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X-Linked Hypophosphatemia in 4 generations due to an exon 13-15 duplication in *PHEX*, in the absence of the c.*231A>G variant

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Abstract

X-Linked hypophosphatemia is the most common cause of inherited rickets, due to inactivating variants of *PHEX*. More than 800 variants have been described to date and one which consists of a single base change in the 3' untranslated region (UTR) (c.*231A>G) is reported as prevalent in North America. Recently an exon 13-15 duplication has been found to occur in concert with the c.*231A>G variant, and thus it is unclear whether the pathogenicity is solely a function of the

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UTR variant. We present a family with XLH who harbors the exon 13-15 duplication but does not carry the 3'UTR variant, providing evidence that the duplication itself is the pathogenic variant when these two variants are found in *cis*.

Keywords

X-Linked hypophosphatemia (XLH); Phosphate-regulating gene with homologies to endopeptidases on the X chromosome (PHEX); rickets

1. Introduction

X-Linked Hypophosphatemia (XLH) is the most common cause of inherited rickets [1] with an estimated prevalence of 1 in 20,000 to 1 in 60,000 individuals [2]. The disorder is caused by inactivating mutations in *PHEX* (phosphate-regulating gene with homologies to endopeptidases on the X chromosome)[3]. *PHEX* encodes a zinc metallopeptidase predominantly expressed in bone and teeth [4], and reduces expression of FGF23 through mechanisms that are unclear. Inactivating mutations in *PHEX* cause elevated circulating levels of FGF23, resulting in renal phosphate-wasting, impaired conversion of 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D and consequent impaired bone mineralization [5]. At this time 870 mutations in *PHEX* have been described [6]. In 2008, a novel single-base change (c. 231A>G) located 3 base pairs upstream of the putative polyadenylation (pA) signal (AATAAA) in the gene's 3'-untranslated region (UTR) was reported to cause XLH in eight patients from seven unrelated kindreds living primarily in the Midwest of the USA [7]. This variant would presumably impact posttranscriptional transport and translation of the mRNA, but functional studies have not been performed [8]. Subsequently this non-coding point mutation was found almost exclusively to be associated with an exon 13-15 duplication suggesting that these two variants co-segregate and constitute a single allele [6,9] and it is thought to be the most prevalent variant across the United States regardless of race [6]. Because one patient carried the duplication without the c.*231A>G variant it has been postulated that the duplication could contribute to pathogenesis itself [9]. The major clinical features of children affected with XLH are rickets, osteomalacia, growth failure, and dental abscesses whereas adults manifest with osteoarthritis, enthesopathy [1,2]. However, a milder phenotype was observed in patients with the c.*231A>G variant than with mutations disrupting the coding region of *PHEX*, especially among women who were often underdiagnosed [8,10]. More recently a wide range of severity has been described [9,11]. Here, we report a family in whom 3 generations have been demonstrated to harbor the duplication without the c.*231A>G variant.

2. Clinical cases

2.1 Case 1 (Proband)

This 70-year-old male (Patient 1, table 1) was evaluated at our center late in his course. He reported wearing braces on both legs from 2 to 6 years of age, and at age 9 was diagnosed with vitamin D resistant rickets. He underwent triple osteotomies in both lower extremities at that time, but was never treated medically. He had multiple dental abscesses

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during adolescence resulting in loss of multiple teeth. In his mid 50s he developed lower extremity radiculopathy and at age 65 underwent 4 laminectomies for ossification of the posterior longitudinal ligament. He also developed enthesopathy at his ankles, knees and hips, and Dupuytren contractures with calcifications. He was diagnosed with bilateral carpal tunnel syndrome. He had no history of fractures; his bone mineral density of the spine and hip were normal per dual energy X-ray absorptiometry (DXA) scanning. Comorbidities included hypertension and hyperlipidemia. His family history is notable in that his mother was thought to be affected with rickets, and his brother had mildly bowed legs. His niece (per this brother) was of normal height (170 cm) but her serum phosphorus (P) was low (2.0 mg/dl) and the tubular reabsorption of phosphate (TRP) and TmP/GFR (threshold maximum for phosphate reabsorption expressed per glomerular filtration rate) were 88% and 2.0 mg/dl respectively, having been investigated at the time her son was diagnosed with XLH (See below, Case 2, table 1). Case 1 also had a clinically unaffected daughter and a maternal cousin with bowed legs. At the time of his physical evaluation at age 70, height was 167 cm, weight 75.7 kg, BMI 27.1, Blood Pressure (BP) 149/87. Biochemical tests were consistent with XLH (Table 1). His circulating FGF23 level was 35 pg/ml (normal range 15-52, however inappropriately normal for the hypophosphatemia). Radiographic studies showed multiple areas of degenerative changes and mineralizing enthesopathy throughout the skeleton. His echocardiogram was normal.

2.2 Case 2

The son of the niece of Case 1 was diagnosed with XLH at age 23 months (Patient 2, table 1). He had a history of bowed legs from the age of 5-6 months which became more pronounced when he began walking. His diet provided adequate mineral via milk intake. His perinatal history was unremarkable, and his developmental milestones were normal. At the time of diagnosis, his height was 81.5 cm (2nd centile, −1.97 SDS), weight 12.25 kg (55th centile, 0.55 SDS), BP 91/69. The rest of his physical examination was normal except for the presence of marked genu varus with an inter-popliteal distance of 8 cm. His gait was functional, but demonstrated in-toeing bilaterally. Laboratory tests were concordant with XLH and treatment with phosphate and calcitriol was initiated. He developed multiple cavities and dental abscesses. Circulating FGF23 level was subsequently measured and found to be elevated (114 pg/ml). At age 11, he discontinued calcitriol and phosphate, and began burosumab therapy. His serum phosphorus levels have been maintained in normal range on relatively low doses of burosumab (most recent at 0.3 mg/kg every 14 days). His height at age 19 is 164.8cm (~ 5th centile).

2.3 Case 3

The younger brother of Case 2 was initially evaluated at age 2 months and found to have normal serum P and TMP/GFR, however he was serially monitored in view of the family history. Serum P and TMP/GFR were low at age 2 yr 1 month, which prompted initiation of phosphate and calcitriol therapy. Subsequently, circulating FGF23 level was found to be elevated (111 pg/ml). He was treated in this manner until age 8, when he began burosumab. He experienced dental abscess early in his course but none after age 5, and had an episode of PFAPA syndrome (periodic fever, aphthous stomatitis, pharyngitis, adenitis) at age 14, which resolved following tonsillectomy and adenoidectomy at that age. Overall, he has done

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well without significant musculoskeletal complications. At age 16 his height is 176.9 (~ 65th centile). As with his brother, serum phosphorus levels have been maintained in normal range on relatively low doses of burosumab (most recent at 0.4 mg/kg every 14 days).

3. Materials and Methods

After clinical panels were uninformative, the family was referred and consented to the Gene Discovery Core of the Manton Center for Orphan Disease Research for further evaluation. Whole exome sequencing (Psomagen, Maryland) and genome sequencing (Broad Institute, Cambridge, MA) were performed. Analysis was performed in Genuity Science (Reykjavik, Iceland) as described previously [12,13]. Sanger sequencing was used to confirm the absence of the c.*231A>G variant, according to previously published protocols.

Ultra-high molecular weight DNA collection

For cases 2 and 3 and their mother, researchers used frozen EDTA blood as a substrate with Bionano Saphyr technology. DNA was isolated using the SP Blood and Cell Culture DNA Isolation Kit v2 (catalog #80042) coupled with the Bionano Prep SP Frozen Human Blood DNA Isolation Protocol v2 (document #30395), revision B.

Samples passed all QC thresholds for white blood cell counts on HemoCue and isolation, and labeling on Qubit Flex. Stained DNA samples were added to chips for scanning. The scan parameters are set to collect a maximum of 1500Gbp using hg38 as the reference genome. The Bionano Saphyr collected 802 GBP of data from case 2. All values on the Molecule Quality Report were passing (N50 >150kbp – 212.82 kbp; Map Rate – 70%; Effective Coverage – 98). Both the De Novo Assembly and Rare Variant Analysis pipelines were run on Bionano Access version 1.7.1.1., according to the manufacturer's recommendations. Only DNA molecules with a minimum length of 150 kbp were used for bioinformatics analysis. No BED file filtering was used.

4. Results

As exome and genome data did not show a c.*231A>G variant or any other variation in *PHEX*, further genetic investigation was pursued. Bionano Saphyr optical genome mapping revealed an approximately 52,000 base pair tandem duplication of exons 13-15 in the *PHEX* gene in case 2, case 3, and their mother (Figure 2), with the unfiltered structural variant (SV) calls listed in Supplemental table 1. The duplication was not identified in 300 control samples. Using the publicly available tool Franklin by Genoox (https://www.genoox.com/the-genoox-platform/) which automates the American College of Medical Genetics and Genomics guidelines [14], the duplication is predicted to be "Likely Pathogenic" (evidence includes that breakpoint(s) are within established haploinsufficient genes, and segregation/inheritance-consistency of copy number within the patient's family). Sanger sequencing confirmed the absence of a c.*231A>G variant in case 2, case 3 and their mother.

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5. Discussion

We describe a family with individuals from 4 generations affected with XLH, albeit with considerably variable severity. When initial genetic evaluation employing Sanger sequencing of the *PHEX* coding region did not reveal any significant variants, we considered that structural or intronic regulatory variants may be present and further analysis was performed. Optical genome mapping revealed a tandem exon 13-15 duplication in *PHEX,* but of note, the reportedly linked c.*231A>G variant was not evident. While it is possible that the endpoints of this duplication differ from the previously published duplication, as Bionano Saphyr data produces approximate structural variation endpoints and not exact, clinical and biochemical manifestations of XLH were observed, supporting the argument that the duplication, rather than the UTR base change, is the major contributor to pathogenesis in the predominant number of cases where both variants are evident. A previous study showed that 5 boys who carried the 3′-UTR change had higher fasting serum P and TmP/GFR compared with mutations elsewhere in *PHEX*. Interestingly, the majority of the mothers of these boys were clinically unaffected but manifest normal or slightly low fasting serum P concentrations and/or TmP/GFR [10], consistent with the mother of cases 2 and 3. Another study compared 30 individuals harboring the 3'- UTR variant with 30 XLH patients without this defect, showing a milder clinical and biochemical phenotype in the group with 3'-UTR variant. This was particularly evident in females, who were often not diagnosed with XLH [8], as with the family presented here. Hence, it has been postulated that the heterozygous state could modulate the expression of XLH in females [8,10]. On the other hand, limited data have shown lower circulating FGF23 levels in some patients who carried c.*231A>G variant as compared to those with other *PHEX* variants, which may explain the milder abnormalities in serum P and TmP/GFR observed in the former group [8], consistent with the FGF23 value reported in the proband of our family. However, his late complications of disease, particularly the extent of enthesopathy, would not be considered mild. In contrast, the 65 patients with the c.*231A>G, Exl3–15 dup variant reported in another study were not clinically or biochemically different from patients with other *PHEX* variants. Furthermore, this study identified one proband harboring the duplication but not the c.*231A>G variant, suggesting that the duplication alone can contribute to the disease [9]. Another recently published study described a 5-generation American kindred of 22 individuals harboring the c.*231A>G; exon 13-15 duplication variant, including 15 women and 7 men, highlighting the frequent lack of diagnosis, or misdiagnosis as ankylosing spondylitis. Predominant clinical features included poor dentition, enthesopathies, and other bone and joint complications. These findings became more prevalent with age and one-third of the patients showed 4-6 characteristics of XLH [11]. The predominance of dental findings is concordant with the history of our patients.

Of note, although the males presented with more XLH manifestations, occasionally females were severely affected [11]. Indeed, sexual dimorphism may be relevant not only with respect to disease severity but also as relates to frequency of diagnosis. One study found equal numbers of male and female cases in families with the 3′-UTR mutation [10] in contrast to the 2:1 female:male ratio expected for an X-linked dominant disease, likely related to the underdiagnosis of women with a milder phenotype. However, the expected

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female:male ratio was observed in a more recent study [11]. Further studies are needed to clarify this matter and it is also possible that genetic testing of relatives of probands, especially women, could contribute to an increase in the diagnosis and incidence of this condition leading to a prompt treatment as needed. Finally, more extensive evaluation may now be occurring in the context of increased awareness of the disease.

This report provides extends evidence that the exon 13-15 duplication in *PHEX* can be pathogenic in isolation and can present with variable degrees of severity, as has been suggested in reports of kindreds with the combined segregation of this duplication and the 3'-UTR variant. Additionally, we emphasize the importance of long-term monitoring for late-onset complications of XLH, even if thought to harbor variants associated with mild disease. We will look forward to further studies of the natural history of this variant in a larger group of patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- A common *PHEX* variant identified in families with XLH, originally reported as c.*231A>G in the 3' untranslated region of the gene, was recently found to occur in concert with an exon 13-15 duplication on the same allele
- We describe several members of a family affected with XLH who were found to carry the exon 13-15 duplication without the 3' untranslated region variant
- This report provides evidence supporting the notion that the exon 13-15 duplication in *PHEX* is pathogenic for XLH, and may be the major contributor in cases where both the duplication and the c.*231A>G variants occur together. Moreover the variant can manifest a broad spectrum of clinical severity.

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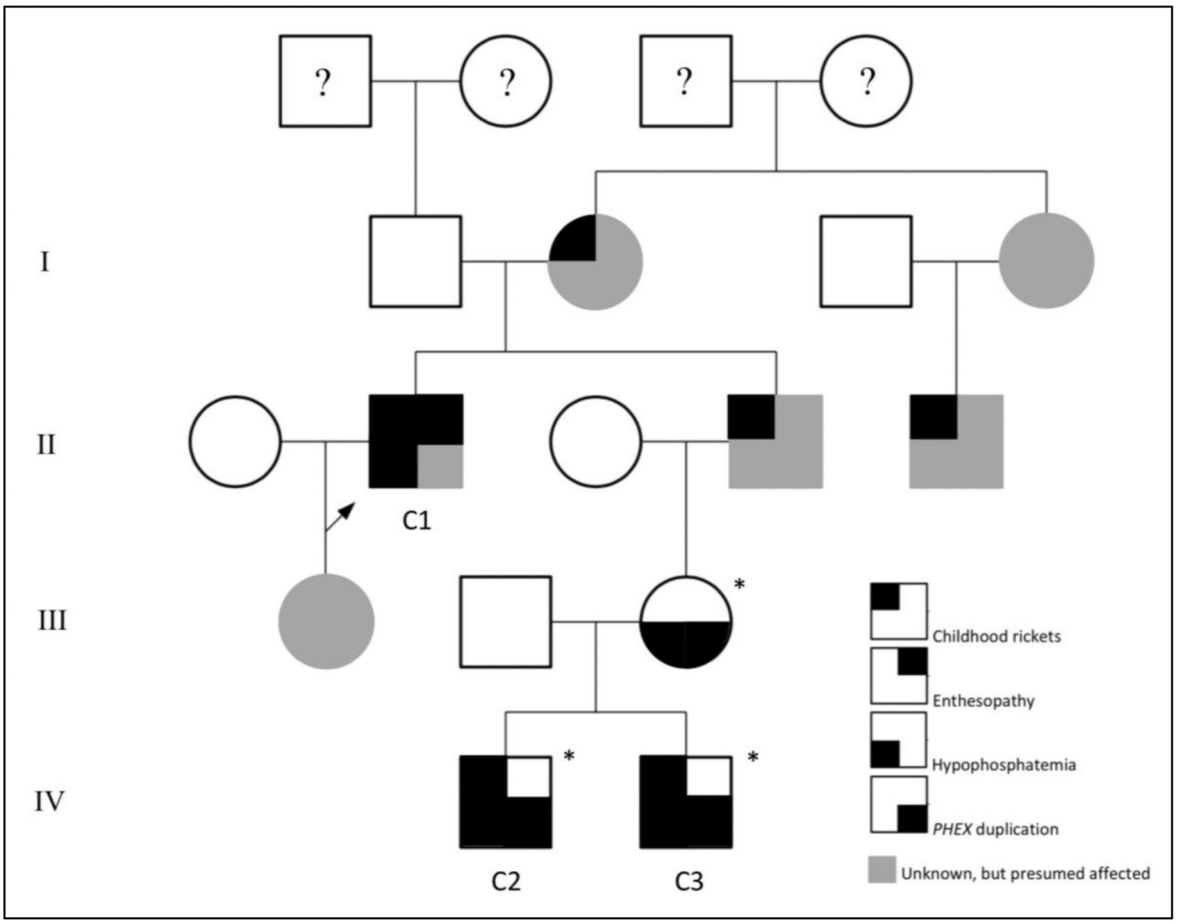

Figure 1.

Pedigree of the four-generation family with XLH due to a tandem duplication in *PHEX*. Clinical and biochemical findings are depicted in family members in whom information was available. An asterisk identifies the family members investigated for the presence of the duplication.

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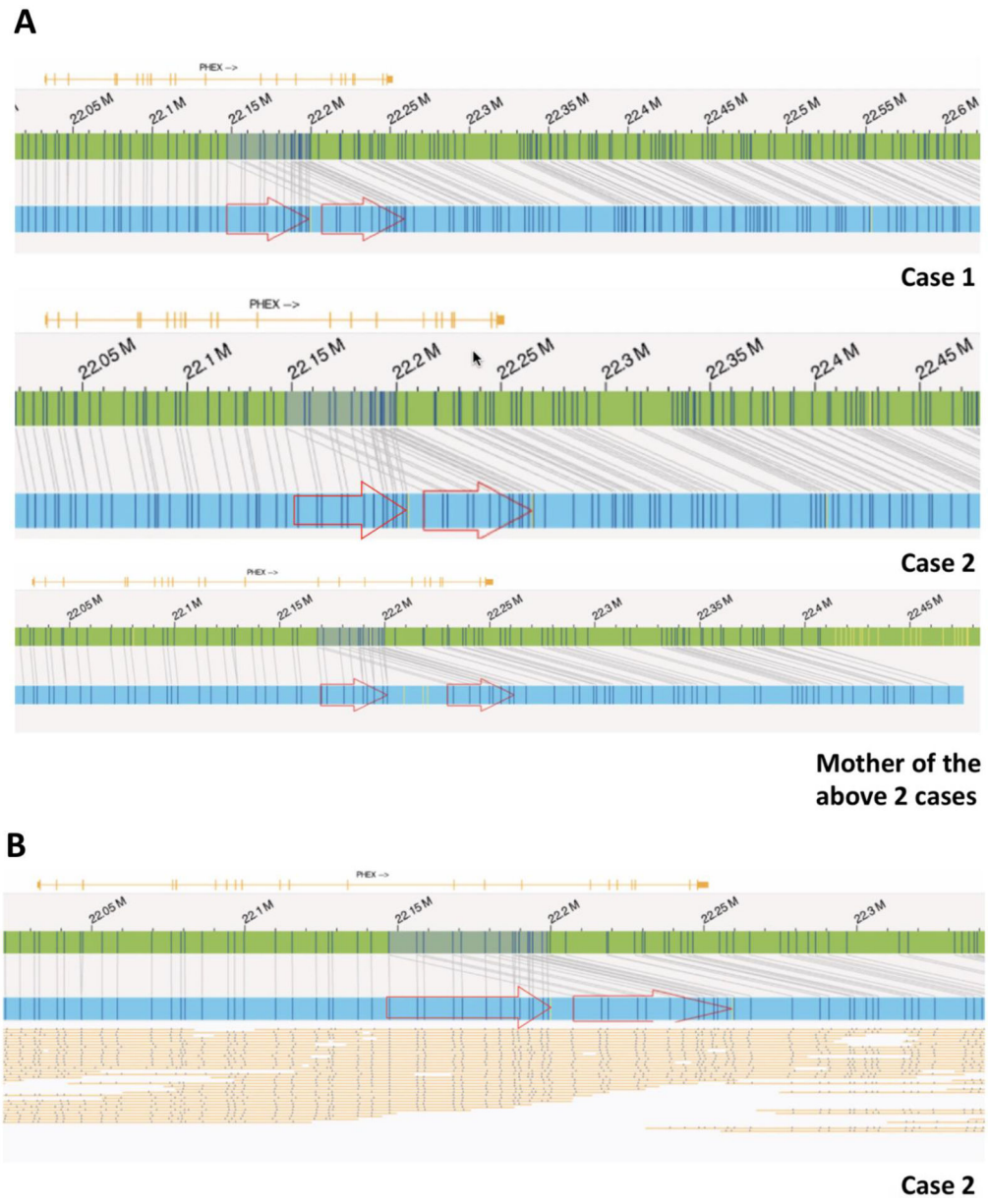

Figure 2. A tandem duplication of exons 13-15 is demonstrated for Cases 1 and 2, and their mother.

A, Optical genome mapping data showing the duplication of PHEX exons 13-15 for all three individuals. The green bar in each panel represents the reference genome, and the respective blue bars represent the sample consensus maps showing that there is a tandem duplication of exons 13–15, indicated by the red arrows and duplicated banding pattern in the blue map. *B,* Optical genome mapping from Case 2 showing the duplication of exons 13-15 has high molecule support spanning the duplication breakpoints.

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Table 1.

Available biochemical values of family members

	Serum Phosphorus (mg/dL)	**Serum Calcium (mg/dL)**	**Serum Alkaline Phosphatase (U/L)**	**Serum Creatinine (mg/dL)**	**Serum 25-OHD (ng/mL)**	**Serum 1,25 $(OH)_2D$ (pg/ml)**	**Serum PTH (nLeq/mL)**	**Intact FGF23 (pg/ml)**	**TmP/GFR (mg/dl)**
C1 (Male)	2.3 (2.5-4.5)	9.0 (8.8-10.2)	63 (30-130)	0.9 (0.5-1.2)	25 (20-45)		21 (10-25)	35 (<50)	
C2 Age 23 mos (Male)	3.1 (3.5-6.5)	9.3 (8.8-10.2)	358 (50-480)	0.4 (0.2-0.8)	42 (20-45)	41 (25-66)	38 (10-25)		2.5 (3.2-5.5)
C2 Age 11 yr								114 (<50)	
C3 Age 2 mos (Male)	4.4 (3.5-6.5)	10.6 (8.8-10.2)	471 (50-480)	0.4 (0.2-0.8)					4.2 (3.2-6.2)
C3 Age 2yr	2.9 (3.5-6.5)	9.0 (8.8-10.2)	284 (50-480)	0.2 (0.2-0.8)					2.5 (3.2-5.5)
C3 Age 8 yr								111 (<50)	
Mother of C2/P3	2.3 (2.5-4.5)			0.7 (0.5-1.2)					2.0 (2.6-3.8)

Reference ranges (age-specific) are shown in parentheses after values. TMP/GFR normative values from references 15 and 16 are presented in the final column.