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Research Article

Prevalence of chondrocalcinosis and calcium pyrophosphate deposition disease in a cohort of adult patients with low alkaline phosphatase levels and a positive versus negative genetic *ALPL* study

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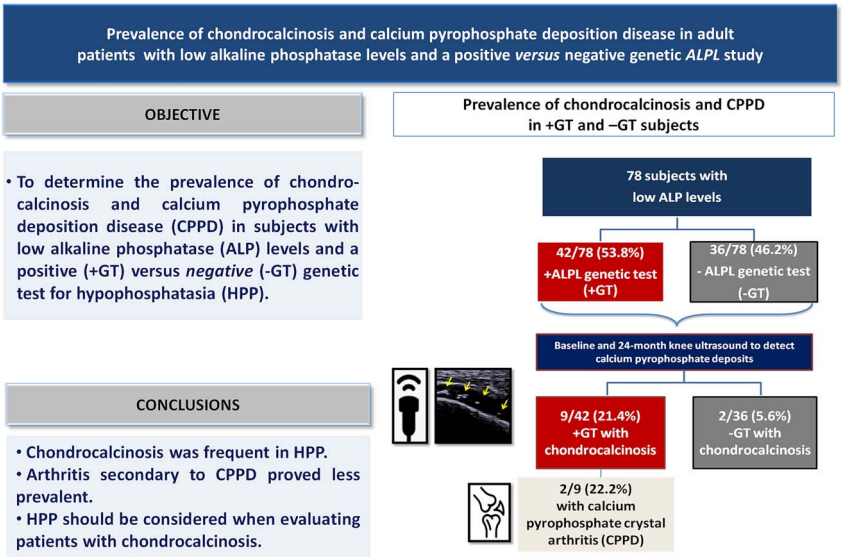
Abstract

Objectives: To estimate the prevalence of chondrocalcinosis and calcium pyrophosphate dihydrate deposition disease (CPPD) in patients with low alkaline phosphatase (ALP) levels and a positive *ALPL* genetic study (+GT) for hypophosphatasia (HPP) compared to those with the same biochemical abnormality and a negative genetic test (−GT). As a secondary objective, to analyze the biochemical factors associated with its presence in subjects with *ALPL* variants. Methods: Seventy-eight subjects with persistently low ALP levels and *ALPL* genetic test were included. Baseline and 24-mo knee ultrasounds were performed in 42 + GT and 36 −GT subjects, in whom the fibrocartilage, hyaline cartilage of menisci, tendons, and synovial fluid were scanned to detect calcium pyrophosphate deposits. A MyLabTwice ultrasound machine (Esaote) with a multifrequency linear array transducer (4–13 MHz) was used. Results: A higher percentage of chondrocalcinosis was observed in the +GT group [9/42 (21.4%)] compared to the −GT group [2/36 (5.6%), p=.045)]. Two patients (4.76%), both in the +GT group, had arthritis secondary to CPPD. No new cases were identified at the 24-mo control. When comparing +GT patients with and without chondrocalcinosis, ALP levels were lower, and pyridoxal-5′-phosphate (PLP) and phosphate levels were higher in the former group (p<.05). Logistic regression analysis revealed that higher PLP levels are associated with the presence of chondrocalcinosis (OR: 1.1; 95% confidence interval, CI, 1.001–1.012). Conclusions: Chondrocalcinosis was a frequent ultrasonographic finding in HPP. Arthritis secondary to calcium pyrophosphate deposits, however, proved less prevalent. Genetic causes, such as HPP, should be considered when evaluating patients with chondrocalcinosis in clinical practice.

Keywords: osteomalacia and rickets, disorders of calcium/phosphate metabolism, radiology and genetic research

Graphical Abstract



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Introduction

Hypophosphatasia (HPP) is a rare condition affecting bone mineral metabolism, characterized by low serum alkaline phosphatase (ALP) activity due to defects in the *ALPL* (ALP-liver) gene, located on chromosome 1p36.1-p34. The latter encodes non-tissue-specific alkaline phosphatase (TNSALP), which is expressed in bone, liver, and kidney. This enzyme plays a fundamental role in the development and maintenance of healthy bones and the metabolism of calcium and phosphorus in the organism.[1,2] The clinical spectrum of HPP is extremely broad, ranging from death in utero, with a severe absence of skeletal mineralization, to forms with exclusively dental involvement. Six main forms are currently recognized: perinatal lethal, prenatal benign, infantile, childhood, adult, and odontohypophosphatasia.[3] The main biochemical characteristic of the disease is the presence of decreased ALP levels. Inorganic pyrophosphate (PPi), pyridoxal-5′-phosphate (PLP), and phosphoethanolamine (PEA) are considered to be its natural substrates. Recent studies suggest that adenosine triphosphate (ATP), di-phosphoryl liposaccharide, and phosphorylated osteopontin may also be natural substrates.[2]

The most significant consequences of TNSALP deficiency stem from defective bone and tooth mineralization. TNSALP hydrolyzes PPi and generates inorganic phosphorus (Pi), enabling the formation of hydroxyapatite (HA) crystals and promoting mineralization. During the initial phase of mineralization, at the level of the matrix vesicles, both calcium (Ca^{2+}) and Pi accumulate. During the second phase, the vesicles' membranes break down and the HA crystals are exposed to extracellular fluids, allowing their propagation and deposition in the collagenous matrix. The concentration ratio of Pi to PPi is crucial in the mineralization process, because although the former is necessary for the formation of HA crystals, PPi acts as an inhibitor.[3–6] This process is regulated by the interaction of promoter and inhibitor factors: TNSALP, phosphatase orphan 1 (PHOSPHO1), and nucleoside pyrophosphohydrolase-1, which play a role in regulating the Pi/PPi ratio.[7] In mice and humans with HPP, electron microscopy has revealed that bone matrix vesicles contain HA, as has been shown by the fact that PHOSPHO1 is normally expressed in matrix vesicles.[8] Nevertheless, the extravesicular growth of crystals is blocked by the extracellular accumulation of PPi,[2] which hinders teeth and bone mineralization,[9] leading to dental abnormalities, early tooth loss, and recurrent poorly healing fractures or pseudofractures observed in adult HPP.[10] In addition, PPi can also accumulate in these patients to form calcium pyrophosphate dihydrate crystals.[9] The deposition of these crystals is often seen in knees, wrists, symphysis pubis, elbows, or hips, frequently asymptomatic and manifests as articular chondrocalcinosis on imaging studies. However, it may also associate with arthritis, in relation to calcium pyrophosphate deposition disease (CPPD).[11,12] Furthermore, in HPP, HA periarticular and tendinous calcifications affecting the shoulder (rotator cuff), hips, elbows, and knees have also been described and symptoms associated include acute or chronic pain, swelling, and restricted movement in the affected area. To differentiate HA from crystal pyrophosphate dyhidrate deposition, imaging studies are key: radiography and ultrasound can reveal HA deposits as amorphous calcifications in the soft tissues, while chondrocalcinosis is characterized by linear or punctate calcifications within the joint cartilage.[12] Additionally, synovial fluid analysis can differentiate both entities.[13] These rheumatologic manifestations have been described mainly in case series,[14–19] and scarce evidence exists about associated factors for chondrocalcinosis in subjects with *ALPL* variants. In this context, the aim of this work was to estimate the prevalence of ultrasound chondrocalcinosis and arthritis secondary to calcium pyrophosphate dihydrate crystals in a cohort of patients with persistently low ALP levels and a positive *ALPL* genetic test (+GT) compared to those with the same biochemical abnormality and a negative genetic study (−GT). As a secondary objective, we sought to analyze the association between biochemical parameters related to bone metabolism and TNSALP substrates (serum PLP and urinary PEA) and chondrocalcinosis in patients with a positive genetic *ALPL* study.

Materials and methods

Study population and design

This study enrolled adults (≥18 yr) identified from the biochemical database of Hospital La Paz, displaying at least 2 ALP measurements below 35 IU/L and none above 45 (normal range: 45–125 IU/L) and in whom secondary causes of persistent hypophosphatasaemia had been previously discarded.[15] Eighty-five patients decided to participate in a cross-sectional study in 2016 and signed the informed consent for *ALPL* genetic testing. Subsequently, these subjects were invited to participate in a longitudinal prospective study for 24 mo, as also were 7 patients later diagnosed with before September 2018 in our Rheumatology Department. Details regarding the recruitment process and genetic assessment are detailed in previous publications by our group.[20,21] For this work, we studied 78 subjects included in the longitudinal study (42 with a positive genetic test and 36 with a negative genetic test) who underwent clinical follow-up every 6 mo and a targeted ultrasound study to detect chondrocalcinosis at baseline and after 24 mo of follow-up. Patients were required to sign an informed consent form, and the protocol was approved by the hospital's Ethics Committee (PI-2295).

Clinical evaluation of calcium pyrophosphate arthropathy and ultrasound assessment of chondrocalcinosis

A clinical assessment every 6 mo during the 24-mo study period was performed to detect the presence of arthropathy secondary to CPPD. For the ultrasound assessments targeted to detect chondrocalcinosis, the hyaline cartilage and fibrocartilage of the medial and lateral menisci, tendons (quadriceps and patellar), and synovial fluid of both knees were explored to detect elemental lesions suggestive of calcium pyrophosphate deposits. To obtain the maximum longitudinal cartilage surface exposure with the knee in maximum flexion, the menisci in flexion (30°) and in full extension, as well as the knee tendons (quadriceps and patellar tendons) were evaluated with the joint in semi-flexion by transverse and longitudinal scans. The ultrasound features associated with calcium pyrophosphate dihydrate crystal deposition were assessed according to the definitions of the OMERACT ultrasound working group: hyperechoic deposits in menisci, hyaline cartilage or synovial fluid, as well as hyperechoic lines of calcification running parallel to tendon fibers.[22,23] For reliability assessment, videos were recorded by a rheumatologist

experienced in ultrasonography and reviewed by an expert in this field. An interobserver reliability analysis was performed between the 2 independent readers, blinded to clinical data, on the recorded videos of the ultrasound examination. A MyLabTwice ultrasound scanner (Esaote) with a multifrequency linear array transducer (4–13 MHz) was used in all patients.

Laboratory methods

For this study, ALP levels and TNSALP substrates (serum PLP and urinary PEA) were assessed at baseline and then yearly during the 24-mo study period. The Laboratory used a Siemens Healthineers (Advia 2400 chemistry system) to measure serum ALP activity. The technical competence of our Clinical Laboratory Service has been accredited with the Internacional Organization for Standardization (ISO) 15189:2013 standard, and the measurement of ALP activity fell within this scope. This method measured ALP activity using a kinetic rate method in which p-nitrophenyl phosphate (a colorless organic phosphate ester substrate) was hydrolyzed by ALP to the yellow-colored product p-nitrophenol and phosphate at pH 10.3. The enzymatic activity of ALP was directly proportional to changes in absorbance at 410 nm. A commercial kit was employed for its measurement in our laboratory, and the normal adult's range is 45 to 116 IU/L according to the reference interval limits given by the manufacturer in the instructions for use document. For PLP, an HPLC method involving a commercial kit from Chromsystems (Teknokroma) was used. PEA measurements were carried out using an in-house liquid chromatography/tandem mass spectrometry (LC–MS/MS) method adapted for quantifying amino acids without derivatization. A normal adult's range is 15–73 nmol/L for PLP and < 70 μmol/g creatinine for PEA, according to intervals recommended for adults by our reference laboratory, which performed the analysis. Patients were required to suspend multi-supplement vitamin intake for at least 1 wk before the analysis, to avoid any interference with the TNSALP substrate measurements, and to fast overnight. Bone metabolism-related parameters such as serum calcium and phosphate 24-hurinary calcium and phosphate excretion were measured by conventional techniques and intact PTH) and 25OHD by chemiluminescent immunoassay on an automated immunoassay and clinical biochemistry analyzer (Atellica-Solution, Siemens Healthineers GmbH).

Statistical analysis

A descriptive analysis of the demographic (age, gender, body mass index) and biochemical variables (ALP, PLP, PEA, parathormone, vitamin D, serum calcium and phosphate, 24-h urinary calcium and phosphate excretion, and serum magnesium) was performed. Continuous variables were described as median (interquartile range, IQR), and categorical variables as absolute number and relative percentage. For the analysis of biochemical parameters, the median was calculated at baseline, 12 and 24 mo in patients with low ALP and a positive and negative *ALPL* genetic test. Comparisons between the 2 groups were performed for continuous variables using a Student's *t*-test for unpaired data if the distribution was normal or a Mann–Whitney U test otherwise. The difference in statistical significance between groups for categorical variables was calculated using a Chi-square test or a Fisher's exact test, as appropriate. Logistic regression models adjusted for confounders were employed to investigate the association between biochemical factors and the presence of chondrocalcinosis in +GT patients. Cohen's kappa coefficient was used for the inter-reader reliability analysis of the ultrasound studies. The level of statistical significance was set at $p<.05$. Statistical analyses were performed with IBM SPSS Statistics 23.0 for Windows.

Results

Seventy-eight subjects with persistently low ALP levels underwent baseline and 24-mo ultrasound screening for chondrocalcinosis. Forty-two of these patients had *ALPL* variants in their genetic assessments and 36 had a negative genetic study. Thirty-nine (92.9%) patients with *ALPL* disease-causing variants (+GT) presented with symptoms potentially related to HPP. No patient received ALP replacement therapy during the study, as we did not have access to treatment. Table 1 includes demographic characteristics and biochemical parameters of those patients with low ALP levels and a positive versus negative *ALPL* genetic test. Table S1 details clinical features in subjects with *ALPL* disease-causing variants.

Among the subjects who had variants in the *ALPL* gene, 35 (83.3%) were heterozygous for pathogenic variants, 5 (11.6%) for a probable pathogenic variant, and 2 (4.7%) patients, who had severe forms of the disease, displayed 2 pathogenic variants in compound heterozygosity. Most of the variants—located in exons 5, 6, and 9—were predicted to have a damaging effect in silico pathogenicity prediction tools and were absent or present at extremely low frequencies in gnomAD. Of the 44 variants found, 35 (79.5%) were missense, 5 (11.4%) duplications, 3 (6.8%) deletions, and 1 (2.3%) an insertion. The most frequent variant was p.(Thr115_Ala116dup), present in 5 patients; p.(Glu291Lys), p.(Gly112Arg), p.(Val128Met), and c.473-2A > G were observed in 3 patients each; and p.(Glu191Lys), p.(Thr166Ile), p.(Asp378Gly), p.(Gly491Arg), and p.(Arg163His) in 2 subjects each. The remaining variants were observed in a single subject.

Excellent reliability between the 2 independent readers was found (Cohen's Kappa: 0.89 for baseline and 24-mo ultrasonography). At baseline, the percentage of ultrasound chondrocalcinosis was higher in the +GT group (9/42 [21.4%]) compared to the −GT group (2/36 [5.4%]; p=.045), and no new cases were detected in the ultrasound evaluation at 24 mo. Chondrocalcinosis was also observed on conventional radiology performed according to clinical practice in 3 of 6 patients (50%) with mutations in *ALPL* and in 1 of 2 (50%) patients without. Two of the patients included in the +GT group (4.8%) suffered from arthritis. One of the patients had been diagnosed with CPPD several years before inclusion in the study, in the presence of knee arthritis and compatible imaging (radiography and ultrasonography) and synovial fluid study. He was treated with colchicine and periodic infiltrations and achieved sustained disease control. The other patient first presented oligoarthritis during follow-up. Subsequently, clinical, analytical, immunological, crystal identification, and imaging evaluations were performed before the patient was finally diagnosed with CPPD. No patient in the −GT group had arthritis. Calcium pyrophosphate deposits and calcifying periarthritis of the shoulder due to HA crystals coexisted in the imaging evaluation of 4 patients (9.5%), who presented a positive genetic test, a finding not observed in the −GT group.

Table 1. Comparative demographic and biochemical data of patients with low alkaline phosphatase levels and a positive versus negative genetic *ALPL* test.

	NR	Total $n=78$	+GT ($n=42$)	−GT ($n=36$)	p value
Age, yr	–	47.7 (40–56.7)	52.8 (38.3–59.6)	47 (40–49.9)	.231
Female sex, *n* (%)	–	60 (76.9%)	29 (69%)	31 (86.1%)	.08
BMI (kg/m ^{2})	–	24.1 (21.9–27.6)	25.5 (22.9–29.2)	22.7 (20.6–24.6)	**<.01**
ALP (UI/L)	45–116	28 (24.9–33)	26.3 (21.8–30)	31 (27–36.9)	**<.001**
PLP (nmol/L)	15–73	145.9 (73.4–284)	278 (186.3–437)	79.5 (57–128.7)	**<.001**
PEA (μmol/g Cr.)	<70	23 (14.8–42.8)	35.5 (23–63)	15 (10.8–21.3)	**<.001**
PTH (pg/mL)	18.5–88	44.9 (38–56.11)	44 (37.1–56.6)	45.5 (38.5–56)	.588
25-hydroxyvitamin D (ng/mL)	30–100	27 (22–34.5)	27 (22.24–34.5)	27.7 (22–35.3)	.55
Calcium (mg/dL)	8.6–10.2	9.52 (9.3–9.8)	9.6 (9.4–9.8)	9.5 (9.2–9.7)	.149
Phosphate (mg/dL)	2.5–4.5	3.7 (3.4–4.1)	3.8 (3.5–4.5)	3.5 (3.4–3.9)	**<.01**
Magnesium (mg/dL)	1.6–2.6	2 (1.8–2.1)	2 (1.9–2.1)	2 (1.8–2.1)	.459
24-h calcium exc. (mg/24 h)	<250	151.5 (113.8–201)	171.5 (134.5–236)	130 (103.9–176.14)	**.043**
24-h phosphate exc. (mg/24 h)	400–1300	724 (629–831.6)	717 (633–871)	714.5 (549–822.5)	.195

Quantitative variables are expressed as median (interquartile range, IQR) and qualitative variables as numbers (percentages). For biochemical determinations, the median (IQR) of the values obtained at 3 visits (baseline, at 12, and 24 mo) was calculated. *P* values in bold are statistically significant. Abbreviations: ALP, alkaline phosphatase; Cr, creatinine; Exc, excretion; NR, biological reference interval; PEA, urinary phosphoethanolamine; PLP, serum pyridoxal-5′-phosphate; PTH, parathyroid hormone.

Table 2. Comparative demographic and biochemical data of patients who had a positive genetic *ALPL* test with and without chondrocalcinosis.

	NR	Presence of chondrocalcinosis ($n=9$)	Absence of chondrocalcinosis ($n=33$)	p value
Age, yr	–	56.3 (39.2–61.3)	50.9 (37.97–60.4)	.487
Female sex, *n* (%)	–	5 (55.6%)	24 (72.7%)	.323
ALP (UI/L)	45–116	17 (11.4–23)	27 (23–30.5)	**<.01**
PLP (nmol/L)	15–73	663.6 (437–863)	260.5 (162.5–310.5)	**<.01**
PEA (μmol/g Cr.)	<70	77.5 (24.5–120)	33.5 (22.25–55)	.096
PTH (pg/mL)	18.5–88	45 (32.15–59)	44 (36.5–56.7)	.786
25-hydroxyvitamin D (ng/mL)	30–100	25 (21–33)	27 (23–35.3)	.631
Calcium (mg/dL)	8.6–10.2	9.6 (9.5–9.9)	9.6 (9.35–9.9)	.695
Phosphate (mg/dL)	2.5–4.5	4.7 (3.8–5.2)	3.8 (3.5–4.3)	**.016**
Magnesium (mg/dL)	1.6–2.6	2 (1.9–2.1)	2 (1.8–2.1)	.984
24-h calcium exc. (mg/24 h)	<250	184.6 (164–195.2)	184.6 (164.3–195.2)	.505
24-h phosphate exc. (mg/24 h)	400–1300	724 (598–937)	724 (598.5–937.5)	.824

Quantitative variables are expressed as median (interquartile range, IQR) and qualitative variables as numbers (percentages). For biochemical assessment, the median (IQR) of the values obtained at 3 visits (baseline, 12, and 24 mo) was calculated. *P* values in bold are statistically significant. Abbreviations: ALP, alkaline phosphatase; Cr, creatinine; Exc, excretion; NR, biological reference interval; PEA, urinary phosphoethanolamine; PLP, serum pyridoxal-5′-phosphate; PTH, parathyroid hormone.

When comparing demographic and biochemical characteristics of those patients who had a positive genetic *ALPL* test with and without chondrocalcinosis (Table 2), age was slightly higher in the first group, although statistically significant differences were not observed (56.3 [39.2–61.3] vs 50.9 [37.9–60.4]; p=.487). The biochemical assessment showed ALP levels were lower (17 [11.4–23] vs 27 [23–30.5]; $p<.01$), while PLP (663.6 [437–863] vs 260.5 [162.5–310.5]; $p<.01$) and serum phosphate levels (4.7 [3.8–5.2] vs 3.8 [3.5–4.3]; p=.016) were significantly higher in the +GT group with chondrocalcinosis ($p<.05$). This trend was also observed for PEA levels (77.5 [24.5–120] vs 33.5 [22.3–55]; p=.096). Sixty-six percent of the +GT subjects with chondrocalcinosis had ALP levels ≤25 IU/L. Excluding from this analysis the 2 subjects with *ALPL* variants in compound heterozygosity, significant differences in ALP levels between the 2 groups were maintained. Logistic regression analysis adjusted for confounders (Table 3) revealed that higher PLP levels were associated with the presence of chondrocalcinosis (Odds Ratio, OR: 1.1; 95% confidence interval: 1.001–1.012), and a trend was observed for lower ALP levels (OR: 0.84 [95% CI], 0.696–1.009). In patients with a positive genetic *ALPL* study and chondrocalcinosis, the pathogenic variants (p.Gly112Arg) and p.(Thr115_Ala116dup) were identified in 2 patients each, and the rest of the mutations in one patient each: p.(Glu291Lys), p.(Gly456Arg), (p.Gly476Lys), p.(Val128Met), p.(Val331Met), (p.Glu191Lys), and (p.Asp378Gly).

Table 3. Results of the logistic regression model adjusted for possible confounders (age and gender) showing the association between biochemical factors and the presence of chondrocalcinosis in +GT patients.

	Beta	Odds ratio	95% CI
ALP levels (UI/L)	−0.18	0.84	0.696–1.009
PLP levels (nmol/L)	0.007	1.1	1.001–1.012
Age, yr	0.05	1.05	0.971–1.143
Female sex	0.39	1.5	0.145–14.917

For biochemical assessment, the median (interquartile range, IQR) of the values obtained at 3 visits (baseline, 12, and 24 mo) was calculated. Abbreviations: ALP, alkaline phosphatase; CI, confidence interval; PLP, serum pyridoxal-5'-phosphate.

Discussion

Chondrocalcinosis and CPPD are clinical manifestations classically related to adult HPP,[24] although there is scarce evidence of their prevalence and biochemical associated factors in the

setting of this pathology. In our work, we observed that ultrasonographic chondrocalcinosis was a distinctive imaging feature of patients with a positive genetic *ALPL* study and was present in up to 21.4% of subjects. Arthropathy secondary to calcium pyrophosphate crystals was, however, less frequent.

In our cohort, with a median (IQR) age of 52.8 (38.3–59.6) yr in the +GT patients and 47 (40–49.9) in the −GT group, the prevalence of chondrocalcinosis was 21.4% and 5%, respectively. Although ultrasound was also employed in this study for its diagnosis, interestingly, the prevalence in subjects with *ALPL* variants in this study was significantly higher than that found in other studies for the general population after adjusting for age (3.7% in patients aged 55–59 yr in the UK[25] and 7% in patients aged 60–69 yr in Spain.[26] On the other hand, data from the Global HPP Registry, an observational, prospective, multinational study including subjects with *ALPL* variants, showed a lower prevalence of chondrocalcinosis (4.4%) and pseudogout (5.8%) in adults.[27] By contrary, Schmidt et al.[17] detected a higher prevalence (21%) and also Berseth et al., with an estimated prevalence of chondrocalcinosis and pseudogout of 27% and 13.6%, respectively.[16] In another study conducted in our country,[28] a prevalence of 14.3% of symptomatic chondrocalcinosis was found among subjects with *ALPL* variants. In our cohort, before applying a specific ultrasound protocol for its detection, the prevalence was lower (7.1%), while its use increased the diagnostic capacity of subclinical forms by almost 15%, proving to be of great utility for this purpose.

The formation of crystals of calcium pyrophosphate is mainly limited to collagen-formed tissues, such as fibrocartilage, hyaline cartilage, tendons, and capsules.[18] A greater biochemical impact of the disease, represented by higher PLP and a trend to lower ALP levels, was associated with the presence of chondrocalcinosis in HPP. The deposition of calcium pyrophosphate crystals probably resulted from extracellular excesses of PPi, together with the failure of TNSALP to dissolve the calcium pyrophosphate crystals.[29] These crystals, once formed, can mediate tissue damage through several mechanisms, such as activating inflammasome components, generating inflammation, and the catabolic effect on chondrocytes and synoviocytes, ultimately worsening joint damage by attaching to the cartilage surface in the form of wearing particles.[30] The presence of knee deposits of calcium pyrophosphate crystals has been associated with knee osteoarthritis.[30] In addition, arthritis flares can result in temporary but profound disability, affecting patients' ability to perform routine activities.[31] These manifestations must also be taken into account when treating adults with HPP, as they may contribute to deteriorations in physical function, worsening both the ability to perform basic activities of daily living and quality of life, which has already been described in this disease.[32]

Moreover, in our study, 4 patients also presented calcifying periarthritis in tandem with chondrocalcinosis. Two of these patients were members of the same family and displayed the same *ALPL* variant in heterozygosis (p.Thr115_Ala116dup). Although the genotype–phenotype correlation in HPP has been difficult to establish, due to the wide spectrum of mutations among other factors,[33,34] cases of calcifying periarthritis[29] or chondrocalcinosis[14] in several members of the same affected family have been described, as well as in this study, showing that familial aggregation should alert clinicians to the possible presence of an underlying metabolic bone disease.

The limitations of this study include the small sample size, although it was nevertheless considerable for such a rare disease. In addition, most patients harbored heterozygous variants, which could be related to milder forms. Among the strengths, we find that this is one of the largest cohorts featuring subjects with persistent hypophosphatasemia and a positive or negative genetic test, as well as the possibility of having performed the TNSALP substrate measurements and a genetic study in this population. Furthermore, to our knowledge, this is the first study to incorporate an ultrasound scanning protocol to detect chondrocalcinosis in HPP, which seems to detect a higher number of calcium pyrophosphate deposits than conventional radiology.[35,36] The limitations of this study include the small sample size, although considerable for such a rare disease, and that most of the patients included harbored heterozygous variants, which could be related to milder forms of the disease. Another limitation related to the ultrasound protocol is that it included the evaluation of calcium pyrophosphate deposits only in the knees, the most frequently involved joint,[37] but other locations, such as the triangular ligament of the wrist, were not explored, due to a greater degree of uncertainty and lower reliability and sensitivity, especially in the early stages of the disease, with low calcium pyrophosphate deposits. Therefore, it is possible that the inclusion of other locations could have reported a slight increase in the prevalence of chondrocalcinosis.[38,39] This study reveals that chondrocalcinosis was a frequent finding in our cohort of subjects with *ALPL* variants, detected in up to 21.4% of patients. Arthritis secondary to calcium pyrophosphate deposits, however, proved less prevalent. A greater biochemical impact of the disease, represented by higher serum PLP and a trend to lower ALP levels, was associated with the presence of chondrocalcinosis in these patients. Our results confirm that HPP should be considered as a possible metabolic cause in patients with chondrocalcinosis and should prompt clinicians to look for the distinctive features of the disease to achieve an early diagnosis.

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Author contributions

Pilar Aguado conceived the idea for this study and participated in its design and coordination, supervising all the process. Carolina Tornero, Pilar Aguado, and Alejandro Balsa recruited and evaluated the subjects. Carolina Tornero and Eugenio de Miguel evaluated ultrasound studies. Carolina Tornero wrote the original draft. Carolina Tornero, Pilar Aguado, Victoria Navarro-Compán , Eugenio de Miguel, and Alejandro Balsa interpreted the data and participated in the review and editing of the draft. All authors were involved in reviewing the manuscript, gave final approval of the version to be published and agreed to be accountable for all aspects of their work.

Carolina Tornero (Data curation, Formal analysis, Investigation, Methodology, Writing—original draft, Writing—review & editing), Eugenio de Miguel (Investigation, Methodology, Writing—review & editing), Victoria Navarro-Compán (Investigation, Methodology, Writing—review & editing), Alejandro Balsa (Investigation, Methodology, Writing—review & editing), and Pilar Aguado (Conceptualization, Investigation, Methodology, Supervision, Writing—review & editing).

Supplementary material

Supplementary material is available at *JBMR Plus* online.

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Conflicts of interest

P.A. and C.T. have received fees for advisory board participation from Alexion Pharmaceuticals and have been recipients of a research grant from this biopharmaceutical company, but not for this original manuscript. The rest of authors declare that they do not have any other conflict of interest regarding the publication of this article.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The study adhered to the tenets of the Declaration of Helsinki and approval was obtained from La Paz University Hospital's ethics committee. Each subject provided written informed consent prior to inclusion.

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