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Uncovering genetic causes of hypophosphatemia

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Background. Chronic hypophosphatemia can result from a variety of acquired disorders, such as malnutrition, intestinal malabsorption, hyperparathyroidism, vitamin D deficiency, excess alcohol intake, some drugs, or organ transplantation. Genetic disorders can be a cause of persistent hypophosphatemia, although they are less recognized. We aimed to better understand the prevalence of genetic hypophosphatemia in the population.

Methods. By combining retrospective and prospective strategies, we searched the laboratory database of 815,828 phosphorus analyses and included patients 17–55 years old with low serum phosphorus. We reviewed the charts of 1287 outpatients with at least 1 phosphorus result ≤2.2 mg/dL. After ruling out clear secondary causes, 109 patients underwent further clinical and analytical studies. Among them, we confirmed hypophosphatemia in 39 patients. After excluding other evident secondary causes, such as primary hyperparathyroidism and vitamin D deficiency, we performed a molecular analysis in 42 patients by sequencing the exonic and flanking intronic regions of a panel of genes related to rickets or hypophosphatemia (CLCN5, CYP27B1, dentin matrix acidic phosphoprotein 1, ENPP1, FAM20C, FGFR1, FGF23, GNAS, PHEX, SLC34A3, and VDR).

Results. We identified 14 index patients with hypophosphatemia and variants in genes related to phosphate metabolism. The phenotype of most patients was mild, but two patients with X-linked hypophosphatemia (XLH) due to novel PHEX mutations had marked skeletal abnormalities.

Conclusion. Genetic causes should be considered in children, but also in adult patients with hypophosphatemia of unknown origin. Our data are consistent with the conception that XLH is the most common cause of genetic hypophosphatemia with an overt musculoskeletal phenotype.

Keywords: familial hypophosphatemia, fibroblast growth factor 23, phosphate, vitamin D, X-linked hypophosphatemia

Introduction

Phosphate plays several functions in the homeostasis of the human body. Many intracellular activation cascades commonly add phosphate groups and accumulate ATP to store energy for various cellular processes. Extracellular phosphate must be available to form the mineral phase of calcified tissues, such as bone and teeth, and to maintain their material properties. Therefore, hypophosphatemia may impair various cellular functions and the structural properties of the skeleton [1].

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Hypophosphatemia is not uncommon. It frequently accompanies severe acute disorders. It may be present in approximately 5% of hospitalized patients and up to 60% of those admitted to intensive care units. Acute hypophosphatemia may cause muscle weakness, respiratory insufficiency, myocardial dysfunction, and neurological and hematological disorders. Thus, hypophosphatemia is associated with poor patient outcomes [2, 3]. In chronic hypophosphatemia, skeletal manifestations may appear, such as bone pain and deformity, and complete and incomplete fractures [4–6]. However, most "routine" metabolic panels do not include phosphate. Therefore, hypophosphatemia may go unrecognized without proper awareness of this diagnosis by clinicians facing patients with musculoskeletal complaints and other nonspecific conditions.

Parathyroid hormone (PTH), calcitriol (1,25-dihydroxyvitamin D; $1,25(OH)_2D$), and several phosphatonins—in particular, fibroblast growth factor 23 (FGF23)—maintain phosphate balance by regulating intestinal absorption and renal reabsorption. PTH and FGF23 tend to lower serum phosphate, whereas the actions of $1,25(OH)_2D$ in the intestine and the kidney increase serum phosphate levels. In turn, serum phosphate can regulate the production of PTH, $1,25(OH)_2D$, and FGF23 [7–10] by feedback mechanisms.

Chronic hypophosphatemia may be caused by a variety of acquired disorders, such as malnutrition, intestinal malabsorption, and alcoholism. Sometimes, the decrease in serum phosphate has genetic causes [1, 11, 12]. Those cases are usually diagnosed in infancy because they typically show retarded growth and rickets manifestations. X-linked hypophosphatemia (XLH) is considered the most common genetic hypophosphatemia, with a prevalence of about 1/20,000 [4, 12, 13]. However, the generalization of new DNA sequencing technologies is revealing that a significant proportion of hereditary diseases may present a mild phenotype that may become evident only in adulthood [14–16]. Thus, hypophosphatemia may go unrecognized, and genetic causes may be disregarded, particularly in adult patients [1, 17]. The motivation for this study was to obtain a better knowledge of the prevalence of genetic hypophosphatemia in the population.

Materials and methods

Study design and subjects

We conducted the study at the Hospital Universitario Marqués de Valdecilla, a tertiary care center in Cantabria, a region in Northern Spain with a population of 550,000. The hospital serves as the primary care center for a population of approximately 320,000 and as the reference center for the rest of the region, which is also served by two community hospitals.

The study included both a retrospective and a prospective arm. In the retrospective arm, we performed a computerized search of the laboratory database between October 2002 and January 2020. We sought patients 17–55 years old with at least one determination of serum phosphorus <2.4 mg/dL. We excluded inpatients and outpatients from medical services likely having secondary hypophosphatemia (eating disorders and alcohol disorder units, gastrointestinal surgery, transplant, antiretrovirals drugs, and iron infusions).

We conducted the prospective arm between January 2020 and March 2021. Within that period, a phosphorus test was added to all lab requests from patients between 17 and 55 years old. At this stage, we included all lab requests in the region of Cantabria, and inclusion criteria were equal to the retrospective arm. Thus, besides Hospital Marqués de Valdecilla, other public community hospitals participated in the study. This was done by implementing an algorithm within the common system for ordering lab tests.

We identified patients with hypophosphatemia from the retrospective and prospective arms, and we reviewed clinical records, paying particular attention to previous phosphorus tests, musculoskeletal manifestations, and other causes of secondary hypophosphatemia. Finally, those with persistent hypophosphatemia (i.e., at least one phosphorus ≤2.2 mg/dL and none within the reference range of 2.4–5.1 mg/dL), in the absence of disorders or drug therapies known to cause hypophosphatemia, were invited to attend our outpatient clinic for clinical evaluation and additional biochemical or genetic tests. This invitation was issued by telephone, or through a letter, if it was not feasible to contact them by phone.

Laboratory procedures

Serum samples were obtained in the morning after an overnight fast. We analyzed creatinine, calcium, phosphorus, alkaline phosphatase, albumin, and intact PTH in the Atellica CH&IM platform (Siemens Healthcare Diagnostics, Malvern, PA, USA), 25-hydroxycholecalciferol (calcidiol) levels by an automated competitive chemiluminescence assay (Liaison XL, DiaSorin Inc, Stillwater, MN, USA), and FGF23 levels by Human FGF23 (Intact) ELISA (Immunotopics International, San Clemente, CA, USA). This FGF23 ELISA kit has a sensitivity <1 pg/mL and 3.5% intra-assay and 6.3% inter-assay reproducibility, and it does not have cross-reactivity with N-terminal (25–179) or C-terminal (180–251) fragments. We included nine subjects (mean age 39 ± 12) with normal phosphorus levels as a control group for FGF23 results. We used the following formula to calculate tubular reabsorption of phosphate (TRP): 1− [(urine P × serum creatinine)/(serum P × urine creatinine)] × 100, and the Walton–Bijvoet nomogram to estimate tubular maximum reabsorption of phosphate per volume of filtrate (TmP/GFR) [18].

In patients undergoing genetic study, we analyzed a custom panel of 12 genes related to phosphorus metabolism (ALPL, CLCN5, CYP27B1, dentin matrix acidic phosphoprotein 1 [DMP1], ENPP1, FAM20C, FGF23, FGFR1, GNAS, PHEX, SLC34A3, and VDR). We prepared DNA libraries with the SureSelect QXT kit (Agilent) and sequenced them in the MiSeq platform (Illumina). Base calling was established with a Q30 > 90%, and reads were aligned to the GRCh37 reference genome. For the filtering and analysis of variants, we used the Alissa platform (Agilent), and variants were classified according to the criteria of the American College of Medical Genetics and Genomics (ACMG) [19]. We performed copy number variation analysis with DECoN v1.0.2 software [20]. We used the combined annotation-dependent depletion (CADD) as a predictor algorithm to measure the deleteriousness of genetic variants. A CADD score >15 is expected to identify the potentially deleterious variants in the genome [21].

Ethical statements

The study was approved by the institutional review board Comité de Ética de Investigación con Medicamentos de Cantabria. All patients evaluated at the clinic signed informed consent.

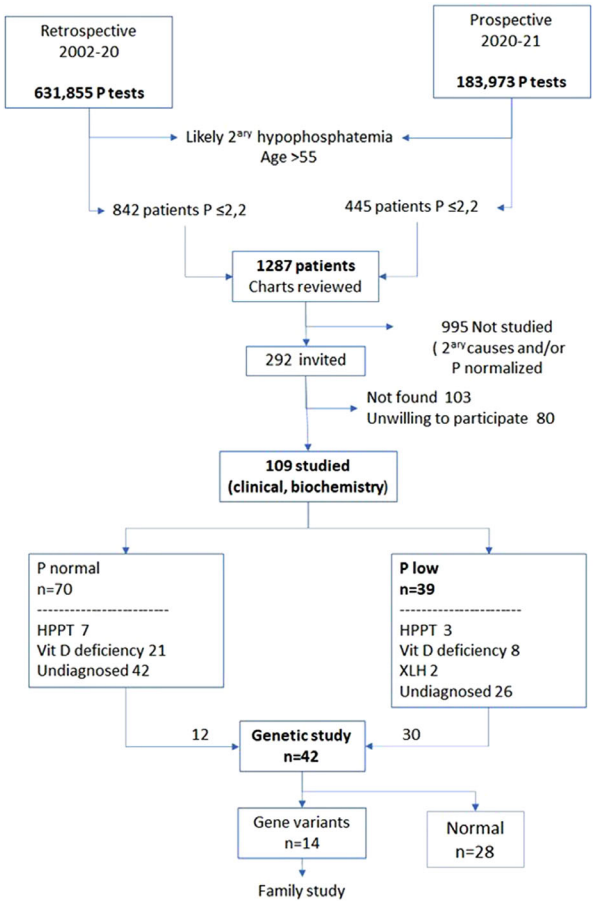

Fig. 1 *Study flow. HPPT, hyperparathyroidism; P, serum phosphate; XLH, X-linked hypophosphatemia.*

Results

In the retrospective phase, we reviewed 631,855 analyses of serum phosphorus (performed in 208,245 patients between October 2002 and January 2020). Among them, 2928 analyses in 1350 patients who fulfilled the inclusion criteria were below the 2.4 mg/dL lower limit of the normal range; 842 patients had a phosphorus level ≤2.2 mg/dL at least once.

In the prospective study, the phosphorus tests performed as part of this study, along with other phosphorus tests directly requested by attending physicians, included 183,973 phosphorus analyses. Among those tests, we identified 445 patients with at least 1 phosphorus test ≤2.2 mg/dL (Fig. 1).

Thus, we reviewed the charts of 1287 patients with at least 1 phosphorus ≤2.2 mg/dL. The

Table 1. *Characteristics of patients with hypophosphatemia at the study visit*

Parameter	*N* (%)
Sex	
Male	30 (77)
Female	9 (23)
Alcohol	
Daily consumption	10 (26)
Weekly consumption	12 (31)
Former drinker	17 (43)
History of fracture	24 (61)
Nontraumatic fracture	1 (3)
Dental problems	15 (39)
Hypercalcemia (>10.4 mg/dL)	1 (3)
High PTH (>88 pg/mL)	7 (18)
25 hydroxyvitamin D, ng/mL	
>30	6 (15)
30–20	18 (46)
<20	15 (39)
1,25 hydroxyvitamin D, pg/mL	
≥66	0
66–26	15 (39)
≤25	13 (33)
Fibroblastic grow factor, pg/mL	
>50	7 (18)
31–50	7 (18)
≤30	16 (41)

Abbreviation: PTH, parathyroid hormone.

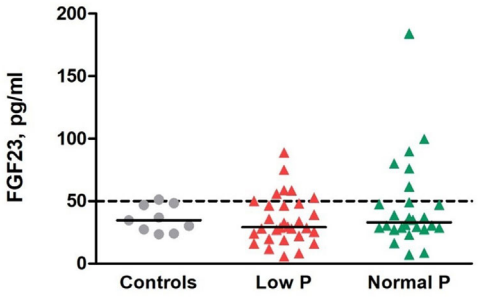

Fig. 2 *Fibroblast growth factor 23 (FGF23) levels in patients attending the study visit. A control group is shown for comparison.*

Table 2. Index patients and relatives with mutations.

Gene	Index patients	Relatives
SLC34A3	3	3
VDR	3	1
PHEX	2[a]	
CYP27B1	1	2
DMP1	1	1
CLCN5	1	1
SMAD3	1	
FGFR1	1	
ENPP1	1	
Total	14	8

Abbreviations: DMP1, dentin matrix acidic phosphoprotein 1; XLH, X-linked hypophosphatemia.
[a]Two sisters with known XLH.

mean age of patients was 45 ± 8 years. In 995 cases, other phosphorus analyses were within the reference range, or existing underlying disorders explained hypophosphatemia. The most common causes were hyperparathyroidism, vitamin D deficiency, excess alcohol intake, and organ transplantation. We selected the remaining 292 patients for clinical interviews, physical examinations, and further tests. We were not able to contact 103 patients by telephone or postal mail (most of them from the retrospective study arm), and 80 declined to participate.

Finally, 109 patients with prior hypophosphatemia attended the clinic for the study visit (Fig. 1). The new analyses revealed normal levels of serum phosphorus in 70 cases and confirmed hypophosphatemia in 39 patients. Of the 70 patients with normal serum phosphorus, 7 were diagnosed with primary hyperparathyroidism and 21 with vitamin D insufficiency. Among the 39 patients with hypophosphatemia (Table 1), 3 had primary hyperparathyroidism, 8 had vitamin D deficiency, 2 had a previous clinical diagnosis of XLH, and the cause of hypophosphatemia was unknown in 26 cases. We measured serum FGF23 in 54 patients. In 26 cases, it was above 30 pg/mL, which is usually regarded as the upper limit of the normal range [22, 23]. However, there was a marked overlap between groups (Fig. 2).

Overall, 42 patients underwent genetic analysis (30 with low serum phosphorus at the study visit and 12 with normal phosphorus at the study visit but previously unexplained low phosphorus levels). In 28 cases, the results were normal, whereas 13 patients carried potentially pathogenic (likely pathogenic or unknown significance) variants of genes related to phosphorus metabolism (including two with normal serum phosphorus at the study visit). The genes with allelic variants are shown in Table 2, and individual biochemical and genetic details are shown in Table 3. Variants of

Table 3. Index patients with genetic variants.

Family	Sex	Age	Gene	Variant	CADD score	ACGM classification	Serum phosphorus at study visit, mg/dL	Lowest serum phosphorus, mg/dL	Serum calcium, mg/dL	PTH, ng/mL	25(OH)D, mg/mL	$1,25(OH)_2D$, pg/mL	FGF23, pg/mL
1	F	52	SLC34A3	Tyr414Ter	40	LP	2.1	2.1	10	95	25	21	38
2	M	52	VDR	Ala303Ala[a]	–	VUS	2.3	2.3	10.7	69	30	46	52
3	M	49	CYP27B1	Arg104Trp	24.2	VUS	2.6	2.1	9.4	62	12	12	200
4	M	51	VDR	Asp65Gly	23.6	VUS	2.2	1.7	9.4	95	23	30	46
5	F	53	SLC34A3	Arg485His	27.2	LB	1.8	1.6	10.3	47	21	14	20
6	M	49	DPM1	Asp478Ala	22.7	VUS	2	1.9	9.2	56	31	–	6
7	M	43	SLC34A3	c.1094-14G > A (p.?)[a]	–	VUS	2.1	1.8	10.3	55	16	7	29
8	F	43	SMAD3	Glu52Lys	25.9	VUS	2.7	2.1	9.7	59	36	–	7
9	M	41	CLCN5	Val699Ile	22.2	VUS	2	1.8	9.5	58	24	23	53
10	M	27	VDR	Met1	24	VUS	1.5	1.5	9.5	52	11	33	22
11	M	50	FGFR1	Ser219Phe	26.8	VUS	1.8	1.8	10.2	55	11	35	28
12a	F	32	PHEX	Phe654Ser		LP	2.2	2.2	9.6	85	8	19	89
12b[b]	F	33	PHEX	Phe654Ser	29.7	LP	1.7	1.9	9.2	100	25	28	50
13	F	44	ENNP1	His535Arg	25.5	VUS	2.3	2.1	8.1	38	26	71	36

Abbreviations: CADD, combined annotation-dependent depletion; LB, likely benign; LP, likely pathogenic; PTH, parathyroid hormone; VUS, variant of unknown significance.

[a]Possible splicing interference.

[b]Patients 12a and 12b are siblings.

SLC34A3, VDR, and *PHEX* were the most common, each being present in three and two patients. One patient with low serum phosphorus without clear cause had an *SLC34A3* variant classified as likely benign (Table 3). We also studied 10 relatives of six patients with gene variants; 8 of them also carried the variant present in the index individual (Table 2). Pedigree details are shown in Table S1 and Fig. S1.

Most patients were asymptomatic or had mild manifestations, including mild dental problems ($n = 10$), osteoarthritis ($n = 5$), and tendinopathy ($n = 1$). One patient with an *SLC34A3* variant had mild hypercalciuria and a small kidney stone. One patient with an *SMAD* variant had suffered a hip fracture after a minor trauma.

The two sister patients with PHEX mutations had a severe skeletal phenotype with short stature (132 and 135 cm, respectively) and, in one case, severe leg deformities that required corrective surgery. We observed a mild increase in FGF23 levels (50 and 89 pg/mL, respectively). We presumed that the father had transmitted the variant to his offspring because their mother had a normal phenotype, whereas the father was referred to as having short stature, but we could not study him. These two patients had a novel PHEX missense variant (Phe654Ser) that had not been previously reported. Several bioinformatic predictors considered it damaging, and no predictor considered it benign. In addition, an alternative variant at the same residue (Phe654Ile) has been reported in ClinVar as likely pathogenic. Therefore, the Phe654 variant was classified as likely pathogenic.

Discussion

The main objective of this study was to uncover genetic causes of hypophosphatemia in adults by performing a retrospective and prospective search of laboratory databases targeting over 200,000 patients in a region with roughly 470,000 individuals over 17 years of age. By using a stepwise approach, we identified 14 individuals with persistent hypophosphatemia presumably related to allelic variants of genes related to phosphate metabolism (Table 2).

Allelic variants in *SLC43A3* and *VDR* were the most frequent (each found in three patients). *SLC43A3* encodes the renal sodium–phosphate cotransporter NPTC2. Biallelic mutations cause hypophosphatemic rickets with hypercalciuria, whereas heterozygous individuals may show mild hypophosphatemia or hypercalciuria [24].

The possibility of performing segregation analyses was limited. Nevertheless, one 17-year-old son of the index patient of family 1 carried the SLC34A3 nonsense variant and had a phosphate level of 3.5 mg/dL, which may be difficult to interpret before finishing the growth period. Two relatives of the index patient of family 7 carried the same SLC34A3 mutation and had serum phosphate close to the lower limit of the normal range (2.5 and 2.7 mg/dL). TRP was inappropriately normal, and TmP/GFGR was below normal limits. We could not study any relative of the index patient in family 5, who carried a missense variant.

VDR encodes the vitamin D receptor, which mediates the classical effects of vitamin D metabolites—specifically $1,25(OH)_2D$, the most active one. Biallelic pathogenic variants of *VDR*, either single-nucleotide or structural, cause vitamin D-dependent rickets type 2A [25]. Three patients with mild hypophosphatemia were heterozygous carriers of variants of unknown significance (VUS) in *VDR*, without other relevant clinical manifestations or compensatory increases in $1,25(OH)_2D$. Thus, the exact role of the heterozygous genetic variants is unclear. A son of the patient of family 4 had normal serum phosphate despite carrying the allelic variant, a result that does not support its functional relevance. The insufficient 25-hydroxyvitamin D levels in one patient may have contributed to hypophosphatemia.

CYP27B1 encodes the 1 α-hydroxylase that converts 25-hydroxyvitamin D into $1,25(OH)_2D$. Pathogenic variants of *CYP27B1* cause the autosomal recessive disease called vitamin D-dependent rickets type 1A. In our series, one patient (in family number 2) carried a monoallelic VUS in this gene. The low circulating levels of $1,25(OH)_2D$ are consistent with diminished enzymatic activity. However, FGF23 serum levels were increased, which was unexpected, as $1,25(OH)_2D$ is a positive regulator of FGF23 expression [26]. By contrast, two family members who carried the same mutation had normal phosphate and FGF23 levels. Although the exact pathogenic mechanism of hypophosphatemia in this patient is unclear, it could be FGF23-mediated, in relation to an unidentified disorder causing high FGF23 levels. It should be highlighted that we did not find any evidence of

oncogenic osteomalacia, a disorder usually related to excess FGF23 [7].

Homozygous mutations of the DMP1 gene cause autosomal recessive hypophosphatemic rickets type 1 and may be associated with enthesopathy. DMP1 is critical for osteoblast–osteocyte differentiation, which is important for mineralization. DMP1 deficiency increases FGFG23 levels by poorly understood mechanisms [27]. The index patient in family 6 had a *DMP1* missense variant of unknown significance (Arg478Ala). Serum FGF23 levels were within the normal range, and a sibling carrying the same variant had normal phosphate and FGF23 levels. Both findings argue against the pathogenic role of the Arg478Ala variant.

Ectonucleotide pyrophosphatase/phosphodiesterase 1 *(ENPP1*) catalyzes the hydrolysis of ATP/GTP to AMP/GMP, thus generating pyrophosphate. Alkaline phosphatase hydrolyzes pyrophosphate to form two phosphate molecules that the mineralizing osteoid incorporates. Overall, *ENPP1* activity increases phosphate levels by providing a source of phosphate, as well as decreasing its urinary elimination by inhibiting FGF23. Pathogenic variants of *ENPP1* may have various phenotypic consequences, such as arterial calcification, hearing loss, ossification of the posterior longitudinal ligament, pseudoxanthoma elasticum, or autosomal recessive hypophosphatemic rickets type 2[28].

It has been recently described that ENPP1 exerts a gene dose effect that may be responsible for a mild disease with hypophosphatemia and early onset osteoporosis in individuals heterozygous for ENPP1 variants [29]. That might be the case for one patient in our series, but it was not possible to perform a segregation analysis.

Dent disease type 1 is renal tubular dysfunction, characterized by proteinuria, hypercalciuria, nephrocalcinosis, nephrolithiasis, and hypophosphatemia, among other manifestations [30]. It is caused by pathogenic variants of CLCN5. This gene encodes a voltage-dependent chloride/proton exchanger, and it is located on Xp11.23. As an X-linked disease, it manifests in hemizygous men. Female carriers are usually asymptomatic, but some may have mild manifestations due to skewed X-chromosome inactivation. An index patient in this series and his brother, both with persistent hypophosphatemia, carried a CLCN5 variant. However, another noncarrier brother had normal serum phosphorus. Thus, although classified as VUS, the missense Val699Ile variant seems to be functionally relevant.

TGFβ is known to have complex effects on skeletal homeostasis, and *SMAD3* mutations have been associated with the Loeys–Dietz syndrome and other skeletal phenotypes [31–33]. A 43-year-old woman with spondyloarthropathy and an atraumatic hip fracture carried a VUS in the *SMAD3* gene, which encodes a protein involved in TGFβ signaling. The patient in this series presented intermittent hypophosphatemia, with serum values between 2.1 and 2.7. A patient's daughter who did not carry the variant had normal phosphate. Thus, although there is no reported association between *SMAD3* variants and hypophosphatemia, this issue merits further investigation.

Pathogenic variants of *FGFR1* have been associated with several skeletal phenotypes [34]. An Ser219Phe variant of the fibroblast growth factor receptor 1 *(FGFR1*) was observed in a 50-year-old man with asymptomatic persistent hypophosphatemia (1.8–2.4 mg/dL) and low TRP. The variant was present in 16% of reads, thus suggesting mosaicism. It had not been previously reported and was classified as VUS. Some patients also present hypophosphatemia, due to increased urinary losses. The mechanism has not been fully elucidated, but it may include the klotho-induced conversion of FGFR1 into a FGF23 receptor [35]. Mosaic status in this patient might explain the mild phenotype.

PHEX gene is located on the short arm of the X chromosome and encodes an endopeptidase expressed mainly in osteoblasts, osteocytes, and teeth. More than 800 different mutations have been found. In most cases, it is inherited with an X-linked dominant pattern, but about one third of patients may be due to "de novo" mutations, as they lack a family history [36, 37]. Two patients with typical XLH due to *PHEX* mutations were also identified in our study. They had short stature and variable bone deformities. They had been diagnosed in infancy but had been lost to follow-up. The Phe654Ser variant, presumably of paternal origin, had not been previously reported.

Most of the patients in this series, other than those with PHEX mutations, either had no symptoms or had minor ailments such as enthesopathy and slightly premature tooth loss, which

cannot be linked with certainty to hypophosphatemia. Thus, the clinical relevance of the mutations found is unclear despite their association with low serum phosphate. Nevertheless, we do not know if those may be evolving disorders with more clinical manifestations appearing with aging.

Two patients with XLH showed important clinical manifestations, including short stature, bone deformities, and musculoskeletal pain because of rickets, despite having been treated with active vitamin D and phosphate supplements since childhood. Burosumab availability will certainly improve the prognosis of patients in the future [38]. XLH is considered the most frequent genetic origin of hypophosphatemia. Given our data, this concept seems questionable. However, XLH certainly seems to be the most common genetic cause of clinically significant hypophosphatemia. The frequency has been estimated at 1/25,000 live births [37], but the prevalence would be considerably lower in our study (roughly 1/200,000). Although we may have missed some patients, the number is unlikely to rise until those figures are retrieved. Therefore, XLH is less frequent in our region than in other regions, such as the United Kingdom, with an estimated prevalence of 15 cases per million people [39]. However, it is not very different from prevalence reported in other countries, such as Norway, where Rafaelsen et al. identified about four cases per million inhabitants (1 per 60,000 children) [40].

Interestingly, several individuals with hypophosphatemia intermittently showed serum phosphate levels within the normal range (although usually within the lower region of the reference range). Serum phosphate depends on meal ingestion and other factors. So, we do not know if those changing values are due to incomplete fasting or reflect changes in the homeostatic status. Whatever the cause may be, it has important practical implications, both for the clinic and further epidemiological studies, as isolated phosphate values within the normal range should not be considered a strong argument against a genetic abnormality or other underlying disorder causing hypophosphatemia.

A major strength of this study derives from the large database, which included various individuals identified over a long period. However, it also has some limitations. The retrospective nature of the first phase and the pragmatic approach of the prospective one limited the clinical data available. A significant proportion of patients were lost to follow-up or elected not to participate in the study. None of them had severe skeletal manifestations that could have suggested the existence of overt rickets history or osteomalacia. In addition, some patients lacked biochemical urinary parameters because we focused on serum biochemical tests and genetic analysis, given the primary objective of the study. Moreover, the absence of enough relatives with and without hypophosphatemia limited the possibility of performing segregation analysis and the elucidation of the pathogenicity of some allelic variants, consequently.

In conclusion, after an extensive search of laboratory databases, we identified 14 index patients with hypophosphatemia and variants in genes related to phosphate metabolism. The phenotype of most patients was mild, but two patients with XLH due to novel PHEX mutations had marked skeletal abnormalities. Although the frequency of XLH seems to be lower in our region than in other populations, our data are consistent with the conception that XLH is the most common cause of genetic hypophosphatemia with an overt musculoskeletal phenotype, and other mild phenotype hereditary diseases are being uncovered thanks to the use of new DNA sequencing techniques.

Author contributions

Supervision; data curation; investigation; formal analysis; resources; writing—original draft; writing—review and editing; visualization; project administration: Nuria Puente-Ruiz. *Data curation; investigation; formal analysis; resources; writing—review and editing; visualization*: Pablo Docio. *Conceptualization; methodology; supervision; writing—review and editing; visualization; funding acquisition; project administration*: María T. García Unzueta. *Data curation; resources; writing—review and editing; visualization*: Bernardo A. Lavín. *Investigation; writing—review and editing; visualization*: Ainhoa Maiztegi. *Data curation; investigation; writing—review and editing; visualization*: Ana Isabel Vega. *Resources; writing—review and editing; visualization*: María Piedra. *Conceptualization; methodology; writing—review and editing; visualization*: Leyre Riancho-Zarrabeitia. *Investigation; writing—review and editing; visualization*: Fátimay Mateos. *Data curation; writing—review and editing; visualization*: Domingo Gonzalez-Lamuño.

Writing—review and editing; visualization: Carmen Valero. *Conceptualization; methodology; supervision; formal analysis; writing—original draft; writing—review and editing; visualization; funding acquisition; project administration*: José A. Riancho.

Conflict of interest statement

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