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Identification of a Novel Dentin Matrix Protein-1 (DMP-1) Mutation and Dental Anomalies in a Kindred with Autosomal Recessive Hypophosphatemia

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Abstract

An autosomal recessive form of hypophosphatemia (ARHP) was recently shown to be caused by homozygous mutations in *DMP1*, the gene encoding dentin matrix protein-1 (DMP-1), a non-collagenous bone matrix protein with an important role in the development and mineralization of bone and teeth. Here, we report a previously not reported consanguineous ARHP kindred in which the three affected individuals carry a novel homozygous *DMP-1* mutation. The index case presented at the age of 3 years with bowing of his legs, and showed hypophosphatemia due to insufficient renal phosphate retention. Serum alkaline phosphatase activity was elevated, with initially normal PTH. FGF23 was inappropriately normal at an older age while being treated with oral phosphate and 1,25 $(OH)_2D$. Similar clinical and biochemical findings, except for elevated FGF23 levels, were present in his 16 month-old brother and his 12.5 year-old female cousin; the parents of the three affected children are first-degree cousins. Nucleotide sequence analysis was performed on PCR-amplified exons encoding DMP-1 and flanking intronic regions. A novel homozygous frame-shift mutation (c. 485Tdel; p.Glu163ArgfsX53) in exon 6 resulting in a premature stop codon was identified in all effected individuals. The parents and available unaffected siblings were heterozygous for c.485Tdel. Tooth growth and shape were normal for the index case, his affected brother and cousin, but their permanent and deciduous teeth displayed enlarged pulp chambers. The identified genetic mutation underscores the importance of *DMP-1* mutations in the pathogenesis of ARHP. Furthermore, *DMP-1* mutations appear to contribute, through yet unknown mechanisms, to tooth development.

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Keywords

dentin matrix protein-1; fibroblast growth factor-23 (FGF-23); hypophosphatemia; rickets; tooth

Introduction

Dentin matrix protein-1 (DMP-1), an acidic non-collagenous phosphoprotein, is a member of the SIBLING (small integrin binding ligand N-linked glycoprotein) family, which includes osteopontin (OPN), bone sialoprotein, dentin sialophosphoprotein (DSPP), enamelin, and matrix extracellular phosphoglycoprotein (MEPE), which are thought to play an important role in tissue mineralization (10-12,27). DMP-1, which is produced by osteoblasts and osteocytes, regulates cell attachment (16) and cell differentiation (14,22), activates matrix metalloproteinase-9 (7), and affects biomineralization (35). Mice that are null for *Dmp-1* develop a bone phenotype characterized by hypomineralization, short and wide vertebrae, long bones with delayed and malformed secondary ossification centers, and abnormal osteocyte morphology associated with loss of dendritic extensions (36). *Dmp-1*-null mice furthermore show increased osteocytic FGF23 expression and elevated FGF23 levels, leading to hypophosphatemia and reduced $1,25(OH)_2D$ levels, leading to mild hypocalcemia and secondary hyperparathyroidism (9,17).

Homozygous *DMP-1* mutations were recently identified in the affected members of several unrelated families with an autosomal recessive form of hypophosphatemia (ARHP) (9,19). The clinical, biochemical, and bone histomorphometric parameters of these individuals are indistinguishable from those observed in patients with X-linked hypophosphatemia (XLH) (9,19). Similar to the findings in *Dmp-1*-null mice, ARHP patients have furthermore normal or low-normal serum calcium levels with high to normal PTH levels, and slightly elevated or inappropriately normal FGF23 levels resulting in diminished $1,25(OH)_2D$ levels (9,19). Tooth abnormalities have thus far not been described in humans with homozygous *DMP-1* mutations, yet *Dmp-1* null mice show increased width of predentin with reduced dentin wall thickness and dentin hypomineralization resembling human disease dentinogenesis imperfecta type II and III (35). Here, we report clinical, laboratory, and dental findings of the affected members of an ARHP kindred, in whom we identified a novel homozygous *DMP-1* mutation.

Materials and Methods

Serum and urine concentrations of calcium, phosphorus, creatinine, and serum alkaline phosphatase activity were measured at the Marmara University Hospital using standard methods. Serum intact PTH concentrations were determined by a solid phase, two-site chemiluminescent enzyme-labeled immunometric assay (Immulite® 2000). Plasma FGF23 levels were measured with an ELISA that detects intact as well as C-terminal FGF23 (Immutopics) (13). TmP/GFR was calculated according to the Walton and Bijvoet nomogram (33) using a fasting 2 hour urine sample and a serum sample that was obtained 1 hour after starting the urine collection.

The blood samples for DNA extractions were collected after informed consent was obtained; the study was approved by Massachusetts General Hospital Institutional Review Board. Nucleotide sequence analysis of the PCR-amplified exons encoding DMP-1 and the flanking intronic regions were performed at the Harvard-Partners Center for Genetics and Genomics (HPCGG). To confirm the presence of the identified mutation, *DMP-1* exon 6 was amplified by using 5′-CGGTTCCTGGAATACTGACC-3′ as forward primer and 5′-TCTTTGGCTGTGTTCTGGTG-′3 as reverse primer to obtain a 1619 bp PCR product, which was then incubated with the endonuclease *Eco*NI.

Patients and Results

The index case (#5) (Figure 1, panel A; arrow) was 3 years old when he presented with bowing of both legs that had become apparent during late infancy after he started walking; his height was 91.1 cm (−0.9 SDS) and his weight was 18.0 kg (+1.8 SDS). Besides genua varum, he had rachitic rosaries on physical examination. Laboratory investigations revealed hypophosphatemia (2.8 mg/dl), low tubular phosphate reabsorption (TMP/GFR: 3.0), elevated serum alkaline phosphate activity (964 U/L), and a normal serum PTH level (48 pg/ml) with normal serum calcium (9.6 mg/dl) (Figure 1, panel B). Treatment with oral phosphate (40 mg/kg/day) and $1,25(OH)_2D$ (0.25 μg/day) was initiated, which led to an improvement of his serum chemistries. After a period of five years without follow-up and treatment, the patient presented again with hypophosphatemia (2.2 mg/dl), hypocalcemia (8.5 mg/dl), and secondary hyperparathyroidism (PTH: 74 pg/ml); his height was 119.3 cm (−2.3 SDS) at that time (Table 1). Treatment with phosphate and $1,25(OH)_2D$ was re-initiated, and while under treatment, his plasma FGF23 level was at the upper end of the normal range (see Figure 1, panel B).

Similar clinical and biochemical findings were observed for his brother (#6) at the age of 16 months and for his female cousin (#2) at the age of 12.5 years. Their laboratory data at first presentation are shown in Figure 1, panel B; note that the brother #6 had at the age of 9 years, during treatment with oral phosphate and $1,25(OH)_2D$, an elevated serum FGF23 level of 167 RU/ml; the cousin #2 had not received any treatment and at the age of 20 years, her FGF23 level was elevated to 257 RU/ml. All other family members were healthy and had normal blood chemistries and normal FGF23 levels. The parents of the three affected children are first-degree cousins (see Figure 1, panel A).

The autosomal recessive mode of inheritance made it unlikely that the hypophosphatemic disorder in this family is caused by mutations in either *PHEX* (mutated in patients with XLH) (5) or FGF23 (mutated in patients with autosomal dominant hypophosphatemic rickets, ADHR) (1), and we therefore analyzed *DMP-1*. Nucleotide sequence analysis of the PCR-amplified exons encoding DMP-1 and the flanking intronic regions led in the index case #5 to the identification of a novel homozygous mutation in exon 6 (c.485Tdel) that resulted in a frame-shift replacing the 335 conserved amino acids within the carboxyl-terminal region with 53 unrelated amino acids (p.Glu163ArgfsX53) (Figure 2); the same homozygous mutation was also identified in the affected brother of the index case (#6) and the affected cousin (#2). The parents of #5 and #6 (i.e. #4 and #8; see Fig. 1, panel A), the mother of #2 (i.e. #3) and the two available unaffected siblings (#1 and #7) were heterozygous for c.485Tdel; results of nucleotide sequence analyses were confirmed by digesting PCR-amplified DNA with the restriction endonuclease *Eco*NI (Figure 1, panel C); using this method, none of the 148 alleles from healthy controls, which included 36 alleles from healthy Turkish individuals, carried the c.485Tdel mutation.

Upon treatment with oral phosphate and $1,25(OH)_2D$, serum phosphorous levels of index case (#5) and his effected brother (#6) increased to 2.8 mg/dl and 3 mg/dl, respectively, calcium and PTH levels normalized. Despite improved serum phosphorous levels, the index case #5 and his affected brother #6 showed no catch-up growth over a two-year observation period as their height standard deviation score (SDS) remained at −2.4 to −2.5 and −4.2 to −3.9, respectively. Both of the patients were prepubertal during this period. Their effected female cousin (#2) was diagnosed at the age of 12.5 years during early pubertal development (breast Tanner stage II) and her height improved slightly from an SDS of −4.5 at the age of 12.5 years to −4.05 after 1 year of treatment. She then discontinued treatment, but her SDS improved to −2.86, which could be related to a constitutional delay of pubertal development and thus late fusion of the growth plates (menarche at age 15.5 years). Nevertheless, her final height was only 143.1 cm, which remains 5 cm below the midparenteral height (Table 1).

Upper-to-lower segment ratio (US/LS) of the two younger affected individuals (#5 and #6) was more than +2 SD above the mean of the Turkish reference range (32). There was a further increase in US/LS ratio over a two-year observation period while on therapy with oral phosphate and $1,25(OH)_2D$, as indicated by the further loss in lower segment SDS to −0.8 and −0.9 SDS, respectively, and a further gain in the upper segment to +0.3 and +0.8 SDS, respectively; a similar growth pattern was also observed in their untreated cousin #2 (Table 1). These findings indicate that our ARHP patients increased their height, regardless of treatment, mainly because of an increase in upper body segment, i.e. the vertebral column. Arm span height difference was within the reference range for #5 and #6 (albeit slightly higher than +2 SDS for #5) when the diagnosis was established and did not change during treatment. In addition, the younger brother (#6) had limited arm extension at elbow and mild cubitus valgus deformity. The intercondylar distance had decreased from 15.5 cm to 8 cm in older brother (#5) and from 10 cm to 5 cm in more severely affected brother (#6) during the 2 years treatment period; the final intercondylar distance of the cousin (#2) was 5 cm.

Radiographs the lower extremities of patient #6 revealed cupping, metaphyseal fraying and bowing of the femurs, which improved after treatment (Figure 3). The medial aspect of the femurs and showed evidence for osteosclerotic changes; similar findings were made for patients #2 and #5 (data not shown). The hand radiographs of patients #5 and #6 revealed bone ages that were consistent with their chronological ages. However, the metacarpals and proximal phalangial bones were short and broad (>2 SD below the mean of healthy controls (26); and additional broading and shortening of the distal phalangial bones, most prominent for the 1^{st}, 3^{rd} and 4^{th} finger of the more severely effected brother (#6), became apparent during the 2 years of treatment (Figure 4). An increase in bone mineral density was detected in patient #2 at the age of 20 years (T-score for L1-L4: +9.4; at the femoral neck: +5.1).

Review of the dental history of the index case (#5) revealed that the loss of deciduous central incisors had occurred at the age of 4.5 years after moderate trauma to the chin. The growth of his permanent teeth was normal. However, after another minor trauma, his lower central incisor fractured, leading to recurrent dental abscesses. Dental examination at the age of 10.5 years revealed transverse fissures on upper central incisors, giving the impression of traumatic tooth injury. Furthermore, a sinus tract on the buccal gingiva of the left mandibular central incisor was noted. The affected younger brother (#6) and the affected cousin (#2) had, at the age of 9 and 20 years, respectively, unremarkable dental histories, although each showed decay of at least one tooth. The panoramic radiographs of affected individuals #2, #5, and #6 revealed wider pulp chambers, thinner dentin, and thinner enamel in erupted teeth in comparison to healthy controls, whereas the shape and the size of each tooth and its root were normal (Figure 5). While enamel thicknesses of unerupted teeth were normal in affected individuals, the enamel of erupted teeth was thinner, indicating rapid post-eruption attrition. Retroalveolar graphs of patient #6 showed, in comparison to healthy controls and to her 21 year old healthy sister (heterozygous carrier #1) wider pulp chambers, thinner dentin, and thinner enamel and a possible mineralization defect (Figure 6). Additionally, a panoramic radiograph revealed the absence of the lower third molar in patient #2; individual #1 had one remaining deciduous tooth due to the absence of the root of the permanent tooth.

Discussion

Human DMP-1 is encoded by an mRNA with an open reading frame of 1539 nucleotides, and the translated pre-protein of 513 amino acids undergoes extensive post-translational modifications. The resulting 94-kDa mature DMP-1 rapidly undergoes cleavage by subtilisin-like proprotein convertases to generate a 57 kDa and a 37 kDa fragment (20). Homozygous *DMP-1* mutations are the cause of ARHP (9,19), a rare autosomal recessive disorder with

laboratory and skeletal changes similar to those observed in XLH (5), and are furthermore consistent with the findings in *Dmp-1*-ablated mice (9,17).

The three affected individuals of a previously not reported consanguineous ARHP kindred carry a novel homozygous *DMP-1* mutation (c.485Tdel) that results in a frame-shift replacing most of the conserved carboxyl-terminal portion of DMP-1 with 53 unrelated amino acid residues (Figure 7). The mutation thus eliminates the entire 57 kDa DMP-1 fragment as well as the carboxyl-terminal portion of the 37 kDa fragment. Like the c.485Tdel mutation identified in our kindred, two previously identified nucleotide changes, c.362delC and c.del1484-1490, are also frame-shift mutations (9,19). While the c.362delC mutation eliminates even more of the protein than does c.485Tdel, the c.del1484-1490 mutation results only in the loss of the most carboxyl-terminal 18 amino acid residues. However, affected individuals carrying either of the previously described deletions presented at similar ages as our patients, and they showed a similar degree of hypophosphatemia and inappropriately elevated renal phosphate excretion.

The fourth DMP-1 mutation leading to ARHP is a homozygous c.1A>G change that results in substitution of the initiator methionine with valine (M1V) (9,19). Patients carrying this mutation show clinical and laboratory abnormalities that are similar to those observed for patients with mutations affecting the carboxyl-terminal portion of DMP-1. However, one of the patients with the M1V mutation presented at 8 years of age and with a milder phenotype than observed in the other children affected by ARHP, and he showed an excellent response to treatment, reaching a normal adult height (3 cm above to target height) (9). Two other patients carrying the same mutation presented at adult ages and both had a subnormal final height, which is probably related to their long-standing untreated disease (19). These findings could imply a genotype-phenotype correlation, namely that patients carrying the M1V mutation might respond more favorably to early and continuous treatment than patients with any of the three other mutations. However, one of the 3 patients with c.del 1484-1490 mutations reached her genetic target height, and final heights of the affected individuals in the kindred presented herein showed considerable variations, making it likely that other factors affect growth.

Bone radiographs of our patients demonstrated findings typical for rickets, i.e. metaphyseal fraying, cupping and bowing of long bones. However, shortened and widened long bones, metacarpal and phalangial bones, as well as dysplastic distal phalanges were detected in our more severely effected cases, and these latter findings are reminiscent of the chondrodysplastic bone changes observed in *Dmp-1*-null mice (36). The bone histomorphological changes of *Dmp-1*-null mice develop postnatally and are similar to those observed in *Hyp* and VDR-ablated mice (28,36). Hypophosphatemia is thus likely to contribute significantly to the bone changes in *Dmp-1*-null mice, but may not be the only etiologic factor for bone findings in ARHP. In fact, normalization of serum phosphate levels in *Dmp-1*-null mice maintained on a high phosphate diet corrected the growth plate defects and thus prevents the appearance of rickets, but did not completely normalize osteoid mineralization (9). This is similar to the findings in phosphate-treated *Hyp* mice and in patients with XLH, who show persistent bone abnormalities even when treated aggressively with oral phosphate (6,18,21,30). *Dmp-1* null newborn mice showed no apparent early tooth phenotype, even though this protein is expressed in odontoblasts and is involved in dentin formation (8). Postnatally these animals develop a profound tooth phenotype characterized by enlarged pulp chambers, increased width of predentin zone with reduced dentin and enamel thickness, and hypomineralization (35). This tooth phenotype is strikingly similar to that observed in *Dspp*-null mice (29) and shares some features of dentinogenesis imperfecta type II and III, a human disease characterized by discoloration, opalescent tooth, rapid attrition, ranging from pulpal obliteration to normal or enlarge pulp chambers, but without obvious bone changes (15). These dental findings that are similar to those observed in *Hyp* mice and are reminiscent, but appear to be less severe than those encountered patients with XLH (2-4,24,25,31,34).

In conclusion, the identified genetic mutation underscores the importance of DMP-1 in the pathogenesis of ARHP, as this protein appears to negatively regulate FGF23 expression under normal physiological conditions, thus playing an important role in the regulation of phosphate homeostasis. It remains to be clarified whether the described bone changes and growth characteristics of ARHP patients are related to the hypophosphatemia, to direct actions of FGF23 on bone, or to other abnormalities involving bone matrix proteins. Likewise, it remains to be determined whether the observed teeth findings, which appear to be similar, yet milder than those in patients with *PHEX* mutations, are caused by the hypophosphatemia and/or by increased FGF23 levels, or whether DMP-1 has a direct role in dental development and the formation of dental abscesses.

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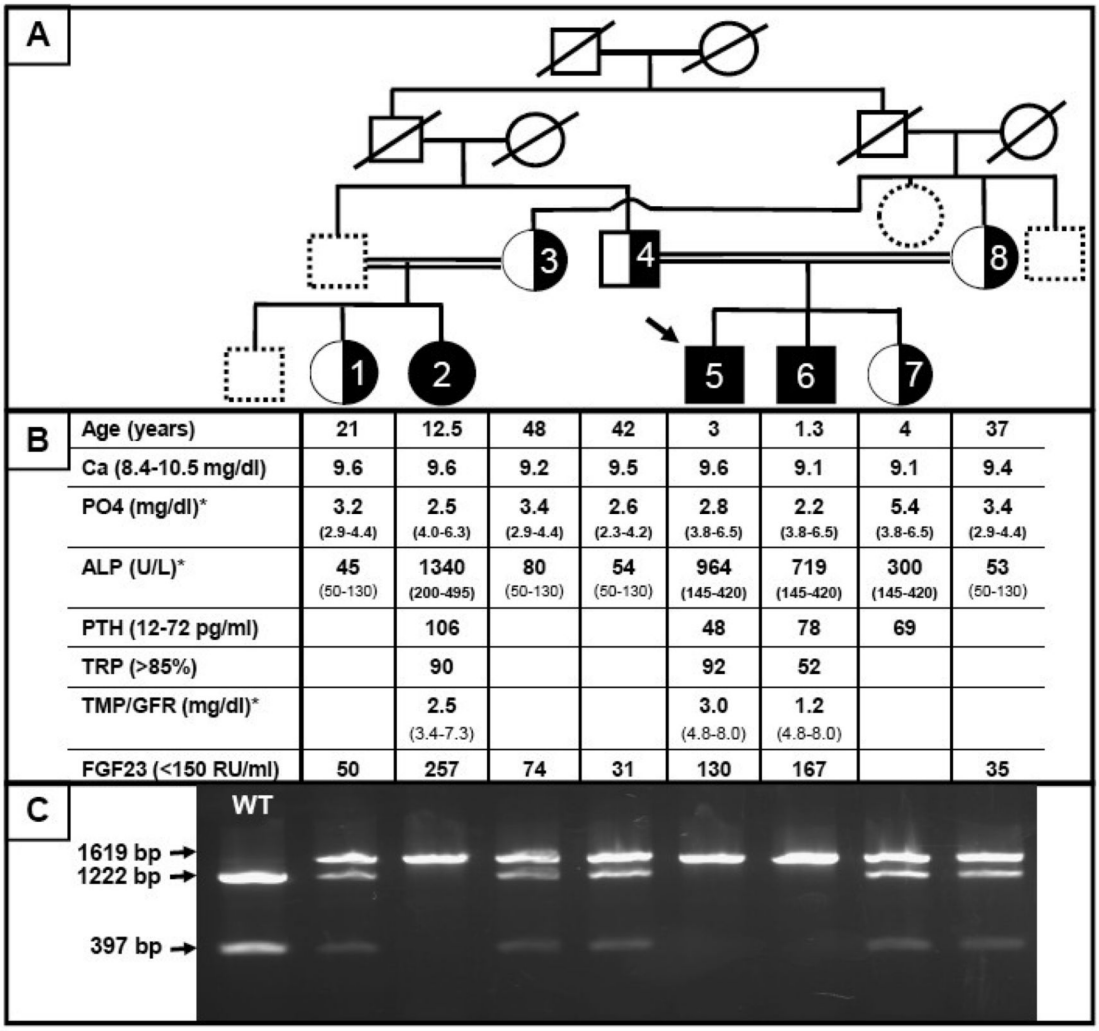

Age (years)	21	12.5	48	42	3	1.3	4	37
Ca (8.4-10.5 mg/dl)	9.6	9.6	9.2	9.5	9.6	9.1	9.1	9.4
PO4 (mg/dl)*	3.2 (2.9-4.4)	2.5 (4.0-6.3)	3.4 (2.9-4.4)	2.6 (2.3-4.2)	2.8 (3.8-6.5)	2.2 (3.8-6.5)	5.4 (3.8-6.5)	3.4 (2.9-4.4)
ALP (U/L)*	45 (50-130)	1340 (200-495)	80 (50-130)	54 (50-130)	964 (145-420)	719 (145-420)	300 (145-420)	53 (50-130)
PTH (12-72 pg/ml)		106			48	78	69	
TRP (>85%)		90			92	52		
TMP/GFR (mg/dl)*		2.5 (3.4-7.3)			3.0 (4.8-8.0)	1.2 (4.8-8.0)		
FGF23 (<150 RU/ml)	50	257	74	31	130	167		35

Figure 1.

Panel A: Pedigree of the kindred with autosomal recessive hypophosphatemia (ARHP). The index case is indicated by the arrow; solid black symbols depict affected individuals; half-filled symbols represent unaffected, obligate carriers, and healthy siblings shown to be carriers of the identified *DMP-1* mutation; stippled boxes represent healthy family members, who were not investigated; /, deceased family members. Panel B: Age at the time of laboratory measurements of affected and unaffected individuals. *Age- and sex-specific reference ranges are given in parenthesis just below the each individual's values for PO_4,, ALP and TMP/GFR. Conversion factors: Ca, 1 mg/dl = 0.25 mmol/L; PO_4, 1 mg/dl = 0.323 mmol/L. Panel C: Confirmation of the identified single nucleotide deletion (c.485delT) by digestion of PCR-amplified genomic DNA (1619 bp) with the endonuclease *Eco*NI. The PCR product derived from the wild-type allele yields two bands of 397 and 1222 bp, respectively, while the product carrying the identified mutation is not reduced in size by digestion with *Eco*NI.

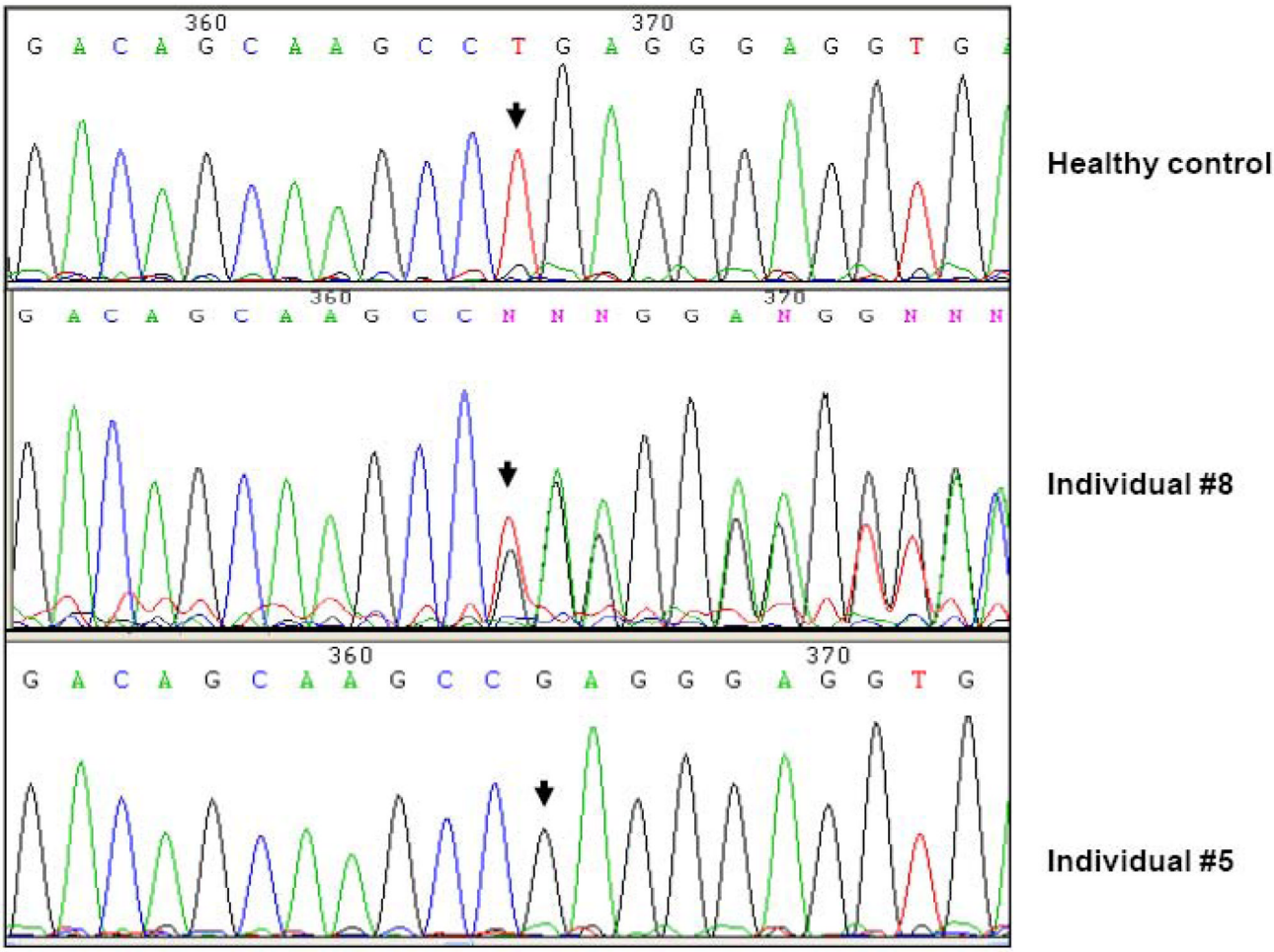

Figure 2.

Nucleotide sequence analysis of DNA from a healthy control (upper), from the unaffected family member #8 carrying a heterozygous *DMP-1* mutation in exon 6 (c.485Tdel), and from the affected family member #5 carrying a homozygous mutation.

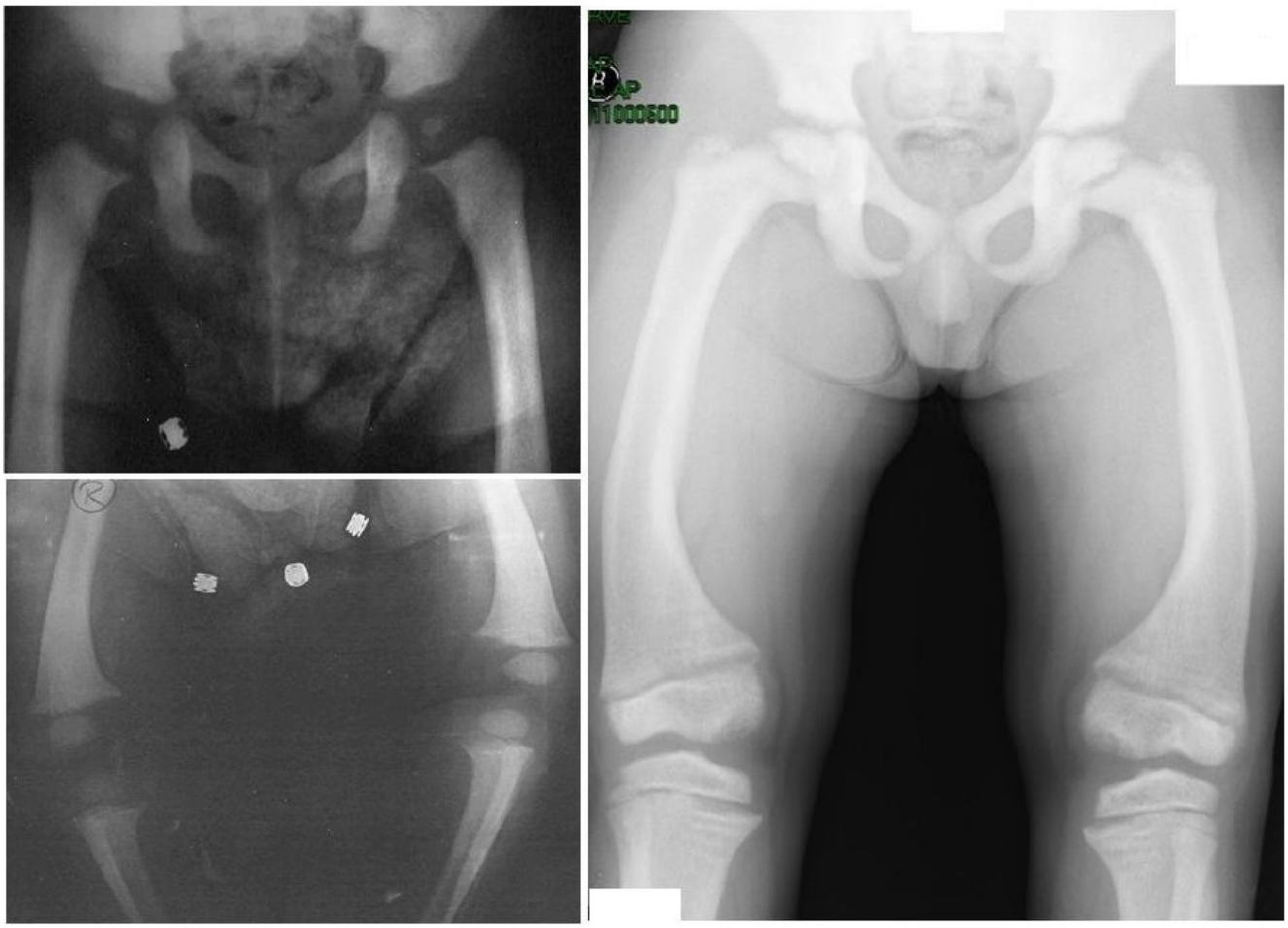

Figure 3.

Radiograph of the lower extremities of patient #6 at the age of 16 months before treatment (lower and upper left panel), which showed metaphyseal fraying, cupping and bowing. At the age of 9.5 years, after 2 years of treatment with oral phosphate and $1,25(OH)_2D$, the radiographs continued to show some metaphyseal fraying and severe bowing of the femur as well as osteosclerosis of medial aspect of femur pelvic bones (right panel).

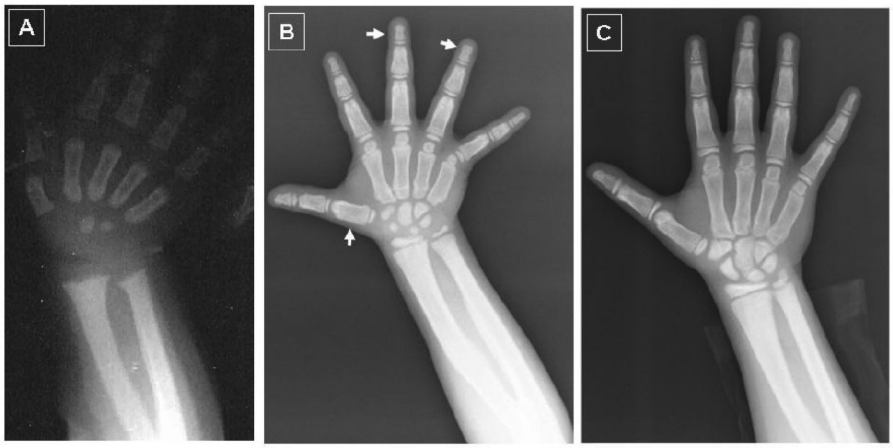

Figure 4.

Radiographs of the left hand of patient #6 at the age of 16 months (Panel A) and at the age of 9.5 years (Panel B). The metatarsals and phalangial bones were short and broad, the distal phalanges were dysplastic, and ulna and fibula were broad (Panel B) and showed an increased thickness of the growth plates, some metaphyseal fraying, and bowing (Panel A). Patient #5 (Panel C) showed at the age of 11 years, broad bones and bowing of radius.

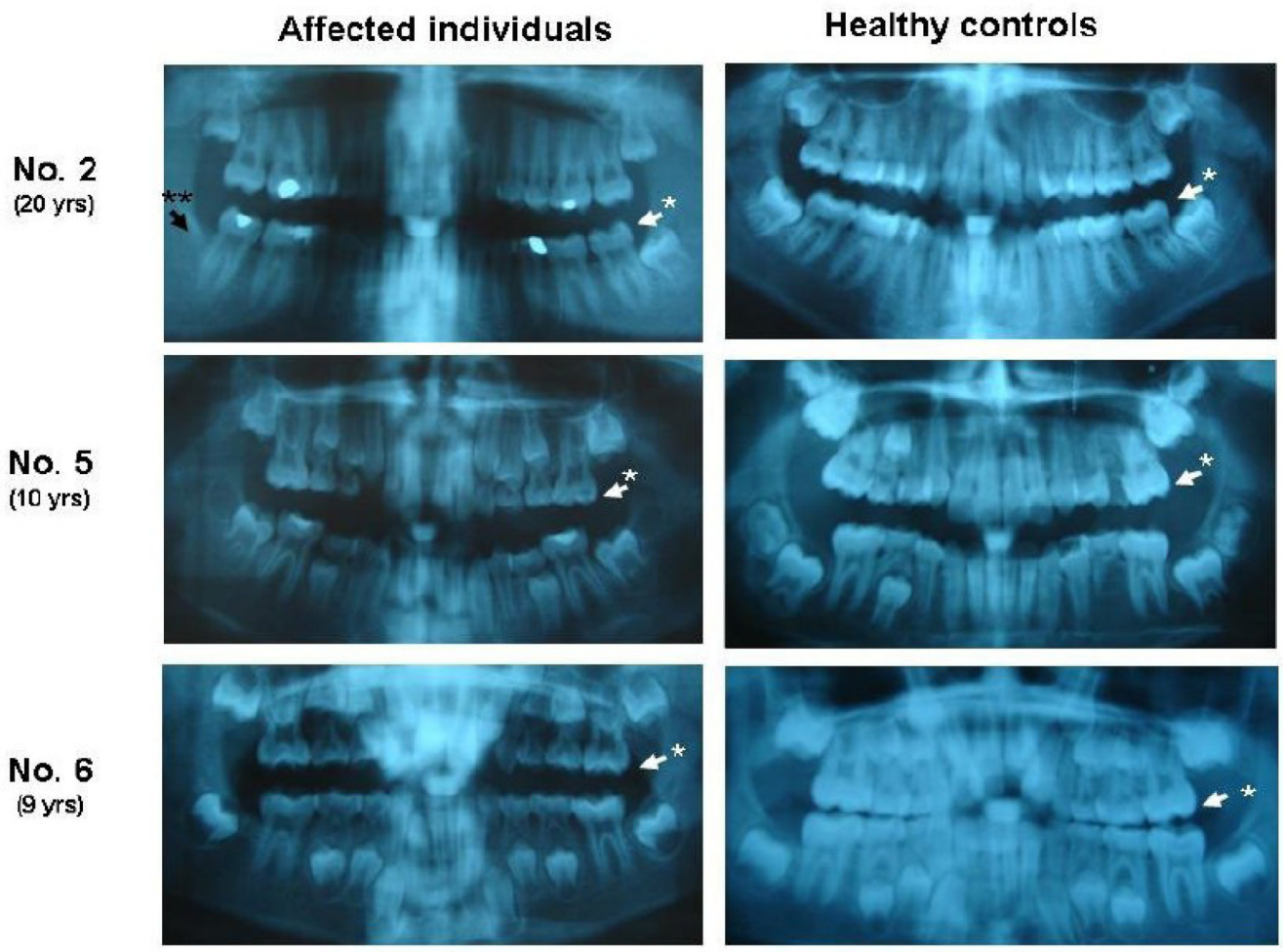

Figure 5.

Panoramic radiographs of the three affected individuals (left panel), which show in comparison to the findings in an age-matched healthy controls (right panel), enlarged pulps, decreased dentin and enamel layers (*), as well as the absence of the lower third molar (**) in individual #2.

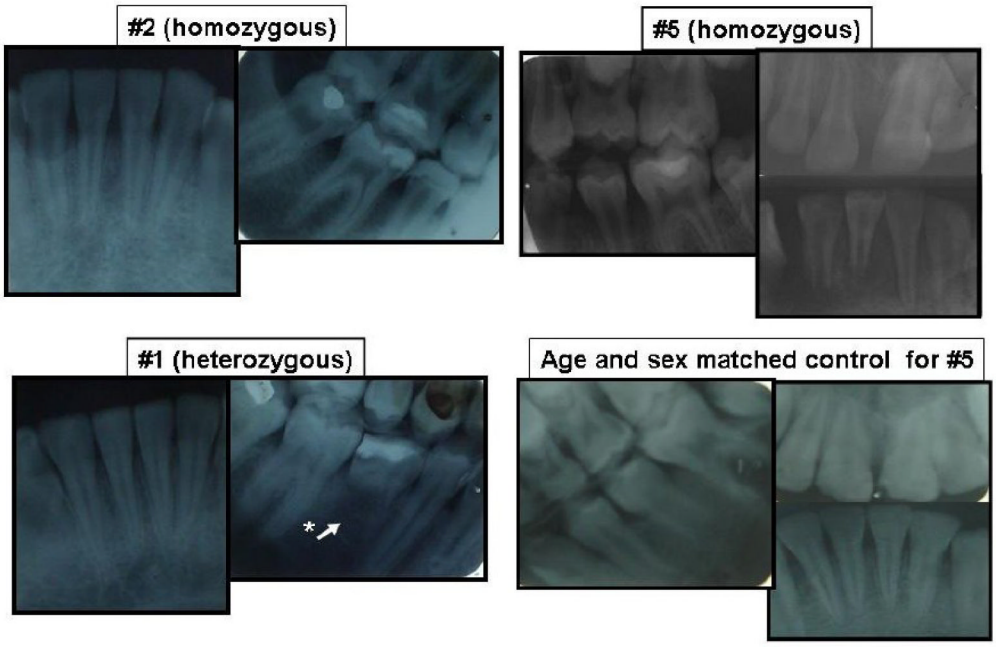

Figure 6.

Retroalveolar radiographs of the two affected individuals #2 and #5 at the ages of 20 and 11 years, respectively (upper left and right panel). The films showed, in comparison to the findings in a healthy heterozygous carrier of the mutation (#1; lower left panel) and an 11 years old age-matched healthy controls (lower right panel), hypomineralization, enlarged pulps and decreased layers of dentin and enamel; patient #1 had one remaining deciduous tooth due to the absence of the root of the permanent tooth (*)

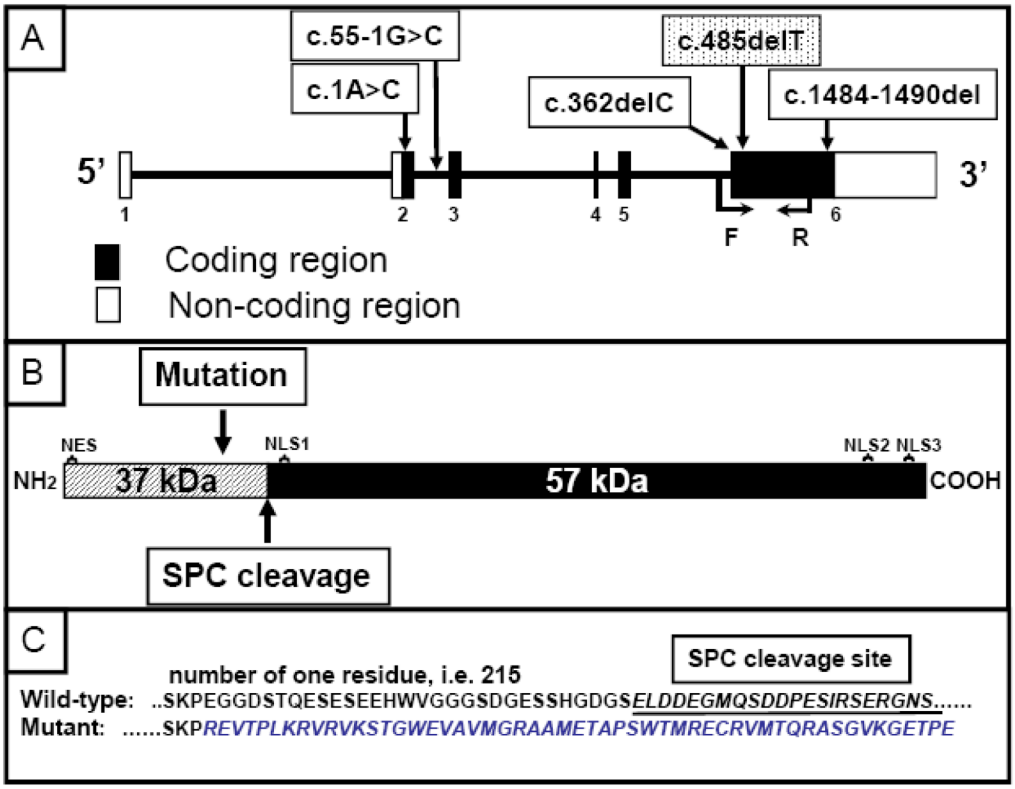

Figure 7.

Panel A: Schematic presentation of the gene encoding *DMP-1* showing the approximate locations of the *DMP-1* mutation identified in this report (c.485delT) and of previously defined mutations (9,19). Coding exons are shown as black boxes and non-coding exonic regions as white boxes. The approximate locations of the oligonucleotide primers (F, forward and R, reverse) that were used for DNA amplification are indicated.

Panel B: Schematic representation of the wild-type DMP-1 protein, which undergoes extensive post-translational modifications (glycosylation and phosphorylation) resulting in a full-length 94 kDa protein that is cleaved into a 37 kDa (stippled bar) and a 57 kDa fragment (black bar) by subtilisin-like proprotein convertases (SPC); the locations of Nuclear Export Signal (NES) and of Nuclear Localization Signals-1, -2 and -3 (NLS-1, -2 and -3) are indicated above the schematic protein molecule; arrows points to the location of the identified mutation and of SPC cleavage site.

Panel C: Partial amino acid sequences of wild-type and mutant DMP-1 proteins, number above indicates the amino acid residue; the novel unrelated amino acid sequence derived from the mutant allele that comprises 53 residues is shown in italics; the site for SPC cleavage in the wild-type protein is shown in underlined italics.

Table 1

Laboratory and growth parameters expressed as standard deviation scores (SDS) according to Turkish growth charts (23,32) at first presentation (1st) and second presentation (2nd) for patients #2, #5, and #6, and at the most recent follow-up visits for patients #5 and #6 during their second year of treatment.

	Patient #2		Patient #5			Patient #6		
	1st	**2nd**	**1st**	**2nd**	**Follow-up**	**1st**	**2nd**	**Follow-up**
Age (years)	12.5	20.0	3.0	9.2	11.4	1.3	7.5	9.8
Ca (mg/dl)	9.6	9.4	9.6	8.5	*8.6-9.3	9.1	9.1	*8.9-10.1
PO4 (mg/dl)	2.5 (2.9-5.4)	2.2 (2.7-4.7)	2.8 (3.8-6.5)	2.2 (3.7-5.6)	*2.1-2.8 (3.7-5.6)	2.2 (3.8-6.5)	2.4 (3.7-5.6)	*2.1-3.0 (3.7-5.6)
ALP (U/L)	1340 (200-495)	514 (50-130)	964 (145-420)	890 (145-420)	*790-850 (145-420)	719 (145-420)	950 (145-420)	*605-850 (145-420)
PTH (12-72 pg/ml)	106	94	48	74	*28-101	78	75	*31-60
Height SDS	−4.5	−2.86	−0.9	−2.4	−2.5	−1.8	−4.2	−3.9
Arm span SDS	−2.2	−2.2	NA	−0.6	−0.9	NA	−3.0	−2.9
Arm-Height SDS	3.5	2.1	NA	2.4	2.2	NA	−0.5	0.0
Upper Segment SDS	−2.5	−0.96	NA	−0.7	−0.4	NA	−1.8	−1.0
Lower Segment SDS	−3.4	−3.5	NA	−2.0	−2.8	NA	−3.2	−4.1
US/LS SDS	2.0	3.0	NA	2.5	3.8	NA	3.2	5.5

* Ranges are given for the laboratory findings obtained during the 2 years treatment period.