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The role of DMP1 in autosomal recessive hypophosphatemic rickets

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Heritable disorders of disturbed phosphate handling are the most common causes of rickets and osteomalacia in developed countries. However, the molecular mechanisms underlying skeletal mineralization are complex, and our current models are far from complete. Isolated renal phosphate wasting and subsequent low serum phosphate concentrations may result from a number of genetic disorders that include: autosomal dominant hypophosphatemic rickets (ADHR), X-linked hypophosphatemic rickets (XLH), and hereditary hypophosphatemic rickets with hypercalciuria (HHRH).An acquired disorder of isolated renal phosphate wasting with similar phenotypes to these heritable disorders has also been recognized, tumor-induced osteomalacia (TIO).

Seminal findings have confirmed an important role for the endocrine molecule, Fibroblast growth factor-23 (FGF23), identified in autosomal dominant hypophosphatemic rickets (ADHR), as playing a central role in X-linked hypophosphatemic rickets (XLH; due to inactivating mutations in the PHEX gene)[1]. Unexpectedly, mice lacking dentin matrix protein-1 (Dmp1, a non-collagenous matrix protein) were shown to have an overlapping phenotype with ADHR and XLH patients, as well as the XLH model, the Hyp mouse, manifesting hypophosphatemic rickets/osteomalacia and elevated FGF23.

Building upon our work regarding the molecular genetics of disorders involving FGF23, we undertook a study to identify a novel disorder of phosphate homeostasis. We identified two unrelated consanguineous kindreds (Families 1 and 2) with hypophosphatemic rickets in the absence of hypercalciuria (thus not HHRH) and a family history consistent with a recessive mode of inheritance[2].

FGF23 and PHEX mutational analyses were negative in both families. The consanguineous nature of the kindreds, as well as the negative genotyping for the known dominant hypophosphatemic disorders, led us to provisionally designate both families as having autosomal recessive hypophosphatemic rickets (ARHR). Due to the fact that the Dmp1-null mouse has biochemical and skeletal phenotypes similar to ADHR and XLH patients, we assessed the ARHR families for DMP1 mutations. We determined by direct DNA sequencing of the five coding DMP1 exons 2-6 (exon 1 is non-coding), that the affected members from Family 1 had a biallelic deletion of seven base pairs (deleted nucleotides 1484-1490, or 1484-1490del) within the 3′ portion of the last coding exon, exon 6 (Figure 1a). This change led to a frame shift in the DMP1 cDNA, which would result in loss of the wild type stop codon, translation of nucleotides within the 3′UTR, and the formation of a new stop codon. Thus an additional 33 residues comprising non-native polypeptide are added to the 3′ end of DMP1 indicating that the highly conserved, C-terminal portion of DMP1 is critical for normal cellular activity.

In ARHR Family 2, sequence analysis of exon 2 indicated that the proband had a homozygous mutation in the DMP1 start codon. In this regard, the adenine nucleotide in position 1 of the start codon, ATG (methionine), was changed to GTG (valine) (1G>A). This substitution created the amino acid change Methionine1Valine (M1V). The localization of the initial methionine and the signal sequence, comprised of residues Met1 through Ala16, is highly conserved across species, and is required for proper DMP1 secretion into the extracellular milieu (Figure 1b). Both mutants were not found in 200 control alleles.

DMP1 is a member of the 'short integrin-binding ligand interacting glycoprotein' (SIBLING) family of matrix proteins including osteopontin (OPN), bone sialoprotein (BSP), dentin sialophosphoprotein (DSPP), and matrix extracellular phosphoglycoprotein (MEPE/osteogenic factor 45)[3]. Full

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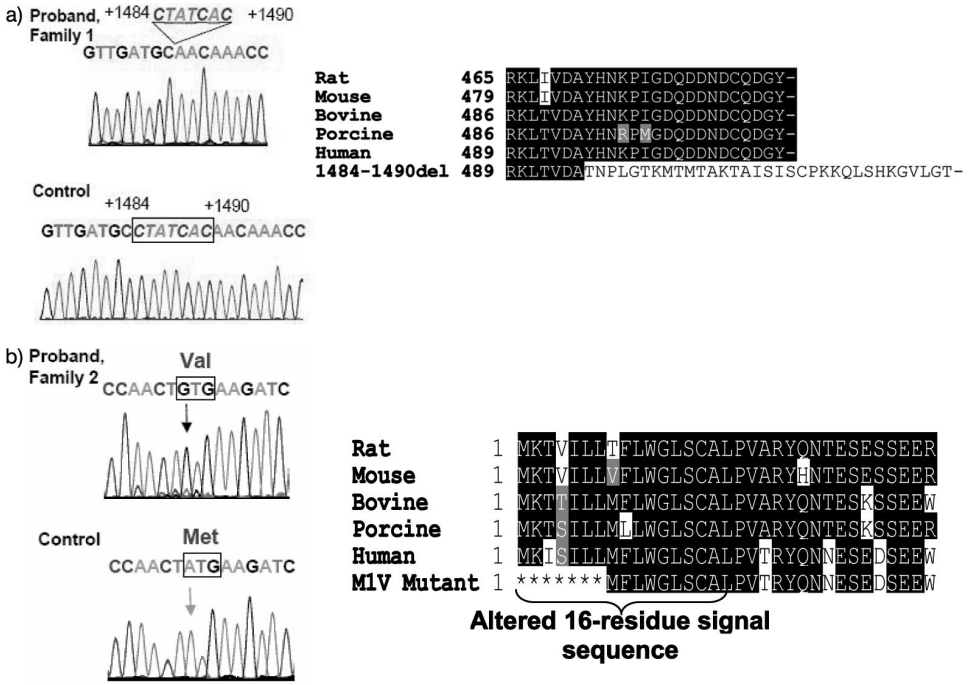

Figure 1a,b. a.1484-1490del DMP1 mutations. Family 1 Proband and Control sequence traces showing deleted nucleotides (left); DMP1 protein alignment (right). Additional residues due to the frame shift are shown in the '1484-1490del' sequence; stop codons indicated by "-". **b. M1V DMP1 mutation.** Left, Family 2 proband and control sequence traces showing A1G (M1V) change; Right, protein alignment showing the conserved DMP1 signal sequence. (Reproduced with permission from reference (2).

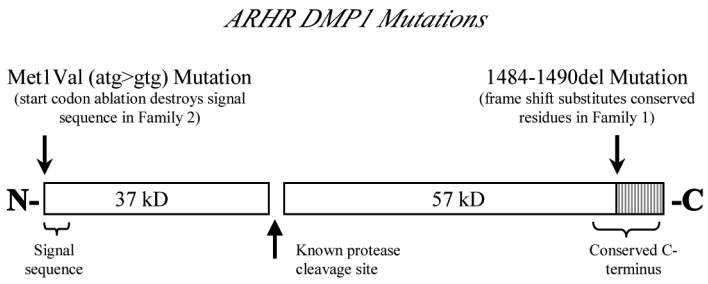

Figure 2. DMP1 is cleaved into secreted N-terminal 37 kD, and C-terminal 57 kD fragments. The sites of the DMP1 ARHR mutations in are shown relative to the known protease cleavage site.

-length DMP1 is comprised of 513 residues but is secreted in bone as 37 kD N-terminal, and 57 kD C-terminal fragments from a 94 kD full-length precursor[4] (Figure 2).

To investigate the effects of the ARHR mutations on DMP1, expression plasmids containing wild type, M1V, or 1484-1490del DMP1 were transfected into HEK293 cells. Western analyses of wild type protein demonstrated that DMP1 is normally expressed equally in the cellular lysates and in the media. In contrast, the ARHR 1484-1490del mutant was primarily detected in the media, whereas the M1V mutant was only detected in the cellular lysates, demonstrating the ARHR mutations affected DMP1 trafficking.

The last 18 amino acids of DMP1 are highly conserved and are replaced by 33 novel residues due to a frameshift caused by the 1484-1490del mutation (Figure 1a). We deleted the last 18 DMP1 residues to determine the role of the C-terminus in processing. Expression of this truncated species was the same as the wild type DMP1, and had balanced intra- and extracellular expression. Therefore, the unbalanced expression of 1484-1490del DMP1 is a function of the 33 novel residues, and not a consequence of deleting the last 18 native residues.

To investigate the effects of the ARHR mutations on DMP1 intracellular trafficking, transfected cells were fluorescently co-stained for DMP1 in parallel with staining for the

trans-golgi network (TGN), ER, and nucleus. Wild type and 1484-1490del mutant DMP1 localized to the TGN, consistent with their cellular secretion. The M1V mutant did not localize to the TGN but rather filled the entire cell due to loss of the highly conserved DMP1 signal peptide, consistent with the western analyses described above. We obtained the same results with these constructs produced as fusion proteins with V5 tags or untagged, in HEK293, UMR106, and MC3T3 cells.

In summary, loss-of-function mutations in DMP1 cause the novel disorder ARHR. Our studies of the ARHR DMP1 mutants illustrate that modifications to the signal peptide and to the C-terminus of DMP1 affect cell processing. The alterations in DMP1 secretion due to the ARHR mutations provide a unique insight into domains of DMP1 critical for normal biological function.

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