is usually characterized by unspecific signs, is probably caused by *ALPL* gene haploinsufficiency [16]. In general, in moderate forms of HPP, about 50% of the patients have 2 pathogenic *ALPL* variants (compound heterozygote or homozygote), whereas 40% to 45% have 1 identified pathogenic variant, as in the present case. In milder forms of the disease, usually only 1 pathogenic *ALPL* gene variant is detected [15,17]. In Japanese HPP patients, the most frequent pathogenic variant is a frameshift mutation in the *ALPL* gene (c.1559delT) [18]. The frequency of c.1559delT carriers is reported to be 1 in 480 in the Japanese population [19]. To our knowledge, the natural history of these carriers has not reported to date. Moreover, no data on the detection rate of carriers using gene-targeted deletion/duplication analyses, such as performed in this study, is currently available [20].

The present patient was diagnosed as having HPP because of frequent fractures since childhood, and low BMD. His mutation has been registered with ClinVar by 4 facilities as "Pathogenic/Likely pathogenic." One case of adult HPP and 1 case of infantile HPP has been described in ClinVar, but no detailed clinical information is provided (https://www.ncbi.nlm.nih.gov/clinvar/variation/504232). Mornet et al. [21] analyzed a cohort of 424 HPP patients, and classified pathogenic *ALPL* gene variants according to their DNE and their severity using 3D modeling and functional tests. Although homozygosity mutations were an aggravating factor of symptom severity, and moderate alleles were rare both in number and frequency, sixty percent of adult HPP patients who were analyzed were heterozygous for an *ALPL* variant showing no DNE, suggesting another mechanism of dominance, such as haploinsufficiency, as in our case. They suggested that adults with dominant HPP without DNE represent a new clinical entity, characterized by nonspecific symptoms and a low ALP level, and for which a high prevalence is expected [21]. Taillandier et al. [22] reported novel mutations in patients with severe HPP. They reported 1 infantile HPP patient with a compound heterozygous mutation (c.1171delC and Met45Leu) [22]. In addition, in an *in vitro* experiment, when a deletion (c.1171delC) was inserted into this position (c.1171), the protein became (Arg391Valfs12), and was assumed to undergo NMD, resulting in no enzyme activity [23]. However, c.1171dup was first reported in patients with mild form of HPP. This mutation results in a stop codon 12 amino acids downstream of exon 11, at position 386. Thus, c.1171dup is assumed to be a pathogenic mutation causing mild HPP (probably the pediatric type).

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Yokoi et al. [24] reported 5 Japanese patients from 3 families with mild HPP. Analysis of the *ALPL* gene revealed compound heterozygous mutations, including Ile395Val and Leu520Argfs in family 1, Val95Met and Gly491Arg in family 2, and a dominant missense mutation (Gly456Arg) in family 3. All of these mutations were at the homodimer interface [24]. The novel mutation identified in this study is a frameshift, which might be located in the homodimer interface in the 3D figure (Figure 1). Genetic analysis of the patient's family members are needed to conclude the mode of inheritance of this patient's disease.

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Ethics approval and consent to participate:

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki declaration.

Consent for publication: Written informed consent was obtained from the patient for publication of this case report and any accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

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