A Novel *PHEX* Gene Mutation in a Patient with Sporadic Hypophosphatemic Rickets

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Phosphate regulating gene with homologies to endopeptidases on the X-chromosome (PHEX) is a common cause of X-linked hypophosphatemic (XLH) rickets. Diverse *PHEX* gene mutations have been reported; however, gene mutations in sporadic rickets are less common than in XLH rickets. Herein, we describe a 50-year-old female patient with sporadic hypophosphatemic rickets harboring a novel splicing-site mutation in the *PHEX* gene (c.663+1G>A) at the exon 5-intron 5 boundary. The patient had recently suffered from right thigh pain and an aggravated waddling gait. She also presented with very short stature, generalized bone pain, and muscle weakness. Despite low serum phosphate levels, her phosphate reabsorption rate was lower than normal. Additionally, her 1,25-dihydroxyvitamin D_3 concentration was lower than normal, although FGF23 level was normal. After treatment with alfacalcidol and elemental phosphate, her rachitic symptoms subsided, and callus formation was observed in the fracture site on the right femur.

Keywords: Phosphate regulating gene with homologies to endopeptidases on the X-chromosome; Rickets, hypophosphatemic; Fibroblast growth factor 23

INTRODUCTION

Hypophosphatemic rickets consists of inherited and acquired forms, which share common pathophysiology and clinical features [1]. In hereditary cases, X-linked hypophosphatemic rickets (XLH) with inactivating mutations of the phosphate regulating gene with homologies to endopeptidases (*PHEX*) gene is the most common [2]. Autosomal dominant hypophosphatemic rickets and autosomal recessive hypophosphatemic rickets have also been reported [3,4]. Acquired hypophosphatemic rickets includes tumor-induced osteomalacia (TIO) [5] and sporadic hypophosphatemic rickets [6].

PHEX is located on the X chromosome at Xp22.1 [7]. PHEX has been observed in cartilage, bone, and teeth osteoblasts [8] and is thought to act on substrates that suppress phosphate excretion in the proximal renal tubule [9]. Recently, several mutational analyses of the *PHEX* gene in XLH rickets and sporadic hypophosphatemic rickets reported deletion, insertion, missense, nonsense, and splicing site mutations [10]. Here, we present the first report of a new splicing-site *PHEX* gene mutation in a Korean patient with sporadic hypophosphatemic rickets.

CASE REPORT

A 50-year-old woman visited the hospital for evaluation of right thigh pain. She had recently suffered from generalized bone pain and generalized weakness, which were aggravated after a fall 6 months prior. Additionally, her waddling gait had worsened. She was born at full term, and her body weight and height were within normal ranges at birth. She had short stature and difficulty in walking when she was a teenager but was not referred to the Department of Pediatrics for evaluation or treatment because she experienced no pain or weakness at that time. She had very short stature (129 cm, just below the 10th percentile for her age) and bowing leg deformities. She was born from healthy parents of normal heights. Her daughter and son have normal stature (her daughter is 160 cm and her son is 180 cm), and the rest of her family is healthy. DNA samples of the family were not available.

She presented with hypophosphatemia 2.0 mg/dL (normal range, 2.5 to 4.7). Her measured serum calcium level was 9.0 mg/dL (normal range, 8.7 to 10.6), alkaline phosphatase was elevated (139 IU/L; normal range, 42 to 98), parathyroid hormone was 45.68 pg/mL (normal range, 10.00 to 65.00), 25-hydroxyvitamin D_3 was 11.10 ng/mL (normal range, 10.00 to 30.00), and 1,25-dihydroxyvitamin D_3 was 21.6 pg/mL (normal range, 25.1 to 66.1). Nephrologic evaluation demonstrated normal diuresis (1,000 mL/24 hours) and normal renal function (estimated glomerular filtration rate of 95 mL/min/1.73 m^2). The tubular reabsorption rate of phosphate was 81% (normal range, 80 to 100). The maximum tubular capacity of phosphate per unit volume of glomerular filtrate was decreased to 0.51 mM (normal range, 0.88 to 1.42 for her age group), which revealed insufficient reabsorption of phosphorus in the kidneys. We also evaluated her serum fibroblast growth factor 23 (FGF23) level using sandwich enzyme-linked immunosorbent assay (ELISA) and found it was 109 RU/mL (normal range, <189 for her age group). The interindividual coefficient of variation (CV) for this ELISA assay is 3.8% to 6.4%, and the intraindividual CV is 2.5% to 6.1%.

Her plain radiograph presented diffuse bowing deformities in the upper and lower extremities and osteoporotic changes in the extremities, spine, and pelvis. There was a right subtrochanteric fracture that included the cortex of one long bone. If she had preferred surgery, it might have been possible. However, the symptoms were not severe, and she could walk by herself without orthoses. Furthermore, multiple pseudofracture lines were also observed in the right proximal femur, left

iliac bone, and proximal shaft of the both fibulas with anterolateral bowing (Fig. 1). Dual energy X-ray absorptiometry measurement showed that the mean bone mineral density in the lumbar spine (L1 to L4) was 0.845 g/cm^2, and that in the femoral neck was 0.661 g/cm^2, resulting in T-scores of –2.7 and –2.3, respectively. After obtaining informed consent from the patient, genomic DNA was extracted from peripheral blood leukocytes using QuickGene Blood kit (Fujifilm, Tokyo, Japan). For *PHEX* gene analyses, all 22 exons and exon-intron junctions of the *PHEX* gene were amplified using polymerase chain reaction (PCR) with the appropriate primers (Table 1). Subsequently sequence analyses were performed with BigDye terminator version 3.1 (Applied Biosystems, Foster City, CA, USA) and the same primers used for PCR amplification. We found a novel splicing mutation in the exon 5-intron 5 boundary of *PHEX*, a heterozygote of the c.663+1G>A variation, which had not been previously reported (Fig. 2).

In chromosomal analyses of 20 cells in metaphase which were performed using 72-hour culture and G banding stain of peripheral blood, mosaicism for trisomy X, and tetrasomy X were unusually expressed. We investigated serum tumor markers and whole body bone scintigraphy using Tc-99m hydroxy methylene diphosphonate (Fig. 3) to identify neoplastic lesions and found no evidence of TIO.

The patient was treated using alfacalcidol and elemental phosphate (Joulie's solution; 10 mL of 4.9 mmol phosphate in 1 mL water) four times a day. After 22 days of outpatient care, her measured serum phosphate level increased to 3.6 mg/dL (normal range, 2.5 to 4.7), alkaline phosphatase decreased to 124 IU/L (normal range, 42 to 98), and her generalized bone pain was decreased to allow daily walking. In addition, follow up X-ray findings of her right femur showed improved fracture union (Fig. 4).

DISCUSSION

XLH is the main cause of hypophosphatemic rickets. The gene that causes XLH was identified on Xp22.1, which was found to be *PHEX* [7]. Although a physiologically relevant PHEX function has not been established, several recent studies indicate that PHEX inactivates a phosphaturic hormone, such as FGF23, or interacts with membrane proteins that modulate phosphorus activity [5]. FGF23 is a representative phosphatonin that stimulates the renal excretion of phosphate in its full-length state by inhibiting sodium-phosphate transport. Physiologically, FGF23 is inactivated by cleavage at the potential
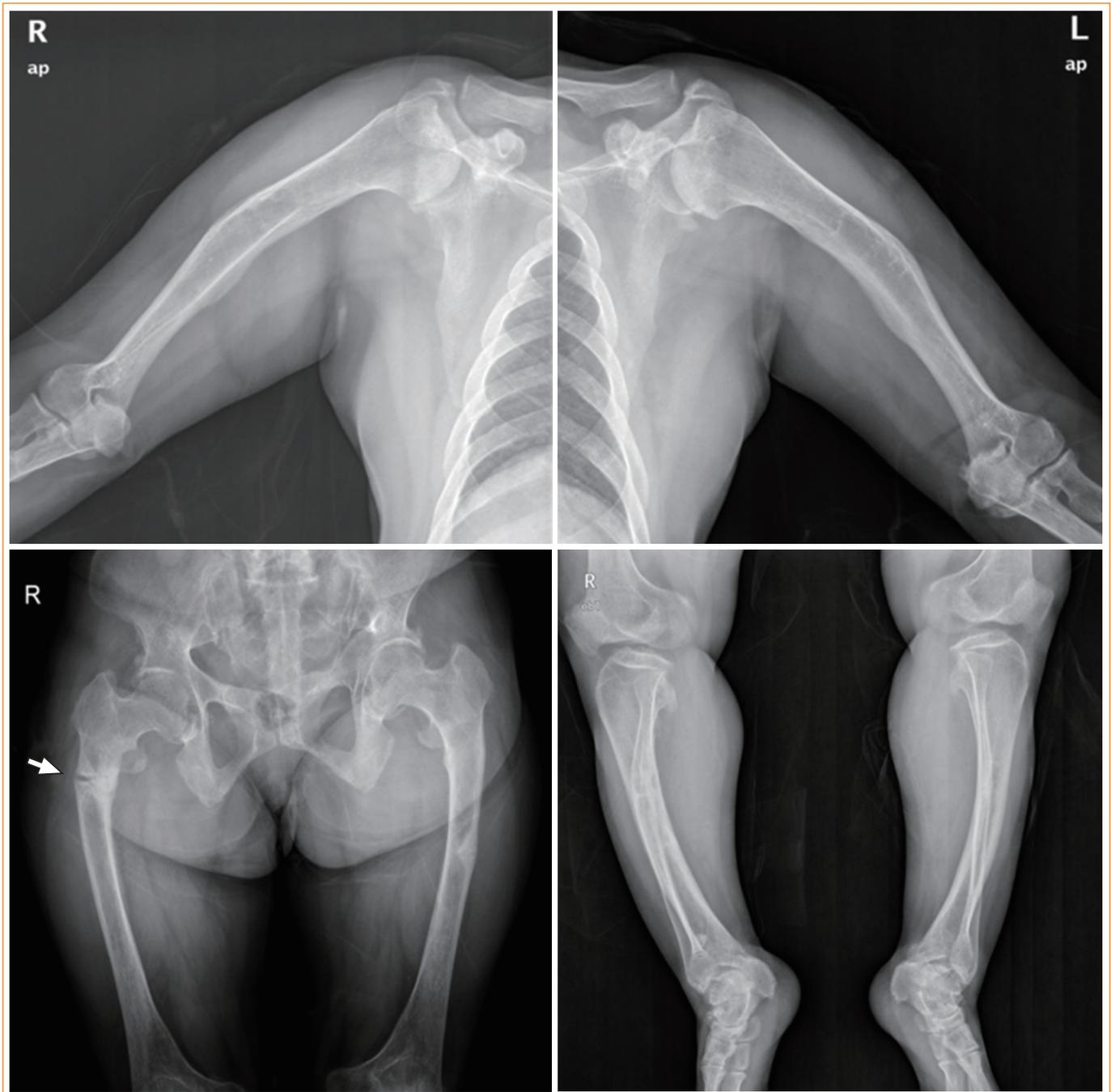
Fig. 1. The plain films of the patient's long bones. Both humeri, femurs, tibias, and fibulas show anterolateral bowing. The arrow indicates the fracture line on the right metaphysis of the femur.

recognition/cleavage sites for the enzymes of the proprotein convertase family (RXXR motif) [11]. Elevated circulating levels of FGF23 were observed in autosomal dominant hypophosphatemic rickets of RXXR motif mutation and also in XLH and TIO [3]. In XLH, an elevated FGF23 level is frequently observed in association with hypophosphatemia.
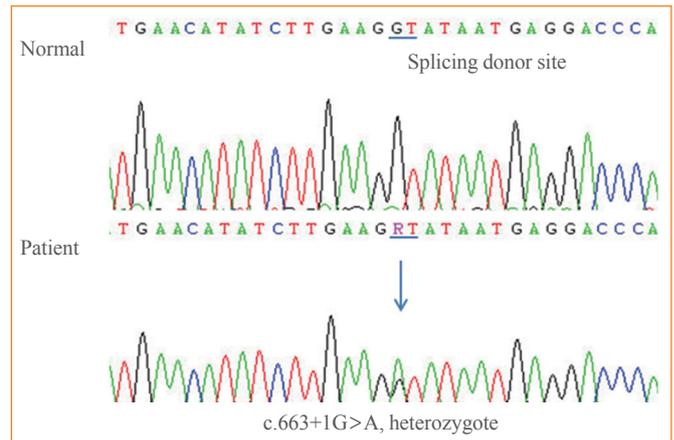
Therefore, some authors have suggested that PHEX controls the inactivation of FGF23, and *PHEX* gene mutations, such as loss of function mutations, increase the circulating level of FGF23 [12]. In *in vitro* experiments, a furin-like enzyme cleaved FGF23, not directly mediated by PHEX using the *Hyp* mouse that is homologous to XLH. However, PHEX inactiva-

Table 1. Sequences of Primers for Polymerase Chain Reaction

	Sense (5'-to-3')	Antisense (5'-to-3')
Exon1	ttagtagaagagcaagaaagcc	ctgtgttatgacaagttttgct
Exon2	tcaaatatcttgcgtatgtttc	tcaaaattgcatttcgtactt
Exon3	ttgttgtttaatttggaaaaga	gcatggtagttaaaaagtgaga
Exon4	ggaggttggaattgtgattat	gaattcttagtagttctcttcttcct
Exon5	gcacttaaattctagtgtgctg	tatttcactaaggcagcatga
Exon6	atggtgacatgagctcaaaata	acactgctcatttccaataaa
Exon7	ctacctgctattttctgctctt	tcattcaggatatactgccttt
Exon8	aatactatgcagatgttttggc	caagactgagagcaggttttac
Exon9	tgaaatcaaaagaaatgaatga	agttttcaaaggatgtgagaag
Exon10	ctgtttctctcaagatgttctg	ccctgtctaatccctaaagatg
Exon11	agactgttttcttcaggttgtt	acaatacccacaggccactac
Exon12	taaagagtcatttctcatgcaa	tatgtattcatccaaaagcaga
Exon13	ttcagtaaatggagttttcagat	atcaccagttttaattgctagg
Exon14	ccttcctatgctgaagtatttt	tactctgacttgaggggaatta
Exon15	cattgttccatgttatctgcta	aatgtcttaccctccatcatag
Exon16	ggtctttgaaacctcagtgtta	ttctttctgctgaataacctttt
Exon17	ctagaaagcagtttatcttggc	atcttcaagctatgatgctgc
Exon18	ctgttatgactgctttttgaag	acagatgctgttgatcttgtaa
Exon19	ttgctataatattgatgcctctt	ttccataatatctaactaactaactcc
Exon20	cgtggcaggagtatatatttg	tttgtttgaaatctttctttga
Exon21	ggttctaaaattgttcctcagt	tttttcagatcacctatctggt
Exon22–23	agcaatattctgctacagtttt	gtctccaggcctaaagcaat

Primers were designed with primer 3 web version 4.0 served from Whitehead Institute (http://primer3.wi.mit.edu/) using sequences from GenBank accession number of NT_167197.1.
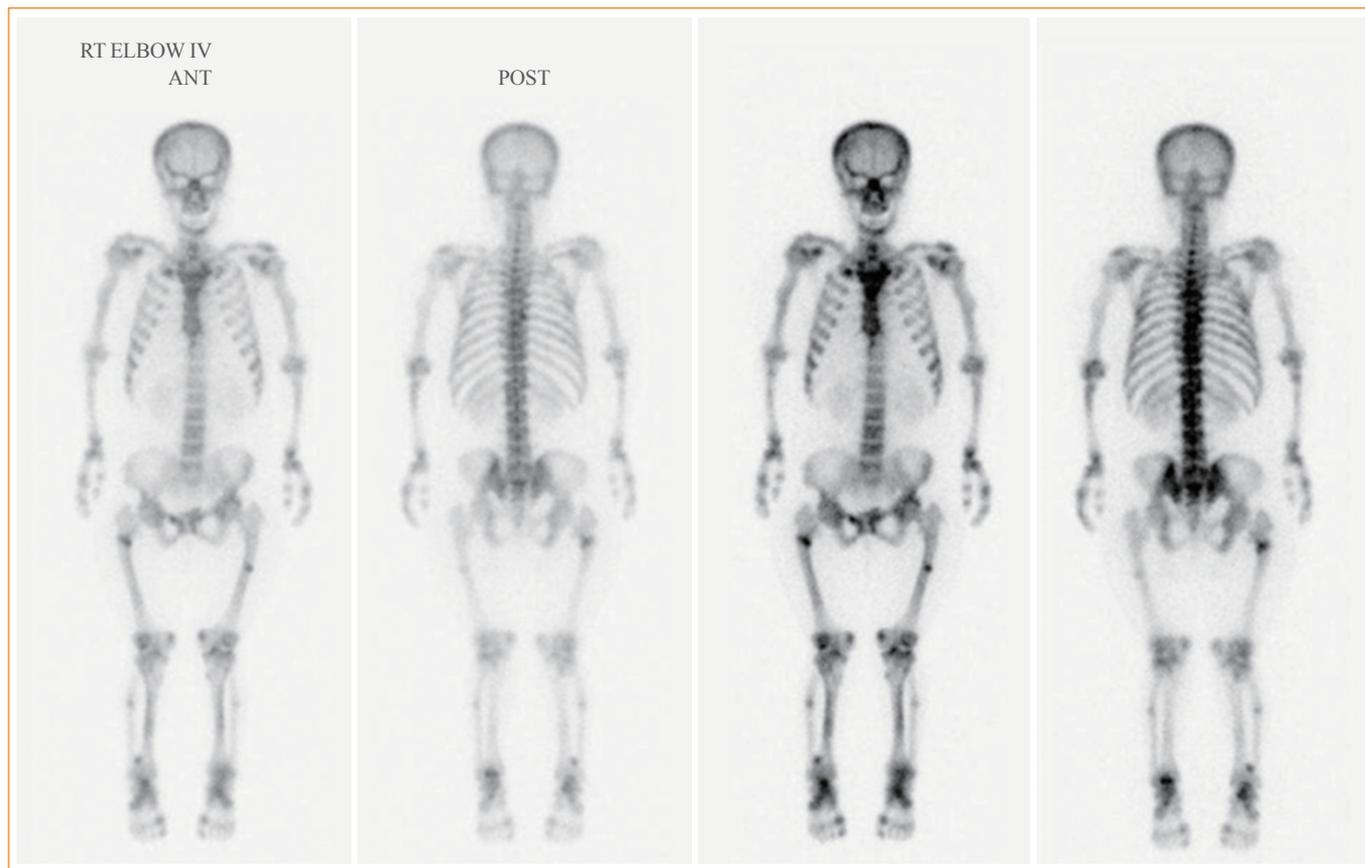

Fig. 2. Partial genomic DNA sequence of the *PHEX* gene. A heterozygote mutation within the splicing donor site, c.663+1G>A was found in the boundary of exon 5 and intron 5 on the *PHEX* gene.

matrix extracellular phosphorglycoprotein (MEPE), and fibroblast growth factor 7 (FGF7). Especially, MEPE transcripts are increased in *Hyp* mice, which have poorly mineralizing bone [15]. An *in vitro* study reported that cathepsin B-dependent cleavage is suppressed by PHEX; therefore, MEPE and FGF have been reported as important phosphatonins affecting phosphate homeostasis and skeletal mineralization [16]. In our case, there is a possibility that another phosphatonin may have led to the resulting rickets phenotype. However, our study is limited in that we did not measure the levels of the other factors like MEPE.

Several studies have identified genotype-phenotype correlations of the *PHEX* gene in hypophosphatemic rickets. In genotype-phenotype analyses of Korean patients with hypophosphatemic rickets, there was no genotype-phenotype correlation, even though skeletal disease is more severe in C-terminal mutations compared to N-terminal mutations [17]. Remarkably, a novel *PHEX* mutation was discovered in the C terminus of exon 5 here, but the rachitic symptoms were less severe compared to other hypophosphatemic rickets diagnosed in childhood or adolescence. The patient did not suffer from bone pain or gait disturbance until middle age.

In previous cases, 124 sporadic mutations of *PHEX* gene have been reported (online PHEX mutation data base; http://www.phexdb.mcgill.ca), and 22 of them are splicing mutations. Table 2 summarizes four splicing mutations in some of the previous cases [6,10,18,19]. As shown in the Table 2, mutation site does not correlate with disease severity. In one case, even though serum FGF23 was elevated to 2,430 pg/mL, the

tion resulted in a tendency to increase FGF23 level in a mouse model. Although FGF23 is not a direct substrate of PHEX, the *PHEX* gene might be associated with FGF23 expression [13]. In our case, normal level of FGF23 and a lower than normal tubular reabsorption rate of phosphate might contribute to the late onset of rachitic symptoms, including generalized bone pain and muscle weakness, despite *PHEX* gene mutation. In the renal phosphate wasting state, an elevated FGF23 level is frequently found. Although the relationship of elevated FGF23 with *PHEX* mutation was not elucidated, most hypophosphatemic rickets present with elevated FGF23. However, Jonsson et al. [12] reported elevated FGF23 levels in 13 of 21 patients. Therefore, not all patients with hypophosphatemic rickets have elevated FGF23. In normal adults and children, the FGF23 levels were definitely normal [12]. Shaikh et al. [14] suggested other circulating factors controlling phosphorous metabolism such as secreted frizzled-related protein (sFRP-4),

Fig. 3. Whole body bone scan of the patient. A hot spot was found in the right femur fracture site and also in multiple pseudofractures in both lower extremities.
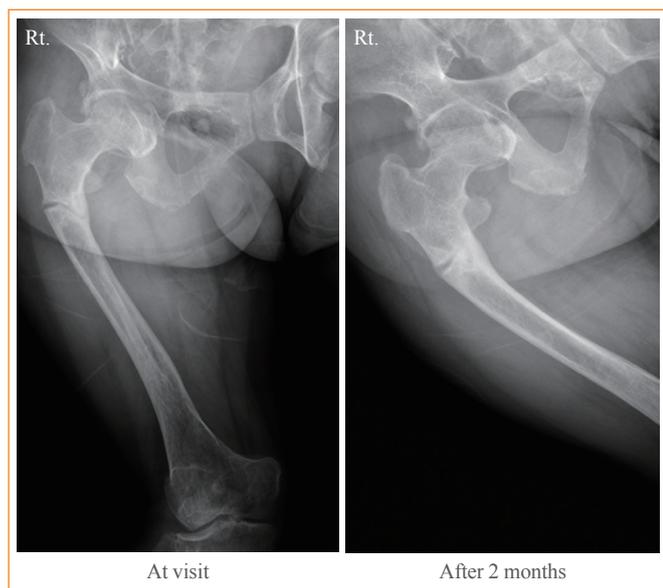
Fig. 4. After 2 months of alfacalcidol and elemental phosphate treatment, callus formation was observed in the fracture site on the right femur metaphysis.

patient had normal height and no bone fractures, and the maximum tubular reabsorptive rate of phosphate decreased to 0.41 mmol/L. In addition, the patient showed a mild phenotype unrelated to FGF23 level, as in our case [10]. Based on these results, we hypothesized that C-terminal splicing mutations of *PHEX* could represent mild clinical phenotypes.

The case we report is also a sporadic gene mutation case that is expressed by a novel splicing mutation (c.663+1G>A) in the exon 5 and intron 5 junction. The radiologic and laboratory findings of the patient were reviewed to exclude TIO, and this is the first sporadic case reported with a splicing site mutation in exon 5 of *PHEX* and a normal FGF23 level. Although morphologic changes including short stature, leg bowing, and waddling gait were found at an early age, the rachitic symptoms were initiated by a traumatic event (falling down) in middle age. After treatment with alfacalcidol and elemental phosphate, the rachitic symptoms subsided.

In conclusion, we have identified a novel splicing site mutation in the *PHEX* gene in a Korean patient with sporadic hypo-

Table 2. Summary of Splicing Mutations of *PHEX* in Patients with Sporadic Hypophosphatemic Rickets

Case no.	DNA	Codon change	Clinical phenotype	Biochemical phenotype	Reference
1	c.118+1g>a	Splice donor	Rickets, height deficiency: –3.76 SD (16 years), –4.55 SD (18 years)	Hypophosphatemia	[18]
2	c.118+1G>A	Loss of entire protein	Leg deformities, height deficiency –3.76 SD	Hypophosphatemia	[19]
3	c.2148-1G>T	Splice acceptor	Male, 23 years, normal height 175 cm (-0.6 SD), skeletal and dental phenotype, 3 HPT surgical removal of 3.5 parathyroid glands, impaired renal function. No fractures, DXA: spine Z-score 1.7 SD	Serum FGF23 2,430 pg/mL, Hypophosphatemia, TmP/ GFR 0.41 mmol/L	[10]
4	c.1483-2A>G	Splice acceptor	Female, 11 years, inability to walk, bowing of legs	Hypophosphatemia	[6]

SD, standard deviation; HPT, height for piston-type imbibition in throat; DXA, dual-energy X-ray absorptionetry; FGF, fibroblast growth factor; TmP/GFR, the maximum tubular reabsorptive rate of phosphate.

phosphatemic rickets. Our results indicate that the inactivating *PHEX* mutation contributes to hypophosphatemia, but is not related to FGF23. Showing a novel mutation in exon 5, these data will provide an opportunity for further analyses of the genotype-phenotype association.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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