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DMP1 mutations in autosomal recessive hypophosphatemia implicate a bone matrix protein in the regulation of phosphate homeostasis

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Abstract

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

Hypophosphatemia is a genetically heterogeneous disease. Here, we mapped an autosomal recessive form (designated ARHP) to chromosome 4q21 and identified homozygous mutations in *DMP1* (dentin matrix protein 1), which encodes a non-collagenous bone matrix protein expressed in osteoblasts and osteocytes. Intact plasma levels of the phosphaturic protein FGF23 were clearly elevated in two of four affected individuals, providing a possible explanation for the phosphaturia and inappropriately normal 1,25(OH)₂D levels and suggesting that DMP1 may regulate FGF23 expression.

Despite its broad biological importance, the control of phosphate homeostasis remains incompletely understood. Most of the genes contributing to its normal regulation have been identified by studying genetic defects leading to different hypophosphatemic disorders. The most frequent of these disorders is X-linked hypophosphatemia (XLH; OMIM 307800), caused by inactivating mutations in a gene encoding a putative endopeptidase (*PHEX*)¹. A much rarer disorder, autosomal dominant hypophosphatemic rickets (ADHR; OMIM 193100) is caused by mutations in *FGF23* that render the phosphaturic factor encoded by this gene resistant to proteolytic cleavage by subtilisin-like proprotein convertases^{2,3}. Wild-type FGF23 is abundantly expressed in certain mesenchymal tumors, causing increased serum FGF23 levels that induce renal phosphate-wasting and osteomalacia (tumor-induced osteomalacia or TIO)⁴⁻⁶.

We have now investigated three multiplex families in which the affected individuals showed clinical, biochemical and histomorphometric parameters that were similar to those observed in XLH and ADHR (Table 1 and Supplementary Methods online). However, inspection of the pedigrees suggested an autosomal recessive mode of inheritance (hence the abbreviation ARHP), thus excluding XLH and ADHR. In order to identify the molecular defect, we performed a genome-wide linkage analysis using SNP array genotyping. Assuming that the disease alleles could be identical by descent in each family, we analyzed the data by homozygosity mapping and identified a 4.6-Mb candidate region on chromosome 4q21 between SNPs rs340204 and rs722937. Parametric LOD score calculations under the conservative assumption of second-cousin marriages in family 1 and 2 resulted in maximum LOD scores of 3.1 and 2.4, respectively, and 4.2 in family 3 with established consanguinity.

The candidate region for ARHP contained a cluster of genes coding for a class of tooth and bone noncollagenous matrix proteins that are referred to as SIBLING proteins (small integrin-binding ligand, N-linked glycoproteins). Members of this protein family include dentin sialophosphoprotein (DSPP), dentin matrix protein 1 (DMP1), integrin-binding sialoprotein (IBSP), matrix extracellular phosphoglycoprotein (MEPE) and osteopontin (also named secreted phosphoprotein 1, SPP1). These polyanionic proteins are believed to have key biological roles in mineralization of osteoid and dentin⁷ and thus were plausible candidates. We therefore searched by direct sequencing for mutations in their exons and relevant flanking intronic regions (except for the last exon of *DSPP* and *MEPE*).

In the affected members of all three investigated families, we identified different homozygous, presumably loss-of-function mutations in *DMP1*; each of the unaffected parents was heterozygous for the respective mutation (Fig. 1 and Supplementary Fig. 1 online). We observed only known sequence variations in *DSPP* and *IBSP* but did not identify

any variations in *MEPE* and *SPP1* (Supplementary Table 1 online). The affected siblings in family 1 carried a homozygous 1-bp deletion in exon 6 (362delC) leading to a premature stop codon after 120 unrelated amino acids. The two affected brothers in family 2 had a homozygous mutation in the canonical splice acceptor sequence (55-1G→C) of intron 2. The affected individuals in family 3 carried a missense mutation in exon 2 that changed the initiator codon ATG to GTG (1A→G, leading to M1V). Protein blot analysis of the supernatant of HEK293 cells transiently expressing a plasmid encoding a His-tagged DMP1 carrying this mutation showed a 23-kDa protein band but not the 80-kDa and 57-kDa bands that were observed in cells expressing His-tagged wild-type DMP1. Furthermore, we searched for *DMP1* mutations in 18 individuals with hypophosphatemia for whom *PHEX* and *FGF23* mutations had been excluded and in three individuals with tumoral calcinosis for whom *FGF23* and *GALNT3* mutations had been excluded. In addition to several common sequence variants (Supplementary Table 1), we identified a non-synonymous, heterozygous variant (349G→A, D117N) in an individual with tumoral calcinosis that was not present in 666 normal control chromosomes.

DMP1 and its mouse and rat orthologs are expressed in tooth, bone, brain and salivary gland. Targeted ablation of both *Dmp1* alleles in mice resulted in shorter bones and vertebrae, a highly expanded zone of proliferating and hypertrophic chondrocytes in the growth plate of younger mice and broad sclerotic long bones in older animals⁸. These skeletal findings, which were initially thought to represent a form of chondrodysplasia, led to the conclusion that DMP1 is required for normal postnatal bone and tooth formation. Recently, however, decreased serum phosphate and calcium levels have been observed⁹, demonstrating the similarity of this phenotype with different hypophosphatemia-induced forms of rickets.

Various *in vitro* and *in vivo* studies suggested that the genes mutated in different forms of hypophosphatemia constitute a previously unrecognized pathway involved in regulating phosphate homeostasis. In brief, the endopeptidase PHEX seems to indirectly regulate levels of the phosphaturic protein FGF23, which affects expression and internalization of the renal sodium-phosphate cotransporters SLC34A1 and SLC34A3. It is not yet known how DMP1 is involved in regulating phosphate homeostasis. However, it has been shown that DMP1 is processed at four different cleavage sites¹⁰ and that cleavage can be achieved by BMP1/tolloid-like proteinases *in vitro*¹¹. Considering that PHEX and DMP1 are coexpressed (Supplementary Fig. 2 online) and that PHEX and BMP1 require an aspartate at the P₁' position of the cleavage site¹², it appeared conceivable that DMP1 might be processed by PHEX¹⁰. To investigate this hypothesis, we expressed human C-terminally tagged (DMP1/His) and mouse N-terminally tagged (Flag/Dmp1) fusion proteins in HEK293 cells and incubated the proteins with a recombinant secreted and soluble form of PHEX (secPHEX)¹². Under these *in vitro* conditions, none of the tagged DMP1 or Dmp1 proteins showed specific cleavage or substantial degradation in the presence of secPHEX (Supplementary Fig. 2). However, we cannot exclude the possibility that intact DMP1 or DMP1 fragments are PHEX substrates under physiological conditions. Previous studies have described that PHEX binds to the C-terminal part of MEPE, another member of the SIBLING family, without cleaving it¹³. To investigate whether DMP1 binds to PHEX protein in a similar manner, PHEX was incubated with a PHEX substrate, PTHrP_{107–139} (ref. 12). The addition of DMP1 or Dmp1

did not alter the extent of degradation of PTHrP107–139 by PHEX (Supplementary Fig. 2), suggesting that physical interactions between DMP1 and PHEX do not affect PHEX activity.

DMP1 has been shown to be expressed more abundantly in TIO tumors than FGF23 (ref. 5). However, unlike FGF23, DMP1 showed no phosphaturic effect *in vivo*⁵ and did not inhibit phosphate uptake *in vitro*¹⁴, suggesting that it does not have a direct role in renal phosphate handling. Because inactivating DMP1 mutations, as shown in this report, lead to hypophosphatemia, we asked whether the lack of DMP1 increases plasma or serum FGF23 levels as in individuals with XLH, TIO and ADHR⁶. Plasma and serum were available from four individuals with homozygous *DMP1* mutations, and two independently collected samples from two individuals showed clearly elevated FGF23 levels when measured by an ELISA that detects intact FGF23 alone and showed slightly elevated levels in one of these individuals when using an ELISA that detects C-terminal and intact FGF23 (Table 1). In the other two individuals, intact FGF23 was slightly elevated or in the upper normal range. These findings are similar to those observed in XLH, in which many but not all affected individuals show elevated FGF23 levels. The phosphaturia observed in ARHP could thus also be FGF23 dependent. As FGF23 also inhibits 1- α -hydroxylase, elevated FGF23 concentrations could furthermore provide an explanation for the inappropriately normal 1,25(OH)₂D levels observed in all affected individuals with ARHP whom we investigated (Table 1). The details of the underlying regulatory mechanisms are not yet known. It is interesting in this regard that DMP1 has been reported to have different biological roles. If phosphorylated, it is exported into the extracellular matrix, where it regulates nucleation of hydroxyapatite. Otherwise, it is transported to the nuclear compartment, where it acts as a transcription factor¹⁵. With the identification of *DMP1* mutations as the cause of ARHP, we have added a further component to the growing list of genes involved in the regulation of phosphate homeostasis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

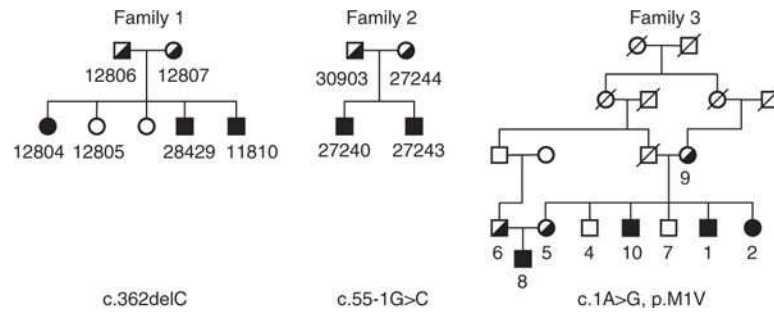
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**Figure 1.**

DMP1 mutations in three families with autosomal recessive hypophosphatemia. All affected individuals were homozygous for the mutated allele segregating in the corresponding family. The parents were heterozygous for the respective mutation. Affected individuals are indicated by filled symbols and heterozygous carriers by half-filled symbols. Radiographs for affected members of each family are shown in Supplementary Figure 3 online. Morphometric parameters of an iliac bone biopsy specimen from an affected individual in family 3 are shown in Supplementary Table 3 online. Primer sequences used for sequence analysis are listed in Supplementary Table 4 online. Informed consent was obtained from all study participants. The study was approved by the institutional review boards of the Medical Department of the Technical University of Munich and Massachusetts General Hospital.

Table 1

Chemical and clinical data

Individual	Family 1			Family 2		Family 3			Reference ranges
	12804	28429	11810	27240	27243	1	2	8	
FGF23 (intact) (pg/ml)	56			102,105	100,118		46		10–50
FGF23 (C-t) (RU/ml)	43			162,132	193,114		51		<120–150
Ca (mmol/l)	2.36	2.3	2.4	2.29	2.19	2.29, 2.41	2.39	2.53	2.15–2.64
P (mmol/l)	0.74 (0.87–1.45)	0.37 (0.71–1.33)	0.43 (0.71–1.33)	1.03 (1.51–2.48)	1.00 (1.06–1.77)	0.66, 0.68 (0.76–1.46)	0.64 (0.8–1.50)	1.05 (1.3–1.8)	
AP (U/l)	117 (35–104)	318 (35–104)	232 (35–104)	445 (150–300)	431 (80–187)	236, 253 (32–92)	175 (50–136)	375 (145–320)	
PTH (pg/ml)	105	87	65	26–50	47–104	90, 100	102	66	12–65
25OHD (ng/ml)	6 (20–45)	3.5 (20–45)	18 (20–45)	77.8 (11–70)	57.9 (11–70)	<4, 8.8 (>10)	6.8 (>10)	18.4 (8–32)	
1,25(OH) ₂ D (pg/ml)	28 (19.9–67)	40 (19.9–67)	37 (19.9–67)	24 (16–56)	50 (16–56)	37, 22 (15–54)	16 (15–54)	45 (9.6–39)	
U Ca/Cr (mg/mg)	0.006		0.2	0.02–0.06	0.03–0.06	<0.1	<0.15	<0.016	< 0.21
TRP (%)	83			65	65	88	89		83–95
TmP/GFR (mmol/l)	0.65			0.61	0.46	0.50	0.46		0.8–1.4
Urine deoxypyridinoline (nmol/mmol creatinine)						32.2	29.6		M: 1.9–3.1 F: 3.2–4.0
Age (in years and months)	35 yrs	26 yrs	30 yrs	8 yrs 5 mo	15 yrs 9 mo	32 yrs	30 yrs	1 yr 10 mo	
Weight (kg)	48.8 (9)	64	72	29.4 (80)	52.3 (40)	70.6 (50)	46.9 (10)	15.3 (90) ^a	
Height (cm)	140 (<3)	157	160	122.7 (40)	159.7 (30)	158 (<3)	144 (<3)	89 (25) ^a	
Rocaltrol (µg/day)	1.0	0.75		0.5	0.25–0.5				
Phosphorus (mg/day)	2000	2000		1.875	1.875				

Age- and sex-dependent reference values are given in parentheses. FGF23 plasma levels were measured after 2 d of withdrawal of 1,25 vitamin D and phosphate substitution; for some affected individuals, the results of two or more measurements are provided. FGF23: fibroblast growth factor 23, Ca: calcium, P: phosphorus, AP: alkaline phosphatase, PTH: parathyroid hormone, 25OHD: 25-hydroxyvitamin D, 1,25(OH)₂D: 1,25-dihydroxyvitamin D, U Ca/Cr: urine calcium/creatinine ratio, TRP: tubular reabsorption of phosphate, TmP/GFR: maximal tubular phosphate reabsorption to glomerular filtration rate. The centiles for weight and height are given in parentheses.

^aAt age 2 years 6 months. Chemical data for the heterozygous parents in Family 2 are shown in Supplementary Table 2 online.