COMMENTARIES

DMP1 and Phosphate Metabolism – Matrix Proteins Go Systemic

Caroline Silve

INSERM U 773 and Laboratoire de Biochimie Hormonale et Génétique, Faculté de Médecine et Hôpital Bichat - Claude Bernard, Paris, France

Commentary on:

- Feng JQ, Ward LM, Liu S, Lu Y, Xie Y, Yuan B, Yu X, Rauch F, Davis SI, Zhang S, Rios H, Drezner MK, Quarles LD, Bonewald LF, White KE. Loss of DMP1 causes rickets and osteomalacia and identifies a role for osteocytes in mineral metabolism. *Nat Genet.* 2006 Nov;38(11):1310-5.

- Lorenz-Depiereux B, Bastepe M, Benet-Pages A, Amyere M, Wagenstaller J, Muller-Barth U, Badenhoop K, Kaiser SM, Rittmaster RS, Shlossberg AH, Olivares JL, Loris C, Ramos FJ, Glorieux F, Vikkula M, Juppner H, Strom TM. DMP1 mutations in autosomal recessive hypophosphatemia implicate a bone matrix protein in the regulation of phosphate homeostasis. *Nat Genet.* 2006 Nov;38(11):1248-50.

The identification of defects in the PHEX and FGF23 genes as the causes of X-linked and autosomal dominant hypophosphatemic rickets (XLH and ADHR), respectively, has led to the discovery of a novel pathway that regulates phosphate metabolism. In this pathway, the endopeptidase PHEX is thought to *indirectly* regulate the circulating levels of FGF23, a "phosphate-depleting" hormone that both reduces the expression of the sodium-phosphate cotransporters SLC34A1 and SLC34A3 at the brush-border membrane of renal tubular cells (thereby increasing phosphate excretion) and decreases the synthesis of the 1α-hydroxylase necessary for 1,25(OH)$_2$ vitamin D synthesis (thereby decreasing intestinal absorption of phosphate and calcium) (1;2). Two recent papers now report the presence of inactivating mutations in the dentin matrix protein-1 (*DMP1*) gene in patients affected with autosomal recessive hypophosphatemia (ARHP), a rare disorder whose clinical hallmarks are highly reminiscent of those seen in XLH and ADHR: i) hypophosphatemia and renal phosphate wasting in the absence of hypercalciuria,

ii) rickets and/or osteomalacia, and iii) inappropriately normal calcitriol levels. Given the similarity of the phenotypes observed in XLH, ADHR, and ARHP, (and tumor-induced osteomalacia), it is satisfying to find that the absence of DMP1 also leads to an increase in circulating levels of FGF23. Indeed, both Feng *et al*. (3) and Lorenz-Depiereux *et al*. (4) report high normal or elevated levels of FGF23 in their patients with ARHP, and FGF23 levels are shown by Feng *et al*. to be increased in the *Dmp1* knockout mouse. Thus, excessive signaling through FGF23 appears to play an important role in causing the bone mineralization defects, hypophosphatemia and inappropriately normal levels of calcitriol seen in these diseases, and suggests that DMP1 is another active participant in this signaling pathway. Although its importance is now clearly established by considerable clinical and experimental work, this multi-step pathway has only been partially deciphered, and is the focus of considerable ongoing effort.

Using different strategies, the two groups have identified homozygous mutations in

DMP1 that most likely result in a loss of functional protein in kindreds affected with ARHP. Using a candidate gene approach based on the known phenotype of *Dmp1*-null mice, Feng *et al.* identified a missense mutation affecting the initiator ATG in the affected member of a consanguineous family, and a homozygous 7 bp deletion (1484-1490del) in exon 6 of *DMP1* that resulted in the replacement of the conserved C-terminal 18 amino acids with 33 unrelated residues in a second kindred. Lorenz-Depiereux *et al.* performed a genome-wide linkage analysis using SNP array genotyping in three multiplex families, and identified a different homozygous defect in each family: a 1-bp deletion in exon 6 of *DMP1* generating a premature stop codon; a mutation in a canonical splice acceptor sequence of the second intron; and the same missense mutation affecting the initiator ATG codon (M1V) found by Feng *et al.* In both studies, the mutations segregated with the disorder, were not found in control alleles, and were also not identified in individuals with hypophosphatemia with no known history of consanguinity and for whom *PHEX* and *FGF23* mutations had been excluded. In both studies, it was demonstrated that DMP1 carrying the M1V mutation was not secreted, consistent with the loss of signal peptide, and a defect in the secretion of the 1484-1490del DMP1 mutant was also demonstrated (3).

The identification of a new player in the FGF23 pathway is welcome news. Herbert Spencer once quipped, however, that when a man's knowledge is not in order, the more of it he has, the greater will be his confusion. Since our understanding of the FGF23 pathway remains far from complete, the question arises whether the identification of DMP1 has added clarity or confusion to this emerging story. At the least, this important new information raises as many questions as it answers, and further emphasizes the lacunae that remain in our understanding of the regulation of phosphate metabolism. Several of these murky areas are discussed below.

DMP1 Mutations and Skeletal Abnormalities

The results suggesting that DMP1 plays a role in regulating systemic phosphate metabolism certainly expand our thinking about the function of this protein. DMP1 belongs to the Small Integrin-Binding Ligand N-linked Glycoprotein (SIBLING) family of proteins, many members of which are involved in the regulation of biomineralization (5;6). DMP1 is abundantly expressed in cells of the osteoblast/osteocyte lineage and is a component of the extracellular matrix of bones and teeth. Since its identification in 1993, most studies of DMP1 have focused on the potential role of this protein during bone development and mineralization. Numerous activities have been described, including roles as a transcription factor regulating the expression of osteoblast-specific genes such as osteocalcin, the control of osteoblast/osteocyte differentiation, and the initiation of mineralization of the extracellular matrix, an activity that may require both extensive phosphorylation of DMP1 and proteolytic cleavage by BMP1/Tolloid-like proteases, and possibly other proteinases (7-9).

It was therefore surprising to find that *Dmp1* knockout mice had only subtle abnormalities in bones and teeth *in utero* or at the time of birth (10-12). Similarly, in humans, the time at which the diagnosis of ARHP has been made is variable, but is often delayed until late infancy or even adulthood. These observations are difficult to reconcile with the protein having a critical role in bone cell differentiation or mineralization. Certainly the most striking phenotypic abnormality in *Dmp1* knockout mice and patients with *DMP1* mutations is the presence of rickets and osteomalacia, which develops progressively after birth. The striking abnormalities in mineral metabolism associated with the *DMP1* mutations described by Feng *et al.* (3) and Lorenz-Depiereux *et al.* (4) provide a ready explanation for these findings. Other pathological findings associated with inactivation of *DMP1* are more difficult to ascribe solely to imbalances in calcium

and/or phosphate. For example, osteocytes in *Dmp1* knockout mice express the expected marker E11:gp38, but inappropriately express the osteoblast-specific markers alkaline phosphatase and type I collagen, and abnormalities in osteocyte lacunae and the bone canalicular system are seen in *Dmp1* knockout mice (3). Similarly, Feng *et al.* (3) demonstrate that a high phosphate diet corrects the mineralization defect at the level of the growth plate, but only partially reverses the osteomalacia. Thus, it remains to be established to what extent inactivation of *DMP1* causes abnormalities in calcified tissues that are independent of changes in mineral metabolism. It is also noteworthy that perilacunar osteocytic abnormalities have also been reported in XLH (13;14). It will be interesting to determine the extent to which similar lesions in osteocyte lacunae and/or canaliculae occur in individuals with *DMP1* mutations and XLH.

How and Where Does DMP1 Interact with the FGF23 Pathway?

Current evidence suggests that the effect of DMP1 on phosphate homeostasis is ultimately mediated through the FGF23 pathway (3;4). Although high level expression of DMP1 has been observed in tumors inducing hypophosphatemia, direct effects of DMP1 on phosphate metabolism have not been observed (15). DMP1 does not have a phosphaturic effect when administered *in vivo*, and does not inhibit phosphate uptake by cells expressing sodium-phosphate co-transporters *in vitro*. Furthermore, the increase in circulating FGF23 levels seen in *Dmp1* null mice is associated with a marked increase in FGF23 gene expression in osteocytes, suggesting that DMP1 operates locally on cells of the osteoblast/osteocyte lineage, and normally inhibits FGF23 production, rather than promoting degradation or sequestration within bone matrix (3).

DMP1, PHEX and FGF23 are all expressed in cells of the osteoblast lineage, but the relationship between these proteins remains elusive. The activities of both FGF23 and DMP1 are regulated in part by proteolytic cleavage (4;6;16;17). FGF23 does not appear to be a substrate for PHEX, however, indicating that PHEX is upstream of FGF23, and moderates FGF23 indirectly. It has also been suggested that DMP1 might be a substrate for PHEX (6), but attempts by Lorenz-Depiereux *et al.* (4) to demonstrate cleavage of recombinant DMP1 by soluble PHEX *in vitro* were unsuccessful. Like PHEX, DMP1 appears to decrease the circulating levels of FGF23, but whether these two proteins are linked in a common pathway remains unclear.

Indeed, it may be worth asking if there is any direct link between DMP1/PHEX and FGF23. Although FGF23 expression is increased in osteocytes from *Dmp1* null mice, it should be remembered that these osteocytes are not normally differentiated, as demonstrated by the concomitant expression of type I collagen, alkaline phosphatase and E11:gp38, and that FGF23 expression in normal osteocytes is quite low. Thus it is conceivable that DMP1 (or PHEX) may primarily influence the developmental steps that control osteoblast to osteocyte differentiation, and defects in these proteins cause this process to be stalled at a stage characterized by high FGF23 production, as has been reported for osteoblasts present at sites of active bone formation (including bone repair sites) (18). Further work characterizing the state of differentiation of osteocytes in other models, including *Hyp* mice and TIO, should clarify whether defects in osteoblast maturation play a causative role in FGF23 overproduction or represent an effect of chronic stimulation by FGF23 on cells of this lineage.

It should also be remembered that although DMP1 and FGF23 can be highly expressed by cells of the osteoblast/osteocyte lineage, both proteins are also expressed in other tissues. For instance, DMP1 is expressed along the nephron, including both proximal convoluted tubules and the distal nephron, where it co-localizes with matrix metalloproteinase-9, a protease that DMP1 specifically activates (19). Thus, the DMP1 phenotype may result from abnormalities in tissues other than bone, and may produce more generalized defects. Given the

importance of calcium and phosphate for mineralization, the prominence of the bone phenotype is not surprising.

FGF23 and Calcium Metabolism

Hypocalcemia has not been reported in patients carrying *DMP1* mutations, either in the heterozygous or homozygous state. In contrast, heterozygous *Dmp1*-inactivated mice are significantly hypocalcemic but normophosphatemic, and homozygous mice are more severely hypocalcemic and hypophosphatemic (3;11). These observations also raise a series of questions. First, why would *Dmp1* knockout mice be hypocalcemic? Perhaps interplay between the FGF23 and PTH pathways may play a role. The parathyroid gland expresses FGF receptors and Klotho, and therefore can respond to FGF23 signaling (20), and FGF23 administration has been shown to transiently decrease serum PTH levels (21) . These findings raise the possibility that chronically increased FGF23 levels in *Dmp1*(-/-) mice reduce the responsiveness of the parathyroid gland to signals eliciting PTH release. Through its action on $1,25(OH)_2D$ synthesis and intestinal calcium absorption, FGF23 may simultaneously cause a fall in serum calcium, which would solicit a PTH response. Together, these effects would be expected to lead to a stimulation of PTH production, but the response might be insufficient to maintain a normal level of serum calcium. Indeed, *Dmp1*-knockout mice have abnormally high circulating PTH levels but low serum calcium (3;11). Patients with *DMP1* mutations, like *Dmp1*-knockout mice, have abnormally high circulating PTH levels (3;4).

Fuller Albright noted many years ago that cases of osteomalacia include those where there is compensatory over-activity of the parathyroids sufficient to maintain serum calcium at a normal level, and those where there is compensatory over-activity of the parathyroids but where this is insufficient to maintain normal serum calcium (22). But why should patients with *DMP1* mutations fall into the first category, and *Dmp1*-knockout mice fall into the second? Numerous possibilities come to mind,

including "species differences," potential effects of dietary calcium, and the possibility that the *DMP1* mutations described to date may only disrupt some of the functions of this protein, and therefore have a phenotype distinct from total disruption. More information on calcium metabolism under conditions of controlled calcium intake will be helpful in sorting out this question, as will information addressing the correlations between the genotype and phenotype of *DMP1* mutations. Based on the findings in *Dmp1* knockout mice, the possibility that individuals carrying heterozygous mutations in *DMP1* may be susceptible to hypocalcemia deserves further attention. In this context, it is noteworthy that abnormally increased levels of PTH, despite normal serum calcium levels, have been observed in some patients with XLH and older *Hyp* mice. It has been proposed that increased PTH levels in this setting result from intermittent dietary phosphate loading. The finding of a similar profile in patients with *DMP1* mutations supports the alternative possibility that syndromes characterized by overproduction of FGF23 chronically activate the PTH pathway (23).

These are interesting times for those interested in phosphate metabolism. The FGF23 pathway is leading us somewhere, but the trail is obscured by unresolved questions and missing data. Ultimately, we will understand what is being controlled by this pathway (intracellular and/or extracellular phosphate and/or calcium?); where the sensors are located and how they work; and how many effector mechanisms contribute to this task. Feng *et al.* and Lorenz-Depiereux *et al.* offer important help, both by providing new information and helping to formulate more clearly questions that remain to be answered.

Conflict of Interest: The author reports that no conflict of interest exists.

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