

Case report

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A novel *de novo* mutation within *PHEX* gene in a young girl with hypophosphatemic rickets and review of literature

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X-linked hypophosphatemia (XLH) is the most common form of familial hypophosphatemic rickets and it is caused by loss-of-function mutations in the *PHEX* gene. Recently, a wide variety of *PHEX* gene defects in XLH have been revealed; these include missense mutations, nonsense mutations, splice site mutations, insertions, and deletions. Recently, we encountered a 2-year-9-month-old female with sporadic hypophosphatemic rickets. She underwent osteotomy, dental abscess was evident, and there was severe bowing of the legs. A low serum phosphorus level in combination with elevated serum alkaline phosphatase activity and normal serum calcium is suggestive of hypophosphatemic rickets. *PHEX* gene analysis revealed a splice acceptor site mutation, c.934-1G > T (IVS8⁻¹G > T), at the intron8 and exon9 junction. To the best of our knowledge, this mutation is novel and has not been reported. The results of this study expand and improve our understanding of the clinical and molecular characteristics and the global pool of patients with sporadic hypophosphatemic rickets.

Keywords: Hunam PHEX protein, Mutation, Hypophosphatemic rickets

Introduction

X-linked hypophosphatemia (XLH) is the most common form of familial hypophosphatemic rickets (FHR) and it is caused by loss-of-function mutations in the *PHEX* gene (phosphate regulating gene with homologies to endopeptidases on the X chromosome) with a prevalence of 1 in 20,000^{1,2}. It is inherited as a dominant disorder and characterized biochemically by hypophosphatemia due to renal phosphate wasting with inappropriately low or normal 1,25-dihydroxyvitamin D concentrations¹. Autosomal dominant hypophosphatemic rickets is known to be associated with mutations in *FGF23* and autosomal recessive hypophosphatemic rickets has been reported by different groups³. Individuals with the features of FHR, but with no family history of rickets (sporadic cases) are also common, and many subsequently transmit the phenotype in an X-linked dominant manner consistent with hypophosphatemia^{3,4}. Despite the fact that *PHEX* mutations cause renal phosphate wasting, *PHEX* is not expressed in the kidney. Instead, *PHEX* is predominantly expressed in bones (osteoblasts and osteocytes) and teeth (odontoblasts)². Clinical manifestations of hypophosphatemic rickets include short stature, bone pain, dental anomalies, lower extremity deformities, and radiographic evidence of rickets in children⁵.

Laboratory findings of the disease include low serum phosphorus levels, normal serum calcium levels, increased activity of serum alkaline phosphatases, normal or increased parathyroid hormone (PTH) levels, and inappropriately normal or decreased levels of 1,25-dihydroxyvitamin D₃^{5,6}. The *PHEX* gene, located on Xp22.1, consists of 22 exons that translate into a 749 amino acid protein, which is a member of the peptidase M13 family of

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membrane-bound metalloproteases^{7,8}). The *PHEX* gene consists of a cytoplasmic region, a transmembrane, and an extracellular domain⁶). Since the identification of the *PHEX* gene, over 351 mutations have been described in patients with XLH; those mutations include nonsense mutations, missense mutations, splicing site mutations, insertions, and deletions in both coding and noncoding regions of the gene^{7,9}). All published mutations have been catalogued in the *PHEX* mutation database (<http://data.mch.mcgill.ca/phexdb>). Only 15 mutations of the *PHEX* gene in Korean patients with hypophosphatemic rickets have been reported so far^{3,6,10}). Here, we report a sporadic case of hypophosphatemic rickets for which genetic analysis has revealed a novel mutation in *PHEX*, and we present a review of the literature.

Case report

The proband was a 2-year-9-month-old Korean female who was referred to our pediatric endocrinology clinic for bilateral bowleg. She had been born at term to a 30-year-old woman whose pregnancy was uneventful. The proband's birth weight was 3,850 g and her body length was 49 cm. She was born of a nonconsanguineous marriage at full term. On admission, her height was 85.3 cm (3th–5th percentile) and her body weight was 13.5 kg (50th–75th percentile). Physical examination

revealed genua vara and café au lait spots on the abdomen. Dental defects, including formation of abscesses and recurrent extractions, were shown in the patient. Her vision and hearing were normal. Laboratory analyses of serum revealed the following values (normal range): inorganic phosphate, 2.5 mg/dL (3.8–6.5 mg/dL); calcium, 9.4 mg/dL (8.8–10.8 mg/dL); intact-PTH, 46.53 pg/mL (9–65 pg/mL); alkaline phosphatase, 1,634 IU/L (129–291 IU/L); 25-hydroxyvitamin D3, 27.30 ng/mL (9.7–52.8 ng/mL); and 1,25-dihydroxyvitamin D3, 38.0 pg/mL (19.6–54.3 pg/mL). Radiologic findings showed widening of the proximal tibial metaphysis with medial bowing (Fig. 1). A low serum phosphorus level in combination with elevated serum alkaline phosphatase activity and normal serum calcium is suggestive of hypophosphatemic rickets and molecular analysis was done. Informed consent was obtained and genomic DNA was extracted from the peripheral blood leukocytes using a QuickGene Blood kit (Fujifilm, Tokyo, Japan). *PHEX* gene analysis revealed a splicing mutation, c.934-1G > T (IVS8⁻¹G > T), which was a novel mutation (Fig. 2). However, the proband's mother had no *PHEX* gene mutation. All members of the proband's family were healthy.

She was treated with calcitriol 0.5 µg/day and elemental phosphorus 2 g/day in four divided dosages. The alkaline phosphatase levels were nearly normalized (492 IU/L) within two years. At the 22-month follow-up, she was 4 years and

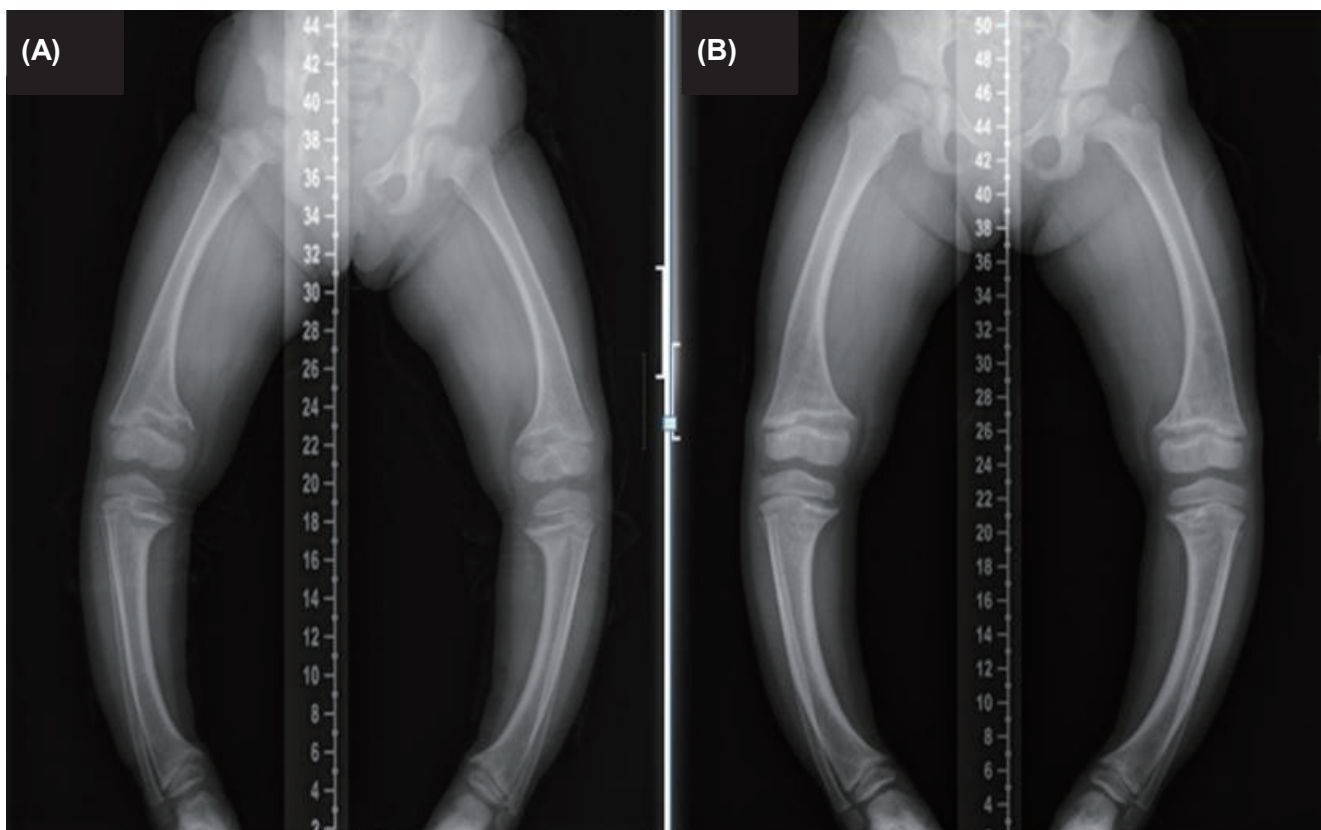


Fig. 1. (A) Initial radiological exam shows severe symmetric shortening and deformation of both legs. (B) At the 22-month follow-up, radiologic finding still shows widening of the proximal tibial metaphysis with medial bowing.

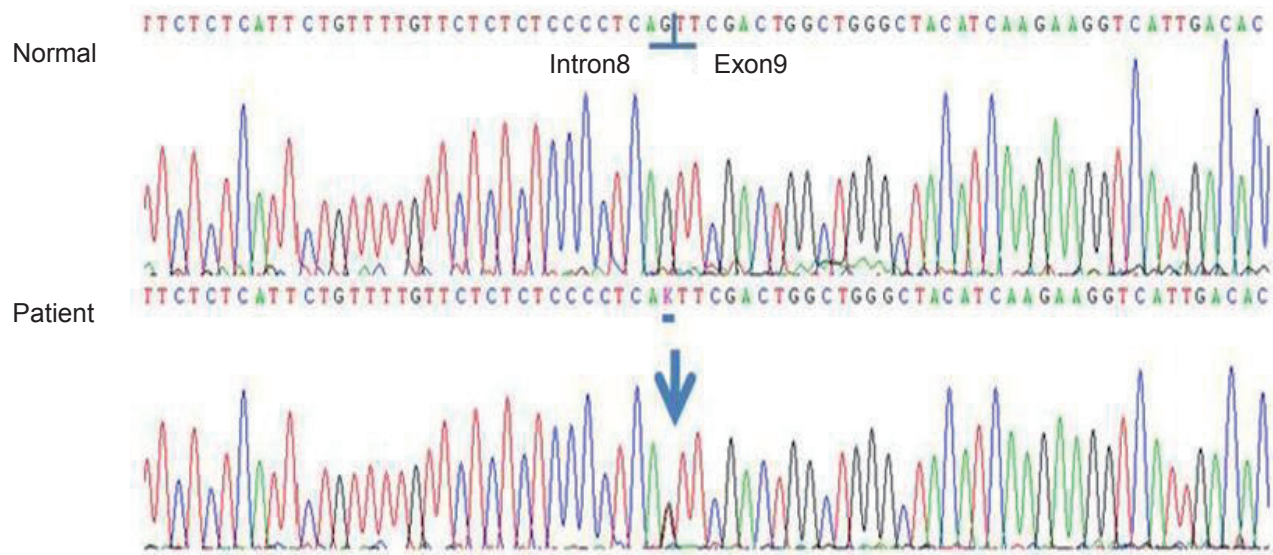


Fig. 2. Partial genomic DNA sequence of the *PHEX* gene of the patient: Novel splice site mutation, c.934-1G > T (IVS8⁻¹G > T), in the *PHEX* gene

7 months old. At that time, her height was 98.6 cm (3th–5th percentile) and her body weight was 17.8 kg (50th–75th percentile). Complications associated with medications, such as hypercalciuria, hypercalcemia, nephrocalcinosis, renal glycosuria, and hyperparathyroidism, were not detected. Though she had been treated with medications for two years, the bowing in her legs had not improved. Therefore, the patient recently underwent surgical correction for *genua vara*.

Discussion

In the present study, we report a splice acceptor site mutation in the *PHEX* gene, c.934-1G > T (IVS8⁻¹G > T), at the intron8 and exon9 junction. To the best of our knowledge, this mutation is novel and has not been reported. The described mutation was located in the extracellular domain in the *PHEX* gene, predicting that it would cause exon9 skipping. In our patient, this mutation at the exon-intron border caused subsequently severe clinical symptoms, including severe bowing of the legs, short stature, and dental defects, such as formation of abscesses and recurrent extraction. This suggests that the exon-intron border is significant in the formation of functional *PHEX* proteins and the mutation in this region probably contributes to the severe phenotypes. Thus, although a functional study is necessary, this mutation likely impairs production of the *PHEX* protein. The functional consequences of novel splice variants were predicted with the Berkeley Drosophila Genome Project (<http://www.fruitfly.org/>). Eighty-four *de novo* mutations reported in the *PHEX* gene in patients with sporadic hypophosphatemic rickets are summarized in Table 1. Among the 84 patients reported with sporadic hypophosphatemic rickets, including our case, splice site mutation was frequently observed (17/84, 20%). Among the 13 patients reported with

sporadic hypophosphatemic rickets in Korea, including our case, splice site mutation was most frequently observed (4/13, 30.8%). Nonsense mutations (3/13, 23.1%) and missense mutations (3/13, 23.1%) were found with the same frequencies. A large number of mutations occur randomly across mainly the extracellular domain, which is encoded by exons2–22, but a mutation in the 3'-untranslated region has also recently been identified². Moreover, the frequency of *de novo* mutations has been estimated to be approximately 20%¹¹⁻¹³. These findings suggest that the *PHEX* gene appears to be particularly prone to mutations for unknown reasons¹³. In the present study, the patient was female and the *de novo* mutation was found to be a sporadic case. The female dominance in sporadic cases is also observed in other ethnic backgrounds, including Asians, Europeans, and Americans⁷. Given that most sporadic patients are female, it is likely that mutated *PHEX* alleles resulted from mutagenesis in the X chromosome of the paternal germ cells, and *PHEX* mutagenesis in paternal germ cells is a frequent event in sporadic patients⁷. Therefore, further study is needed to prove this inference at the protein level with a biopsy to obtain cells for culture.

The phenotype was assessed as severity of skeletal disease (mild vs. moderate-to-severe) and severity of dental disease (mild vs. moderate-to-severe) using the following objective criteria: history of osteotomy, degree of bowing, and history of dental abscesses⁴. In our patient, the truncating mutation predicts a severe clinical occurrence. The girl underwent osteotomy, dental abscess was evident, and there was severe bowing of the legs, thus meeting all the major criteria set forth by Holm et al.⁴ to be classified as a severe skeletal phenotype. No significant correlation was found between the severity of the skeletal or dental disease and the mutation type and the location of the mutation^{4,10,14}. However, in one study, skeletal disease was more severe in the group with a mutation in the C-terminal

Table 1. Summary of *de novo* *PHEX* mutations in patients with sporadic hypophosphatemic rickets

Case	Sex	Nucleotide change	Protein change	Ethnic origin	Reference
1	Female	IVS8 ⁻¹ G > T		Korean	This case
2	Female	c.1586_1586+1delAG	p.E529GfsX41	Korean	[3]
3	Male	c.1715G > A	p.G572D	Korean	[10]
4	Female	c.58C > T	p.R20X	Korean	[10]
5	Female	c.2239C > T	p.R747X	Korean	[10]
6	Female	c.1952-1963del12	p.R651-R655del4	Korean	[10]
7	Female	c.2171-2172delTT	p.F724X	Korean	[10]
8	Female	c.64G > T	p.A22S	Korean	[10]
9	Female	c.160C > T	p.P534L	Korean	[14]
10	Female	c.466_467insAC	p.L156HfsX76	Korean	[14]
11	Female	IVS10 ⁺¹ G > A		Korean	[14]
12	Female	IVS10 ⁺¹ G > A		Korean	[14]
13	Female	IVS17 ⁺⁵ G > A		Korean	[14]
14	Female	c.830T > A	p.L277X	American	[4]
15	Female	IVS13 ⁺¹ G > C		American	[4]
16	Female	IVS13 ⁺¹ G > C		American	[4]
17	Female	c.496C > T	p.R166C	American	[4]
18	Female	c.1133T > C	p.L378P	American	[4]
19	Female	c.1601C > T	p.P534L	American	[4]
20	Female	c.1736G > T	p.G579V	American	[4]
21	Female	c.2090delGA	p.R697TfsX19	Taiwanese	[15]
22	Female	c.670C > T	p.Q224X	Taiwanese	[15]
23	Female	c.1217G > A	p.C406Y	Turkish	[7]
24	Female	c.2078G > T	p.C693F	Turkish	[7]
25	Female	IVS15 ⁺¹ G > A		Turkish	[7]
26	Female	IVS13-2A > G		Turkish	[7]
27	Female	c.1217G > A	p.C406Y	Turkish	[7]
28	Female	c.784G > C	A262P	Spanish	[16]
29	Female	c.1105A > T	R369X	Spanish	[16]
30	Female	c.1529G > C	R510P	Spanish	[16]
31	Female	c.1639C > T	Q547X	Spanish	[16]
32	Female	c.1645C > T	R549X	Spanish	[16]
33	Female	c.1645C > T	R549X	Spanish	[16]
34	Female	c.1543C > T	Q515X	Spanish	[16]
35	Female	c.1735 G > C	G579R	Spanish	[16]
36	Female	c.18251830delAAAAG	E609X	Spanish	[16]
37	Female	c.1952G > C	R651P	Spanish	[16]
38	Female	c.2104C > T	R702X	Spanish	[16]
39	Female	c.2239C > T	R747X	Spanish	[16]
40	Female	c.897_898delAA	K299N	Spanish	[16]
41	Female	c.188_349del		Spanish	[16]
42	Female	c.395_398dup	p.Q133HISfsX14	Spanish	[16]
43	Female	c.1405_1645del		Spanish	[16]
44	Female	c.1412_2250del	p.I495WfsX20	Spanish	[16]
45	Male	c.1646_2250del		Spanish	[16]
46	Female	c.1701_2070del		Spanish	[16]
47	Male	c.1768+174_1768+177dupTAAG		Spanish	[16]
48	Female	c.1936_1938delGAT	p.D646del	Spanish	[16]
49	Female	c.2138_2139dupCT	p.Q714LfsX27	Spanish	[16]
50	Female	c.2168dupA	p.N723KfsX3	Spanish	[16]
51	Female	IVS04 ⁺³ G > C		Spanish	[16]
52	Female	IVS10 ⁺¹ G > A		Spanish	[16]
53	Female	IVS13 ⁺⁴ delA		Spanish	[16]

Table 1. Summary of *de novo* *PHEX* mutations in patients with sporadic hypophosphatemic rickets (continues)

Case	Sex	Nucleotide change	Protein change	Ethnic origin	Reference
54	Male	IVS15 ⁺¹ G > A		Spanish	[16]
55	Female	IVS19 ⁺¹ G > A		Spanish	[16]
56	Female	c.1601.C > T p.P534L		Spanish	[16]
57	Male	c.565C > T	Q189X	Spanish	[16]
58	Female	c.1601C > T p.P534L		German	[17]
59	Female	c.1735G > A p.G579R		German	[17]
60	Female	c.1735G > A	p.G579R	German	[17]
61	Male	c.2239C > T	p.R747X	German	[17]
62	Female	c.1559delG	p.W520CfsX1	German	[17]
63	Female	c.1991insTGACTGAC	p.R664SfsX2	German	[17]
64	Male	Exon 9 del		German	[17]
65	Female	c.2137–2141.del/ins	p.713NfsX1	Swiss	[17]
66	Female	c.242T > C	p.F81S	European	[14]
67	Female	c.413T > C	p.L138P	European	[18]
68	Female	c.710A > G	p.D237G	European	[18]
69	Male	c.832G > T	p.E278X	European	[18]
70	Female	IVS9 ⁺² T > G		European	[18]
71	Female	c.1351G > T	p.E451X	European	[18]
72	Female	c.1363G > T	p.E455X	European	[18]
73	Female	c.1728C > A	p.A573D	European	[18]
74	Female	c.1736G > A	p.G579R	European	[18]
75	Male	c.2064T > A	p.Y688X	European	[18]
76	Female	c.2198G > C	p.C733S	European	[18]
77	Female	c.2238C > G	p.C746W	European	[18]
78	Male	c.2239C > T	p.R747X	European	[18]
79	Female	c.1504insGACT	p.Y502fsX	European	[18]
80	Female	c.1986insTGAC	p.D663fsX1	European	[18]
81	Male	c.2162insCCCT	p.S722PfsX	European	[18]
82	Male	IVS14 ⁺³ delGAGT		European	[18]
83	Female	IVS19 ⁺¹ G > A		European	[18]
84	Male	IVS21 ⁻² A > C		European	[18]

half of the protein¹⁴). Among patients with a family history of hypophosphatemic rickets, there was a trend toward more severe skeletal disease in patients with truncating mutations⁴). Hearing defect was correlated with mutations in the beginning fragment of the gene, and dental disease and increased head length was correlated with the mutations in the beginning and the terminal fragment of the gene¹⁰).

Without treatment, clinical manifestations begin in the first year of life, and affected children suffer progressive growth failure and bony deformities, such as tibial bowing and frontal bossing¹⁴). However, treatment with vitamin D and phosphate resulted in only a partial growth improvement in most cases, and was frequently complicated by hypercalciuria, hypercalcemia, nephrocalcinosis, or hyperparathyroidism¹⁰). Despite the fact that the affected individual was treated with vitamin D and phosphate, rickets and growth catch-up were not completely improved in the patient, and finally surgical correction had to be done at 55 months of age. This investigation suggest that early initiated vitamin D and phosphate treatment are not sufficient to correct hypophosphatemic rickets caused by severe loss-of-function mutations, and that an effective therapeutic approach

might be required in such circumstances based on new concepts of the molecular mechanism of the disorder. In conclusion, we report a novel *de novo* splicing mutation in the *PHEX* gene. The results of this study expand and improve our understanding of the clinical and molecular characteristics and the global pool of patients with sporadic hypophosphatemic rickets.

Conflict of interest

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

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