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ORIGINAL ARTICLE

Congenital hypophosphataemia in adults: determinants of bone turnover markers and amelioration of renal phosphate wasting following total parathyroidectomy

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Abstract

Congenital hypophosphataemia (CH) is a collection of disorders that cause defective bone mineralisation manifesting with rickets in childhood and osteomalacia in adulthood. Bone turnover markers (BTMs) are surrogate measures of metabolic bone disease severity. We explored the utility of BTMs in 27 adults with CH: 23 had X-linked hypophosphataemia (XLH), of whom 2 were hypoparathyroid post-total parathyroidectomy (PTx); 2 had autosomal dominant hypophosphataemic rickets (ADHR), and 2 had none of the known mutations. We measured the renal tubular maximum reabsorption rate of phosphate (TmP/GFR), C-terminal fibroblast growth factor 23 (FGF23), parathyroid hormone (PTH), ionised calcium, 1,25-dihydroxy-vitamin D [1,25$(OH)_2$D], and a panel of BTMs: serum bone-specific alkaline phosphatase (bone ALP), osteocalcin (Oc), total procollagen type I amino-terminal propeptide (PINP), and carboxy-terminal telopeptide of type I collagen (CTX); and urine amino-terminal telopeptides of type I collagen (uNTX). After excluding 2 patients with XLH and PTx, the frequency of abnormal elevation in BTMs was: bone ALP (96%); CTX (72%); PINP (52%); uNTX (48%); Oc (28%). The strongest association with bone ALP was TmP/GFR. Those patients receiving phosphate supplements and alfacalcidol had significant elevation in CTX. The 2 patients with XLH and PTx had normalisation of TmP/GFR and near normalisation of BTMs post-operatively, despite marked elevation in both C-terminal and intact FGF23. In conclusion, BTMs in our CH patients indicated that most have abnormalities consistent with osteomalacia and many have mild secondary hyperparathyroidism; and the normalisation of TmP/GFR after total PTx in 2 cases of XLH remains unexplained, but possible causes are speculated.

Keywords Hypophosphataemia · Rickets · Secondary hyperparathyroidism · FGF23

Introduction

Chronic hypophosphataemia results in defective mineralisation of bone that manifests as rickets in childhood and as osteomalacia in adults [1]. The principal regulators of phosphate homeostasis are PTH, activated vitamin D, and FGF23 [2]. In healthy individuals, an increase in serum inorganic phosphate concentration induces secretion of both FGF23 and PTH, resulting in reduced expression of the sodium-dependent phosphate co-transporters, NaPi-2a and NaPi-2c, in the proximal renal tubule increasing urinary phosphate excretion [3]. Calculation of TmP/GFR using serum and urine creatinine and phosphate measurements in timed paired fasting samples facilitates discrimination between renal or non-renal mediated hypophosphataemia [4, 5]. While it is straightforward to diagnose renal phosphate wasting by measurement of TmP/GFR, further testing is needed to discriminate between the potential causes by estimation of FGF23 and by genetic testing. Most CH cases are diagnosed in early childhood with the most common diagnosis being XLH. ADHR has a variable phenotype that may not manifest until adulthood [1]. Both XLH and ADHR are usually associated with elevated FGF23 [1]. Genetic

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testing distinguishes between these two conditions as well as identifying ever rarer mutations, but there are still a small number of cases with unknown mutations.

Adults with CH still have the same metabolic aberrations as in childhood with reduced TmP/GFR, reduced serum phosphate, inappropriately low/normal serum $1,25(OH)_2D$, and osteomalacia. The management and surveillance of CH in adulthood is poorly studied. It is debatable whether adults with CH benefit from the standard treatments that are administered to children, namely activated vitamin D and phosphate supplements [6–8]. Indeed, Shanbhogue et al. in a longitudinal study suggested that long-term treatment with alfacalcidol and phosphate in adults with XLH may be harmful due to stimulation of increased bone resorption as indicated by elevated CTX [7]. Management of adults with CH is on the cusp of dramatic change with the forthcoming use of burosumab, which is a recombinant human IgG1 monoclonal antibody that binds to FGF23 blocking its activity [9, 10]. It has proven effectiveness in both childhood [11] and in adults with respect to TmP/GFR, $1,25(OH)_2D$, quality of life, stiffness, rickets severity, and fracture healing [12–15]. Short of having bone histomorphometry, biochemical indices of bone remodelling activity should be the best measure of the need for treatment and of efficacy, but there are only three reports of BTM measurement in XLH [7, 9, 16]. We sought to evaluate BTMs in the assessment of adults with CH.

Materials and methods

Patients

Our case series included 27 patients with CH: XLH ($n=23$); ADHR ($n=2$); none of the known mutations ($n=2$). The latter 2 patients had onset during childhood with classical phenotype of bowed long bones, short stature, low TmP/GFR, and normal indices of calcium metabolism. Two of the XLH had complete hypoparathyroidism following total PTx for severe tertiary hyperparathyroidism with severe osteitis fibrosa cystica and renal impairment, one of whom developed post-operative severe hungry bone disease and was reported previously [17]. Data were collected prospectively starting in 2011 with patients having fasting blood and urine tests as part of our standard clinic practice, when they attended their annual review visits. Following approval by the St. Vincent's University Hospital Research Ethics Committee, informed consent was obtained from patients for genetic testing.

Biochemistry

Fasting morning bloods were taken from all subjects into serum and EDTA plasma tubes during a 2-h urine collection. Blood and urine samples were centrifuged at 3000 rpm for 10 min, and serum, plasma and urine aliquots stored at room temperature or at − 80 °C as appropriate pending analysis as part of usual laboratory service. Serum samples were used for the analysis of ionised calcium, PTH, 25OHD and BTMs, and EDTA plasma for C-terminal and intact FGF23. Serum and urine samples for phosphate and creatinine were analysed on a Roche Cobas 8000 automated chemistry system. TmP/GFR was calculated as previously described [18]. Serum measurements of 25OHD, PTH, CTX, PINP, and Oc were determined using the Cobas Roche e602 immunoassay system; samples for ionised calcium were analysed on an ABL 800 flex blood gas analyser. Bone ALP, C-terminal FGF23 and uNTX were analysed using BAP Ostase, Immunotopics, and Osteomark® uNTX ELISA kits, respectively, on the Grifols Triturus® automated ELISA system. $1,25(OH)_2D$ was measured on serum samples using either the immunodiagnostics radioimmunoassay (2011–July 2017) or the DiaSorin Liaison chemiluminescent assay (July 2017 onwards). In the two cases of CH with hypoparathyroidism post-total PTx, intact FGF23 was also measured using the supra-regional bone marker assay service provided by Norfolk and Norwich University Hospital using the Kainos ELISA method. Biochemical results were interpreted against reference intervals sourced from each assay manufacturer's published instructions for use. Reference intervals were independently tested for transference to our study population prior to the analysis of clinical samples according to the Clinical and Laboratory Standards Institute Guideline EP28-A3C [19]. 25OHD was interpreted in accordance with the 2011 Institute of Medicine report on dietary reference intakes for calcium and vitamin D [20].

Genetic analysis

EDTA blood samples were taken from all cases of CH and sent to the Molecular Genetics Department of the Royal Devon and Exeter Hospital. Genomic DNA was extracted from peripheral leukocytes using standard procedures. The full coding region of the *PHEX* gene including the intron–exon boundaries was amplified by PCR; unidirectional sequencing was performed using an ABI 3730 Genetic Analyzer (Applied Biosystems, Foster City, CA). Sequences were compared to the published sequence (GenBank Accession no NM_000444.4) using Mutation Surveyor (SoftGenetics, State College, PA). Dosage analysis

of the *PHEX* gene was also performed using multi-ligation-dependent probe amplification (MLPA) kit P223-B1 (MRC Holland). Patients who did not have a mutation in *PHEX* were then sequenced at coding regions and splice sites following amplification by PCR for gene mutations in the following order: *FGF23* (NM_020638.2), *DMP1* (NM_004407.2), *SLC34A3* (NM_080877.2), and *ENPP1* (NM_006208.2).

Statistical analysis

Descriptive statistics are presented as number and percent. Kolmogorov–Smirnov was used as test of normality for scale variables. Differences between groups were tested by Mann–Whitney *U* test. Associations between the biochemical indices were explored using Pearson correlation coefficients. Hierarchical linear regression analysis was performed to explore associations in more detail. Statistics were performed using IBM SPSS for Windows version 24 (Armonk, NY, USA).

Results

Clinical information

In total there were 27 cases: 23 with XLH from 18 kindreds, 2 unrelated cases of ADHR, and 2 cases in whom no mutation was identified. Individual data are given (Tables 1, 2, Supplemental Table 1). There were 19 women and 8 men with ages ranging from 19 to 62 years; height was below the 2nd centile in 52%, below the 25th centile in 85%, and below the 50th centile in 100%. All had symptoms ranging from minimal stiffness to daily bone pain; one patient with chronic pain in both groins had radiographic evidence of refractory bilateral Looser zones on the medial surface of both upper femurs (case 18). Some of the laboratory results

Table 1 Descriptive characteristics of the case series

Case ID	Diagnosis	Age (years)	Gender	Height (cm)	BMI (kg/m)	1-α (μg/day)	P (g/day)	Number of samples
1	XLH	19	F	150	39.5	0.5	2	2
2	XLH	20	M	166	26.5	2	2	3
3	XLH	45	F	144	30.5	0	0	4
4	XLH	19	F	142	21.7	0.5	2	1
5	XLH	50	M	170	29.7	0.5	2	3
6	XLH	19	F	162	29.5	0.5	2	3
7	XLH	46	F	149	28.5	0	0	5
8	XLH	34	F	152	24.5	0.5	1.5	3
9	XLH	51	F	158	24	0	0	2
10	XLH	62	M	151	27.6	1	0	4
11	XLH	27	F	159	23.4	0.5	0	1
12	XLH	20	F	161	22	2.5	2	1
13	XLH	23	F	159	21.8	0	0	1
14	XLH	20	M	160	30.2	2	4	3
15	XLH	21	M	163	29	2	2	3
16	XLH	46	F	143	35.6	0	0	2
17	XLH	23	F	147	26	0	0	1
18	XLH	20	M	175	25.7	3	2	6
19	XLH	43	F	142	32.7	1	0	3
20	XLH	18	F	139	28.7	1	2	1
21	XLH	36	F	153	21.9	1	1.5	4
22	Unknown	30	F	153	40.3	2	2	4
23	Unknown	19	M	155	27.7	1	0	3
24	ADHR	18	M	173	44	0	0	1
25	ADHR	32	F	158	21.2	1	0	2
26	XLH PTX	36	F	159	35.2	1.25	0	8
27	XLH PTX	37	F	155	26.2	0.25	0	1

1-α alfacalcidiol, *P* phosphate supplementation

Table 2 Biochemistry results

Case ID	Diagnosis	PO4 mmol/L (0.8–1.5)	TmP/GFR mmol/L (0.84–1.48)	C-terminal FGF23 RU/mL (< 100)	Intact FGF23 pg/mL (10–50)	Ca ionised mmol/L (1.19–1.35)	PTH pmol/L (1.6–6.9)	25OHD nmol/L (30–125)	$1,25(OH)_2D$ pmol/L (43–168)	Urine Ca:Creat 0.07–0.41	eGFR mL/min
1	XLH	0.61	0.38	150	–	1.35	9.4	41.5	84.7	0.31	127
2	XLH	0.46	0.23	289	–	1.26	6.4	80.3	86.0	0.18	148
3	XLH	0.5	0.4	482	–	1.22	8.3	53.1	94.4	0.04	109
4	XLH	0.64	0.58	–	–	1.25	9.3	26.3	–	0.15	98
5	XLH	0.57	0.39	246	–	1.26	6.3	49.6	45.0	0.13	106
6	XLH	0.69	0.56	153	–	1.27	5.1	29.3	60.0	0.13	116
7	XLH	0.79	0.81	87	–	1.23	4.1	48.3	54.3	0.09	102
8	XLH	0.5	0.44	120	–	1.30	9.9	64.4	–	0.08	107
9	XLH	0.79	0.62	120	–	1.25	6.6	43.4	52.7	0.19	107
10	XLH	0.5	0.34	543	–	1.29	9.1	49.5	–	0.20	63
11	XLH	0.54	0.47	96	–	1.30	9.2	45.9	106.0	0.05	127
12	XLH	0.6	0.56	88	–	1.26	2.8	130.3	84.6	0.05	134
13	XLH	0.75	0.48	155	–	1.27	5.6	100.7	106.6	0.02	123
14	XLH	0.5	0.38	90	–	1.25	6.0	32.5	144.3	0.25	131
15	XLH	0.47	0.33	142	–	1.30	6.3	32.3	115.4	0.10	111
16	XLH	0.6	0.48	370	–	1.27	4.7	65.2	123.4	0.25	107
17	XLH	0.49	0.41	109	–	1.24	4.7	62.7	136.6	< 0.06	131
18	XLH	0.36	0.35	127	–	1.31	5.3	68.2	153.3	0.33	124
19	XLH	0.59	0.57	203	–	1.22	7.5	17.3	88.1	0.09	116
20	XLH	0.61	0.2	208	–	1.30	10.2	53.1	80.0	0.03	127
21	XLH	0.4	0.36	78	–	1.28	7.6	117.7	108.0	0.19	109
22	Unknown	0.6	0.58	106	–	1.25	8.9	54.0	108.8	0.15	119
23	Unknown	0.6	0.54	95	–	1.31	9.5	47.0	235.0	0.05	152
24	ADHR	0.77	0.63	88	–	1.25	3.3	17.5	156.2	0.57	123
25	ADHR	0.86	0.78	23	–	1.23	2.5	61.6	129.5	0.38	92
26	XLH PTX	1.48	1.1	10015	19033	1.17	< 0.6	92.4	68.0	0.49	39
27	XLH PTX	1.16	0.92	4310	7440	1.24	< 0.6	79.2	136.6	0.35	28

are based on the mean of multiple samples from consecutive outpatient appointments, with 19 patients having had more than one sample (Table 1); there were few missing samples: C-terminal FGF23 ($n=1$); $1,25(OH)_2D=3$. Results on the 2 cases of XLH and complete postsurgical hypoparathyroidism are given in Table 2 and depicted in Fig. 1, but their data are excluded from the statistical analysis, as discussed in more detail below. Of the remaining patients ($n=25$), 5 were treated with alfacalcidol only, and 13 of that 18 were also treated with both alfacalcidol (doses ranging from 0.25 to 3 µg daily) and phosphate supplements (ranging from 1.5 to 2.0 g daily) (Table 1).

Genetic results

Of the twenty-seven subjects suspected to have a congenital disorder of phosphate homeostasis, genetic analysis was performed on twenty-three subjects (Supplemental Table 1). The four patients who did not undergo genetic testing were diagnosed to be obligate carriers by reason of X-linked inheritance. Case 2 is the male offspring of an affected mother (case 3), cases 8 and 21 are daughters of an affected male (case 10), and case 9 is the mother of two affected daughters (cases 12 and 13). Analysis of the remaining twenty-three patients demonstrated fourteen different pathogenic mutations in the *PHEX* gene consistent with a diagnosis of X-linked hypophosphataemia (XLH), including 3 novel mutations at the time of analysis. Two patients had mutations in the *FGF 23* gene, one novel at the time of analysis and one previously reported, confirming autosomal dominant hypophosphataemic rickets (ADHR). Two unrelated subjects (cases 24 and 25) had no identified mutation in the *PHEX, FGF23*, *DMP1*, *SLC34A3*, or *ENPP1*. The three novel *PHEX* gene mutations were as follows: case 26 was found to have a heterozygous mutation for a novel deletion/insertion mutation (c.2166delinsGG) in exon 22, resulting in a premature termination at codon 725 (p.S722Rfs*4); case 10 demonstrated a novel missense mutation on sequencing analysis in exon 17 (p.G572S), resulting in the substitution of serine for glycine (p.G572S), the same location as a previously reported pathogenic mutation [21]; case 27 had a splicing mutation in intron 21 (c. 2147+1G>A) of *FGF23* gene, resulting in the substitution of glycine for arginine (p.R176G). Case 24 had a novel heterozygous missense mutation at exon 3 of the *FGF23* gene (c.535C>T), resulting in the substitution of tryptophan for arginine (p.R176G).

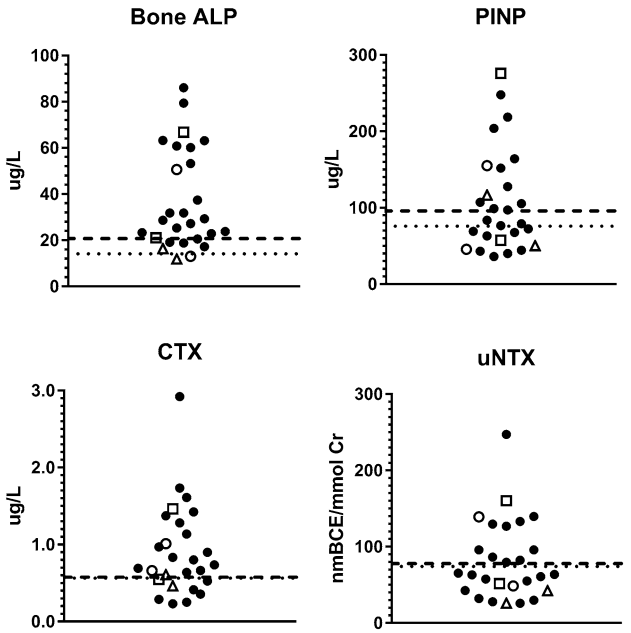

Fig. 1 Bone turnover markers in individual cases: XLH, solid circles; XLH with hypoparathryoidism after PTx, open triangles; ADHR, open circles; and unknown mutations, open squares. Gender-specific reference ranges for BTMs for men (dashed line) and women (dotted line) as follows: PINP, 22.1–96.2 and 17.3–83.4 ug/L; Oc, 14–42 and 11–43 ug/L; bone ALP 3.7–20.9 and 2.9–14.5 ug/L; CTX, 0.016–0.584 AND 0.025–0.573 ug/L, and uNTX, 13–78 and 14–74 nmBCE/mmol Cr. Reference ranges were sourced from each assay manufacturers' published instructions for use before being individually verified byt the laboratory prior to the analysis of clinical samples

Biochemical findings

TmP/GFR was low in all cases, by definition, but was varied widely (Table 2). The frequency of biochemical abnormalities was as follows: C-terminal FGF23 was elevated in 16 of 24 (67%), ionised calcium was normal in all; PTH was elevated in 10 of 25 (40%), but only mildly so; 25OHD was below 30 nmol/L in 2 of 25 (8%); $1,25(OH)_2D$ was not low in any case, but elevated in case 23 without a known genetic mutation; and urinary calcium:creatinine ratio was elevated in one case with ADHR (Table 2). Regarding BTMs (Fig. 1), bone ALP was elevated in 24 of 25 cases (96%); PINP was elevated in 13 of 25 (52%); Oc was elevated in 7 of 25 cases (28%); CTX was elevated in 18 of 25 cases (72%); uNTX was elevated in 12 of 25 cases (48%). One woman was postmenopausal (case 9) that would contribute to her higher BTMs. There was evidence of lower disease severity in the 2 cases of ADHR as reflected by higher TmP/GFR, lower C-terminal FGF23, and lower BTMs (Fig. 1). Those treated with both phosphate supplements and alfacalcidol ($n=13$) compared to the remainder ($n=12$) had lower TmP/GFR ($p=0.030$) and higher CTX concentrations ($p=0.040$).

C-terminal FGF23, bone ALP and PINP needed log-transformation to pass the test of normality. There were very strong direct correlations between the five BTM measurements with r values ranging from 0.636 to 0.918 (Supplemental Table 2). TmP/GFR correlated inversely with log C-terminal FGF23 ($r=-0.564$, $p=0.004$), with PTH ($r=-0.448$, $p=0.025$), with ionised calcium ($r=-0.503$,

$p = 0.010$), with Oc ($r = -0.409$, $p = 0.013$), and with log bone ALP ($r = -0.564$, $p = 0.004$). Log C-terminal FGF23 did not correlate with any of the BTMs. PTH correlated directly with ionised calcium ($r = 0.463$, $p = 0.020$), but not with any of the BTMs. There were no correlations between 25OHD and any other variables; $1,25(OH)_2D$ only correlated with urinary calcium:creatinine ratio after excluding outlier case 23 with high $1,25(OH)_2D$ ($r = 0.506$, $r = 0.019$).

In exploring determinants of TmP/GFR and BTMs, a few confounder variables were considered. Age correlated positively with log C-terminal FGF23 ($r = 0.405$, $p = 0.049$) and negatively with all the BTMs (ranging from − 0.407 to − 0.488). Renal function, as measured by eGFR, only correlated positively with uNTX ($r = 0.427$, $p = 0.033$). In men compared to women, bone ALP, PINP, Oc, and CTX were significantly higher. Sex was deemed to have a suppressor effect rather than a confounder effect in XLH because men have a hemizygote mutation and women have a heterozygote mutation; subsequent analyses were not adjusted for sex. In view of the significant correlation between TmP/GFR and its three main determinants (log C-terminal FGF23, PTH, and ionised calcium), a hierarchical multiple regression was computed: age (model 1); adding log C-terminal FGF23 (model 2); adding PTH (model 3); adding ionised calcium (model 4); and adding the interaction term (model 5). The interaction term for PTH and log C-terminal FGF23 was the product of their centred values to avoid multicollinearity. The overall model summary was significant (adjusted $R^2 = 0.499$, $p < 0.001$), but the only significant predictor was log C-terminal FGF23 ($\beta = -0.668$, $t = -3.58$, $p = 0.002$); thus, the model accounted for 49.9% of the variance in TmP/GFR. Regarding prediction of BTMs after adjusting for age, only TmP/GFR was significantly associated with bone ALP (R^2 change = 0.165, $\beta = -0.411$ $p = 0.030$), and Oc (R^2 change = 0.180, $\beta = -0.429$, $p = 0.019$); thus, hierarchical multiple regression analysis was not conducted.

Two patients with XLH (case 26 and case 27) had tertiary hyperparathyroidism with onset in childhood that was intractable and severe [17]. In view of severe parathyroid bone disease and deteriorating renal function, both had total PTx that was followed by near normalisation of BTMs (Table 2; Fig. 1) coupled with marked symptomatic improvement such that both had subsequent successful pregnancies. After PTx in both patients, PTH was undetectable and TmP/GFR at 1.10 mmol/L and at 0.92 mmol/L was within the normal reference range (Table 1), despite extremely high C-terminal FGF23 (upper threshold < 100 RU/mL) in case 26 with multiple estimations (4790 RU/mL; 6690 RU/mL; 13030 RU/mL; 6690 RU/mL, 6830 RU/mL, and 8350 RU/mL) and in case 27 with a single estimation (4310 RU/mL). Intact FGF23 (reference interval 10–50 pg/mL) was markedly elevated in case 26 (12340 pg/mL; 20340 pg/mL; 21450 pg/mL; and > 22000 pg/mL) and in case 27 (7440 pg/mL). Serum creatinine concentrations were elevated at 149 µmol/L in case 26 and 191 µmol/L in case 27, giving an eGFR of 39 mL/min/1.73 m^2 and 28 mL/min/1.73 m^2, respectively [22]. Neither C-terminal nor intact FGF23 concentrations had been measured prior to PTx. Once both patients recovered from expected hungry bone disease [17], BTMs returned to near normal in both cases (Fig. 1), musculoskeletal symptoms ameliorated, and both had successful pregnancies without incident.

Discussion

In a cohort of Irish adults with CH, we found that BTMs were high. Most patients had elevated bone ALP, which is located on the outer surface of osteoblasts and is key to bone mineralisation [23]. Half of the cohort had elevated PINP, which is a marker of collagen formation [23], whereas one-quarter had elevated Oc which reflects mature osteoblast activity [23]. Nearly three-quarters had elevated CTX and nearly one-half had elevated uNTX; since both reflect collagen degradation, the findings suggest an increase in bone resorption [23]. Several of our patients had mild secondary hyperparathyroidism. Our BTM findings are consistent with hypophosphataemic osteomalacia and are suggestive of mild hyperparathyroid bone disease. C-terminal FGF23 was the principal determinant of TmP/GFR, but both PTH and ionised calcium were also significantly associated. In modelling analysis combining all three known determinants, a significant association was observed only for C-terminal FGF23. Regarding associations with BTMs, only TmP/GFR predicted bone ALP and Oc; PTH was not associated with any BTMs.

Definitive diagnosis of bone disease requires dynamic bone histomorphometry [24]. Two studies of bone in XLH, in the era prior to BTMs, showed osteomalacia with increased bone volume as expected but also demonstrated increased bone turnover; both studies recorded secondary hyperparathyroidism [25, 26]. To date, only three other studies have reported BTMs in XLH. In the first reported study by Ros et al. in a cases series of 5 patients with XLH, bone ALP was high in 4 patients, PINP was high in 3 patients, and both CTX and uNTX were high in 1 patient. In a multicentre phase 1/2 trial of burosumab in XLH, Imel et al. monitored BTMs (bone ALP, PINP, Oc, and CTX) as safety parameters; at baseline PTH was elevated in 64% of subjects with clear elevations in bone ALP, but the frequency of abnormalities in BTMs was not recorded [13]. Most recently, Shanbhogue et al. monitored ionised calcium, PTH, TmP/GFR, intact FGF23, bone ALP, PINP, and CTX in a prospective cohort study of 28 patients with XLH comparing treated (on alfacalcidol and phosphate) with untreated patients. In treated patients compared to untreated patients at the start of

their study, they recorded that ionised calcium was higher, phosphate was lower, TmP/GFR was lower, intact FGF23 was higher, CTX was higher, PINP was not different, and areal bone mineral density was not different. Although there was no difference in PTH between the two groups, PTH was elevated in 35% of treated patients and in 25% of untreated patients [7]. After 6 years follow-up, they noted a significant rise in CTX. They speculated that phosphate supplementation accounted for an increase in bone resorption due to PTH that was the best explanation for their prior observation of increased cortical porosity in treated XLH [27]. We noted that patients on phosphate supplementation had higher CTX compared to those not on supplementation, in keeping with the findings of Shanbhogue et al., supporting the hypothesis that long-term phosphate treatment in adults with XLH may increase bone resorption [7]. Given that secondary hyperparathyroidism in the setting of XLH is well recognised due to phosphate supplementation [6], it is axiomatic to infer that increased bone remodelling activity might be consequent upon sustained secondary hyperparathyroidism.

Regarding the effects of burosumab on BTMs, Imel et al. in a small phase 1/2 trial of burosumab in adults with XLH demonstrated that bone ALP, PINP, and Oc increased, but there were no data on resorption markers [13]. PTH remained stable, but mean serum calcium increased slightly with 2 of 28 having episodes of hypercalcaemia and 5 of 28 having spells of hypercalciuria. In a second study with a phase 3 design including a much larger number of adults with XLH ($n = 134$), both PINP and CTX tended to be elevated prior to burosumab and then had a mean increase of 81% and 38%, respectively, remaining elevated at the end of 24 weeks. PTH declined on burosumab, but increased on placebo. Thus, it is apparent that careful surveillance of calcium/PTH status and BTM status will be needed after initiation of burosumab to maximise the benefit and minimise the risk of any potential harm. Having an accurate record at baseline bone and mineral status will be essential for monitoring response to burosumab.

In our CH cohort, we included two cases of XLH with hypoparathyroidism after PTx; neither had renal phosphate wasting or hypophosphataemia despite marked elevation in C-terminal FGF23 and intact FGF23, and both had near normalisation of BTMs. The reason for this observation was not investigated but it is likely related, in part, to the abatement of excessive PTH on renal phosphate wasting and on bone turnover. A confounding factor in both our cases was CKD. Both patients had CKD: case 26 had CKD stage 3b with eGFR of 39 mL/min/1.73 m^2; and case 27 had CKD stage 4 with eGFR of 28 mL/min/1.73 m^2. C-terminal FGF23 increases with advancing CKD [28]. One of our XLH patients (case 10) with a reduced eGFR of 63 mL/min/1.73 m^2 had a C-terminal FGF23 of 525 RU/mL. Gutierrez et al. reported the following range for C-terminal FGF23 according to eGFR: 86.2 ± 61.4 RU/mL for eGFR > 60 mL/min; 136.2 ± 69.1 RU/mL for eGFR 45–60 mL/min; 224.6 ± 200.1 RU/mL for eGFR 30–45 mL/min; and 436.0 ± 493.8 RU/mL for eGFR < 30 mL/min [28]. C-terminal FGF23 concentrations in both post-PTx XLH cases were at least tenfold higher than the average expected according to Gutierrez et al. Thus, the findings in our two cases suggest that elevation in both C-terminal and intact FGF23 concentrations seems to be out of proportion to the degree of CKD. This suggests an interaction between PTH and FGF23 with respect to renal phosphate handling.

There is anecdotal evidence of an interaction effect from two clinical case reports. A patient with tumour-induced osteomalacia and tertiary hyperparathyroidism had a total PTx. After PTx, TmP/GFR normalised despite persistently high C-terminal FGF23 [29]. In 1969, Riggs et al. reported a case of adult-onset hypophosphataemic bone disease that was labelled with the old term, vitamin D-resistant rickets. The patient had a total PTx in 1948 with evidence of normal parathyroid glands. Two decades later, the patient remained well without any evidence of renal phosphate wasting or of osteomalacia. Riggs proposed a permissive effect of PTH on renal phosphate handling in hypophosphataemic disease [30]. Neither case had CKD like our two cases.

Some animal studies have explored this interaction. Shimada et al. investigated the effect of FGF23 in normal and parathyroidectomised rats [31]. They demonstrated unequivocally that FGF23 regulates NaPi-2a independent of PTH, but serum phosphate post-FGF23 was higher than baseline in sham-operated rats. Thus, the ameliorating effect of PTx on renal phosphate wasting cannot be explained by a direct interaction of PTH on the renal tubular effect of FGF23. Karaplis and colleagues studied parathyroid function in Hyp mice, which is the animal model for XLH [32]. They generated a PTH knockout mouse (Pth−/−) that was also hemizygous for Hyp. They observed early lethality due to hypocalcaemia that could be ameliorated by administration of PTH1-34. Of note, they also observed that the Pth−/−Hyp mice did not develop hypophosphataemia. Although FGF23 concentrations were not measured, it suggests that the absence of PTH may ameliorate renal phosphate wasting in Hyp mice.

Our study has limitations. Sample size was the main limitation, particularly with respect to multivariable analyses that explored the determinants of TmP/GFR and of BTMs. The CH subjects consisted of 19 women and 8 men, with 5 XLH subjects being recruited from the same family that may have influenced the distribution of BTMs in our sample. On the other hand, rigorous sampling methodology was applied such that the potential pitfalls of BTM measurement relating to time of sampling and fasting status were minimised. In addition, many of the results were composite of results from multiple visits. The heterogeneity of the groups with

respect to treatment (with or without either phosphate supplements or alfacalcidol) and the degree of secondary hyperparathyroidism curtail the utility of BTMs in understanding the nature of bone disease in our cohort.

In conclusion, we observed that BTMs are invariably abnormal in CH, in part reflecting a mineralisation defect as a consequence of reduced TmP/GFR, but also as a consequence of secondary hyperparathyroidism. Monitoring BTMs is likely to be even more pertinent given the advent of burosumab. The reason for the amelioration of renal phosphate wasting post-PTx in XLH and consequent near normalisation of BTMs, while welcome, is unexplained and warrants further investigation.

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Compliance with ethical standards

Conflict of interest Research support from Ultragenyx Pharmaceutical: MMcK, RC.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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