Phosphate matters when investigating hypercalcemia: a mutation in SLC34A3 causing HHRH

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Summary

Hereditary hypophosphatemic rickets with hypercalciuria (HHRH) is a rare, autosomal recessive disorder caused by mutations in the SLC34A3 gene that encodes the renal sodium-dependent phosphate cotransporter 2c (NaPi-IIc). It may present as intermittent mild hypercalcemia which may attract initial diagnostic attention but appreciation of concomitant hypophosphatemia is critical for consideration of the necessary diagnostic approach. A 21-year-old woman was assessed by adult endocrinology for low bone mass. She initially presented age two with short stature, nephrocalcinosis and mild intermittent hypercalcemia with hypercalciuria. She had no evidence of medullary sponge kidney or Fanconi syndrome and no bone deformities, pain or fractures. She had recurrent episodes of nephrolithiasis. In childhood, she was treated with hydrochlorothiazide to reduce urinary calcium. Upon review of prior investigations, she had persistent hypophosphatemia with phosphaturia, low PTH and a high-normal calcitriol. A diagnosis of HHRH was suspected and genetic testing confirmed a homozygous c.1483G>A (p.G495R) missense mutation of the SLC34A3 gene. She was started on oral phosphate replacement which normalized her serum phosphate, serum calcium and urine calcium levels over the subsequent 5 years. HHRH is an autosomal recessive condition that causes decreased renal reabsorption of phosphate, leading to hyperphosphaturia, hypophosphatemia and PTH-independent hypercalcemia due to the physiologic increase in calcitriol which also promotes hypercalciuria. Classically, patients present in childhood with bone pain, vitamin D-independent rickets and growth delay. This case of a SLC34A3 mutation illustrates the importance of investigating chronic hypophosphatemia even in the presence of other more common electrolyte abnormalities.

Learning points:

• Hypophosphatemia is an important diagnostic clue that should not be ignored, even in the face of more common electrolyte disorders.
• HHRH is a cause of PTH-independent hypophosphatemia that may also show hypercalcemia.
• HHRH is a cause of hypophosphatemic nephrocalcinosis that should not be treated with calcitriol, unlike other congenital phosphate wasting syndromes.
• Some congenital phosphate wasting disorders may not present until adolescence or early adulthood.
Background

Hereditary hypophosphatemic rickets with hypercalciuria (HHRH (OMIM 251530)) is rare disease of phosphate homeostasis caused by mutations in the SLC34A3 gene. This gene encodes the proximal renal tubule sodium phosphate cotransporter (NaPi-IIc) which results in excessive renal losses of phosphate (1). Through a cascade outlined in Fig. 1, loss of function of NaPi-IIc leads to hypophosphatemia and hypercalciuria (1). Untreated HHRH can lead to osteomalacia, fractures, nephrolithiasis, nephrocalcinosis, chronic kidney disease (1) and distal renal tubular acidosis (2).

It is essential to differentiate HHRH from other renal phosphate wasting disorders such as autosomal dominant hypophosphatemic rickets (ADHR), autosomal recessive hypophosphatemic rickets (ARHR) and X-linked hypophosphatemic rickets (XLH). Although they have similar clinical and biochemical presentations, treatment is vastly different. ADHR, ARHR and XLH are treated with phosphate and calcitriol supplementation. However, in patients with HHRH, calcitriol supplementation may worsen hypercalciuria and increase the risk of nephrolithiasis (3).

The diagnosis of HHRH can be challenging owing to lack of recognition and a variety of clinical and biochemical presentations (1). Additionally, individuals within the same family and with the same mutations may have different phenotypes (1). Furthermore, confounding features such as 25-hydroxyvitamin D deficiency (4) and renal failure (5) can alter the biochemical presentation. We present a case of HHRH presenting with nephrocalcinosis but not diagnosed until adulthood due to the dominant focus upon her hypercalemia and delayed development of hypophosphatemia.

Case presentation

The patient provided written consent for this report. A 2-year-8-month-old female presented to a pediatrician for failure to thrive. She was found to have nephrocalcinosis and intermittent, mild hypercalcemia. From age 2–11 years, she was taking a daily multivitamin containing elemental calcium 100 mg, phosphate 10 mg and cholecalciferol 600IU. Further work-up is summarized in Table 1 and revealed a high-normal calcium of 2.51 mmol/L (normal 2.18–2.54 mmol/L) with hypercalcuria of 0.15 mmol/kg/day (normal <0.10). At presentation, her serum phosphate was in the age-specific normal range at 1.39 mmol/L (normal 1.29–1.94 mmol/L) and remained normal up to age 11 years when she was taken off her multivitamin due to hypercalcemia of 2.63 mmol/L. Her phosphate was low at 0.84 mmol/L (normal 1.00–1.90 mmol/L) and her parathyroid hormone (PTH) undetectable.

The patient was born at 38-week gestation following an unremarkable pregnancy. Birth weight was 3.66 kg (40th percentile, Z-score −0.3). Throughout her life, she was consistently below the first percentile for height and weight (Table 1). She had delayed thelarche at age 15 years and did not undergo menarche until age 17 years. There was no history of cognitive developmental delay. Details about the family history are limited as this patient was adopted in infancy. There was no known consanguinity. Her biological mother had a history of constitutional pubertal delay and reached normal adult height of 162.5 cm. The patient’s father was reported to have a normal height.

She was initially referred to Pediatric Endocrinology for possible William’s Syndrome but no chromosomal abnormality was identified. She was labeled with idiopathic hypercalciuria and was treated with hydrochlorothiazide from age 14 to 21 years.

Investigation

The patient had consistently delayed bone age on serial radiographs with evidence of osteopenia. Bone mineral density at age 19 years showed low bone mineral density. In the lumbar spine, BMD was 0.601 g/cm^3 with a Z-score of −4.1. In the femoral neck, BMD was 0.597 g/cm^3 with a Z-score of −2.27. She was referred to adult

Figure 1
Pathophysiology of HHRH. Mutations in SLC34A3 result in excessive renal losses of phosphate. The resultant hypophosphatemia stimulates production of calcitriol which, in turn, suppresses parathyroid hormone (PTH) production and increases intestinal calcium absorption. The net effect is absorptive hypercalciuria.
Table 1  Summary of the investigations and clinical course of the HHRH patient.

| Age          | Notes                                      | Height (Z-score) | Weight (Z-score) | Ca (ref mmol/L) | PO4 (ref mmol/L) | Creatinine (ref μmol/L) | PTH (ref ng/L) | 25-OH vitamin D (ref nmol/L) | 1,25-OH vitamin D (ref pmol/L) | 24-h urine Ca (mmol/kg/day) | 24-h urine PO4 (mmol/kg/day) |
|--------------|--------------------------------------------|------------------|------------------|-----------------|-----------------|------------------------|---------------|--------------------------------|---------------------------------|-----------------------------|-------------------------------|
| 2 years, 8 months | Daily multivitamin                          | 79.0 (−3.2)      | 8.7 (−4.5)       | 2.51 (2.18-2.54)| 1.39 (1.29-1.94) | 17 (10-50)             |               |                                |                                  |                             |                               |
| 3 years, 1 year | Daily multivitamin                          | 84.0 (−2.6)      | 9.5 (−3.9)       | 2.58 (2.18-2.54)| 1.48 (1.29-1.94) | 1.44 (0.15)           |               |                                |                                  |                             |                               |
| 5 years, 1 month | Daily multivitamin                         | 91.0 (−3.9)      | 11.0 (−4.9)      | 2.54 (2.18-2.54)| 1.11 (0.91-1.59) | 44 (24-58)            |               |                                |                                  |                             |                               |
| 11 years, 5 months | Off multivitamin                            | 120.0 (−3.6)     | 20.0 (−4.3)      | 2.63 (2.18-2.54)| 0.84 (1.00-1.90) | 60 (30-70)            | <1 (13-54)    |                                |                                  |                             |                               |
| 11 years, 9 months |                                      | 121.7 (−3.6)     | 20.2 (−4.5)      | 2.65 (2.20-2.65)| 1.1 (1.00-1.90)  | 69 (30-70)            | 4 (13-54)     |                                |                                  |                             |                               |
| 14 years, 11 months | Started on HCTZ 25 mg OD                 | 140.0 (−3.4)     | 27.6 (−5.5)      |               |                 | 5.49 (0.2)           |               |                                |                                  |                             |                               |
| 15 years, 8 months | HCTZ 25 mg                                | 142.1 (−3.2)     | 29.2 (−5.8)      | 2.84 (2.20-2.60)| 0.74 (0.90-1.60) | 84 (45-100)           |               |                                |                                  |                             |                               |
| 15 years, 6 months | HCTZ 25 mg                                |               |                 | 2.77 (2.20-2.60)| 0.91 (0.90-1.60) | 69 (45-100)           |               |                                |                                  |                             |                               |
| 18 years     | HCTZ 50 mg; Spironolactone 25 mg; Calcium 500 mg; Vitamin D 100 IU | 147.7 (−2.4)     | 38.8 (−3.3)      | 2.66 (2.10-2.55)| 0.66 (0.90-1.60) | 87 (35-100)           | 10 (13-54)    |                                |                                  |                             |                               |
| 21 years     | Off spironolactone, HCTZ                  | 38.0             | 2.48 (2.10-2.55)| 0.56 (0.80-1.50)| 86 (35-100)     | 56.7 (80.0-200.0)    | 144 (55-190) |                                |                                  |                             |                               |
| 23 years     | Phosphate 500 mg PO BID                    | 37.5             | 2.36 (2.10-2.55)| 0.75 (0.80-1.50)| 78 (35-100)     | 21 (13-54)            |               |                                |                                  |                             |                               |
| 24 years     | AKI after calcium oxalate and uric acid renal stone | 2.4 (2.10-2.55) | 0.33 (0.80-1.50) | 126 (35-100)    |               |                       |               |                                |                                  |                             |                               |
| 27 years     | Phosphate 500 mg PO BID                    | 2.54 (2.10-2.55)| 1.03 (0.80-1.50) | 92 (35-100)     |               |                       |               |                                |                                  |                             |                               |

Ref denotes age-specific normal ranges.
endocrinology for reassessment. There were no obvious skeletal abnormalities besides short stature with a height of 147.2 cm (0.9th percentile, Z-score –2.4) and a weight of 38.0 kg. She had evidence of PTH-independent hypercalcemia with an elevated calcium of 2.66 mmol/L (normal 2.10–2.55 mmol/L), a low PTH of 10 ng/L (normal 13–54 ng/L) and low phosphate of 0.66 mmol/L (normal 0.90–1.60 mmol/L). Serum albumin levels were repeatedly normal, between 40 and 46 g/L. Off diuretics, she had hypophosphatemia of 0.56 mmol/L with inappropriate phosphaturia and a low maximal tubular reabsorption rate for phosphate (TmP/GFR 0.384 mmol/L (normal 0.96–1.44)). Calcium was normal at 2.48 mmol/L, PTH was normal at 21 ng/L and 24-h urine calcium was elevated at 8.53 mmol/day (normal 2.5–7.5 mmol/day). Her 25-hydroxyvitamin D was adequate at 56.7 nmol/L (normal >50 nmol/L) and her calcitriol was normal at 14 pg/mL (normal 55–190 pg/mL). Multiple alkaline phosphatase levels measured between ages 19 and 24 were normal according to age-specific reference ranges.

Consideration was initially given to Jansen Syndrome (activating mutation of the PTH receptor) (6), but her urinary cyclic AMP was normal at 1.3 nmol/dL (normal 1.2–2.7 nmol/dL). FGF23 was 70 RU/mL (normal <230 RU/mL) which ruled out ADHR, ARHR and XLH.

Given the ongoing intermittent PTH-independent hypercalcemia and phosphaturia with no evidence of proximal tubular dysfunction from Fanconi syndrome, she underwent genetic testing for HHRH. Sequencing of the SLC34A3 gene revealed homozygosity for a missense mutation c.1483G>A (p.G495R) which was predicted to be pathogenic.

**Treatment**

Subsequently, she was treated with oral phosphate 500 mg twice per day which resulted in normalization of her serum and urinary calcium and phosphate.

**Outcome and follow-up**

The patient has been followed for 8 years; at age 24 years, she had an episode of nephrolithiasis.

**Discussion**

We describe a case of a woman who presented in early childhood with growth delay, hypercalciuria and nephrocalcinosis. This case highlights the varied presentations of HHRH. Although she manifested nephrocalcinosis early on, this patient did not develop hypophosphatemia until much later in her disease course. The patient did not develop any rachitic deformities or bone pain despite chronic hypophosphatemia.

HHRH can be difficult to recognize for a variety of reasons. HHRH is incredibly uncommon: since it was first described in 1985 among a consanguineous Bendouin kindred (7), less than 150 patients have been reported (1). Despite a well-characterized pathophysiological mechanism, not all patients present with hypophosphatemia, osteomalacia and hypercalciuria (1). 25-hydroxyvitamin D deficiency can result in near-normalization of the urinary calcium excretion and calcitriol levels (4). Concomitant renal failure can result in hyperphosphatemia, PTH elevation, as well as reductions in urinary calcium and phosphate excretion (5). Earlier use of hydrochlorothiazide may have helped uncover the frank hypercalcemia which is not always uniformly present.

The novel c.1483G>A (p.G495R) missense mutation in exon 13 encodes the C-terminal transmembrane helix and would result in the substitution of an uncharged amino acid (glycine) for one with a positively charged side chain (arginine) (8). Across species, this region is highly conserved for hydrophobic amino acid residues (Table 2). *In vitro* experiments offer a few possible mechanisms. Under normal conditions, NaPi-IIc is an electrogenic sodium-dependant phosphate cotransporter expressed in the renal proximal tubules. The last intracellular loop in NaPi-IIc is vital to the function of the transporter; it binds PEX19 and allows for internalization of NaPi-IIc mediated by PTH (9). As a result, a protein change (p.R468) in a region adjacent to the mutation in this case results in a disruption to the last intracellular loop of NaPi-IIc and retention of the transporter within the endoplasmic reticulum (9). A protein change in another transmembrane helix (p.S138F) results in appropriate trafficking to the apical membrane but rapid lysosomal degradation due to protein instability (9). Both mechanisms could explain the loss of function in NaPi-IIc due to the c.1483G>A (p.G495R) missense mutation as the transporter does not reach the proximal tubule, resulting in renal phosphate wasting (Fig. 1). In most other forms of congenital hypophosphatemia, there are high levels of FGF23 causing both renal phosphate wasting and inhibition of 1-alpha-hydroxylase activity, resulting in low calcitriol levels. However, HHRH is not related to an abnormality of FGF23 action and levels are typically normal/low and as a consequence, calcitriol
production is not inhibited such that hypercalciuria and sometimes mild intermittent hypercalcemia may result.

This case highlights the variability in the biochemical profile and presentation of HHRH. Many clinicians are trained to investigate hypercalcemia which is far more common than hypophosphatemia. In the present patient, extensive investigations to explain the hypercalcemia failed to arrive at a diagnosis until a differential diagnosis including hypophosphatemic disorders was considered. Chronic hypophosphatemic disorders in children are rare but important and definitive diagnosis is possible with appropriate biochemical and genetic testing. Adult endocrinologists should be aware that some adults may have late-onset hypophosphatemia previously unexplained and yet of a congenital nature, related to germline mutations in \textit{SLC34A3} (10). This case also highlights the importance of continued adherence to phosphate supplementation to prevent acute nephrolithiasis and long-term complications of HHRH. Long-term studies are lacking to show whether oral phosphate supplementation alone is enough to prevent renal calcifications and bone loss. Regardless, it is critical to differentiate HHRH from other causes of hypophosphatemic rickets; calcitriol is elevated in HHRH and calcitriol supplementation can potentiate further hypercalciuria, resulting in nephrolithiasis/nephrocalcinosis (1). Other forms of FGF-23-mediated hypophosphatemia would likely respond to burosumab therapy, but this treatment would not be appropriate in FGF-23 independent hypophosphatemia such as HHRH (11). It is critically important to include measurement of serum phosphate levels with investigation of any abnormality when assessing suspected disorders of bone or calcium homeostasis.

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