Case Report
J Genet Med 2022;19(1):27-31
https://doi.org/10.5734/JGM.2022.19.1.27
ISSN 1226-1769 (Print) 2383-8442 (Online)

JGM Journal of Genetic Medicine

A novel variant of *PHEX* in a Korean family with X-linked hypophosphatemic rickets

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X-linked dominant hypophosphatemic rickets are the most common form of familial hypophosphatemic rickets resulting from hypophosphatemia caused by renal phosphate wasting, which in turn is a result of loss-of-function mutations in *PHEX*. Herein, we report a 39-year-old female with short stature and skeletal deformities and 12-month-old asymptomatic daughter. The female has a history of multiple surgical treatments because of lower limb deformities. Her biochemical findings revealed low serum phosphorus levels with elevated serum alkaline phosphatase activity and normal serum calcium levels, suggesting presence of hypophosphatemic rickets. To identify the molecular causes, we used a multigene testing panel and found a mutation, c.667dup (p.Asp223GlyfsTer15), in *PHEX* gene. To the best of our knowledge, this is a novel mutation. A heterozygous form of the same variant was detected in daughter, who showed no typical symptoms such as bow legs, frontal bossing, or waddling gate, but presented early signs of impaired mineralization in both X-ray and biochemical findings. The daughter was initiated onto early medical treatment with oral phosphate supplementation and an active vitamin D analog. Because the daughter was genetically diagnosed based on a family history before the onset of symptoms, appropriate medical management was possible from early infancy.

Key words: Familial hypophosphatemic rickets, Genetic testing, Genetic counseling, High-throughput nucleotide sequencing.

Introduction

Familial hypophosphatemic rickets (FHR) are disorders resulting from hypophosphatemia caused by a defect in renal phosphate transport, leading to phosphate wasting. There are four inherited forms of FHR, the most common being the X-linked dominant hypophosphatemic rickets (XLH; OMIM no. 307800), which results from a loss-of-function mutation in the *PHEX*. *PHEX* is a phosphate-regulating gene with homologies to endopeptidases located on the X chromosome. The estimated incidence of XLH is 1 in 20,000 live birth. Although *PHEX* mutations cause renal phosphate wasting, *PHEX* is expressed mainly in osteoblasts and osteocytes in bone (and odontoblasts in teeth) and not in the kidney [1,2].

Traditional diagnosis of XLH includes clinical manifestations, laboratory findings, and radiologic findings. The clinical features of XLH include hypophosphatemia, radiological evidence of rickets in childhood, deformity of the lower limb, decreased growth velocity, short stature, bone pain, and dental problems such as tooth abscesses. Laboratory findings include low serum phosphate levels secondary to renal phosphate wasting, normal calcium levels, increased activity of serum alkaline phosphatase, normal or increased parathyroid hormone levels, and normal or low 1, 25-dihydroxyvitamin D levels. Along with these observations, the percentage of tubular reabsorption of phosphate (TRP) decreases, and urinary phosphate excretion increases [3].

Received: 14 December 2021, Revised: 6 May 2022, Accepted: 8 May 2022, Published: 30 June 2022
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Conflict of interest: The authors declare that they do not have any conflicts of interest.

Radiologic findings include evidence of rickets (metaphyseal widening, cupping, and fraying of the wrist and lower limbs) and valgus or varus deformities of the knee.

Genetic testing has been used to confirm the diagnosis of XLH. Recent advances in molecular technologies have provided a cost-effective and rapid approach for the molecular diagnosis of XLH through extensive and simultaneous evaluation of a group of disease-causing genes. The integration of clinical findings with genetic information allows for improved diagnosis and prognosis and helps cascade family testing to identify and manage risk. Here, we report a Korean family with XLH exhibiting a novel pathogenic variant, c.667dup (p.Asp223GlyfsTer15), of the *PHEX*, detected using genetic diagnosis and advantages of early intervention in patients with hypophosphatemic rickets (HR). This study was approved by the Institutional Review Board (IRB) of Dong-A University Hospital (IRB no. DAUHIRB-21-184). The written informed consent was waived by IRB.

Case

Two female patients visited the pediatric endocrinology clinic for genetic counseling and evaluation of their health status. The first patient, proband, was a 39-year-old female and the second patient was her 12-months-old daughter. The mother exhibited short stature, and her measured height was 137.4 cm. She had a history of surgical correction for right genu valgum and left genu varum at the age of 25 years (Fig. 1). After being admitted for orthopedic surgery, her laboratory serum analysis revealed hypophosphatemia (0.8 mg/dL; normal range, 2.5-4.5 mg/dL), normal calcium level (8.6 mg/dL; normal range, 8.2-10.5 mg/dL), and increased alkaline phosphatase level (126 IU/L; normal range, 30-120 IU/L). The urine phosphorus was unchecked. The above findings and surgical history suggested presence of HR. Through additional questions about her family, we found that her mother's height was also 137 cm. However, the mother had no findings of skeletal dysplasia other than short stature. Except for the mother, other family members answered that they did not have short stature or abnormalities in appearance (Fig. 2). For genetic confirmation of the diagnosis and to differentiate the diagnosis from diseases with similar phenotypes, we performed multigene panel sequencing associated with skeletal dysplasia. Written consent was obtained from the patient and her family for the blood samples. The testing revealed the presence of a heterozygote of c.667dup (p.Asp223GlyfsTer15) variation in the *PHEX*. This variant was novel and classified as pathogenic according to the American College of Medical Genetics and Genomics and the Association for Molecular Pathology guideline [4]: frame-shift null variant in gene *PHEX* for which loss-of-function is a known mechanism of disease (PVS1); not found in gnomAD and Korean Reference Genome Database (PM2); the patient's phenotype or family history is highly specific for a disease with a single genetic etiology (PP4).

Her daughter was born via cesarean section at 37 weeks of

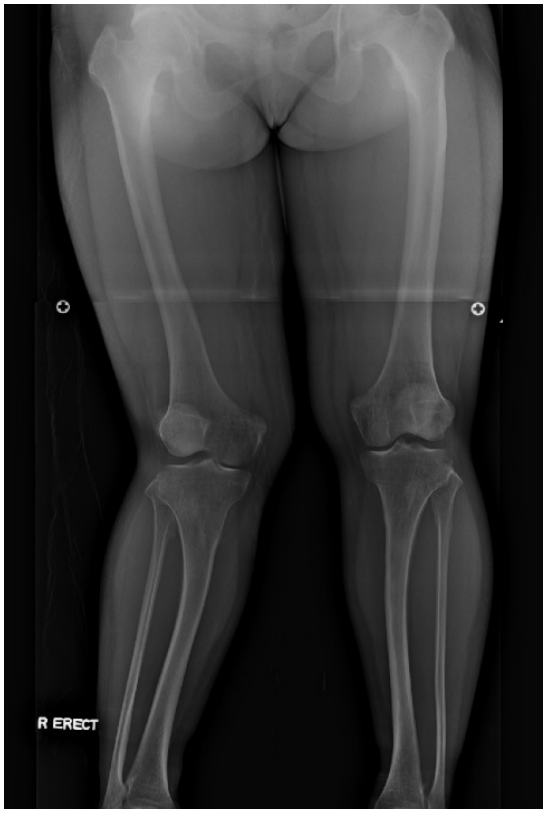

Fig. 1. The female's radiograph taken at the age of 25 years showed right genu valgum and left genu varum.

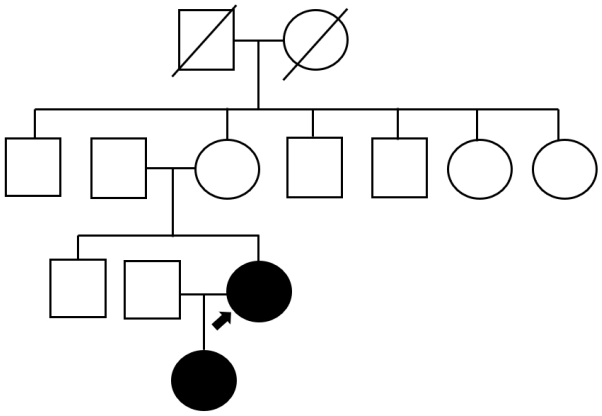

Fig. 2. Pedigree showing that the proband (arrow) and her daughter have hypophosphatemic rickets and that the grandmother might have the same disease. The remaining family members are healthy.

gestation; birth weight was 2,670 g (–0.37 standard deviation score [SDS]) and height was 51 cm (+1.37 SDS). At the outpatient clinic, her weight and height were 10.1 kg (+0.98 SDS) and 74.2 cm (+0.07 SDS), respectively. Physical examination revealed no obvious bowed legs or frontal bossing. She could walk by holding someone's hand but was unable to walk by herself, and no waddling gait was seen. She had 4 for upper deciduous teeth and 2 for lower deciduous teeth; there were no dental problems. Laboratory tests revealed hypophosphatemia (2.9 mg/dL; normal range, 4.3-7.2 mg/dL), normal calcium level (10.4 mg/dL; normal range, 9.5-10.6 mg/dL), and increased alkaline phosphatase level (1,468 IU/L; normal range, 144-475 IU/L). The intact parathyroid hormone level was 20.6 pg/mL (normal range, 12-75 pg/mL), 25-hydroxyvitamin D3 level was 42.9 ng/mL (normal range 30.1-100 ng/mL), and 1,25 dihydroxyvitamin D3 level was 87.19 pg/mL (normal range, 19.6-54.3 pg/mL). To estimate the renal capacity of phosphate reabsorption, TRP was measured in random urine samples and was 99.8% (normal range, over 90%). Radiological findings showed widening, fraying, and cupping of the proximal tibial metaphysis and distal radial and ulnar metaphysis (Fig. 3). Hypophosphatemia with elevated alkaline phosphatase activity and normal serum calcium level suggested presence of HR like her mother, which was confirmed by direct sequencing analysis of the *PHEX*. We found that she carried the same novel mutation in the *PHEX*, a heterozygote of the c.667dup (p.Asp223GlyfsTer15) variation, as seen in her mother (Fig. 4).

The daughter started treatment with alfacalcidol 0.5 μg/day (48 ng/kg/day) and elemental phosphorus 47 mg/kg/day in four divided dosages. After 3 months of follow-up, at the age of 15 months, her height was measured to be 79.2 cm (+0.21 SDS), and the weight was 12 kg (+1.59 SDS). The alkaline phosphatase levels still showed increased (1291 IU/L; normal range, 144-475 IU/L) and still showed hypophosphatemia (2.9 mg/dL; normal range, 4.3-7.2 mg/dL). She exhibited no complications associated with medication, such as hypercalcemia, hyperparathyroidism, hypercalciuria, and renal glycosuria. Kidney ultrasonography showed normal echogenicity, size, and contour except both mild hydronephrosis.

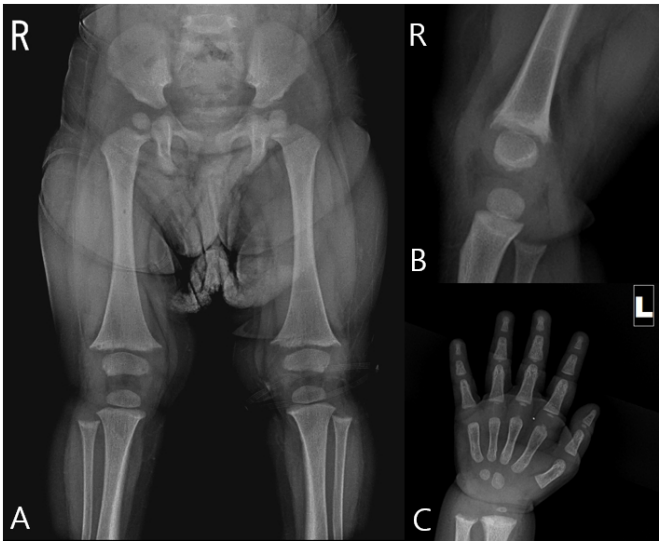

Fig. 3. A radiographic of the daughter at the age of 12 months. (A) Diffuse bone contour change with fraying, cupping, and widening of proximal tibial metaphysis. (B) Distal femoral metaphysis. (C) Distal radial and ulnar metaphysis.

Discussion

The present case identified a mutation in the *PHEX* gene, c.667dup (p.Asp223GlyfsTer15), which was inherited from a mother exhibiting short stature and deformities in lower limbs to her daughter, who looked healthy but presented rachitic change upon radiologic and biochemical findings. To the best of our knowledge, this variant is novel and has not yet been reported. To date, only 418 disease-causing mutations associated with XLH have been reported in the Human Gene Mutation Database (*PHEX*, access date: Aug 2021). In Korea, 20 *PHEX* mutations

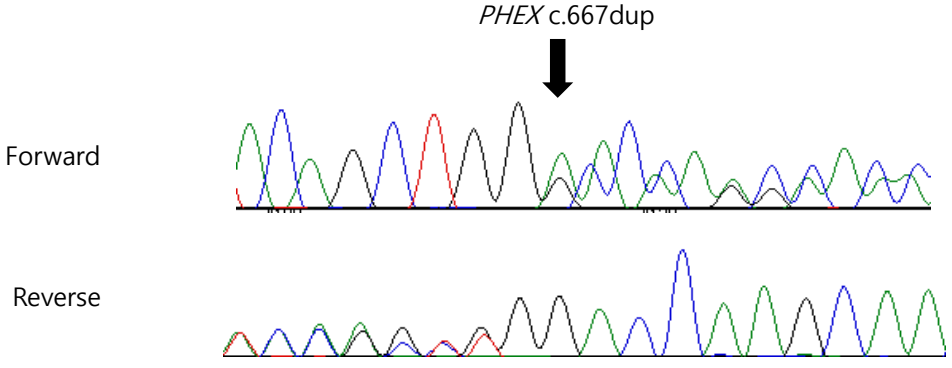

Fig. 4. Sanger sequencing electropherogram of the daughter. Heterozygous duplication variant c.667dup (arrow) was found in *PHEX* gene.

have been reported to cause XLH [3,5-8], and the most common mutation type is splice-site mutation (35%).

Several studies have reported genotype-phenotype correlations in XLH, but there is no definite correlation between disease severities and the type or location of the mutations [2,3,9]. However, one study reported that skeletal disease was more severe in the group with a mutation in the C-terminal half of the protein [10]. Another study reported a trend between truncating mutations and more severe skeletal disease in patients with history of FHR [2].

Meanwhile, skeletal disease and dental problems tend to present a milder phenotype in later generations, which may be attributed to the earlier onset of treatment owing to early mutation analysis and more effective treatment with the 1, 25-dihydroxy vitamin D in later generations [2]. Although persistent hypophosphatemia without treatment causes bone deformity and growth retardation within a year after birth, early medical treatment with oral phosphate supplementation and active vitamin D analogs (alfacalcidol or calcitriol), especially before the onset of clinical manifestation, improves height outcome and decreases disease activity [9]. Normal growth can be achieved in *PHEX*-deficient patients when treated with combined calcitriol and oral phosphate despite persistent hypophosphatemia. Those who start treatment early (before 1 year old) can maintain normal growth (−0.7 SDS, −1.5 SDS to +0.3 SDS), whereas height SDS lost at treatment onset in the late group (after 1 years old) could only marginally be regained (−2.0 SDS, −2.3 SDS to −1.0 SDS) [10]. Despite adequate vitamin D and oral phosphate administration, some patients did not achieve complete growth catch-up or had to undergo surgical treatment because of no improvement in bone deformity, suggesting that medical treatment does not completely normalize bone metabolism in XLH [9,11,12]. It is also necessary to monitor serum calcium, phosphate, vitamin D, parathyroid hormone, and urinary calcium/creatinine ratio every 3-6 months, and perform ultrasonography of the kidney every year while administering medications.

On the other hand, in the case of XLH in adults, there is no clear consensus regarding indications for treatment, but empirically, it is believed that in some cases, as in children, supplementation of calcitriol and phosphate would be beneficial [13]. In this case, the female did not suffered from spontaneous fracture, bone pain and there was no biochemical evidence of osteomalacia, so only cholecalciferol was taken.

Recently, a phase 3/4 open label trial using a human monoclonal antibody against FGF23 (burosumab) has been reported. In this study, fifteen children aged 1 to 12 years with XLH were included from 4 Japanese medical centers and received burosumab (starting with 0.8 mg/kg; maximum 2 mg/kg) via subcutaneous injection every 2 weeks for average 121.7 weeks. Burosumab had a favorable safety profile, increased serum phosphorus, decreased serum alkaline phosphatase, and improved radiographic changes in rickets [14].

In conclusion, we identified a novel variant, c.667dup (p.Asp223GlyfsTer15), in the *PHEX* gene of a Korean family using multigene panels associated with skeletal dysplasia. The mutation was identified in a mother exhibiting clinical symptoms of HR. In addition, we identified the same mutation in her daughter who was asymptomatic but had radiological rachitic changes. Early diagnosis helped initiate early medical treatment for the daughter.

Acknowledgements

This work was supported by the Dong-A University research fund in 2021.

Authors' Contributions

Conception and design: Sungsoo Kim. Acquisition of data: Sejin Kim. Analysis and interpretation of data: NK. Drafting the article: Sejin Kim. Critical revision of the article: NK. Final approval of the version to be published: all authors.

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