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Investigation of *ALPL* variant states and clinical outcomes: An analysis of adults and adolescents with hypophosphatasia treated with asfotase alfa

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ABSTRACT

Background: Hypophosphatasia (HPP), a rare metabolic disease, can be inherited in an autosomal recessive (biallelic) or an autosomal dominant (monoallelic) manner. Most of the severe, early-onset, frequently lethal HPP in infants is acquired through recessive inheritance; less severe, later-onset, typically nonlethal HPP phenotypes are acquired through either dominant or recessive inheritance. HPP's variable clinical presentation arises from >400 identified *ALPL* pathogenic variants with likely variable penetrance, especially with autosomal dominant inheritance. This post hoc analysis investigated the relationship between *ALPL* variant state (biallelic and monoallelic) and clinical outcomes with asfotase alfa in HPP.

Methods: Data were pooled from two phase 2, randomized, open-label studies in adolescents and adults with HPP; one study evaluated the efficacy and safety of different doses of asfotase alfa ($n = 25$), and the other assessed the pharmacodynamics and safety of asfotase alfa ($n = 19$). Patients were grouped by *ALPL* variant state (biallelic or monoallelic). Available data from both studies included *ALPL* pathogenic variant state, Baseline characteristics, HPP-specific medical history, and Baseline TNSALP substrate levels (inorganic pyrophosphate [PPi] and pyridoxal 5′-phosphate [PLP]) concentrations). Clinical outcomes over 5 years of treatment were available from only the efficacy and safety study.

Results: In total, 44 patients with known variant status were included in the pooled analysis (biallelic, $n = 30$; monoallelic, $n = 14$). The most common pathogenic variant was c.571G > A (p.Glu191Lys) in biallelic patients (allele frequency: 19/60) and c.1133A > T (p.Asp378Val) in monoallelic patients (allele frequency: 7/28). Median (min, max) Baseline PPi concentrations were significantly higher in patients with a biallelic vs monoallelic variant state (5.3 [2.2, 12.1] vs 4.3 [3.5, 7.4] μM; $P = 0.0113$), as were Baseline PLP concentrations (221.4 [62.4, 1590.0] vs 75.1 [28.8, 577.0] ng/mL; $P = 0.0022$). HPP-specific medical history was generally similar between biallelic and monoallelic patients in terms of incidence and type of manifestations; notable exceptions included fractures, which were more common among monoallelic patients, and delayed walking and bone deformities such as abnormally shaped chest and head and bowing of arms or legs, which were more common among biallelic patients. Data from the efficacy and safety study ($n = 19$) showed that median PPi and PLP concentrations were normalized over 5 years of treatment in patients with both variant states. Median % predicted distance walked on the 6-Minute Walk Test remained within the normal range for monoallelic patients over 4 years of treatment, and improved from below normal (<84%) to normal in biallelic patients.

Conclusions: Although patients with biallelic variants had significantly higher Baseline PPi and PLP levels than monoallelic variants, both groups generally showed similar pretreatment Baseline clinical characteristics. Treatment with asfotase alfa for up to 5 years normalized TNSALP substrate concentrations and improved functional outcomes, with no clear differences between biallelic and monoallelic variant states. This study suggests that patients with HPP have significant disease burden, regardless of *ALPL* variant state.

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1. Introduction

Hypophosphatasia (HPP) is a rare, inherited, metabolic disease characterized by low tissue-nonspecific alkaline phosphatase (TNSALP) activity caused by pathogenic variants in the *ALPL* gene [1]. Low TNSALP activity results in extracellular accumulation of the TNSALP substrates inorganic pyrophosphate (PPi) and pyridoxal 5′-phosphate (PLP). Impaired bone mineralization due to PPi accumulation can lead to rickets, osteomalacia, and fractures [2,3]. The accumulation of PLP, the major circulating form of vitamin B6 and a necessary cofactor for neurotransmitter synthesis in the brain, leads to a heightened risk of pyridoxine-responsive seizures in infants and potentially other reported neurologic symptoms in adults [4,5].

More than 400 *ALPL* pathogenic variants have been identified [6] and considerable clinical variability has been observed, with manifestations that can present at any time from in utero and infancy through adulthood [7]. Certain manifestations may be more common or frequently reported in patients depending on their age or age of HPP onset [8]. Children and adolescents with HPP may present with bone deformities, rickets, fractures, motor development delays, and/or premature tooth loss [3,7,8]. Adults with HPP commonly present with muscle weakness/fatigue, musculoskeletal pain, recurrent or poorly healing fractures/pseudofractures, rheumatologic manifestations, mobility difficulties, and/or dental complications [3,7–10]. Initial findings from the Global HPP Registry indicate that adults with HPP have a substantial burden of illness that is associated with reduced patient-reported health-related quality of life [11] and frequently experience substantial delays in diagnosis because of the heterogeneous presentation of clinical signs and symptoms of their disease [12].

HPP can be inherited in an autosomal recessive or autosomal dominant manner. In autosomal recessive disease, either 2 different pathogenic variants (biallelic variants) are present in *trans* (compound heterozygous) or the same pathogenic variant is present on both alleles (homozygous). In autosomal dominant disease, only one pathogenic variant (monoallelic variant) is present. Autosomal recessive inheritance of *ALPL* variants accounts for most of the severe, early-onset, frequently lethal HPP observed more commonly in perinatal and infantile forms of the disease, whereas the less severe, later-onset, typically non-lethal phenotypes have been associated with either dominant or recessive inheritance [13–16]. However, these general trends have no known predictive value [17]. Among patients who inherit *ALPL* variants in a dominant manner, penetrance can vary between variants as well as between patients with the same variant, even within the same family, further contributing to the clinical variability of HPP [18].

Asfotase alfa (Strensiq®, Alexion Pharmaceuticals, Inc., Boston, MA) is the human recombinant TNSALP enzyme replacement therapy approved for the treatment of HPP [19]. In a phase 2 randomized controlled study that evaluated the safety and efficacy of asfotase alfa in adolescents and adults with HPP, plasma concentrations of PPi and PLP normalized and physical function improved over 5 years of treatment [20]. In a separate phase 2a, randomized, open-label study, the pharmacodynamic effectiveness of the approved dose of asfotase alfa (6 mg/kg/week) was confirmed in adults ≥18 years of age with pediatric-onset HPP [21]. To better understand whether currently reported genotype/phenotype relationships in perinatal and infant patients with severe, early-onset forms of HPP extend to adolescents and adults, the current pooled post hoc analysis assessed the relationship between the *ALPL* variant state and the clinical manifestations of HPP and asfotase alfa treatment outcomes.

2. Methods

2.1. Studies included and patient population

Data for this post hoc analysis were pooled from two phase 2, randomized, open-label studies in adolescents and adults with HPP; detailed study methodology and results for both studies have been previously published [20,21]. In brief, the first study (ClinicalTrials.gov: NCT01163149; EudraCT: 2010–019850-42; Study 009; $n = 19$) evaluated the efficacy and safety of different doses of asfotase alfa in patients 13 to 66 years of age with HPP onset at any age [20]. Diagnosis of HPP was based on low age-adjusted serum alkaline phosphatase (ALP) activity, high plasma PLP concentration (≥2 times the upper limit of normal with no vitamin B6 administered ≥1 week before specimen collection), osteopenia on bone radiographs, and osteomalacia documented by bone biopsy with a mineralization lag time *Z*-score of +2 or more. The study consisted of a 6-month primary treatment period followed by an open-label extension phase. In the primary treatment period, patients were randomized to receive asfotase alfa at 0.3 or 0.5 mg/kg/day or no treatment. During the extension phase, all patients received asfotase alfa at 0.5 mg/kg/day for 6 to 12 months and 1 mg/kg/day for 6 days/week. thereafter. The second study included in the current analysis (ClinicalTrials.gov: NCT02797821; EudraCT: 2015–003131-35; Study 208; $n = 25$) assessed the pharmacodynamics and safety of asfotase alfa in patients ≥18 years of age with pediatric-onset HPP (first signs or symptoms of HPP before 18 years of age) [21]. Patients with a documented history of HPP-related skeletal abnormalities also had to have: (1) documented *ALPL* pathogenic variant(s) and/or (2) serum concentrations of ALP below the age-adjusted reference range in addition to plasma PLP concentrations above the upper limit of normal at Screening or a historically elevated circulating PLP result. Eligible patients were randomized 1:1:1 to receive a single dose of asfotase alfa (0.5, 2.0, or 3.0 mg/kg) during Week 1, then 3 times per week (equivalent to 1.5, 6.0, or 9.0 mg/kg/week, respectively) from Weeks 3 to 9.

Both studies complied with the World Medical Association Declaration of Helsinki and the International Conference on Harmonisation E6 Guideline for Good Clinical Practice. Approval was obtained from the institutional review board or research ethics board at each investigative site. All patients or their legal guardians provided informed consent or assent.

Genetic analysis data for patients were captured from medical records at the study site by the investigator. If results were not available in the medical records, blood samples for *ALPL* variant analysis were collected at the Baseline visit and analyzed at Connective Tissue Gene Tests (Allentown, PA, USA) for the efficacy and safety study and during Screening or the Run-in period and analyzed at Centogene AG (Rostock, Germany) for the pharmacodynamic study. There were no limitations on the type of technique used.

2.2. Assessments

Data available from both studies that were pooled for analysis include *ALPL* pathogenic variants, Baseline patient demographics and characteristics, HPP-specific medical history, and pretreatment Baseline plasma concentrations of TNSALP substrates (PPi and PLP).

Clinical outcomes over 5 years of treatment with asfotase alfa were collected only in the efficacy and safety study ($n = 19$). These included plasma concentrations of TNSALP substrates (PPi and PLP), which were measured during asfotase alfa treatment at prespecified visits (Weeks 6, 12, 24, 30 [control group only], 36, and 48 and then every 6 months thereafter). Change in walking ability was assessed with the 6-Minute Walk Test (6MWT) performed in accordance with American Thoracic Society guidelines [22]. The % predicted value for the 6MWT was defined as % of normal predicted distance walked based on age, sex, and height and was calculated if the patient walked the full 6 min and was ≤65 years of age [23,24]. The normal range for healthy age-, sex-, and height-matched peers is 84% to 112% [25]. Patient-reported functional disability was assessed with the Lower Extremity Functional Scale (LEFS), which is scored from 0 to 80, with a higher score denoting better lower extremity functioning [26]. Patient-reported pain was assessed using a modified version of the Brief Pain Inventory-Short Form

(BPI-SF), a validated tool scored from 0 to 40 based on 4 pain severity items, with lower scores indicating less pain [27,28].

All data are summarized by variant state as either biallelic (2 pathogenic *ALPL* variants) or monoallelic (1 pathogenic *ALPL* variant).

2.3. Statistical analyses

Baseline values represent the last assessment before the first dose of asfotase alfa. Data were summarized with descriptive statistics. The biallelic and monoallelic variant state groups for Baseline PPi and PLP were compared using a Wilcoxon test.

3. Results

3.1. ALPL pathogenic variants and baseline characteristics (pooled data; N = 44)

In total, 44 adolescents and adults were included in this pooled analysis; of these, 30 had a biallelic variant state and 14 had a monoallelic variant state. Baseline patient characteristics and *ALPL* variant state are summarized in Table 1. The median (min, max) age of onset of HPP signs and symptoms was 1 (0, 4) years in biallelic patients and 4 (0, 36) years in monoallelic patients. Median Baseline ALP activity was similar between the two groups.

The most common pathogenic variant in biallelic patients was c.571G > A (p.Glu191Lys), present in 19 patients (allele frequency: 19/60). Only 1 patient in the biallelic group had a homozygous variant state (c.530C > T). The most common pathogenic variant in monoallelic patients was c.1133A > T (p.Asp378Val), present in 7 patients (allele frequency: 7/28). Fig. 1 shows the location of the variants within a ribbon structure of the TNSALP dimer homology model [29] and indicates whether those variants were found to produce a dominant negative effect (DNE; defined as mutation/wild-type activity <40% relative to wild-type [29]), had low enzymatic activity (defined as ≤25% relative to wild-type [29]) but did not have a DNE, or had neither a DNE nor low enzymatic activity. Variants were dispersed throughout the protein, with the majority of those with DNE in the active site or crown domain. All of the variants in patients with a monoallelic state had low in vitro activity (2.15%–7.76%) and, with the exception of 2 variants (c.1001G > A and c.1034C > T), were located in the active site or crown domain. Variants in patients with a biallelic state showed greater heterogeneity. The in vitro activity and DNE of the variants are summarized in **Supplemental Table 1**.

3.2. HPP-specific medical history (pooled data; N = 44)

HPP-specific medical history was generally similar in terms of incidence and type of manifestations between those with biallelic or monoallelic variant states (Table 2). A large percentage of patients in both variant states experienced severe bone pain, unusual gait, and fractures (nonhealing fractures, pseudofractures, and vertebral fractures). Manifestations, such as abnormally shaped chest or head, bowing of the arms or legs, and delayed walking, were more common among patients with a biallelic variant state. Patients with a monoallelic variant state had a higher number of fractures (median: 10) than those with a biallelic variant state (median: 6), although comparisons were limited by the small sample size of patients with available data.

3.3. TNSALP substrate concentrations (pooled data; N = 44)

Overall, median (min, max) Baseline PPi concentrations were significantly higher in the biallelic variant state group than the monoallelic variant state group (5.3 [2.2, 12.1] vs 4.3 [3.5, 7.4] μM; $P = 0.0113$), as

Table 1
Summary of *ALPL* pathogenic variants and baseline characteristics ($N = 44$).

Patient	Base Change(s)		Amino Acid Change(s) or Functional Prediction		Variant Type	Sex	Age at Enrollment, (y)	Age at First HPP Sign/ Symptom	ALP Activity (U/L)	PPi (μM)[a]	PLP (ng/mL)[b]
Biallelic variant state (N=30)											
Overall Median (min, max)						Female: 57%	44.5 (13, 77)	1 (0, 4)[c]	18 (18, 45)	5.3 (2.2, 12.1)	221.4 (62.4, 1590)
1	c.550C>T	c.571G>A	p.Arg184Trp	p.Glu191Lys	Missense	F	56	1 mo	<18	2.2	1270
2	c.526G>A	c.1015G>A	p.Ala176Thr	p.Gly339Arg	Missense	M	13	2 mo	37	7.5	906
3	c.526G>A	c.881A>C	p.Ala176Thr	p.Asp294Ala	Missense	F	14	2 mo	<18	5.1	673
4	c.151G>T	c.400_401 AC>CA	p.Ala51Ser	p.Thr134His	Missense	M	15	0	<18	5.2	1590
5	c.526G>A	c.984_986del CTT	p.Ala176Thr	p.Phe327del	Missense/ deletion	M	37	0.1 y	18	6.0	1059.6
6	c.571G>A	c.1001G>A	p.Glu191Lys	p.Gly334Asp	Missense	M	64	4 y	<18	8.2	171
7	c.571G>A	c.1001G>A	p.Glu191Lys	p.Gly334Asp	Missense	M	26	9 mo	<18	6.6	199
8	c.571G>A	c.1001G>A	p.Glu191Lys	p.Gly334Asp	Missense	F	16	6 mo	<18	4.3	141
9	c.550C>T	c.571G>A	p.Arg184Trp	p.Glu191Lys	Missense	F	55	2 y	<18	6.3	370
10	c.571G>A	c.1132G>C	p.Glu191Lys	p.Asp378His	Missense	M	16	6 mo	21	4.2	275
11	c.526G>A	IVS6 c.648+1G>A	p.Ala176Thr	N/A	Missense/ splice	F	45	2 y	<18	6.9	508
12	c.571G>A	c.1250A>G	p.Glu191Lys	p.Asn417Ser	Missense	F	54	3 y	26	5.2	196
13	c.526G>A	IVS6 c.648+1G>A	p.Ala176Thr	N/A	Missense/splice	F	58	4 y	21	5.9	474

Patient	Base Change(s)		Amino Acid Change(s) or Functional Prediction		Variant Type	Sex	Age at Enrollment, (y)	Age at First HPP Sign/ Symptom	ALP Activity (U/L)	PPi (μM)[a]	PLP (ng/mL)[b]
30	c.1171delC	c.571G>A	p.Arg391Valfs	p.Glu191Lys	Frameshift/ missense	F	55	N/A	18	4.4	62.4
Monoallelic variant state, N=14											
Overall Median (min, max)						Female: 71%	49 (24, 69)	4 (0, 36) y	19 (18, 80)	4.3 (3.5, 7.4)	75.1 (28.8, 577)
31	c.551G>A		p.Arg184Gln		Missense	M	57	2 y	35	7.4	28.8
32	c.1001G>A		p.Gly334Asp		Missense	F	44	36 y	19	4.1	34.5
33	c.1133A>T[e]		p.Asp378Val		Missense	F	53	3 y	<18	3.8	90.3
34	c.1328C>T		p.Ala443Val		Missense	F	66	13 y	<18	4.6	577
35	c.1133A>T[e]		p.Asp378Val		Missense	F	57	1.1 y	27	5.5	160
36	c.1133A>T[e]		p.Asp378Val		Missense	F	30	0	20	3.5	35.4
37	c.1133A>T[e]		p.Asp378Val		Missense	F	69	14 y	18	5.7	152
38	c.1133A>T[e]		p.Asp378Val		Missense	M	45	4 y	19	6.4	521.8
39	c.1250A>G		p.Asn417Ser		Missense	M	24	4 y	23	3.8	44.2
40	c.1250A>G		p.Asn417Ser		Missense	F	67	12 y	80	3.9	41.1
41	c.1133A>T[e]		p.Asp378Val		Missense	F	27	0.8 y	18	4.3	515.2
42	c.1034C>T		p.Ala345Val		Missense	F	29	8 y	19	4.6	59.8
43	c.1171C>T		p.Arg391Cys		Missense	F	61	7 y	29	3.6	28.8
44	c.1133A>T[e]		p.Asp378Val		Missense	M	29	3 y	18	4.2	93.4

Patient	Base Change(s)		Amino Acid Change(s) or Functional Prediction		Variant Type	Sex	Age at Enrollment, (y)	Age at First HPP Sign/ Symptom	ALP Activity (U/L)	PPi (μM)[a]	PLP (ng/mL)[b]
30	c.1171delC	c.571G>A	p.Arg391Valfs	p.Glu191Lys	Frameshift/ missense	F	55	N/A	18	4.4	62.4
Monoallelic variant state, N=14											
Overall Median (min, max)						Female: 71%	49 (24, 69)	4 (0, 36) y	19 (18, 80)	4.3 (3.5, 7.4)	75.1 (28.8, 577)
31	c.551G>A		p.Arg184Gln		Missense	M	57	2 y	35	7.4	28.8
32	c.1001G>A		p.Gly334Asp		Missense	F	44	36 y	19	4.1	34.5
33	c.1133A>T[e]		p.Asp378Val		Missense	F	53	3 y	<18	3.8	90.3
34	c.1328C>T		p.Ala443Val		Missense	F	66	13 y	<18	4.6	577
35	c.1133A>T[e]		p.Asp378Val		Missense	F	57	1.1 y	27	5.5	160
36	c.1133A>T[e]		p.Asp378Val		Missense	F	30	0	20	3.5	35.4
37	c.1133A>T[e]		p.Asp378Val		Missense	F	69	14 y	18	5.7	152
38	c.1133A>T[e]		p.Asp378Val		Missense	M	45	4 y	19	6.4	521.8
39	c.1250A>G		p.Asn417Ser		Missense	M	24	4 y	23	3.8	44.2
40	c.1250A>G		p.Asn417Ser		Missense	F	67	12 y	80	3.9	41.1
41	c.1133A>T[e]		p.Asp378Val		Missense	F	27	0.8 y	18	4.3	515.2
42	c.1034C>T		p.Ala345Val		Missense	F	29	8 y	19	4.6	59.8
43	c.1171C>T		p.Arg391Cys		Missense	F	61	7 y	29	3.6	28.8
44	c.1133A>T[e]		p.Asp378Val		Missense	M	29	3 y	18	4.2	93.4

Individual base changes occurring in >1 patients are each shaded with a similar color.
[a]PPi reference range: age 13–18 y: <0.8–4.8 μM; age > 18 y: 1.0–5.8 μM.
[b]PLP reference range: 5–18 y: 5.7–61.2 ng/mL; >18 y: 2.8–26.7 ng/mL.
[c]As the age of first HPP sign/symptom was not available for 11 patients in the biallelic group, and the overall median (min, max) was calculated from 19 patients with available data; however, all patients had confirmed pediatric-onset HPP.
[d]Variant not reported as of March 22, 2020 [6].
[e]This is a known pathogenic variant with null residual activity that shows a dominant negative effect.
ALP = alkaline phosphatase; N/A = not available; PLP = pyridoxal 5′-phosphate; PPi = inorganic pyrophosphate.

were Baseline PLP concentrations (221.4 [62.4, 1590.0] vs 75.1 [28.8, 577.0] ng/mL, respectively; $P = 0.0022$). Individual Baseline plasma PPi and PLP concentrations are shown in Fig. 2. The highest concentrations were generally observed in patients with a biallelic state, and the lowest concentrations were observed in patients with monoallelic variant state.

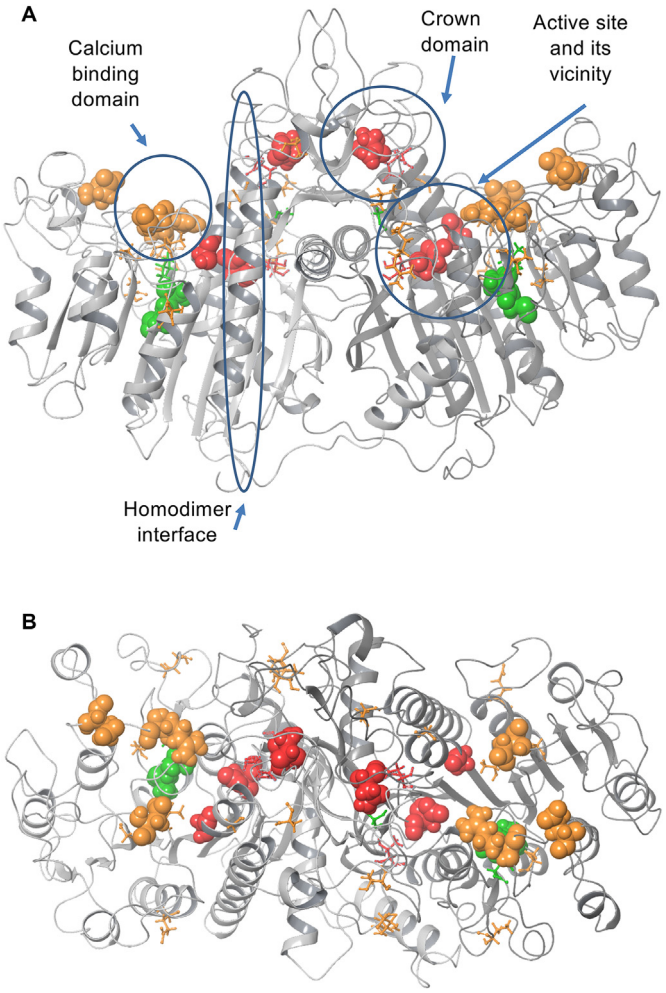

Fig. 1. Location of *ALPL* variants within ribbon structure of a TNSALP homology model: (**A**) front symmetrical view and (**B**) top view. Colors: Red represents variants found to cause dominant negative effect (mutation/wild-type activity <40% relative to wild-type), orange represents variants with low enzymatic activity (defined as activity ≤25% relative to wild-type) but not dominant negative effect, and green represents variants with neither dominant negative effect nor low enzymatic activity. Atom sizes: Solid spheres represent residues where variants appear more than once and thin tubes where variants appear only once. Mirror residues on the opposite chain are not marked with arrows/circles. TNSALP = tissue-nonspecific alkaline phosphatase.

3.4. Clinical outcomes (collected from efficacy and safety study only; n = 19)

Regardless of variant state, data collected from the efficacy and safety study indicate that median PPi concentrations generally decreased to levels within the normal reference range within 6 months of treatment with asfotase alfa and were maintained within the normal range through 5 years (Fig. 3A). PLP concentrations followed a similar trend, with the exception of those in biallelic patients, for whom concentrations also decreased from Baseline but remained higher than normal up to 1 year after treatment, after which levels normalized (Fig. 3B).

Treatment with asfotase alfa over 5 years improved % predicted distance walked (6MWT) from below normal (<84%) to within the normal range for patients with a biallelic variant state (Fig. 4). The % predicted distance walked for patients with a monoallelic variant state was within the normal range at Baseline and was maintained within the normal range through Year 4 (Fig. 4). One patient with a monoallelic variant state had a total knee replacement before the Year 5 visit, which affected the 6MWT distance walked.

Table 2
HPP-specific bone- and developmental-related medical history by *ALPL* variant state (pooled data; N = 44).

Variable	Biallelic variant state (N = 30)	Monoallelic variant state (N = 14)
Number of fractures, median (min, max)	6 (1,30)[a]	10 (5, 14)[b]
Bone, n (%)		
Abnormally shaped chest	6 (20.0)	0 (0)
Abnormally shaped head	7 (23.3)	1 (7.1)
Bone pain severe enough to limit activity	29 (96.7)	11 (78.6)
Bone pain severe enough to require pain medication	26 (86.7)	11 (78.6)
Bowing of arms or legs	17 (56.7)	1 (7.1)
Club foot deformity	2 (6.7)	0 (0)
Fractures that won't heal	12 (40.0)	8 (57.1)
Knock knees	7 (23.3)	3 (21.4)
Pseudofractures	17 (56.7)	6 (42.9)
Unusual gait or way of walking/running	26 (86.7)	10 (71.4)
Vertebral fracture	13 (43.3)	8 (57.1)
Developmental, n (%)		
Delayed talking	2 (6.7)	1 (7.1)
Delayed walking	6 (20.0)	0 (0)
Difficulty gaining weight	8 (26.7)	2 (14.3)

HPP = hypophosphatasia.
[a] n = 13.
[b] n = 5.

Patient-reported functional disability, as measured with the LEFS, improved after 5 years of treatment with asfotase alfa in biallelic (median [min, max] total score: Baseline: 35 [17, 78]; Year 5: 49 [16, 80]) and monoallelic (Baseline: 29 [18, 71]; Year 5: 58 [25, 69]) patients. However, responses in both groups showed high variability, with large ranges for change from Baseline at each timepoint. Pain scores on the BPI-SF also improved in patients with both a biallelic and monoallelic variant state after 5 years of treatment with asfotase alfa. The median (min, max) change from Baseline in total score was −3.0 (−20, 5) for biallelic patients and −4.0 (−7, 4) for monoallelic patients.

4. Discussion

As shown in our pooled analysis of two phase 2, randomized, open-label studies in adolescents and adults with HPP, *ALPL* variant state (biallelic or monoallelic) generally does not appear to impact the burden of HPP disease. Although pretreatment Baseline PPi and PLP concentrations were higher in biallelic patients, concentrations of both substrates decreased to levels within the normal reference range following treatment with asfotase alfa in both variant groups. Further, variant state did not appear to influence treatment outcomes with asfotase alfa in the long-term efficacy and safety study.

To date, more than 400 *ALPL* pathogenic variants have been identified [6], resulting in the highly variable clinical expression of HPP. As observed in our analysis, the majority of *ALPL* pathogenic variants are missense mutations, leading to variable residual TNSALP activity and presumably variable ability to metabolize substrates, potentially contributing to disease heterogeneity [13]. Site-directed mutagenesis and computer-aided modeling studies have been used to characterize individual pathogenic variants and assess for potential genotype-phenotype relationships, and have shown that pathogenic variants associated with severe disease affect residues localized to areas crucial for protein function (active site, active site vicinity, and homodimer interface) [18,30].

The most severe forms of the disease (perinatal and infantile) are more likely to be inherited as an autosomal recessive trait and other forms are associated with autosomal recessive or autosomal dominant inheritance [14,31,32]. For instance, in adult patients with HPP, both autosomal dominant and autosomal recessive inheritance have been

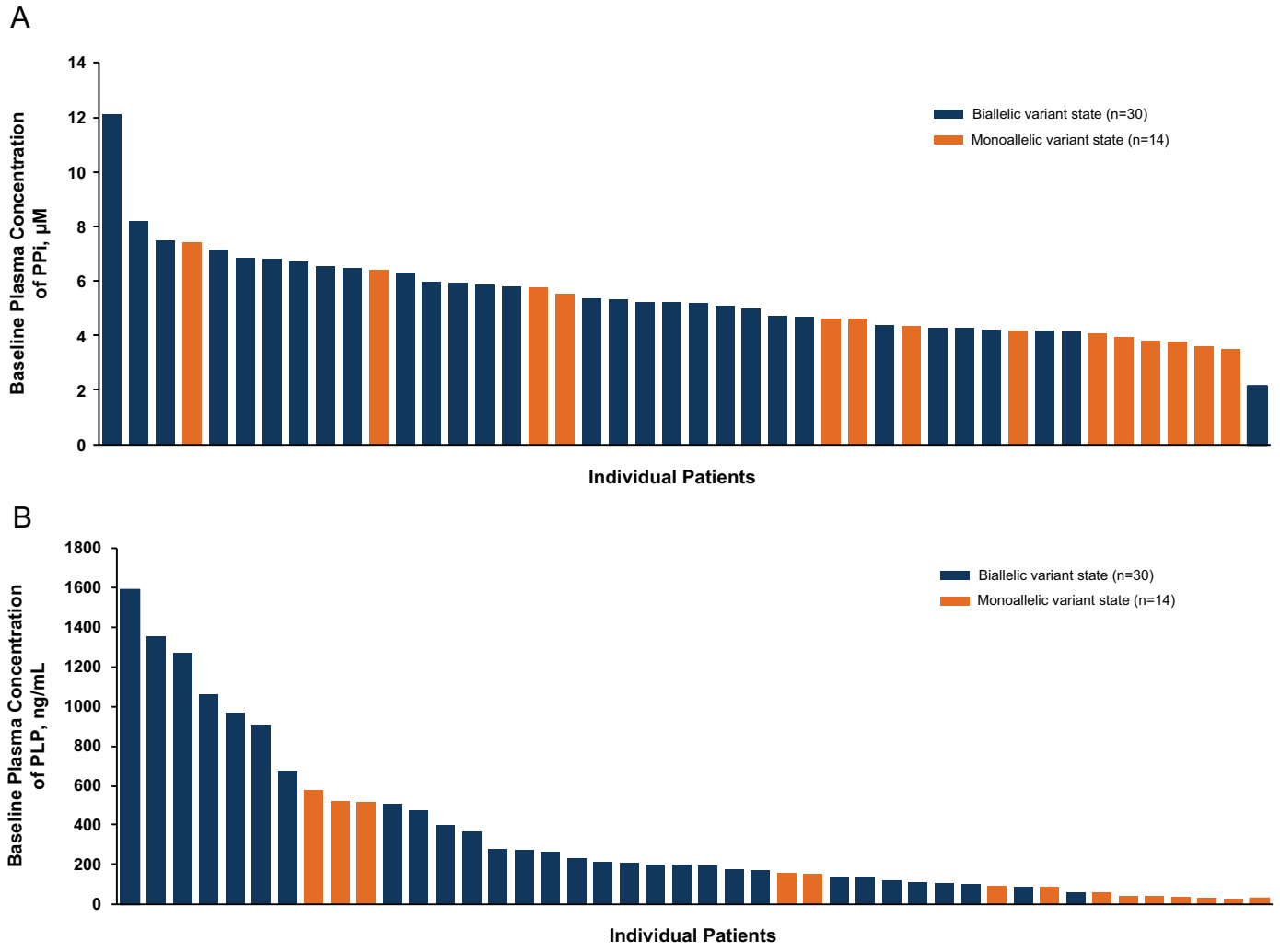

Fig. 2. Individual patient plasma concentrations of PPi (**A**) and PLP (**B**) at Baseline by *ALPL* variant state. PLP = pyridoxal 5′-phosphate; PPi = inorganic pyrophosphate.

reported, with variable disease burden between variant states [33]. Additionally, studies in patients with less severe HPP have suggested that this phenotype results largely from heterozygous mutations with a DNE, in which the mutant protein inhibits the activity of the wild-type enzyme [13,34,35]. However, the DNE has been shown to be variable, which can also lead to significant clinical variability [36]. Further, >50% of heterozygous adult patients with what was deemed to be mild HPP had *ALPL* variants that did not exert a DNE, suggesting the possibility of other mechanisms of disease, such as modifier genes [37]. Mornet et al. recently reported haploinsufficiency as a new mechanism of dominance in HPP, defined as variants that do not have DNE but still have a dominant effect in patients with autosomal dominant inheritance [16]. In our analysis, 3 variants (c.1171C > T, c.551G > A, and c.1328C > T) in patients with a monoallelic variant state met the criteria for haploinsufficiency (i.e., low enzymatic activity of 2.26%–7.76%, but no DNE). Collectively, these data, along with the results of our analysis, suggest that the clinical presentation and disease burden for all patients with HPP should be closely monitored, regardless of the pathogenic variant(s).

The most prevalent pathogenic variant in our patients was c.571G > A (p.Glu191Lys), present in 19 biallelic patients. In our analysis, this variant was not reported in any patient with a monoallelic variant state. This was not unexpected, as this variant is known to be present frequently in heterozygous state in patients with biallelic disease [16,34,38] and has previously been shown to have increased prevalence in Caucasian populations [16,39,40]. Based on site-directed mutagenesis studies, this variant produces significant residual TNSALP activity [30]. The pathogenic variant c.1001G > A (p.Gly334Asp) was present in both biallelic patients ($n = 5$) and a monoallelic patient ($n = 1$). This variant has been reported to have low residual TNSALP activity and have a DNE [41]. The pathogenic variant c.1250A > G (p.Asn417Ser) was also present in both biallelic ($n = 2$) and monoallelic (n = 2) patients. This variant, first reported by Sergi et al. in 2001 [42], has been shown to have almost negligible residual TNSALP activity (~3% of wild-type) and a strong DNE (27%–32% of residual activity when a mutant/wild-type mixture was cotransfected at a 50:50 ratio) [29,34]. The pathogenic variant c.1133A > T (p.Asp378Val), first reported by Henthorn et al. in 1992 [43], was present in 7 monoallelic patients. This variant has also been reported to have almost negligible residual TNSALP activity (1%–2% of normal) and a strong DNE (20%–28% of residual activity when a mutant/wild-type mixture was cotransfected at a 50:50 ratio) [29,44]. In our analysis, this variant was not reported in patients with a biallelic variant state.

Adults and adolescents with HPP experience a substantial burden of illness, regardless of inheritance pattern or variant state (biallelic or monoallelic). The skeletal manifestations of HPP were generally similar between patients with a biallelic or monoallelic variant state with the exception of fractures, which were more common among monoallelic patients, and abnormally shaped chest and head, bowing of the arms or legs, and delayed walking, which were more common in biallelic

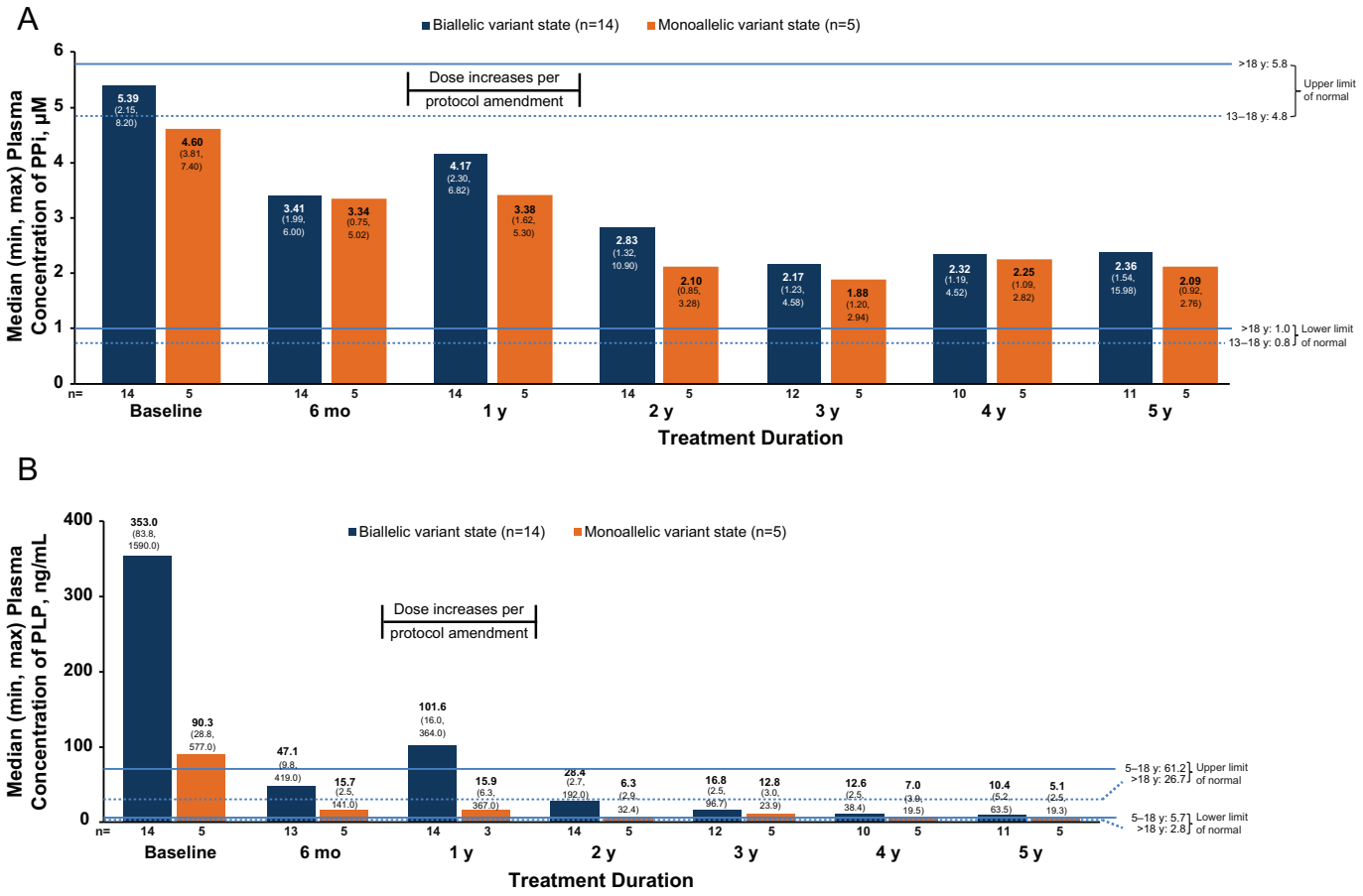

Fig. 3. Plasma concentrations of PPi (**A**) and PLP (**B**) over 5 years of treatment with asfotase alfa by *ALPL* variant state (efficacy and safety study only). HPP = hypophosphatasia; PLP = pyridoxal 5′-phosphate; PPi = inorganic pyrophosphate.

patients. The differences observed in the biallelic patients may be driven by the younger age at presentation of HPP (1 year for biallelic patients and 4 years for monoallelic patients) rather than *ALPL* variant state. Additionally, the number of fractures is not a perfect measure of disease severity, as it is likely that the mechanism of injury and type of fracture are different (i.e., vertebral, nonvertebral, or nonhealing fractures or pseudofractures).

Further, long-term data from the efficacy and safety study ($n = 19$) showed that treatment with asfotase alfa for up to 5 years normalized TNSALP substrate levels and improved functional outcomes, with no

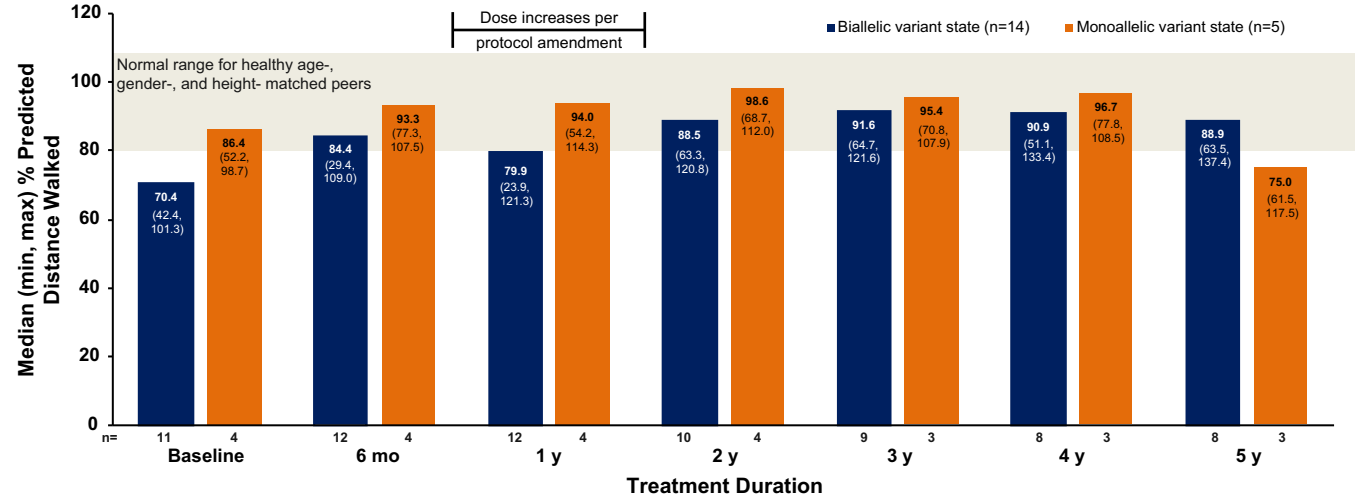

Fig. 4. Percent predicted distance walked on the 6MWT by *ALPL* variant state (efficacy and safety study only). Percent predicted value, defined as percentage of normal predicted distance walked based on age, sex, and height, was calculated in patients aged ≤65 years who walked the full 6 minutes. Three patients with biallelic variant state were unable to walk 6 minutes at Baseline due to physical/cognitive limitations, and one patient with a monoallelic variant state was older (66 years old) than the cutoff for the calculation (65 years). One patient with a monoallelic variant state had a total knee replacement before Year 5 visit that affected 6MWT distance (% predicted distance walked: 61.5%). 6MWT=6-Minute Walk Test; HPP= hypophosphatasia.

clear differences between variant states. Overall, our findings confirm the high clinical burden of disease experienced by patients with HPP [10,45,46] and further show that this burden or response to treatment with asfotase alfa is not influenced by variant state.

A key limitation of the current analysis is that it was conducted post hoc, which may lead to a bias in interpretation. The small sample size of the study and patient underrepresentation in variant states limited the interpretation of biochemical and functional outcomes, particularly because in patients with HPP a large number of variant types are known. Although data on clinical outcomes with asfotase alfa treatment were not available, the *ALPL* variant and baseline characteristics information available for the additional 25 adults from the pharmacodynamics study of asfotase alfa support the observation that HPP creates a substantial disease burden necessitating treatment in adults with all *ALPL* variant states. A larger population will be required to understand genotype-phenotype correlation in a more meaningful manner. Further, given the geographic location of the study sites, the genetic diversity was extremely limited with one of the more frequently seen variants (c.571G > A) being more common in the Caucasian population and the other more commonly seen variant (c.1001G > A) being most common in the Mennonite population. Additionally, there were limitations on the type of genetic analysis technique used and the timeframe in which the genetic analysis had been conducted. Thus, it may be possible that a patient has a sequence variant in a region of the gene (e.g., an intron or regulatory region) not covered by the laboratory's test or that a patient may have a sequence variant that cannot be detected by the sequence analysis (e.g., a large deletion). Lastly, the variant state (biallelic or monoallelic) is only one aspect of genotype-phenotype correlation. Our analysis does not assess for the influence of genetic polymorphisms, other modifier genes, or epigenetic or environmental factors on HPP phenotype [13,32,34,37], which was outside the scope of our analysis.

In summary, this analysis suggests that *ALPL* pathogenic variant state (biallelic or monoallelic) does not impact burden of disease or asfotase alfa treatment outcomes in adults and adolescents with HPP.

Declaration of Competing Interest

PSK is a clinical study investigator and has received consulting fees and travel support from Alexion Pharmaceuticals, Inc., for consulting and participation on advisory boards. **GdA** is an employee of Alexion Pharmaceuticals, Inc., the study sponsor, and may own stock options in the company. **SZ** is an employee of Covance, Inc., which is under contract to Alexion Pharmaceuticals, Inc., and provided statistical services for this analysis. **ETR** has received consulting fees from Alexion Pharmaceuticals, Inc., for consulting, speaking, and participation on advisory boards.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ymgme.2021.03.011.

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