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ORIGINAL ARTICLE

A novel nonsense mutation in the *DMP1* gene in a Japanese family with autosomal recessive hypophosphatemic rickets

Ryusuke Koshida · Hideki Yamaguchi · Koji Yamasaki · Wakaba Tsuchimochi · Tadato Yonekawa · Masamitsu Nakazato

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Abstract Autosomal recessive hypophosphatemic rickets (ARHR) is an extremely rare disorder of autosomal recessive inheritance, characterized by hypophosphatemia resulting from renal phosphate wasting. Dentin matrix protein 1 (DMP1), a noncollagenous extracellular protein, plays critical roles in bone mineralization and phosphate homeostasis. Recently, loss-of-function mutations in *DMP1* gene have been identified as the molecular cause of ARHR. Here, we describe a Japanese family that includes two ARHR-affected siblings carrying a novel mutation of the *DMP1* gene. The patients were a 53-year-old woman and a 50-year-old man with short stature and skeletal deformities who were the offspring of a first-cousin marriage. Biochemical examination revealed hypophosphatemia with renal phosphate excretion and low levels of $1,25(OH)_2D$. Serum calcium, parathyroid hormone, and urinary calcium excretion were within the normal range, leading to clinical diagnosis of ARHR. Sequence analysis of peripheral leukocytes from the patients revealed that they carried a novel homozygous nonsense mutation in the *DMP1* gene (98G>A, W33X), which leads to a truncated DMP protein with no putative biological function. Unaffected family members were heterozygous for the mutation. This is the first report of a Japanese family with ARHR carrying a novel mutation of the *DMP1* gene.

R. Koshida · H. Yamaguchi · K. Yamasaki · W. Tsuchimochi · T. Yonekawa · M. Nakazato (✉)
Division of Neurology, Respirology, Endocrinology and Metabolism, Department of Internal Medicine, Miyazaki Medical College, University of Miyazaki, 5200 Kihara, Kiyotake, Miyazaki 889-1692, Japan
e-mail: nakazato@med.miyazaki-u.ac.jp

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Introduction

Hypophosphatemic rickets is a disorder associated with impaired bone mineralization caused by phosphate deficiency, resulting in skeletal deformity and growth retardation in childhood. Inherited hypophosphatemic rickets is caused by genetic defects that lead to renal phosphate wasting. To date, four types of hypophosphatemic rickets have been identified: X-linked hypophosphatemia (XLH), autosomal dominant hypophosphatemic rickets (ADHR), hereditary hypophosphatemic rickets with hypercalciuria (HHRH), and autosomal recessive hypophosphatemic rickets (ARHR) [1]. Fibroblast growth factor 23 (FGF23), a phosphaturic hormone, has been found to be elevated in XLH [2], ADHR [3, 4], and ARHR [5–7] and is involved in hypophosphatemia in these disorders.

ARHR (OMIM 241520) is an extremely rare disorder of autosomal recessive inheritance, with only a few families of Middle Eastern and European origins reported. ARHR patients exhibit growth retardation, lower-extremity deformities, and dental defects. Later in life, some patients also develop enthesopathy. Patients also show hypophosphatemia as a consequence of renal phosphate wasting and low levels of serum 1,25-dihydroxyvitamin D [$1,25(OH)_2D$], whereas serum calcium, parathyroid hormone (PTH), and urinary calcium excretion are normal. Notably, circulating levels of FGF23 are elevated in patients with ARHR [5–7]. ARHR shares clinical and biochemical features with XLH and ADHR, but recent genetic studies have shown that mutations in the *PHEX* [8], *FGF23* [9], and *DMP1* [5, 6] genes underlie XLH, ADHR, and ARHR,

respectively, enabling us to distinguish ARHR from other disorders.

Dentin matrix protein 1 (DMP1) is a noncollagenous extracellular matrix protein that is a member of the short integrin-binding ligand-interacting glycoprotein (SIBLING) family [10]. DMP1 is highly expressed in bone and teeth [11] and promotes mineralization [12] and odontogenic differentiation [13]. It has recently been reported that mutations in the *DMP1* gene, which is important for phosphate homeostasis, cause ARHR [5, 6].

Here, we examined the *DMP1* gene in a Japanese family with ARHR and identified a novel nonsense mutation. This is the first report describing a Japanese family with ARHR carrying a novel mutation of the *DMP1* gene.

Patients and methods

Patients

Patient 1 (II-3, Fig. 1) was a 53-year-old woman who had genu varum and growth retardation in childhood. She received no medications, including phosphorus or vitamin D supplementation. At the age of 44 years, she was referred to a hospital because of cervical myelopathy caused by ossification of the posterior longitudinal ligament (OPLL) and was diagnosed with hypophosphatemic rickets. She was 130 cm tall, and had hypophosphatemia (2.2 mg/dl; normal range, 2.7–4.5) with renal phosphate excretion [tubular maximum rate for phosphate reabsorption per glomerular filtrate (TmP/GFR), 1.9 mg/dl; normal range, 2.3–4.5] and low $1,25(OH)_2D$ level (16 pg/ml; normal range, 20–60) (Table 1). Serum calcium, intact PTH, and urinary calcium excretion were within the normal range. She underwent laminoplasty for OPLL and was treated with alfacalcidol for hypophosphatemia. At the age of 53 years, she was admitted to our hospital for hypercalcemia (15.6 mg/dl) and renal insufficiency (serum creatinine, 3.34 mg/dl). She had mild bowing of her legs and

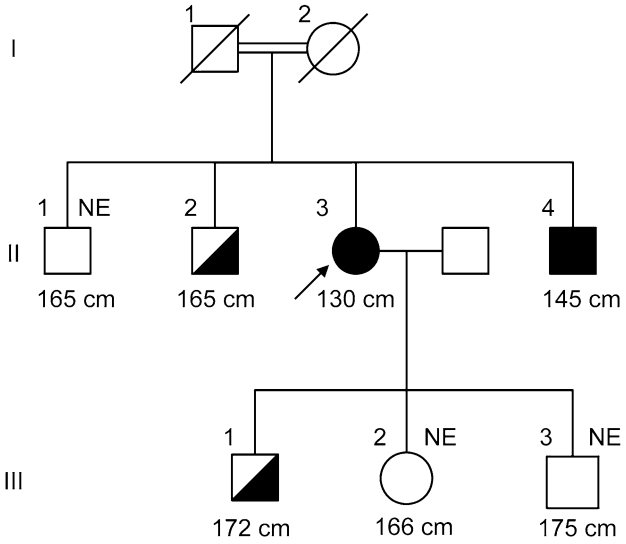

Fig. 1 Pedigree of the Japanese family with autosomal recessive hypophosphatemic rickets (ARHR). *Black symbols* indicate affected individuals. An *arrow* indicates the proband (patient 1). *Half symbols* indicate heterozygous individuals. *Double line* shows a consanguineous marriage. Final heights are shown below the individual symbols. *NE*, not examined for mutations

Table 1 Laboratory data

Individual	Patient 1 II-3	Patient 2 II-4	Unaffected sibling II-2	Unaffected child III-1	Reference ranges
Age (years)	53	50	57	18	
Sex (M/F)	F	M	M	M	
Height (cm)	130	145	165	172	
Serum P (mg/dl)	2.2	2.3	3.6	3.2	2.7–4.5
Serum Ca (mg/dl)	8.9	9.8	9.2	9.8	8.5–9.8
Intact PTH (pg/ml)	25	17	22		10–65
$1,25(OH)_2D$ (pg/ml)	16[a]	22	26		20–60
$25(OH)_2D$ (ng/ml)	9[a]	11			10–55
TmP/GFR (mg/dl)	1.9[a]	2.2			2.3–4.3
Urine Ca/Cr (mg/mg)	0.07[a]	0.04			0.06–0.18
Serum Cr (mg/dl)	1.15	0.40			M: 0.47–0.79
					F: 0.61–1.04
BUN (mg/dl)	24.0	14.1			8.3–22.2
CCr (ml/min/1.73 m^2)	24.4	159.1			64.6–165.3
Intact FGF23 (pg/ml)	4540.1	31.8[b]	15.7		10.0–50.0

P, phosphorus; Ca, calcium; PTH, parathyroid hormone; $1,25(OH)_2D$, 1,25-dihydroxyvitamin D; $25(OH)_2D$, 25-hydroxyvitamin D; TRP, tubular reabsorption of phosphate; TmP/GFR, maximal tubular phosphate reabsorption to glomerular filtration rate; Ca/Cr, calcium/creatinine ratio; BUN, blood urea nitrogen; CCr, creatinine clearance

[a] At age 44 years of patient 1 on admission

[b] Blood samples were collected separately from other biochemical data

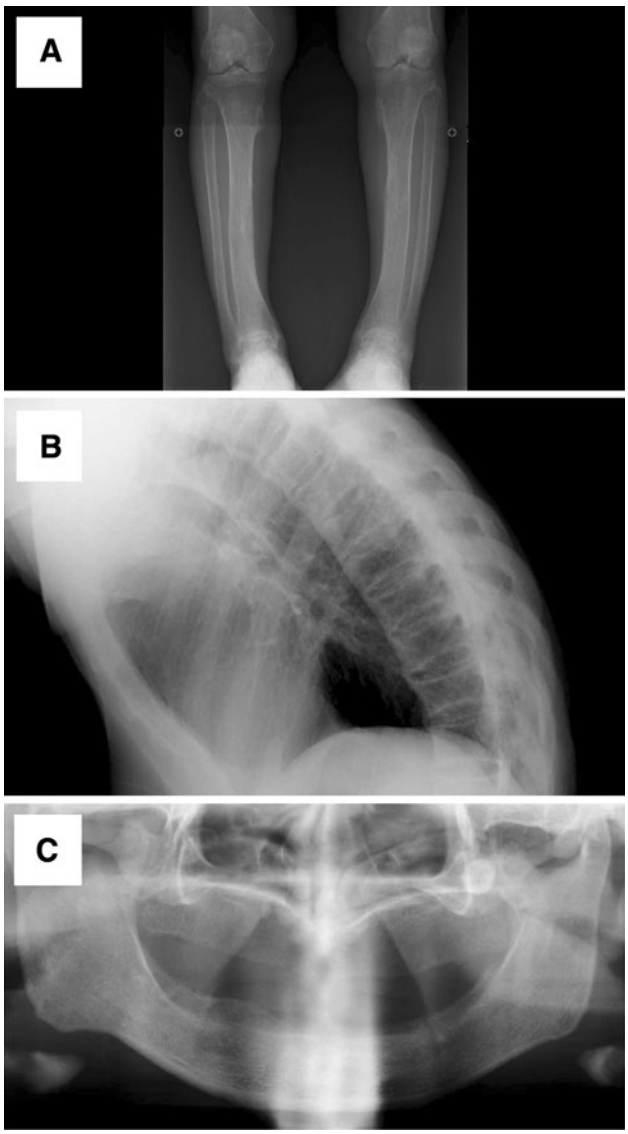

Fig. 2 Skeletal and dental findings in patient 1 at the age of 53 years. **a**, **b** Radiographs show mild bowing of her legs and severe kyphosis. **c** A panoramic radiograph shows loss of all her teeth

severe kyphosis and had lost almost all her teeth (Fig. 2a–c). The high level of serum calcium returned to the normal range, but renal dysfunction was still present (creatinine clearance, 24.4 ml/min) (see Table 1). The serum level of intact FGF23 in patient 1 was extremely high (4540.7 pg/ml; reference range, 10.0–50.0), while the serum creatinine level had declined to 1.15 mg/dl (Table 1).

Patient 2 (II-4), a 50-year-old man, was the brother of patient 1. He had genu varum and growth retardation in childhood but received no medications. At the age of 40 years, he was referred to a hospital because of cervical myelopathy caused by OPLL and was diagnosed with hypophosphatemic rickets. He was 145 cm tall, and had hypophosphatemia (2.3 mg/dl) with renal phosphate excretion (TmP/GFR, 2.2 mg/dl) and normal level of $1,25(OH)_2D$ (22 pg/ml). Serum calcium, intact PTH levels, and urinary calcium excretion were within the normal range (see Table 1). He underwent laminoplasty for OPLL and was treated with alfacalcidol for hypophosphatemia. At the age of 50 years, his serum intact FGF23 level was within the normal range (31.8 pg/ml).

The parents of patients I-1 and I-2 were first cousins. The siblings of the patients did not show any clinical evidence of rickets or osteomalacia. Laboratory data of an unaffected sibling (II-2) and a child (III-1) confirmed normophosphatemia (see Table 1). Based on the clinical features, autosomal recessive inheritance, and the absence of hypercalciuria, the patients were diagnosed with ARHR.

Urinary calcium and phosphorus levels were measured after the patients included approximately 500 mg/day calcium and 1000 mg/day phosphorus in their diets for 1 week. Tubular maximum rate for phosphate reabsorption per glomerular filtrate (TmP/GFR) was calculated using a nomogram [14]. Serum intact FGF23 levels were measured as described previously [15].

Mutation analysis

Genomic DNA was extracted from blood samples using the QIAamp DNA Mini Kit (QIAGEN, Tokyo, Japan), according to the manufacturer's protocol. The five coding *DMP1* exons 2–6 were amplified by polymerase chain reaction (PCR) with intronic primers designed with Primer3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi), as described previously [16] (Table 2). PCR products were electrophoresed on an agarose gel, purified from the gel using the UltraClean 15 DNA Purification Kit (MO BIO Laboratories, Solana Beach, CA, USA), and directly sequenced by a BigDye Terminator v3.1 Cycle Sequencing Kit and the ABI PRISM 3100 Genetic Analyzer (Applied Biosystems Japan, Tokyo, Japan). The study was approved by the Ethical Committee of Miyazaki Medical College, and written informed consent was obtained from all subjects before participation.

Results

We performed sequence analysis of *DMP1*, the causative gene of ARHR. We found that patients 1 and 2 carried a novel homozygous mutation, an A-to-G substitution at nucleotide 98 in exon 3 (98G>A; Fig. 3a). This substitution introduces a premature termination codon (PTC) at codon 33 that replaces the wild-type tryptophan residue

(W33X; Fig. 3a). An unaffected sibling (II-2) and the child of patient 1 (III-1) were heterozygous for the mutation.

Discussion

DMP1 was originally identified as an extracellular matrix protein [17] and belongs to the SIBLING family, which includes sialoprotein (BSP), osteopontin (OPN), matrix extracellular phosphoglycoprotein (MEPE), and dentin sialophosphoprotein (DSPP) [10]. DMP1 contains a large number of acidic domains that play an important role in mineralization by nucleating the formation of hydroxyapatite crystals [12]. In addition, DMP1 plays a regulatory role in odontogenic differentiation [13]. Recent studies showed that *DMP1* null mice exhibit hypophosphatemic rickets, indicating that DMP1 is important for phosphate homeostasis in vivo [5]. In humans, loss-of-

Table 2 Polymerase chain reaction (PCR) and sequencing primers for *DMP1* gene

Exon	Forward primer	Reverse primer	Product size (bp)
2	CATTCCAATGTGGCCTTTTT	AGCCATCTTGGTTCATAGCC	419
3	TTTAACAAGGCCTCCCAGTG	GTCATGCATCCCCTGGTACT	429
4	GTTGTATGAACGGCCAGGTT	CTCCTGAAAGGGGAAAAATG	500
5	ACCAACACCACCCTTGGTAA	GCCAGCAATCTAGGCTCAGT	463
6-1	TTGGCAGCATTGAGTGAAGT	TTTCCTACTGGGATGCTCCA	844
6-2	TGCAGAGTGATGACCCAGAG	GCTGAGCTGCTGTGAGACTG	676
6-3	GGCCAACCTGTCATCTCAAG	GTCCAGCAATTCCTTTCAGG	653

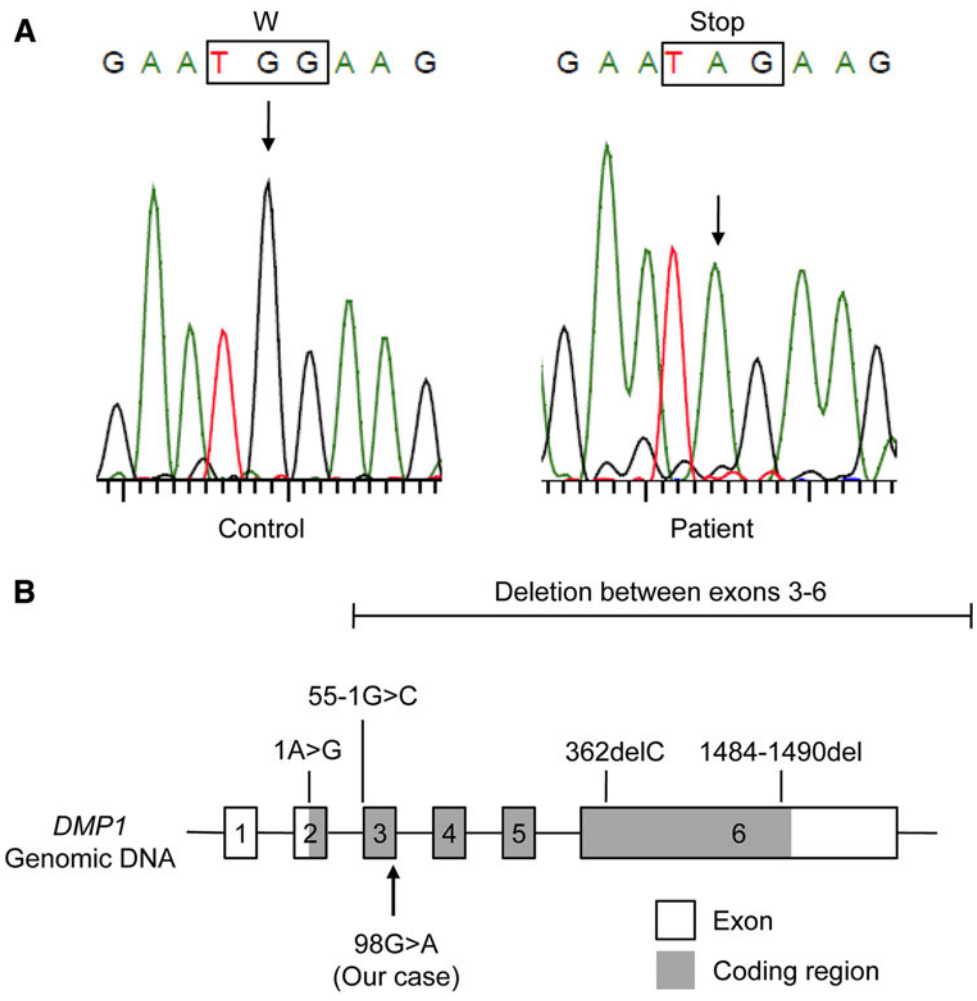

Fig. 3 Sequence analysis of the *DMP1* gene. **a** DNA sequencing reveals a homozygous A-to-G substitution at nucleotide 98 (98G>A, *arrows*) in ARHR patients (*right panel*). This substitution introduces a premature termination codon at codon 33 (*boxed*) that replaces the wild-type tryptophan residue (W33X). **b** Schematic structure of the *DMP1* gene. Mutations that were previously reported and identified in our study are shown

function mutations in *DMP1* gene were identified as the molecular cause of ARHR, but only a few pathogenic mutations have been reported (Fig. 3b) [5–7].

Here, we identified a novel nonsense mutation (98G>A, W33X) in the *DMP1* gene in a Japanese family. It is plausible that the W33X mutation leads to loss of function, because the majority of mRNAs containing premature termination codons (PTCs) are degraded by nonsense-mediated mRNA decay (NMD) to prevent the production of deleterious truncated proteins [18]. As a general rule, PTCs are located more than 50–55 nucleotides upstream of the most 3′ exon–exon junction of the mRNA trigger NMD. In our case, the PTC introduced by the 98G>A mutation is located 84 nucleotides upstream of the most 3′ exon–exon junction, which is between nucleotide 183 (exon 5) and 184 (exon 6). Therefore, the mutant mRNA is likely degraded by NMD. Even if the mutant mRNA is translated, the truncated protein containing only 32 of the 513 amino acids in the full-length protein is assumed to be nonfunctional because it lacks integrin/DNA-binding domains and essential domains for hydroxyapatite nucleation [12, 17, 19].

FGF23 is a bone-derived hormone that stimulates renal phosphate excretion, inhibits the production of $1,25(OH)D_2$ [20], and is involved in phosphate disorders. Tumor-induced osteomalacia, a paraneoplastic syndrome causing hypophosphatemia, is caused by excess circulating FGF23 derived from mesenchymal tumors [2, 21]. On the other hand, the inactivation of FGF23 results in hyperphosphatemia, such as tumoral calcinosis [22, 23]. Serum phosphate is a major regulator of circulating FGF23, and serum FGF23 levels are markedly elevated in patients with chronic kidney disease [24]. In patient 1, high levels of serum FGF23 levels might be caused by renal insufficiency. However, the serum level of phosphate was inappropriately low in spite of renal sufficiency and treated with oral administration of vitamin D. According to the previous report [25], circulating FGF23 was suppressed to undetectable levels in patients with FGF23-independent hypophosphatemia, such as vitamin D deficiency and Fanconi's syndrome. Hypophosphatemia among patients with XLH, ADHR, is also suggested to be caused by excess plasma levels of FGF23 [20]. The relatively high circulating FGF23 level in patient 2 and extremely high circulating FGF23 level in patient 1 with hypophosphatemic ARHR suggested the possibility that the high level of circulating FGF23 was a primary origin of hypophosphatemia in patients 1 and 2 with ARHR. A normal level of circulating FGF23 was reported in the patients with ARHR [5, 6], suggesting that circulating FGF23 is not always suppressed in patients with ARHR.

According to previous reports, ARHR is likely to have a wide spectrum of severity. The patients with ARHR in this family were not diagnosed with hypophosphatemia until adult life, probably because their symptoms were mild. On the other hand, other patients underwent repeated osteotomy [6] and developed complications associated with nerve deafness and learning disability. However, a large deletion encompassing at least 49 kb was found in these patients, suggesting the phenotype is not simply caused by the loss of DMP1 [7]. Despite receiving no medications, including vitamin D or phosphorus supplementation, they showed mild skeletal and dental symptoms deformities during adolescence. In addition, patient 1 was able to give birth to three healthy children. The ARHR patients reported here had a mild clinical course and normal fertility without any treatment.

In conclusion, this is the first report of a Japanese family with ARHR. We identified a novel nonsense mutation in the *DMP1* gene as the likely cause of the disease. Further accumulation of ARHR cases is necessary to analyze genotype–phenotype correlation.

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