CASE REPORT

Familial hypophosphatemic rickets caused by a large deletion in *PHEX* gene

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Abstract

Context: X-linked hypophosphatemic rickets/osteomalacia (XLH), autosomal dominant and recessive hypophosphatemic rickets/osteomalacia (ADHR and ARHR) share common clinical features including high fibroblast growth factor 23 (FGF23) levels. These diseases are caused by mutations in phosphate regulating endopeptidase homolog, X-linked (*PHEX*), *FGF23*, and dentin matrix acidic phosphoprotein 1 (*DMP1*) gene respectively. It remains unclear whether these diseases can be clinically discriminated.

Objective: To clarify the underlying mechanism of patients with hypophosphatemic rickets whose parents showed no physical findings suggesting rickets.

Design and patients: The proband is a 39-year-old woman. She and her 37-year-old brother show the same clinical features such as bowing of legs together with hypophosphatemia (sister: P 1.8 mg/dl, brother: P 1.6 mg/dl) and high FGF23 levels (sister: 542 pg/ml, brother: 96 pg/ml). Physical findings of their parents are normal and ARHR was suspected.

Results: Sequencing of all coding exons and exon–intron junctions of *DMP1* and *FGF23* genes showed no mutation. Subsequent analysis revealed that there is a deletion of 52 143 bp including exons 1–3 in *PHEX* gene in the brother. His sister was found to be a heterozygote for the same deletion indicating that they are suffering from XLH. The same deletion was detected in the mother. However, the amount of the wild-type allele was more and that of the mutant one was less in genomic DNA from the mother compared with those from the sister. Single nucleotide polymorphism (SNP) analysis indicated that the mother has three kinds of *PHEX* alleles suggesting a somatic mosaicism.

Conclusion: Careful genetic analysis is mandatory for correct differential diagnosis of hypophosphatemic rickets with high FGF23 levels.

European Journal of Endocrinology 161 647–651

Introduction

Hypophosphatemia is a well-known cause of rickets/osteomalacia. There are several kinds of hypophosphatemic rickets/osteomalacia, which share common clinical features. X-linked hypophosphatemic rickets/osteomalacia (XLH), autosomal dominant and recessive hypophosphatemic rickets/osteomalacia (ADHR and ARHR) are characterized by hypophosphatemia associated with impaired proximal tubular phosphate reabsorption. In addition, hypophosphatemia usually enhances 1,25-dihydroxyvitamin D ($1,25(\text{OH})_2\text{D}$) production and increases serum $1,25(\text{OH})_2\text{D}$ level. However, $1,25(\text{OH})_2\text{D}$ remains to be low to low normal in patients with these diseases (1). Recent investigations indicated that excess actions of fibroblast growth factor 23 (FGF23) underlie the pathogenesis of these hypophosphatemic diseases (2–6). FGF23 suppresses proximal tubular phosphate reabsorption by reducing

the expression of type 2a and 2c sodium–phosphate cotransporters, which mediate physiological phosphate reabsorption (7). FGF23 also decreases serum $1,25(\text{OH})_2\text{D}$ at least in part by suppressing $1,25(\text{OH})_2\text{D}$ production (7). Responsible genes for XLH, ADHR, and ARHR have been shown to be phosphate regulating endopeptidase homolog, X-linked (*PHEX*), *FGF23*, and dentin matrix acidic phosphoprotein 1 (*DMP1*) respectively (4–6, 8).

While patients with XLH, ADHR, and ARHR have similar clinical and biochemical features, these diseases show different modes of inheritance. Therefore, it is anticipated that these diseases can be clinically discriminated, especially in familial cases. Here, we report familial cases of hypophosphatemic rickets/osteomalacia. Our case indicates the difficulty of proper differential diagnosis of hypophosphatemic rickets/osteomalacia by clinical analysis alone.

Subjects and methods

Patient description

The proband is a 39-year-old female. She was born from nonconsanguineous parents who showed no signs of metabolic bone diseases. She visited a hospital because she showed bowing of legs when she was 21 months old. She started to take oral vitamin D₃ and phosphate. She switched to active vitamin D₃ and phosphate when she was about 10 years old. She received 2 g of phosphate per day at most. She received wedge osteotomy of tibia at the age of 14. Hypercalcemia appeared when she was 16 years old and she received parathyroidectomy and transplantation of a part of her parathyroid glands for tertiary hyperparathyroidism when she was 20. Since then, intact PTH levels have been within the reference range with active vitamin D₃ treatment.

Her 37-year-old brother also showed bowing of legs when he was 8 months old and started to take oral vitamin D₃ and phosphate. The maximal dose of phosphate was 2.5 g/day. He later developed hypercalcemia with increased PTH levels when he was 13 years old and he stopped taking vitamin D₃. However, hypercalcemia from tertiary hyperparathyroidism persisted and he started 0.1 µg/kg per day of 1,25(OH)₂D₃ when he was 17 years old with a partial reduction in PTH levels. He finally had parathyroidectomy and transplantation of a part of his parathyroid glands. Since then, he has taken oral active vitamin D₃ and his symptoms have been stable except for mild renal dysfunction (estimated glomerular filtration rate of 55.5 ml/min per 1.73 m² from the formula established by the Japanese Society of Nephrology).

Their mother is 68 years old and physical examination revealed no signs of rickets/osteomalacia. Their father showed no physical findings of rickets/osteomalacia, either, and did not approve to give blood samples for analysis. Biochemical parameters and clinical features of this family are summarized in Table 1. Because parents of these two patients showed no physical signs suggesting rickets/osteomalacia, both patients were presumed to have ARHR caused by mutations in *DMP1* gene.

Sample collection and biochemical measurement

After written informed consent was obtained, blood samples were collected and frozen at -20 °C until analysis. Serum phosphate and creatinine were measured by autoanalyzer. Serum FGF23 was evaluated using FGF23 ELISA kit that detects only full-length, biologically active FGF23 (Kainos, Tokyo, Japan). This study was approved by the institutional review board of the University of Tokyo.

Table 1 Clinical and biochemical features of the family.

	Mother	Sister	Brother	Reference range
Year	68	39	37	
Height (cm)	156.3	147.5	167.0	
Weight (kg)	57.2	54.7	66.5	
Lower limb (cm)	82	73	83	
Arm span (cm)	157	155	175	
Serum Ca (mg/dl)	9.1	9.1	9.9	8.5–10.2
Serum phosphate (mg/dl)	2.4	1.8	1.6	2.5–4.5
Intact PTH (pg/ml)	59	44	54	10–65
TRP (%)	77	73	79	80–90
1,25(OH) ₂ D (pg/ml)	59.1	22.0	28.0	20–60
FGF23 (pg/ml)	61.5	95.7	541.6	10–50
Estimated GFR (ml/min per 1.73 m ²)	91.2	63.0	55.5	

GFR, glomerular filtration rate.

Sequencing *DMP1*, *FGF23*, and *PHEX* genes

Genomic DNA was extracted from peripheral blood leukocytes using a DNA extraction kit (QIAamp DNA Blood Mini Kit, Qiagen). We amplified all the coding exons and exon–intron junctions of *DMP1*, *FGF23*, and *PHEX* genes using PCR; primer sequences can be obtained upon request. PCR products were separated by electrophoresis with 1% agarose gel, purified using Wizard SV Gel and PCR Clean-Up system (Promega), and directly sequenced with the same primers by dye deoxy termination cycle sequencing.

Analysis of 5' region of *PHEX* gene

Initial study suggested the presence of a deletion in 5' region of *PHEX* gene in the male patient (see Results). It is known that spermine synthase (*SMS*) gene is the adjacent one present in the 5' region of *PHEX* (9). Therefore, we prepared several primers corresponding to the intervening sequence between *SMS* and *PHEX*, and analyzed 5' region of *PHEX* gene by PCR.

Semi-quantitative PCR

Semi-quantitative PCR of genomic DNA was conducted to compare the amount of wild-type and mutant *PHEX* alleles. Primer pairs amplifying either the deleted or undeleted regions were used to amplify the mutant or wild-type allele respectively. PCR was done using the same amount of genomic DNA (1 µg/tube) and 25 cycles were chosen not to saturate the amplification.

SNP analysis

We have selected eight SNPs that exist between exons 1 and 3 of *PHEX* gene (rs178711, rs178712, rs2027822, rs2027824, rs2027825, rs178715,

rs2301304, and rs178716) and prepared five sets of primers to cover these SNPs. PCR products were directly sequenced to analyze haplotypes of *PHEX* gene.

Results

Mutational analysis

Direct sequencing of PCR products using genomic DNA from the proband and her brother identified no disease-causing mutation in all coding exons and the exon–intron junctions of *FGF23* or *DMP1* gene. In addition, no missense mutation in *PHEX* gene was found in PCR products from genomic DNA of the proband. However, no PCR products corresponding to exons 1–3 were obtained from genomic DNA of the male patient (Fig. 1a). Because PCR products corresponding to exons 1–3 were obtained using DNA from the proband (Fig. 1a), these results suggested that there is a deletion including exons 1–3 of *PHEX* gene in DNA of the male patient.

Analysis of deleted region of *PHEX* gene

In order to confirm that there is a deletion in *PHEX* gene, we tried to identify the actual deleted region using PCR from DNA of the male patient. The intervening sequence between *SMS* and *PHEX* is about 38 kb, and intron 3 of *PHEX* gene is about 29 kb. We prepared several pairs of primers covering these regions that were expected to amplify PCR products of about 200–400 bps (Fig. 2). PCR using these primer pairs indicated that the starting point of the deletion exists between DNA regions corresponding to the reverse primer for PCR product of 5c and the forward primer of 5d. Similarly, the deletion ends between DNA regions corresponding to the reverse primer for PCR product of 3c and the forward primer for PCR product of 3d. Genomic PCR from the brother using the forward primer for PCR product of 5c and the reverse primer for PCR product of 3d produced a PCR product of about 2600 bp (Fig. 1b). Direct sequencing of the PCR product showed that there is a 52 143 bp deletion including exon 1–3 in this

patient. Because there are identical nucleotide sequence of seven bases in the 5' and 3' deleted regions (Fig. 2), it was impossible to accurately specify the starting and the ending nucleotide of the deletion.

The same deletion was observed in DNA from the proband (Fig. 1b). These results indicated that the male patient is hemizygous, while the female patient is heterozygous for the same deletion of *PHEX* gene and these patients are suffering from XLH.

Semi-quantitative PCR

However, it is considered to be quite unlikely that the same mutations develop *de novo* in two patients of this family. Therefore, we examined whether the mother of these patients has the same deletion of *PHEX* gene. While she showed no physical findings suggesting XLH, the same deletion was also observed in DNA obtained from the mother (Fig. 1b). Furthermore, semi-quantitative PCR suggested that the amount of the mutant allele in the mother is less than those of the patients (Fig. 3a). In addition, comparison of *PHEX* gene in the mother and her daughter indicated that the amount of wild-type allele corresponding to the deleted region (exons 2 and 3) in the daughter is less than that of the mother. In contrast, there is no difference in the amount of wild-type allele not affected by the mutation (exon 7) in these subjects (Fig. 3b). These results indicated that the ratio of the wild-type and the mutant allele is different between the mother and her children, even between the mother and the daughter. Because the daughter is considered to be a heterozygote, these results suggested that the mother is not a heterozygote for the mutation.

SNP analysis

The above results suggested that the mother has more wild-type allele than the proband who is a heterozygote for the mutation. In order to evaluate this possibility, we examined SNPs existing in the deleted region of *PHEX* gene. It was found that there are two alleles in DNA from the mother in one of the eight SNPs (rs178712 T and C). These results indicate that there are two wild-type alleles and one deleted mutant allele in genomic DNA from the mother.

Discussion

XLH is the most common cause of vitamin D-resistant hypophosphatemic rickets/osteomalacia. Since the identification of *PHEX*, more than 100 kinds of mutations in *PEHX* gene have been reported (10). Various mutations including missense mutations, insertions, and deletions have been reported in *PHEX* gene (11). Recent investigations have indicated that mutant *PHEX* in bone underlie the pathogenesis of XLH

Figure 1 Analysis of *PHEX* gene by genomic PCR. (a) While PCR products corresponding to exons 1–3 were obtained using genomic DNA from the proband (P), exons 1–3 of *PHEX* gene were not amplified from the brother (B) suggesting that these exons are deleted. (b) Primers corresponding to the 5' and 3' regions of the deleted region produced the PCR product of about 2600 bp from DNA of the proband, her brother and their mother (Mo). In this experiment, PCR was conducted for 45 cycles.

Figure 2 Determination of the deleted region of *PHEX* gene. We prepared 16 pairs of primers covering the 5' region of exon 1 and intron 3 of *PHEX* gene, which were expected to produce PCR products of about 200–400 bps (5a–h, 3a–h). Although 5c and 3d were amplified by PCR using genomic DNA from the male patient, 5d and 3c were not amplified. In addition, PCR using the forward primer for PCR product of 5c and the reverse primer for PCR product of 3d produced a PCR product of about 2600 bp (Fig. 1b). Direct sequencing of this 2600 bp PCR product showed that there is a 52 143 bp deletion including exons 1–3. It was impossible to accurately specify the starting and the ending nucleotides of the deletion because there are identical nucleotide sequences of seven bases in the 5' and 3' ends of the deleted region (underlined). SMS, spermine synthase.

probably through enhancing the production of FGF23 (12, 13). We have identified a 52 143 bp deletion including exons 1–3 of *PHEX* gene. While a deletion containing exons 1–3 has been already reported in one family (14), the precise length and location of this deletion were not described. It is unclear whether the present deletion is the same as the previously reported one or not.

While the proband and her brother showed bowing of legs and had been treated with vitamin D metabolites under the diagnosis of hypophosphatemic rickets, the mother did not show any physical features of rickets. Her height is normal and her arm span is almost equal to her height. Her legs are straight without any treatment. Therefore, ARHR was suspected in this family. However, genetic analysis indicated that the mother also has the same deletion in the *PHEX* gene as that of her children. This indicates that the deletion is present in germ cells of the mother. Further analysis indicated that the mother is heterozygous for one SNP located in the deleted region of *PHEX* gene showing

that she has one mutant allele and two kinds of wild-type alleles of *PHEX* gene. A possible explanation of this would be mosaicism developed in the early stage of the zygote. The lack of family history of rickets in the ancestors of the mother supports this possibility. Already, mosaicism of mutations in *PHEX* gene has been reported in several papers (15, 16). In one report, the mosaicism was described to cause a hypophosphatemic disease mimicking an autosomal dominant inheritance (15). Otherwise, she may be suffering from triple X syndrome. Because she declined further chromosomal analysis, we could not discriminate these two possibilities.

It is of interest that the mother with a deletion in *PHEX* gene has no physical signs of XLH. XLH is an X-linked dominant disease with almost complete penetrance (17). This means that loss of one allele of wild-type *PHEX* gene causes XLH phenotype. As shown in the table, the mother was mildly hypophosphatemic with slightly elevated FGF23. These results could be explained by a small number of mutant cells in bone, which resulted in marginal increase in FGF23 if she is mosaic. Because semi-quantitative PCR indicated that the amount of the mutant allele in the mother is less than that of the heterozygous proband, the percentage of the mutant cells would be < 50%. If she is suffering from triple X syndrome, one-third of the cells are expected to express the mutant allele because of lyonization. In any case, these results suggest that there is a threshold in the amount of mutant *PHEX* allele to produce XLH phenotype.

While XLH is by far more frequent than ADHR or ARHR and patients with hypophosphatemic rickets with high FGF23 are most likely to be suffering from XLH (18), mutations in *PHEX* gene have not been

Figure 3 Semi-quantitative PCR for comparison of the amount of wild-type and mutant alleles. Genomic PCR was conducted for 25 cycles. In this condition, the amount of the same mutant PCR product as that shown in Fig. 1b was less in the mother compared with that in the hemizygous (B) or heterozygous (P) patient. (a) While there is no difference in the amount of wild-type allele in the nondeleted region (exon 7), PCR indicated that the amount of the wild-type allele in the deleted region (exons 2 and 3) in the proband was less than that of the mother (b).

detected in all clinically diagnosed patients with XLH (14, 19–22). Usual direct sequencing of PCR products for all exons in *PHEX* gene failed to detect any mutation in the mother and the proband in this family; therefore, it would have been impossible to identify the deletion in this family if there were no male patients.

In conclusion, we have reported a family with hypophosphatemic rickets. While ARHR was clinically suspected, genetic analysis indicated there is a deletion in *PHEX* gene. Careful genetic analysis is mandatory for correct differential diagnosis of hypophosphatemic rickets with high FGF23 levels.

Declaration of interest

There is no conflict of interest that could be perceived as prejudicing the impartiality of the scientific work reported.

Funding

This study was supported in part by grants from the Ministry of Education, Culture, Sports, Science and Technology, and from the Ministry of Health, Labour and Welfare, Japan.

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Received 15 May 2009

Accepted 6 July 2009