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Atypical Presentation of Hereditary Hypophosphatemic Rickets with Hypercalciuria due
to Digenic Mutations

A Thesis Submitted to the Yale University School of Medicine
in Partial Fulfillment of the Requirements for the Degree of Doctor of Medicine

by

Bryan Bo-Ran Ho, 2022

Atypical Presentation of Hereditary Hypophosphatemic Rickets with Hypercalciuria due to Digenic Mutations

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Abstract:

Loss-of-function (LOF) mutations in *SLC34A3* cause renal phosphate wasting due to loss of sodium phosphate co-transporter NPT2c, generally presenting in childhood with hereditary hypophosphatemic rickets with hypercalciuria (HHRH). Described here are two individuals with atypical presentations of HHRH due to additional heterozygous mutations in *SLC34A1* and *NPHP4*, genetic causes of idiopathic infantile hypercalcemia type 2 and nephronophthisis, respectively. The first case has a 10-year history of well-controlled ulcerative colitis and atrial fibrillation. He wore braces briefly at age 5 for lower extremity bowing, which resolved spontaneously. He was without fractures until age 41, when he sustained a tibial plateau fracture after falling from his treadmill. He presented at age 44 with several outside emergency department visits for weakness and dizziness before presenting to this institution following an episode of light-headedness and rapid heart rate of 160 beats per minute. His physical exam was notable for the absence of lower extremity bowing, dental malformations, and bone tenderness, and his family history was unremarkable for skeletal disorders. The second case initially presented with asymptomatic pyuria and microscopic hematuria at age 7. Renal ultrasound at this age revealed bilateral nephrocalcinosis, and she was started on potassium citrate. At age 19, she was found to have an elevated creatinine of 1.3 mg/dL

and diagnosed with mild hypertension, and she was treated with lisinopril and hydrochlorothiazide. She is of normal stature without pathologic fractures, and physical examination was notable for mild knee knock consistent with a history of mild childhood rickets. Whole exome sequencing of the first patient revealed a novel het.*SLC34A1*.c.847G>A,p.G292S and a previously reported pathogenic het.*SLC34A3*.c.1402C>T,p.R468W variant. The second patient possesses a known pathogenic hom.*SLC34A3*.c.575C>T,p.S192L variant, consistent with a diagnosis of HHRH, and a cis-inherited het.*NPHP4*.c.2579G>A,p.G860E; c.133C>T,p.Q45X variant. The first case is the second report of di-genic heterozygous mutations in *SLC34A1* and *SLC34A3*, and the second case is the first report of di-genic mutations in *SLC34A3* and *NPHP4*. Mild bone phenotypes as a child that resolved spontaneously and an absence of hypercalciuria and renal calcifications in the first case, and presence of nephrocalcinosis with early onset renal failure in the second case illustrate the high degree of phenotypic variability caused by *SLC34A3* mutations and suggest a polygenic contribution to disease severity.

I would like to acknowledge my primary adviser, Clemens Bergwitz, for the years of guidance, advice, and mentorship he has provided throughout this and previous research projects, in both the art of discovery in scientific research and in the practice of medicine. I would also like to acknowledge Diana Athonvarangkul and Karl Insogna for their help with the initial evaluation of Case A, Chi-Yuan Hsu for the initial evaluation and referral of Case B, and Jordan Nestor for her help with the genetic work-up of Case B.

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Introduction

Phosphate is a ubiquitous metabolite that is essential for a wide variety of cellular functions, including cellular signaling, regulation of enzymes by phosphorylation and dephosphorylation, nucleic acid synthesis and replication, energy metabolism, and skeletal growth and mineralization¹. The body stores about 90% of total phosphorus in bone mineral as calcium phosphate crystals called hydroxyapatite, with the extracellular fluid and serum containing less than 1% of total phosphorus in the form of inorganic phosphates².

The primary source of serum phosphate is through dietary phosphate absorption and the influx of phosphate from reabsorption of calcium phosphate from bone mineral stores, along with reabsorption of filtered phosphate in the kidneys, while phosphate excretion is primarily through decreased proximal tubule reabsorption of filtered phosphate³. The typical diet includes phosphorus additives within process foods, and daily intake ranges between 1,500 and 1,700 mg of phosphate per day for men and 1,000 to 1,200 mg for women. Roughly 50 to 70% of bioavailable phosphate is absorbed in the small intestine, and is inversely proportional to age. Unless a disorder in phosphate homeostasis is present, serum phosphate remains relatively stable with roughly 13 mg/kg/day net intestinal absorption and an equal amount excreted in the urine. Hyperphosphatemia has been associated with increased cardiovascular morbidity and mortality, including myocardial hypertrophy and vascular calcifications, in individuals with chronic kidney disease (CKD), whereas hypophosphatemia has been associated with skeletal and cardiac myopathies along with skeletal and dental mineralization defects⁴.

Phosphate is primarily recycled by two SLC34 solute carrier family proteins, the two sodium-coupled phosphate co-transporters NPT2a, encoded by *SLC34A1*, and NPT2c, encoded by *SLC34A3*⁵. Additionally, a member of the SLC20 solute carrier family transporters, the inorganic phosphate-sodium transporter PIT2, encoded by *SLC20A2*, has been shown to be expressed in the proximal tubules and may also mediate renal phosphate reabsorption⁶. An additional member of the SLC34 solute carrier family, the sodium-coupled phosphate co-transporter NPT2b, encoded by *SLC34A2*, is expressed in the intestines rather than the kidney and is responsible for intestinal phosphate absorption⁷.

Phosphate homeostasis is governed by the actions of three hormones: parathyroid hormone (PTH), 1,25-dihydroxy-vitamin D (1,25-(OH)₂D), and fibroblast growth factor 23 (FGF23)⁸. In particular, FGF23 is a phosphaturic hormone that, along with PTH, regulates proximal tubular phosphate reabsorption and the renal synthesis of 1,25-(OH)₂D⁹. FGF23 is a 32kDa protein that is post-transcriptionally modified by phosphorylation by the extracellular kinase family member 20C (FAM20C), making FGF23 susceptible to proteolysis by the subtilisin-like proprotein convertase FURIN due to prevention of O-glycosylation by polypeptide N-acetylgalactosaminyltransferase 3 (GALNT3). O-glycosylation by GALNT3 prevents phosphorylation by FAM20C, leading to the secretion of intact active FGF23¹⁰.

FGF23 increases phosphate excretion by reducing membrane levels of NPT2a and NPT2c by promoting internalization and degradation of membrane-bound phosphate transporters¹¹. Likewise, FGF23 induces 25-hydroxyvitamin-D-24-hydroxylase, encoded by *CYP24A1*, which catalyzes the degradation of active 1,25-(OH)₂D and inactive 25-(OH)D into 24,25-dihydroxy-vitamin D (24,25-(OH)₂D)¹². FGF23 also indirectly reduces

intestinal phosphate absorption by acting in the proximal tubule to suppress 1-alpha-hydroxylase, which is encoded by *CYP27B1* and converts inactive 25-hydroxy-vitamin D (25-(OH)D) into active 1,25-(OH)₂D^{9,13}. FGF23 has also been shown to reduce PTH secretion by the parathyroid gland^{14,15}, which may be due to a reduced set point for calcium in the parathyroid gland¹⁶. FGF23 also increases calcium reabsorption in the distal tubule through increased membrane expression of calcium channel protein transient receptor potential vanilloid 5 (TRPV5)¹¹.

FGF23 also has been associated with a number of non-renal effects, including inducing left ventricular hypertrophy^{17,18}, negatively regulating erythropoiesis¹⁹, and inhibiting bone mineralization by suppression of the tissue-specific phosphatase TNAP²⁰. The manner of FGF23 signaling, through its interaction with Fibroblast Growth Factor Receptor (FGFR) and Klotho (KL), and a further discussion on the downstream effects of FGF23 has been recently reviewed²¹. Interesting, recent *ex vivo* studies using isolated murine hearts showed FGF23 induced premature ventricular beats and increased calcium levels in left ventricular cardiomyocytes²².

PTH also promotes phosphate excretion by reduction of activity of NPT2a and NPT2c²³, while 1,25-(OH)₂D appears to decrease phosphate excretion by acting on NPT2a²⁴. 1,25-(OH)₂D instead promotes phosphate absorption in the intestine by increasing membrane expression of NPT2b^{7,25}.

Because the majority of phosphate is stored within bone, disorders of phosphate homeostasis resulting in hypophosphatemia may result in metabolic bone disease and defective bone mineralization. Hereditary hypophosphatemic rickets encompasses a group of rare renal phosphate wasting disorders, often requiring genetic testing for diagnosis, with

an estimated prevalence of 3.9 per 100,000 live births^{26,27}. Hereditary hypophosphatemic rickets can further be divided into two groups, FGF23-dependent and FGF23-independent hereditary rickets. FGF23-dependent hereditary rickets include X-linked hypophosphatemic rickets, which is the most common cause of hereditary hypophosphatemic rickets and is caused by mutations in phosphate-regulating endopeptidase (PHEX); autosomal dominant hypophosphatemic rickets (FGF23); autosomal recessive hypophosphatemic rickets type 1 (dentin matrix acidic phosphoprotein 1, DMP1); and autosomal recessive hypophosphatemic rickets type 2 (ectonucleotide pyrophosphatase/phosphodiesterase 1, ENPP1), among others²⁸. Treatment is targeted at improving bone mineralization and reducing the burden of skeletal deformities and consists of lifetime phosphate and calcitriol supplementation to achieve a low-normal serum phosphate level and high-normal serum ALP level²⁹.

In contrast, FGF23-independent hereditary rickets can be caused by loss-of-function mutations in *SLC34A1* and *SLC34A3*. Hereditary hypophosphatemic rickets with hypercalciuria (HHRH) is an autosomal recessive disease caused by bi-allelic loss-of-function mutations in *SLC34A3*, resulting in a reduced ability to recycle phosphate in the proximal tubules and thus renal phosphate wasting³⁰. HHRH typically presents in childhood with an inability to recycle phosphate leads to rickets or osteomalacia. 1-alpha-hydroxylase is upregulated to increase 1,25-(OH)₂D synthesis to compensate for phosphate wasting by increasing intestinal phosphate absorption, which also increases intestinal calcium excretion resulting in hypercalciuria; however, PTH and FGF23 is often normal or suppressed. Renal calcifications, either nephrocalcinosis or nephrolithiasis, may be also be present, but renal failure due to renal calcifications from HHRH has not been

described^{30,31}. A literature review revealed that for individuals with HHRH, roughly 50% exhibit bone symptoms, 17% exhibit renal symptoms, and 18% exhibit both bone and renal symptoms, with 9% of individuals exhibiting no symptoms at all³². Individuals who carry a heterogenous loss-of-function mutation in *SLC34A3* show a milder phenotype, typically exhibiting idiopathic hypercalciuria and low bone density, but remain at risk of developing renal calcifications³³. Whether any biochemical parameters may be predictive of symptoms of HHRH is not well understood; one study suggests that increased serum phosphate, increased serum 1,25-(OH)₂D, and decreased tubular reabsorption of phosphate (TRP) may be predictive of renal calcifications³³.

Biallelic loss-of-function mutations in *SLC34A1* cause idiopathic infantile hypercalcemia type 2, an autosomal recessive disorder presenting in infancy with hypercalcemia and hypercalciuria due to an inappropriate increase in 1,25-(OH)₂D caused by renal phosphate wasting³⁴. Individuals who carry a heterozygous loss-of-function mutation in *SLC34A1* exhibit a milder phenotype marked by hypophosphatemia, renal calcification, and osteoporosis known as hypophosphatemic nephrolithiasis/osteoporosis 1 (NHLOP1)³⁵. A related disorder, idiopathic infantile hypercalcemia type 1, is an autosomal recessive disorder caused by homozygous loss-of-function mutations in *CYP24A1* presenting similarly with hypercalcemia and suppressed PTH, though hypophosphatemia is absent³⁶.

Because of the number of genotypes belonging to a diagnosis of hereditary hypophosphatemic rickets and their overlapping phenotypes and presentations, genetic testing for suspected cases of hereditary hypophosphatemic rickets is necessary for a definitive diagnosis. For example, a cross-sectional study of the genetic evaluation of 319

individuals with provider-reported clinical diagnosis of X-linked hypophosphatemia revealed roughly 19% of individuals had no XLH-causing mutation in *PHEX*³⁷. As with FGF23-dependent hereditary rickets, treatment of HHRH and idiopathic infantile hypercalcemia consists of lifelong phosphate supplementation. In contrast, calcitriol supplementation is not necessary and in fact may be contradictory as serum 1,25-(OH)₂D is already elevated, and exogenous sources of vitamin D may further increase hypercalciuria and subsequently risk of renal calcification³⁸.

Homozygous or compound heterozygous loss-of-function mutations in *SLC34A3* are required for a diagnosis of HHRH and presentation with rickets and renal phosphate wasting. Recently, however, the first report of a family with apparent autosomal dominant transmission of HHRH was reported³⁹. Genetic evaluation revealed that affected individuals carried digenic heterozygous mutations in both *SLC34A3* and *SLC34A1*, while family members carrying the heterozygous mutation in *SLC34A3* but not in *SLC34A1* were clinically less affected, suggesting a synergistic deleterious effect resulting in a HHRH phenotype. A polygenic contribution to disease severity has also been proposed in individuals who carry heterozygous mutations in *SLC34A3* and *CYP24A1*, who presented with symptomatic neonatal hypercalcemia and hypercalciuria along with a hypersensitivity to vitamin D⁴⁰.

While the hormonal and metabolic imbalances in HHRH is quite well understood, the phenotypic variability in patients with HHRH is not as well understood. Patients may present with severe rickets, osteoporosis, or no apparent bone phenotype; similarly, renal calcifications may or may not be present. As such, genetic evaluation of patients may be delayed, and difficulty in arriving at a diagnosis of HHRH versus other forms of

hypophosphatemic rickets may also result in harmful treatment, as both calcium and vitamin D can worsen hypercalciuria. Further complicating the workup of hereditary hypophosphatemic rickets is a lack of consensus on an established approach of using molecular genetic testing to determine the diagnosis⁴¹. Thus, understanding the underlying genetic causes of the phenotypic variability present in HHRH and a consensus approach to genetic evaluation suspected causes of hereditary hypophosphatemic rickets may facilitate earlier diagnosis, proper treatment, and integration of multi-disciplinary specialists in the care of the patient.

Statement of Purpose

Two individuals whose genetic evaluation revealed loss-of-function mutations in *SLC34A3* were evaluated at Yale-New Haven Hospital, one of whom had presented with cardiac arrhythmia in the setting of hypophosphatemia, and the second of whom had presented to an outside institution with asymptomatic pyuria and microscopic hematuria who later developed early onset renal failure and presented to Yale for management of her HHRH diagnosis. Cardiac arrhythmia and early onset renal failure have not yet been reported in association with HHRH, and I hypothesize there is a polygenic contribution to disease severity that may explain these atypical presentations. The purpose of this thesis is to elucidate any additional genetic causes of atypical symptoms associated with *SLC34A3* mutations and to propose an approach to the genetic evaluation of individuals with suspected hereditary hypophosphatemic rickets.

Methods

Human Subjects

This study was approved by the institutional review board of Yale University (Yale HIC 1501015216). Written informed consent was obtained from the two index cases by Clemens Bergwitz, who were recruited due to a genetic diagnosis of HHRH; family members across three generations were recruited as available. The index cases were individually evaluated and clinically managed by Clemens Bergwitz, either in-person or over telehealth due to health and safety limitations by the COVID-19 pandemic. Prospective clinical, biochemical, and molecular data was collected, and retrospective clinical, biochemical, radiographical, and molecular data was reviewed as available in the electronic medical record. Not all subjects were available for all of the proposed analyses or data collection performed. Chart review of patients and family members available for the study was performed by Bryan Ho.

Genetic Analyses

Genetic evaluation of the index cases was performed by Next Generation Sequencing of leukocyte DNA. Related family members were evaluated by mutation-specific polymerase chain reaction assays and Sanger Sequencing of leukocyte DNA, specifically targeting *SLC34A1*, *SLC34A3*, and *NPHP4*, as appropriate. Variants detected by whole exome sequencing were checked against reported mutations as listed in the National Center for Biotechnology Information dbSNP database (<https://www.ncbi.nlm.nih.gov/snp/>). Variants were screened using the ENSEMBL Variant Effect Predictor (VEP) to calculate a PhyloP 46-way conservation score and predict the impact of novel variants. Allele frequency of novel variants were checked using

the data browser of the Exome Variant Server of the National Heart, Lung, and Blood Institute Exome Sequencing Project (<https://evs.gs.washington.edu/EVS/>). Position of intronic variants were located using the University of California, Santa Cruz, Genome Browser (<https://genome.ucsc.edu/>) against the February 2009 human reference sequence (GRCh37). Evolutionary conservation was also determined manually using available compiled orthologue sequences of placental mammals obtained from Ensembl (<https://useast.ensembl.org/index.html>). Interpretation of variants was performed by Bryan Ho and Clemens Bergwitz.

Genetic evaluation for Case A specifically targeted 21 genes associated with disorders of phosphate metabolism, including *FGF23*, *PHEX*, *DMPI*, *ENPP1*, and *FAM20C*, and 41 genes associated with renal tubulopathies. A list of 55 genes associated with hereditary causes of cardiac arrhythmias was also reviewed⁴². Genetic evaluation for Case B specifically targeted a manually-curated list of 625 genes associated with renal failure, as reported by Groopman et al⁴³.

Biochemical Analyses

Biochemical serum and urine analyses were performed using standard clinical assays at either Yale-New Haven Hospital or Quest Diagnostics. Adult reference ranges are provided as reported by the resulting laboratory. Serum concentrations of 1,25-(OH)₂-vitamin D and 25-(OH)-vitamin D were measured by the Mineral Metabolism Lab at the Yale School of Medicine. Serum FGF23 levels were measured as c-terminal FGF23 by enzyme-linked immunosorbent assay at Mayo Clinic Laboratory. %TRP was calculated by the following formula:

$$\%TRP = \left(1 - \frac{\text{Urine Phos} \times \text{Serum Cr}}{\text{Urine Cr} \times \text{Serum Phos}} \right) \times 100$$

Case Presentations

Case A

Case A (Fig. 1, kindred A/II-1) first presented at age 5 with bowing of the lower extremities and required braces, but no diagnosis of hypophosphatemic rickets was made at the time. His height and weight tracked within the normal range, with an adult height of 5'8". He was diagnosed with ulcerative colitis at age 31, which has been in remission since being treated with prednisone and infliximab. Per pre-Epic records, at age 33, he was diagnosed with intermittent atrial fibrillation; an electrocardiogram at this time is not available. Trans-esophageal echocardiogram at the time showed a left atrial thrombus, and he was started on sotalol and warfarin, with resolution of his palpitations at the time. He had had no fractures until age 41, when he fell from his treadmill and sustained a right tibial plateau fracture.

At age 44, he was referred to and evaluated in the emergency room of Yale-New Haven Hospital for generalized weakness, palpitations, and orthostatic symptoms. His evaluation in the emergency room did not reveal any abnormalities on electrocardiogram. He returned twice more to the emergency room, again with weakness, palpitations, and orthostatic symptoms. On his third visit, his laboratory studies was notable for a severely reduced serum phosphorus of 0.9 mg/dL (reference range 2.2 – 4.5 mg/dL, Fig. 1). The rest of his work-up was notable for a high-normal 1,25-(OH)₂D of 49 pg/mL (reference range 25 – 66 pg/mL), normal 25-(OH)D of 42 ng/mL (reference range 30 – 100 ng/mL), normal PTH of 33.5 pg/mL (reference range 15 – 65 pg/mL), non-suppressed circulating FGF23 (cFGF23) of 101 RU/mL (reference range <180 RU/mL), and normal calcium of 9.3 mg/dL (reference range 8.8 – 10.2 mg/dL). Urine studies revealed a normal urine

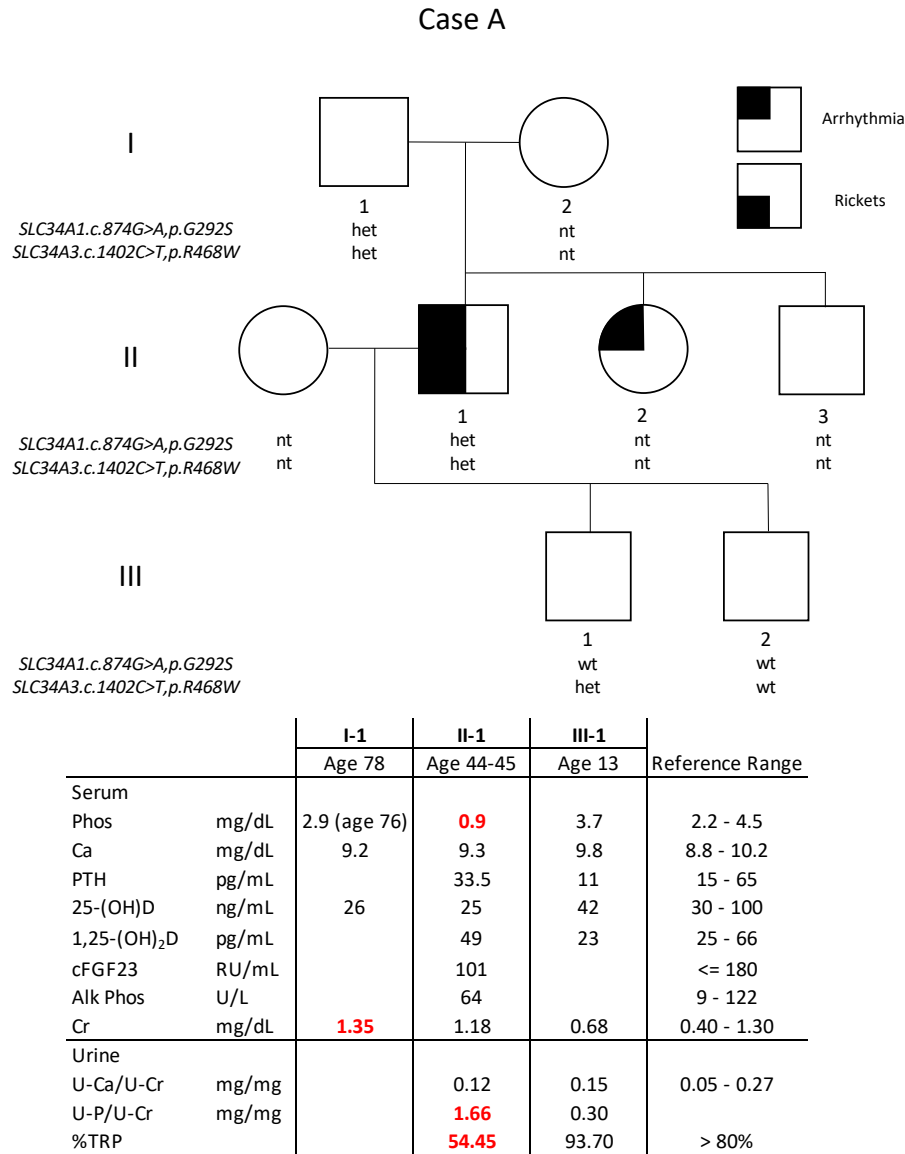


Figure 1. Three generation family pedigree for Case A. The phenotype and genotype for each individual in the kindred of Case A is presented. Subject A/II-1 is the index case who presented with cardiac arrhythmias in the setting of severe hypophosphatemia. Below the pedigree, serum and urine parameters of calcium and phosphorus homeostasis, along with serum measurements of phosphate-regulating hormones, are presented for the index case at presentation and family members. Circles denote females; squares denote males. wt = wild-type; het = heterozygous; nt = not tested; Phos = phosphorus; Ca = calcium; PTH = parathyroid hormone; 25-(OH)D = 25-hydroxy-vitamin D; 1,25-(OH)₂D = 1,25-dihydroxy-vitamin D; cFGF23 = circulating fibroblast growth factor 23; Alk Phos = alkaline phosphatase; Cr = creatinine; U-Ca/U-Cr = urine calcium-to-creatinine ratio; U-P/U-Cr = urine phosphorus-to-creatinine ratio; TRP = tubular reabsorption of phosphate.

calcium-to-creatinine ratio of 0.12 and an elevated urine phosphate-to-creatinine ratio of 1.66, with a decreased total reabsorption of phosphorus (TRP) of 54.45%.

Because of his non-suppressed FGF23 given his hypophosphatemia, there was suspicion for tumor-induced osteomalacia (TIO), but an FDG-PET-CT did not show any bony lesions concerning for TIO. He was started on phosphate supplementation with 250 mg/pack potassium/sodium phosphates (PhosNaK), 10 packs divided over five doses daily, and also initially with 0.25 mcg calcitriol three times daily. His serum phosphorus subsequently normalized, and he no longer experienced any palpitations. Calcitriol was briefly stopped due to hypercalciuria, upon which his palpitations recurred. Further cardiology work-up included several ECGs and 24-hour Holter monitoring that showed only premature ventricular contractions. Resumption of calcitriol again resolved his palpitations.

Bone densitometry performed at age 46 after approximately two years of phosphate and calcitriol supplementation revealed a lumbar spine bone mineral density of 1.187 g/cm² (Z-score 1.1) and total hip bone mineral density of 1.010 g/cm² (Z-score 0.1) (Fig. 2), both of which were in the normal range. His medical history is negative for nephrolithiasis, and renal ultrasound performed at age 45 and repeated at age 47 was normal with no evidence of nephrolithiasis. His ECGs in the interim has continued to show normal sinus rhythm.

Repeat and routine laboratory testing has shown normalization of his serum calcium and phosphorus levels while on PhosNaK and calcitriol treatment. However, even brief cessation of phosphate supplementation for five days resulted in a reduction in his serum phosphorus to 1.9 mg/dL. His cFGF23 remained non-suppressed, and his 1,25-(OH)₂D

Age	Kindred A, II-1					Kindred B, II-1						Reference Range
	44 (presentation)	45	45	45	46 - 47	7	19	23	35	36	36	
Symptom(s)	Palpitations	None	None	None	None	Asymptomatic hematuria	None	None	None	None	None	
Therapy												
Phosphate		PhosNaK 250mg 10 packs qd	PhosNaK paused overnight	PhosNaK paused 5 days	PhosNaK 250mg 10 packs qd						KPhos Original 500mg bid	
Vitamin D	Cholecalciferol 1000U qd	Cholecalciferol 1000U qd, calcitriol 0.25mcg tid	Cholecalciferol 1000U qd, calcitriol 0.25mcg tid	Calcitriol paused overnight	Cholecalciferol 1000U qd, calcitriol 0.25mcg bid							
Citrate						Urocit K 6480mg bid	Urocit K 6480mg bid	Discontinued	Discontinued	Discontinued		
Other	Sotalol 80mg bid	Sotalol 80mg bid	Sotalol 80mg bid	Sotalol 80mg bid	Sotalol 80mg bid	Lisinopril 7.5mg, HCTZ 50mg	Lisinopril 7.5mg, HCTZ 50mg	Discontinued	Lisinopril 2.5mg	Lisinopril 2.5mg		
Serum												
Na	mmol/L	137			140		139		138	140		136 - 144
K	mmol/L	3.4			4.2		4.8		4.2	4.8		3.4 - 4.8
Cl	mmol/L	98			106		100		99	101		98 - 107
Cr	mg/dL	1.18	1.27	1.28	1.24		1.2		1.45	2.41	1.36	0.4 - 1.3
Ca	mg/dL	9.3	8.9	9.6	9.0		9.5		9.7	9.9	9.8	8.8 - 10.2
Mg	mg/dL	1.8			1.8				2.1	2.0	2.0	1.7 - 2.4
Phos	mg/dL	0.9	3.1	2.0	1.9		3.0		2.3	2.6	2.9	2.2 - 4.5
Alk Phos	U/L	64	65	59							54	50 - 480
PTH	pg/mL		33.5	19.1	27.7				15	19.6	19.6	15 - 65
25-(OH)D	ng/mL	18	25	25					42.7	33	33	30 - 100
1,25-(OH) ₂ D	pg/mL		49		57				45.5	133	133	25 - 66
cFGF23	RU/mL		101		77					<14	<14	<=180
Urine												
U-Ca	mg/dL		10	14.6							4.4	
24-hr U-Ca	mg/day			550	321							100 - 300
U-P	mg/dL		272.9	71.4	49.4						30.4	
U-Citrate	mg											
U-Cr	mg/dL		203	208	100		290				59	16 - 326
U-Ca/U-Cr	mg/mg		0.05	0.07			0.09				0.07	0.05 - 0.27
U-Protein/U-Cr	mg/mg						0.13		0.85	0.34	0.20	<0.18
U-P/U-Cr	mg/mg		1.34	0.34	0.49						0.52	
%TRP			44.93	78.03	67.76						75.84	<80
Bone Densitometry												
Lumbar Spine	gm/cm ²				1.187			0.937		0.959		
Lumbar Spine Z-score					1.1			-0.9		-0.7		
Total Hip	gm/cm ²				1.01					0.843		
Total Hip Z-score					0.1					-0.7		
Imaging/Diagnostics												
Electrocardiogram		Normal sinus rhythm	Normal sinus rhythm	Normal sinus rhythm	Normal sinus rhythm							
Renal ultrasound			No nephrolithiasis, hydro-nephrosis, mass, or cyst noted.		No nephrolithiasis, hydro-nephrosis, mass, or cyst noted.	Bilateral medullary nephrocalcinosis, no hydro-nephrosis		Bilateral medullary nephrocalcinosis, no hydro-nephrosis			Bilateral medullary nephrocalcinosis, mild dilatation of R renal pelvis, 1.2 cm cyst R superior pole	

Figure 2. Clinical, biochemical, and imaging characteristics of index cases. Symptoms and laboratory values of serum and urine parameters are presented for the index cases of case A and case B along with symptoms and therapy taken at time of measurement. Bone densitometry, electrocardiogram, and renal ultrasound results are provided as available. Values outside the normal range are bolded in red. Na = sodium; K = potassium; Cl = chloride; Cr = creatinine; Ca = calcium; Mg = magnesium; Phos = phosphorus; Alk Phos = alkaline phosphatase; PTH = parathyroid hormone; 25-(OH)D = 25-hydroxy-vitamin D; 1,25-(OH)₂D = 1,25-dihydroxy-vitamin D; cFGF23 = circulating fibroblast growth factor 23; U-Ca = urine calcium; U-P = urine phosphate; U-Cr = urine creatinine; TRP = tubular reabsorption of phosphate.

remained in the high-normal range at 57 pg/mL even when calcitriol was paused overnight prior to measurement.

His family history is notable for a 10-years older sister (Fig. 1, A/II-2) who shares a diagnosis of ulcerative colitis and a cardiac arrhythmia of unknown type that is being treated with metoprolol but not warfarin. His oldest son was diagnosed with neuroblastoma at age 1 that was treated with doxorubicin and has been in remission since. His father reached an adult height of 5'8" and has a history of type 2 diabetes mellitus, hypertension, and hyperlipidemia, with a mildly decreased 25-OH(D) of 26 ng/mL and a mildly elevated urine albumin-to-creatinine ratio of 1512 mg/g Cr attributed to diabetes nephropathy. His mother is healthy with an adult height of 5'2". His younger son is reportedly healthy. None of his family members have any history of hypophosphatemia, nephrolithiasis, short stature, lower extremity bowing, pathologic fractures, or evidence of muscle weakness.

Case B

Case B (Fig. 3, kindred B/II-1) first presented to an outside institution for evaluation after developing persistent asymptomatic pyuria and microscopic hematuria at age 7. Renal ultrasound performed at the time revealed bilateral medullary nephrocalcinosis without evidence of hydronephrosis. She was started on potassium citrate 1080 mg/tab, 6 tabs daily; no laboratory values were available for review.

At age 19, she transitioned her care to her current adult nephrologist when she had a rise in serum creatinine to 1.2 mg/dL (reference range 0.4 – 1.3 mg/dL) (Fig. 2). Her serum calcium and phosphorus were both within the normal range at 9.5 mg/dL and 3 mg/dL, respectively. Her urine studies revealed a normal urine calcium-to-creatinine,

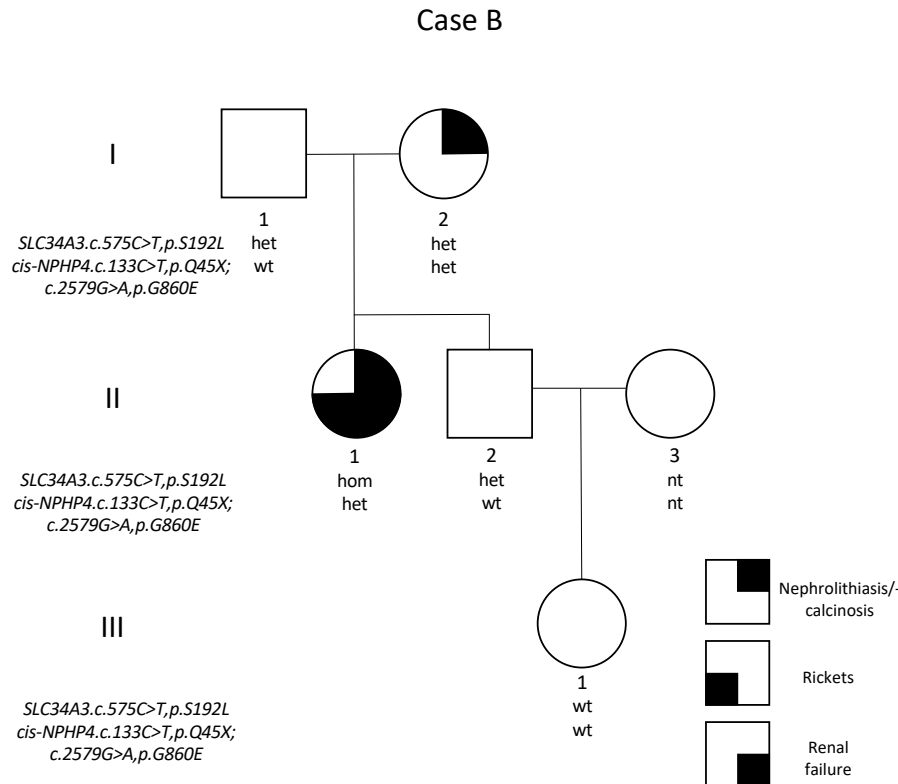


Figure 3. Three generation family pedigree for Case B. The phenotype and genotype for each individual in the kindred of Case B is presented. Subject B/II-1 is the index case who presented with bilateral nephrocalcinosis and chronic kidney disease stage 3 with physical examination findings of knee knock consistent with mild childhood rickets. Circles denote females; squares denote males. wt = wild-type; het = heterozygous; nt = not tested.

urine protein-to-creatinine ratio, and urine citrate of 0.09, 0.13, and 290 mg, respectively.

Vitals measured was notable for a systolic blood pressure in the 130s, and she was diagnosed with mild hypertension. She was subsequently started on lisinopril 7.5 mg daily and hydrochlorothiazide 50 mg daily. Her first bone mineral densitometry was performed at age 23 showing a low-normal lumbar spine bone mineral density of 0.937 g/cm² (Z-score -0.9). However, at age 25 she self-discontinued the lisinopril and hydrochlorothiazide because it caused fatigue and dizziness, and she only continued taking the potassium citrate tablets, which she slowly self-reduced the dosage and dosing across the next several years. By age 30 she had stopped taking the potassium citrate tablets completely. Total urine

calcium measured between ages 26 to 28 were in the normal range between 130 and 210 mg per 24 hours, and urinary pH was between 6.4 and 7.

By age 35, her kidney function had declined to CKD stage 3, with a serum creatinine of 1.45 mg/dL. Her other laboratory values, including a calcium of 9.7 mg/dL, phosphorus of 2.3 mg/dL, 25-(OH)D of 42.7, and 1,25-(OH)₂D, were within the normal range, although her PTH was low-normal at 15 pg/mL (Fig. 2). Urine studies were notable for an elevated urine protein-to-creatinine ratio of 0.85, concerning for significant proteinuria. She was restarted on lisinopril 2.5 mg daily.

She had reached an adult height of 169 cm, with a mid-parental height of 167.5 cm; however, on examination at our institution, her physical exam was notable for mild knee knock that was consistent with a history of childhood rickets. Aside from the nephrocalcinosis, she had never developed symptomatic nephrocalcinosis, pathologic fractures, or evidence of muscle weakness. In addition to the lisinopril, she was also taking bupropion 200mg daily and sertraline 25 mg daily for anxiety, depression, and insomnia, along with magnesium-oxalate suspension 1000 mg daily. She had not taken any calcium, phosphate, or vitamin D supplementation.

Repeat laboratory testing at age 36 continued to show an elevated creatinine with a high of 2.41 mg/dL. Hormonal testing showed a suppressed cFGF23 of <14 pg/mL, low-normal PTH of 19.6 pg/mL, low-normal 25-(OH)D of 33 ng/mL, and an elevated 1,25-(OH)₂D of 133 pg/mL. Urine testing showed a urine protein-to-creatinine ratio that had decreased to 0.20, with a normal urine calcium-to-creatinine ratio of 0.07 and urine phosphorus-to-creatinine ratio of 0.52 with a low TRP of 75.84%. Her bone mineral densitometry reading at age 36 again showed normal range lumbar bone mineral density

of 0.959 (Z-score -0.7) and total hip mineral density of 0.843 (Z-score -0.7). Renal ultrasound again showed bilateral medullary nephrocalcinosis, with new mild dilatation of the right renal pelvis and 1.2 cm cyst on the superior pole of the right kidney (Fig. 2). She was then started on potassium phosphate (KPhos Original) 500 mg twice daily.

Her family history is notable for a reported episode of symptomatic nephrolithiasis, proteinuria, and a parathyroid abnormality in her mother. Her maternal aunt was diagnosed with osteoporosis and has had reported jaw and finger fractures. Her father and brother are both generally healthy, without a history of hypophosphatemia, nephrolithiasis, short stature, lower extremity bowing, pathologic fractures, or evidence of muscle weakness.

Results

Genetic Analysis

Genetic testing of Case A (A/II-1) was conducted because of suspicion for hereditary rickets, which showed two heterozygous mutations in two different genes: a C to T substitution at position 1402 in exon 13 of *SLC34A3* resulting in a missense mutation from arginine to tryptophan at residue 468 (*SLC34A3.c.1402C>T,p.R468W*) that has been previously reported as a pathogenic variant³⁰, and a G to A substitution at position 874 in exon 8 of *SLC34A1* resulting in a missense mutation from glycine to serine at residue 292 (*SLC34A1.c.874G>A,p.G292S*) that is a novel variant of unknown significance. Comparison with orthologue sequences of 98 placental mammals revealed a conserved G residue at residue 292 in 98/98 (100%) of available sequences.

Because of Case A's sensitivity to calcitriol and re-occurrence of his palpitations when he briefly paused calcitriol, his exome was further studied for mutations in genes

regulating the metabolism of vitamin D, which did not reveal any suspicious variants. Further examination of whole exome sequencing showed a four nucleotide GATA duplication within intron 15, 161 base pairs downstream of the exon14/intron 15 boundary, in proprotein convertase subtilisin/kexin type 5 (*PCSK5.c.1900+181_1900+185dupGAATA*, Fig. 4), with a predicted high impact consequence by the ENSEMBL Variant Effect Predictor.

Further review of 55 genes associated with hereditary causes of cardiac arrhythmias revealed three splice-site variants: a single nucleotide A deletion at a position 5 nucleotides upstream of the boundary of intron 45 and exon 46 of titin (*TTN*) (*TTN.c.10798+1080delA*), a single nucleotide A deletion at a position 3 nucleotides upstream of the boundary of intron 108 and exon 109 of *TTN* (*TTN.c.13858+39165delA*), and a single nucleotide A insertion at a position 5 nucleotides upstream of the boundary of intron 4 and exon 5 of the calcium voltage-gated channel auxiliary subunit $\alpha 2\delta 1$ (*CACNA2D1*) (*CACNA2D1.c.10798+1080delA*). One intron variant was also reported, a two nucleotide AA deletion at a position 13 nucleotides upstream of the boundary of intron 2 and exon 3 of triadin (*TRDN*) (*TRDN.c.233-14_233-13delAA*). All four of these variants were, however, predicted to be of low impact (Fig. 4).

Genetic testing of available family members revealed that the father of Case A (A/I-1) carries the same di-genic mutations in *SLC34A1* and *SLC34A3* as the A/II-1 (Fig 1). His oldest son, A/III-1, is also a heterozygous carrier of the *SLC34A1.c.874G>A.,p.G292S* mutation, but not of the mutation in *SLC34A3*. His younger son, A/III-2, did not possess the two variants in *SLC34A1* and *SLC34A3*. The mother and older sister, A/II-2, were not available for genetic testing.

Gene	Full Name	Variant	Consequence	VEP Impact	pLI	PhyloP 46-way Score	Population Frequency
PCSK5	Proprotein convertase subtilisin/kexin type 5	c.1900+181_1900+185dupGAATA	Frameshift/intron variant	High	0.0002	-2.482	0
TTN	Titin	c.13858+39165delA	Splice region deletion	Low	1.211E-32	3.003	0
		c.10798+1080delA	Splice region deletion	Low	1.211E-32	-1.187	0
CACNA2D1	Calcium voltage-gated channel auxiliary subunit	c.355-5dupA	Splice region insertion	Low	0.99999	-0.455	0
TRDN	Triadin	c.233-14_233-13delAA	Intron variant deletion	Low	1.816E-10	-0.935	0

Figure 4. Variants of interest from whole exome sequencing of A/II-1. Whole exome sequencing was performed on case A/II-1, and results targeted for genes associated with hereditary forms of cardiac arrhythmias and disorders of phosphate and vitamin D metabolism. Impact score was predicted by ENSEMBL Variant Effect Predictor (VEP), of which a gene that may be involved in post-translational processing of FGF23, PCSK5, generated a high impact prediction. 3 genes associated with hereditary causes of cardiac arrhythmia, TTN, CACNA2D1, and TRDN, were predicted to have a low impact. Tolerance to loss-of-function is predicted and scored by probability of loss of function intolerance (pLI), and evolutionary conservation was estimated using the PhyloP 46-way base-wise conservation scoring.

Genetic testing of Case B (B/II-1, Fig. 3) was conducted because of a suspicion for a genetic cause of her early onset renal failure and childhood bilateral nephrocalcinosis. Review of 625 genes⁴³ associated with hereditary forms of renal and urogenital diseases revealed homozygous known pathogenic mutations in *SLC34A3*: a C to T substitution at position 575 in exon 7 causing a serine to leucine shift at residue 192 (*SLC34A3.c.575G>A,p.S192L*), resulting in a genetic diagnosis of HHRH³⁰. She was also found to possess two heterozygous mutations in nephrocystin 4 (NPHP4). A G to A substitution at position 2579 in exon 19 results in a glycine to glutamate change at residue 860 (*NPHP4.c.2579G>A,p.G860E*) that was classified as a variant of unknown significance, with 97/98 (99%) of orthologue sequences of placental mammals having a conserved G residue at site 860. She also possessed a known nonsense mutation substituting C to T at position 133 in exon 2 resulting in an early termination at residue 45 (*NPHP4.c.133C>T,p.Q45X*)⁴⁴. These mutations were later determined to be contained on the same allele and thus cis-inherited.

Evaluation of available family members revealed that the mother is a heterozygous carrier of the cis-inherited *NPHP4* mutations (Fig. 3). Because the index case B/II-1 is homozygous for the *SLC34A3*.c.874G>A,p.S192L variant, both parents are obligate carriers of at least one allele containing the same *SLC34A3* variant. Her brother, B/II-2, is a heterozygous carrier of the *SLC34A3*.c.874G>A,p.S192L variant, but not of the *NPHP4* variant. Her niece, B/III-1, was also tested and did not possess any pathogenic variants in *SLC34A3* or *NPHP4*.

Discussion

A presentation of childhood rickets or early-onset osteoporosis, with or without renal calcifications, that appears to be inherited in an autosomal recessive manner is most consistent with a diagnosis of HHRH; however, as had been recently reviewed³², roughly half of patients do not present with apparent bone symptoms. Bone disease may also not be readily present and may manifest only through careful review of a patient's medical history for childhood bone pain, lower extremity bowing, fractures, or an adult height below the predicted mid-parental height. Furthermore, patients may present with renal calcifications without any apparent bone disease, or even no symptoms at all. The two index cases, who both possess *SLC34A3* variants, presented here presented not with readily apparent bone disease, but rather, a cardiac arrhythmia in Case A and early-onset renal failure in Case B, suggesting an even broader range of symptoms and effects possibly caused by HHRH.

Carriers of heterozygous pathogenic *SLC34A3* variants typically do not cause the bone disease and full HHRH phenotype as seen in individuals with homozygous

pathogenic *SLC34A3* variants³³, although a recent report by Gordon et al did suggest a polygenic contribution to a disease phenotype similar to that of HHRH, as individuals who were carriers of heterozygous *SLC34A3* and *SLC34A1* variants seemed to show a HHRH-like phenotype but in an autosomal dominant fashion³⁹. Case A likewise is a heterozygous carrier of a known pathogenic *SLC34A3* variant and novel *SLC34A1* variant of unknown significance, who has a history of childhood rickets and symptomatic hypophosphatemia consistent with HHRH, which offers further support that compound heterozygous mutations across these two phosphate transporters may cause a HHRH-like phenotype. However, the phenotype in this case is not as severe as those reported by Gordon et al, whose proband presented with a combination of childhood rickets, short stature, hypercalciuria, and bilateral nephrocalcinosis; this may be a product of a higher degree of loss-of-function, as the reported variant, *SLC34A3.c.1561dupC,p.L521Profs*72*, is a predicted pathogenic variant due to a frameshift mutation with an early termination 72 amino acids downstream, whereas the *SLC34A3* variant, although previously reported as pathogenic, carried by Case A is a missense mutation.

The low population frequency and high evolutionary conservation of the *SLC34A1* variant raises the risk that this may be a disease-causing variant and is contributing to the observed phenotype. NPT2a consists of eight transmembrane domains, with two large amino acid repeats in the “N”- and “C”- halves of the protein joined by a large extracellular loop, which includes the substituted amino acid at residue 292, between the third and fourth transmembrane domains.⁴⁵ Although the function of this large extracellular loop is not well understood, alteration of this large extracellular loop may impair proper substrate access for transmembrane transport or joining of the two protein halves.

In Case A, initial evaluation in the emergency room for subjective symptoms of palpitations revealed an ECG in normal sinus rhythm, and his biochemical studies were only notable for a severe hypophosphatemia. Although he had a history of atrial fibrillation, this had been well-controlled on sotalol with normal sinus rhythm on electrocardiograms in the interim. It has been well-described that hypophosphatemia has been associated with new-onset supraventricular arrhythmias in septic and critically-ill patients⁴⁶; a small study comparing incidence of arrhythmias in critically-ill patients with and without intravenous phosphorus replacement showed a significant decrease in incidence of arrhythmias after IV phosphorus replacement⁴⁷. However, whether hypophosphatemia in non-critically-ill patients can lead to new cardiac arrhythmias is less well understood, although in patients with myocardial infarction, hypophosphatemia is a significant predictor of ventricular tachycardias in the first 24 hours⁴⁸, and thus can disrupt myocardial contractility and cardiac function with increased left ventricular stroke work⁴⁹. His palpitations did resolve with timely phosphate supplementation and correction, hinting that his hypophosphatemia may be contributing to his arrhythmias. Initial evaluation of patients with new cardiac arrhythmias may benefit from prompt measurement of serum phosphorus and replacement as necessary, especially in patients with suspected phosphaturic disorders.

Calcitriol is not recommended for treatment of HHRH due to the risk for increased hypercalciuria and subsequent risk of renal stones. Case A was advised to decrease his calcitriol usage, but due to a recurrence of his palpitations when calcitriol was paused, he continued on calcitriol. It is unclear whether vitamin D deficiency itself is a cause of palpitations; some studies have shown a correlation between atrial fibrillation and vitamin

D deficiency in a relatively younger population (<58 years old), and that an increasing degree of vitamin D deficiency may increase the risk of post-operative atrial fibrillation⁵⁰.

Furthermore, the hormone studies, revealing a non-suppressed but rather elevated cFGF23, normal 1,25-(OH)₂D, and a low-normal 25-(OH)D, is not wholly consistent with HHRH, where 1,25-(OH)₂D is often elevated and FGF23 is normal or suppressed³⁰, which was initially suspicious for TIO, a paraneoplastic syndrome marked by FGF23-secreting mesenchymal tumors and subsequent reduced 1,25-(OH)₂D, renal phosphate wasting, bone pain, and muscle weakness. Work-up and management uses functional imaging such as FDG-PET-CT, phosphate, and calcitriol supplementation⁵¹. Case A was thus started on phosphate and calcitriol, but his imaging was negative for suspicious bony lesions. Given his eventual genetic testing revealing a known pathogenic *SLC34A3* variant, I suspected that his atypical hormone presentation may have an underlying genetic cause. Review of his whole exome sequencing for causes of disorders in vitamin D metabolism revealed a variant in intron 15 of *PCSK5*; this intron is flanked by exons encoding a cysteine-rich domain that is essential for cell-surface anchoring and protein-protein interactions such as binding of tissue inhibitors of metalloproteinases.⁵² Intronic variants, including within intron 15, have been shown to have a deleterious effect on *PCSK5* in regulation of high-density lipoprotein cholesterol levels⁵³; it is unclear what effect this intronic variant has on splicing but may interfere with the intron enhancer region or loop formation. Aberrant splicing of an exon in the cysteine-rich domain would likely disrupt cell-surface anchoring or protein-protein interactions such as FGF23 processing as discussed below, and thus decreases its activity.

Further, at least two isoforms of PCSK5 are known, PC5 and PC6, although only the transcript of PC6 is fully known.^{54,55} An alternative transcript (uc004ajy.2) incorporates this intron as exon and would result in an p.R688E and p.I689Y substitutions, with preservation of the termination codon 8 amino acids downstream. In vitro studies in a murine pituitary tumor cells show that the Pc5 isoform is sorted for either secretion or trans-membrane inclusion by a -COOH tag⁵⁶; the two amino acid substitution in an alternative splice variant may alter appropriate sub-cellular trafficking and thus decrease the activity of the final protein product.

FGF23 is post-transcriptionally modified by phosphorylation by FAM20C, resulting in eventual proteolysis by subtilisin-like proprotein convertase FURIN, whereas O-glycosylation by GALNT3 prevents FAM20C phosphorylation and results in the secretion of active FGF23. Loss-of-function mutations in PCSK5, a related subtilisin/kexin proprotein convertase, would be expected to result in reduced proteolysis and thus inactivation of FGF23 and could explain the patient's non-suppressed FGF23 levels. Studies in the Hyp mouse model of X-linked hypophosphatemia, marked by elevated Fgf23 levels due to a mutation in Phex, showed decreased Pcsk5 and Galnt3 in bone, suggesting that reduced post-translational processing may contribute to the elevated FGF23 seen in XLH⁵⁷.

To rule out hereditary forms of cardiac arrhythmias, Case A's exome was screened for 55 genes associated with hereditary causes of cardiac arrhythmias. Heterozygous intronic variants were reported in TTN, causing arrhythmogenic right ventricular cardiomyopathy; TRDN, causing long QT syndrome; and CACNA2D1, causing Brugada and short QT syndrome, all in an autosomal recessive manner^{42,58}. Given the heterozygous

carrier status and low predicted impact score by the Ensembl Variant Effect Predictor, along with a normal echocardiogram with no structural heart disease, hereditary forms of cardiac arrhythmias were ruled out, further supporting a role of hypophosphatemia as a source of his palpitations.

Case A's father, A/I-1, possesses the same heterozygous *SLC34A1* and *SLC34A3* mutations as the index case, A/II-1. Chart review did not reveal any signs of HHRH in the father, such as hypophosphatemia, hypercalciuria, childhood rickets, renal calcifications, or nephrolithiasis, which would argue against a genotype/phenotype relationship that the *SLC34A1* and *SLC34A3* mutations contributes to or severely modifies the HHRH phenotype in an autosomal dominant fashion. However, whole exome sequencing and measurements of serum 1,25-(OH)₂D and FGF23 was not available, and as in HHRH his asymptomatic status may be a result of variable expressivity. Notably, his serum 25-(OH)D is mildly decreased, but a 1,25-(OH)₂D level was not measured. Determination of whether he possess any variants in the metabolism of vitamin D, as in A/II-1, is warranted; future whole exome sequencing analysis of the father may not, for example, reveal a *PCSK5* mutation and thus could explain a reduced expressivity of the *SLC34A1* and *SLC34A3* mutations. Similarly, a sister, A/II-2, also possesses a cardiac arrhythmia; she was unavailable for genetic testing, and precludes segregation analysis of the arrhythmia phenotype with the heterozygous *SLC34A1* and *SLC34A3* mutations.

In Case B, childhood bilateral nephrocalcinosis, development of an elevated creatinine to 1.2 mg/dL by age 19, and worsening proteinuria led to a work-up for genetic causes of renal failure, which revealed homozygous known pathogenic *SLC34A3* variant, confirming a genetic diagnosis of HHRH, and a heterozygous cis-inherited *NPHP4* variant.

Homozygous or compound heterozygous *NPHP4* mutations cause an autosomal recessive cystic kidney disease, nephronophthisis, which is a ciliopathy that is the most common genetic cause of childhood renal failure⁵⁹; adolescent nephronophthisis presents with a median age of the onset of renal failure of 19 years⁶⁰. *NPHP4* belongs to a group of nephrocystins that form the ciliary axenome, of which *NPHP4*, along with *NPHP1* and *NPHP8*, forms the transition zone⁶¹. Because of their effects on cilia function, *NPHP4* variants may also be associated with extra-renal manifestations, including the ocular findings retinitis pigmentosa or Leber congenital amaurosis, which along with renal failure constitutes Senior-Loken syndrome. Heterozygous carriers are asymptomatic without risk of developing early onset renal failure⁶². Thus, development of early onset renal failure may be due to the combined gene burden of the heterozygous *NPHP4* mutation and the homozygous *SLC34A3* mutations.

Initial evaluation of the renal defects in Case B did not include urinary phosphate studies; her hypocitraturia, mild hypercalciuria, and non-acidified urinary pH of 6.4 to 7, along with nephrocalcinosis, suggested she had an incomplete renal tubular acidosis, which could have explained her nephrocalcinosis. She had been started on potassium citrate and hydrochlorothiazide to increase urinary citrate excretion and decrease urinary calcium excretion, respectively. She also did not have readily apparent signs of childhood rickets, with a mild knee knock the only sign of a history of rickets on physical exam. Lack of urinary phosphate studies, presentation with subtle signs of rickets, and emerging early onset renal failure likely delayed a suspicion for hereditary rickets and genetic evaluation, which later revealed the correct genetic diagnosis. Careful physical examination and consideration of including urinary phosphate measurements with other urinary parameters

such as calcium and citrate may be beneficial in the work-up of early onset renal failures. Subsequent laboratory workup at age 36 revealed an elevated 1,25-(OH)₂D and suppressed FGF23 consistent with a diagnosis of HHRH. Because the hypercalciuria seen in HHRH is caused by inappropriately elevated 1,25-(OH)₂D, phosphate rather than citrate supplementation was advised; the patient was started on phosphate supplementation with KPhos Original, which concomitantly increases urinary pH to reduce stone formation and raises urinary citrate excretion⁶³.

The mother of Case B, B/I-2, was found to be a heterozygous carrier of both the *SLC34A3* and the cis-inherited *NPHP4* mutations. Her history was notable for a reported episode of nephrolithiasis. The brother, B/II-2, was revealed to be a heterozygous carrier of the *SLC34A3* mutation but not the *NPHP4* mutations, and did not have any signs of a bone or renal phenotype. Similarly, the father, B/I-1, is a heterozygous carrier of the *SLC34A3* mutation but not the *NPHP4* mutations, and did not exhibit a bone or renal phenotype. There appears to be a correlation between gene burden and disease severity, as presence of the *NPHP4* variant along with one copy of the *SLC34A3* variant appears to predispose to nephrolithiasis, and when combined with two copies of the *SLC34A3* variant, early onset renal failure and childhood rickets. In this kindred, possessing only one allele of the *SLC34A3* with lack of the *NPHP4* variant does not seem to predispose to a renal phenotype.

Early onset renal failure has not yet been attributed to HHRH, although it is possible that the renal calcifications due to HHRH may result in kidney damage. A literature review of reported cases of HHRH revealed 2 individuals who had elevated serum creatinine, evidence of renal calcifications, and *SLC34A3* mutations. Areses-Trapote et al reported a

50 year old male with a history of rickets, nephrolithiasis, and bilateral nephrocalcinosis who possessed homozygous intronic *SLC34A3* variants (*SLC34A3.c.448+5G>A*)⁶⁴. Notably, his serum creatinine was 1.66 mg/dL with a calculated Z-score of 5.68, and a mild glomerular proteinuria and incomplete renal tubular acidosis was detected, which was attributed to the renal calcifications and not the underlying *SLC34A3* variant. Dasgupta et al reported an 11 year old female who presented with nephrolithiasis and bilateral nephrocalcinosis without any bone phenotype, and was found to possess compound heterozygous mutations in *SLC34A3* (*SLC34A3.c.575C>T,p.S192L;c.367delC*)³³. Her serum creatinine was 1 mg/dL with a calculated Z-score of 6.00. Notably, management of her nephrolithiasis and nephrocalcinosis included lithotripsy and pig-tail placement, and such manipulation may itself worsen kidney function. A third case reported by Yu et al presented a 15 year old male who was asymptomatic possessing a heterozygous *SLC34A3.c.1764C>G,p.Y588X* mutation, with no evidence of nephrocalcinosis⁶⁵. His creatinine was 0.89 mg/dL with a calculated Z-score by Dasgupta et al of 3.36³³; however, upon further review, the recommended reference range used was for a pediatric group, whereas Mayo Labs recommends 15 year old males to be evaluated with the adult reference range⁶⁶, which would have placed his serum creatinine in the normal range.

Elevated serum phosphate and 1,25-(OH)₂D, along with decreased TRP, has been suggested to be predictive of renal calcifications in patients with HHRH³³. B/II-1 had a reduced TRP and significantly elevated 1,25-(OH)₂D, but a serum phosphate within the normal range, which offers support for TRP and 1,25-(OH)₂D as predictors of nephrocalcinosis. Whether these parameters are independent predictors of

nephrocalcinosis or a by-product of the underlying genotype warrants further study, as these parameters may help guide therapy to manage or prevent renal calcifications.

This study is limited by its small sample size of two index cases, and limited participation and availability of family members, which precludes a statement of definitive causality of among the *SLC34A1*, *SLC34A3*, and *NPHP4* variants described here. That these variants are either known pathogenic or deleterious variants, or novel variants with highly conserved residues, along with functional and segregation analysis, implies that a genotype-to-phenotype correlation exists among variants in these three genes.

HHRH is a rare disorder, with fewer than 50 cases so far reported, and of those cases reported, only the initial presentation and evaluation are available. The variable expressivity and shared phenotype with other forms of hereditary rickets also delays or precludes appropriate genetic work-up, suggesting HHRH may be under-diagnosed. Follow-up and long-term studies of HHRH and response to treatment are not as well studied. It would be important to understand, for example, whether long-term phosphate supplementation affects cardiovascular morbidity, or if phosphate requirements decrease as patients age, as seen in autosomal dominant hereditary rickets⁶⁷. Efforts to recruit family members of these cases for a genetic evaluation are ongoing, in order to attempt to generate statistically significant logarithm of the odds (LOD) score.

In conclusion, reported herein are the second case of an individual with di-genic heterozygous *SLC34A1* and *SLC34A3* mutations and the first known case of an individual with di-genic homozygous *SLC34A3* mutations and heterozygous *NPHP4* mutation, who presented with cardiac arrhythmias and early onset renal failure, respectively. HHRH and *SLC34A3* variants present with variable expressivity, which may be explained by a

polygenic contribution to disease severity. An approach to suspected cases of HHRH with atypical presentation should include a targeted genetic evaluation with careful consideration and interpretation of variants in a diverse group of proteins related to a specific symptom, response, or pathophysiologic pathway.

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