A De Novo Mosaic *PHEX* Variant Causing Sporadic X-Linked Hypophosphatemic Rickets in a 2-Year-Old Girl

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Abstract

Pathogenic variants in the *PHEX* gene are causative of X-linked hypophosphatemic rickets (XLH). We present a case of a 2-year-old girl with hypophosphatemic rickets with genu varum and short stature without any family history of XLH. Next generation sequencing of the *PHEX* gene identified a splice donor variant, NM_000444.6:c.1173 + 5G > A in intron 10. This variant had a mosaic pattern with only 22% of the sequence reads showing the variant allele and was not present in the girl's parents, both of whom had a normal phenotype. This is a sporadic case of a de novo mosaic splice-site variant in the *PHEX* gene.

Keywords
- *PHEX*
- X-linked hypophosphatemic rickets
- mosaic

Introduction

Hereditary hypophosphatemic rickets, dependent on the causative gene defects, is known to be associated with different modes of inheritance, including autosomal dominant, autosomal recessive, X-linked dominant, and X-linked recessive. X-linked dominant hypophosphatemic rickets (XLH) was first described in 1937[1,2] and is a rare genetic disorder with an incidence of approximately 1 in 20,000.[3]

Background

XLH caused by loss-of-function variants in the PHosphate regulating gene with homology to Endopeptidases on the X-chromosome (*PHEX*) gene is the most common hereditary form of hypophosphatemic rickets.[3] Lack of PHEX protein leads to overproduction of fibroblast growth factor 23 (FGF23) by osteocytes, and this results in hypophosphatemia due to reduced renal tubular phosphate reabsorption and decreases synthesis of the active form of vitamin D (1,25-$[OH]_2$D).[3] However, the mechanism by which PHEX deficiency causes FGF23 elevation remains unclear. Further, it has been suggested that inactivating mutations in the *PHEX* gene increase the FGF23 expression by induction of acidic serine aspartate-rich-matrix extracellular phosphoglycoprotein, which leads to increased release of FGF23 into the serum and FGF23-mediated signaling.[4]

Clinical Manifestation

Common clinical manifestations of hypophosphatemic rickets include hypophosphatemia, growth retardation,

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childhood rickets, skeletal deformities, and dental abscesses. Biochemical manifestations include low serum phosphate, low to normal 1,25-$(OH)_2$D, and elevated serum alkaline phosphatase.[5] XLH commonly presents in the first year and a half of life with difficulty walking and deformities of the lower limbs.[3]

Diagnosis

Diagnosis of sporadic cases with hypophosphatemic rickets solely based on clinical and laboratory findings can be challenging in pediatric patients without performing molecular analysis. The *PHEX* gene has a broad mutational spectrum that extends from small variants to large copy number variants.[4] These germline mutations in the *PHEX* gene are identified using the commonly used capillary sequencing and multiplex ligation-dependent probe amplification (MLPA) techniques in the molecular diagnostic laboratories. There has been a growing body of literature reporting genetic mosaicism in XLH cases[6–10] in which existing technologies could potentially miss the pathogenic variants with low allelic frequency. A recent consensus statement from clinical experts recommends molecular genetic analysis for the confirmation of XLH diagnosis whenever possible.[11]

Management

Until recently, the therapeutic approach mainly relied on vitamin D metabolites and phosphate replacement to correct biochemical abnormalities. However, this does not correct the underlying pathophysiology of XLH. Recently, a monoclonal antibody to FGF23, burosumab has been approved for the treatment of XLH and showed improved renal phosphate reabsorption and reduced severity of rickets.[5] This new advance in XLH therapy, together with early genetic diagnosis, enables good long-term outcomes.

Case Presentation

Here we describe a case of a 2-year-old girl with an initial clinical and biochemical diagnosis of hypophosphatemic rickets. The patient had a delayed onset of walking at age 2 years and presented at the age of 2.5 years with genu varum and a waddling gait. She managed with bracing for presumed developmental dysplasia of the hips. Besides motor delay in walking and abnormal gait, there were no other developmental problems or personal medical history. There was no hearing loss. Her parents are not consanguineous and there was no family history of rickets in the family.

On physical examination, the patient had marked bowing with a femoral intercondylar distance of 10 cm as well as frontal bossing. She was not dysmorphic but had short stature with a height on the 1st percentile in the context of a midparental height on the 25th percentile. Biochemical investigations showed low serum phosphate at 0.9 mmol/L (1.1–1.8 mmol/L), elevated alkaline phosphatase (ALP) of 450 IU/L (100–300 IU/L) and a marginally elevated parathyroid hormone (PTH) level of 7.3 pmol/L (1.3–6.8). Her calcium was normal at 2.15 mmol/L (2.1–2.6 mmol/L) and her 25 hydroxyvitamin D level was normal at 67 nmol/L (50–160 nmol/L). The calculated fractional excretion of phosphate (urine phosphate/serum phosphate X serum creatinine/urine creatinine) was 18%. Based on this, the renal tubular reabsorption of phosphate was 82.0%, and the renal threshold for phosphate concentration (TmPO4/GFR) was 0.74 mmol/100 mL glomerular filtration rate using the Walton and Bijvoet normogram. The renal ultrasound did not reveal significant nephrocalcinosis. Hypophosphatemic rickets was clinically diagnosed in this patient, and she was started on phosphate replacement of 30 to 40 mg/kg/day divided over five doses with calcitriol 0.25 mcg daily (0.015 mcg/kg).

Molecular investigation of the *PHEX* gene, which is the most commonly mutated gene for hypophosphatemic rickets, was subsequently performed to determine the genetic etiology in this girl with the informed consent of the parents. Multiplex polymerase chain reactions (PCRs) were performed on the patient's genomic DNA with previously published primers.[12] PCR amplicons were pooled to generate a library using the Nextera DNA Library Prep Kit (Illumina). Next-generation sequencing (NGS) was performed by using MiSeq Reagent Nano Kit v2 (Illumina) on a MiSeq instrument (Illumina). Variant call format files generated from the MiSeq Reporter v2.3 software were filtered and annotated by using VariantStudio v3.0 (Illumina). Integrative genomics viewer (IGV) v2.4.9 was used to examine the reads based on hg19 human sequence version. Analyses of copy number variations, large deletion, and duplication of the *PHEX* exons were also performed with MLPA with SALSA MLPA Probemix P223 *PHEX* kit (MRC-Holland).

A NGS depth plot for the targeted genomic region of the *PHEX* gene is shown in ►**Fig. 1A**. The NM_000444.6:c.1173 + 5G > A variant was identified within the splice-donor region in intron 10 of the *PHEX* gene (►**Fig. 1B**). NGS analysis shows that this variant has a variant frequency of 22% (288 reads/1,341 total reads), suggesting it is a mosaic variant as a typical germline heterozygous variant in a female with 2 X chromosomes has a variant frequency close to 50%. Targeted capillary sequencing confirmed this variant to be bona fide. This finding raised the suspicion of mosaicism. Subsequent targeted capillary sequencing in her parents also confirmed the de novo status of this variant in the patient since this variant was not detected in both her parents (►**Fig. 1C**). MLPA analysis excluded abnormal gene dosage (►**Fig. 1D**), one of the frequent genetic defects in the *PHEX* gene. The genetic diagnosis enabled her to be recruited into a trial for burosumab for which a dose of 10 mg (0.568 mg/kg) was given every 2 weeks.

Discussion

We identified a mosaic splice variant in a girl with XLH with phenotypically normal parents. The NM_000444.6:c.1173 + 5G > A variant has been reported in a recent study[13] in which the capillary sequencing technique was employed. Unfortunately, the clinical presentation of the case with the NM_000444.6:c.1173 + 5G > A variant was not described.[13]

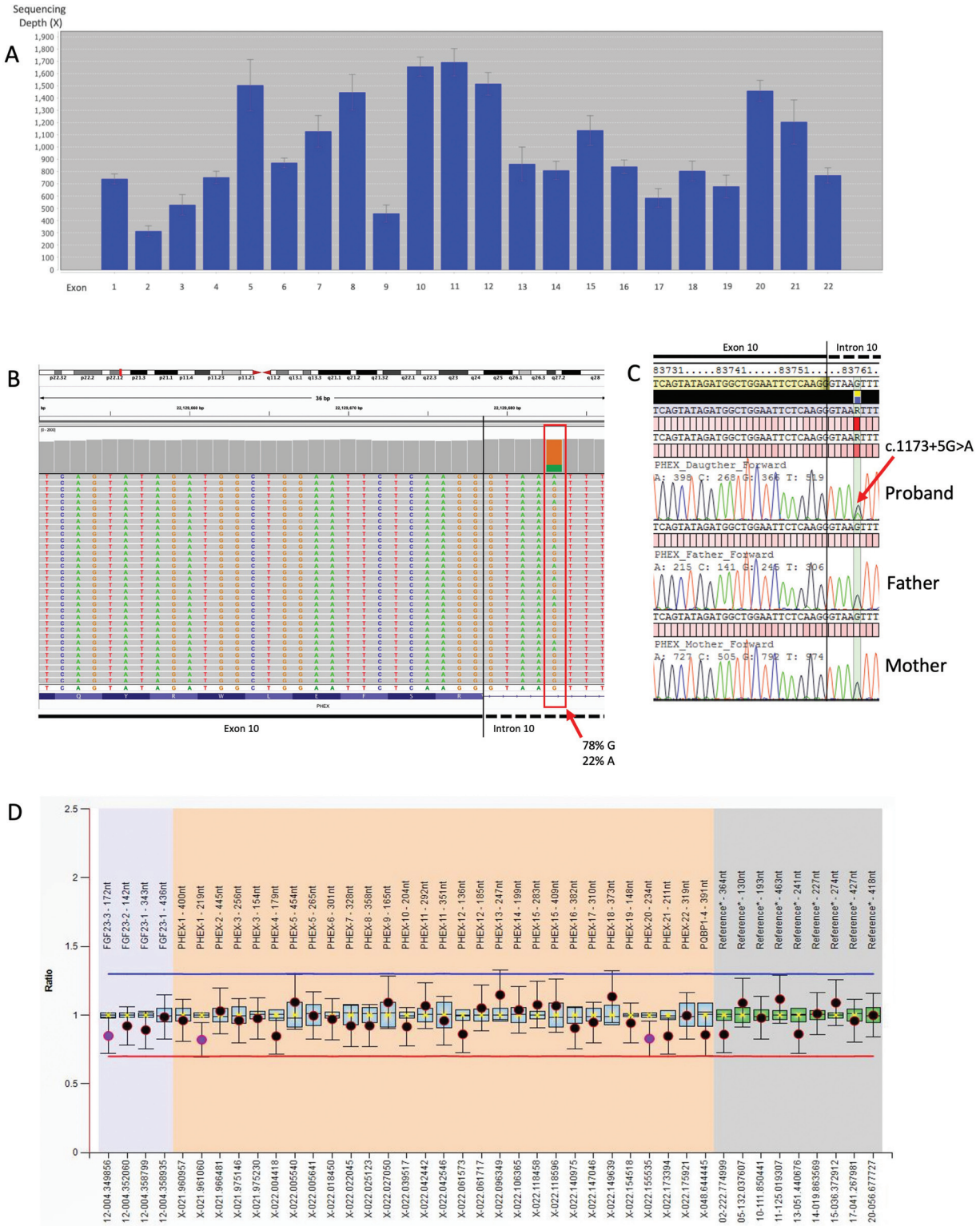

Fig. 1 (**A**) Average sequencing depth for individual amplicons for 22 *PHEX* exons in the proband. (**B**) Integrative Genomic Viewer visualization of next-generation sequencing reads harboring the NM_000444.6:c.1173 + 5G > A variant (red box) in the proband. In total, 22% variant allele frequency was detected (indicated by a red arrow). (**C**) Sanger electropherograms show the partial nucleotide sequences at the *PHEX* exon 10-intron 10 boundaries of proband, her father and mother. The 1173 + 5G > A variant (indicated by a red arrow), which was identified in the proband was not found in the proband's parents. The findings suggest the variant occurred de novo in the proband. (**D**) Ratio chart of *PHEX* and *FGF23* multiplex ligation-dependent probe amplification probes. Copy number ratio relative to the normal control is indicated as normal (black dot) for regions with dosage quotient between 0.80 (red line) and 1.20 (blue line).The findings suggest no copy number variants in the *FGF23* and *PHEX* genes.

Hence we are unable to compare with our case. The absence of this variant in population databases such as the gnomAD database, dbSNP, and Exome Aggregation Consortium reduces the likelihood that this variant is a benign polymorphism. This variant was analyzed by Human Splicing Finder 3.1,[14] which is a splice site prediction software recommended by the American College of Medical Genetics and Genomics (ACMG)/College of American Pathologists (CAP) guidelines[15] to aid pathogenicity interpretation. The G to A nucleotide change is predicted to result in the loss of the donor site and lead to abnormal splicing and aberrant mRNA transcript. However, the current report's limitation is the lack of in vitro functional evidence to verify the in silico prediction outcomes for this variant. Aberrant mRNA transcript could be sought by reverse-transcription PCR (RT-PCR) to ascertain the splicing defect from this variant. Unfortunately, the RNA material from this patient was not available for such a study. Apart from this limitation, the identified variant is still classified as likely pathogenic in the context of XLH according to the current ACMG/CAP guidelines, as the variant was detected de novo in our patient with the disease and no family history (PS2, PM2, PP5). Another limitation in this study is that only the *PHEX* gene was sequenced and not a panel of genes implicated in hypophosphatemic rickets. However, mutations in the *PHEX* gene are the most common cause of hypophosphatemic rickets. Other earlier findings of mosaicism in XLH have also been seen.[6–10] Capillary sequencing is currently the gold standard method to detect germline variants. Mosaic variants had been previously detected mainly in XLH males with capillary sequencing,[8,13] when the "heterozygous" variant appeared in the chromatograms of the X-linked *PHEX* gene. Mosaicism of *PHEX* variants is likely to be overlooked by the "qualitative" capillary sequencing analysis since females have two *PHEX* alleles. With the advance of NGS, variant allele frequency could be derived from the counting of the sequencing reads harbouring the variant of interest. The high sensitivity of NGS allows the detection of low-level mosaicism. Before this, mosaicism of the *PHEX* variant was investigated by TA cloning[7] which is a laborious technique not routinely available in the diagnostic laboratory setting. There was also a case of gonadal mosaicism in the sperm of a phenotypically normal father of a girl with confirmed XLH with a *PHEX* variant.[16] Our case's causative variant is likely of "postzygotic" origin since somatic mosaicism was detected in her blood sample. Identifying the mechanism of mosaicism has important clinical implications for genetic counseling and estimation of recurrence risk. However, the investigation is challenging because additional testing specimens of multiple tissue origins from the proband and parents is required.

Conclusion

In conclusion, genetic testing of the *PHEX* gene revealed a mosaic splice-site mutation (NM_000444.6:c.1173 + 5G > A) consistent with the clinical diagnosis of sporadic X-linked hypophosphatemic rickets in the proband. A timely genetic diagnosis enabled her to be recruited into a trial for burosumab therapy. This case illustrates the importance of sequencing the *PHEX* gene by NGS in patients with hypophosphatemic rickets who are still undiagnosed after initial Sanger sequencing, considering the possibility of a de novo mosaic mutation.

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Conflict of Interest
None declared.

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