

Case report

Novel Variant of *SLC34A3* in a Compound Heterozygous Brazilian Girl with Hereditary Hypophosphatemic Rickets with Hypercalciuria**Valadares and Carvalho. Hypophosphatemic Rickets with Hypercalciuria in a Brazilian Girl**Luciana Pinto Valadares¹, Daniel Rocha de Carvalho²¹Unit of Osteometabolic Disorders, SARAH Network of Rehabilitation Hospitals, Brasília, Brazil.²Medical Genetics Unit, SARAH Network of Rehabilitation Hospitals, Brasília, Brazil.**What is already known on this topic?**

HHRH is caused by loss-of-function variants of the sodium-phosphate co-transporter NPT2c and is an FGF23-independent disorder that causes rickets. Phosphate supplementation alone is standard of care. Because 1,25(OH)₂D is already elevated, active vitamin D analogs are not indicated. The best approach for managing hypercalciuria has not yet been established.

What this study adds?

We report a novel variant of *SLC34A3* gene in compound heterozygosity causing HHRH in a Brazilian girl. We discuss treatment strategies and observed that thiazide diuretics may be useful as adjunctive therapy to lower urinary calcium excretion.

Abstract

Hereditary hypophosphatemic rickets with hypercalciuria (HHRH) is a rare FGF23-independent disorder caused by biallelic variants in the *SLC34A3* gene. The disease severity varies, and patients have an increased risk of developing renal complications. Phosphate supplementation is standard of care and active vitamin D analogs are not indicated as they could worsen the hypercalciuria. We report a Brazilian girl with HHRH who presented with knee pain and progressive genu valgum deformity that became apparent later in childhood (at age 8). Nephrocalcinosis was also identified at age 13. Next-generation sequencing (NGS) target panel directed to inherited forms of rickets detected compound heterozygous pathogenic variants in *SLC34A3*, including a novel missense variant c.1217G>T (p.Gly406Val).

Compliance to oral phosphorus therapy was suboptimal and adjunctive chlorthalidone therapy improved hypercalciuria. Our case highlights the phenotypic variability of patients with HHRH and expands the growing list of *SLC34A3* variants associated with this disorder. An accurate diagnosis is crucial for proper treatment, and a thiazide diuretic may be useful as adjunctive therapy for controlling hypercalciuria.

Keywords: hereditary hypophosphatemic rickets with hypercalciuria; *SLC34A3* pathogenic variants; hypercalciuria.

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Introduction

Hereditary hypophosphatemic rickets with hypercalciuria (HHRH) is a rare disorder caused by biallelic mutations in *SLC34A3*, the gene encoding the sodium-phosphate co-transporter type 2c (NPT2c)(1). NPT2c is expressed in the renal proximal tubule cells and mediates renal phosphate resorption. HHRH is a fibroblast growth factor 23 (FGF23)-independent disorder, and pathogenic variants of *SLC34A3* lead to hypophosphatemia due to excessive urinary phosphate wasting. Circulating levels of 1,25 di-hydroxyvitamin D [1,25(OH)₂D] are appropriately elevated, leading to increased intestinal calcium resorption, hypercalciuria, and parathyroid hormone (PTH) suppression (1,2). The clinical spectrum of skeletal disease varies, and renal complications such as nephrolithiasis and nephrocalcinosis may occur in approximately half of the affected subjects (2). Accurate diagnosis is crucial for appropriate therapy, as patients should receive only oral phosphate treatment. Active vitamin D analogs should not be used, as they may exacerbate hypercalciuria and increase the risk of renal complications (2).

We describe a Brazilian girl with HHRH who presented with progressive genu valgum deformity that became apparent after 8 years of age. Genetic analysis detected compound heterozygous pathogenic variants in *SLC34A3*, including a novel pathogenic variant. Here, we discuss the clinical spectrum of the disease and its treatment strategies.

This study was approved by the Ethics Committee of the SARAH Network of Rehabilitation Hospitals (Certificate of Presentation for Ethical Appreciation number 36961620.9.0000.0022) and performed in accordance with the tenets of the Declaration of Helsinki. Informed consent was obtained from all subjects.

Case presentation

A 16-year-old Brazilian girl was admitted for orthopedic evaluation at the age of 10 because of knee pain and genu valgum deformity that became apparent after the age of 8.

She was born healthy to non-consanguineous parents. No dental, hearing problems or other signs of rickets such as muscle weakness, widening of knees or wrists, rachitic rosary or cranial abnormalities (dolichocephaly, craniosynostosis) were observed. Pubertal development was normal, and menarche occurred at 12 years of age. At age 16, Tanner Stage was 4 for both breast development and pubic hair, and her height (144.5 cm; SDS -2.8) was below her parental target height (father was 170 cm, and mother was 159 cm of height) and near the final height prediction (145.4 ± 0.8). She had no family history of bone disease; however, her father reported a history of nephrolithiasis.

Laboratory data at the first orthopedic evaluation at age 10 showed elevated alkaline phosphatase (ALP), low-normal serum PTH, normal calcium and phosphorous levels, 25-hydroxy vitamin D (25OHD) value of 29.54 ng/mL, and elevated urinary calcium excretion (calcium/creatinine ratio of spot urine: 407 mg/g) (Table 1). Blood gas analysis did not indicate acidosis, and the urinalysis results were negative for aminoaciduria and glycosuria. Skeletal radiographs showed marked genu valgum deformity (Figure 1), and she underwent bilateral distal femoral epiphysiodesis at age 11.

Biochemical analysis at age 13 revealed an elevated ALP and C-telopeptide of type I collagen (CTX), hypophosphatemia with a reduced TmP/GFR (maximum rate of renal tubular reabsorption of phosphate per glomerular filtration rate) indicating urinary phosphate wasting, and elevated 24-h urinary calcium excretion

(up to 8 mg/kg/day), with normal serum calcium and low PTH levels, raising suspicion for HHRH (Table 1). Renal ultrasonography revealed a discrete bilateral increase in the renal medullary echogenicity, suggesting nephrocalcinosis. Bone mineral density (BMD) obtained by dual-energy X-ray absorptiometry (DXA) showed decreased BMD in the lumbar spine (Z-score -2.7) and total body (Z-score total body less head -3.3).

A targeted next-generation sequencing (NGS) panel for 13 genes associated with inherited forms of rickets (*ALPL*, *CLCN5*, *CYP27B1*, *CYP2R1*, *DMP1*, *ENPP1*, *FAH*, *FGF23*, *KL*, *PHEX*, *SLC34A1*, *SLC34A3*, and *VDR*) was performed using genomic DNA extracted from oral mucosa. A compound heterozygous mutation in *SLC34A3* was detected (Refseq NM_080877.3). The novel *missense* variant c.1217G>T (p.Gly406Val) was identified in the paternal allele, and classified as a variant of uncertain significance. Glycine at position 406 of the protein is highly conserved among different species (figure 2), and this variant was predicted to be deleterious or disease-causing by *in silico* analysis (PolyPhen and Mutation Taster). The second variant c.1058G>T (p.Arg353Leu), inherited from the mother, was previously described in patients with HHRH and classified as likely pathogenic (1).

Phosphate therapy (20 mg/kg of elemental phosphorus per day) was initiated after genetic diagnosis, with poor tolerance due to gastrointestinal symptoms and suboptimal compliance. A thiazide diuretic (chlorthalidone 25 mg/day) was initiated as an adjunctive treatment for hypercalciuria at age 15. Bone turnover markers decreased, and urinary calcium excretion improved following treatment (Table 1). No adverse effects of thiazide diuretic, such as hypercalcemia or electrolytic abnormalities, were reported during the follow-up period. She required right femoral osteotomy at age 15 and left femoral osteotomy at age 16 to correct lower limb deformities. Serum calcium, phosphorous, ALP, and PTH levels of her parents were normal. The 24-h urinary calcium excretion of the father was normal, but he exhibited concurrent vitamin D deficiency (Table 1).

Discussion

This study describes the clinical and biochemical features of a Brazilian girl with HHRH caused by compound heterozygous variants in *SLC34A3*, expanding the knowledge of the phenotype and genetic variants associated with this rare metabolic disorder. HHRH is rare, with an estimated prevalence of 1:250,000, which is approximately 10-fold less frequent than X-linked hypophosphatemia (XLH), the most common form of inherited hypophosphatemic rickets (2).

Pathogenic variants in *SLC34A3* result in hypophosphatemia due to urinary phosphate wasting from NPT2c dysfunction. FGF-23 is downregulated in response to hypophosphatemia, leading to the compensatory up-regulation of renal 1- α -hydroxylase. Thus, patients present with hypophosphatemic rickets/osteomalacia, increased 1,25(OH) $_2$ D levels, and hypercalciuria (2,3). These biochemical findings differentiate HHRH from FGF23-mediated disorders (4).

Skeletal abnormalities typically occur in childhood, but some patients may exhibit late-onset clinical features such as early-onset osteoporosis, recurrent fractures, and renal stones (5–7). Our patient presented with knee pain and progressive lower-limb deformity that became apparent after 8 years of age. This contrasts with the XLH phenotype, in which bone involvement is present in the first years of life. In our case, the laboratory findings were not entirely consistent with hypophosphatemic rickets at the first evaluation, with normal phosphorus levels. Subsequent biochemical evaluation revealed hypophosphatemia with a low Tmp/GFR, indicating renal phosphate wasting. Although serum 1,25(OH) $_2$ D and FGF-23 levels could not be measured, concurrent findings of hypercalciuria and low serum PTH were not expected in FGF-23-mediated disorders, raising the suspicion of HHRH. Klemke et al. also reported a case in which hypophosphatemia was absent at the first evaluation, suggesting that serum phosphorous levels may fluctuate in this condition, and repeated biochemical evaluation may be necessary to establish the diagnosis (8).

Individuals with HHRH carry homozygous or compound heterozygous pathogenic variants of *SCLA34A3* (1,2). A single heterozygous pathogenic variant is associated with isolated hypercalciuria, which increases the risk of nephrocalcinosis and nephrolithiasis without apparent bone disease (2,3). However, skeletal abnormalities, including osteomalacia, predominant cortical loss, and osteoporosis, have been observed in subjects with monoallelic *SCLA34A3* variants (9).

Rickets is more prevalent in homozygous patients than in those with compound heterozygous pathogenic variants in *SCLA34A3* (10). The milder phenotypes of subjects carrying heterozygous variants are probably related to a lower degree of urine phosphate loss, related to increased residual activity of NPT2c and higher serum phosphate levels (11). The variability in the age of onset might be related to the incomplete penetrance of variants and complex interactions with environmental and nutritional factors.

Accurate diagnosis of HHRH is crucial for precise therapeutic intervention, as a presumptive diagnosis of XLH or other FGF-23-mediated disorders could lead to inappropriate therapy with calcitriol, worsening hypercalciuria and increasing the risk of renal complications (2,4). Target genetic panels directed to inherited forms of rickets are relevant for accurate diagnosis and facilitate correct treatment.

In our patient, a targeted NGS panel identified a previously reported pathogenic heterozygous variant in the maternal allele of *SLC34A3* (p.Arg353Leu) and a novel heterozygous mutation in the paternal allele (p.Gly406Val). Although we did not perform *in vitro* functional tests, the glycine at position 406 of the protein is highly conserved among different species (figure 2), and the p.Gly406Val variant was predicted to be deleterious or disease-causing by *in silico* analysis. A rare variant in the same codon (c.1217G>A, p.G406E, rs139408872) is described in dbSNP, and like the p.G406V is predicted to be pathogenic (<https://www.ncbi.nlm.nih.gov/snp>, accessed December 04, 2022). In addition, the heterozygous father manifested nephrolithiasis, which reinforces the probable pathogenicity of this *SLC34A3* variant. Although hypercalciuria was not detected in the father, the increased level of urinary calcium excretion may have been concealed by vitamin D deficiency (8).

The standard treatment of HHRH consists of monotherapy with oral inorganic phosphate (Pi), which improves skeletal bone disease and hypercalciuria, presumably by reducing 1,25(OH) $_2$ D (2,4,11). However, Pi therapy can cause several adverse events, including gastrointestinal symptoms and, with chronic treatment, secondary or tertiary hyperparathyroidism, and nephrocalcinosis (12). Long-term medical compliance to oral Pi can be a considerable clinical problem. In addition, data regarding the long-term safety of Pi therapy for renal calcification are unknown, and the best approach for managing hypercalciuria has not been established.

It is also unclear whether a biochemical parameter or genetic factor (variant type) could be associated with an increased risk of renal complications in HHRH. Dasgupta et al. found that serum 1,25(OH) $_2$ D, low serum phosphate and decreased tubular resorption of phosphate (TRP) may be positive predictors of renal calcifications (13). Recently, Stürznickel et al. found that urinary calcium excretion and 1,25(OH) $_2$ D levels, but not TRP levels, were associated with nephrocalcinosis, and urinary calcium excretion was suggested as a therapeutic target (9).

In patients with idiopathic hypercalciuria, thiazide is used to decrease urinary calcium excretion and may prevent or delay the progression of renal complications (14). Thiazide diuretics can be used to reduce calciuria in patients with familial hypomagnesemia with hypercalciuria and nephrocalcinosis, a rare disorder characterized by renal magnesium wasting, hypercalciuria, nephrocalcinosis and kidney failure (15). Hydrochlorothiazide also decreases calciuria and may prevent the sonographic progression of nephrocalcinosis in patients with XLH (16). Therefore, it is plausible that thiazide diuretics may be useful in other metabolic disorders with hypercalciuria, such as HHRH.

In our patient compliance to Pi therapy was suboptimal, and we could not evaluate if higher doses of Pi alone would lead to resolution of hypercalciuria. However, considering the risk of nephrocalcinosis progression and deterioration of renal function with persistent hypercalciuria, adjunctive thiazide diuretic was initiated. We observed that oral Pi therapy with a thiazide diuretic led to improved serum markers of rickets and adequate control of hypercalciuria. Longitudinal follow-up and additional studies are required to evaluate whether this treatment strategy protects against or slows the progression of nephrocalcinosis (9).

Conclusion

In summary, we described a Brazilian girl with HHRH whose skeletal abnormalities became apparent later in childhood. Genetic analysis revealed compound heterozygous variants in *SLC34A3*, including a novel variant. An accurate diagnosis is crucial for proper treatment, and thiazide diuretics may be useful as adjunctive therapy for controlling hypercalciuria.

CONFLICT OF INTEREST

The authors have nothing to disclose.

REFERENCES

1. Lorenz-Depiereux B, Benet-Pages A, Eckstein G, Tenenbaum-Rakover Y, Wagenstaller J, Tiosano D, et al. Hereditary hypophosphatemic rickets with hypercalciuria is caused by mutations in the sodium-phosphate co-transporter gene *SLC34A3*. *Am J Hum Genet*. 2006;78(2):193–201. doi: 10.1086/499410
2. Bergwitz C, Miyamoto KI. Hereditary hypophosphatemic rickets with hypercalciuria: pathophysiology, clinical presentation, diagnosis and therapy. *Pflugers Arch Eur J Physiol*. 2019;471(1):149–63. doi: 10.1007/s00424-018-2184-2
3. Ichikawa S, Sorenson AH, Imel EA, Friedman NE, Gertner JM, Econs MJ. Intronic deletions in the *SLC34A3* gene cause hereditary hypophosphatemic rickets with hypercalciuria. *J Clin Endocrinol Metab*. 2006;91(10):4022–7. doi: 10.1210/jc.2005-2840
4. Chen A, Ro H, Mundra VRR, Joseph K, Brenner D, Carpenter TO, et al. Description of 5 Novel *SLC34A3*/*NPT2c* Mutations Causing Hereditary Hypophosphatemic Rickets With Hypercalciuria. *Kidney Int Reports*. 2019;4(8):1179–86. doi.org/10.1016/j.ekir.2019.05.004
5. Dhir G, Li D, Hakonarson H, Levine MA. Late-onset hereditary hypophosphatemic rickets with hypercalciuria (HHRH) due to mutation of *SLC34A3*/*NPT2c*. *Bone*. 2017;97(April):15–9. doi: 10.1016/j.bone.2016.12.001
6. Hasani-Ranjbar S, Ejtahed HS, Amoli MM, Bitarafan F, Qorbani M, Soltani A, et al. *SLC34A3* Intronic Deletion in an Iranian Kindred with Hereditary Hypophosphatemic Rickets with Hypercalciuria. *JCRPE J Clin Res Pediatr Endocrinol*. 2018;10(4):343–9. doi: 10.4274/jcrpe.0057
7. Colazo JM, Reasoner SA, Holt G, Faugere MCM, Dahir KM. Hereditary Hypophosphatemic Rickets with Hypercalciuria (HHRH) Presenting with Genu Valgum Deformity: Treatment with Phosphate Supplementation and Surgical Correction. *Case Rep Endocrinol*. 2020;2020. doi: 10.1155/2020/1047327
8. Kremke B, Bergwitz C, Ahrens W, Schutt S, Schumacher M, Wagner V, et al. Hypophosphatemic rickets with hypercalciuria due to mutation in *SLC34A3*/*NaPi-IIc* can be masked by vitamin D deficiency and can be associated with renal calcifications. *Exp Clin Endocrinol Diabetes*. 2009;117(2):49–56. doi: 10.1055/s-2008-1076716
9. Stürznickel J, Heider F, Delsmann A, Gödel M, Grünhagen J, Huber TB, et al. Clinical spectrum of Hereditary Hypophosphatemic rickets with Hypercalciuria (HHRH). *J Bone Miner Res*. 2022;00(00):1–12. doi: 10.1002/jbmr.4630
10. Bhadada SK, Sridhar S, Dhiman V, Wong K, Bennetts B, Naot D, et al. Hypophosphatemic Rickets with Hypercalciuria: A Novel Homozygous Mutation in *SLC34A3* and Literature Review. *AACE Clin Case Reports*. 2020;6(3):e105–12. doi: 10.4158/ACCR-2019-0456
11. Abe Y, Nagasaki K, Watanabe T, Abe T, Fukami M. Association between compound heterozygous mutations of *SLC34A3* and hypercalciuria. *Horm Res Paediatr*. 2014;82(1):65–71. doi: 10.1159/000360291
12. Florenzano P, Cipriani C, Roszko KL, Fukumoto S, Collins MT, Mimsola S, et al. Approach to patients with hypophosphataemia. *Lancet Diabetes Endocrinol* [Internet]. 2020;8(2):163–74. doi.org/10.1016/S2213-8587(19)30426-7
13. Dasgupta D, Wee MJ, Reyes M, Li Y, Simm PJ, Sharma A, et al. Mutations in *SLC34A3*/*NPT2c* are associated with kidney stones and nephrocalcinosis. *J Am Soc Nephrol*. 2014;25(10):2366–75. doi: 10.1681/ASN.2013101085
14. Habbig S, Beck BB, Hoppe B. Nephrocalcinosis and urolithiasis in children. *Kidney Int* [Internet]. 2011;80(12):1278–91. doi.org/10.1038/ki.2011.336
15. Edvardsson VO, Goldfarb DS, Lieske JC, Beara-Lasic L, Anglani F, Milliner DS, et al. Hereditary causes of kidney stones and chronic kidney disease. *Pediatr Nephrol*. 2013;28(10):1923–42. doi: 10.1007/s00467-012-2329-z
16. Seikaly MG, Baum M. Thiazide diuretics arrest the progression of nephrocalcinosis in children with X-linked hypophosphatemia. *Pediatrics*. 2001;108(1):4–7. doi: 10.1542/peds.108.1.e6.

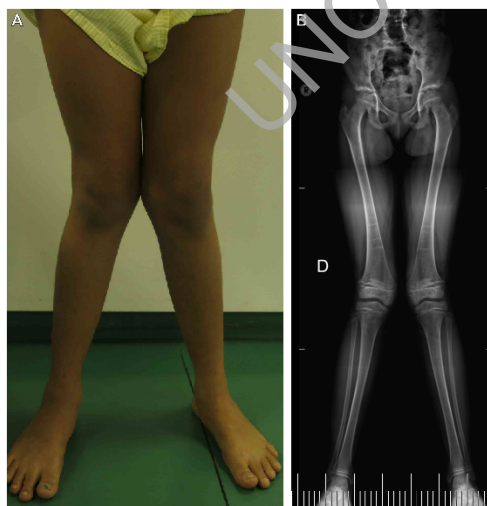


Figure 1: (A) Clinical photographs of lower limbs showing marked genu valgum; (B) Panoramic radiograph of lower limbs showing diffuse bone demineralization, marked valgus deviation of the knees, coxa valga, and sclerotic and irregular contour of the acetabulum.

HUMAN LGWLGGYLAVLAGAGLTFALQSSSVFTA⁶AVVPLMGV¹GVISLDRAYP
RAT FGWLSGYLAILVGAGLTFLLQSSSVFTA⁶AIVPLMGV¹GINLERAYP
MOUSE FGWLSGYLAILVGAGLTFLLQSSSVFTA⁶AIVPLMGV¹VIDLERAYP
CATTLE FGWLSGYLAMLVGAGLTFALQSSSVFTA⁶ATVPLIGV¹GISLERAYP
DOG FSWLSGYLAILVGAGLTFVLQSSSVFTA⁶AIVPLMGV¹GISLERAYP
RHESUS MONKEY LGWLGGYLALLAGAGLTFALQSSSVFTA⁶AIVPLMGV¹GVISLDRAYP
CHIMPANZEE LGWLGGYLAVLAGAGLTFALQSSSVFTA⁶AVVPLMGV¹GVISLDRAYP
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Figure 2: Multiple amino acid alignment of human NPTC2 protein (**Sodium-dependent phosphate transport protein 2C**) with other mammalian **SLC34A3** proteins. p.406G is shown in red and is highly conserved among different species. Sequence alignment was performed with BLAST/UniProt (www.uniprot.org). Human (*Homo sapiens*, Q8N130); Rat (*Rattus norvegicus*, G3V7E1); Mouse (*Mus musculus*, Q80SU6); Cattle (*Bos taurus*, G3MXY5), Dog (*Canis lupus familiaris*, A0A813PKB4), Rhesus monkey (*Macaca mulatta*, A0A5F8AJ48), Chimpanzee (*Pan troglodytes*, A0A2J8N9J5). Alignment data (*): identical and conserved; (:): Strongly similar; (.): weakly similar.

Table 1: Laboratory data of the patient and her parents

Biochemical Parameters	10-year-old (Age at presentation)	13-year-old	16-year-old	Mother	Father	Reference Range
Serum						
Calcium (mg/dL)	9.22	9.15	8.68	8.99	8.86	8.5-10.1
Phosphorous (mg/dL)	3.41 (3.2-5.7)	2.61 (2.9-5.1)	2.53 (2.7-4.9)	3.01 (2.5-5.1)	2.87 (2.5-5.1)	^a
25OHD (ng/mL)	29.54	27.0	21.84	24.51	19.3	> 20
PTH (pg/mL)	16.1	12.92	20.1	52.62	49.35	15-65
ALP (U/L)	486 (51-332)	249 (50-162)	157 (47-119)	56 (42-98)	64 (53-128)	^a
CTX (ng/mL)	-	2.61 (0.144-1.202)	1.07 (0.048-0.579)	0.429 (0.025-0.573)	0.868 (0.016-0.584)	^a
Creatinine (mg/dL)	0.76	0.62	0.68	0.71	0.96	0.46-0.81 >18y: 0.70-1.30
eGFR	120	136	129	122	104	>90
Urine						
Calcium/Creatinine (mg/mg)	0.407	0.333	-	0.108	0.102	< 0.200
Urine calcium excretion	-	8.2mg/kg/d	3.4mg/kg/d	115mg/d	118mg/d	Child < 4mg/kg/d Adult Female <250mg/d Male <300mg/d
TRP (%)	-	84	82	-	-	>85
TmP/GFR (mg/dL)	-	2.19 (2.9-6.5)	2.07 (2.9-6.5)	-	-	^a

Laboratory data were obtained after fasting overnight; ^a Reference ranges according to age and sex; Urinary calcium/creatinine: analyzed in spot urine; TRP: Tubular resorption of phosphate; TmP/GFR: ratio of the maximal renal phosphate reabsorption to glomerular filtration rate; 25OHD: 25 hydroxyvitamin D; PTH parathyroid hormone; ALP alkaline phosphatase; CTX: C-telopeptide of type I collagen; eGFR: estimation of glomerular filtration rate according to the CKD-EPI equation. Dashes: data not available.