



Article

Clinical and Genetic Characteristics of Pediatric Patients with Hypophosphatasia in the Russian Population

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Abstract: (1) Hypophosphatasia (HPP) is a rare inherited disease caused by mutations (pathogenic variants) in the *ALPL* gene which encodes tissue-nonspecific alkaline phosphatase (TNSALP). HPP is characterized by impaired bone mineral metabolism due to the low enzymatic activity of TNSALP. Knowledge about the structure of the gene and the features and functions of various *ALPL* gene variants, taking into account population specificity, gives an understanding of the hereditary nature of the disease, and contributes to the diagnosis, prevention, and treatment of the disease. The purpose of the study was to describe the spectrum and analyze the functional features of the *ALPL* gene variants, considering various HPP subtypes and clinical symptoms in Russian children. (2) From 2014–2021, the study included the blood samples obtained from 1612 patients with reduced alkaline phosphatase activity. The patients underwent an examination with an assessment of their clinical symptoms and biochemical levels of TNSALP. DNA was isolated from dried blood spots (DBSs) or blood from the patients to search for mutations in the exons of the *ALPL* gene using Sanger sequencing. The PCR products were sequenced using a reagent BigDye Terminator 3.1 kit (Applied Biosystems). Statistical analysis was performed using the GraphPad Prism 8.01 software. (3) The most common clinical symptoms in Russian patients with HPP and two of its variants ($n = 22$) were bone disorders (75%), hypomyotonia (50%), and respiratory failure (50%). The heterozygous carriage of the causal variants of the *ALPL* gene was detected in 225 patients. A total of 2 variants were found in 27 patients. In this group ($n = 27$), we identified 28 unique variants of the *ALPL* gene, of which 75.0% were missense, 17.9% were frameshift, 3.6% were splicing variants, and 3.6% were duplications. A total of 39.3% (11/28) of the variants were pathogenic, with two variants being probably pathogenic, and 15 variants had unknown clinical significance (VUS). Among the VUS group, 28.6% of the variants (7/28) were discovered by us for the first time. The most common variants were c.571G > A (p.Glu191Lys) and

c.1171del (Arg391Valfs*12), with frequencies of 48.2% (13/28) and 11% (3/28), respectively. It was found that the frequency of nonsense variants of the *ALPL* gene was higher ($p < 0.0001$) in patients with the perinatal form compared to the infantile and childhood forms of HPP. Additionally, the number of homozygotes in patients with the perinatal form exceeded ($p < 0.01$) the frequencies of these genotypes in children with infantile and childhood forms of HPP. On the contrary, the frequencies of the compound-heterozygous and heterozygous genotypes were higher ($p < 0.01$) in patients with infantile childhood HPP than in perinatal HPP. In the perinatal form, residual TNSALP activity was lower ($p < 0.0005$) in comparison to the infantile and childhood ($p < 0.05$) forms of HPP. At the same time, patients with the heterozygous and compound-heterozygous genotypes (mainly missense variants) of the *ALPL* gene had greater residual activity (of the TNSALP protein) regarding those homozygous patients who were carriers of the nonsense variants (deletions and duplications) of the *ALPL* gene. Residual TNSALP activity was lower ($p < 0.0001$) in patients with pathogenic variants encoding the amino acids from the active site and the calcium and crown domains in comparison with the nonspecific region of the protein.

Keywords: hypophosphatasia; clinical forms of hypophosphatasia; tissue-specific alkaline phosphatase; *ALPL* gene; SNP; residual activity of *ALPL* mutant alleles

1. Introduction

Hypophosphatasia (HPP, OMIM: 146300, 241500, 241510) is a rare inherited mineral metabolism disorder caused by low enzymatic activity in the tissue nonspecific alkaline phosphatase (TNSALP) as a result of mutations (pathogenic variants) in the *ALPL* gene that is localized on chromosome 1p36.12 [1]. The prevalence of HPP is variously estimated at 1/100,000–1/900,000 liveborn [2]. The incidence of pathogenic variants in the *ALPL* gene in the Russian population is 4.2% [3]. HPP was first mentioned in 1948, and the first pathogenic variant of c. 711A > G (Ala162Thr) in the *ALPL* gene was detected in 1988 [4,5].

A high level of TNSALP expression is observed in the liver, kidneys, and bones [6]. TNSALP is involved in the dephosphorylation of pyridoxal-5-phosphate (PLP) and inorganic pyrophosphate (PPi) in these organs and tissues. A decrease in its serum activity leads to the accumulation of PPi mineralization inhibitors in tissues, which causes bone and tooth defects [7]. It has been demonstrated that a decrease in TNSALP activity and the concentration of its substrates correlate with the severity of the clinical symptoms of HPP, defining a heterogeneous phenotype of the disease [8]. However, the activity of TNSALP can decrease under the influence of drugs, the lack of zinc, magnesium (in celiac disease), achondroplasia, and anemia and lead to an increase in liver diseases and osteogenesis imperfecta [9].

HPP is usually classified into six clinical forms: perinatal, prenatal benign, infantile, childhood, odonto-HPP, and adult [10]. The most severe perinatal form of HPP manifests in utero or can be obviously diagnosed at birth based on profound skeletal hypomineralization, caput membraceum, shorted and deformed limbs, and pyridoxine-dependent seizures. These patients have a very high risk of death in the first days of life due to the hypomineralization of the ribs, pulmonary hypoplasia, and asphyxia [11]. The infantile type is characterized by respiratory complications, total demineralization, skeletal deformities, and rickets, with symptoms present in the first six months of life [12]. The childhood form of HPP appears between 6 months and 18 years of age, with mineralization defects, bone deformities, premature loss of deciduous teeth, delayed walking, short stature, brain damage, delayed motor development and skill formation, and the progression of convulsions [13]. Odonto-HPP is typical in early isolated damage to the dentition, with the premature loss of deciduous teeth, impaired teeth growth and structure, and periodontal disease. Adult HPP expresses skeletal hypomineralization leading to fractures in the metatarsal bones and femur, with premature tooth loss, muscle and joint pain, arthropathy, pseudogout and enthesopathy, and neurological symptoms [12].

The clinical symptoms and severity of HPP, however, can vary considerably in different patients, even within the same form of the disease, which is associated with the heterogeneous pathogenic variants in the *ALPL* gene [14,15]. For this reason, the diagnosis of HPP involves measuring TNSALP activity levels in the serum according to age and gender and searching for mutations in the *ALPL* gene (NM_000478.6, 12 exons, and a length of about 70,000 bp) [16].

By February 2022, more than 500 pathogenic variants of the *ALPL* gene were registered in the Global Variome Shared International Database [17]. Among all *ALPL* genetic variants, missense mutations account for 71.2%, deletions 11.0%, splice mutations 4.9%, nonsense mutations 4.6%, insertions 3.4%, large deletions/duplications 2.9%, insertions/deletions 1.5%, and regulatory mutations 0.2% [18].

The missense variants of the *ALPL* gene can affect the level of expression, modification, folding, membrane transport, and dimerization of TNSALP, which is phenotypically indicated by different levels of its residual enzymatic activity [19]. At the same time, it has been established that the severity of HPP depends on the inheritance type. In mild forms, there is a functional loss of a single copy of the *ALPL* gene, which is reflected in the autosomal-dominant inheritance of the disease, with varying expression and incomplete penetrance. The perinatal and infantile forms are characterized by recessive inheritance [10]. Meanwhile, the pathogenic variants of the *ALPL* gene can exhibit a dominant-negative effect (DNE) due to the mutant monomer inhibiting the interaction between the wild-type monomers or due to the sequestering of the wild-type protein by the mutant monomer, preventing its transport to the membrane, thereby explaining the presence of the dominant forms of the disease [20]. Some results demonstrate that the in vitro residual enzymatic activity of the mutant protein and/or DNE value correlates with the clinical symptoms of HPP [21]. Study objective: to describe the spectrum and analyze the functional features of the variants of the *ALPL* gene with regard to different HPP subtypes and clinical symptoms in Russian children.

2. Results

2.1. Clinical Features and Forms of Hypophosphatasia

The clinical symptoms of HPP in children with variant(s) in the *ALPL* gene in the Russian population were most fully described in only 56 of the patients, and clinical information was available for 22 compound heterozygotes carriers of the causal variants in these patients (Table 1, Figure 1).

Table 1. Clinical forms of HPP in patients with two variants of the *ALPL* gene ($n = 22$).

HPP Onset	Number of Patients	Age at the Time of Diagnosis, Years	TNSALP Level in Serum, U/L
Prenatal	4	up to 1 year	18.7 ± 6.0
Infantile	8	2.0 ± 1.2 (1–4)	31.6 ± 10.8
Childhood	10	5.3 ± 5.0 (1–18)	40.1 ± 16.5

Among children with perinatal HPP ($n = 4$), the most frequent symptoms were bone deformity (75%), craniosynostosis (50%), pectus excavatum (50%), bone hypomineralization (50%), limb shortening (50%), skull bone deformity (75%), skull hypomineralization (75%), hypotension (50%), and respiratory failure (50%) (Figure 2).

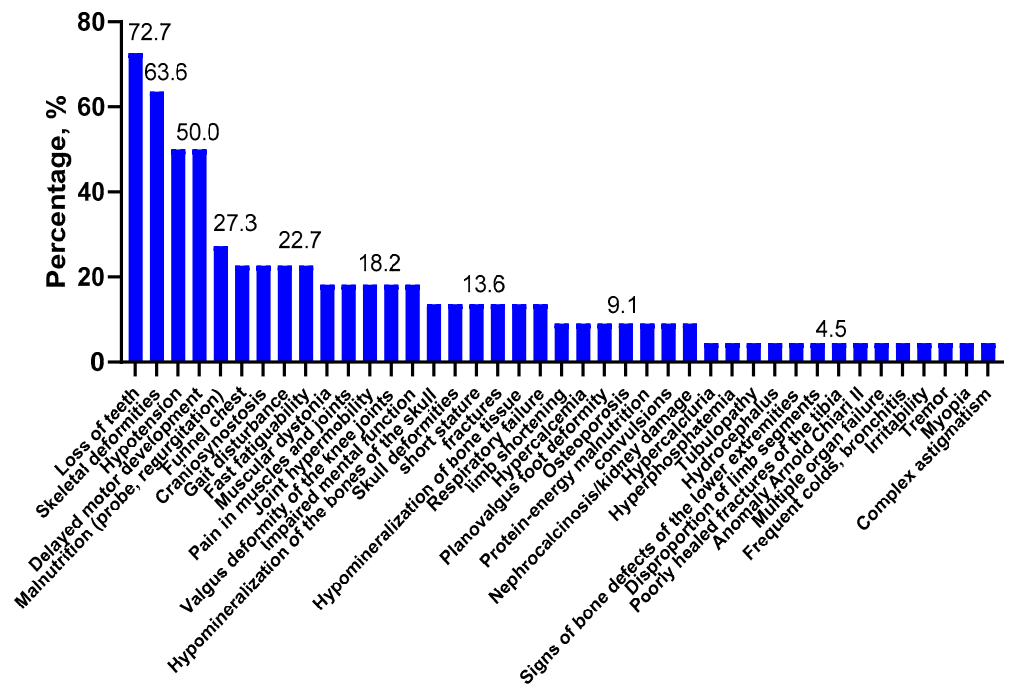


Figure 1. Clinical symptoms of HPP in the *ALPL* gene for patients with two pathogenic variants and within the Russian population ($n = 22$). The numbers above the columns indicate the percentage of the manifestation of the clinical symptom.

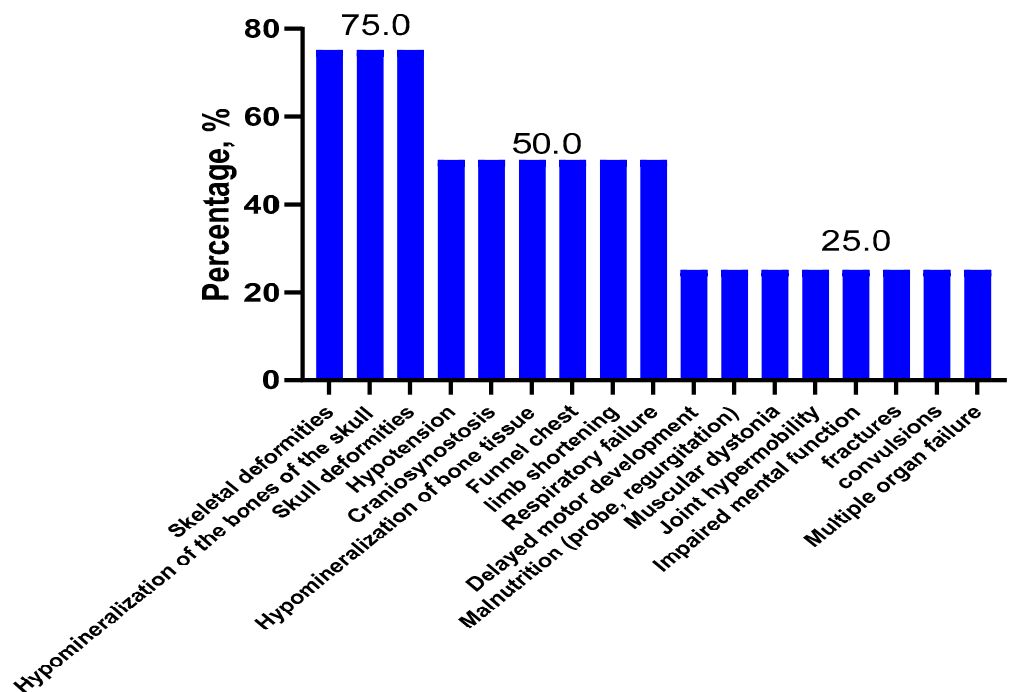


Figure 2. Clinical symptoms of HPP in children with perinatal HPP ($n = 4$).

For children with infantile HPP ($n = 8$), the most common symptoms were skeletal deformities (87.5%), tooth loss (75.0%), neuropsychomotor developmental delay (75.0%), and malnutrition (50.0%) (Figure 3).

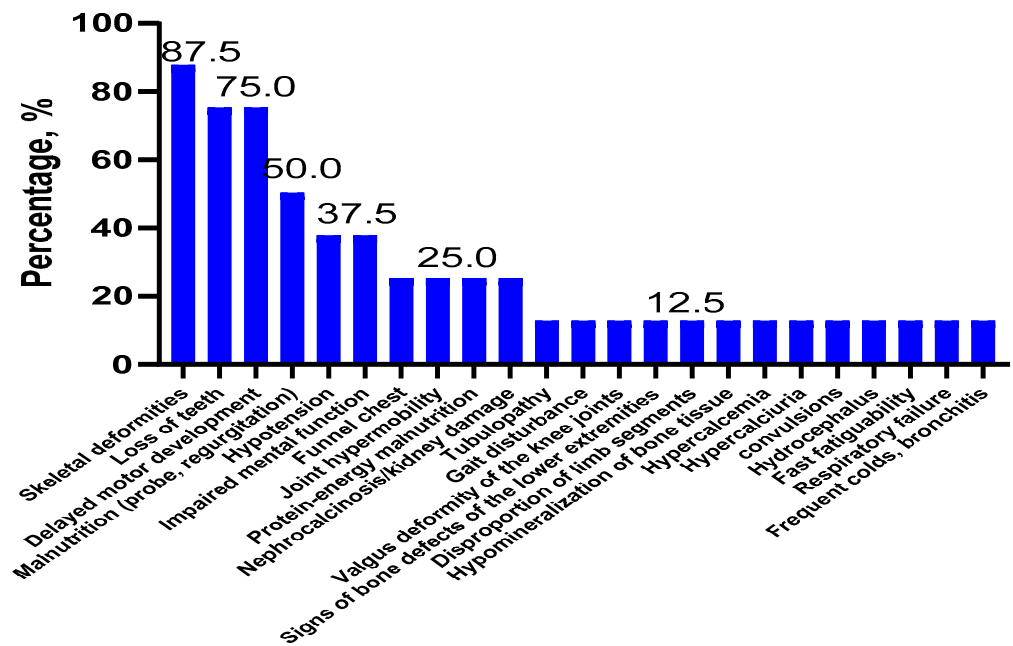


Figure 3. Clinical symptoms of HPP in children with infantile HPP ($n = 8$).

In patients with childhood HPP ($n = 10$), the most frequent symptoms were tooth loss (100.0%), hypomyotonia (60.0%), skeletal deformity (40.0%), gait disorder (40.0%), muscle and joint pain (40.0%) neuropsychomotor developmental delay (40.0%), and fatigue (40.0%) (Figure 4).

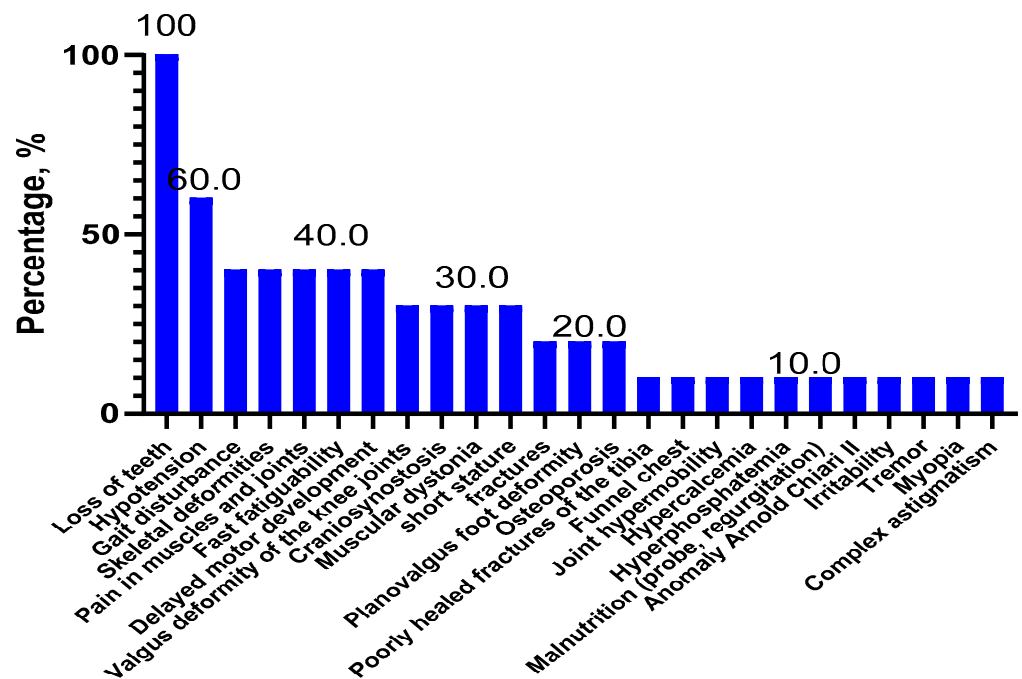


Figure 4. Clinical symptoms of HPP in patients with childhood HPP ($n = 10$).

2.2. Variant Frequencies in the ALPL Gene at HPP

Compound heterozygotes were found in 27 of the patients with HPP. A total of 28 different genetic variants were found in this group of patients, including some previously undescribed in other populations (Table 2). The variants were classified according to the American College of Medical Genetics and Genomics (ACMG) guidelines (Richards et al., 2015).

Table 2. Genetic variants of the ALPL gene in 27 compound heterozygotes and homozygotes HPP patients.

Allele 1	Allele 2	Amino Acid Substitution	Variant Type	Number of Patients	Clinical Variant Classification
c.182G > A	c.571G > A	Gly61Glu/Glu191Lys	Missense/Missense	1	Pathogenic/Pathogenic
c.211C > T	c.1101_1103del	Arg71Cys/Ser368del	Missense/In frame	1	Pathogenic/Pathogenic
c.228G > T	c.571G > A	Gln76His/Glu191Lys	Missense/Missense	1	Pathogenic/Pathogenic
c.253A > C	c.571G > A	Thr85Pro/Glu191Lys	Missense/Missense	1	VUS/Pathogenic
c.302A > G	c.571G > A	Tyr101Cys/Glu191Lys	Missense/Missense	1	VUS/Pathogenic
c.303C > A	c.314C > T	Tyr101Ter/Ala105Val	Nonsense/Missense	1	Pathogenic/VUS
c.303C > A	c.571G > A	Tyr101Ter/Glu191Lys	Nonsense/Missense	1	Pathogenic/Pathogenic
c.340G > A	c.571G > A	Ala114Thr/Glu191Lys	Missense/Missense	1	Pathogenic/Pathogenic
c.382G > A	c.871G > C	Val128Met/Glu291Gln	Missense/Missense	1	Pathogenic/VUS
c.508A > G	c.508A > G	Asn170Asp	Missense	1	Pathogenic
c.526G > A	c.1375G > A	Ala176Thr/Val459Met	Missense/Missense	1	Pathogenic/Pathogenic
c.571G > A	c.144_148dup	Glu191Lys/ Val50AlafsTer20	Missense/Frameshift	1	Pathogenic/VUS
c.571G > A	c.662delG	Glu191Lys/ Gly221Valfs*56	Missense//Frameshift	2	Pathogenic/Pathogenic
c.571G > A	c.1259G > T	Glu191Lys/Gly420Val	Missense/Missense	1	Pathogenic/Pathogenic
c.571G > A	c.1157_1175inv	Glu191Lys/GGYTPRG386_392ATGGVST	Missense/Frameshift	1	Pathogenic//Pathogenic
c.571G > A	c.1309 + 2T > C	Glu191Lys/	Missense/Splicing	2	Pathogenic/Pathogenic
c.984_986delCT	c.371A > G	Phe328del/Asn124Ser	In frame/Missense	1	Pathogenic/VUS
c.1112C > A	c.1447G > A	Thr371Asn/Val483Met	Missense/Missense	2	Pathogenic/VUS
c.1163A > G	c.1163A > G	Tyr388Cys	Missense	1	VUS
c.1171del	c.1171del	Arg391ValfsTer12	Frameshift	1	Pathogenic
c.1171delC	c.302A > G	Arg391Valfs*12/ Tyr101Cys	Fameshift/Missense	1	Pathogenic/VUS
c.1171delC	c.571G > A	Arg391Valfs*12/Glu191Lys	Fameshift/Missense	1	Pathogenic/Pathogenic
c.1349G > A	c.1349G > A	Arg450His	Missense	1	Pathogenic
c.1364G > A	c.571G > A	Glu455Asp/Glu191Lys	Missense/Missense	1	Pathogenic/Pathogenic

In bold are the variants that are not represented in the Global Variome shared LOVD ALPL database as of 1 August 2022 (VUS: a variant of uncertain (or unknown) significance).

The most commonly found variants among those patients with two variants were c.571G > A(p.Glu191Lys) and c.1171del (Arg391Valfs*12) with frequencies of 48.2% (13/28) and 11% (3/28), respectively. These variants are localized in exons 6 and 10, correspondingly. According to the ACMG classification [15], for this group of patients, 71.4% (20/27) of the genetic variants were identified as pathogenic, and 8 variants (28.6%) were of an uncertain (or unknown) significance (VUS). Among these variants, 25.0% (7/28: 4 pathogenic and 3 VUS) were absent from the LOVD and dbSNP databases and were initially identified in our study (Table 2).

The heterozygous carriage of the variants was detected in 225 of the total 1612 patients with decreased alkaline phosphatase activity. A total of 66 unique genetic variants were identified (Table 3).

Table 3. Genetic variants of the *ALPL* gene in 225 heterozygote HPP patients.

Variant	Amino Acid Substitution	Variant Type	Allelic	Clinical Variants
			Frequency, %	Interpretation
c.61G > A	Glu21Lys	Missense	0.22 (1/450)	Pathogenic
c.98C > T	Ala33Val	Missense	0.22 (1/450)	Pathogenic
c.119C > T	Ala40Val	Missense	0.22 (1/450)	Pathogenic
c.144_148dup	Val50Alafs*20	Frameshift	0.22 (1/450)	Pathogenic
c.182G > A	Gly61Glu	Missense	0.22 (1/450)	VUS
c.188G > T	Gly63Val	Missense	0.44 (2/450)	Pathogenic
c.202_204delACG	Thr68del	In frame	0.22 (1/450)	VUS
c.203C > T	Thr68Met	Missense	0.44 (2/450)	Pathogenic
c.205G > A	Ala69Thr	Missense	1.11 (5/450)	VUS
c.211C > T	Arg71Cys	Missense	0.67 (3/450)	Pathogenic
c.211C > A	p.Arg71Ser	Missense	0.22 (1/450)	Pathogenic
c.212G > A	p.Arg71His	Missense	0.22 (1/450)	Pathogenic
c.214A > G	Ile72Val	Missense	0.22 (1/450)	Likely Pathogenic
c.216C > A	p.(=)	Synonymous	0.22 (1/450)	VUS
c.250G > A	Glu84Lys	Missense	0.22 (1/450)	Pathogenic
c.253A > C	Thr85Pro	Missense	0.22 (1/450)	VUS
c.297 + 1G > A	-	Splicing	0.44 (2/450)	Pathogenic
c.298-2A > G	-	Splicing	0.22 (1/450)	Pathogenic
c.302A > G	Tyr101Cys	Missense	0.22 (1/450)	VUS
c.303C > A	Tyr101Ter	Nonsense	1.11 (5/450)	Pathogenic
c.305A > C	Asn102Thr	Missense	0.44 (2/450)	VUS
c.314C > T	Ala105Val	Missense	0.22 (1/450)	VUS
c.331_332insCCGGCA	p.T113_A114insGT	In frame	0.22 (1/450)	VUS
c.368C > A	Ala123Asp	Missense	0.44 (2/450)	Pathogenic
c.371A > G	Asn124Ser	Missense	0.22 (1/450)	VUS
c.389del	Val130GlufsTer35	Frameshift	0.22 (1/450)	Pathogenic
c.407G > A	Arg136His	Missense	0.22 (1/450)	Pathogenic
c.436G > A	Glu146Lys	Missense	0.67 (3/450)	VUS
c.455G > A	Arg152His	Missense	3.33 (15/450)	VUS
c.500C > T	Thr167Met	Missense	0.22 (1/450)	Pathogenic
c.508A > G	Asn170Asp	Missense	0.44 (2/450)	Pathogenic
c.571G > A	Glu191Lys	Missense	12.7 (57/450)	Pathogenic
c.595C > T	Gly199*	Nonsense	0.44 (2/450)	Pathogenic
c.648 + 1G > A	-	Splicing	0.22 (1/450)	Pathogenic
c.659G > T	Gly220Ala	Missense	0.67 (3/450)	Pathogenic
c.662_663insG	Gly222Trpfs	Frameshift	0.44 (2/450)	Pathogenic
c.815G > T	Arg272Leu	Missense	0.22 (1/450)	Pathogenic
c.818C > T	Thr273Met	Missense	0.88 (4/450)	VUS
c.889T > A	Tyr297Asn	Missense	0.44 (2/450)	VUS
c.902delG	Asn302Thrfs	Frameshift	0.67 (3/450)	Pathogenic
c.928_929delTC	Ser310Argfs	Frameshift	0.44 (2/450)	Pathogenic
c.968A > T	Asn323Ile	Missense	0.22 (1/450)	VUS
c.979T > C	Phe327Leu	Missense	0.22 (1/450)	Pathogenic
c.984_986delCTT	Phe328del	In frame	0.22 (1/450)	Pathogenic
c.997 + 2T > A	-	Splicing	0.44 (2/450)	Pathogenic
c.998-1G > A	-	Splicing	0.67 (3/450)	Pathogenic
c.1001G > A	Gly334Asp	Missense	0.44 (1/225)	Pathogenic
c.1066G > A	Asp356Asn	Missense	0.22 (1/450)	VUS
c.1072G > A	Ala358Thr	Missense	0.22 (1/450)	VUS
c.1101_1103del	Ser368del	In frame	0.22 (1/450)	Pathogenic
c.1130C > T	Ala377Val	Missense	0.22 (1/450)	Pathogenic
c.1163A > G	Tyr388Cys	Missense	0.22 (1/450)	VUS
c.1171del (16)	Arg391Valfs*12	Frameshift	3.11 (14/450)	Pathogenic
c.1190-2_1190-1delinsCT	-	Splicing	0.44 (2/450)	Pathogenic
c.1216G > A	Asp406Asn	Missense	0.22 (1/450)	VUS
c.1247G > T	Gly416Val	Missense	0.22 (1/450)	VUS

Table 3. Cont.

Variant	Amino Acid Substitution	Variant Type	Allelic	Clinical Variants
			Frequency, %	Interpretation
c.1259G > T	Gly420Val	Missense	0.44 (2/450)	VUS
c.1276G > A	p.Gly426Ser	Missense	0.44 (2/450)	Pathogenic
c.1309 + 2T > C	-	Splicing	0.44 (2/450)	Pathogenic
c.1310C > T	Ala437Val	Missense	0.22 (1/450)	VUS
c.1328C > T	Ala443Val	Missense	0.22 (1/450)	Pathogenic
c.1349G > A	Arg450His	Missense	0.67 (3/450)	Pathogenic
c.1364G > A	Glu455Asp	Missense	0.44 (2/450)	Pathogenic
c.1447G > A	Val483Met	Missense	1.11 (5/450)	VUS
c.1483G > A	Gly495Ser	Missense	0.22 (1/450)	VUS
c.1489T > C	Cys497Arg	Missense	0.22 (1/450)	VUS

In bold are variants that are not represented in the Global Variome shared LOVD *ALPL* database as of 1 August 2022 (VUS a variant of uncertain (or unknown) significance). All genetic variants are presented in Table S1 in the Supplementary Materials.

The most common variants in heterozygous patients were also c.571G > A (p.Glu191Lys), 1171del (Arg391Valfs*12), and c.455G > A (Arg152His) with frequencies of 12.7, 3.1, and 3.3% (57/450, 14/450, 15/450), respectively. These variants were localized in exons 5, 6, and 10. According to the ACMG [17], for the heterozygous patients, 60.6% (40 of 66) of the genetic variants were pathogenic, one variant (1.5%) was likely pathogenic or pathogenic, and 25 variants (37.9%) were VUS. The 18 other variants (27.3%, 18/66) were not previously described in the LOVD and dbSNP databases and literature and were first identified in this research.

2.3. Analysis of the Variants Depending on Type and Localization in the *ALPL* Gene

Compound heterozygotes in the *ALPL* gene were detected in 27 of the individuals. The majority of them were missense (75.0%), with 17.9% frameshift and in frame, 3.6% splice variants, and 3.6% duplications (Figure 5).

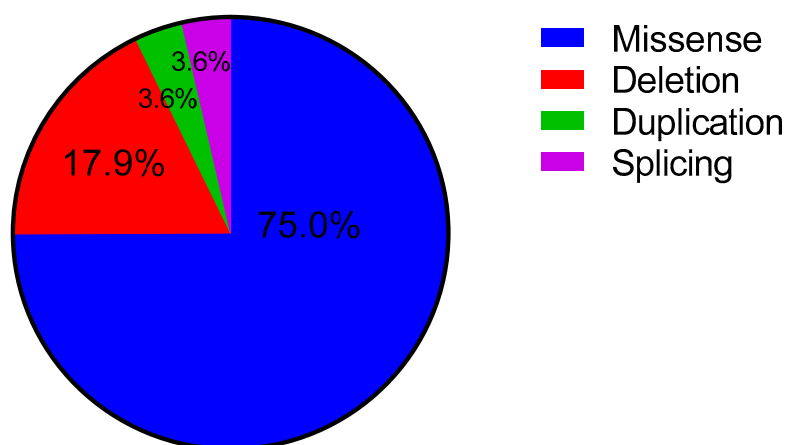


Figure 5. Distribution of variants depending on the type in the *ALPL* gene.

Interestingly, 50.0% of the variants (14/28) in the patients with two variants were localized in the 5th (6 variants), 10th (4 variants), and 12th (4 variants) exons of the *ALPL* gene (Figure 6).

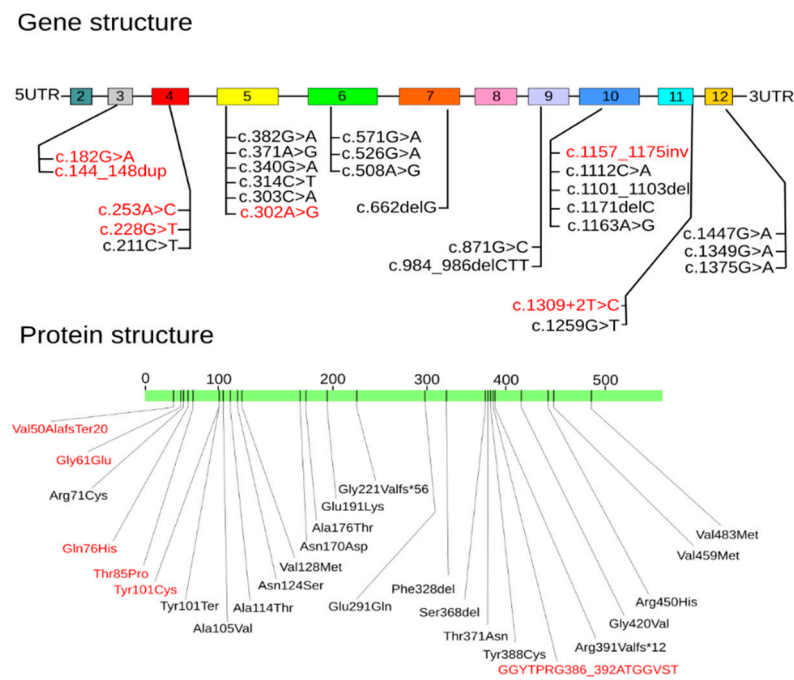


Figure 6. Distribution of genetic variants in the *ALPL* gene and amino acid substitutions in the TNSALP protein in Russian patients (compound heterozygotes and homozygotes only). Variants are highlighted in red, identified for the first time in the Russian population.

The distribution of amino acid substitutions in the TNSALP protein domains in patients with the two variants is illustrated in Figure 7.

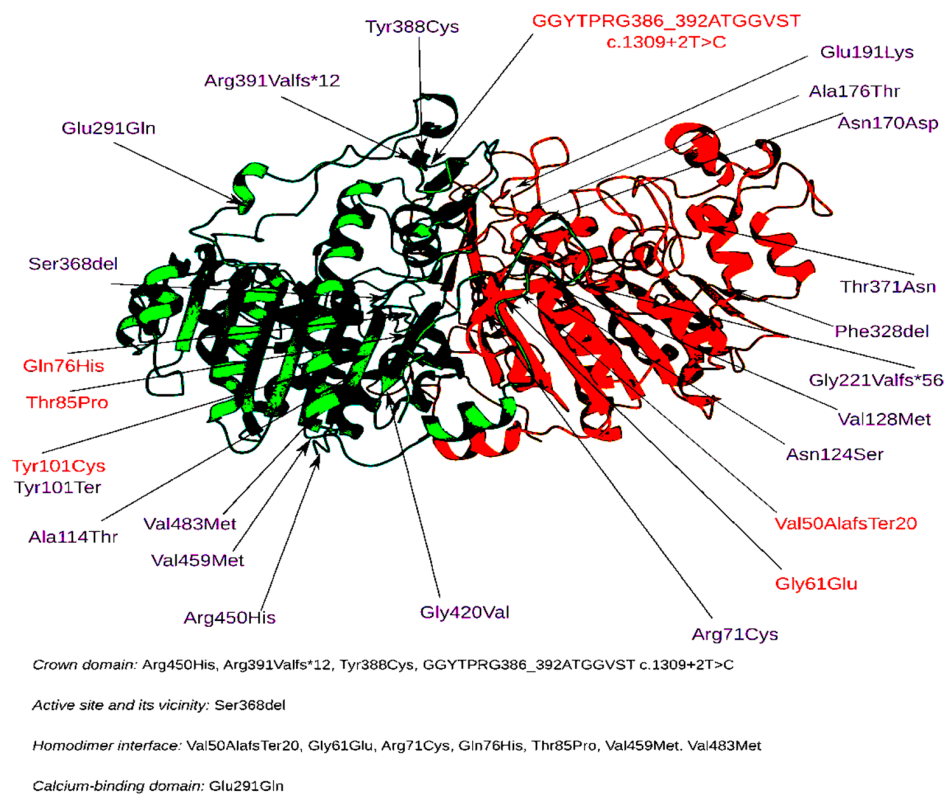




Figure 7. Localization of amino acids in TNSALP protein domains in Russian patients (compound heterozygotes only). The altered amino acids identified in the Russian population are highlighted in red.

Table 4. Cont.

Clinical Symptoms	Patients, Forms of HPP																					
	Perinatal			Infantile						Childhood												
Hypercalciuria																						
Hyperphosphatemia																						
Tubulopathy																						
Hydrocephalus																						
Signs of bone defects																						
Poorly healed fractures of the tibia																						
Anomaly Arnold Chiari II,																						
Multiple organ failure																						
Frequent colds, bronchitis																						
Irritability																						
Tremor																						
Myopia																						
Complex astigmatism																						
Genotype																						
	c.1163A>G/c.1163A>G	c.1171delC/c.1171delC	c.1171delC/c.302A>G	c.508A>G/c.508A>G	c.571G>A/c.1259G>T	c.303C>A/c.314C>T	c.253A>C/c.571G>A	c.571G>A/c.1157_1175inv	c.984_986delCT/c.371A>G	c.526G>A/c.1375G>A	c.211C>T/c.1101_1103del	c.1364G>A/c.571G>A	c.340G>A/c.571G>A	c.303C>A/c.571G>A	c.571G>A/c.144_148dup	c.571G>A/c.1309+2T>C	c.571G>A/c.1309+2T>C	c.382G>A/c.871G>C	c.571G>A/c.662delG	c.571G>A/c.662delG	c.302A>G/c.571G>A	c.1171delC/c.571G>A

Note: Color  indicates homozygotes, —compound heterozygotes.

A total of three out of four patients with the perinatal form, according to the data in Table 4, were homozygotes (two in the missense and one in the nonsense variants) and had bone disorders and skull hypomineralization; one child had hypotonia and another had respiratory disorders. Of the eight patients with the infantile form, six were compound heterozygotes and two were heterozygotes of the missense and nonsense variants. This group of patients was predominated by skeletal deformities (87.5%), loss of teeth (75.0%), and neuropsychomotor developmental delay (75.0%). Of the ten patients with the pediatric form, seven were compound heterozygotes, two were splice heterozygotes, and one was nonsense heterozygote. Tooth loss (100.0%) and hypotonia (60.0%) prevailed in this group of patients. It is noteworthy that the homozygous patients, regarding missense variants or compound heterozygotes for nonsense variants, had severe clinical symptoms.

Further, we compared the frequencies of missense and nonsense variants depending on the form of HPP (Table 3, Figure 8).

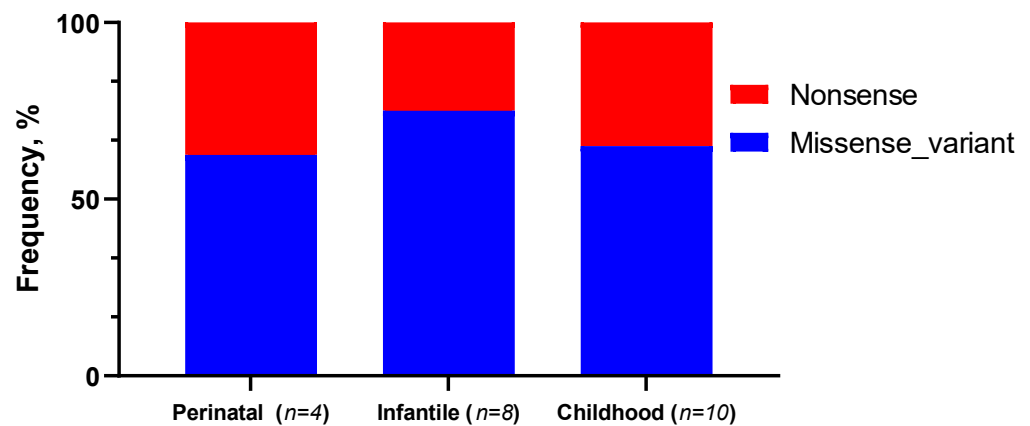


Figure 8. Frequencies of genetic variants in the ALPL gene depending on the HPP forms in the patients.

As follows from the data presented in Figure 4, there is not a statistically significant ($p > 0.05$) predominance of the nonsense mutations of the ALPL gene in patients with the perinatal form compared with the infantile and childhood forms of HPP.

We also compared the frequencies of the homozygous and heterozygous variants of the ALPL gene in different forms of HPP (Table 3, Figure 9).

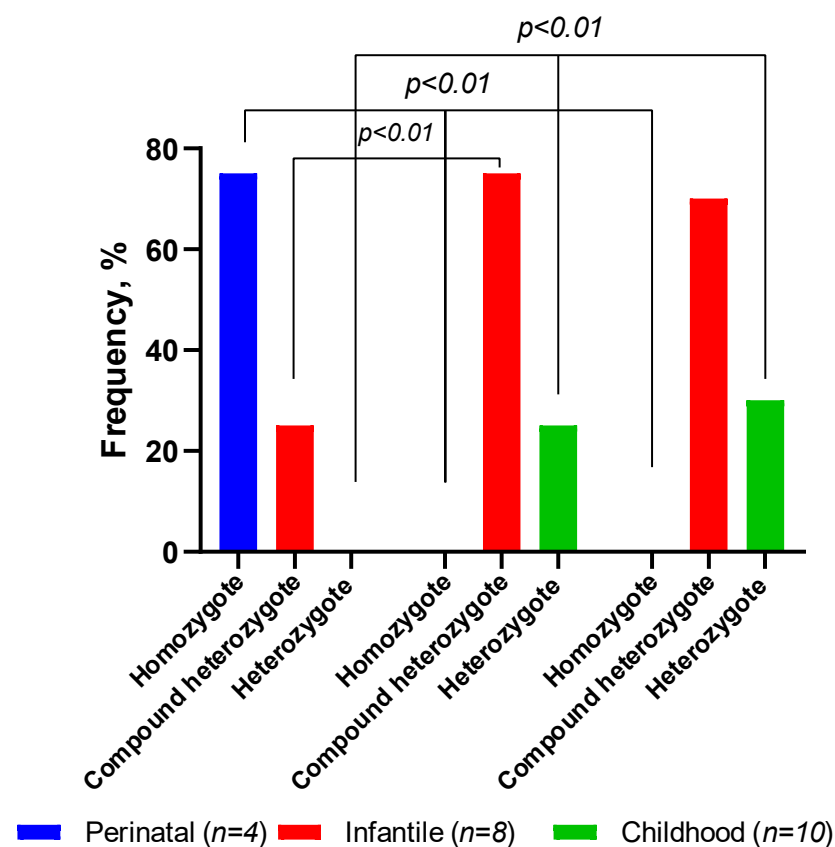


Figure 9. Frequency of the homozygous, compound-heterozygote, and heterozygote genotypes of the ALPL gene in patients, depending on the form of HPP.

The number of homozygotes with the perinatal form was significantly ($p < 0.01$) higher than the number of these genotypes in patients with the infantile and childhood forms of HPP. On the contrary, the frequency of the compound heterozygotes and heterozygotes

was higher ($p < 0.01$) in patients with the infantile and childhood forms when compared to the perinatal form of HPP.

2.5. Analysis of TNSALP Activity Depending on the Form, Type of Variants, and the Genotype of the Patients

We analyzed the TNSALP activity, depending on the form of HPP (Table 3, Figures 10 and 11).

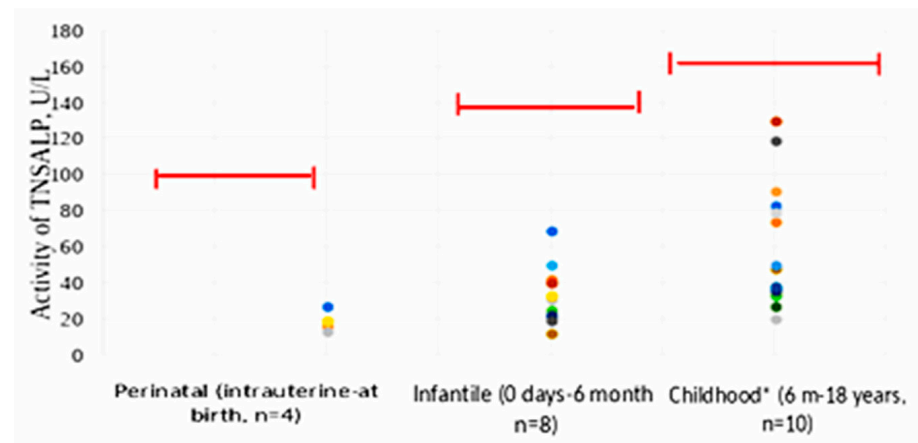


Figure 10. TNSALP activity, depending on the form of HPP in patients ($n = 22$). Each colored dot in the figure explain one patient. Note: each point is one patient. Red and black indicate the lower limit of normal TNSALP at this age. * Most patients with the childhood form were diagnosed at the age of 2–9 years. The middle limit of normal was taken from the study [23].

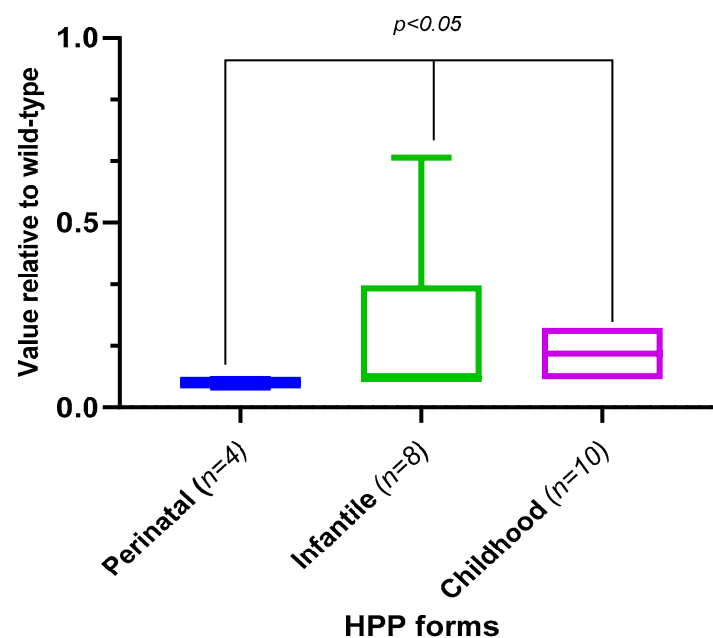


Figure 11. Residual activity of TNSALP variants relative to their wild-type, according to HPP subtype [21].

Based on the works of [21], we compared the theoretical residual activity of the variants in our group of patients according to its TNSALP domain localization (Figure 11).

The results presented in Figures 10 and 11 establish that residual TNSALP activity in the perinatal form was statistically significantly ($p < 0.0005$) lower than in the infantile and pediatric ($p < 0.05$) forms of HPP.

However, the theoretically calculated (the real data for these groups of patients are partially available), heterozygotes and compound heterozygotes, with variants in the *ALPL* gene (Table 4, Figure 12A), have higher residual protein activity compared to the homozygotes. We did not detect differences between the real age-related changes and the theoretically-calculated activity of the TNSALP for different genotypes (Figure 12B).

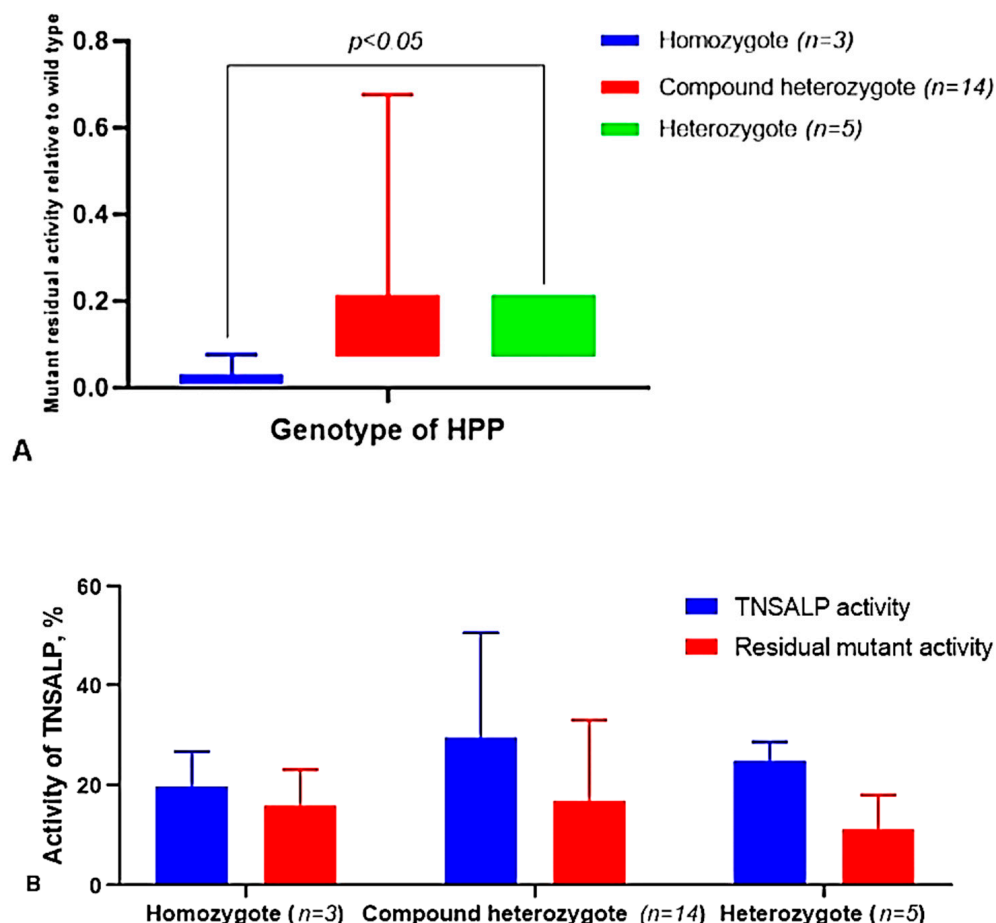


Figure 12. The residual mutant activity of the different variants of the *ALPL* gene in relation to wild-type activity deepened according to genotype. (A) Comparison of the real TNSALP activity in the serum with the theoretically calculated values, depending on the genotypes of the patients with HPP ((B), see the Material and Methods section).

2.6. Analysis of the Activity of the *ALPL* Gene Variants Depending on Their Domain Localization and Origin

It was found that the activity of TNSALP for those variants encoding the amino acids in the “active center” and corona domain was statistically significantly ($p < 0.05$) lower than the activity in the variants at the homodimeric interface (nonspecific sites of the protein). This implies that these sites in the protein structure are crucial for the preservation of enzyme activity.

We compared the activity of the *ALPL* gene variants in our group of patients depending on their domain localization (Figure 13).

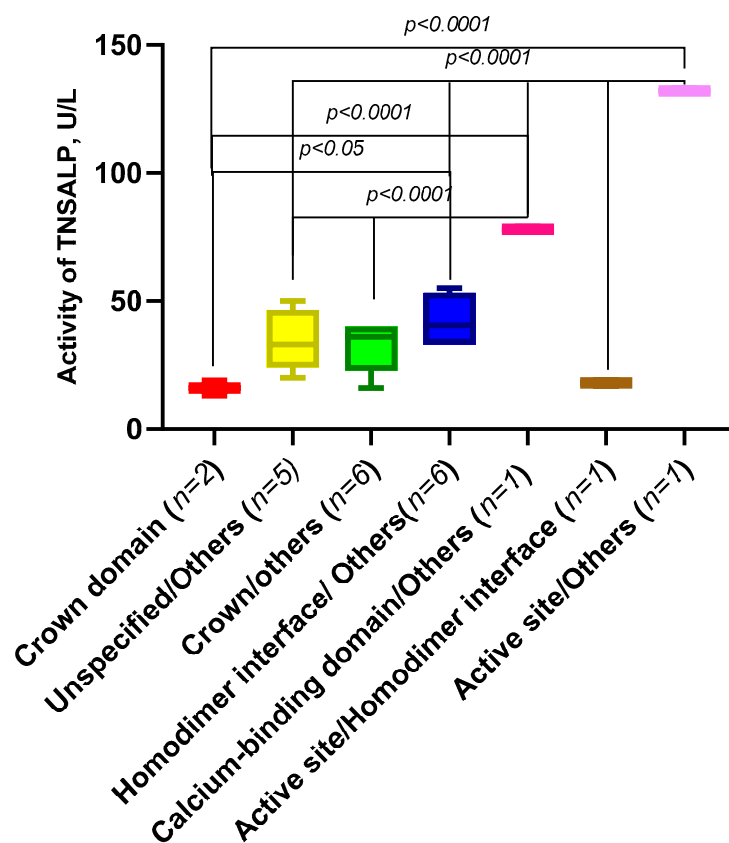


Figure 13. Activity of *ALPL* gene variants, depending on the protein domain location.

3. Discussion

In our study of Russian children with HPP, the most frequent symptoms were tooth loss (72.7%), skeletal deformities (63.6%), hypotonia (50.0%), and neuropsychomotor developmental delay (50%) (Figure 1). Whereas, in the European and American childhood populations ($n = 121$), the main symptoms of HPP were premature tooth loss (48.2%), bone disorders (44.2%), craniosynostosis (26.7%), and pain syndrome (19.3%) [24]. Among the four Russian children with perinatal HPP, the most frequent symptoms were bone deformities (75%), skull bone deformity (75%), skull hypomineralization (75%), hypotonia (50%), craniosynostosis (50%), pectus excavatum (50%), bone hypomineralization (50%), limb shortening (50%), and respiratory failure (50%) (Figure 2). Whereas, for Canadian and American children with perinatal HPP ($n = 30$), the most frequent symptoms were fractures (40.0%), skull abnormalities (40.0%), and general motor difficulties (23.3%) [25]. Notably, there are common clinical features for different populations, as well as specific ones. The most common symptoms in the eight Russian children with infantile HPP were skeletal deformities (87.5%), loss of teeth (75.0%), neuropsychomotor developmental delay (75.0%), and nutritional disorders (50.0%) (Figure 3). In contrast, in a group of Canadian and American children with infantile HPP ($n = 102$), the leading symptoms were loss of teeth (61.8%), cranial malformations (43.1%), and motor and mobility disorders (40.2%) [25]. Among the 10 Russian patients with childhood HPP, the most frequent symptoms were loss of teeth (100.0%), hypotonia (60.0%), skeletal deformity (40.0%), gait disorders (40.0%), muscle and joint pain (40.0%), motor retardation (40.0%), and fatigue (40.0%) (Figure 4). In Canadian and American children with childhood HPP ($n = 87$), the main symptoms were loss of teeth (70.1%), fractures (34.5%), and pain syndrome (43.7%) [25]. Note that those patients with more severe symptoms (skeletal deformities, loss of teeth, etc.) in the Russian, American, and European populations have a homozygous genotype or have nonsense variants (deletions or splice variants) in the genotype. In general, the low frequency of

HPP detection in the Russian population is due to the fact that the main criterion for selecting patients was not the presence of specific clinical symptoms but a recorded low level of alkaline phosphatase, which could not be associated with HPP. The difference in the frequency of the clinical symptoms in Russian patients compared with foreign data is due to (1) a small sample of our patients with a confirmed diagnosis, for whom the detailed clinical data were available, (2) the retrospective analysis of clinical data from the medical record, and (3) a lack of uniform criteria for assessing some symptoms (for example, if a child did not complain of pain by their own accord, they could not be asked about it).

Additionally, we identified a total of 79 unique variants of the *ALPL* gene, characterized by residual enzymatic activity, from 252 Russian patients with HPP. Among them, patients with two pathogenic variants had 28 different variants. Of these, 75% of the patients had missense variants, 17.9% had frameshift deletions, 3.6% had duplications, and 3.6% had splicing variants. American and French researchers obtained similar frequencies for their patients. They found a total of 155 variants of the *ALPL* gene in 345 patients with HPP. Missense variants accounted for 146 (94.2%) patients, nonsense variants (variants with a broken chain) for five (3.2%), variants with a reading frame shift for two (1.3%), and deletions for two (1.3%) patients [23]. It should be noted that our group of patients was less characterized by missense variants, while the reading frameshift deletions were more representative. This is probably associated with the fact that our group included patients both with two variants and/or VUS variants and with a more severe form of HPP.

The c.571G > A(p.Glu191Lys) variant in the 6th exon in the *ALPL* gene was the most frequent among the compound-heterozygote and heterozygotes in the Russian population at 28.89% (73/252). According to the study by del Angel G. et al., the frequency of this c.571G > A/p.Glu191Lys variant is more than 10^{-3} [23]. The c.571G > A/p.Glu191Lys variant is also the most common (with a frequency of up to 55%) in patients with HPP of a European origin [26], and the initial frequency of this variant was 0.08 in infants, children, and patients with odonto-HPP [6]. The c.571G > A/p.Glu191Lys variant has been demonstrated to reduce TNSALP enzymatic activity by up to 68% (207.1 ± 39.3 U/ μ g) when compared with wild-type enzyme activity (302.5 ± 102.2 U/ μ g) [26].

The other variants, such as c.1001G > A (p.Gly334Asp), both in our study and in the study by del Angel G. et al. [23], were detected only in patients with HPP at a carrier frequency of less than 1%, which corresponds to the frequency of this variant in Manitoba Mennonites [27]. Finally, it should be noted that some variants can be detected with a higher frequency in a particular population. For example, the frequency of the c.787T > C/p.Tyr263His variant in the Japanese population is 0.31–0.55, and in the gnomAD database, 0.1833 [28,29] of the p.Asp378Val and p.Leu520ArgfsX86 variants are most common in the USA [6] and Japan [30], respectively. The majority of the mutations in the *ALPL* gene were localized in the 5th, 7th, 10th, and 3rd exons [18].

In the study by del Angel G. et al. (2020), the pathogenic variants (reading frame shift, deletions, and splicing variants) exhibited close to zero residual enzymatic activity. An interesting fact is that, whereas low residual enzymatic activity can serve as a predictor of pathogenicity, the converse statement is incorrect. High residual activity values for the variants are not necessarily a predictor of their benignity. In calculating the activity within patients with HPP, it was found that extremely low in vitro values correlate with perinatal lethal or infantile HPP subtypes (Figure 7). At the same time, there was an overlap in activity between patients with HPP and healthy donors ($\geq 50\%$) [21]. Consequently, the prognostic value of low TNSALP enzymatic activity is limited by extremely low values in the most severe HPP subtypes. Data obtained by gnomAD (version 2.1.1) [6] show that 844 out of 923 genetic variants are rare, with allele frequencies less than $<10^{-4}$ in the population, independent of the residual activity level, which is compatible with the rare nature of HPP, especially in its perinatal and infantile subtypes [15,31]. Meanwhile, variants with frequencies of more than 10^{-3} have higher residual TNSALP activity [21]. This is related to evolutionary pressure, which results in variants with low activity occurring at low population frequencies. This conclusion is supported by the comparison of variant residual

activity with clinical significance [32]. In addition, high values of residual enzymatic activity (>50% of WT values) can also be associated with the clinical subtype of HPP. Notably, pathogenic variants in the *ALPL* gene can have a dominant-negative effect (DNE) due to the inhibition of the interaction with the wild-type monomers by mutant monomers or as a result of the sequestration of the wild-type protein by the mutant monomer, preventing its transport to the membrane [33]. In our study, the residual enzymatic activity of TNSALP was most decreased for those variants associated with amino acid substitutions in the active center, corona domain, and the homodimeric interface.

We have determined residual enzymatic activity in 22 patients, including 14 patients (66.7%) with residual TNSALP activity of less than $\leq 25.0\%$, six (28.6%) with activity between 25–50%, and one patient with activity above 50%. In a study by del Angel G. et al., 58% (90/155) of the variants had a residual activity of less than $\leq 25\%$, 34% (52/155) of the variants exhibited an activity of more than $\geq 50\%$, and 8% (13/155) had activity between these levels [21].

Another study noted that pathogenic variants are localized at the calcium-binding site [34]. In our study, curiously, the residual activity of TNSALP at the homodimer interaction site covered a wide range. Consequently, the clinical significance of the variants in this domain may depend on the positional interactions and the specific amino acid changes involved. This conclusion is confirmed by comparing the residual activity of a variant to its clinical significance [32]. At the same time, high values of residual enzymatic activity (>50% of WT values) may also be associated with the clinical subtype of HPP. In addition, it is suggested that some alleles in patients with severe types of HPP may not reduce enzymatic activity but impair its transport and the localization of the TNSALP dimer in the extracellular domain or by impairing the creation of aggregates that are improperly cleaved in the proteasome [19,35].

4. Materials and Methods

4.1. Patients with Hypophosphatasia

Genetic testing was performed on 1612 patients, with decreased activity of alkaline phosphatase in the blood plasma from different cities and regions of the Russian Federation (Moscow, Saint Petersburg, Yekaterinburg, Surgut, Khabarovsk, Cheboksary, Krasnodar, etc.) from 2014–2021. The inclusion criteria for the patients were a detected low level of alkaline phosphatase, clinical symptoms, radiographic changes, and genetic variants in the *ALPL* gene. Heterozygous carriage of pathogenic and likely pathogenic variants in the *ALPL* gene was detected in 225 patients. Compound heterozygotes and homozygotes were found in 27 patients. The sex ratio of all subjects was approximately 3:1 (males to females). The average age of onset was 7 months (from 2 h after birth to 14 years old). The mean age of diagnosis was 2.43 years (2 h after birth to 19 years old).

4.2. Biochemical Studies

Patients included in this study were tested for screening diagnostic purposes. Fasting blood samples were collected from all patients to obtain serum. TNSALP activity in the serum was measured according to reference values, depending on age and sex using kinetic and colorimetric methods and an alkaline phosphatase kit (BioSystems S.A. biochemical analyzer) (Costa Brava, Barcelona Spain) [36]. The TNSALP concentration in the serum samples was calculated using the following formula:

$$\Delta A \times \frac{V_t \times 106}{\epsilon \times l \times V_s}$$

where $\Delta A/\text{min}$ is the average absorption difference per minute, ϵ is the molar absorption coefficient of 4-nitrophenol at 405 nm, which is 18450, the optical path (l) is 1 cm, the total

reaction volume (V_t) is 1.02, the sample volume (V_s) is 0.02, and 1 U/L is 0.0166 $\mu\text{kat/L}$. The following factors were used to calculate enzyme activity.

$$\Delta A \times 2764 = \text{U/L or } \times 46.08 = \mu\text{kat/L}$$

TNSALP values in patients with two variants in the *ALPL* gene in different clinical forms of HPP are presented in Table 1.

4.3. Molecular Genetic Analysis of a *ALPL* Gene

The search for mutations in the exons of the *ALPL* gene was performed using Sanger sequencing. DNA was isolated from dry blood spots or whole blood by phenol-chloroform extraction. Special primers were developed for each exon in the *ALPL* gene. PCR conditions were selected individually for each exon. The synthesized PCR products were purified with 5 m NH₄Ac and 96% ethanol, and then 70% ethanol, dried at 60 °C and dissolved in 10 μL of deionized water. Further, the purified PCR products were sequenced using the reagent BigDye Terminator 3.1 kit (Applied Biosystems, Waltham, MA, USA). Then, capillary electrophoresis was performed in a genetic analyzer 3500xl (Applied Biosystems, Waltham, MA, USA). The obtained exon sequences were analyzed using the Sequence Scanner (version 2.0, Applied Biosystems, Waltham, MA, USA) software. The presence of the mutation was confirmed by visual inspection of the sequencing chromatograms.

4.4. Predicting Theoretical Activity of Patient Genotypes

Predicting theoretical TNSALP residual activity in Russian HPP patients ($n = 252$) depended on genotype (homozygotes, heterozygotes, and compound heterozygotes) and was calculated as a fraction of WT activity for each patient. The TNSALP residual activity for the homozygous patients, which had a variant with complete loss of function (nonsense, splice, or frameshift), was assumed to be null. The TNSALP residual activity for the heterozygotes was detected as 50% from the activity of the homozygotes. In patients with a single LoF variant, the TNSALP residual activity was 0.5, with the suggestion that the LoF allele would not express any protein. The TNSALP residual activity for the compound heterozygotes was calculated as the average activity of both alleles. If an allele had a dominant negative effect (DNE), the TNSALP activity was determined as “activity divided by four”, or leaving only 25% from total TNSALP activity, with two copies of allele one [21].

4.5. Statistical Analysis

The statistical data were analyzed using GraphPad Prism 8.01 software. The Kolmogorov–Smirnov test was used to determine the type of data distribution. ANOVA, with posthoc (usually Tukey’s honest significant difference, HSD) used to find the differences between several normally-distributed samples. When pairwise comparing data with a distribution other than normal, the Mann–Whitney U test was used [37].

4.6. Bioinformatic Analysis

Variant frequencies in the population were analyzed using the gnomad v2.1.1 database, as well as reference datasets, such as 1000 Genome [38], ExAC [39], and the proprietary Russian exome database [40–42].

5. Conclusions

From 2014–2021, we performed genetic testing on 1612 patients from various regions of the Russian Federation, with decreased alkaline phosphatase activity in their blood plasma. Among them, we identified 75 variants of the *ALPL* gene characterized by residual enzymatic activity in 252 Russian patients with HPP. A total of 75% of these patients had missense variants, and the others had nonsense variants. Among them, 71.4% (20/28) of the genetic variants were identified as being pathogenic, and eight variants (28.6%) were of uncertain (or unknown) significance (VUS). Among these variants, 25.0% (7/28: four

pathogenic and three VUS) were absent from the database and were initially identified by us.

The high frequency of hypotension in children from the Russian population is notable in the clinical picture, whereas the other clinical symptoms were similar. Among the heterozygous patients, 60.6% (40 of 66) of the genetic variants were pathogenic, one variant (1.5%) was likely pathogenic or pathogenic, and 25 variants (37.9%) were VUS. A total of 18 variants (27.3%: 18/66) were not previously described in the LOVD and dbSNP databases and the literature and were first identified in this research. The frequencies of the nonsense variants and low TNSALP enzymatic activity were higher in patients with the perinatal form of HPP compared to the infantile and childhood forms. Residual activity was detected as a function of the HPP subtype, variant type, and protein domain localization. TNSALP enzymatic activity cannot be used to assess the severity of HPP, but in combination with clinical symptoms, it will contribute to a better understanding of the phenotypic spectrum of HPP.

Supplementary Materials: The supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/ijms232112976/s1>.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patients to publish this paper.

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References

1. Fenn, J.S.; Lorde, N.; Martin Ward, J.; Borovickova, I. Hypophosphatasia. *J. Clin. Pathol.* **2021**, *74*, 635–640. [[CrossRef](#)] [[PubMed](#)]
2. Conti, F.; Ciullini, L.; Pugliese, G. Hypophosphatasia: Clinical manifestation and burden of disease in adult patients. *Clin. Cases Miner. Bone Metab.* **2017**, *14*, 230–234. [[CrossRef](#)] [[PubMed](#)]
3. RUSeq. Available online: <http://ruseq.ru/#/gene/249> (accessed on 20 September 2022).
4. Rathbun, J.C. Hypophosphatasia; a new developmental anomaly. *Am. J. Dis. Child.* **1948**, *75*, 822–831. [[CrossRef](#)] [[PubMed](#)]
5. Weiss, M.J.; Cole, D.E.; Ray, K.; Whyte, M.P.; Lafferty, M.A.; Mulivor, R.A.; Harris, H. A missense mutation in the human liver/bone/kidney alkaline phosphatase gene causing a lethal form of hypophosphatasia. *Proc. Natl. Acad. Sci. USA* **1988**, *85*, 7666–7669. [[CrossRef](#)] [[PubMed](#)]
6. Whyte, M.P.; Zhang, F.; Wenkert, D.; McAlister, W.H.; Mack, K.E.; Benigno, M.C.; Coburn, S.P.; Wagay, S.; Griffin, D.M.; Ericson, K.L.; et al. Hypophosphatasia: Validation and expansion of the clinical nosology for children from 25 years experience with 173 pediatric patients. *Bone* **2015**, *75*, 229–239. [[CrossRef](#)] [[PubMed](#)]
7. Mao, X.; Liu, S.; Lin, Y.; Chen, Z.; Shao, Y.; Yu, Q.; Liu, H.; Lu, Z.; Sheng, H.; Lu, X.; et al. Two novel mutations in the ALPL gene of unrelated Chinese children with Hypophosphatasia: Case reports and literature review. *BMC Pediatr.* **2019**, *19*, 456. [[CrossRef](#)] [[PubMed](#)]
8. Bangura, A.; Wright, L.; Shuler, T. Hypophosphatasia: Current Literature for Pathophysiology, Clinical Manifestations, Diagnosis, and Treatment. *Cureus* **2020**, *12*, e8594. [[CrossRef](#)]

9. Ignatovich, O.N.; Namazova-Baranova, L.S.; Margieva, T.V.; Yakhyaeva, G.T.; Zhurkova, N.V.; Savostyanov, K.V.; Pushkov, A.A.; Krotov, I.A. Osteogenesis Imperfecta: Diagnostic Feature. *Pediatr. Pharmacol.* **2018**, *15*, 224–232. [[CrossRef](#)]
10. García-Fontana, C.M.; Villa-Suárez, J.; Andújar-Vera, F.; González-Salvatierra, S.; Martínez-Navajas, G.; Real, P.J.; Gómez Vida, J.M.; de Haro, T.; García-Fontana, B.; Muñoz-Torres, M. Epidemiological, Clinical and Genetic Study of Hypophosphatasia in A Spanish Population: Identification of Two Novel Mutations in The Alpl Gene. *Sci Rep.* **2019**, *9*, 9569. [[CrossRef](#)] [[PubMed](#)]
11. Baranov, A.A.; Namazova-Baranova, L.S.; Savostianov, K.V.; Margieva, T.V.; Vishneva, E.A.; Yakhyaeva, G.T. CLinical Recommendation to the Diagnostics and Treatment of Hypophosphatasia in Children. *Pediatr. Pharmacol.* **2016**, *13*, 539–543. (In Russian) [[CrossRef](#)]
12. Martos-Moreno, G.A.; Calzada, J.; Couce, M.L.; Argente, J. Hypophosphatasia: Clinical manifestations, diagnostic recommendations and therapeutic options. *An. Pediatr. (Engl. Ed.)* **2018**, *88*, e1–e356. [[CrossRef](#)]
13. Collmann, H.; Mornet, E.; Gattenlöhner, S.; Beck, C.; Girschick, H. Neurosurgical aspects of childhood hypophosphatasia. *Childs Nerv. Syst.* **2009**, *25*, 217–223. [[CrossRef](#)] [[PubMed](#)]
14. Kishnani, P.S.; Rush, E.T.; Arundel, P.; Bishop, N.; Dahir, K.; Fraser, W.; Harmatz, P.; Linglart, A.; Munns, C.F.; Nunes, M.E.; et al. Monitoring guidance for patients with hypophosphatasia treated with asfotase alfa. *Mol. Genet. Metab.* **2017**, *122*, 4–17. [[CrossRef](#)]
15. Mornet, E. Genetics of hypophosphatasia. *Arch. Pediatr.* **2017**, *24*, 5s51–5s56. [[CrossRef](#)]
16. 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*, 572. [[CrossRef](#)] [[PubMed](#)]
17. Global Variome Shared LOVD ALPL (Alkaline Phosphatase, Liver/Bone/Kidney). Available online: <https://databases.lovd.nl/shared/variants/ALPL/unique> (accessed on 20 September 2022).
18. Liu, M.; Liu, M.; Liang, X.; Wu, D.; Li, W.; Su, C.; Cao, B.; Chen, J.; Gong, C. Clinical and genetic characteristics of hypophosphatasia in Chinese children. *Orphanet. J. Rare Dis.* **2021**, *16*, 159. [[CrossRef](#)]
19. Brun-Heath, I.; Lia-Baldini, A.S.; Maillard, S.; Taillandier, A.; Utsch, B.; Nunes, M.E.; Serre, J.L.; Mornet, E. Delayed transport of tissue-nonspecific alkaline phosphatase with missense mutations causing hypophosphatasia. *Eur. J. Med. Genet.* **2007**, *50*, 367–378. [[CrossRef](#)] [[PubMed](#)]
20. Mornet, E. Molecular genetics of Hypophosphatasia and phenotype-genotype correlations. *Subcell. Biochem.* **2015**, *76*, 25–43. [[CrossRef](#)]
21. 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*, 1250–1262. [[CrossRef](#)]
22. Whyte, M.P.; Greenberg, C.R.; Salman, N.J.; Bober, M.B.; McAlister, W.H.; Wenkert, D.; Van Sickle, B.J.; Simmons, J.H.; Edgar, T.S.; Bauer, M.L.; et al. Enzyme-replacement therapy in life-threatening hypophosphatasia. *N. Engl. J. Med.* **2012**, *366*, 904–913. [[CrossRef](#)]
23. Adeli, K.; Higgins, V.; Trajcevski, K.; White-Al Habeeb, N. The Canadian laboratory initiative on pediatric reference intervals: A CALIPER white paper. *Crit. Rev. Clin. Lab. Sci.* **2017**, *54*, 358–413. [[CrossRef](#)] [[PubMed](#)]
24. Högler, W.; Langman, C.; da Silva, H.G.; Fang, S.; Linglart, A.; Ozono, K.; Petryk, A.; Rockman-Greenberg, C.; Seefried, L.; Kishnani, P.S. Diagnostic delay is common among patients with hypophosphatasia: Initial findings from a longitudinal, prospective, global registry. *BMC Musculoskelet. Disord.* **2019**, *20*, 80. [[CrossRef](#)] [[PubMed](#)]
25. Szabo, S.M.; Tomazou, I.C.; Petryk, A.; Powell, L.C.; Donato, B.M.K.; Zarate, Y.A.; Tiulpakov, A.; Martos-Moreno, G.A. Frequency and age at occurrence of clinical manifestations of disease in patients with hypophosphatasia: A systematic literature review. *Orphanet. J. Rare Dis.* **2019**, *14*, 85. [[CrossRef](#)]
26. 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*, 1160–1164. [[CrossRef](#)]
27. Greenberg, C.R.; Evans, J.A.; McKendry-Smith, S.; Redekopp, S.; Haworth, J.C.; Mulivor, R.; Chodirker, B.N. Infantile hypophosphatasia: Localization within chromosome region 1p36.1-34 and prenatal diagnosis using linked DNA markers. *Am. J. Hum. Genet.* **1990**, *46*, 286–292.
28. Genome Aggregation Database. Available online: <https://gnomad.broadinstitute.org/> (accessed on 20 September 2022).
29. Okawa, R.; Kokomoto, K.; Kitaoka, T.; Kubota, T.; Watanabe, A.; Taketani, T.; Michigami, T.; Ozono, K.; Nakano, K. Japanese nationwide survey of hypophosphatasia reveals prominent differences in genetic and dental findings between odonto and non-odonto types. *PLoS ONE* **2019**, *14*, e0222931. [[CrossRef](#)] [[PubMed](#)]
30. Michigami, T.; Tachikawa, K.; Yamazaki, M.; Kawai, M.; Kubota, T.; Ozono, K. Hypophosphatasia in Japan: ALPL mutation analysis in 98 unrelated patients. *Calcif. Tissue Int.* **2020**, *106*, 221–231. [[CrossRef](#)]
31. Taillandier, A.; Domingues, C.; Dufour, A.; Debiais, F.; Guggenbuhl, P.; Roux, C.; Cormier, C.; Cortet, B.; Porquet-Bordes, V.; Coury, F.; et al. Genetic analysis of adults heterozygous for ALPL mutations. *J. Bone Miner. Metab.* **2018**, *36*, 723–733. [[CrossRef](#)]
32. 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. [[CrossRef](#)]
33. Landrum, M.J.; Lee, J.M.; Benson, M.; Brown, G.R.; Chao, C.; Chitipiralla, S.; Gu, B.; Hart, J.; Hoffman, D.; Jang, W.; et al. ClinVar: Improving access to variant interpretations and supporting evidence. *Nucleic Acids Res.* **2018**, *46*, D1062–D1067. [[CrossRef](#)]

34. Brun-Heath, I.; Taillandier, A.; Serre, J.-L.; Mornet, E. Characterization of 11 novel mutations in the tissue non-specific alkaline phosphatase gene responsible for hypophosphatasia and genotype-phenotype correlations. *Mol. Genet. Metab.* **2005**, *84*, 273–277. [[CrossRef](#)] [[PubMed](#)]
35. Lia-Baldini, A.S.; Brun-Heath, I.; Carrion, C.; Simon-Bouy, B.; Serre, J.L.; Nunes, M.E.; Mornet, E. A new mechanism of dominance in hypophosphatasia: The mutated protein can disturb the cell localization of the wild-type protein. *Hum. Genet.* **2008**, *123*, 429–432. [[CrossRef](#)] [[PubMed](#)]
36. Schumann, G.; Klauke, R.; Canalias, F.; Bossert-Reuther, S.; Franck, P.F.H.; Gella, F.-J.; Jørgensen, P.J.; Kang, D.; Lessinger, J.-M.; Panteghini, M.; et al. IFCC primary reference procedures for the measurement of catalytic activity concentrations of enzymes at 37 °C. Part 9: Reference procedure for the measurement of catalytic concentration of alkaline phosphatase International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) Scientific Division, Committee on Reference Systems of Enzymes (C-RSE) (1). *Clin. Chem. Lab. Med.* **2011**, *49*, 1439–1446. [[CrossRef](#)] [[PubMed](#)]
37. van Belle, G.; Fisher, L.D.; Heagerty, P.J.; Lumley, T. *Biostatistics: A Methodology for the Health Sciences*; Fisher, L.D., van Belle, G., Eds.; John Wiley and Sons Inc.: Hoboken, NJ, USA, 2004.
38. 1000 Genomes Project Consortium; Auton, A.; Brooks, L.D.; Durbin, R.M.; Garrison, E.P.; Kang, H.M.; Korbel, J.O.; Marchini, J.L.; McCarthy, S.; McVean, G.A.; et al. A global reference for human genetic variation. *Nature* **2015**, *526*, 68–74. [[CrossRef](#)] [[PubMed](#)]
39. Lek, M.; Karczewski, K.J.; Minikel, E.V.; Samocha, K.E.; Banks, E.; Fennell, T.; O'Donnell-Luria, A.H.; Ware, J.S.; Hill, A.J.; Cummings, B.B.; et al. Analysis of protein-coding genetic variation in 60,706 humans. *Nature* **2016**, *536*, 285–291. [[CrossRef](#)] [[PubMed](#)]
40. Barbitoff, Y.A.; Skitchenko, R.K.; Poleshchuk, O.I.; Shikov, A.E.; Serebryakova, E.A.; Nasykhova, Y.A.; Polev, D.E.; Shuvalova, A.R.; Shcherbakova, I.V.; Fedyakov, M.A.; et al. Whole exome sequencing provides insights into monogenic disease prevalence in Northwest Russia. *Mol. Genet. Genom. Med.* **2019**, *7*, e964. [[CrossRef](#)]
41. Barbitoff, Y.A.; Khmelkova, D.N.; Pomerantseva, E.A.; Slepchenkov, A.V.; Zubashenko, N.A.; Mironova, I.V.; Kaimonov, V.S.; Polev, D.E.; Tsay, V.V.; Glotov, A.S.; et al. Expanding the Russian allele frequency reference via cross-laboratory data integration: Insights from 6096 exome samples. *MedRxiv* **2021**. [[CrossRef](#)]
42. Shikov, A.E.; Barbitoff, Y.A.; Glotov, A.S.; Danilova, M.M.; Tonyan, Z.N.; Nasykhova, Y.A.; Mikhailova, A.A.; Bespalova, O.N.; Kalinin, R.S.; Mirzorustamova, A.M.; et al. Analysis of the Spectrum of ACE2 Variation Suggests a Possible Influence of Rare and Common Variants on Susceptibility to COVID-19 and Severity of Outcome. *Front. Genet.* **2020**, *11*, 551220. [[CrossRef](#)]