JBMR Plus, 2025, **9**, ziae152
https://doi.org/10.1093/jbmrpl/ziae152
Advance access publication: November 19, 2024
Research Article

Unusual *PHEX* variants implicate uncommon genetic mechanisms for X-linked hypophosphatemic rickets

Lama Alzoebie[1], Dong Li[2,3], Xiang Wang[3], David R. Weber[1,4], Michael A. Levine[1,4,*]

[1]Division of Endocrinology and Diabetes, The Children's Hospital of Philadelphia, Philadelphia, PA 19104, United States
[2]Division of Human Genetics, The Children's Hospital of Philadelphia, Philadelphia, PA 19104, United States
[3]Center for Applied Genomics, The Children's Hospital of Philadelphia, Philadelphia, PA 19104, United States
[4]Center for Bone Health, The Children's Hospital of Philadelphia, Philadelphia, PA 19104, United States

*Corresponding author: Michael A. Levine, Division of Endocrinology and Diabetes, The Children's Hospital of Philadelphia, ARC 510A, 3615 Civic Center Boulevard, Philadelphia, PA 19104, United States (levinem@chop.edu).

Abstract

X-linked hypophosphatemic rickets (XLH), the most common form of hereditary rickets, is characterized by renal phosphate wasting and abnormal vitamin D metabolism due to elevated circulating levels of the phosphatonin fibroblast growth factor 23 (FGF23). Dominant inactivating variants of the phosphate regulating endopeptidase homolog, X-linked (*PHEX*), gene are present in patients with XLH, and more than half of affected patients carry de novo variants. We report on 3 families in whom affected members had highly unusual *PHEX* pathogenic variants. In 1 family we identified a previously described deep intronic *PHEX* variant (c.1768 + 173A>G) in the proband and her affected son. This variant is also near a previously reported PHEX variant (c.1768 + 177_1768 + 180dupGTAA) and is predicted to affect splicing by SpliceAI (delta score: 0.95) through creation of a new donor splice site. In a second proband we identified 2 pathogenic de novo and novel *PHEX* variants, c.2083delT (p.Ser695Profs∗45) and c.2085delC (p.Tyr696Thrfs∗44), that were present on different alleles, consistent with mosaicism for 3 *PHEX* alleles. The third proband also carried 2 *PHEX* variants (c.755 T>C [p.Phe252Ser] and c.759G>A [p.Met253Ile]), but in this case both variants were present on the same *PHEX* allele. These studies expand the molecular catalog of pathogenic *PHEX* variants in XLH and emphasize the importance of deep intronic sequencing and comprehensive family studies. Conventional approaches to genetic diagnosis may not be adequate to identify or characterize the disease-causing variants in the *PHEX* gene in some patients with likely XLH.

Keywords: hypophosphatemia, rickets, XLH, next-generation sequencing, FGF23, PHEX

Lay Summary

X-linked hypophosphatemic rickets (XLH), due to mutation in the *PHEX* gene, is the most common form of inherited rickets. The availability of burosumab, a novel antibody therapy that is approved for XLH as disease-specific treatment, makes definitive diagnosis critical. Genetic testing can provide confirmation of the clinical diagnosis and facilitate early recognition of affected individuals who have not yet developed rickets so that treatment can be optimized. Here we show that conventional approaches to genetic diagnosis may not be adequate to identify or characterize the disease-causing variants in the *PHEX* gene and describe unusual genetic findings.

Introduction

X-linked hypophosphatemic rickets (XLH; OMIM 307800) is characterized by renal phosphate wasting, decreased absorption of phosphate and calcium from the intestine, and abnormal vitamin D metabolism due to elevated circulating levels of the phosphatonin fibroblast growth factor 23 (FGF23).[1–4] Affected patients develop early-onset rickets and osteomalacia with skeletal deformities that principally affect the lower extremities, reduced growth velocity with short stature, bone pain, craniosynostosis, and dental abnormalities.[5–8] Adults with XLH have osteomalacia and can manifest continuing challenges with dental abscesses, osteoarthritis, vertebral ankylosis, enthesopathy, and rarely, spinal stenosis.[8–10] In XLH, elevated serum concentrations of FGF23 (1) impair vitamin D metabolism by reducing conversion of 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D [1,25(OH)2D], the active form of vitamin D, and increasing production of 24,25-dihydroxyvitamin D [24,25(OH)2D] and (2) reduce expression and function of the type 2 sodium-dependent phosphate cotransporters Npt2a (*SLC34A1*) and Npt2c (*SLC34A3*), thereby leading to hypophosphatemia.[4,11] X-linked hypophosphatemic rickets is the most common form of inherited rickets, with an estimated prevalence of 1 in 20 000 to 1 in 60 000 individuals and is due to dominant inactivating variants of the phosphate regulating endopeptidase homolog, X-linked (*PHEX*), gene on Xp22.11.[2,6,12–14] X-linked hypophosphatemic rickets is one of a growing number of inherited forms of hypophosphatemic rickets in which levels of FGF23 are elevated and which have been attributed to pathogenic variants in genes that encode proteins for regulation of proximal renal tubular phosphate transport (Table 1).[3,6,15] Elevated circulating levels of FGF23 are also present in other inherited forms of hypophosphatemic rickets that are caused by autosomal dominant variants in *FGF23*

Received: November 1, 2024. **Revised:** November 12, 2024. **Accepted:** November 15, 2024

Table 1. Common Forms of Hereditary Hypophosphatemic Rickets.

	Gene				
	PHEX	*FGF23*	*DMP1*	*ENPP1*	*SLC34A1*
OMIM number	307 800	605 380	241 520	173 335	182 309
Term	X-linked hypophosphatemic rickets	Autosomal dominant hypophosphatemic rickets	Autosomal recessive hypophosphatemic rickets, 1	Autosomal recessive hypophosphatemic rickets, 2	Hereditary hypophosphatemic rickets with hypercalciuria
Encoded protein	Transmembrane endopeptidase	Fibroblast growth factor proteins	Dentin matrix acidic phosphoprotein 1	Type II transmembrane glycoprotein	Sodium phosphate cotransporter
Transmission	X-linked dominant	Autosomal dominant	Autosomal recessive	Autosomal recessive	Autosomal recessive
Type of variant	Loss of function	Gain of function	Loss of function	Loss of function	Loss of function
Serum FGF23 concentration	Elevated	Elevated	Elevated	Elevated	Low

Abbreviations: *DMP1*, dentin matrix protein 1; *ENPP1*, ectonucleotide pyrophosphatase/phosphodiesterase 1; *FGFG23*, fibroblast growth factor 23; OMIM, Online Mendelian Inheritance in Man; *PHEX*, phosphate regulating endopeptidase homolog, X-linked.

and autosomal recessive variants in dentin matrix protein 1 (*DMP1*), ectonucleotide pyrophosphatase/phosphodiesterase 1 (*ENPP1*), and family with sequence similarity 20, member C (*FAM20C*)[16] (Table 1). By contrast, biallelic loss-of-function variants in *SLC34A3* directly inactivate the principal sodium-phosphate co-transporter Npt2c, and cause a form of hypophosphatemic rickets in which serum concentrations of FGF23 are suppressed, thereby leading to elevated serum concentrations of 1,25(OH)2D and hypercalciuria that is termed hereditary hypophosphatemic rickets with hypercalciuria (Table 1).[13]

To date, over 1000 *PHEX* variants have been identified in patients with XLH and more than half of patients with XLH carry de novo variants.[16] Remarkably, a wide range of clinical features have been described in affected patients (eg, craniosynostosis), emphasizing the importance of genetic testing in the evaluation of patients with hypophosphatemic rickets.[16] Here we describe patients with XLH from 3 unrelated families with novel and/or unusual *PHEX* gene defects.

Materials and methods

Patients and identification of variants

We studied 3 families (see below) that included members affected with hypophosphatemic rickets. Genomic DNA was extracted from peripheral blood leukocytes or saliva. Targeted sequencing and deletion/duplication analysis of genes known to be associated with hypophosphatemic rickets was performed using a next-generation sequencing panel (*ALPL, CLCN5, CTNS, CYP2R1, CYP27B1, DMP1, ENPP1, FAH, FAM20C, FGF23, FGFR1, GNAS, OCRL, PHEX, SLC34A1, SLC34A3*, and *VDR;* Invitae Laboratories, test code 72039).[3,16] In addition, in some cases (see below), we performed whole-exome sequencing (WES)[17] and whole-genome sequencing (WGS) with paired-end 100-bp reads,[18] as previously described. Libraries were generated from genomic DNA using the Illumina TruSeq DNA PCR-Free Library Prep Kit (Illumina, San Diego, CA). All of the raw reads were aligned to the reference human genome using the Burrows-Wheeler Aligner (BWA-Mem), and single-nucleotide variants (SNVs) and small insertions/deletions (INDELs) were captured using the Genome Analysis Tool Kit (GATK). ANNOVAR and SnpEff were subsequently used to functionally annotate the variants. The confirmatory genotyping of the identified variant in kindred A was performed by Sanger sequencing with primers 5′ACCGGTTCATTTTATAAGCTGCT3′ and 5′AATCATCGTTGGAGCAGAGC3′. The protocol was approved by the Children's Hospital of Philadelphia Institutional Review Board, and informed consent/assent was obtained as appropriate.

Results

Kindred A

The proband of family A (Figure 1A, II-2) is a 47-year-old White female who presented with bowed legs at the age of 2 years. She was treated with orthopedic braces until the age of 7 years when she was diagnosed with hypophosphatemic rickets. Treatment with oral phosphate and calcitriol was initiated. The proband had an affected son (III-1) and unaffected daughter (III-2); all other relatives were unaffected. Her affected son was 11 years old at the time of our evaluation; he was diagnosed with hypophosphatemic rickets when he presented with bilateral bowing of the legs at the age of 13 months. Examination of the affected son at age 3 years was significant for bilateral lower extremity varus deformity and tibial torsion and absent upper incisors with normal gums. A summary of their biochemical results is presented in Table 2. Neither affected patient had hypercalciuria or nephrocalcinosis. The proband had normal sequences for the *PHEX*, *FGF23*, *DMP1*, and *ENPP1* genes on the Invitae Hypophosphatemic Rickets panel. Whole-exome sequencing analysis of the family did not reveal a candidate gene. By contrast, WGS followed by Sanger sequencing revealed a de novo deep intronic *PHEX* variant (c.1768 + 173A>G; Figure 2) in the proband that had been previously reported in Clinvar (variation ID: 829888; rs1602402549) in an individual with XLH. The proband's affected son also carried this variant. By contrast, her unaffected parents, daughter, and husband showed only wild-type *PHEX* sequences. The c.1768 + 173A>G variant is also near a previously reported *PHEX* variant (c.1768 + 177_1768 + 180dupGTAA).[19] The c.1768 + 173A>G variant is not present in publicly available databases and is predicted to affect splicing by SpliceAI (delta score: 0.95) through creation of a new donor splice site.

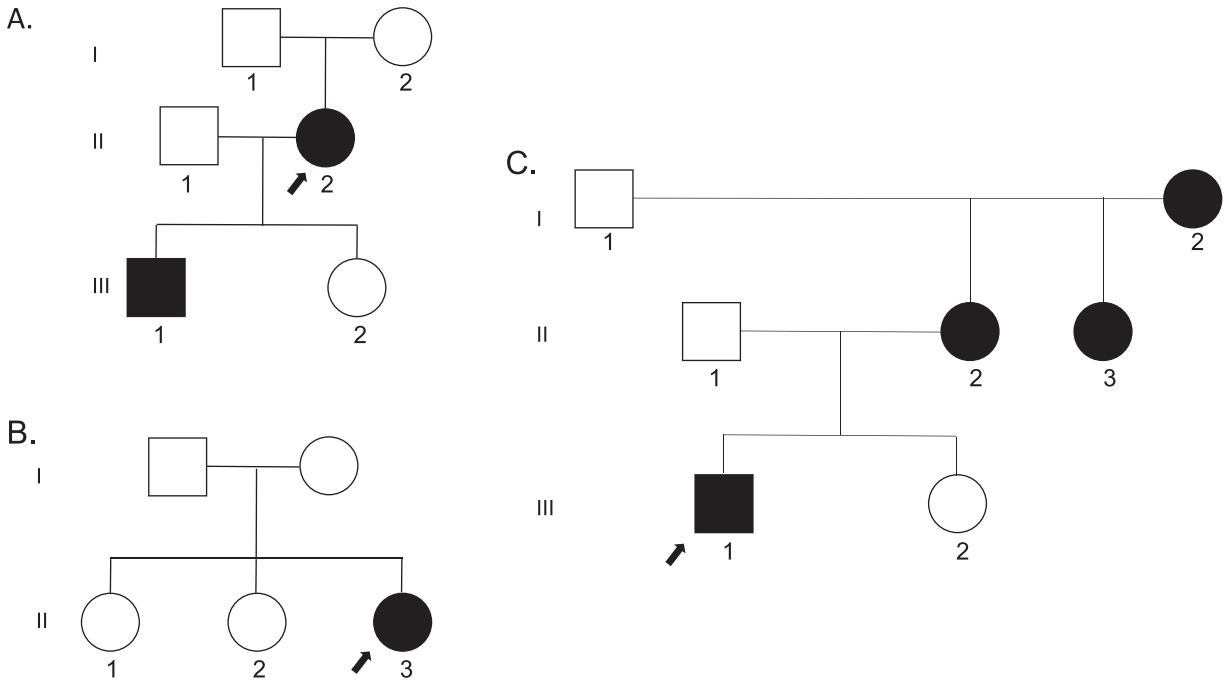

Figure 1. Pedigrees analyzed in the current study. Pedigrees corresponding to kindreds A, B, and C are represented in the corresponding panels. A black square (male) or circle (female) represents an affected individual, while white symbols represent unaffected individuals. Each proband is depicted by an arrow.

Table 2. Laboratory Data.

	Kindred A		Kindred B	Kindred C
	Subject I-2 at age 40 y	Subject II-1 at age 13 mo	Subject II-3 at age 5 y	Subject III-1 at age 3–11 mo
Serum phosphate (mg/dL) **Adult** **Child**	1.8	2.3	2.0	3.7
Serum calcium (mg/dL) **8.7–10.5 mg/dL**	9.2	9.8	9.5	10
Serum ALP (U/L) **Adult: 30–128 U/L** **Child**	70	671	537	829
Serum 25(OH)D **20–96 ng/mL**	89	32	26.7	34
Serum PTH (pg/mL) **16–87 pg/mL**	25.3	152	135.4	70
Serum FGF23 (RU/mL) **44–215 RU/mL**	—	—	150	—
Serum creatinine (mg/dL) **0.2–1.3 mg/dL**	0.8	0.4	0.2	0.2
TRP (%) **85%–95%**	70	91.5	81	72
TmP/GFR (mg/dL) **Normal (mg/dL)**	1.24 2.60-3.80	2.98 3.00-5.08	2.17 3.25-5.51	2.39 3.16-5.82

Abbreviations: ALP, alkaline phosphatase; FGFG23, fibroblast growth factor 23; PTH, parathyroid hormone; RU, Relative units/mL; TmP/GFR, ratio of tubular maximum reabsorption of phosphate (TmP) to glomerular filtration rate (GFR) represents the tubular maximum phosphate reabsorption capacity (normal reference ranges for age are from references [33–35]); TRP, tubular reabsorption of phosphate; 25(OH)D, 25-hydroxyvitamin D.

Kindred B

A 13-year-old female (Figure 1B, II-3) was referred to our endocrine clinic after having been diagnosed with hypophosphatemic rickets (Table 2) at the age of 5 years when she was evaluated for bowed legs and tibial torsion. She was born to nonconsanguineous parents from Morocco and Algeria. Her parents and both sisters were unaffected (Figure 1B). She did not have hypercalciuria or renal calcifications. She was initially managed with phosphorus and calcitriol and was subsequently transitioned to burosomab injections every 2 weeks, with improvement in biochemistries and radiological healing of rickets; however, tibial torsion was not improved. At age 15 5/12 years she had completed her growth and had a height of 147 cm (1st percentile) compared with a midparental height prediction of 160 cm. Targeted sequencing with the Invitae Hypophosphatemic Rickets panel revealed 2

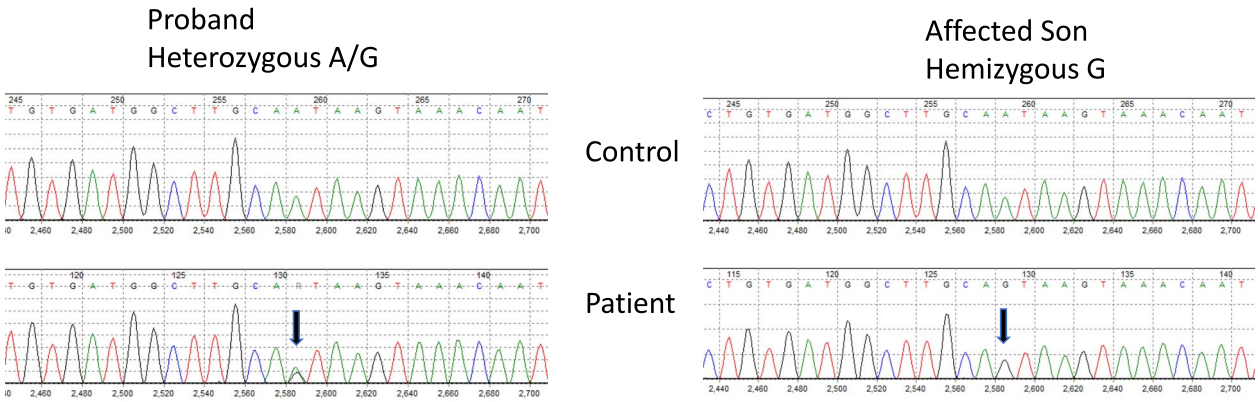

Figure 2. DNA sequencing electropherograms for kindred A. A DNA sequencing electropherogram is a graphic display of the results of DNA sequencing that shows peaks in colors that correspond to specific nucleotides. In this figure the results of sequencing DNA from 2 controls are shown in the upper panels and demonstrate an A at the position corresponding to c.1768 + 173. The lower panels reveal that the proband (left), a female, is heterozygous at this site (an A and a G are shown by the arrow), while her affected, hemizygous son (right) has only a G at the site (arrow), in both cases corresponding to a de novo deep intronic *PHEX* variant (c.1768 + 173A>G). Abbreviation: *PHEX*, phosphate regulating endopeptidase homolog, X-linked.

pathogenic de novo and novel *PHEX* variants, c.2083delT (p.Ser695Profs*45) and c.2085delC (p.Tyr696Thrfs*44), that were not present in other unaffected parents or siblings. Next-generation sequencing of DNA from peripheral blood cells revealed wild-type sequences as well as unique variant sequences indicating that the 2 variants were present on different alleles, consistent with mosaicism for 3 *PHEX* alleles. Our WES showed that 69 reads (29.7%) were converted from wild-type to c.2083delT and 29 reads (12.5%) were converted to c.2085delC, while the remaining 134 reads (57.8%) were unchanged, suggesting a complex mosaic status (Figure 3).

Kindred C

The proband (Figure 1C; III-1) was a 16-year-old male who was diagnosed with hypophosphatemic rickets at the age of 3 months (Table 2). He had an extensive family history for hypophosphatemic rickets in his mother, maternal aunt, and maternal grandmother, all of whom manifested similar degrees of biochemical and clinical severity as the proband. The patient was managed with calcitriol and phosphorus and follow-up clinic visits revealed gross motor delay in infancy associated with hypotonia and early bowing of legs. At the age of 2 years, he had severe scaphocephaly requiring reconstruction and had developed bilateral nephrocalcinosis. He had also experienced several dental abscesses starting from the age of 5 years. Genetic analyses using an NGS targeted panel revealed 2 *PHEX* variants: c.755 T>C (p.Phe252Ser) and c.759G>A (p.Met253Ile), which were both present in the mother and maternal grandmother as well (Figure 1C). An unaffected sister and father did not carry either variant. Exome sequencing revealed that both variants were present on the same *PHEX* allele (Figure 4). These 2 tandem cis variants had been previously described in related patients with familial XLH using a different numbering system as c.748 T>C (p.Phe249Ser) and c.752G>A (p.Met250Ile).[20]

Discussion

Dominant loss-of-function variants in the *PHEX* gene are the most common cause of XLH. The *PHEX* gene, which is located on Xp22.1, spans over 220 kb and consists of 22 exons. As of the most recent analysis, over 1000 different variants have been identified throughout the gene and affecting almost all exons or their adjacent introns.[21] However, the main densities of *PHEX* missense variants are not randomly distributed and are located within consensus motifs containing substrate-interacting residues and the zinc binding site in exons 15 and 19, respectively.[22,23] When standard genetic testing procedures are utilized, detection rates of pathogenic *PHEX* variants vary from 51% to 86% for familial cases and from 22% to 57% for sporadic cases in most populations.[23–28] Exceptionally, 1 Finnish study reported a variant detection rate of 100% for familial cases and 93% for sporadic cases.[26] Recent evidence supports the hypothesis that genetic phenocopies could account for a nonnegligible fraction of patients who are diagnosed with XLH based solely on biochemical testing or who are misclassified as nongenetic upon standard genetic testing. These observations encourage a more comprehensive understanding of the overall genetic background of each single patient and for the elucidation of potential biochemical biomarkers that can distinguish between the various genetic disorders of phosphate metabolism (Table 1).

The relatively low variant detection rates in familial cases of XLH may reflect limitations in the conventional, targeted techniques used to detect variants, which interrogate the coding regions and flanking intron-exon boundaries of *PHEX*.[3] The development of "second" or "next-generation" sequencing technology (also referred to as NGS) has extended the application of genomic analysis not only in research but also in clinical settings. NGS is based on massive parallel sequencing—that is, the simultaneous analysis of multiple genes in a single run. They can include a selection of genes of interest (targeted panels), all coding DNA regions (WES) or the entire genome (WGS). This was particularly relevant in kindred A, in which the pathogenic *PHEX* variant, c.1768 + 173A>G, was not identified by an NGS targeted panel but was only disclosed after we performed WGS, which revealed a deep intronic variant that is predicted to affect splicing. Splice-site variants have been shown to be responsible for 17% of all reported *PHEX* variants.[29] Of all the known types of splicing variants, the rarest and most difficult to predict are those that activate pseudo-exons, also known as cryptic exons. Pseudo-exon variants appear to create entirely new exons within introns.[30] In kindred A, the intron 17 variant

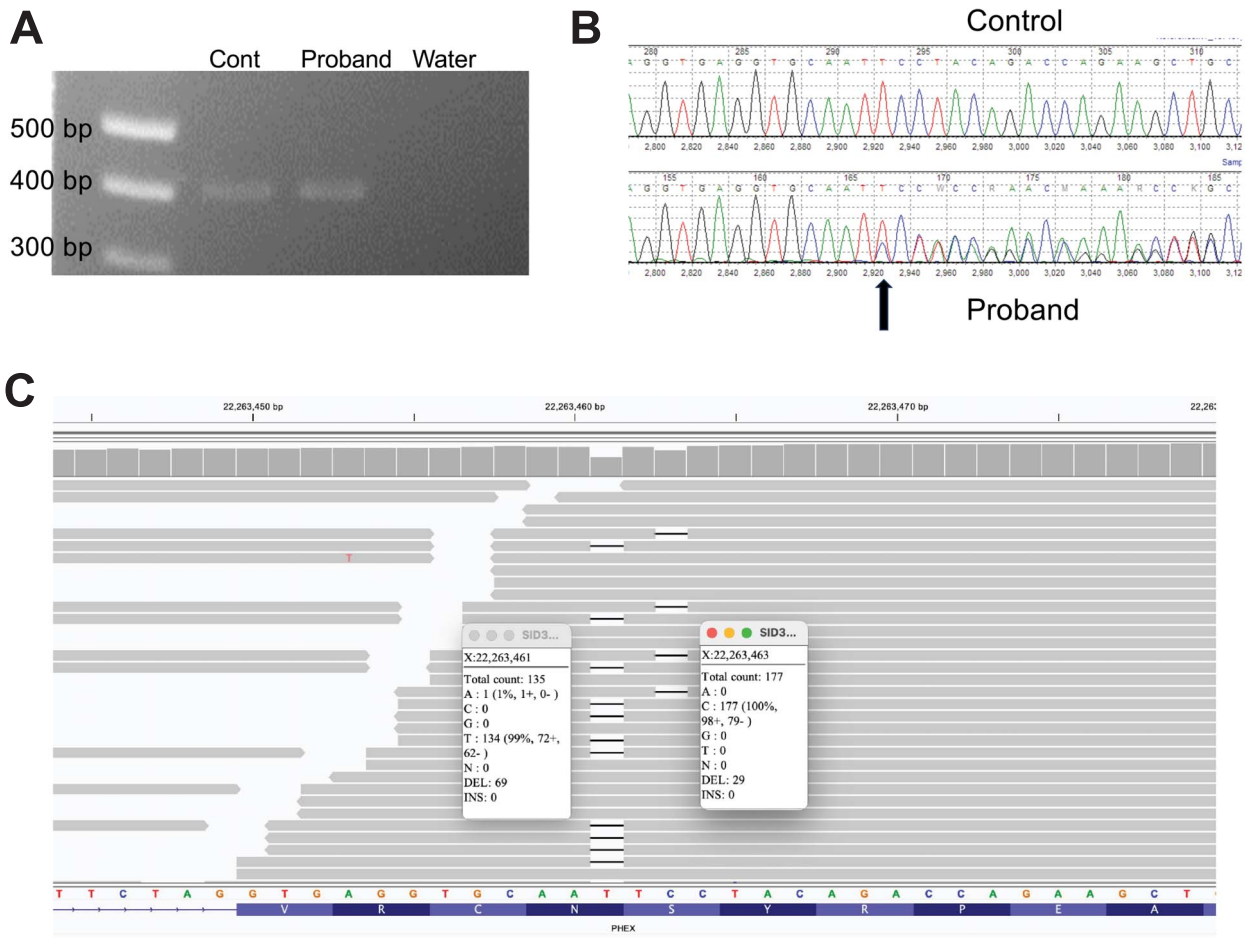

Figure 3. *PHEX* analysis for kindred B. A. This panel shows the polyacrylamide gel electrophoresis of products generated by a PCR reaction of sequences that flank the *PHEX* variant using DNA from a control subject and affected subject II-2. Both the control subject and the affected subject show an expected band of 400 bp (based on sizes shown on the DNA fragment ladder on the left). B. The PCR products shown in panel A were subjected to Sanger sequencing and the resulting electropherograms show the normal sequence in the control and a deletion of either c.2083delT or c.2085delT (arrow) in 1 allele that leads to double sequence (2 or more peaks in the same location) in proband. C. Exome integrative genomics viewer (IGV) view of 2 somatic *PHEX* variants showed that the T (left) and C (right) nucleotides are present on different DNA fragments, thereby indicating that the 2 variants are on different alleles. Abbreviations: Cont, control; PCR, polymerase chain reaction; *PHEX*, phosphate regulating endopeptidase homolog, X-linked.

is predicted by in silico analyses to create a new donor splice site that induces pseudo-exon inclusion, likely resulting in the introduction of a premature termination codon. This case highlights the utility of WGS in identifying deep intronic variants as targeted testing and WES could not identify the variant. Remarkably, this variant is close to a previously reported variant, (c.1768 + 177_1768 + 180dupGTAA).[19]

Mosaicism is uncommon in XLH and is more frequent in males than in females, many of whom are 47,XXX. We identified 2 mosaic variants, c.2083delT (p.Ser695Profs*45) and c.2085delC (p.Tyr696Thrfs*44), located within the same site of the *PHEX* gene in the proband of kindred B that were absent in her parents and unaffected brother. The proband carried each variant on a unique allele in addition to a wild-type allele. A de novo variant generally occurs in the sperm or egg of 1 parent or a fertilized egg and, once inherited, is present in all cells. In this patient, the mosaic pattern for a variant probably appeared in the early postzygotic period, so that a variant allele is present in most but not all tissues. We suggest that these 2 variants occurred due to replication slippage on both newly synthesized strands between repeat motifs of microhomology. This is the first description to our knowledge of 2 nearby de novo mosaic *PHEX* deletion variants in XLH and is reminiscent of a recently described XLH female with 2 mosaic variants located in the same site of the *PHEX* gene, c.1809 = /G>T/G>A.[31] Our experience with this case emphasizes the need to consider mosaicism in the assessment of genetic variants and the importance of appropriate detection methods and cutoff values used during the bioinformatic analysis.

We also identified 2 *PHEX* variants in the proband of kindred C (Figure 1C) in which transmission of hypophosphatemic rickets was consistent with a dominant pattern of inheritance. Both *PHEX* variants, c.755 T>C (p.Phe252Ser) and c.759G>A (p.Met253Ile), were previously described as pathogenic variants.[21] Remarkably, genetic analysis of the affected mother and grandmother showed that both of these relatives also carried the same 2 *PHEX* variants. To confirm that these 2 variants were located in cis, we analyzed WES reads in this region using DNA from both the mother and the proband, which revealed that the 2 nearby nucleotide transitions were located on the same allele (Figure 4), thereby excluding an unusual maternal meiotic crossover event between the X chromosomes. The presence

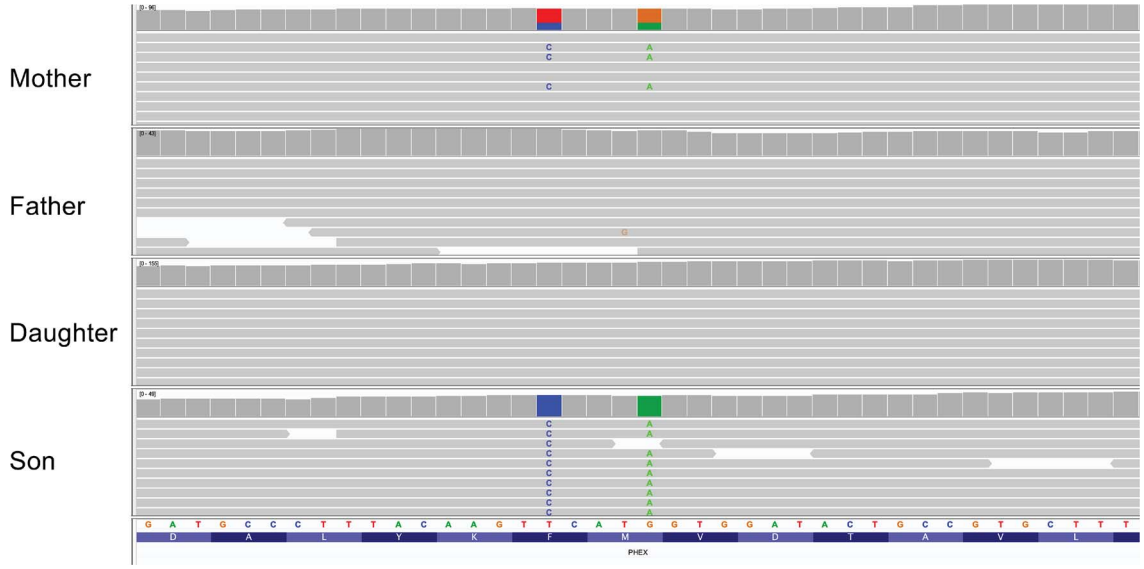

Figure 4. Integrative genomics viewer (IGV) view of *PHEX* variant analysis in kindred C. Genetic analyses revealed 2 *PHEX* variants, c.755 T>C (p.Phe252Ser) and c.759G>A (p.Met253Ile), in the proband, his mother, and his maternal grandmother (Figure 1C). An unaffected sister and father did not carry either variant. Next-generation sequencing revealed that both variant nucleotides (C and A) were present on the same DNA fragments of *PHEX*, where the XX mother is heterozygous at both sites (T/C and G/A; only variant nucleotide is shown on the viewer tracing) and the son is hemizygous (only C and A). These results indicate that both variants are present on the same allele. Color was used to highlight the alternative allele. Abbreviation: *PHEX*, phosphate regulating endopeptidase homolog, X-linked.

of 2 variants on a single allele, both of which are pathogenic in isolation, has been previously reported in XLH as a single-base change (c. 231A>G) located 3 base pairs upstream of the putative polyadenylation (pA) signal (AATAAA) in the *PHEX* gene's 3′-untranslated region that is found almost exclusively to be associated with an exon 13–15 duplication.[16,21,32] This single allele is considered to be the most common *PHEX* variant in the United States.

In conclusion, this report expands the complex molecular genetics of XLH. Our cases emphasize the importance of using appropriate methods to analyze potential alterations in *PHEX* through detection methods that can identify variants within introns, recognize mosaicism, and assign allelic phase.

Author contributions

Lama Alzoebie (Conceptualization, Investigation, Writing—original draft, Writing—review & editing), Dong Li (Data curation, Formal analysis, Investigation, Methodology, Writing—review & editing), Xiang Wang (Methodology, Writing—review & editing), David R. Weber (Conceptualization, Formal analysis, Methodology, Writing—review & editing), and Michael A. Levine (Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Writing—original draft, Writing—review & editing). All authors have read and agreed to the published version of the manuscript.

Funding

Financial support was received from the Children's Hospital of Philadelphia Stokes Research Institute and the National Institutes of Health (R01 DK112955 from the National Institute of Diabetes and Digestive and Kidney Diseases [NIDDK] to M.A.L.). The funding sources were not involved in study design; in the collection, analysis and interpretation of data, in the writing of the manuscript, or in the decision to submit the paper for publication.

Conflicts of interest

None declared.

Data availability

The dataset is available upon reasonable request.

References

1. Alhamoudi KM, Alghamdi B, Alswailem M, et al. A unique mechanism of a novel synonymous PHEX variant causing X-linked hypophosphatemia. *J Clin Endocrinol Metab*. 2022;107(10):2883-2891. https://doi.org/10.1210/clinem/dgac435
2. Jagtap VS, Sarathi V, Lila AR, Bandgar T, Menon P, Shah NS. Hypophosphatemic rickets. Indian J. *Endocrinol Metab*. 2012;16(2):177-182. https://doi.org/10.4103/2230-8210.93733
3. Jo HY, Shin JH, Kim HY, et al. Identification of a novel variant in the PHEX gene using targeted gene panel sequencing in a 24-month-old boy with hypophosphatemic rickets. *Ann Pediatr Endocrinol Metab*. 2020;25(1):63-67. https://doi.org/10.6065/apem.2020.25.1.63
4. Munns CF, Yoo HW, Jalaludin MY, et al. Asia-Pacific consensus recommendations on X-linked hypophosphatemia: diagnosis, multidisciplinary management, and transition from pediatric to adult care. *JBMR Plus*. 2023;7(6):e10744. https://doi.org/10.1002/jbm4.10744
5. Yamamoto A, Nakamura T, Ohata Y, Kubota T, Ozono K. Phenotypes of a family with XLH with a novel PHEX mutation. *Hum Genome Var*. 2020;7(1):8. https://doi.org/10.1038/s41439-020-0095-1
6. Pavone V, Testa G, Gioitta Iachino S, Evola FR, Avondo S, Sessa G. Hypophosphatemic rickets: etiology, clinical features and treatment. *Eur J Orthop Surg Traumatol*. 2015;25(2):221-226. https://doi.org/10.1007/s00590-014-1496-y
7. Baroncelli GI, Mora S. X-linked hypophosphatemic rickets: multisystemic disorder in children requiring multidisciplinary management. *Front Endocrinol (Lausanne)*. 2021;12:688309. https://doi.org/10.3389/fendo.2021.688309

8. Mindler GT, Stauffer A, Kranzl A, et al. Persistent lower limb deformities despite amelioration of rickets in X-linked hypophosphatemia (XLH)—a prospective observational study. *Front Endocrinol (Lausanne)*. 2022;13:866170. https://doi.org/10.3389/fendo.2022.866170
9. Herrou J, Picaud AS, Lassalle L, et al. Prevalence of enthesopathies in adults with X-linked hypophosphatemia: analysis of risk factors. *J Clin Endocrinol Metab*. 2022;107(1):e224-e235. https://doi.org/10.1210/clinem/dgab580
10. Herrou J, Fechtenbaum J, Rothenbuhler A, et al. Development of spinal enthesopathies in adults with X-linked hypophosphatemia. *J Clin Endocrinol Metab*. 2023;108(12):e1524-e1531. https://doi.org/10.1210/clinem/dgad383
11. Ohata Y, Ishihara Y. Pathogenic variants of the PHEX gene. *Endocrine*. 2022;3(3):498-511. https://doi.org/10.3390/endocrines3030040
12. Francis F, Hennig S, Korn B, et al. A gene (PEX) with homologies to endopeptidases is mutated in patients with X-linked hypophosphatemic rickets. The HYP Consortium. *Nat Genet*. 1995;11(2):130-136
13. 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;2020:1-7. https://doi.org/10.1155/2020/1047327
14. Quarles LD. FGF23, PHEX, and MEPE regulation of phosphate homeostasis and skeletal mineralization. *Am J Physiol Endocrinol Metab*. 2003;285(1):E1-E9. https://doi.org/10.1152/ajpendo.00016.2003
15. Razali NN, Hwu TT, Thilakavathy K. Phosphate homeostasis and genetic mutations of familial hypophosphatemic rickets. *J Pediatr Endocrinol Metab*. 2015;28(9-10):1009-1017. https://doi.org/10.1515/jpem-2014-0366
16. Rush ET, Johnson B, Aradhya S, et al. Molecular diagnoses of X-linked and other genetic hypophosphatemias: results from a sponsored genetic testing program. *J Bone Miner Res*. 2022;37(2):202-214. https://doi.org/10.1002/jbmr.4454
17. Li D, Gordon CT, Oufadem M, et al. Heterozygous mutations in TBX1 as a cause of isolated hypoparathyroidism. *J Clin Endocrinol Metab*. 2018;103(11):4023-4032. https://doi.org/10.1210/jc.2018-01260
18. Li D, Bupp C, March ME, Hakonarson H, Levine MA. Intragenic deletions of GNAS in pseudohypoparathyroidism type 1A identify a new region affecting methylation of exon a/B. *J Clin Endocrinol Metab*. 2020;105(9):e3197-e3206. https://doi.org/10.1210/clinem/dgaa286
19. Morey M, Castro-Feijoo L, Barreiro J, et al. Genetic diagnosis of X-linked dominant hypophosphatemic rickets in a cohort study: tubular reabsorption of phosphate and 1,25(OH)2D serum levels are associated with PHEX mutation type. *BMC Med Genet*. 2011;12(1):116. https://doi.org/10.1186/1471-2350-12-116
20. Holm IA, Huang X, Kunkel LM. Mutational analysis of the PEX gene in patients with X-linked hypophosphatemic rickets. *Am J Hum Genet*. 1997;60(4):790-797
21. Sarafrazi S, Daugherty SC, Miller N, et al. Novel PHEX gene locus-specific database: comprehensive characterization of vast number of variants associated with X-linked hypophosphatemia (XLH). *Hum Mutat*. 2022;43(2):143-157. https://doi.org/10.1002/humu.24296
22. Ishihara Y, Ohata Y, Takeyari S, et al. Genotype-phenotype analysis, and assessment of the importance of the zinc-binding site in PHEX in Japanese patients with X-linked hypophosphatemic rickets using 3D structure modeling. *Bone*. 2021;153:116135. https://doi.org/10.1016/j.bone.2021.116135
23. Gaucher C, Walrant-Debray O, Nguyen TM, Esterle L, Garabedian M, Jehan F. PHEX analysis in 118 pedigrees reveals new genetic clues in hypophosphatemic rickets. *Hum Genet*. 2009;125(4):401-411. https://doi.org/10.1007/s00439-009-0631-z
24. Puente-Ruiz N, Docio P, Unzueta MTG, et al. Uncovering genetic causes of hypophosphatemia. *J Intern Med*. 2023;293(6):753-762. https://doi.org/10.1111/joim.13635
25. Holm IA, Nelson AE, Robinson BG, et al. Mutational analysis and genotype-phenotype correlation of the PHEX gene in X-linked hypophosphatemic rickets. *J Clin Endocrinol Metab*. 2001;86(8):3889-3899. https://doi.org/10.1210/jcem.86.8.7761
26. Tyynismaa H, Kaitila I, Nanto-Salonen K, Ala-Houhala M, Alitalo T. Identification of fifteen novel PHEX gene mutations in Finnish patients with hypophosphatemic rickets. *Hum Mutat*. 2000;15(4):383-384. https://doi.org/10.1002/(SICI)1098-1004(200004)15:4<383::AID-HUMU18>3.0.CO;2-#
27. Cho HY, Lee BH, Kang JH, Ha IS, Cheong HI, Choi Y. A clinical and molecular genetic study of hypophosphatemic rickets in children. *Pediatr Res*. 2005;58(2):329-333. https://doi.org/10.1203/01.PDR.0000169983.40758.7B
28. Lin X, Li S, Zhang Z, Yue H. Clinical and genetic characteristics of 153 Chinese patients with X-linked hypophosphatemia. *Front Cell Dev Biol*. 2021;9:617738. https://doi.org/10.3389/fcell.2021.617738
29. BinEssa HA, Zou M, Al-Enezi AF, et al. Functional analysis of 22 splice-site mutations in the PHEX, the causative gene in X-linked dominant hypophosphatemic rickets. *Bone*. 2019;125:186-193. https://doi.org/10.1016/j.bone.2019.05.017
30. Christie PT, Harding B, Nesbit MA, Whyte MP, Thakker RV. X-linked hypophosphatemia attributable to pseudoexons of the PHEX gene. *J Clin Endocrinol Metab*. 2001;86(8):3840-3844. https://doi.org/10.1210/jcem.86.8.7730
31. Lin Y, Zhang W, Huang X, et al. Two De novo mosaic variants within the same site of PHEX gene in a girl with X-linked hypophosphatemic rickets. *Calcif Tissue Int*. 2022;110(2):266-271. https://doi.org/10.1007/s00223-021-00909-1
32. Soto Barros J, Sanchez SI, Cabral K, et al. X-linked hypophosphatemia in 4 generations due to an exon 13-15 duplication in PHEX, in the absence of the c.*231A>G variant. *Bone*. 2023;172:116763. https://doi.org/10.1016/j.bone.2023.116763
33. Stark H, Eisenstein B, Tieder M, Rachmel A, Alpert G. Direct measurement of TP/GFR: a simple and reliable parameter of renal phosphate handling. *Nephron*. 1986;44(2):125-128. https://doi.org/10.1159/000184216
34. Brodehl J, Gellissen K, Weber HP. Postnatal development of tubular phosphate reabsorption. *Clin Nephrol*. 1982;17(4):163-171.
35. Brodehl J. Assessment and interpretation of the tubular threshold for phosphate in infants and children. *Pediatr Nephrol*. 1994;8(5):645. https://doi.org/10.1007/BF00858154

JBMR Plus, 2025, 9, ziae152
https://doi.org/10.1093/jbmrpl/ziae152
Research Article