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REVIEW

Vitamin D/dietary calcium deficiency rickets and pseudo-vitamin D deficiency rickets

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This review describes the pathogenesis, clinical presentation and biochemical perturbations found in privational (nutritional) rickets and pseudo-vitamin D deficiency rickets (PDDR), an autosomal recessive condition with loss of function mutations in *CYP27B1*. It may seem strange to combine a discussion on privational rickets and PDDR as a single topic, but privational rickets and PDDR present with similar clinical signs and symptoms and with similar perturbations in bone and mineral metabolism. Of interest is the characteristic lack of features of rickets at birth in infants with PDDR, a finding which has also been reported in infants born to vitamin D-deficient mothers. This highlights the independence of the fetus and neonate from the need for vitamin D to maintain calcium homeostasis during this period. The variable roles of vitamin D deficiency and dietary calcium deficiency in the pathogenesis of privational rickets are discussed and the associated alterations in vitamin D metabolism highlighted. Although PDDR is a rare autosomal recessive disorder, results of long-term follow-up are now available on the effect of treatment with calcitriol, and these are discussed. Areas of uncertainty, such as should affected mothers breastfeed their infants, are emphasized.

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Introduction

The metabolism and action of Vitamin D have been amply discussed in other parts of this special issue. Deficiencies or abnormalities in the vitamin D pathway have a major impact on skeletal growth and health. This review will address two such situations namely simple deficiency in vitamin D and/or calcium, and heritable inactivation of the gene encoding the 25-hydroxyvitamin D-1α-hydroxylase enzyme (*CYP27B1*). In both instances, rickets and osteomalacia will develop early in life. Even though clinical features are similar in both instances, biochemical abnormalities and therapeutic approaches clearly differ.

Vitamin D and/or Dietary Calcium Deficiency Rickets

Renewed and often heated discussions concerning the definition of vitamin D deficiency and the relevant cutoffs of serum 25-hydroxyvitamin D (25OHD) to be used as a biomarker of vitamin D deficiency, have resulted from the release of the report on the dietary requirements of vitamin D by the Institute of Medicine[1] and of the clinical practice guidelines by the Endocrine Society.[2] These discussions have highlighted the marked divergence of opinion among researchers as to what is the optimal concentration of 25OHD needed to prevent nutritional rickets in children.

The appropriate level of serum 25OHD below which there is an increased risk of developing rickets is governed by a number of environmental and genetic factors, the most important of which being the role that vitamin D has in the prevention of the development of nutritional (privational) rickets. Although this seems to be rather obvious, an understanding of the pathogenesis of nutritional rickets is essential if we are to discuss the possibility that two different etiologies exist—those of vitamin D deficiency and dietary calcium deficiency as has been suggested by a number of researchers, including the authors of this review.[3,4] Complicating the discussion is the fact that it is likely that dietary calcium deficiency and vitamin D deficiency might combine to varying degrees in individual patients to have variable but often significant joint roles in the pathogenesis of privational (nutritional) rickets.

Vitamin D Deficiency Rickets

There appears to be general consensus around the pathogenesis of vitamin D deficiency rickets, which is depicted in **Figure 1a**. As a consequence of falling circulating 25OHD levels following on reduced intake or skin synthesis of vitamin D, a

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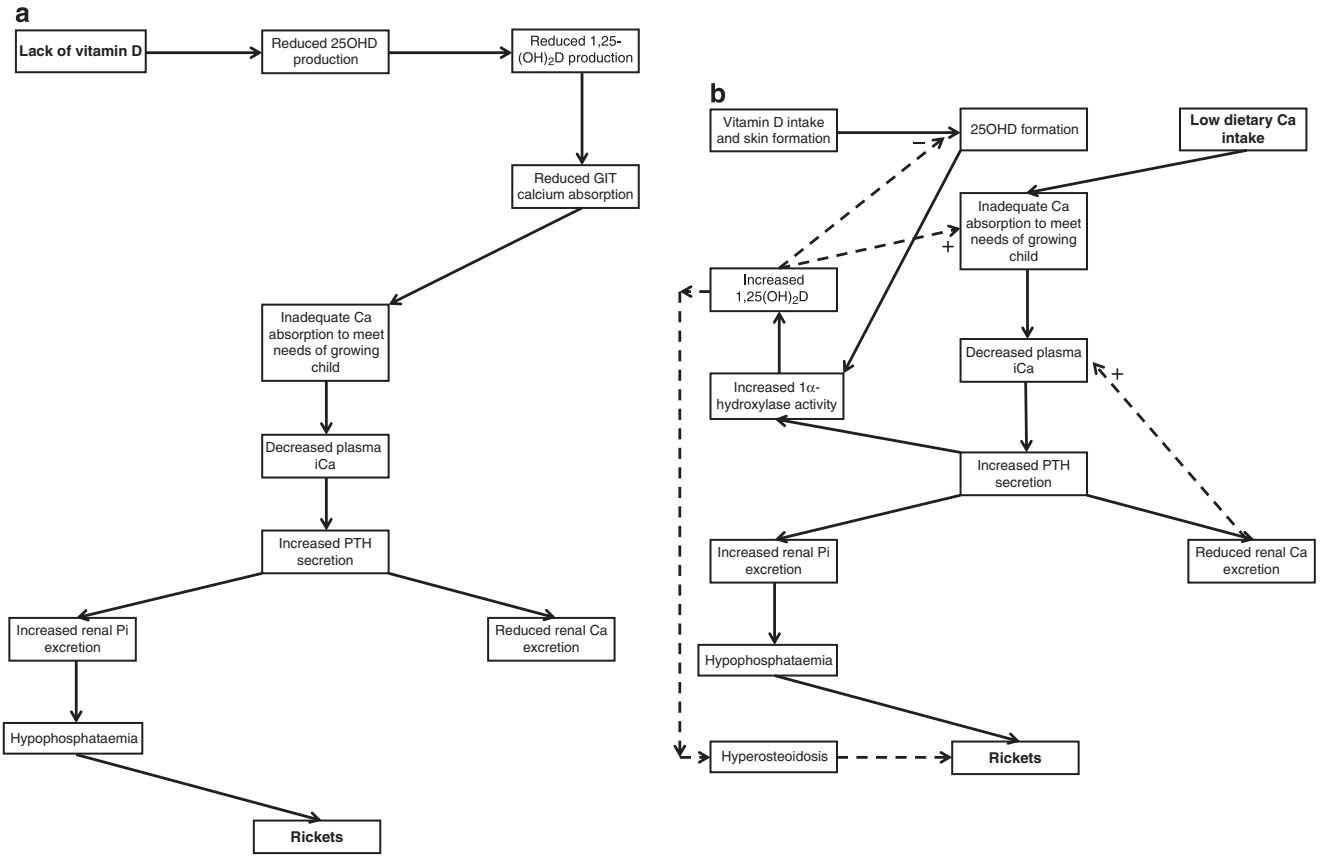

Figure 1 (**a**) Schematic representation of the pathogenesis of vitamin D deficiency rickets. (**b**) Schematic representation of the pathogenesis of dietary calcium deficiency rickets.

point is reached at which 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) concentrations can no longer be maintained at levels needed to sustain appropriate intestinal calcium absorption to meet the needs of the growing child, despite what would normally be considered an adequate dietary calcium intake. The resultant fall in circulating ionized calcium (iCa) levels stimulates PTH (parathyroid hormone) secretion, which increases renal 1α-hydroxylase activity and not only increases circulating $1,25(OH)_2D$ to levels required to return intestinal calcium absorption to that needed for normal calcium homeostasis, but also increases renal phosphate loss resulting in a decline in serum Pi concentrations. Thus a new steady state is reached associated with a mild increase in PTH, slightly lower iCa levels and an increase in renal Pi excretion associated with an increase in mineral resorption from bone.[5] With progressive worsening of vitamin D status, 25OHD levels continues to fall, such that secondary hyperparathyroidism is unable to maintain renal $1,25(OH)_2D$ production at concentrations necessary for adequate intestinal calcium absorption or for the mobilization of mineral from bone. At this stage hypocalcemia ensues. With the progressively increasing PTH concentrations, renal phosphate reabsorption is reduced further and hypophosphatemia develops, which results in the impairment of apoptosis of hypertrophied chondrocytes at the growth plate and of mineralization of matrix vesicles in osteoid, and the histological and radiological features of rickets and osteomalacia develop.[6]

Thus, the critical feature in the pathogenesis of vitamin D deficiency rickets is a reduction in intestinal calcium absorption due to inadequate $1,25(OH)_2D$ concentrations in the face of what is considered to be an adequate dietary calcium intake. Some researchers believe that 25OHD also has a role in optimizing intestinal calcium absorption.[7] However, evidence for 25OHD having more than a minimal direct role is not supported by other studies.[8] There are limited data on the levels of 25OHD and $1,25(OH)_2D$ at which calcium absorption becomes impaired, but this is probably dependent on the bioavailability of calcium in the diet and on the calcium demands of the individual among many other factors. In a cross-sectional study of osteoporotic adults, $1,25(OH)_2D$ levels and intestinal calcium absorption were only adversely affected once 25OHD concentrations fell below 10 nmol l^{-1}.[9] In adult subjects with vitamin D deficiency osteomalacia, $1,25(OH)_2D$ concentrations have been reported to be low[10] or normal[11] in association with very low levels of 25OHD.[12] Parfitt states that abnormal mineral homeostasis is only seen in privational vitamin D deficiency when 25OHD fall below 5 ng ml^{-1} (12.5 nmol l^{-1}).[12] Similar data are available for infants and toddlers with presumed vitamin D deficiency rickets. In the pediatric studies, $1,25(OH)_2D$ levels have been reported to be low,[13] normal[13] or high[14] prior to commencement of therapy. Of interest is the finding by Kruse[14] that elevated levels of $1,25(OH)_2D$ were only present in those untreated infants who were categorized as being in stage 2 rickets (with normocalcemia with hypophosphatemia). In the entire cohort, serum Ca values were directly associated with $1,25(OH)_2D$ levels despite all the children in the study having 25OHD < 12.5 nmol l^{-1}. The finding of elevated

$1,25(OH)_2D$ in stage 2 vitamin D deficiency rickets begs the question as to why the values are above concentrations which would generally be considered adequate to maintain an appropriately positive calcium balance in vitamin D replete subjects, and yet in vitamin D deficient patients do not correct the features of rickets and osteomalacia. It would suggest that the elevated $1,25(OH)_2D$ levels are being maintained by secondary hyperparathyroidism and that the resultant hypophosphatemia has an important role in the pathogenesis and maintenance of clinical and radiological rickets.[15]

The question that is important to answer if we suggest that privational rickets may be due to vitamin D deficiency, dietary calcium deficiency or a combination of both, is 'is it possible to separate vitamin D deficiency from dietary calcium deficiency based on serum 25OHD concentrations?' Many researchers indicate that 25OHD concentrations are typically lower than 10 ng ml^{-1} (25 nmol l^{-1}) in children with vitamin D deficiency rickets.[16] In a large study of rachitic children in Australia, 94% of those who were hypocalcemic (52% of the sample) had 25OHD <20 nmol l^{-1}, whereas in those who were normocalcemic only 51% has levels <20 nmol l^{-1}; it is possible that this latter group was not homogeneous and might reflect children whose dietary calcium intakes were low or who had been exposed to small doses of vitamin D/sunlight prior to 25OHD being measured.[17]

Dietary Calcium Deficiency Rickets

A number of studies have indicated the importance of low dietary calcium intake in the pathogenesis of nutritional rickets in children particularly in those living in communities in developing countries,[18–23] where calcium intakes are characteristically low and hours of daily sunshine adequate. Furthermore, a single report from the USA suggests that low dietary calcium intake might be responsible for nutritional rickets in toddlers whose dairy intakes are limited.[24] Isolated reports have also highlighted the importance of low dietary calcium intakes in the pathogenesis of rickets associated with inappropriate infant feeding, such as soy based milks not formulated for infants[25] and macrobiotic diets.[26] Mean dietary calcium intakes have been estimated to be $\sim$200 mg/day in a number of studies of children with dietary calcium deficiency rickets in different part of the world.[4,20] In the UK, low dietary calcium intakes associated with high phytate containing vegetarian diets are considered to have a major role in the pathogenesis of vitamin D deficiency in children of immigrants from the Indian subcontinent,[27] however in this group of patients vitamin D deficiency is always an associated finding.

The biochemical features that have been used to differentiate dietary calcium deficiency rickets from vitamin D deficiency rickets are 25OHD concentrations above values generally associated with vitamin D deficiency, and consistently elevated $1,25(OH)_2D$ levels (**Table 1**). It is apparent from **Table 1** that there are several characteristic features of the vitamin D metabolite levels in these children; first, mean 25OHD levels are consistently above the level defined by the Institute of Medicine[1] below which there is an increasing risk of vitamin D deficiency (30 nmol l^{-1}); second, 25OHD levels in patients are approximately a half to one-third lower than concentrations in control subjects; and third, mean $1,25(OH)_2D$ values are consistently 50–100% higher than in control subjects.

There are several possible explanations for the lower 25OHD levels in the rachitic patients than the controls, the most obvious being differences in vitamin D intake or skin synthesis. In none of the studies in which vitamin D intake has been measured were differences found between patients and controls.[28] As in most developing countries with limited access to vitamin D fortified foods, the dietary intake of vitamin D has been reported to be low, while sunlight exposure and skin coverage are reported to be similar between rachitic patients and controls.[19,20] Thus, the usual environmental and lifestyle factors responsible for determining the vitamin D status of individuals do not appear to account for the differences in 25OHD between patients and controls. Genetic variants in genes responsible for cholesterol synthesis, and vitamin D metabolite hydroxylation and transportation have been shown to influence serum 25OHD concentrations, but their roles in the pathogenesis of the lower 25OHD levels in children with dietary calcium deficiency rickets are not known. A plausible explanation for the differences in 25OHD between the two groups is provided by the faster catabolism of vitamin D and its metabolites induced by the consistently elevated concentrations of $1,25(OH)_2D$ in rachitic subjects, which was shown over 25 years ago by Clements in elegant animal experiments.[29] He showed the deleterious effects of low dietary calcium intakes and elevated $1,25(OH)_2D$ on the biological half-life of 25OHD, and postulated that these mechanisms played a role in inducing vitamin D deficiency in the Asian community in the UK.[26] Despite these findings, we still await comparative data on the half-life of 25OHD between children with presumed dietary calcium deficiency rickets and controls, which we believe will support the hypothesis that

Table 1 Vitamin D metabolite concentrations in children diagnosed as having dietary calcium deficiency rickets

Reference	*25OHD (nmol l^{-1})*		*1,25-dihydroxyvitamin D (pmol l^{-1})*		*Country*
	Controls	*Active rickets*	*Controls*	*Active rickets*	
Prentice *et al.*[22]	95 (75–115)[a]	42 (29–56)	185 (152–227)	362 (270–485)	The Gambia
Thacher *et al.*[19]	50 (42–62)	32 (22–60)	278 ± 91[b]	322 ± 96	Nigeria
Oginni *et al.*[23]	69 ± 22	36 ± 28	369 ± 134	568 ± 317	Nigeria
Fischer *et al.*[21]	62 (40–87)	50 (17–162)	182 (55–360)	327 (195–475)	Bangladesh
Pettifor *et al.*[18]	37–135[c]	62 ± 22	92 ± 30	202 ± 25	South Africa
Aggarwal *et al.*[20]	48 (31–61)	34 (25–45)			India
DeLucia *et al.*[24]	>37.5[c]	52 ± 29	75–225[c]	287 ± 112	USA

The measurements were obtained before treatment.
[a]Median and interquartile range.
[b]Mean ± s.d.
[c]Normal range.

increased catabolism of vitamin D is responsible for the lower 25OHD.

Despite lower 25OHD levels in children with dietary calcium deficiency than in controls, convincing evidence that these children are not classically vitamin D deficient is provided by the intestinal calcium absorption studies conducted in Nigerian children[30] and many years earlier in older South African children.[31] Vitamin D deficiency is characterized by low intestinal calcium absorption with 10–15% of dietary intake being reported to be absorbed;[32] however, in children with active dietary calcium deficiency rickets and markedly elevated $1,25(OH)_2D$ concentrations fractional intestinal calcium absorption was $61 \pm 20\%$,[33] which was no different from the fractional absorption in age-matched control children.

Thus the results of the calcium absorption studies do not support the contention that these children are suffering from vitamin D deficiency, however their $1,25(OH)_2D$ response to a bolus of vitamin D is similar to that found in subjects who are vitamin D deficient. Even though baseline $1,25(OH)_2D$ concentrations are elevated by some 50–100% in children with dietary calcium deficiency when compared with controls, the provision of a single bolus of vitamin D_2 or D_3 (50 000 IU) results in a further elevation of $1,25(OH)_2D$ of nearly twofold by day 3. In the control group of children, a bolus of vitamin D had no effect on $1,25(OH)_2D$ concentrations.[34]

Pseudo-Vitamin D Deficiency Rickets

The clinical features of pseudo-vitamin D deficiency rickets (PDDR) (OMIM264700) also referred to as vitamin D dependent rickets type I resemble those of simple vitamin D deficiency. They include rickets, failure to thrive, hypotonia and growth retardation.[35] Characteristically the newborn looks healthy and the symptoms appear in the second 6 months of life. At that time rickets is evident clinically (rosary, enlarged metaphyses, long bone bowing) and on radiographic films (diffuse osteopenia and metaphyseal changes). Tooth eruption is delayed, and erupted teeth show evidence of enamel hypoplasia.

Hypocalcemia is the main biochemical feature. It triggers secondary hyperparathyroidism and hypophosphatemia (less severe than in X-linked hypophosphatemic rickets). Serum levels of 25OHD and $24,25(OH)_2D$ are normal in line with vitamin D status. The hallmark is low circulating levels of $1,25(OH)_2D$[36] caused by a defective 1α-hydroxylation of 25OHD detectable at birth. It leads to hypocalcemia, hyperparathyroidism and hypophosphatemia (**Figure 1a**) causing a severe mineralization defect in the zone of provisional calcification in the growth plates (rickets) and accumulation of osteoid in cancellous and cortical bone (osteomalacia).

PDDR is a rare autosomal recessive disorder with about 100 patients reported in the literature. It has been identified in multiple ethnic groups with the highest prevalence in the French Canadian population where a Founder effect has been clearly established.[37] Understanding of the molecular etiology of PDDR came through the cloning of the gene encoding the 1α-OHase (*CYP27B1*), from man[38] and mouse.[39] The human gene was also cloned, sequenced and mapped to chromosome 12q13.1–13.3 by fluorescence *in situ* hybridization,[40] consistent with the earlier mapping of the disease by linkage analysis. The definite proof that mutations in the 1α-OHase gene are responsible for the PDDR phenotype comes from the identification of such mutations in PDDR patients and obligate carriers. To date, many mutations in the *CYP27B1* gene that codes for the 1α-OHase enzyme have been identified, including missense mutations, deletions, duplications and splice mutations. These mutations are dispersed throughout the CYP27B1 gene, affecting all exons. Most mutations associated with PDDR lead to a total loss of 1α-OHase activity when expressed *in vitro*.[41]

Early on, PDDR patients were treated with high doses of vitamin D (20 000–100 000 IU/d) in an attempt to overcome the 1α-OHase deficiency. Under such treatment, circulating levels of 25OHD increase sharply, with only minor changes in the levels of $1,25(OH)_2D$. It is likely that massive concentrations of 25OHD were able to bind to the VDR and induce the response of the target organs to normalize calcium homeostasis. Such a view is supported by the report that the cyp27b1-deficient phenotype in mice is rescued by high doses of vitamin D that drive 25OHD levels very high (250–375 nmol l^{-1}) with no changes in $1,25(OH)_2D$ levels.[42] However, because such therapy leads to progressive accumulation of vitamin D in fat and muscle tissues, adjustment in case of overdose was difficult, and slow to come into effect. Furthermore, the therapeutic doses were close to the toxic doses and placed the patient at risk for nephrocalcinosis and impaired renal function.

On the basis of the understanding of the molecular defect, the treatment of choice is replacement therapy with $1,25(OH)_2D$ (calcitriol). Before the compound became available from commercial sources, several investigators used the monohydroxylated analog 1α-OHD (1α-OHD), which requires only liver hydroxylation at the 25 position (a step not affected by the PDDR mutation) to fully mimic $1,25(OH)_2D$.[43] The response was rapid with healing of rickets in 7–9 weeks, requiring a daily dosage of 2–5 μg with the maintenance dose being about half the initial dose. Withdrawal induces a reappearance of symptoms within 3 weeks. Thus, long-term compliance is a more important consideration than in the case of vitamin D treatment. On a weight basis, $1\alpha\text{-}OHD_3$ is about half as potent as $1,25(OH)_2D$, nullifying any possible economic advantage in favor of the monohydroxylated form. The reason for this difference in potency has not been investigated, but may be related to a difference in intestinal absorption or to a variable degree of 25-hydroxylation of $1\alpha\text{-}OHD_3$. As calcitriol became commercially available in 1973, the treatment of choice for PDDR patients is replacement therapy. Treatment (either in liquid form or in capsules) is started at a dose of 1 μg per day, given in two doses of 0.5 μg. The aims of the treatment are to achieve normocalcemia, to maintain PTH levels within normal limits and to avoid hypercalciuria. Replacement therapy results in rapid and complete correction of the abnormal phenotype. Muscle tone is restored within 1 week, and radiographic healing of rickets and normalization of lumbar spine areal BMD within 3 months just as efficiently as described in children and adults who are treated for vitamin D deficiency.[44] The rapidity of the increase in BMD suggests that calcitriol treatment initially leads to the mineralization of pre-existing unmineralized osteoid rather than the production of new bone matrix. With time, the calcitriol dose is adjusted on the basis of growth rate and variations in biochemical parameters. Histological evidence of osteomalacia healing has been documented in PDDR patients. With early replacement therapy normal adult stature is achieved.[45] Follow-up over more than 35 years has

documented the safety of this therapy with only mild nephrocalcinosis on ultrasound with no alteration of renal function in 4/39 patients. Clearly, replacement therapy will have to be maintained throughout the life of the affected individual. Its safety, efficacy and simplicity will make it unlikely that therapeutic approaches based on the correction of the basic hydroxylase defect will be considered anytime soon. However, in normal individuals, endogenous synthesis of calcitriol is tightly controlled to allow for rapid and precise adaptation to changes in calcium needs and availability. In contrast, calcitriol administration (once or twice daily) in PDDR patients bypasses this feedback mechanism. Long-term regular follow-up is thus mandatory.

Adequately treated female PDDR subjects develop normally and are fertile. In our series, nine women had a total of 19 documented pregnancies.[45] During pregnancy, treatment was adjusted to maintain normocalcemia. Thus calcitriol doses were increased during 12 of the 19 pregnancies. After delivery, dosages were returned to prepregnancy levels. All pregnancies were without complications, in particular, there was no case of intrauterine growth retardation and all newborns were normocalcemic at birth. Infants born from PDDR mothers are healthy carriers of a PDDR mutation. Whether they can be safely breastfed is not established. In a single case we observed hypercalcemia developing at 4 days of age in a neonate breastfed by a mother treated with calcitriol (2 μg/day). Shifting to an infant formula rapidly normalized calcemia. The question was not further investigated but it appears prudent not to recommend breastfeeding in such circumstances.

Overall the lack of clinical findings in term PDDR infants is in line with the placenta providing calcium to the fetus without relying on vitamin D metabolites as hypocalcemia and skeletal changes of rickets are not present at birth but only develop days to months afterward. This is due to the fact that in the neonate intestinal calcium absorption is largely passive, nonsaturable and not dependent on $1,25(OH)_2D$. As postnatal age increases, enterocytes express higher levels of the vitamin D receptor and intestinal calcium absorption changes from a nonsaturable, passive process to an active, saturable, $1,25(OH)_2D$-dependent process.[46]

Initially thought to be limited to the nephron it is now established that CYP27B1 is also expressed in skeletal and non-skeletal tissues. It has been detected in chondrocytes, osteoblasts, keratinocytes, macrophages, monocytes, placenta decidual cells, vasculature endothelial cells, enterocytes and pancreas islets.[47] This array of sites suggests that calcitriol may have important autocrine and paracrine roles in various physiological functions, and there is a growing body of information along those lines. It can be assumed that in PDDR both systemic and local production of calcitriol is disrupted by the *CYP27B1* mutations. However, the follow-up of patients on replacement therapy over many years (35 years in some instances) continues to show complete rescue of the PDDR phenotype. Skeletal homeostasis is maintained and there is no clinical evidence of functional abnormalities in other tissues. One may conclude that in PDDR systemic availability of calcitriol is necessary and sufficient for overall health.

Conflict of Interest

The authors declare no conflict of interest.

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