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Research Article

Hypophosphatasia: low penetrance of pathogenic and likely-pathogenic *ALPL* variants identified through an unselected biorepository

Kathryn M. Dahir[1,*], Jennifer E. Below[2], Jinyuan Liu[3], Amir Javid[3], Guancho Wang[3], Lisa Bastarache[4]

[1]Program for Metabolic Bone Disorders, Division of Endocrinology, Vanderbilt University Medical Center, Nashville, TN 37232, United States
[2]Division of Genetic Medicine, Vanderbilt Genetics Institute, Vanderbilt University Medical Center, Nashville, TN 37232, United States
[3]Department of Biostatistics, Vanderbilt University Medical Center, Nashville, TN 37232, United States
[4]Department of Biomedical Informatics, Vanderbilt University Medical Center, Nashville, TN 37232, United States

*Corresponding author: Kathryn M. Dahir, Program for Metabolic Bone Disorders at Vanderbilt, Division of Endocrinology and Diabetes, Vanderbilt University Medical Center, 8210 Medical Center East, 1215 21st Avenue South, Nashville, TN 37232, United States (kathryn.dahir@vumc.org).

Abstract

Hypophosphatasia (HPP) is a heritable multisystem disorder caused by pathogenic variants in the tissue nonspecific alkaline phosphatase (ALP)-coding gene *ALPL*. The genotype–phenotype correlation in heterozygous adults with HPP remains incompletely understood. In this genotype-based study, we aimed to measure the prevalence of pathogenic or likely-pathogenic *ALPL* variants and to test the hypothesis that HPP penetrance is low in adult carriers. A total of 37 147 genomes from unselected individuals visiting a tertiary care, academic medical center were investigated. Variants classified as pathogenic or likely-pathogenic were observed with a prevalence of 0.3% ($n = 109$) or 1/341. Variant c.571G>A was most frequent (67.9%). A subset of 70 individuals had linked electronic health records (EHRs) and were termed *ALPL*+. All 70 *ALPL*+ individuals showed mild, mainly neurological, symptoms often reported in adults with HPP. However, low serum ALP, a hallmark of HPP, was found in only 65.7% (38/70) of *ALPL*+ individuals, and 12.9% (9/70) met the diagnostic criteria for HPP based on consensus guidelines, thus complete penetrance was low. Compared to controls lacking pathogenic or likely-pathogenic variants (*ALPL*−), the *ALPL*+ individuals had a higher probability of progression for mobility issues (median age 73 yr *ALPL*+ vs 82 yr *ALPL*−, $p = .03$), as well as a similar probability of progression for fatigue, arthritis, or dental problems. Unexpectedly, 3.4% (5/148) of individuals in the *ALPL*- group met the diagnostic criteria for HPP, possibly due to unidentified variants or non-*ALPL* genetic factors. Overall, the data support our hypothesis and aids the management of carriers of pathogenic *ALPL* variants.

Keywords: diseases and disorders related to bone, hypophosphatasia, metabolic bone disease, fractures, mobility impairment

Lay Summary

The disease hypophosphatasia (HPP) causes bone and muscle issues as well as pain. Hypophosphatasia results from disease causing variants, that is, DNA mutations, and is believed to be rare. This study showed that more people than previously estimated carry variants that can cause HPP. However, it also showed that far fewer carriers than expected (only about 1 in 10 persons) will develop symptoms consistent with HPP.

Introduction

Hypophosphatasia (HPP) was recognized as a distinct clinical disorder in 1948 by Dr. John Campbell Rathbun in an infant suffering from pronounced skeletal hypomineralization, seizures, respiratory failure, and paradoxically low levels of the enzyme alkaline phosphatase (ALP) in tissues and serum.[1] Since then, it has become evident that low ALP activity is the biochemical hallmark of HPP.[2] The low ALP results from pathogenic variants in the gene *ALPL*, which encodes for the tissue nonspecific isoenzyme of ALP.[3] Currently, more than 480 pathogenic or likely-pathogenic *ALPL* variants have been catalogued (https://alplmutationdatabase.jku.at/, accessed August 10, 2025), and there is an ongoing effort to classify their severity based on the residual enzymatic activity of the homodimeric tissue nonspecific ALP protein.[4] Mutations in the *ALPL* gene are heritable[5,6] and in HPP can reflect autosomal dominant or autosomal recessive inheritance.[7] Recessive inheritance typically underlies the most serious complications from HPP because both copies of the *ALPL* gene are compromised in homozygous or compound heterozygous individuals.[2,7] Heterozygotes are typically less severely affected, but it appears that the genotype–phenotype correlation in HPP is complex. Recent in vitro transfection studies showed that both the severity of an allele, that is, severe

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or moderate reduction in ALP activity, and the recessive or dominant nature of the allele influence the HPP phenotype.[8,9]

Severity, age-of-onset, and manifestation of HPP have broad clinical variability.[2,6] The most severe disease presents perinatally or in infants and, if untreated, has high morbidity and mortality due to skeletal and nonskeletal manifestations.[10] In children, the more severe forms of HPP tend to present earlier in childhood with significant skeletal disease including rickets, while less severe forms of childhood HPP generally present later in childhood with no clinically apparent rachitic disease.[10] Less severe forms of the disease are also seen in odonto-HPP, which only affect teeth, and adult HPP.[7,11]

Adult HPP manifests in both skeletal complications, such as osteomalacia, fractures, or pseudofractures, and nonskeletal symptoms, including pain, fatigue, or dental problems.[10] The severity of these manifestations ranges from severe nonlife threatening to moderate to very mild.[10] This variability is underscored by the observation that a vast majority of adult cases are heterozygous, yet many are likely to stem from a limited number of *ALPL* variants, including c.1250A>G and c.571G>A.[12] The genotype–phenotype correlation for these variants remains poorly defined, in part because published studies have largely characterized individuals with the canonical clinical phenotype, leaving those with atypical or milder presentations underrepresented. Consequently, phenotype-driven research may underestimate the prevalence and spectrum of heterozygous HPP, whereas the recognition of individuals with biochemical hallmarks among carriers of *ALPL* variants may overestimate it.[13–17] Understanding this dual bias is essential for accurately defining prognosis and determining when treatment is warranted. Because there is a paucity of genotype-based studies for the evaluation of phenotypic outcomes in individuals with pathogenic *ALPL* variants, we applied automated screening for unbiased identification of individuals harboring *ALPL* variants to an unselected cross-section of individuals from the general population and tested the hypothesis that penetrance, that is, the proportion of individuals carrying a variant that also manifest disease, is low in adult carriers.

Materials and methods

BioVU

The BioVU biorepository is hosted, maintained, and advanced by the Vanderbilt Institute for Clinical and Translational Research at Vanderbilt University Medical Center (VUMC). It uses an "opt-out" model for sample collection and currently harbors over 300 000 DNA samples collected from blood and tissue samples discarded at our hospital (https://victr.vumc.org/what-is-biovu/). BioVU links DNA data to electronic health records (EHRs) via a de-identified mirror image of the EHR referred to as "synthetic derivative," which at the time of writing mirrors over 3.6 million electronic records with no defined exclusions.[18]

Ethics

In accordance with the ethical framework previously described for BioVU studies,[18] all investigators had VUMC Institutional Review Board determination before accessing BioVU resources and signed a standard data-use agreement. The present study was determined to be nonhuman subjects research by the Institutional Review Board (protocol #222309), as there are no personal health identifiers in the dataset.

ALPL variants

This study was based on a cohort of 37 147 individuals with extant genotyping in BioVU, which included a total of 1759 DNA samples sequenced by whole exome sequencing (WES) and an additional 35 388 DNA samples genotyped on an Exome BeadChip (Illumina). All variants provided on these platforms were annotated using Annovar (http://www.openbioinformatics.org/annovar/), including gene labels, functional annotations, and population allele frequencies from GnomAD (https://gnomad.broadinstitute.org/). Exonic and splice region variants in the *ALPL* gene were identified with maximum allele frequencies of less than 2%. These variants were annotated using ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) and variants with pathogenic or likely-pathogenic classifications selected. Variants other than pathogenic or likely-pathogenic *ALPL* variants were excluded. All *ALPL* variants of unknown significance (VUS) were also excluded.

To ensure the accuracy of the *ALPL* variant calls from the Exome BeadChip, we tested the concordance with calls from a second genotyping platform, that is, the MEGA array. A total of 19 909 of the 35 388 samples genotyped on the Illumina Exome BeadChip were also genotyped on the MEGA array. Concordance was robust between the Exome BeadChip and MEGA array calls. Fifty-six of the 109 carriers of pathogenic or likely-pathogenic *ALPL* variants were genotyped on both the Exome BeadChip and MEGA array. Of these, 52 were identified as heterozygous carriers on the MEGA array and 4 were labeled as "missing," because they did not pass the threshold in quality control.

Diagnostic criteria for HPP

Diagnosis of HPP builds on persistently low serum ALP and a combination of clinical signs and symptoms in 4 categories: musculoskeletal, neurological, renal, and dental.[19] The International Working Group on HPP has ranked symptoms from these categories based on reported prevalence and expert opinion and proposed a diagnostic consensus algorithm based on low serum ALP, 4 major diagnostic criteria, that is, a pathogenic or likely-pathogenic *ALPL* variant, femur or metatarsal fractures, or elevated ALP substrates, and 5 minor diagnostic criteria, including chondrocalcinosis, poor fracture healing, nephrocalcinosis, or early loss of teeth.[14,19] Based on this algorithm, diagnostic criteria for HPP are met in the case of low serum ALP and 2 major criteria or 1 major criterium and 2 minor criteria.[14,19] We applied this algorithm with the following modifications: (1) at least 2 serial measures of low serum ALP; (2) exclusion of ALP substrates because their measurement requires specific protocols not part of routine clinical panels; (3) expansion of minor criteria to adjust for published real-world data on symptoms, including abnormal gait and muscle weakness[20] (Table S1); and (4) match to an International Classification of Diseases code, 9th and 10th revision (ICD-9 and ICD-10). Table S1 details on the applied ICD-9/10 codes. Pain was assessed based on use of pain medications instead of ICD-9/10 codes (Table S1).

Automated screening

Screening for both carriers of pathogenic or likely-pathogenic *ALPL* variants and patients meeting the diagnostic criteria

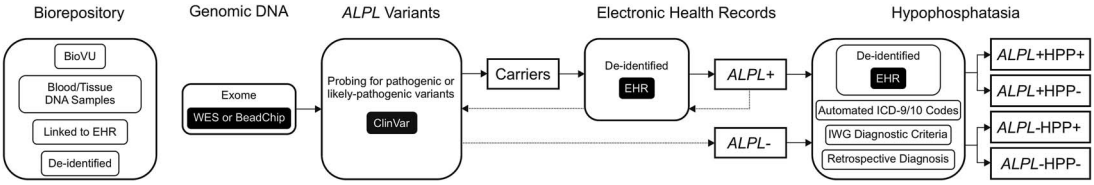

Figure 1. Automated screening for *ALPL* variants and HPP. Screening was based on the biorepository BioVU, which combines genomic DNA obtained from blood or tissue samples with de-identified EHRs. Exosome DNA was interrogated by whole exome sequencing (WES) or bead-based array technology (BeadChip) for the presence of pathogenic or likely-pathogenic *ALPL* variants catalogued in the NIH ClinVar database. Individuals positive for pathogenic or likely-pathogenic *ALPL* variants were termed carriers. Carriers with linked EHRs were pooled as *ALPL*+ cohort. Based on the demographics of the *ALPL*+ cohort, the *ALPL*− control cohort was assembled by random selection of individuals with matching demographics but no pathogenic or likely-pathogenic *ALPL* variant. Retrospective diagnosis of HPP was based on the ICD-9/10 diagnostic codes recorded in the de-identified EHR. Codes were organized by HPP symptom category and a diagnosis made based on the symptom combinations put forth in the diagnostic consensus guidelines proposed by International Working Group (IWG) on HPP. Diagnostic criteria were applied to both the *ALPL*+ and *ALPL*− cohorts. Abbreviations: *ALPL*, gene coding tissue nonspecific ALP; EHR, electronic health record; HPP, hypophosphatasia; ICD, International Classification of Diseases codes; IWG, International Working Group (on HPP).

for HPP was automated to permit efficient, unbiased interrogation of the large BioVU dataset (Figure 1). Carriers of *ALPL* variants were identified as described above. All carriers with de-identified EHRs were grouped as *ALPL*+ cohort (Figure 1). De-identified EHRs were used for automated extraction of demographics, laboratory measurements, including total serum ALP, medication use (bone health, sleep medications, muscle relaxants, seizure medications, antidepressants, anxiety medications, steroids, NSAIDs, and non-opioid and opioid pain medications), and ICD-9/10 codes. The ICD-9/10 codes permitted retrospective diagnosis of HPP based on the diagnostic criteria outlined above. Symptom categories were created by grouping related ICD codes into musculoskeletal, neurological, renal, and dental categories (Table S1). Extracted ICD-9/10 diagnosis codes for HPP (E83.3*, 275.3) were excluded to ensure the cohort contained no previously diagnosed patients. Screening for HPP was seemingly interconnected to the screening for *ALPL* variants but not dependent on the presence of a variant (Figure 1). The *ALPL*− control cohort (Figure 1), which was empirically selected to be about twice the size of the *ALPL*+ cohort, encompassed individuals with no detectable pathogenic or likely-pathogenic *ALPL* variant and was matched to the *ALPL*+ cohort with respect to age, sex, and race, genome cohort (WES or BeadChip), and record length of the de-identified EHR. A random, blinded manual data review of 20% of both *ALPL*+ and *ALPL*− cohorts were performed to validate cohort integrity.

Manual chart review

The de-identified EHR chart was accessed not automatically but via the BioVU interface, which allows for review of the entire medical record. An experienced HPP expert (KMD) reviewed and summarized the clinical presentation of both the 9 *ALPL*+HPP+ and 5 *ALPL*−HPP+ individuals.

Data analyses and statistics

Genetic penetrance was determined as previously described.[21] All analysis were performed using R 4.2.2 and R studio 2024.9.1.394. Statistical tests were 2-sided with $p < .05$ considered significant. Fisher's exact test was used for comparison of proportions between 2 independent groups with categorical outcomes. Modified Kaplan–Meier curves,[22,23] referred to as time-to-symptom curves, were used to characterize the probability of remaining symptom-free by age, stratified by 2 groups. These curves provided nonparametric estimates of the median (range) age at first symptom occurrence and enabled group comparisons. The log-rank test was used to compare the proportion of subjects (*ALPL*+ and *ALPL*−) with symptom progression.

Results

Pathogenic ALPL variants are common

A total of 37 147 genomic DNA samples from the BioVU biorepository were screened through a workflow designed to retrieve, in an anonymous and unbiased fashion, individuals carrying pathogenic or likely-pathogenic *ALPL* variants (Figure 1). A total of 109 individuals (0.3% or 1/341) were found to carry pathogenic or likely-pathogenic variants (Figure 2A). Carriers were mostly White, older adults with a median age (IQR) of 60.0 (22.5-75.0) yr, and comprised an equal proportion of women and men (Table 1). Each carrier harbored 1 of 7 distinct pathogenic or likely-pathogenic *ALPL* variants (Figure 2B). c.571G>A was the most frequent variant (67.9%) followed by c.881A>C (25.7%), the other variants occurred at frequencies of less than 2% (Figure 2B). Comparisons of variants based on previously published in vitro data (Table S3) showed that the predominant variant c.571G>A exhibits a modest reduction in ALP activity, while variant c.881A>C is associated with a greater activity reduction (Figure 2C and Table S3). Apart from variant c.346G>A, none of the variants display a strong dominant negative effect (Figure 2C and Table S3).

Low penetrance of HPP in undiagnosed *ALPL*+ individuals

The recently published consensus guidelines for the diagnosis of HPP in adults built on combinations of symptoms from musculoskeletal, neurological, renal, and dental categories in addition to persistently low serum ALP for age and gender.[14,19] De-identified EHRs were used to recognize individuals with 1 or more symptoms from these categories, even if isolated symptoms or symptom combinations did not meet HPP diagnostic criteria.

Seventy of the 109 carriers had linked EHRs and were designated *ALPL*+. The 70 *ALPL*+ individuals had a median age (IQR) of 65.0 (45.8-78.5) yr and were predominantly White (91.4%) with an about equal proportion of women and

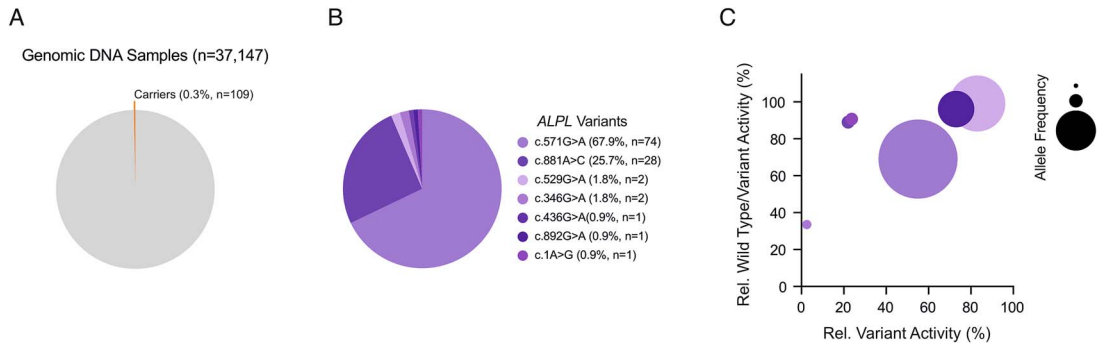

Figure 2. Prevalence of pathogenic or likely-pathogenic *ALPL* variants. (A) Pathogenic or likely-pathogenic *ALPL* variants were observed in 109 carriers with a prevalence of 0.3%. (B) Variants c.571G>A and c.881A>C were most frequent. (C) Residual ALP activity measures from independent sources (Table S3) were plotted as mean values. The majority of variants have a weak dominant negative effect and at least modest residual ALP activity.

men (Table 1). All 70 *ALPL*+ individuals revealed symptoms in, at least, 1 of the 4 symptom categories (Figure 3A and B and Table S4). Neurological (82.9%) and musculoskeletal (57.1%) symptoms were most frequent (Figure 3A), followed by renal issues (20.0%) and less frequent dental problems (2.9%). More than half of the individuals had only 1 symptom and approximately 30% had 2 symptoms (Figure 3B). Neurological symptoms coincided with musculoskeletal symptoms (Figure 3C). A comparison of medication uses between the *ALPL*+ individuals and 148 *ALPL*− controls with no pathogenic or likely-pathogenic variants showed no statistically significant differences for any of the tested medications, suggesting the isolated symptoms were mild (Figure 3D). Overall, there was no significant difference between the *ALPL*+ cohort and the *ALPL*− controls in the number of individuals presenting symptoms (Table S2).

Serum ALP was low in 65.7% of *ALPL*+ individuals vs 23.7% of *ALPL*− controls ($p < .0001$); thus, 34.3% (24/70) of *ALPL*+ individuals did not have low serum ALP (Figure 3E). A total of 60.9% (28/46) and 87.5% (21/24) of individuals with and w/o low serum ALP, respectively, carried variant c.571G>A. The higher frequency of neurological compared to musculoskeletal symptoms in low serum ALP individuals (86.9% vs 52.2%) was less pronounced in individuals w/o low serum ALP (75.0% vs 66.7%) (Figure 3F and G). Interestingly, the cohort w/o low serum ALP had the highest rate of individuals, about 4%, with symptoms in all 4 categories (Figure 3G). Of the 38 low serum ALP individuals, 9 (23.7%, Table 2) had symptom combinations consistent with a HPP diagnosis according to the diagnostic consensus criteria, yielding a penetrance rate of 12.9% (9/70) (Figure 3H).

ALPL+ individuals were likely to develop premature mobility issues

Penetrance often depends on an age threshold.[25] Although our automated screening did not permit direct measure of a potential threshold for HPP, time-to-symptom analyses were performed to compare *ALPL*+ individuals to *ALPL*− controls with respect to the age-dependency of isolated clinical symptoms from the 4 categories described above. Compared to the *ALPL*− individuals, the *ALPL*+ individuals had a higher probability of progression for mobility issues, specifically gait abnormalities (median age 73 yr *ALPL*+ vs 82 yr *ALPL*−, $p = .03$), as well as a similar probability of progression for fatigue, arthritis, or dental problems, including lost and damaged teeth or implants (Figure 4).

ALPL− individuals presented with HPP

The automated screening protocol included HPP screening of the 148 *ALPL*− control individuals. Unexpectedly, 3.4% (5/148) of individuals met the diagnostic criteria for HPP (Table 3), while lacking an associated pathogenic or likely-pathogenic *ALPL* variant. Whereas overall demographics of *ALPL*−HPP+ and *ALPL*+HPP+ individuals were similar (Table 1), 3 specific differences were observed. First, 4 of the 5 *ALPL*−HPP+ individuals were women. Second, while the *ALPL*+HPP+ group constituted only adults 22 yr and older (Table 2), 1 *ALPL*−HPP+ individual was an infant and another one a 5-yr-old child (Table 3); of note were the unusually high serum ALP levels in latter patient (Table 3), which may have suggested ALP enzyme replacement therapy and inadequate genetic testing. This child was ultimately diagnosed with 4q13.2-q22.11 microdeletion syndrome which has considerable phenotypic overlap with infantile HPP and may support an alternative non HPP diagnosis. Lastly, both infant and child presented with seizures, an HPP symptom typically associated with severe, infantile forms of HPP[11,13]; however, the vitamin B6 levels in these individuals could not be determined.

Discussion

This *ALPL* genotype-based study found evidence that pathogenic and likely-pathogenic *ALPL* variants are common, and when associated with disease manifestations have low penetrance. These observations have important implications for real-world clinical dilemmas, as exemplified in the 2 clinical cases below.

Case 1

A 37-yr-old White woman, with a history notable for polycystic ovarian syndrome and endometriosis, presented to an in vitro fertilization clinic for infertility. She was offered preimplantation genetic testing for monogenic disorders, including *ALPL*, along with preimplantation genetic testing for chromosome abnormalities to inform embryo selection and transfer. Testing showed she harbors pathogenic heterozygous *ALPL* variant c.346G>A. The father was tested and found negative for *ALPL* variants. Given her carrier status for HPP, which

Table 1. Demographics of the cohorts.

	Carriers (*n* = 109)	*ALPL*+ (*n* = 70)	*ALPL*+HPP+ (*n* = 9)	*ALPL*+HPP− (*n* = 61)	*ALPL*− (*n* = 148)	*ALPL*−HPP+ (*n* = 5)	*ALPL*−HPP− (*n* = 143)
Age at last EHR, years							
Median (IQR)	60.0 (22.5-75.0)	65.0 (45.8-78.5)	73.0 (60.0-79.0)	64.0 (45.0-77.0)	61.0 (29.8-76.2)	81.0 (17.0-82.0)	61.0 (31.5-75.5)
Min/Max	0/89.0	0/89.0	38.0/89.0	0/89.0	0/89.0	9.0/84.0	0/89.0
Mean (SD)	52.1 (27.0)	60.1 (23.5)	67.8 (17.8)	59.0 (24.2)	54.1 (27.0)	54.6 (38.1)	54.1 (26.7)
Age at first EHR, years							
Median (IQR)	41.0 (13.0-88.0)	52.0 (31.5-64.5)	56.0 (31.0-61.0)	49.0 (33.0-65.0)	41.0 (15.0-62.0)	60.0 (5.0-69.0)	41.0 (17.0-62.0)
Min/Max	0/88.0	0/86.0	22.0/86.0	0/82.0	0/88.0	0/72.0	0/88.0
Mean (SD)	40.3 (26.3)	46.3 (23.4)	51.1 (20.6)	45.6 (23.8)	40.4 (26.8)	41.2 (35.6)	40.4 (26.6)
Sex, *n* (%)							
Female	55 (50.5)	38 (54.3)	6 (66.7)	32 (52.5)	87 (58.8)	4 (80.0)	83 (58.0)
Race, *n* (%)							
White	101 (92.7)	64 (91.4)	8 (88.9)	56 (91.8)	139 (93.9)	5 (100.0)	134 (93.7)
Black	5 (4.6)	4 (5.7)	0 (0)	4 (6.6)	8 (5.4)	0 (0)	8 (5.6)
Black Hispanic	0 (0)	1 (1.4)	1 (11.1)	0 (0)	0 (0)	0 (0)	0 (0)
Unknown	3 (2.7)	1 (1.4)	0 (0)	1 (1.6)	1 (0.7)	0 (0)	1 (0.7)

ALPL+: pathogenic or likely-pathogenic *ALPL* variant present; *ALPL*−: pathogenic or likely-pathogenic *ALPL* variant absent; HPP+: meets HPP diagnostic criteria; HPP−: does not meet HPP diagnostic criteria.
Abbreviations: *ALPL*, gene coding tissue nonspecific ALP; EHR, electronic health record (entry); HPP, hypophosphatasia.

can be transmitted in both autosomal recessive and dominant forms, she was counseled regarding the 50% chance for each child to be affected with HPP. On exam, the patient did not have any signs or symptoms indicative of HPP, and only mild arthritis as reported in her mother raising the question of whether in vitro fertilization with preimplantation genetic testing for embryo selection to reduce the risk of passing the condition to offspring was necessary.

Case 2

A 34-yr-old White woman was incidentally found to have low serum ALP while undergoing treatment with chemotherapy, radiation, leuprolide acetate, and anastrozole for T1cN0 ER+ PR− HER2 breast cancer. Genetic testing found pathogenic heterozygous *ALPL* variant c.657G>T and testing of family members was recommended. Her aromatase inhibitor was thus changed to tamoxifen. One year later, a bone density scan revealed a 3 T score in the spine and a 4%-6% decline in the hip, and anti-osteoclast therapy was recommended by her oncologist but was determined to be contraindicated because it might compound the *ALPL* gene defect and potentially impair bone turnover and thereby formation.

These 2 consult cases from our clinic highlight 3 often underappreciated, multifactorial aspects of HPP management. First, HPP in adults may present as co-morbidity with other diseases. Second, testing for pathogenic or likely-pathogenic *ALPL* variants has become increasingly frequent within all of clinical diagnostics. Third, clinical decision making begins to factor in the presence of pathogenic or likely-pathogenic *ALPL* variants, yet the current HPP knowledge base disproportionately represents the HPP disease burden associated with *ALPL* mutations due to a longstanding reliance on case series drawn from clinically affected individuals and inherently selected for affected cases, potentially inflating estimates of penetrance and clinical expressivity. This motivated us to design a genotype-based study of prevalence and penetrance in a large, unselected cohort of mostly adults with no preexisting HPP diagnosis (Figure 1).

To our knowledge, this is the first report on large-scale genomic screening of pathogenic and likely-pathogenic *ALPL* variants. The study builds on 2 prior reports. A genomic screening study from the UK Biobank validated in 11 individuals that a pathogenic variant associated with infantile HPP correlated with low serum ALP, supporting the value of variant screening for HPP characterization.[26] The second report is a BioVU genome-wide association study from our group that investigated 3 *ALPL* single nucleotide pathogenic variants and established important technical aspects of our automated screening protocol, including the use of ICD codes.[27]

In our large, unselected real-world population, heterozygous pathogenic or likely-pathogenic *ALPL* variants had a prevalence of 0.3% or 1/341. This prevalence provides much anticipated support for a previously described theoretical, allele-based model that estimated a variant frequency of 1/254 for mildly affected adults.[8] With 67.9%, the predominant variant was c.571G>A (Figure 2), the most commonly reported variant in biallelic HPP and the variant recently described as very common but not predominant in a large cohort of United States individuals with a clinical suspicion of HPP.[28,29] Our subgroup analysis showed that c.571G>A was predominant in individuals both with or w/o low serum ALP (data not shown). Low serum ALP, a state often referred to as biochemical hallmark of HPP,[15] was detected in two-third of

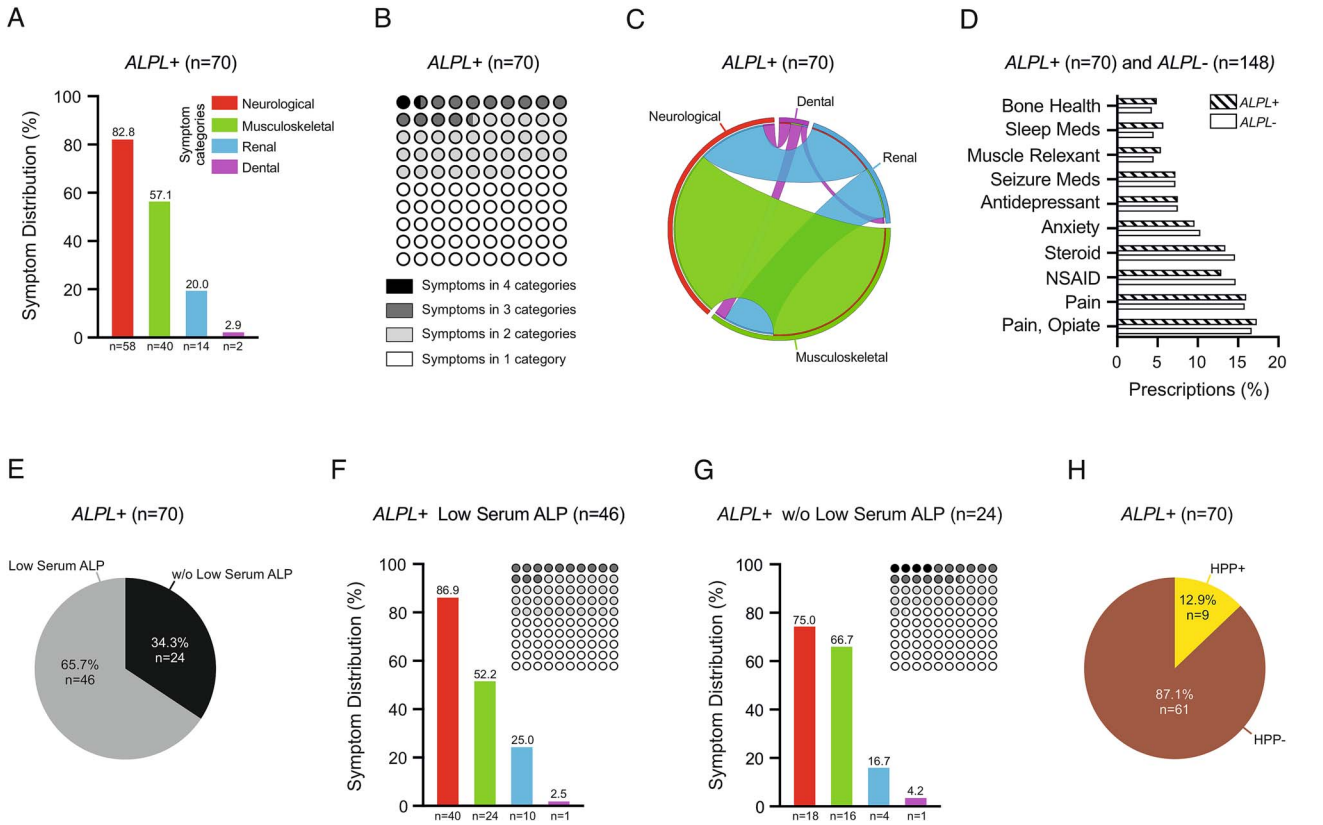

Figure 3. ALPL+ individuals and HPP penetrance. Carriers with linked EHR (*ALPL*+) were assessed for occurrence of one or more symptoms from the musculoskeletal, neurological, renal, and dental categories (A-C), medication use (D), serum ALP (E-G), and HPP penetrance (H). (A) Neurological symptoms were predominant in *ALPL*+ individuals and (B) the majority of *ALPL*+ individuals presented with a single symptom. (C) Neurological symptoms frequently associated with musculoskeletal symptoms. (D) Medication use was similar between *ALPL*+ and *ALPL*− individuals. Statistical comparison based on Fisher's exact test showed no significant difference between the *ALPL*+ and *ALPL*− cohorts for any of the 10 medication categories. (E) About two-third of *ALPL*+ individual had low serum ALP. (F) Symptom distribution and (G) symptom frequencies were dissimilar between the cohorts with or w/o low serum ALP. (H) HPP penetrance was approximately 1 out of 10 *ALPL*+ individuals. Abbreviations: ALP, alkaline phosphatase; *ALPL*, gene coding tissue nonspecific ALP; HPP, hypophosphatasia.

ALPL+ individuals, while the other one-third presented w/o low serum ALP (Figure 3). This finding demonstrates that the strong association between pathogenic *ALPL* variants and the hallmark low serum ALP observed in individuals with clinically established HPP is less stringent in carriers. However, the lifelong impact of abnormal serum ALP due to pathogenic or likely-pathogenic *ALPL* variants is not fully elucidated, and there clearly is an unmet need for longitudinal studies in carriers of pathogenic or likely-pathogenic variants to better understand the progression to low serum ALP and to determine the prognostic significance of decreased serum ALP. Interestingly, more individuals carried c.571G>A in the group w/o low serum ALP; however, c.571G>A was the predominant variant in in the *ALPL*+HPP+ group (Table 2), suggesting c.571G>A is a limited marker for disease manifestation.

In HPP, penetrance has frequently been noted as incomplete, but experimental measures are sparse.[8,30–32] Penetrance in adults with mild disease was only reported in a single study and estimated at about 50% based on a reanalysis of data from 2 prior publications unrelated to penetrance.[8] One study assessed the frequency of *ALPL* mutations in 42 preselected patients with low serum ALP phenotypes of unknown origin,[33] while the other study was the above mentioned population-based, genome-wide association study of 3 *ALPL* single nucleotide polymorphisms.[27] In contrast, the present study used genomic analysis for unbiased, unselected identification of individuals carrying pathogenic or likely-pathogenic *ALPL* variants and applied diagnostic criteria for HPP based on the classic definition of multisystemic disease manifestation. A penetrance of 12.9% was observed; thus, our underlying hypothesis that HPP penetrance is low in adult carriers was supported. However, little is known about the mechanisms governing penetrance. One current working model is based on variant dominance and proposes that in heterozygous individuals a strong dominant negative effect is associated with clinical symptoms and thus high penetrance.[9] Our study supports this model because the two most frequent *ALPL* variants in our study have a limited to modest dominant negative effect in vitro (Figure 2) and, as proposed, yield low penetrance. However, the 9 *ALPL*+HPP+ individuals (Table 2) carry the same variants, which may suggest potential alternative mechanisms influencing penetrance or expressivity.

The penetrance data from this study may aid clinical decision making in cases such as the ones described above. It has previously been postulated that patients initially diagnosed with mild clinical symptoms including dental problems, remain at risk of developing symptoms later in life.[34] Here, we observed low adult penetrance of variants, such as c.571G>A and c.881A>C. A finding that may help carriers to assess their risk of developing HPP and provide guidance to physicians on

Table 2. Clinical presentation of the *ALPL*+HPP+ individuals.

Sex	Age[a]	Variant	HPP symptoms								
			Clinical						Serum ALP (U/L)		
			Neurological	Musculoskeletal		Renal	Dental	Other	Min	Max	Mean	RR[b]
Female	86	c.881A>C	Balance issues	Mobility issues		Renal insufficiency	N/R	N/R	21	24	23	40-150
Female	61	c.571G>A	Fatigue	Humus fracture Arthritis Abnormal gait Impaired mobility Osteoporosis		Hypercalcemia Hypercalciuria	N/R	Weakness	27	47	33	40-150
Female	60	c.571G>A	Neuropathy Depression Anxiety Balance deficit	Vertebral fracture Muscle weakness Arthritis Scoliosis Spinal stenosis	Neck pain Shoulder pain Joint pain Mobility device Wheelchair	N/R	N/R	Sensorineural hearing loss	35	65	53	40-150
Female	54	c.571G>A	Anxiety TIA	Spine fractures Rib fractures Ankle fracture Hip fracture Osteoporosis	Arthritis Joint pain Muscle pain Bone pain	N/R	Poor dentition	Hearing loss	22	51	31	40-150
Female	31	c.571G>A	Depression Anxiety	Tibia fracture Spine disease Arthritis Gait abnormality Back pain	Foot pain	Kidney stones	Fractured teeth Dental extractions Dental implants	N/R	29	82	52	40-150
Female	27	c.881A>C	Depression Headaches	Tibia fracture Chondrocalcinosis Osteopenia Knee pain Back pain		N/R	N/R	N/R	25	28	26	40-150
Male	63	c.571G>A	Neuropathy Fatigue	Muscle weakness Spinal stenosis Gout Joint pain Gait abnormality	Mobility device	Kidney stones	N/R	N/R	35	53	45	40-150
Male	56	c.571G>A	N/R	Arthritis Muscle weakness Bone pain Joint pain Mobility device		Renal disease	N/R	N/R	37	65	47	40-150
Male	22	c.881A>C	Seizure disorder	Muscle weakness Mobility device Tibia fracture Scoliosis		Renal failure	N/R	N/R	7	19	13	40-150

[a] At first electronic health record entry. [b] Sex- and age-adjusted[24]; Vanderbilt Hospital Diagnostic Laboratories CLIA #44D0659066. Abbreviations: ALP, alkaline phosphatase; *ALPL*, gene coding tissue nonspecific ALP; HPP, hypophosphatasia; N/R, not reported; RR, reference range; TIA, transient ischemic attack.

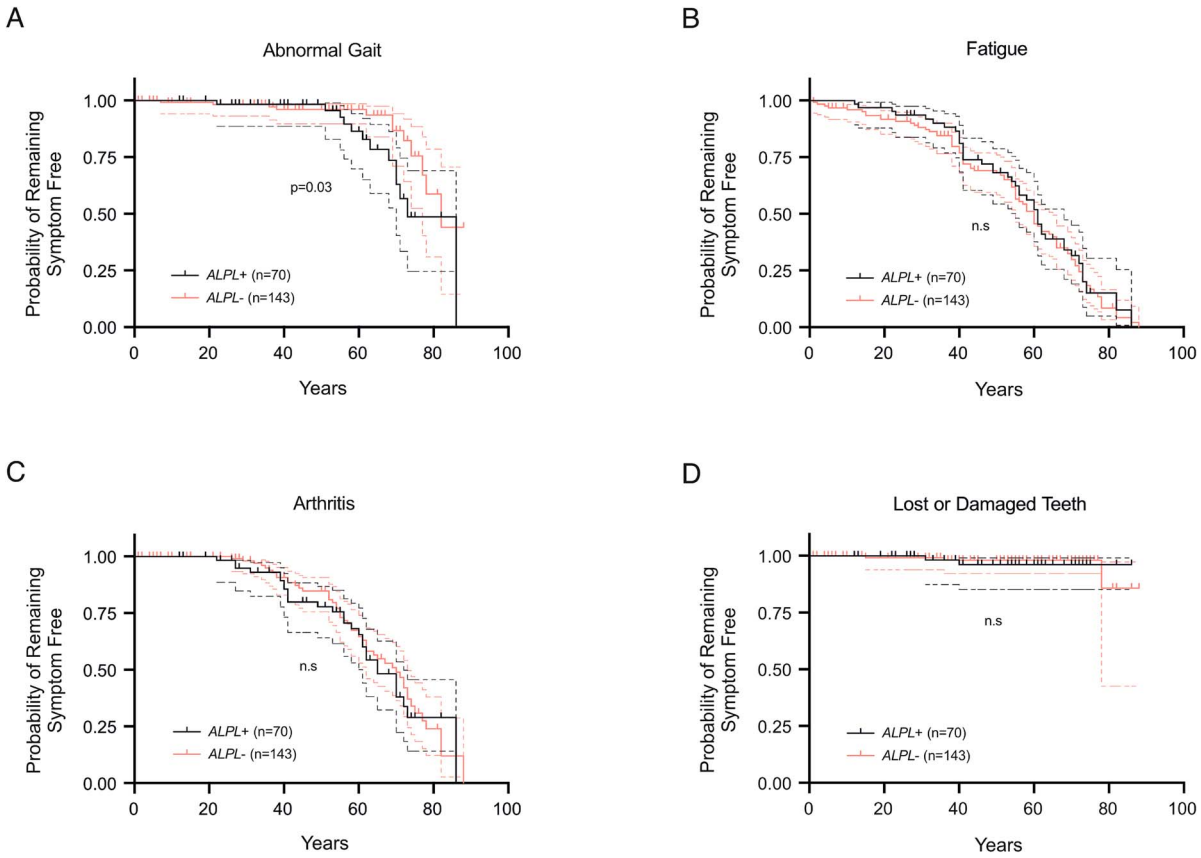

Figure 4. Age-dependency of clinical symptoms in *ALPL*+ individuals. Time-to-symptom curves compared the probability of progression of (A) mobility issues, (B) fatigue, (C) arthritis, and (D) dental problems between *ALPL*+ and *ALPL*− individuals. The 5 HPP+ individuals in the *ALPL*− group were excluded from the analysis. Solid lines show the time-to-symptom curves, while dashed lines denote probability of being event-free between upper and lower 95% CIs. Compared to *ALPL*− control individuals, *ALPL*+ individuals had a higher probability of progression for gait abnormalities. Statistical comparisons were based on the log-rank test. Abbreviations: *ALPL*, gene coding tissue nonspecific ALP; n.s., not significant.

the monitoring protocols of those patients. Both the 12.9% penetrance (Figure 3) and a probability of remaining symptom free that is similar between the *ALPL*+ and *ALPL*− cohorts for three out of 4 symptoms tested (Figure 4), may indicate that adult carriers have a more limited risk of developing HPP. Nevertheless, proper risk determination and monitoring is warranted for all carriers of pathogenic or likely-pathogenic variants and may include an HPP specialist, annual health check-ups, and targeted questioning on common clinical signs of HPP. Our data indicates that latter should include neurological symptoms, which were seen in almost all of the *ALPL*+HPP+ individuals (Table 2) and frequent in carriers (Figure 3). However, isolated HPP symptoms, including neurological and musculoskeletal manifestations, are often unspecific and in agreement we found no significant difference in occurrence between the *ALPL*+ and *ALPL*− cohorts. Therefore, adherence to diagnostic guidelines and accurate differential diagnosis are essential for monitoring carriers. Our study also included a time-to-symptom estimation of symptom risk with age. Comparing *ALPL*+ and *ALPL*− cohorts, we observed an increased risk of mobility issues, that is, gait abnormalities, as compared to fatigue, arthritis or dental problems (Figure 4). This observation must be interpreted with caution because the only direct evidence that the abnormalities were not age related comes from our comparison to the *ALPL*− control cohort. Despite this limitation, the finding suggests that mobility assessments should be a mandatory part of longitudinal monitoring; published monitoring guidelines for HPP patients treated with asfotase alfa may provide some guidance on what monitoring techniques to use.[10] Our study also provided insight into symptom patterns (Figure 3). The co-occurrence of neurological and musculoskeletal symptoms suggests that in clinical practice carriers presenting with either one should be thoroughly examined for the other. We were surprised to find only modest co-occurrences of neurological or musculoskeletal symptoms with dental symptoms. Possible explanations include the lack of access to dental records from both private practice and dental insurance. Similarly, renal complications, such as hypercalcemia and hyperphosphatemia, are common in HPP and further work is needed to fully define symptom patterns in adults with HPP. Lastly, we observed that about 40% of both *ALPL*+ low serum ALP and *ALPL*+ w/o low serum ALP individuals had both neurological and musculoskeletal symptoms (Table S4) but failed to meet diagnostic criteria for HPP. If and how these individuals progress to meet the diagnostic consensus criteria of HPP is at present unknown, which underscores the need for longitudinal studies in carriers with isolated HPP symptoms. Additionally, this serves as a call for modification of the diagnostic criteria to better encompass milder and emerging cases as more of the phenotypic landscape of HPP is discovered.

Implemented as quality assurance, the automated screening included a survey for HPP in the *ALPL*− control cohort

Table 3. Clinical presentation of the *ALPL*−HPP+ individuals.

Sex	Age[a]	HPP symptoms						Serum ALP (U/L)				Exposures lowering serum ALP	Alternative low serum ALP diagnosis in EHR
		Clinical											
		Neurological	Musculoskeletal		Renal	Dental	Other	Min	Max	Mean	RR[b]		
Female	69 (82)	Fatigue	Hip fracture Rib fracture Femur fracture Elbow fracture Arthritis	Scoliosis Osteopenia Wheelchair use	Kidney stones	N/R	N/R	36	89	67	40-150	Glucocorticoids Thyroid replacement therapy	Rheumatoid arthritis Hypothyroidism
Female	60 (81)	Neuropathy	Muscle pain Joint pain Bone pain		Kidney stones	N/R	N/R	35	89	56	40-150	Glucocorticoids	Lupus Mixed connective tissue disease Chronic kidney disease
Female	5 (17)	Seizures Fatigue	Metatarsal fractures Nasal fracture		N/R	Poor dentition	N/R	110	4198[c]	2154[c]	150-500	N/R	5 4q13.2 microdeletion syndrome Growth delay secondary to the microdeletion syndrome
Female	0 (9)	Seizures Headaches	Skull deformity Rhizomelia Short stature Muscle weakness		Kidney stones	N/R	Hearing loss	108	356	234	120-560	Glucocorticoids use Thyroid replacement therapy Blood transfusions	Paroxysmal nocturnal hemoglobinura Premature ovarian failure Hypothyroidism Hypomagnesemia
Male	72 (84)	Aphasia Neuropathy Cognitive Impairment	Joint pain Muscle weakness Mobility device		N/R	N/R	Hearing loss	28	58	39	40-150	Glucocorticoids Thyroid replacement therapy Blood transfusions	Hypothyroidism

[a]At first (last) electronic health record entry. [b]Sex- and age-adjusted[24]; Vanderbilt Hospital Diagnostic Laboratories CLIA #44D0659066. [c]Possibly due to enzyme replacement therapy, records incomplete. Abbreviations: ALP, alkaline phosphatase; *ALPL*, gene coding tissue nonspecific ALP; HPP, hypophosphatasia; N/R, not reported; RR, reference range.

(Figure 1). This led to the identification of 5 individuals that presented with HPP according to the diagnostic consensus criteria but lacked a pathogenic or likely-pathogenic *ALPL* variant (Table 3). A possible explanation is that the underlying *ALPL* variant or variants are currently unknown or were not included on the extant genotyping. This emphasizes the importance of a comprehensive *ALPL* variant catalogue and increased use of whole genome sequencing to definitively determine if any *ALPL*− individual indeed meets diagnostic criteria. Another explanation for the five *ALPL*−HPP+ cases, all of which had low serum ALP, is that they did not stem from *ALPL* mutations per se but from unknown alternations directly or indirectly related to ALP metabolism. Finally, other disorders associated with skeletal burden may have phenotypic overlap with HPP as seen with our 5-yr-old with a 4q13.2-q22.11 microdeletion syndrome.

This study has limitations. While genotyping and sequencing data were processed with standard quality control measures and showed high concordance among individuals genotyped on 2 platforms, the possibility of false positive carriers cannot be eliminated. Analyses were also limited because both ClinVar and the BeadChip array, which analyzed the bulk of genomes, have a limited, prespecified inventory and do not permit de novo discovery. Hence, there is a possibility that an *ALPL* variant included in ClinVar was not detected by the BeadChip and vice versa. It is therefore of considerable interest to expand our analyses using whole genome sequencing, which would also allow for a comprehensive genetic assessment of the *ALPL*−HPP+ individuals. Future whole genome sequencing studies should be combined with expanded ClinVar annotations, including *ALPL* VUS, and we have initiated such studies. Regarding the experimental design, it is important to accept the possibility of incomplete EHRs[35] as a result of limited longitudinal follow-up or fracture or dental care outside the VUMC health system. Also, the retrospective design of our study limited us to standard clinical laboratory measures excluding HPP markers, for example, pyridoxal 5′-phosphate, typically ordered if HPP is suspected. Further, in certain instances, ICD codes are inexact, as exemplified in code 275.3, which captures both HPP and hypophosphatemia. Moreover, any penetrance calculation evidently depends on the HPP diagnostic consensus criteria proposed at the time of data analysis. Because the published consensus guidelines rely on major and minor diagnostic criteria with prevalences greater and smaller 50%, respectively, it is possible they miss overt disease. Misclassification is potentially amplified by (1) the often mild and atypical presentation of HPP in adults, (2) emerging phenotypes, or (3) incomplete EHRs, and can result in underestimation of HPP and thus penetrance. Likewise, the guidelines may incorrectly indicate overt disease. Overestimation could, for instance, result from the difficulty to discriminate HPP fractures from osteoporosis fractures in the absence of a biopsy. Moreover, future changes in diagnostic consensus guidelines have potential to change the measured penetrance value. Penetrance measures are also population dependent.[25] Our study investigated a comparably large, unselected population of individuals seen at a major Southeastern United States tertiary care and referral hospital for the region and nationally. Arguably, this population offers a reasonable cross-section of the regional population and provides a more generalizable penetrance assessment as compared to small phenotypically enriched clinical cohorts. However, the vast majority of the studied individuals were of European ancestry and additional data on individuals from African and other ancestries is clearly needed. Moreover, the studied individuals were seeking medical attention and extrapolations of our findings to other populations require validation and intrinsically limits the unbiased nature of our study. Lastly, HPP penetrance probably depends on additional factors, such as inheritance pattern, variant, age, and sex, and deserves further investigation.

Future directions

The observations from this study lead to the following 6 conclusions: (1) clinical context is more important for decision making than an pathogenic or likely-pathogenic *ALPL* variant in isolation, (2) low or normal serum ALP in isolation is an inadequate biomarker for HPP, (3) longitudinal follow-up is recommend for progression of HPP disease burden, (4) neurologic symptoms are a strong component of the HPP phenotype in our study and currently neither a major nor minor criteria in the diagnostic consensus guidelines for adults, (5) mobility impairment is an important discriminator from aging and warrants functional testing, such as the timed up-and-go or 6-minute walk tests, as part of HPP patient monitoring, and (6) future studies using whole genome sequencing are needed for refined penetrance measures across all forms of HPP.

Additional efforts are needed to further characterize and reclassify VUS, which we excluded from this analysis, and to expand evaluation into non-European ancestry cohorts to understand phenotype expression beyond the populations studied to date. Leveraging large-scale biobanks also represents a promising strategy to identify undiagnosed cases, through ancestry-based clustering approaches. At the same time, diagnostic criteria for HPP must undergo ongoing revision as knowledge of the disease spectrum and penetrance evolves. As clinical and genetics laboratory are expected to support a true diagnosis of HPP, it is the physician, not the laboratory testing, that makes the diagnosis. Future work should integrate genomic and nongenomic factors to further refine risk prediction, improve variant interpretation, and guide more personalized approaches to counseling and management for individuals with pathogenic *ALPL* variants.

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Author contributions

Kathryn M. Dahir (Conceptualization, Methodology, Investigation, Formal analysis, Validation, Resources, Writing, Supervision), Jennifer E. Below (Conceptualization, Methodology, Investigation, Writing—original draft, Writing—review & editing), Jinyuan Liu (Methodology, Investigation, Formal analysis, Validation), Amir Javid (Methodology, Investigation, Formal analysis, Validation), Guancho Wang (Methodology, Investigation, Formal analysis, Validation), and Lisa Bastarache (Methodology, Investigation, Validation, Resources, Writing—original draft, Writing—review & editing, Supervision)

Supplementary material

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Conflicts of interest

No potential conflict of interest relevant to this article was reported.

Data availability

The data supporting this study are available within the article and its supplementary materials. Table S1 is available at: https://doi.org/10.7910/DVN/BMHRAG.

Ethics statement

Nonhuman subjects research as determined by IRB.

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