Journal of Bone and Mineral Research, 2026, **41**, 259–269
https://doi.org/10.1093/jbmr/zjaf124
Advance access publication: September 11, 2025
Research Article

Biochemical phenotype of hypophosphatasia in asymptomatic individuals carrying *ALPL* variants

Rodrigo Montero-Lopez[1,2], Mariam R. Farman[1], Florian Högler[1], Catherine Rehder[3], Theodora Malli[4], Gerald Webersinke[4], Cheryl Rockman-Greenberg[5], Kathryn Dahir[6], Gabriel Ángel Martos-Moreno[7,8,9], Agnès Linglart[10], Keiichi Ozono[11], Lothar Seefried[12], Guillermo del Angel[13], Erica Burner Nading[14], Erin Huggins[14], Eric T. Rush[15,16], Josephine T. Tauer[1], Priya S. Kishnani[14], Wolfgang Högler[1,2,*]

[1]Department of Paediatrics and Adolescent Medicine, Johannes Kepler University Linz, 4021 Linz, Austria
[2]Centre for Growth and Osteology, Department of Paediatrics and Adolescent Medicine, Kepler University Hospital GmbH, 4020 Linz, Austria
[3]Department of Pathology, Duke University Medical Center, Durham, NC 27705, United States
[4]Laboratory for Molecular Genetic Diagnostics, Ordensklinikum Linz, 4010 Linz, Austria
[5]Department of Pediatrics and Child Health Max Rady College of Medicine, Rady Faculty of Health Sciences, Winnipeg, MB R3A 1S1, Canada
[6]Program for Metabolic Bone Disorders, Vanderbilt University Medical Center, Nashville, TN 37232, United States
[7]Departments of Pediatrics and Pediatric Endocrinology, Hospital Infantil Universitario Niño Jesús, IIS La Princesa, 28009 Madrid, Spain
[8]Department of Pediatrics, Universidad Autónoma de Madrid, 28029 Madrid, Spain
[9]CIBER Fisiopatología de la Obesidad y Nutrición. ISCIII, 28029 Madrid, Spain
[10]AP-HP, Paris Saclay University, INSERM, Bicêtre Paris Saclay Hospital, 94270 Le Kremlin-Bicêtre, France
[11]Department of Pediatrics, Osaka University Graduate School of Medicine, Suita, Osaka 565-0871, Japan
[12]Musculoskeletal Center Würzburg, University Hospital Würzburg, 97080 Würzburg, Germany
[13] Centre for Genomics Research, Discovery Sciences, Biopharmaceuticals R&D, AstraZeneca, Boston, MA 02210, United States
[14]Division of Medical Genetics, Department of Pediatrics, Duke University Medical Center, Durham, NC 27705, United States
[15]Division of Clinical Genetics, Children's Mercy Hospital Kansas City, Kansas City, MO 64108, United States
[16]Department of Pediatrics, University of Missouri-Kansas City School of Medicine, Kansas City, MO 64108, United States
*Corresponding author: Wolfgang Högler, Department of Paediatrics and Adolescent Medicine, Johannes Kepler University Linz, Krankenhausstraße 26–30, 4021 Linz, Austria (wolfgang.hoegler@kepleruniklinikum.at).

Abstract

Hypophosphatasia (HPP) is the rare metabolic disorder caused by variants in the *ALPL* gene, resulting in deficient activity of tissue-nonspecific alkaline phosphatase (ALP). This leads to accumulation of substrates contributing to impaired bone mineralization. Hypophosphatasia manifests with a broad clinical spectrum; however, an increasing number of individuals with *ALPL* variants have been identified presenting the hallmark biochemical feature of HPP of low serum ALP activity, with or without elevated serum pyridoxal-5-phosphate (PLP) or urine phosphoethanolamine (PEA), while remaining asymptomatic. These *ALPL* carriers may represent a distinct subgroup within the HPP continuum, prompting the need for clearer classification. Using data from the Global *ALPL* Gene Variant Database, we identified 43 subjects who fulfilled the following criteria: low ALP (adjusted for age/sex), at least one *ALPL* variant, and no overt or reported HPP-related symptoms. Their median age was 29 yr (range 0-64); 23 were female. Serum ALP activity was reduced in all cases, with 76% of subjects showing levels less than 50% below the lower limit of normal. In 19 of 43 individuals, PLP or PEA was also elevated. Thirty distinct genotypes were observed; 79% of subjects were heterozygous, while 21% harbored homozygous or compound heterozygous variants. The identified variants were largely missense (77%), mostly affecting regions without a specific domain (38%). Five variants showed a dominant-negative effect in vitro, yet produced no clinical manifestations. Some identified genotypes were also linked to adult, childhood, or odontohypophosphatasia phenotypes, underscoring significant genotype-phenotype variability. These findings refine our understanding of the HPP spectrum, identifying a cohort of asymptomatic *ALPL* carriers with biochemical phenotype of HPP. Recognizing this group is important for improving diagnostic criteria and preventing overdiagnosis and unnecessary treatment. Longitudinal studies are needed to clarify follow-up strategies and determine whether these individuals develop clinical manifestations later in life or remain asymptomatic.

Keywords: alkaline phosphatase, ALPL, nosology, phenotype, hypophosphatasemia, pyrophosphate, PLP, PEA, rickets, osteomalacia

Lay Summary

Hypophosphatasia (HPP) is a rare inherited condition causing abnormal bone and teeth mineralization, as well as non-skeletal manifestations, due to low levels of an enzyme called alkaline phosphatase (ALP). People with HPP often have weak bones, early tooth loss, muscle weakness, fatigue, and/or chronic pain. However, some individuals reported no obvious symptoms of HPP despite carrying the same genetic change and having low ALP. Our study identified 43 such people, showing that these genetic changes can exist without causing noticeable health problems but still affect important blood markers. Understanding this form will guide medical advice and help determine if these individuals will develop symptoms later. This knowledge will also improve diagnosis, counseling, and care for affected families.

Received: March 15, 2025. **Revised:** August 17, 2025. **Accepted:** September 7, 2025

Introduction

Hypophosphatasia (HPP) is a rare genetic metabolic disorder caused by pathogenic variants in the *ALPL* gene, which encodes tissue-nonspecific alkaline phosphatase (TNSALP).[1] Tissue-nonspecific alkaline phosphatase is a homodimeric cell-surface phosphohydrolase expressed on the cell surface of bone, kidney, and liver.[2] Its deficiency leads to low serum alkaline phosphatase (ALP) activity (hypophosphatasemia) and accumulation of inorganic pyrophosphate (PPi), which inhibits bone mineralization.[2] The combined presence of low ALP activity and the accumulation of other ALP natural substrates, such as pyridoxal-5-phosphate (PLP, a vitamin B6 vitamer) and/or urine phosphoethanolamine (PEA), defines the hallmark biochemical phenotype of HPP and contributes to the broad clinical spectrum of HPP.[3,4]

The *ALPL* gene, located on chromosome 1p36-34, spans 12 exons and demonstrates significant allelic heterogeneity, with more than 480 variants currently identified.[5–7] Hypophosphatasia can follow either an autosomal dominant or recessive inheritance pattern.[1] In dominant cases, heterozygous variants frequently exert a dominant-negative effect (DNE), where mutated TNSALP monomers disrupt the expression or function of WT monomers, resulting in diminished ALP activity. Even though DNE is not a necessary prerequisite for HPP manifestation in heterozygous individuals, this mechanism explains the dominant inheritance pattern of HPP, and the low serum ALP activity observed in some affected heterozygous individuals.[8–10]

Hypophosphatasia exhibits a broad clinical spectrum, ranging from severe perinatal hypomineralization and early mortality to milder or even asymptomatic forms. Traditionally, seven clinical forms have been recognized, classified by age of onset and severity: benign prenatal, perinatal, infantile, severe childhood, mild childhood, adult, and odontohypophosphatasia.[3,8] The most severe, early-onset HPP phenotypes appear at birth or in the first months of life, presenting with bone deformities and mineralization defects, respiratory distress, hypercalcemia, and vitamin B6-dependent seizures. These phenotypes frequently require intensive care, with poor prognosis without enzyme replacement therapy.[11] Moderate late-onset HPP phenotypes, such as mild childhood and adult HPP, are characterized by nonspecific musculoskeletal symptoms, including chronic pain, metatarsal fractures, pseudofractures, and muscle weakness, despite normal serum calcium and inorganic phosphate levels. Odontohypophosphatasia, which manifests as early primary tooth loss before the age of 4, can occur as an early sign of HPP or as an isolated dental condition.[8]

In recent decades, individuals with heterozygous pathogenic *ALPL* variants have been reported as exhibiting the characteristic biochemical phenotype of HPP while remaining clinically asymptomatic. This has often observed among asymptomatic relatives of individuals with severe biallelic HPP or identified through large-scale screening studies.[6,12–15] This subgroup of *ALPL* carriers expands the known spectrum of the disorder and highlights its underlying complexity.[16,17] Although the prevalence of moderate HPP phenotypes has been estimated at approximately 1 in 2430,[14] the carrier frequency of pathogenic or likely pathogenic *ALPL* variants is considerably higher, ranging from 1 in 190 to 1 in 290.[18] This disparity suggests that individuals with *ALPL* variants who display a biochemical trait of HPP but remain asymptomatic may be more common than previously recognized. Characterizing this phenotype is critical to improving diagnostic precision, informing family screening, and refining clinical management, while minimizing the risks of overdiagnosis and unnecessary treatment.

This paper aims to expand and better define the clinical spectrum of HPP by investigating asymptomatic individuals carrying *ALPL* variants who exhibit the biochemical phenotype of HPP. Using data from the *ALPL* Gene Variant Database, we undertook a detailed characterization of this underexplored presentation.

Materials and methods

This study was conducted by reviewing data available in the Global *ALPL* Gene Variant Database (https://alplmutationdatabase.jku.at/) by December 2024. This open-access database currently contains information concerning more than 480 *ALPL* variants and their associated over 1000 genotypes. The project offers a comprehensive and accessible tool for the medical and scientific community to share and utilize information on *ALPL* variants, genotypes, and phenotypes. The *ALPL* Gene Variant Consortium is (re)classifying variants of uncertain significance (VUS) and incorporating newly reported variants, genotypes, and phenotypes from both case submissions and literature mining. Ethical approval for the project was obtained from the Ethics Committee of the Medical Faculty at the Johannes Kepler University Linz, Linz, Austria (EK Nr: 1118/2021).

The methodology of the project is detailed elsewhere.[7] The database integrates information from three main sources: (1) the original Laboratoire SESEP database, curated by Prof. Etienne Mornet (University of Versailles Saint-Quentin-en-Yvelines), which was incorporated in April 2020; (2) case submissions via the project portal from clinicians and researchers; and (3) systematic scientific literature mining conducted by project staff. Each case is analyzed individually using an HPP likelihood score. This scoring system evaluates key biochemical, clinical, and radiological parameters, with particular emphasis on ALP activity and its natural substrate levels (see Table S1). Following this scoring process, all variants are subjected to a comprehensive evaluation that includes phenotype scoring, literature mining, assessment of genetic evidence, and in vitro functional testing where applicable. The *ALPL* Gene Variant Consortium (see Table S2) reviews all available evidence, including the HPP likelihood scores, and conducts the final classification or reclassification of variants based on the rigorous criteria established by the American College of Medical Genetics and Genomics/Association of Molecular Pathology (ACMG/AMP) guidelines.[19]

Asymptomatic carriers with a biochemical phenotype of HPP were identified based on the following criteria: (1) low serum ALP activity, adjusted for age and sex; (2) presence of at least one *ALPL* variant; and (3) documented absence of clinical or radiological signs and symptoms of HPP. Elevated levels of PLP or urinary PEA, when available, were considered supportive but not mandatory, in line with proposed diagnostic criteria that classify these as major, but not essential, biochemical indicators.[20] Individuals without *ALPL* variants or a documented alternative cause for low ALP levels were excluded. Heterozygous individuals without the biochemical phenotype were not included.

Key variables were collected for each subject, covering both genotypic and phenotypic data. Protected health information was not collected. Genotypic data of a subject can include one, two, or more *ALPL* variants. Variant data included the specific *ALPL* variant with base change, its ACMG/AMP classification, affected domain, variant type, and results from in-vitro functional testing (single and co-transfection).[7] Phenotypic data encompassed gender, age, serum ALP, PLP, and urinary PEA concentrations, all reported clinical and radiological features, and the HPP likelihood score if available. Serum ALP, PLP, and urinary PEA were evaluated according to reference ranges provided by submitting centers or otherwise those cited in the literature. Longitudinal data were collected if available.

RML, MRF, FH, and WH cross-referenced genotype and phenotype data, verifying biochemical markers according to project protocols and conducting data curation. Conflicting information was resolved by reviewing relevant publications or directly communicating with the original case submitters or corresponding authors of publications.

Descriptive statistics were used to summarize the clinical and biochemical characteristics of asymptomatic individuals with the biochemical phenotype of HPP. Continuous variables, such as age, were expressed as median and range values. Categorical variables, such as gender and variant classifications (eg, pathogenic, likely pathogenic, VUS), were presented as frequencies and percentages.

Results

A total of 43 asymptomatic individuals with *ALPL* variants and the biochemical phenotype of HPP were identified from the *ALPL* Gene Variant Database. Of these, 10 individuals had been reported directly through the *ALPL* Gene Variant Database submission process, while 33 were identified in the published scientific literature. The median age at the time of reporting was 29 yr (range 0-64 yr; age documented in 33/43), and 23 (53%) were female. Table 1 summarizes the phenotypic and genotypic characteristics of these individuals.

All subjects were reported to have low serum ALP activity, adjusted for age and sex (Figure 1). Among the 34 individuals with ALP numeric data, only 8 (24%) had ALP activity below 50% of the lower limit of normal. Eighteen patients had no available PLP or PEA measurements. Among the 25 patients with natural TNSALP substrate level data available, 15 presented with elevated PLP with or without PEA, while 4 had PEA levels above the upper limit of normal but lacked corresponding PLP measurements. Importantly, no signs or symptoms consistent with HPP were documented in any individuals across the cohort. Furthermore, none of the cases included mention of antiresorptive therapy or any other medications or conditions that might influence serum ALP levels.[8]

All 43 individuals had at least one variant in the *ALPL* gene, with a total of 30 different genotypes identified. Nine subjects exhibited two *ALPL* variants, involving five distinct genotypes: c.[237_238delCA];[455G>A], c.[455G>A];[484G>A], c.[715G>T];[715G>T], c.[787T>C];[787T>C], and c.[787T>C];[1559delT]. For the four subjects with two different variants, sufficient documentation, including genetic information from their parents, confirmed their compound heterozygous status. The remaining 34 subjects (79%) were heterozygous for a single *ALPL* variant.

Among the 31 *ALPL* variants reported, 12/31 (38%) were classified as pathogenic, 11/31 (35%) as likely pathogenic, 5/31 (16%) as VUS, 1/31 (3%) as likely benign, and 2/31 (6%) as benign by the reporting laboratory/institution. Five variants previously reported to exhibit a DNE in in-vitro testing were identified in heterozygous individuals within our cohort.[21]

The identified variants were spread across exons 3-12 and introns 3, 6, and 7 (Figure 2). Exon 4 (5/31 variants), 7 (5/31), 11 (4/31), and 12 (5/31) were the most frequently affected. Twelve variants (38%) affected regions without a specific domain of the homodimeric enzyme, while others impacted the calcium site (5/31) or the homodimeric interface (6/31). Most variants were missense (24/31), followed by frameshift (3/31), with smaller numbers of nonsense, splice site, and in-frame indel variants also identified.

A notable phenotypic overlap was observed between the genotypes in our cohort and those associated with symptomatic HPP phenotypes documented in the *ALPL* Gene Variant Database (Figure 3). Four of the 30 genotypes were linked to both the biochemical phenotype in asymptomatic carriers and childhood/juvenile HPP, while three genotypes were shared with carriers without a biochemical phenotype (3/30). Associations were also observed with milder and late-onset phenotypes of HPP, such as adult HPP (3/30) and odontohypophosphatasia (2/30). Notably, the genotype c.[542C>T];[=] was associated with both asymptomatic carriers with biochemical phenotype and infantile HPP, whereas c.[667C>T];[=] was linked to severe perinatal HPP. The median age of the subgroup with overlapping phenotypes was 25.5 yr (range 0-60 yr).

Longitudinal data were available for three subjects (#1, #2, and #11), with a mean follow-up of approximately 2.5 yr. Subject 1 (male), initially evaluated at age 11 yr, consistently exhibited low ALP and elevated PLP. Subject 2 (female) presented with low ALP and normal PLP at age 8 yr but developed high PLP one year later (data not shown). Since plasma pyridoxic acid was not measured, it cannot be excluded that the initially normal PLP level could potentially reflect vitamin B6 deficiency. Subject 11 (male) had normal ALP activity until nine months of age, but developed the biochemical phenotype of HPP by age 16 mo (Figure 4). Other biochemical parameters, including serum calcium, inorganic phosphate, PTH, 25-OHD, and liver function markers, were consistently normal in these three subjects. All three remained clinically asymptomatic during the observation period.

Discussion

Our study provides the most comprehensive analysis to date of asymptomatic *ALPL* carriers who feature the biochemical phenotype of HPP, identifying 43 individuals with at least one *ALPL* variant and persistently low serum ALP activity, in some cases accompanied by elevated PLP or PEA. Despite this biochemical trait, none of these individuals exhibited clinical signs or symptoms, underscoring the presence of a biochemically defined yet clinically asymptomatic phenotype. The observed genotype-phenotype variability, with both heterozygous and homozygous cases, underscores the complexity of HPP and the challenges in predicting disease

Table 1. Phenotypic and genotypic characteristics of the cohort.

ID	Variant 1	Exon	Class	DNE	Variant 2	Exon	Class	DNE	Gender	Age (yr)	ALP (U/L)	ALP LLN (U/L)[a]	PLP (nmol/L)[b]	PLP ULN (nmol/L)[b]	PEA urine (μmol/gCrea)[b]	PEA ULN (μmol/gCrea)[b]
1	c.83A>G	3	P	NO	WT	–	–	–	Male	13	65	127	418	247	N/A	–
2	c.83A>G	3	P	NO	WT	–	–	–	Female	9	115	156	284	247	N/A	–
3	c.182-2A>G	IVS3	LP	NO	WT	–	–	–	Male	31	29	49	N/A	–	N/A	–
4	c.206C>T	4	VUS	NO	WT	–	–	–	Female	24	31	49	141	108	N/A	–
5	c.237_238delCA	4	LP	NO	c.455G>A	5	B	NO	Female	18	27	48	33 mg/L	50 mg/L	119 mmol/L	48 mmol/L
6	c.244G>A	4	LP	YES	WT	–	–	–	Female	29	52	106	N/A	–	281.6 μmol/L	76.6 μmol/L
7	c.244G>A	4	LP	YES	WT	–	–	–	Female	22	36	106	N/A	–	86.9 μmol/L	76.6 μmol/L
8	c.244G>C	4	LP	NO	WT	–	–	–	Male	2	54	90	692	202	457 μmol/L	342 μmol/L
9	c.244G>C	4	LP	NO	WT	–	–	–	Male	36	30	35	80	202	270 μmol/L	48 μmol/L
10	c.286G>C	4	LP	NO	WT	–	–	–	Male	64	19	40	208	75	N/A	–
11	c.299C>T	5	P	YES	WT	–	–	–	Male	1.3	100	156	471	277	N/A	–
12	c.455G>A	5	B	NO	c.484G>A	6	P	NO	Male	5.9	83	156	477	247	N/A	–
13	c.511C>G	6	LP	YES	WT	–	–	–	Male	64	40	49	N/A	–	N/A	–
14	c.542C>T	6	P	NO	WT	–	–	–	Male	N/A	39	40	N/A	–	N/A	–
15	c.648+1G>A	IVS6	P	NO	WT	–	–	–	Female	N/A	39	50	N/A	–	N/A	–
16	c.667C>T	7	P	NO	WT	–	–	–	Male	38	37	49	157	108	N/A	–
17	c.675_676insCA	7	LP	NO	WT	–	–	–	Female	35	29	49	101	108	300 μmol/L	48 μmol/L
18	c.715G>T	7	VUS	NO	c.715G>T	7	VUS	NO	Female	4	92	250	204	100	433	222
19	c.715G>T	7	VUS	NO	c.715G>T	7	VUS	NO	Female	7.4	62	105	193	100	1627	222
20	c.715G>T	7	VUS	NO	c.715G>T	7	VUS	NO	Female	8.5	90	105	362	100	106	222
21	c.738G>T	7	LP	NO	WT	–	–	–	Male	N/A	Low	N/A	N/A	–	N/A	–
22	c.787T>C	7	B	NO	c.787T>C	7	B	NO	Female	39	90	−2.3 SD[c]	N/A	–	140	155
23	c.787T>C	7	B	NO	c.787T>C	7	B	NO	Male	0.9	404	−2.0 SD[c]	N/A	–	180	222
24	c.787T>C	7	B	NO	c.1559delT	12	P	NO	Female	19	73	−2.8 SD[c]	N/A	–	205	146
25	c.787T>C	7	B	NO	c.1559delT	12	P	NO	Male	3.5	280	−2.0 SD[c]	N/A	–	220	222
26	c.793-30_793-11DEL	IVS7	LP	NO	WT	–	–	–	Male	33	24	40	N/A	–	N/A	–
27	c.793-30_793-11DEL	IVS7	LP	NO	WT				Female	29	28	35	N/A	–	N/A	–
28	c.881A>C	9	P	NO	WT	–	–	–	Female	8	33	156	N/A	–	N/A	–
29	c.881A>C	9	P	NO	WT	–	–	–	Male	10	119	141	N/A	–	N/A	–
30	c.906C>A	9	VUS	NO	WT	–	–	–	Female	25	28	49	126	108	N/A	–
31	c.1015G>A	10	LP	YES	WT	–	–	–	Female	60	33	49	N/A	–	330.5	70
32	c.1225C>T	11	VUS	NO	WT	–	–	–	Female	47	13	49	143.5	130	N/A	–
33	c.1250A>G	11	P	YES	WT	–	–	–	Female	N/A	23	40	N/A	–	N/A	–
34	c.1250A>G	11	P	YES	WT	–	–	–	Female	N/A	24	40	N/A	–	N/A	–
35	c.1250A>G	11	P	YES	WT	–	–	–	Male	N/A	32	65	N/A	–	N/A	–
36	c.1268T>C	11	P	NO	WT	–	–	–	Male	60	17	35	497	110	4 mmol/mol Cr	5.4 mmol/mol Cr
37	c.1292T>A	11	LP	NO	WT	–	–	–	Female	0	52	90	N/A	–	N/A	–
38	c.1323C>A	12	P	NO	WT	–	–	–	Female	33	31	35	163	121	N/A	–
39	c.1333T>A	12	VUS	NO	WT	–	–	–	Female	50	28	40	N/A	–	N/A	–
40	c.1381G>A	12	LB	NO	WT	–	–	–	Male	N/A	Low	N/A	N/A	–	N/A	–
41	c.1402G>A	12	P	NO	WT	–	–	–	Male	N/A	Low	N/A	N/A	–	N/A	–
42	c.1402G>A	12	P	NO	WT	–	–	–	Female	N/A	Low	N/A	N/A	–	N/A	–
43	c.1402G>A	12	P	NO	WT	–	–	–	Male	N/A	Low	N/A	N/A	–	N/A	–

The cohort consisted of 43 individuals with 30 unique genotypes. Thirty-four individuals were heterozygous, five were homozygous, and four were compound heterozygous, confirmed through family history. Age data were unavailable for 10 individuals; of these, five had ALP activity below the lower limit of normal at any age, while in the remaining five cases, publications only described ALP levels as "low" without providing specific values. Abbreviations: ALP, serum alkaline phosphatase activity; B, benign; DNE, dominant-negative effect; LB, likely benign; LLN, lower limit of normal; LP, likely pathogenic; N/A, data not available; P, pathogenic; PEA, urine phosphoethanolamine; PLP, pyridoxal-5-phosphate; ULN, upper limit of normal; VUS, variant uncertain significance. [a]ALP reference values were sourced from either submissions or cited literature. [b]Units from original publications are noted. [c]Standard deviations were exclusively reported in these publications.

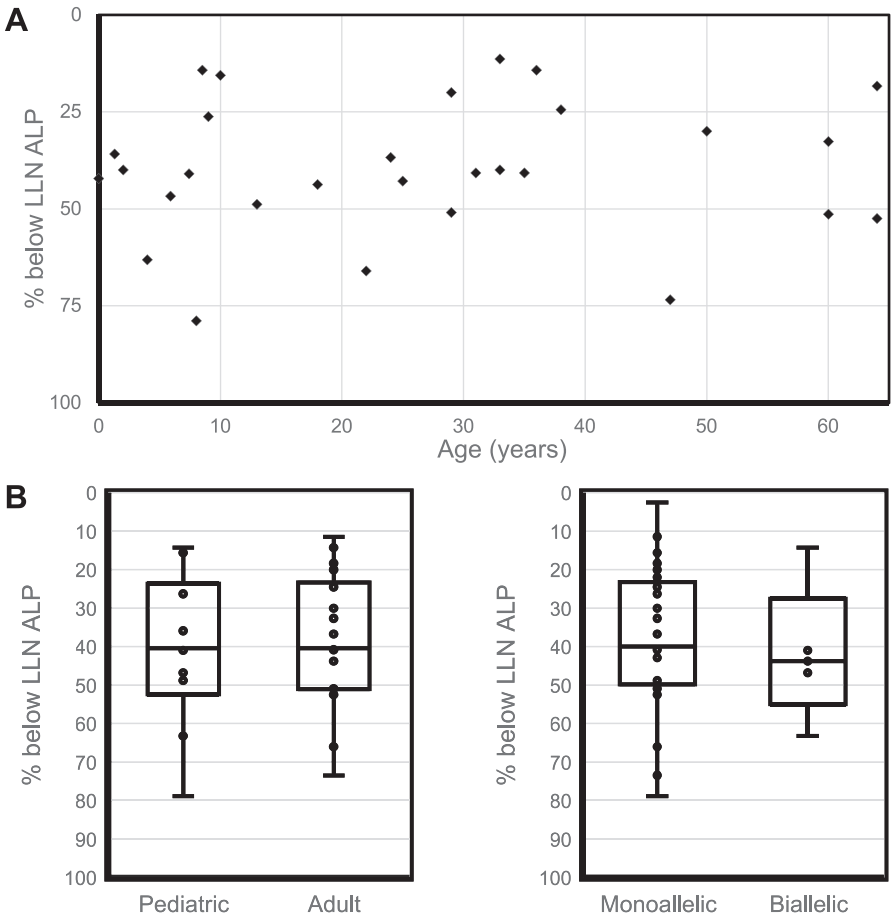

Figure 1. Serum ALP activity in asymptomatic individuals with *ALPL* variants and biochemical HPP phenotype. (A) Scatterplot showing the percentage by which each subject's serum ALP activity falls below the lower limit of normal (LLN), plotted as a function of age. A value of 0% indicates an ALP level at the LLN; higher percentages reflect greater deviation below the LLN. All included individuals had ALP values below their respective LLN. Among the 34 subjects with available data, 76% had ALP levels less than 50% below the LLN. Fourteen individuals were excluded due to missing data on age, ALP activity, or reference ranges. (B) Box plots comparing the percentage below the LLN between subgroups. The left panel shows pediatric versus adult individuals; the right panel compares monoallelic (heterozygous) and biallelic (homozygous or compound heterozygous) cases. No significant differences were observed between groups, with similar median values. The wider range seen in the biallelic group likely reflects the smaller sample size ($n = 5$).

expression.[10,22,23] Notably, some variants linked to asymptomatic *ALPL* carriers with biochemical HPP phenotype were also associated with symptomatic phenotypes. These findings refine the recognized spectrum of HPP, underscoring the need to improve diagnostic criteria and better understand long-term outcomes in this population.

Asymptomatic *ALPL* carriers with biochemical HPP phenotype have been acknowledged in the scientific literature, with early reports from the 1990s describing obligate carriers of heterozygous *ALPL* variants with low serum ALP activity who were asymptomatic.[13,15,24] As mentioned above, this phenotype has been mentioned primarily in the context of asymptomatic relatives of individuals with severe biallelic HPP, such as siblings or parents carrying heterozygous *ALPL* variants, or in large-scale laboratory screening studies.[4,6,25,26] Recently, a study reported a cohort of seven asymptomatic adults with the biochemical phenotype of HPP, although genetic confirmation was lacking.[27] Our study provides robust genetic confirmation by analyzing a much larger cohort, offering a clearer picture of the characteristics of this distinct phenotype.

All subjects in this cohort demonstrated low serum ALP activity, consistent with the obligate biochemical hallmark of HPP. Notably, 76% of the participants had serum ALP activity over 50% of the lower limit of normal. Generally, lower ALP activity has been correlated with more severe symptomatic presentations of HPP.[3] However, this relationship could not be tested in our cohort due to known methodological differences in biochemical testing, which hindered the calculation of precise mean values and limited comparisons with other studies.

Our cohort consisted of young individuals, with a median age of 29 yr (range 0-64). This age is younger than the usually reported age of onset for adult HPP in the literature, which typically ranges from 30 to 70 yr.[28,29] This younger age distribution may indicate that asymptomatic *ALPL* carriers with biochemical HPP phenotype represent an earlier stage of the disease, where symptoms have not yet manifested. Alternatively, it could reflect a selection bias, as many individuals in our cohort were identified through genetic counseling of family members of severely affected individuals, including young parents and siblings. These findings emphasize the need for careful characterization and follow-up of such individuals to clarify their clinical trajectory. In this context, artificial intelligence tools and targeted laboratory screening studies could provide valuable insights, facilitating recognition of

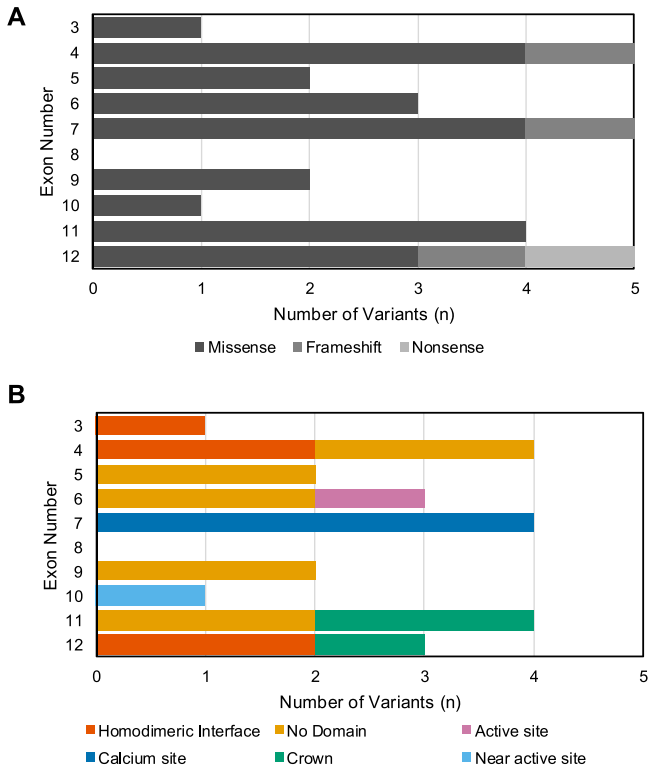

Figure 2. Distribution of *ALPL* variants across exons and functional domains. This figure presents the types and functional domains of *ALPL* variants identified in the cohort. Figure 2A shows the variant types (missense, frameshift and nonsense) distributed across exons 3-12. Missense variants were the most common. Figure 2B highlights the functional domains affected by these variants (only missense variants shown). Most variants were located in non-active regions, while fewer were observed in critical functional domains, such as the homodimeric interface, active site, and calcium-binding site.

carriers with a biochemical phenotype and supporting informed clinical decision-making.

Although currently proposed diagnostic criteria do not strictly require *ALPL* variants for the diagnosis of HPP,[20] and cases with a biochemical phenotype but no detectable *ALPL* variant have been reported,[30] we limited inclusion to individuals with confirmed *ALPL* variants, given that low ALP levels can arise from a large variety of unrelated conditions.[31] From a genetic perspective, our findings show that most individuals (79%) were heterozygous for single *ALPL* variants, with homozygous and compound heterozygous genotypes being less common. This finding aligns with prior studies associating severe phenotypes of HPP with homozygous or compound heterozygous genotypes, and milder phenotypes, with heterozygous states.[9,10,14] Notably, only 24% of heterozygous individuals had variants with DNE, in line with recent literature.[32] The lower prevalence of DNE variants in our cohort likely contributes to the absence of symptoms, as DNE variants are known to exert a stronger impact on enzyme activity and are typically associated with more severe HPP phenotypes than those without DNE.[14,33] However, why heterozygous individuals with DNE variants in our cohort remained asymptomatic is unclear and may reflect either the influence of unknown genetic and/or environmental modifiers (as discussed below), the lack of deep phenotyping (ie, self-reporting from siblings or parents of HPP patients) or the need for longer follow-up time.

For homozygous subjects, the two identified variants (c.715G>T and c.787T>C) are classified as VUS and benign in the *ALPL* Gene Variant Database, respectively. Functional testing indicates that these variants retain relatively high residual enzyme activity, which may account for the absence of a clinical phenotype in the heterozygous state. The c.787T>C is a common variant, with multiple homozygous cases reported in GnomAD.[34] Given that the biochemical phenotype described here lacks clinical manifestations, it is very likely that homozygous individuals with this phenotype are represented in GnomAD. This raises the hypothesis that while c.787T>C may be benign in relation to symptomatic HPP phenotypes, it could act as a risk allele for the biochemical phenotype of HPP in asymptomatic *ALPL* carriers. However, undetected *ALPL* variants in deep intronic or non-coding regions cannot be ruled out in individuals in our cohort. Establishing c.787T>C as a risk allele would require genetic association studies to assess its prevalence and clinical significance in larger populations. Furthermore, benign polymorphisms are typically filtered out in standard genetic reports, which could lead to underrecognition of this asymptomatic biochemical phenotype.

Similarly, the three compound heterozygous genotypes identified in our cohort included the common variants c.455G>A and c.787C>T. We infer that in these individuals, the biochemical phenotype was primarily driven by the second variant in heterozygous state.

Thirty-one variants were identified, predominantly missense (24/31), followed by frameshift, splice site, and nonsense variants. While frameshift and nonsense variants are often linked to severe phenotypes, the predominance of missense variants in our cohort likely supports the milder phenotypic presentation observed.[34] Our findings reinforce prior studies indicating that both the location and type of variants within the *ALPL* gene are key determinants of disease severity.[33] The location of variants across functional regions further supports this conclusion: variants in critical domains were limited, whereas the majority were located in regions without defined functional roles.[9,35,36] Taken together, the genotypic characteristics of the cohort are consistent with what is known about less severe phenotypes of HPP.[26,37]

Further, our study highlights another key finding: several genotypes identified in asymptomatic *ALPL* carriers with a biochemical phenotype of HPP are also linked to other milder, late-onset HPP phenotypes (Figure 3). For instance, the heterozygous c.[1250A>G];[=] genotype has been reported in cases of adult HPP, childhood HPP, prenatal benign HPP, and HPP with massive ectopic calcifications.[21,37] Additionally, we observed associations with early, severe phenotypes of HPP, including severe infantile and perinatal cases.[38] Our findings underscore the inherent variability and unpredictability of genotype-phenotype correlations in HPP, demonstrating that the same genotype can lead to a wide range of clinical presentations, complicating diagnosis, management, and genetic counseling.[25,39] However, these results must be interpreted with caution, as some previous reports originate from studies that analyzed only coding exons via Sanger sequencing. Consequently, we cannot exclude the possibility that undetected intronic or non-coding variants may contribute to these severe presentations in heterozygous individuals.

The influence of disease modifiers may play a significant role in HPP. Epigenetic mechanisms, such as CpG methylation at the *ALPL* promoter, have been shown to regulate TNSALP

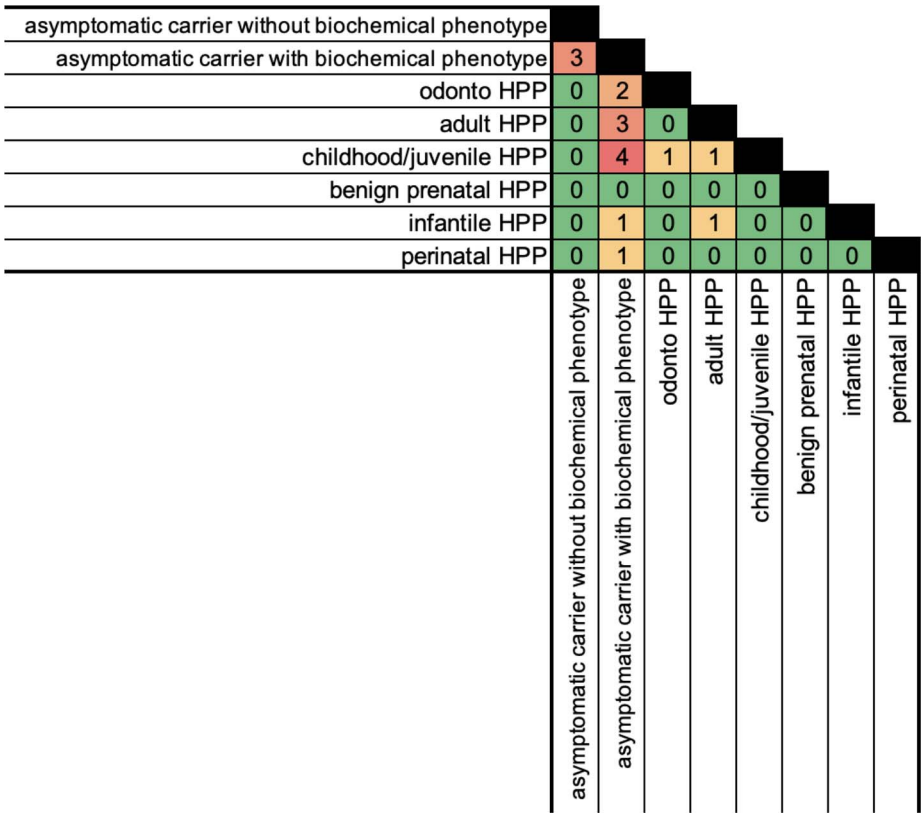

Figure 3. Phenotypic variability caused by *ALPL* genotypes associated with asymptomatic carriers with a biochemical HPP phenotype. The matrix shows that genotypes associated with this phenotype can also cause childhood/juvenile HPP (4), asymptomatic carriers without biochemical phenotype (3), adult HPP (3), odontohypophosphatasia (2), infantile HPP (1), and perinatal HPP (1). Given that infantile and perinatal cases are caused by bi-allelic pathogenic variants, it is likely that further intronic, non-coding or structural *ALPL* variants were missed in these published cases. This highlights the broad phenotypic spectrum and potential progression within the HPP continuum.

expression in bone cells and may influence phenotypic expression.[40] Osteoblast senescence may also play a role in late disease manifestation. In addition, *ALPL* polymorphisms have been associated with reduced serum ALP levels; individuals carrying three or more minor alleles demonstrated lower ALP activity and a higher frequency of metatarsal fractures, suggesting a modifying role in adult HPP.[41] Although several modifying genes have been proposed, none have been conclusively identified.[30,35] Environmental factors, including dietary calcium and phosphate intake, may also influence disease expression.[1] These genetic, epigenetic, and environmental factors—together with incomplete penetrance and variable expressivity—may help explain the phenotypic variability observed in our cohort, including the absence of symptoms in heterozygous individuals harboring DNE variants.[42,43]

Another perspective views HPP as existing along a "continuum," governed by varying *ALPL* expression, ALP activity, and substrate accumulation throughout life. Evidence suggests a dose-dependent relationship between ALP activity and clinical severity, with low ALP potentially leading to excessive PPi accumulation and, in turn, disease manifestations.[3] However, accurate PPi quantification remains difficult, owing to suboptimal measurement methods and discrepancies between serum and renal PPi levels.[3] The concept of age-dependent ALP activity thresholds reflecting physiological needs may explain not only the natural history of HPP but also the emergence of milder, late-onset phenotypes.[39]

Consistent with this continuum view, genotypes, such as c.[715G>T];[715G>T], c.[244G>A];[=], and c.[1333T>A];[=], were observed in our cohort of asymptomatic *ALPL* carriers with a biochemical phenotype of HPP, but have also been reported in carriers without this biochemical trait, potentially reflecting an earlier stage, before biochemical changes evolve.[6,21,44] In contrast, genotypes like c.[542C>T];[=], c.[1268T>C];[=], and c.[1250A>G];[=] were also linked to adult HPP cases, suggesting a later manifesting stage in the disease continuum.[26,37,45] Although limited, longitudinal data from our cohort could further support this continuum model, illustrating that asymptomatic individuals carrying *ALPL* variants may later develop the biochemical trait of HPP (Figure 4). Moreover, the relatively young median age of our cohort (29 yr) raises the prospect that, with time, these individuals may transition from a purely biochemical phenotype to overt clinical symptoms.

Similarly, Szabo et al., summarizing 265 HPP cases with over 1 yr of follow-up, found that clinical features often accumulate over time, suggesting that more symptomatic HPP phenotypes may develop gradually.[29] While some adult cases may represent delayed diagnoses of juvenile phenotypes,[46] the clinical and biochemical transitions preceding symptom onset remain poorly understood. Hypophosphatasia may involve an asymptomatic phase prior to overt disease manifestation, potentially as a long-term result of substrate accumulation such as PPi or pyrophosphate crystals. Prospective studies are needed to characterize these early changes and understand the natural course of HPP.

Diagnosing HPP remains challenging and continues to be researched. Recently proposed diagnostic criteria define HPP

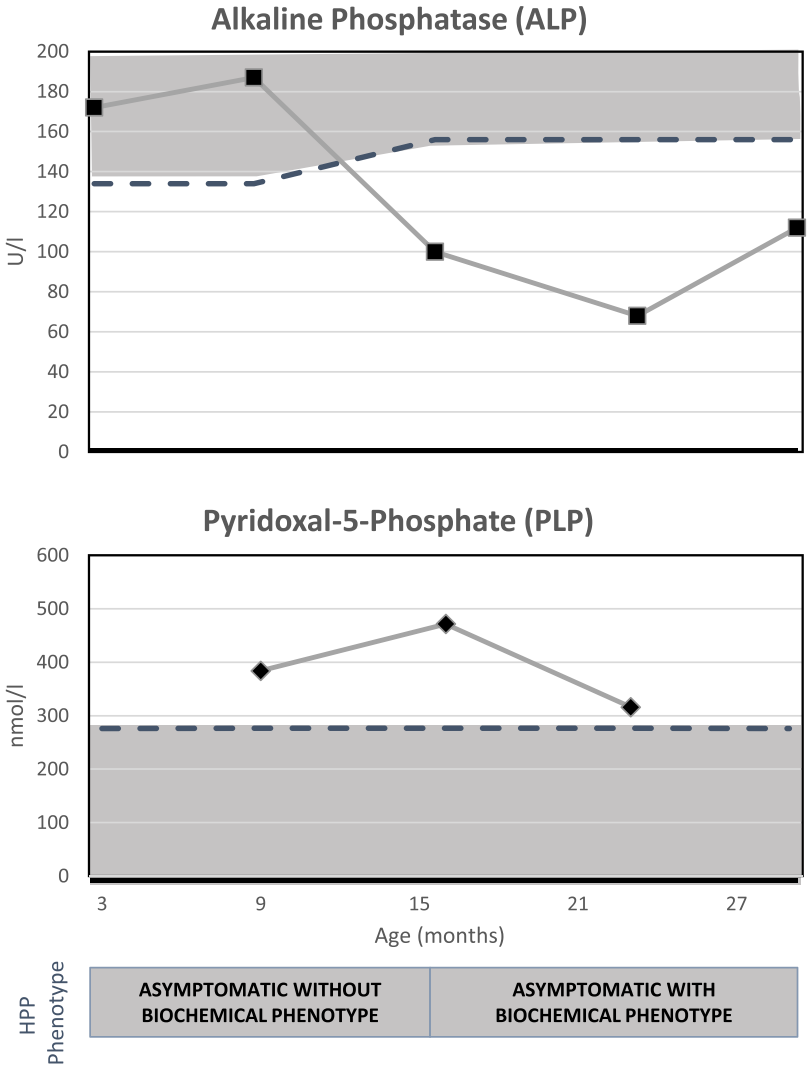

Figure 4. Hypophosphatasia phenotypic shift over time. A 2-yr-old male, monitored due to a family history of juvenile HPP and odontohypophosphatasia caused by a pathogenic *ALPL* variant (c.299C>T), initially exhibited normal serum ALP activity at 8 mo of age. By age 16 mo, he developed a biochemical phenotype (low ALP and high PLP) while remaining asymptomatic.

by persistently low ALP activity plus at least two additional major indicators, such as a pathogenic or likely pathogenic *ALPL* variant and elevated substrates like PLP or PEA.[47] Applying these criteria, 16 individuals in our cohort (37%) met these diagnostic criteria while remaining asymptomatic, raising important concerns regarding potential overdiagnosis. The current inclusion of asymptomatic individuals under this proposed definition of HPP highlights the need for further refinement of diagnostic thresholds that account for disease expressivity and clinical impact.

To address this gap, we refer to these individuals as asymptomatic *ALPL* carriers with a biochemical HPP phenotype, defined by persistently low ALP and an *ALPL* variant in the absence of clinical symptoms. This terminology identifies a distinct phenotype, separating these individuals from asymptomatic carriers without biochemical abnormalities, who may represent a different stage in the same continuum.[18] While individuals with this asymptomatic biochemical phenotype do not require treatment, clinical monitoring may be advisable given the potential for symptom evolution. In particular, caution is warranted with enzyme replacement therapy, such as asfotase alfa, as standard dosing in mild or asymptomatic individuals may elevate the risk of ectopic calcifications.[48,49] Notably, the safety of asfotase alfa in these populations remains unknown, since clinical trials have focused exclusively on individuals with severe, life-threatening manifestations.[50] Recognizing this group may facilitate more precise diagnostic classification and help reduce both overdiagnosis and unnecessary concern. Effective communication and education are essential to avoid misinterpretation and guide appropriate follow-up.

This study represents the largest cohort of asymptomatic ALPL carriers with a biochemical phenotype of HPP to date, providing insights into the wide spectrum of the disease. A strength of our study is the comprehensive collection of genotypic and phenotypic data, which enhances our understanding of HPP in individuals without symptoms. However, several limitations must be acknowledged. The data came from *real-world* submissions and literature, which may be incomplete or inconsistent. Interpretation of ALP activity is further complicated by the artificial nature of current clinical and in vitro assays, which may not accurately reflect TNSALP's physiological function in vivo. Additionally, the heterogeneity in ALP activity measurement methods prevented precise

calculation of mean ALP values, limiting characterization accuracy, in some cases, ALP activity could only be described as "low" without precise quantification. The lack of longitudinal follow-up in the majority of our subjects also restricts understanding of disease progression. The inclusion of VUS, as well as the benign variants for symptomatic HPP phenotypes (c.787T>C and c.455G>A), and the potential for undetected variants in deep intronic or non-coding regions not covered by routine exome sequencing, may have influenced our findings.

In conclusion, our study contributes to a more refined understanding of HPP by documenting a distinct phenotype, which is characterized by at least one *ALPL* variant and the biochemical phenotype of HPP in the absence of reported clinical symptoms. Although underrepresented in the literature, this phenotype may represent an early or mild stage along the HPP disease continuum, emphasizing the importance of phenotypic variability in this disorder. Recognizing this presentation is critical for improving diagnostic accuracy and reducing the risks of overdiagnosis and overtreatment. Future investigations, particularly prospective studies and bone biopsy analyses, are essential to clarify whether these individuals remain asymptomatic, harbor occult osteomalacia, or show evolving disease severity over time.

Accessibility and usage

The *ALPL* Gene Variant Database is publicly accessible for searches at https://alplmutationdatabase.jku.at/. Researchers wishing to submit new variants, including those classified as variants of uncertain significance (VUS), may do so via the submission portal at https://alplmutationdatabase.jku.at/portal/, accessible through the "Submission" button on the main page. Comprehensive submission guidelines are provided within the portal. To report additional genotypes or phenotypes associated with existing variants in the database, please contact the curator at hppresearch@jku.at.

Previous publications

Preliminary findings from this study were previously presented in abstract form at the ASBMR 2024 Annual Meeting in Toronto and the 62nd Annual ESPE Meeting 2024 in Liverpool. These presentations included partial data from the current analysis but did not encompass the full scope or depth of the findings reported in this manuscript.

Statement of ethics

This project has received approval from the Ethics Committee of the Medical Faculty at Johannes Kepler University (JKU), Linz, Austria (EK Nr: 1118/2021). Contributing geneticists and physicians are required to obtain approval from their respective Institutional Review Boards (IRBs), unless an IRB waiver has been granted. Patient information and consent forms are available for download on the project website (https://alplmutationdatabase.jku.at/portal/).

Acknowledgments

This project was funded through an externally sponsored research agreement with Alexion, AstraZeneca Rare Disease. We thank Prof. Etienne Mornet for his dedication in maintaining the database over decades, providing a foundational legacy for this work. Our appreciation also goes to Lukas Micha Binder and Abdulrhman Brighsh (Senior Software Developers, X-Net Services GmbH, Linz, Austria) for their expertise and support in developing the *ALPL* Gene Variant Database. Special thanks to Romana Schiller (Department of Paediatrics and Adolescent Medicine, Johannes Kepler University Linz) for her excellent administrative coordination and assistance with consortium meetings. Lastly, we gratefully acknowledge the many medical professionals who contributed to this project through their case submissions, which have been essential to our progress.

Author contributions

Rodrigo Montero-Lopez (Conceptualization, Data curation, Investigation, Writing—original draft, Writing—review & editing), Mariam R. Farman (Data curation, Investigation, Methodology, Writing—original draft, Writing—review & editing), Florian Högler (Data curation, Investigation, Writing—review & editing), Catherine Rehder (Writing—review & editing), Theodora Malli (Investigation, Writing—review & editing), Gerald Webersinke (Investigation, Writing—review & editing), Cheryl Rockman-Greenberg (Investigation, Writing—review & editing), Kathryn Dahir (Writing—review & editing), Gabriel Ángel Martos-Moreno (Writing—review & editing), Agnès Linglart (Writing—review & editing), Keiichi Ozono (Writing—review & editing), Lothar Seefried (Writing—review & editing), Guillermo del Angel (Writing—review & editing), Erica Burner Nading (Investigation, Writing—review & editing), Erin Huggins (Investigation, Writing—review & editing), Eric T. Rush (Writing—review & editing), Josephine T. Tauer (Writing—review & editing), Priya S. Kishnani (Writing—review & editing), and Wolfgang Högler (Conceptualization, Data curation, Methodology, Writing—original draft, Writing—review & editing)

Supplementary material

Supplementary material is available at *Journal of Bone and Mineral Research* online.

Funding

The *ALPL* Gene Variant Database Project is an investigator-initiated study (Johannes Kepler University Linz, JKU) funded by Alexion AstraZeneca Rare Disease through an externally sponsored research agreement. This agreement funds the research team at JKU and the consortium molecular genetics teams (Ordensklinikum Linz and Duke University).

C.R.-G., K.D., G.Á.M.-M., A.L., K.O., L.S., P.S.K., and W.H. are steering committee members of the HPP global registry sponsored by Alexion, AstraZeneca Rare Disease, have worked as consultants, and received research funding and honoraria from Alexion, AstraZeneca Rare Disease. P.S.K. is a member of the Scientific Advisory Board for Alexion Pharmaceuticals, Inc. E.T.R. has worked as a speaker, consultant, and has received research funding from Alexion AstraZeneca Rare Disease. G.d.A. is a current employee of Alexion, AstraZeneca Rare Disease and may own stock and/or options therein.

Conflicts of interest

P.S.K., G.Á.M.-M., K.D., A.L., C.R.-G., K.O., E.T.R., L.S., and W.H. are consultants for and have received research funding and honoraria from, Alexion, AstraZeneca Rare Disease. C.R., E.B.N., and E.H. have received honoraria from Alexion, AstraZeneca Rare Disease. G.d.A. is an employee of AstraZeneca and may own stock/options thereof. M.R.F. is funded through a research grant from Alexion, AstraZeneca Rare Disease and paid by Johannes Kepler University Linz, Linz, Austria. R.M.-L. received travel funding from Alexion, AstraZeneca

Rare Disease to attend a conference. The other authors have no conflicts of interest to declare.

References

1. Whyte MP. Hypophosphatasia: an overview for 2017. *Bone*. 2017;102:15-25. https://doi.org/10.1016/j.bone.2017.02.011
2. Weiss MJ, Cole DE, Ray K, et al. A missense mutation in the human liver/bone/kidney alkaline phosphatase gene causing a lethal form of hypophosphatasia. *Proc Natl Acad Sci*. 1988;85(20):7666-7669. https://doi.org/10.1073/pnas.85.20.7666
3. Whyte MP, Coburn SP, Ryan LM, Ericson KL, Zhang F. Hypophosphatasia: biochemical hallmarks validate the expanded pediatric clinical nosology. *Bone*. 2018;110:96-106.
4. Saraff V, Narayanan VK, Lawson AJ, Shaw NJ, Preece MA, Högler W. A diagnostic algorithm for children with low alkaline phosphatase activities: lessons learned from laboratory screening for hypophosphatasia. *J Pediatr*. 2016;172(5):181-186.e1. https://doi.org/10.1016/j.jpeds.2016.01.045
5. Greenberg CR, Evans JA, Mckendry-Smith S, et al. Infantile hypophosphatasia: localization within chromosome region lp36.1-34 and prenatal diagnosis using linked DNA markers. *Am J Hum Genet*. 1990;46(2):286-292.
6. Uday S, Matsumura T, Saraff V, Saito S, Orimo H, Högler W. Tissue non-specific alkaline phosphatase activity and mineralization capacity of bi-allelic mutations from severe perinatal and asymptomatic hypophosphatasia phenotypes: results from an in vitro mutagenesis model. *Bone*. 2019;127:9-16. https://doi.org/10.1016/j.bone.2019.05.031
7. Farman MR, Rehder C, Malli T, et al. The global ALPL gene variant classification project: dedicated to deciphering variants. *Bone*. 2024;178:116947. https://doi.org/10.1016/j.bone.2023.116947
8. Whyte MP. Hypophosphatasia-aetiology, nosology, pathogenesis, diagnosis and treatment. *Nat Rev Endocrinol*. 2016;12(4):233-246. https://doi.org/10.1038/nrendo.2016.14
9. Fauvert D, Brun-Heath I, Lia-Baldini AS, et al. Mild forms of hypophosphatasia mostly result from dominant negative effect of severe alleles or from compound heterozygosity for severe and moderate alleles. *BMC Med Genet*. 2009;10(1):51. https://doi.org/10.1186/1471-2350-10-51
10. Kishnani PS, Seefried L, Dahir KM, et al. Disease burden by *ALPL* variant number in patients with non-life-threatening hypophosphatasia in the Global HPP Registry. *J Med Genet*. 2025;62(4):249-257. Available from: https://jmg.bmj.com/lookup/doi/10.1136/jmg-2024-110383
11. Whyte MP, Leung E, Wilcox WR, et al. Natural history of perinatal and infantile hypophosphatasia: a retrospective study. *J Pediatr*. 2019;209:116-124.e4. https://doi.org/10.1016/j.jpeds.2019.01.049
12. Whyte MP. Physiological role of alkaline phosphatase explored in hypophosphatasia. *Ann N Y Acad Sci*. 2010;1192:190-200. https://doi.org/10.1111/j.1749-6632.2010.05387.x
13. Chodirker BN, Evans JA, Seargent LE, Cheang MS, Greenberg CR. Hyperphosphatemia in infantile hypophosphatasia: implications for carrier diagnosis and screening. *Am J Hum Genet*. 1990;46(2):280-285.
14. Mornet E, Taillandier A, Domingues C, et al. Hypophosphatasia: a genetic-based nosology and new insights in genotype-phenotype correlation. *Eur J Hum Genet*. 2021;29(2):289-299. https://doi.org/10.1038/s41431-020-00732-6
15. Whyte MP, Teitelbaum SL, Murphy WA, Bergfeld MA, Avioli LV. Adult hypophosphatasia. Clinical, laboratory, and genetic investigation of a large kindred with review of the literature. *Medicine*. 1979;58(5):329-347. https://doi.org/10.1097/00005792-197909000-00001
16. Seefried L, Genest F, Hofmann C, Brandi ML, Rush E. Diagnosis and treatment of hypophosphatasia. *Calcif Tissue Int*. 2025;116(1):46. https://doi.org/10.1007/s00223-025-01356-y
17. Qiu C, Sigala B, Wu J, Barag S, Saad B. A rare case of subclinical hypophosphatasia in an adult patient. *JOFP-CA*. 2024;03(Fall 2024):28-30 Available from: https://www.acofpca.org/page/JOFPCAFall2024Hypophosphatasia
18. Beck NM, Sagaser KG, Lawson CS, et al. Not just a carrier: clinical presentation and management of patients with heterozygous disease-causing alkaline phosphatase (ALPL) variants identified through expanded carrier screening. *Mol Genet Genomic Med*. 2023;11(1):e2056. http://doi.org/10.1002/mgg3.2056
19. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17(5):405-424. https://doi.org/10.1038/gim.2015.30
20. Khan AA, Brandi ML, Rush ET, et al. Hypophosphatasia diagnosis: current state of the art and proposed diagnostic criteria for children and adults. *Osteoporos Int*. 2024;35(3):431-438. http://doi.org/10.1007/s00198-023-06844-1.
21. The ALPL Gene Variant Consortium. *The ALPL Gene Variant Database*. https://alplmutationdatabase.jku.at/. 2024.
22. Montero-Lopez R, Farman MR, Högler F, Saraff V, Högler W. Challenges in hypophosphatasia: suspicion, diagnosis, genetics, management, and follow-up. *Horm Res Paediatr*. 20245:1-10. https://doi.org/10.1159/000540692
23. Prakash V, Elbabaa S, Banks R, et al. Markedly discordant hypophosphatasia in a young girl. *Bone*. 2025;199:117541. https://doi.org/10.1016/j.bone.2025.117541
24. Whyte MP, Murphy WA, Fallon MD. Adult hypophosphatasia with chondrocalcinosis and arthropathy. *Am J Med*. 1982;72(4):631-641. https://doi.org/10.1016/0002-9343(82)90474-0
25. Kato M, Hattori T, Shimizu T, et al. Intrafamilial phenotypic distinction of hypophosphatasia with identical tissue nonspecific alkaline phosphatase gene mutation: a family report. *J Bone Miner Metab*. 2020;38(6):903-907. https://doi.org/10.1007/s00774-020-01137-7
26. Lefever E, Witters P, Gielen E, et al. Hypophosphatasia in adults: clinical spectrum and its association with genetics and metabolic substrates. *J Clin Densitom*. 2020;23(3):340-348. https://doi.org/10.1016/j.jocd.2018.12.006
27. Berkseth KE, Tebben PJ, Drake MT, Hefferan TE, Jewison DE, Wermers RA. Clinical spectrum of hypophosphatasia diagnosed in adults. *Bone*. 2013;54(1):21-27. https://doi.org/10.1016/j.bone.2013.01.024
28. Feurstein J, Behanova M, Haschka J, et al. Identifying adult hypophosphatasia in the rheumatology unit. *Orphanet J Rare Dis*. 2022;17(1):435. https://doi.org/10.1186/s13023-022-02572-7
29. Szabo SM, Tomazos IC, Petryk A, et al. Frequency and age at occurrence of clinical manifestations of disease in patients with hypophosphatasia: a systematic literature review. *Orphanet J Rare Dis*. 2019;14(1):85. https://doi.org/10.1186/s13023-019-1062-0
30. Seefried L, Petryk A, del Angel G, Reder F, Bauer P. Whole genome sequencing in adults with clinical hallmarks of hypophosphatasia negative for ALPL variants. *Mol Biol Rep*. 2024;51(1):984. http://doi.org/10.1007/s11033-024-09906-7
31. Riancho JA. Diagnostic approach to patients with low serum alkaline phosphatase. *Calcif Tissue Int*. 2023;112(3):289-296. https://doi.org/10.1007/s00223-022-01039-y
32. Kishnani PS, Seefried L, Dahir KM, et al. New insights into the landscape of ALPL gene variants in patients with hypophosphatasia from the Global HPP Registry. *Am J Med Genet A*. 2024;194(11):e63781. http://doi.org/10.1002/ajmg.a.63781
33. del Angel G, Reynders J, Negron C, Steinbrecher T, Mornet E. Large-scale in vitro functional testing and novel variant scoring via protein modeling provide insights into alkaline phosphatase activity in hypophosphatasia. *Hum Mutat*. 2020;41(7):1250-1262
34. Chen S, Francioli LC, Goodrich JK, et al. A genomic mutational constraint map using variation in 76,156 human genomes. *Nature*. 2024;625(7993):92-100. https://doi.org/10.1038/s41586-023-06045-0

35. Taillandier A, Domingues C, Dufour A, et al. Genetic analysis of adults heterozygous for ALPL mutations. *J Bone Miner Metab*. 2018;36(6):723-733. https://doi.org/10.1007/s00774-017-0888-6
36. Farman MR, Malli T, Rehder C, et al. The ALPL gene variant project: results of the first 100 reclassified variants. *JBMR Plus*. 2025;9(6):ziaf044. Available from: https://academic.oup.com/jbmrplus/article/doi/10.1093/jbmrpl/ziaf044/8081574
37. Shajani-Yi Z, Ayala-Lopez N, Black M, Dahir KMC. Urine phosphoethanolamine is a specific biomarker for hypophosphatasia in adults. *Bone*. 2022;163:116504. http://doi.org/10.1016/j.bone.2022.116504
38. Mornet E, Taillandier A, Peyramaure S, et al. Identification of fifteen novel mutations in the tissue-nonspecific alkaline phosphatase (TNSALP) gene in European patients with severe hypophosphatasia. *Eur J Hum Genet*. 1998;6(4):308-314. https://doi.org/10.1038/sj.ejhg.5200190
39. Hofmann C, Girschick H, Mornet E, Schneider D, Jakob F, Mentrup B. Unexpected high intrafamilial phenotypic variability observed in hypophosphatasia. *Eur J Hum Genet*. 2014;22(10):1160-1164. https://doi.org/10.1038/ejhg.2014.10
40. Delgado-Calle J, Sañudo C, Sánchez-Verde L, García-Renedo RJ, Arozamena J, Riancho JA. Epigenetic regulation of alkaline phosphatase in human cells of the osteoblastic lineage. *Bone*. 2011;49(4):830-838. https://doi.org/10.1016/j.bone.2011.06.006
41. Masi L, Marini F, Franceschelli F, et al. Polymorphic variants of alkaline phosphatase gene correlate with clinical signs of adult hypophosphatasia? *Osteoporos Int*. 2021;32(12):2461-2472. https://doi.org/10.1007/s00198-021-05893-8
42. Collins MT, Marcucci G, Anders HJ, et al. Skeletal and extraskeletal disorders of biomineralization. *Nat Rev Endocrinol*. 2022;18(8):473-489.
43. 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(3):439-445. https://doi.org/10.1111/j.1469-1809.2011.00642.x
44. Kato M, Michigami T, Tachikawa K, et al. Novel mutation in the ALPL gene with a dominant negative effect in a Japanese family. *J Bone Miner Metab*. 2021;39(5):804-809. https://doi.org/10.1007/s00774-021-01219-0
45. Tenorio J, Álvarez I, Riancho-Zarrabeitia L, et al. Molecular and clinical analysis of ALPL in a cohort of patients with suspicion of hypophosphatasia. *Am J Med Genet A*. 2017;173(3):601-610. https://doi.org/10.1002/ajmg.a.37991
46. Högler W, Langman C, Gomes Da Silva H, et al. Diagnostic delay is common among patients with hypophosphatasia: initial findings from a longitudinal, prospective, global registry. *BMC Musculoskelet Disord*. 2019;20(1):80. https://doi.org/10.1186/s12891-019-2420-8
47. Khan AA, Brandi ML, Rush ET, et al. Hypophosphatasia diagnosis: current state of the art and proposed diagnostic criteria for children and adults. *Osteoporos Int*. 2024;35(3):431-438; Available from: https://link.springer.com/10.1007/s00198-023-06844-1
48. Gospe SM, Santiago-Turla C, DeArmey SM, Cummings TJ, Kishnani PS, Bhatti MT. Ectopic ocular surface calcification in patients with hypophosphatasia treated with asfotase alfa. *Cornea*. 2019;38(7):896-900. https://doi.org/10.1097/ICO.0000000000001947
49. Amadeu de Oliveira F, Narisawa S, Bottini M, Millán JL. Visualization of mineral-targeted alkaline phosphatase binding to sites of calcification in vivo. *J Bone Miner Res*. 2020;35(9):1765-1771. https://doi.org/10.1002/jbmr.4038
50. Whyte MP, Rockman-Greenberg C, Ozono K, et al. Asfotase alfa treatment improves survival for perinatal and infantile hypophosphatasia. *J Clin Endocrinol Metab*. 2016;101(1):334-342. https://doi.org/10.1210/jc.2015-3462

Journal of Bone and Mineral Research, 2026, 41, 259–269
https://doi.org/10.1093/jbmr/zjaf124
Research Article