Description of 5 Novel SLC34A3/NPT2c Mutations Causing Hereditary Hypophosphatemic Rickets With Hypercalciuria

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Received 1 April 2019; accepted 6 May 2019; published online 17 May 2019

Kidney Int Rep (2019) 4, 1179–1186; https://doi.org/10.1016/j.ekir.2019.05.004
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INTRODUCTION

Hereditary hypophosphatemic rickets with hypercalciuria (HHRH) is a rare autosomal-recessive disorder. The disease was mapped to SLC34A3, the gene encoding renal sodium-phosphate (Pi) cotransporter NPT2c.¹ Hypophosphatemia leads to suppression of fibroblast growth factor 23 (FGF23), increased 1,25-dihydroxy vitamin D (1,25(OH)₂D), and hypercalciuria, the hallmark of HHRH, which is shared by a group of Pi wasting disorders caused by loss-of-function mutations in SLC34A1/NPT2a² but differentiates it from X-linked and autosomal forms of FGF23-dependent hypophosphatemia.³ Typically, patients with homozygous or compound heterozygous SLC34A3/NPT2c mutations present in childhood with metabolic bone disease and less commonly with nephrolithiasis and/or nephrocalcinosis. Conversely, heterozygous carriers present later in life with idiopathic hypercalciuria, often with mild hypophosphatemia and/or elevated 1,25(OH)₂D levels, which set this condition apart from other forms of hypercalciuric nephrolithiasis and/or nephrocalcinosis.³ Bone disease is generally absent in heterozygous carriers. Correct diagnosis of HHRH by genetic testing is essential for timely therapeutic intervention. Here we describe three individuals with HHRH in whom we discovered five novel compound heterozygous SLC34A3/NPT2c mutations, and discuss the differential diagnosis and treatment strategies.

CASE PRESENTATION

Case 1 (Kindred 19)

A 22-year-old man (19/II-1) presented after passing multiple urinary stones. His past medical history included genu varum at age 10 years requiring bilateral corrective knee surgery. Further studies confirmed urinary Pi wasting. He was diagnosed with hypophosphatemic rickets and was prescribed calcitriol and oral sodium and potassium phosphate supplements. He continued treatment until age 19, when he reached adult height. At this presentation (at 22 years of age), he was 5 feet 10 inches (177.8 cm) tall (mother: 5 feet 5 inches, father: 5 feet 10 inches) and weighed 210 lbs (95.25 Kg). His physical examination, including alignment of his lower extremities, was unremarkable. Biochemical investigation showed persistent hypophosphatemia, phosphaturia, and hypercalciuria (Table 1). A computed tomographic scan of the abdomen and pelvis revealed bilateral nonobstructing renal calculi, mildly increased density of renal pyramids consistent with nephrocalcinosis, and 3 cysts in each kidney. Analysis of a recent stone showed 80% calcium phosphate and 20% calcium oxalate. Analysis of a 24-hour urine collection revealed oxalate excretion 26 mg/d (normal: 20—40), citrate 293 mg/d (>450), and uric acid 0.865 g/d (<0.8). His family history revealed nephrolithiasis in the father (19/I-1), but there was no history of cystic kidney disease. His mother (19/I-2) and younger brother (19/II-2) were asymptomatic.
**Table 1. Biochemical evaluation of HHRH patients at initial presentation and following therapy**

|                | 19/II-1 | 24/II-1 | 25/II-1 | 25/II-2 | 33/II-1 |
|----------------|---------|---------|---------|---------|---------|
| Age, yr        | 20–23   | 24–26   | 7       | 9       | 6       |
| Treatment      | None    | Phospho250 Neutral (13 mg/kg daily) | None    | Potassium phosphate (74 mg/kg daily) | None    |
|                | None    | Potassium phosphate (74 mg/kg daily) | None    | Potassium phosphate (40 mg/kg daily) | None    |
|                | None    | Sodium and potassium phosphate (45 mg/kg daily) | None    | Sodium and potassium phosphate (45 mg/kg daily) | None    |
| Blood          |         |         |         |         |         |
| Calcium (mg/dl)| 9.5–10.0 (8.4–10.4) | 9.4–9.7 (8.4–10.4) | 9.7 (8.8–10.2) | 9.6–9.8 (8.9–10.4) | 10.3–10.5 (8.9–10.4) |
|                | 10.6 (8.9–10.4) | 9.9–10.0 (8.8–10.2) | 9.2–9.5 (8.9–10.4) | 9.2–9.5 (8.9–10.4) | 9.2–9.5 (8.9–10.4) |
| Phosphorus (mg/dl) | 1.5–2.1 (2.4–5.0) | 2.1–2.5 (2.4–5.0) | 2.7 (3.5–6.0) | 3.1–3.2 (3.0–6.0) | 3.1 (3.0–6.0) |
|                | 3.3–3.7 (3.5–5.6) | 3.2–4.0 (2.5–5.3) | 3.2–4.0 (2.5–5.3) | 3.2–4.0 (2.5–5.3) | 3.2–4.0 (2.5–5.3) |
| 25-hydroxy vitamin D, total (ng/ml) | 77–87 (18–72) | 65 (18–72) | 164 (25–66) | 100–121 (31–87) | 120–141 (15–90) |
|                | 121 (15–90) | 16–29 (15–90) | 16 (12–90) | 9 (12–90) | 16–97 (12–95) |
| FGF23 (pg/ml)  | N/A     | <50 (<180) | <50 (<180) | <50 (<180) | <50 (<180) |
| Creactine (mg/dl) | 1.1–1.2 (0.7–1.3) | 1.1–1.3 (0.7–1.3) | 0.4 (0.5–1.00) | 0.44 (0.5–1.00) | 0.36–0.43 (0.7–1.3) |
|                | 0.5 (0.7–1.3) | 0.95–1.00 (0.6–1.0) | 0.95–1.00 (0.6–1.0) | 0.95–1.00 (0.6–1.0) | 0.95–1.00 (0.6–1.0) |
| Alkaline phosphatase (units/l) | 82–114 (39–117) | N/A | 696 (50–480) | 333 (50–480) | 410–416 (50–480) |
|                | 312 (96–297) | 352–547 (50–480) | 352–547 (50–480) | 352–547 (50–480) | 352–547 (50–480) |
| Bicarbonate (mmol/l) | 26–31 (22–30) | 28 (22–30) | 24 (22–30) | 20 (22–30) | 24 (18–29) |
| AG (mmol/l)   | 3.0–9.0 (7–17) | 11 (7–17) | 11 (7–17) | 11 (7–17) | 11 (7–17) |
| Urine          |         |         |         |         |         |
| TRP (%)       | 44–71 (>80) | 65–76 (>80) | 77–91 (>80) | 80 (>80) | 81–89 (>80) |
|                | 84 (>80) | 84 (>80) | 84 (>80) | 84 (>80) | 84 (>80) |
| Spot calcium/creatinine (mg/mg) | 0.17 (<0.14) | 0.36 (<0.14) | 0.43 (<0.14) | 0.43 (<0.14) | 0.43 (<0.14) |
|                | 0.45 (0.04–0.7) | 0.05–0.1 (<0.14) | 0.05–0.1 (<0.14) | 0.05–0.1 (<0.14) | 0.05–0.1 (<0.14) |
| 24-h Ca/Cr (mg/mg) | 149 (<140) | 54–114 (<140) | 150–160 (<140) | 150–160 (<140) | 150–160 (<140) |
| 24-h Calcium (mg/d) | 283 (<250) | 236 (<250) | 36.5–54.6 (100–300) | 36.5–54.6 (100–300) | 36.5–54.6 (100–300) |
| AG (mmol/l)   | 69 (<10) | 40 (<10) | 40 (<10) | 40 (<10) | 40 (<10) |

FGF23, fibroblast growth factor 23; N/A, not available; PTH, parathyroid hormone; %TRP tubular reabsorption of phosphorus; urine Ca/Cr, Calcium to Creatinine ratio.
genetic diagnosis with HHRH, he was restarted on monotherapy with Phospha250 Neutral oral tablets (13 mg/kg of elemental phosphorus per day; Ingenus Pharmaceuticals NJ, LL, Fairfield, NJ). Two years later, the patient was free of recurrent nephrolithiasis, and his hypophosphatemia and hypercalciuria had improved. Repeat evaluation on renal ultrasound showed that his right simple cyst in the lower pole, which measured $1.7 \times 1.3 \times 1.3$ cm, and a simple cyst in the right lower pole, which measured up to 2.0 cm, both were grossly unchanged. Likewise, a left medial cyst seen on prior CT was unchanged. Conversely, his left upper pole cyst, which measured up to 4.7 cm on prior CT, was reduced to $3.4 \times 3.2 \times 3.9$ cm, and his simple cyst lower pole, previously up to 4.2 cm on CT, was reduced to 2.7 cm in size.

Case 2 (Kindred 25)
At the age of 6 years, the index case patient 25/II-1 presented with bone pain and muscle weakness which affected his running and walking upstairs, prompting orthopedic evaluation at age 7 years. Examination revealed a genu valgum deformity, and lower extremity radiographs demonstrated classic epiphyseal features of rickets. On the presumptive diagnosis of X-linked hypophosphatemia, therapy with calcitriol and oral phosphate salts was initiated. He remained on this treatment for 5.5 months until exome sequencing identified a genetic mutation in \textit{SLC34A3}, consistent with HHRH, and calcitriol was stopped. In retrospect, his laboratory evaluation (Table 1) showed elevated 1,25(OH)$_2$D levels and hypercalciuria, which are more consistent with HHRH. Phosphate monotherapy improved these parameters. His linear growth was similarly improved with this therapy: at 8 years he was at the 19th centile and by 9 years had increased to the 36th centile.

At age 8 years, bilateral epiphysiodesis was performed to correct his genu valgum. He underwent this guided growth procedure with no complications and had an uneventful postoperative recovery. At age 9, a renal ultrasound demonstrated the presence of medullary nephrocalcinosis. Elevated circulating 1,25(OH)$_2$D levels recurred, and persistent hypercalciuria in the absence of glucosuria and proteinuria was evident. Based on these biochemical findings, his phosphate supplementation was increased from 61 mg/kg to 74 mg/kg of elemental phosphorus daily. Bone mineral density of the lumbar spine determined by dual energy x-ray absorptiometry was low (Z-score: $-1.9$ height adjust Z-score of $-1.75$ using Pediatric Z-Score Calculator from Children’s Hospital of Philadelphia, \url{https://zscore.research.chop.edu/bmdCalculator.php}). Laboratory values after 7 months of this higher phosphate dose (74 mg/kg of elemental phosphorus per day) are shown in Table 1, with persistence of the elevated 1,25(OH)$_2$D levels.

His younger sister 25/II-2 developed leg and hip pain at age 6 years. She has no limb deformities and does not have difficulty walking or climbing stairs. At 2 months of age, her renal sonogram showed that both kidneys were normal in size, contour, position, and echogenicity. Subsequent laboratory evaluation at age 6 years revealed elevated serum 1,25(OH)$_2$D, and alkaline phosphatase levels, renal phosphate leak, hypercalciuria in the absence of glucosuria, and proteinuria were evident (Table 1). A clinical diagnosis of HHRH was made, and she was started on oral phosphate supplementation with K-Phos Neutral (40 mg/kg of elemental phosphorus daily; BEACH PHARMACEUTICALS, Division of Beach Products, Inc., Tampa, FL). Genetic evaluation confirmed that she carries the same compound heterozygous mutations in \textit{SLC34A3}/NPT2c as her brother. Laboratory evaluation after 7 months of the prescribed therapy showed normalization of 1,25(OH)$_2$D levels but persistent hypercalciuria and elevated alkaline phosphatase. Thus, her dosage was increased from 40 mg/kg of elemental phosphorus daily to approximately 50 mg/kg of elemental phosphorus daily.

The index case patient’s mother (25/I-2) had a history of a single kidney stone, and both grandfathers had multiple kidney stones, but no history of growth retardation or deformities. His father 25/I-1 developed a stress fracture as adult but has no history of kidney stones, growth retardation, or deformities consistent with past rickets.

Case 3 (Kindred 33)
The index case of this kindred (33/II-1) presented at age 7 years to the clinic with kidney stones. Her laboratory evaluation showed hypercalciuria, low serum phosphate, low TRP accompanied by low normal PTH, and had a normal alkaline phosphatase (Table 1). Her physical examination was unremarkable except for mild genu valgum. Radiographs of the long bones were normal at diagnosis. A dual energy x-ray absorptiometry scan revealed Z scores: PA spine $-1.9$, left hip $-3.1$, total body $-3.9$. HHRH was suspected and she was started on 750 mg/d of sodium and potassium phosphate (NeutraPhos, BEACH PHARMACEUTICALS, Division of Beach Products, Inc.), which was increased to 2.25g/d over the first year of treatment. Her urinary calcium excretion normalized, and she remained free of recurrent stones. A renal ultrasound was normal, without evidence for nephrocalcinosis. Repeat dual energy x-ray absorptiometry at age 11 showed improvement of the bone density to Z score: PA spine $-1.1$, left hip $-2.2$, total body $-2.8$. Her linear growth was at the 5th to 10th centile at presentation, and accelerated on treatment briefly to the 25th centile when she entered menarche at age 12 years. However,
her final adult height remains at the 5th centile (2 standard deviations below her mid-parental height). She has, aside from removal of a benign 3-cm breast fibroadenoma, remained without other medical problems. Biochemical evaluation at age 17 years shows that she has done well on potassium phosphate treatment.

Gene sequencing of SLC34A3 was ultimately performed at age 17 and confirmed the diagnosis HHRH with compound heterozygous mutations in SLC34A3, one of which was inherited from the mother 33/I-2; the father was unavailable for genetic testing (Figure 1). There is no family history of kidney stones. She has a healthy younger sister 33/II-2, whose screening laboratory test results at 13 years were within normal limits: Calcium 10.1 mg/dl (8.7–10.4), Phos 3.8 mg/dl (2.7–4.5), ionized Ca 5.3 mg/dl (4.8–5.3), 1,25(OH)2D 57 pg/ml (27–71), 25-hydroxy vitamin D (25(OH)D) 32 ng/dl (30–100), and PTH intact 44.6 pg/ml (14–72).

**Whole Exome Sequence Analysis of the Index Case and Genetic Evaluation of Family Members**

Genetic evaluation was performed following informed consent (Yale HIC1501015216) by a combination of Next Generation Sequencing of leukocyte DNA of the index case followed by mutation-specific polymerase chain reaction assays and Sanger Sequencing of leukocyte DNA of the parents and/or siblings. One previously reported (c.448+1G>A) and 5 novel compound heterozygous mutations were detected that have not been reported in dbSNP (https://www.ncbi.nlm.nih.gov/snp, accessed February 2, 2019), the Exome variant server (http://evs.gs.washington.edu/EVS/, accessed February 2, 2019), or the Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/index.php, accessed February 2, 2019) (Figure 1). Mutation Taster predicts that these mutations cause frame shifts in the SLC34A3 mRNA and, as a result, a change in amino acid sequence predicted to cause loss-of-function of the NPT2c sodium-Pi co-transporter. No disease-causing variants were found in SLC34A1, CYP24A1, FGF23, KL, DMP1, FAM20C, and PHEX.

**DISCUSSION**

Presentation with childhood rickets or early-onset osteoporosis along with nephrolithiasis in an individual with family history consistent with autosomal-recessive inheritance is typical for HHRH (Figure 2). However, bone disease may be suggested only by history of childhood bone pain, fractures, bowing, and adult height below expected for mid-parental height. Some individuals with HHRH initially present with nephrolithiasis/nephrocalcinosis, whereas apparent bone disease is missing. Therefore, bone turnover parameters and dual energy x-ray absorptiometry imaging to detect asymptomatic metabolic bone disease in patients initially presenting with renal findings, and renal ultrasound to look for asymptomatic nephrolithiasis/nephrocalcinosis in patients with bone manifestations, should be performed.

Because heterozygous carriers of SLC34A3/NPTc mutations have an increased risk of renal calcifications,7 the family history may be consistent with an autosomal-dominant inheritance of stone disease. Childhood onset should raise suspicion for an inherited disorder (Figure 2).

The next step in the evaluation of this group of individuals is to distinguish primary hypercalciuria from that secondary to renal Pi leaks (Figure 2, Table 2). Serum Pi concentrations are influenced by the time of day, relation to meals, and age of the subject, and none of the methods for determination of tubular...
reabsorption are entirely satisfying. To determine the cause of abnormal serum Pi levels in a patient who has normal parathyroid and renal function, we generally first assess the tubular reabsorption for Pi (%TRP). For this purpose, the patient is asked to collect a 3-hour timed fasting morning urine for Pi (U-P) and creatinine (U-creat) along with the corresponding serum parameters (Serum phosphorus: S-P; Serum creatinine: S-creat). %TRP is calculated according to the formula:

\[ \%TRP = \frac{100 \times (U-P \times S-creat)}{(S-P \times U-creat)} \]

and the tubular maximum of reabsorption for Pi (TmP/GFR) is derived from a nomogram, which was devised by Walton and Bijvoet\(^7\) to correct for the nonlinear relationships of %TRP and TmP/GFR when TRP is higher than 80%. TmP/GFR reflects the threshold of the serum Pi concentration above which Pi is no longer fully reclaimed from the glomerular filtrate in the proximal tubules. Although the TmP/GFR derived from the Walton and Bijvoet nomogram is generally sufficient in adults, the nomogram does not accommodate the higher normal range of serum Pi values in newborns and toddlers. Thus, calculating TP/GFR provides a more accurate assessment of renal Pi handling in the pediatric population using the following formula:

\[ TP/GFR = S-P - (U-P \times S-creat/U-creat) \]

Inappropriately low %TRP in the setting of hypophosphatemia is suggestive of a proximal renal tubular defect, for example resulting from loss-of-function mutations in NPT2c, which cause HRHH. This diagnosis can be further confirmed by determining excess production of 1,25(OH)\(_2\)D, which may lead to increased absorption of calcium in the gut, resulting in hypercalciuria and some suppression of PTH production, and may, in the setting of hypophosphatemia, be diagnostic for HRHH.\(^5\) Vitamin D deficiency may mask these findings and needs to be corrected before the above testing.\(^3\) Conversely, excess production of 1,25(OH)\(_2\)D in the absence of a renal Pi leak should raise suspicion for granulomatous disorders or deficiency of the enzyme 25(OH)-vitamin D 24-hydroxylase (CYP24A1),\(^4\) which normally degrades 1,25(OH)\(_2\)D. Loss-of-function mutations in CYP24A1 cause infantile idiopathic hypercalcemia type 1 (IIH1). In addition, individuals with loss-of-function mutations in SLC34A1/NPT2a present with idiopathic hypercalcemia type 2 (IIH2), whereas, different from HRHH, bone disease may be less apparent in these individuals (Table 2).\(^5\) Circulating FGF23 levels can be determined using several commercially available enzyme-linked immunoassays.\(^6\) None of the currently available assays, however, are sensitive enough to detect suppressed or inappropriately normal FGF23 levels with sufficient confidence, thus limiting their utility for distinguishing FGF23-independent hypophosphatemic disorders such as HRHH from the FGF23-dependent hypophosphatemic disorders.\(^7,8\) The C-terminal FGF23 assay (Immutoxics, Inc., San Clemente, CA) uses antibodies directed against
2 distinct epitopes within the C-terminal region of FGF23 and is currently the only Clinical Laboratory Improvement Amendments (CLIA)–certified assay in the United States. This assay, in our experience, returns FGF23 levels of < 30 RU/ml in FGF23-independent hypophosphatemic disorders such as HHRH or Fanconi syndrome.

| Disorder | Abbreviation | Inheritance | Gene | Mechanism | OMIM | Ref |
|----------|--------------|-------------|------|-----------|------|-----|
| Hypophosphatemic disorders without hypercalcemia | | | | | | |
| Vitamin D deficiency | N/A | N/A | Acquired | Reduced absorption of dietary calcium and Pi, FGF23-independent, secondary hyperparathyroidism contributes to renal Pi losses | N/A | S30, S31 |
| Tumor-induced osteomalacia | TI0 | somatic | | | | |
| Autosomal-dominant hypophosphatemic rickets | ADHR1 | AR | PHEx | FGF23-dependent | #193100 | S33 |
| Autosomal-dominant hypophosphatemic rickets | ADHR2 | AD | FGFR3 | FGF23-dependent, secondary hyperparathyroidism contributes to renal Pi losses | #612089 | S34 |
| Autosomal-recessive hypophosphatemia type 1 | ARHP1 | AR | CLCN16 and 19 | Distal tubular defect of paracellular cation transport | #613312 | S36-S38 |
| Autosomal-recessive hypophosphatemia type 3, Raine syndrome | ARHP3 | AR | FAM20C | FGF23-dependent | #259775 | S36-S38 |
| Vitamin-resistant rickets type 1 | VDDR1 | AR | CYP27B1 | 1.25(OH)2D deficiency, FGF23-independent, secondary hyperparathyroidism contributes to renal Pi losses | #241530 | S22, S23 |
| Vitamin-resistant rickets type 2 | VDDR2 | AR | VDR | 1.25(OH)2D -resistance, FGF23-independent, secondary hyperparathyroidism contributes to renal Pi losses | #277440 | S40 |
| Familial hypocalciuric hypercalcaemia type 1/neonatal severe hyperparathyroidism | FHH1 | AD/AR | CaR | Proximal tubular Pi wasting, PTH-dependent | #145980 | S41 |
| Familial hypocalciuric hypercalcaemia type 2 | FHH2 | AD | GNAS1 | Proximal tubular Pi wasting, PTH-dependent | #145981 | S42-S44 |
| Familial hypocalciuric hypercalcaemia type 3 | FHH3 | AD | AP2S1 | Proximal tubular Pi wasting, PTH-dependent | #3100740 | S41-S44 |
| Hypophosphatemic disorders with hypercalcemia | | | | | | |
| Dietary Pi deficiency | N/A | N/A | Acquired | Reduced absorption of dietary Pi, PTH- and FGF23-independent | N/A | S45 |
| Proximal tubular damage (caused by theophylline, foscarnet, renal tubular acidosis) | N/A | N/A | Acquired | Proximal tubular Pi wasting, PTH- and FGF23-independent | N/A | S46 |
| Hereditary hypophosphatemic rickets with hypercalciuria | HHRH | AR | SLC34A3 | Proximal tubular Pi wasting, PTH- and FGF23-dependent | #241530 | S22, S23 |
| Isolated (idiopathic) infantile hypercalcaemia type 2, Fanconi syndrome, and nephrocalcinosis | IIH2 | AR | SLC34A1 | Proximal tubular Pi wasting, PTH- and FGF23-dependent | #61963 | S5 |
| Isolated (idiopathic) hypercalciuria | IH | AD | Q932.2-p43.2 | Unknown | N/A | S16 |
| Renal tubular acidosis | Multiple | Multiple | Multiple | Renal bicarbonate loss results in secondary Pi wasting and hypercalciuria | Multiple | |
| Primary hyperparathyroidism | PHPT | Somatic mutations | MEN1, CDC73, and yet-unknown genes | PTH-dependent proximal tubular Pi losses | #131100, #607393 | S47-S48 |
| Humoral hypercalcemia of malignancy | HHM | Somatic mutations | Acquired and yet-unknown genes | PTHrP-dependent (and FGF23-dependent?) proximal tubular Pi losses | N/A | S49-S51 |
| Jansen disease | AD | PTHR1 | Const. active PTHR1 contributes to renal Pi losses; FGF23-dependent? | #156400 | S52-S53 |

*Const., constitutively; FGF23, fibroblast growth factor 23; N/A, not available; PTH, parathyroid hormone; Ref, reference.*
Once renal loss of Pi is confirmed as the underlying cause of hypophosphatemia, FGF23-dependent disorders such as X-linked hypophosphatemia (XLH) should be considered if 1,25(OH)2D levels are suppressed, secondary hyperparathyroidism is present, and urine calcium excretion is low (Figure 2). Patients with X-linked hypophosphatemia, autosomal-dominant hypophosphatemic rickets (ADHR), and autosomal-recessive hypophosphatemia type 1 (ARHP1) also develop enthesopathies that have not been reported in HHRH. The term enthesopathy refers to painful or indolent mineral deposits near the insertion sites of tendons usually at the lower extremities, which can be identified on radiographs. Likewise, dental abscess formation, enamel defects leading to tooth decay, craniosynostosis, midfacial hypoplasia, frontal bossing, scaphocephaly, and Chiari I malformation are thought to be due to Klotho-independent activation of canonical FGF receptors by FGF23, and their presence should raise suspicion for X-linked hypophosphatemia, whereas they are absent in HHRH.

One of the Barter syndromes should be considered as a cause of hypercalciuric nephrolithiasis if hypotension is present. Fanconi syndrome should be suspected if other renal functions are impaired and cause glucosuria and/or aminoaciduria. Tachypnea may suggest renal tubular acidosis as the underlying cause. It is important to consider several monogenic disorders other than mutations in SLC34A3/NPT2c as the cause of what formerly has been named “idiopathic” but more properly may be named “isolated” hypercalciuria (Table 2). Some of these genes affect tubular handling of calcium and Pi in a way that is similar to what is observed in our patients with SLC34A3/NPT2c mutations. We will defer to several excellent reviews for a detailed discussion of these disorders.

Although the presence of hypophosphatemia and low C-terminal FGF23 in the setting of hypercalciuric nephrolithiasis/nephrocalcinosis can be suggestive of a proximal tubular Pi leak, these parameters may be normal, as in some of the presented cases, and therefore our approach is to obtain genetic testing. This is increasingly done by whole exome sequence analysis of leukocyte DNA, prepared from an ethylene diamine tetraacetic acid (EDTA) whole blood sample. Whole exome sequence analysis permits screening of a panel of genes shown in Table 2. Yale’s Clinical Genome Research Center provides sufficient coverage of intronic sequence to permit detect the known intronic deletions in SLC34A3. However, single gene testing may be required to detect deletions in the 5’ and 3’UTR or in the larger intron 12 of SLC34A3, which is offered at Yale and a number of commercial laboratories.

As illustrated by the 3 case reports, compound heterozygous loss-of-function mutations in SLC34A3/NPT2c are common in kindreds with HHRH, and it is therefore important to include at least 1 parent in the genetic evaluation to permit segregation analysis and allele assignment consistent with compound heterozygous inheritance.

The correct diagnosis was initially missed in 2 of the 3 cases presented here, and, as a consequence, they were treated with active vitamin D metabolites, which worsened the hypercalciuria and likely contributed to the development of nephrocalcinosis and/or nephrolithiasis. When correctly treated with oral Pi supplementation only, the rachitic bone disease and hypercalciuria of the affected individuals improved quickly. Because the diagnosis was suspected clinically and oral Pi supplementation was used at the onset, recurrent nephrolithiasis and development of nephrocalcinosis was avoided in our third case (33/II-1).

It is still unknown whether oral Pi supplementation is safe in the long term with respect to renal calcifications; whether hyperparathyroidism or enthesopathies can develop as has been described in X-linked hypophosphatemia; whether the renal Pi-leak persists lifelong or whether therapy may be stopped, as, for example, in ADHR; and whether HHRH predisposes to the accelerated bone loss during adulthood, as described in some individuals with SLC34A1/NPT2a or Na+/H+ Exchanger Regulatory Factor (NHERF1) mutations.

It is also unclear which genetic and biochemical criteria best predicts risk for renal calcifications and how oral Pi therapy should be monitored. Based on our initial survey serum Pi levels, excretion of Pi and serum 1,25(OH)2D merit further evaluation as possible nongenetic predictors of renal calcifications. Our studies in Npt2a knockout mice on diets with different Pi contents furthermore suggest that dietary Pi can be harmful under certain conditions, and that oral Pi supplementation to treat bone disease in hypophosphatemic rickets may need to be carefully monitored so as not to cause renal calcifications despite resolution of hypercalciuria on this therapy. Serum 1,25(OH)2D, in our experience, takes a long time to normalize and may therefore be suitable to assess compliance with oral Pi therapy along with 24-hour urine collections for Pi. To our knowledge, renal cysts have not been reported in individuals with HHRH, but could be the result of obstructive microlithiasis in the index case of 19/II-1, as we have observed a similar phenomenon in Npt2a knockout mice fed a high-Pi, high-calcium diet (unpublished observation).

In summary, our cases illustrate the importance of early and correct diagnosis of HHRH, and adds to the growing list of compound heterozygous SLC34A3/NPT2c mutations.
DISCLOSURE

CB has received research funding from Nutricia North America, Bethesda, MD. DVR has received research funding from Reata pharmaceuticals, FAST BioMedical, Retrophin Inc., and Achillion pharmaceuticals. DVR serves on Visterra Inc. advisory board. All the other authors declared no competing interests.

ACKNOWLEDGMENTS

This work was in part supported by the Yale O’Brien Center (Pilot grant to CB, NIH P30DK079310).

AUTHORSHIP

AC and CB performed the segregation analysis of all kindreds and wrote the manuscript. HR performed segregation analysis, VRRM and DVR contributed clinical information for kindred 19, KR and TOC contributed clinical information for kindred 25, and DB contributed clinical information for kindred 33.

SUPPLEMENTARY MATERIAL

Supplementary File (Word)
Supplemental References.

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