# Short- and Long-Term Outcome of Patients with Pseudo-Vitamin D Deficiency Rickets Treated with Calcitriol

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**Background:** Pseudo-vitamin D deficiency rickets (PDDR; OMIM 264700) is a rare autosomal recessive disorder caused by mutations in the *CYP27B1* gene, leading to an inability to synthesize 1$\alpha$,25-dihydroxyvitamin $D_3$ (calcitriol). The long-term (>1 yr) effects of calcitriol replacement treatment have not been reported.

**Materials and Methods:** Thirty-nine patients (20 females) with PDDR received calcitriol for periods of 2.0–26 yr. In 21 patients, data were available at diagnosis and during the first 2 yr of treatment with calcitriol. Twenty-five patients had reached their final height at the time of this analysis.

**Results:** The most common presenting features were active rickets, neurological signs, and short stature. Treatment with calcitriol resulted in the normalization of biochemical parameters and mean lumbar spine areal bone mineral density z-scores within 3 months, whereas height z-scores increased more gradually. As to long-term effects, adult patients who had received calcitriol before the pubertal growth spurt (n = 11) had normal height, whereas patients who were treated with calcitriol only after puberty (n = 14) on average were short (height z-score −2.2). Lumbar spine areal bone mineral density z-scores were normal in all patients who had achieved final height. Nine women had 19 pregnancies, which all were without complications. All newborns were eucalcemic at birth.

**Conclusion:** Treatment with calcitriol started in infancy results in short- and long-term correction of all clinical, biochemical, and radiological abnormalities related to PDDR. (***J Clin Endocrinol Metab* 96: 82–89, 2011**)

Vitamin D is essential for the maintenance of calcium homeostasis and plays an important role in the modulation of proliferation and differentiation of several cell types (1). Vitamin D undergoes two successive hydroxylation reactions, first in the liver in which a hydroxyl group is added on carbon 25 by the activity of the vitamin D-25 hydroxylase, and second, in kidneys in which the 25-hydroxyvitamin D-1$\alpha$ hydroxylase (1$\alpha$-OHase) drives the addition of a hydroxyl group on carbon 1, leading to the formation of 1$\alpha$,25-dihydroxyvitamin $D_3$ [1,25$(OH)_2D_3$], the active hormonal form of vitamin $D_3$.

Pseudo-vitamin D deficiency rickets (PDDR; OMIM 264700; also referred to as vitamin D hydroxylation-deficient rickets type 1A, selective 1-$\alpha$, 25-hydroxyvitamin D3 deficiency, 25-hydroxycholecalciferol-1-hydroxylase deficiency, 1-$\alpha$ hydroxylase deficiency, and vitamin D dependency type 1′) is a rare autosomal recessive disease caused by mutations in the gene encoding 1$\alpha$-OHase (CYP27B1), leading to an inability to synthesize 1,25$(OH)_2D_3$ (2). Patients with PDDR develop growth retardation, rickets, hypotonia, and sometimes present with seizures (3). Apart from low circulating levels of 1,25$(OH)_2D_3$, biochemical features include hy-

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Abbreviations: BMD, Bone mineral density; 1$\alpha$-OHase, 25-hydroxyvitamin D-1$\alpha$ hydroxylase; 1,25$(OH)_2D_3$, 1$\alpha$,25-dihydroxyvitamin $D_3$; 25OH D, 25-hydroxyvitamin D; PDDR, pseudo-vitamin D deficiency rickets.

pocalcemia and secondary hyperparathyroidism, with normal to elevated levels of 25-hydroxyvitamin D (25OH D) (4).

To date, close to 100 patients with PDDR have been reported in the literature (5, 6). Although identified in multiple ethnic groups, PDDR occurs at an unusually high frequency in the French Canadian population of some areas of Québec due to a founder effect (7).

Many mutations in the *CYP27B1* gene have been identified, including missense mutations, deletions, duplications, and splice mutations (5, 6). The two most frequently observed mutations are a deletion of guanine at nucleotide 958 (g.958delG) in exon 2, commonly found in French Canadian patients, and a 7-bp duplication in exon 8, which arose independently in several populations (5). The g.958delG mutation is predicted to result in a frame shift leading to a premature stop codon (8). Most mutations associated with PDDR lead to a total loss of 1$\alpha$-OHase activity when expressed *in vitro* (8).

Historically patients with PDDR were treated with high doses of vitamin D (20,000–100,000 IU/d), in an attempt to overcome 1$\alpha$-OHase deficiency (9). However, after $1,25(OH)_2D_3$ (calcitriol) became commercially available in 1973, the treatment of choice for PDDR patients has been replacement therapy with calcitriol (10–12). In a mouse model of PDDR, this treatment cures rickets and osteomalacia and restores the biomechanical properties of bones (13). In short-term human studies, calcitriol rapidly corrected hypocalcemia, eliminated secondary hyperparathyroidism, and healed rickets (11).

Available reports on the effect of calcitriol treatment of PDDR included less than 10 patients and had follow-up periods of less than 1 yr. The long-term outcome of calcitriol treatment in patients with PDDR has not been documented. In the present study, we present the results of 39 PDDR patients who received calcitriol for a period of 2.0–26 yr.

## Patients and Methods

### Patient population

The study comprises all 31 patients with a diagnosis of PDDR who were followed up at the Shriners Hospital for Children in Montreal as well as eight patients who were followed up at Sainte Justine Hospital (Montréal, Québec) between 1977 and 2010. The data of these 39 patients (20 females, 19 males) were obtained by retrospective chart review. The total duration of follow-up ranged from 2.0 to 33 yr (median 19 yr).

The diagnosis of PDDR was based on the presence of clinical and radiological signs of rickets, hypocalcemia with secondary hyperparathyroidism, and low circulating levels of $1,25(OH)_2D$ with normal to elevated levels of 25OH D. Sequence analyses of the *CYP27B1* gene were performed in 27 individuals and identified mutations in each patient. The remaining 12 patients were not interested in genetic analysis.

For the purpose of the present analysis, two overlapping patient groups were distinguished. The pediatric group consisted of patients in whom data were available at the time of diagnosis in early childhood and during the first 2 yr of treatment with calcitriol. This pediatric group comprised 21 patients (seven females, 14 males). The adult group consisted of the 25 patients (14 females, 11 males) who at last follow-up were at final height.

At the time of the investigations presented here, all patients in the adult group had received calcitriol for 3.5–26.2 yr (median 13.8 yr). Seven of these patients had exclusively been treated with calcitriol from infancy to adulthood (adult group 1). Eighteen older patients had initially been treated with high doses of vitamin D (median dose 50,000 IU/d, range 5,000–100,000 IU/d) but subsequently received calcitriol after calcitriol had become available in Canada. In four of these patients, calcitriol was started before they had undergone the pubertal growth spurt (adult group 2), and in 14 patients calcitriol was commenced after the pubertal growth spurt (adult group 3).

The study was approved by the Shriners and Sainte-Justine Hospital Institutional Review Boards. Informed consent was obtained from the legal guardians and/or patients.

### Follow-up

After starting calcitriol treatment in newly diagnosed children, serum levels of calcium, phosphate, alkaline phosphatase, and PTH, as well as urinary calcium excretion, were evaluated once per month for the first 3 months. Thereafter children were seen every 4 months as long as growth continued. After the growth period, follow-up visits occurred every 6 months. Height and weight measurements were obtained at each visit and were converted to age- and sex-specific z-scores on the basis of reference data published by the Centers for Disease Control and Prevention (14). Renal ultrasound and ophthalmologic examinations were performed every 2 yr to assess for the presence of nephrocalcinosis and corneal calcium deposits. During pregnancy, serum levels of calcium, phosphate, alkaline phosphatase, and PTH as well as urinary calcium excretion were evaluated once every 2 months during the first two trimesters and once per month during the third trimester.

### Biochemical measurements

Serum total calcium, phosphate, and alkaline phosphatase were measured using colorimetric methods (Monarch; Instrumentation Laboratories Inc., Lexington, MA). Initially, serum PTH concentration (fragment 39–84) was determined by RIA (15). Since 2004, another RIA was used to determine active intact PTH (fragment 1–84) (immunoradiometric assay; Diasorin, Stillwater, MN).

25OH D and $1,25(OH)_2D$ were measured with RIAs (Osteo SP; Incstar Corp., Stillwater, MN). Urinary creatinine concentration was quantified colorimetrically. Patients were fasting at the time of blood and urine sampling.

### Dual-energy x-ray absorptiometry

Dual-energy x-ray absorptiometry was performed in the anterior-posterior direction at the lumbar spine (L1-L4) using a Hologic QDR 2000W or 4500 device (Hologic Inc., Waltham, MA). Areal bone mineral density (BMD) results of children were converted to age- and sex-specific z-scores using Canadian ref-

erence data (16, 17). For adults, reference data provided by the manufacturer were used. An estimate of three-dimensional BMD (volumetric BMD, unit milligrams per cubic centimeter) was derived as described by Carter *et al.* (18).

### Sequence analysis of the *CYP27B1* gene

Total genomic DNA was isolated from peripheral blood using the QIAamp DNA blood midi kit (QIAGEN Inc., Mississauga, Ontario, Canada). The coding regions of the *CYP27B1* gene, including the exon-intron boundaries, were amplified by PCR using primers previously described (8). The sequencing reaction was performed using a BigDye Terminator cycle sequencing kit (Applied Biosystems, Foster City, CA). The nucleotide sequence was determined using an Applied Biosystems 3100 DNA sequencer.

Sequence traces were aligned with the GenBank reference sequences of the CYP27B1 genomic DNA (AF027152). Mutations were numbered following the convention (http://www.hgvs.org/mutnomen/recs.html), which starts with the translation initiator methionine as amino acid +1, and the A of the ATG codon as nucleotide +1.

### Statistical analysis

Raw results were transformed to age- and sex-specific z-scores from the average result in the reference population using the published reference data cited in the description of measurement techniques. The expected mean result of these transformed values in a healthy population is 0. The significance of the difference from 0 was calculated by the one-sample *t* test.

Differences between the groups were tested for significance using the Mann-Whitney *U* test for pairwise group comparisons and Kruskal-Wallis test for comparisons between more than two groups. Bonferroni's adjustment was used to adjust for multiple testing. Wilcoxon test was used to analyze changes during treatment. All tests were two tailed and throughout the study $P < 0.05$ was considered significant. These calculations were performed using the SPSS software, version 11.5 for Windows (SPSS Inc., Chicago, IL).

## Results

### Mutations in the *CYP27B1* gene

Homozygous or compound heterozygous mutations were found in all 27 patients who had sequence analysis of the *CYP27B1* gene (Supplemental Table 1). Eighteen patients were homozygous for the g.958delG frame shift in exon 2. One patient was homozygous for the g.3392-3398 duplication in exon 8. All other patients were compound heterozygous. Among these, four patients were compound heterozygous for the g.958delG frame shift and the g.3392-3398 duplication. Compared with the mutations previously published, five new mutations were found (g.913G>C, g.1609delC, g.3311A>G, g.3430G>A, g.3902G>C). The clinical presentation of the four patients with missense mutations (patients 5, 12, 16, and 19) was indistinguishable from that of the patients with frame shift mutations.

### Pretreatment results in young children

One baby (patient 1, Table 1) was assessed at the age of 1 month in the absence of any symptoms because a sibling was affected with PDDR. This infant had a height of 56.7 cm (z-score +0.8), a weight of 4.95 kg (z-score +0.9), and there were no clinical or radiological signs of rickets. Normal results were found for serum levels of total calcium

**TABLE 1.** Clinical characteristics in the pediatric group at diagnosis

| Patient | Gender | Age (yr) | Presenting signs and symptoms | Height (z-score) |
|---|---|---|---|---|
| 1 | M | 0.1 | None (detected by biochemical screening) | 0.8 |
| 2 | M | 0.5 | Hypocalcemic seizure | 2.0 |
| 3 | M | 0.6 | Dyspnea secondary to ribs fractures | −2.1 |
| 4 | F | 0.8 | Hypocalcemic seizure | ND |
| 5 | M | 0.9 | Decreased growth velocity, hepatosplenomegaly, anemia | −2.4 |
| 6 | M | 1.0 | Muscle weakness | ND |
| 7 | M | 1.0 | Hypocalcemic seizure, motor delay (not sitting) | −2.0 |
| 8 | M | 1.1 | Motor delay, not walking, muscle weakness | −2.7 |
| 9 | F | 1.1 | Motor delay, not walking | −0.4 |
| 10 | M | 1.2 | Motor delay, not walking | ND |
| 11 | M | 1.3 | Motor delay, not walking, pain during mobilization | −1.9 |
| 12 | M | 1.4 | Motor delay, not walking (seizure at 10 months without examination) | −3.2 |
| 13 | M | 1.4 | Motor delay, not walking, decreased growth velocity | −2.8 |
| 14 | M | 1.5 | Motor delay, not walking, with stagnation at 15 months | −1.9 |
| 15 | M | 1.6 | Motor delay, not walking | −1.7 |
| 16 | F | 1.7 | Motor delay, not walking, decreased growth velocity | −4.0 |
| 17 | M | 1.8 | Short stature | −3.4 |
| 18 | F | 1.8 | Motor delay, not walking | −2.3 |
| 19 | F | 2.0 | Motor delay, not walking, decreased growth velocity | −2.9 |
| 20 | F | 2.1 | Motor delay (muscle biopsy performed) | −3.2 |
| 21 | F | 2.6 | Motor delay, not walking, with stagnation at 18 months | −4.3 |

ND, Not documented; M, male; F, female.

**TABLE 2.** Results in symptomatic children with PDDR at the time of diagnosis (n = 20)

| | n | Median (range) | Reference range |
|---|---|---|---|
| Number (female/male) | 20 | 20 (7/13) | |
| Age (yr) | 19 | 1.4 (0.5; 2.6) | |
| Height (z-score) | 17 | −2.4 (−4.3; 2.0)[a] | |
| Weight (z-score) | 16 | −1.9 (−3.8; 1.3)[a] | |
| Total calcium (mmol/liter) | 17 | 1.66 (1.20; 2.18) | 2.25–2.63 |
| Phosphate (mmol/liter) | 14 | 1.31 (0.59; 2.60) | 1.23–1.62 |
| Alkaline phosphatase (IU/liter) | 15 | 2514 (833; 3867) | <300 |
| 25OH D (nmol/liter) | 11 | 86 (35; 184) | 34–91 |
| $1,25(OH)_2D$ (pmol/liter) | 8 | 8 (3; 14) | 65–134 |

[a] $P < 0.0001$ for comparison with 0 by one-sample *t* test.

(2.31 mmol/liter), phosphorus (2.49 mmol/liter), alkaline phosphatase (263 U/liter), and 25OH D (107 nmol/liter). However, the serum $1,25(OH)_2D$ level was low (12 pmol/liter), thus establishing the diagnosis of PDDR. This was subsequently confirmed by finding a homozygous g.958delG deletion in the *CYP27B1* gene.

The other 20 patients in the pediatric group were symptomatic at the time of the first evaluation. The majority of patients presented with neurological signs, such as delayed gross motor development and hypotonia (n = 14) or hypocalcemic seizures (n = 3) (Table 1). Clinical and radiological signs of active rickets were found in all of these 20 children. Twelve of the 17 patients (70%) with a documented pretreatment height had short stature (height z-score at or below −2.0), and only two children had a height z-score above −1.

The pretreatment median height and weight z-scores of the 20 symptomatic children were significantly below the average values expected for healthy children (Table 2). Evaluation of pretreatment growth curves, available in 13 children, revealed that growth trajectories started to cross the percentile curves in a downward direction at a median age of 9 months (range 5–21 months). Age at presentation was negatively associated with height z-score ($R^2 = 0.48$, $P = 0.002$) (Fig. 1).

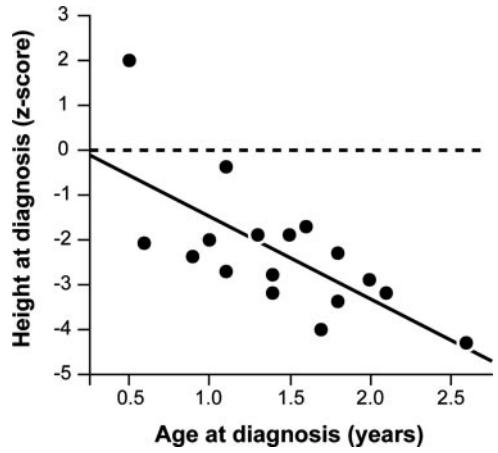


**FIG. 1.** Height z-score according to age at time of diagnosis in young children with PDDR.

At the time of the pretreatment evaluation, all 20 of these children had hypocalcemia and high alkaline phosphatase levels. Serum phosphorus levels, documented in 14 children, were variable, with low values (<1.23 mmol/liter) in five children. Baseline PTH levels, documented in nine children, were very high (on average 8-fold above the upper limit of the assay specific reference range; range 2.8- to 17-fold), indicating secondary hyperparathyroidism. In 12 patients, baseline serum levels of $1,25(OH)_2D$ were not available because they had initially been diagnosed at another hospital. Baseline lumbar spine areal BMD, documented in five children, was very low with a median z-score of −5.2 (range −6.4 to −3.2).

## Evolution during the first 2 yr of calcitriol treatment in newly diagnosed children

Calcitriol solution was started on an outpatient basis at a dose of 1.0 μg/d, given in two doses of 0.5 μg. Subsequently the calcitriol dose was modified according to the results of biochemical analyses. The aims of the treatment were to achieve normocalcemia, to maintain PTH levels within normal limits, and to avoid hypercalciuria. After 3 months of treatment, the median daily calcitriol dose was 0.50 μg/d (range 0.2–1.0 μg). Median daily calcitriol doses were 0.25 μg after 1 yr (range 0.1–1.0 μg) and 0.25 μg after 2 yr (range 0.1–0.5 μg) of treatment.

Treatment with calcitriol resulted in the normalization of serum levels of calcium, phosphate, alkaline phosphatase, and PTH within 3 months. In the five patients with available bone densitometry data, lumbar spine areal BMD z-scores increased markedly in the first 3 months of treatment and then remained stable (Fig. 2). In the 12 patients with a documented pretreatment height, height z-scores increased more gradually.

The onset of walking had been documented in eight children who at the time of referral (median age of 18 months, range 12–24) had never walked. In these children, the median duration for walking after starting calcitriol treatment was 3 months (range 2–6 months).

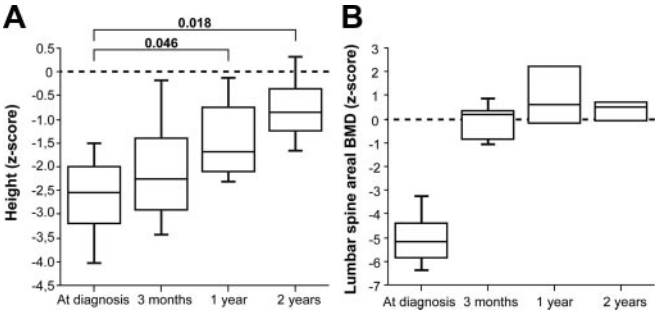


**FIG. 2.** Evolution of height (in 12 patients) and areal BMD of the lumbar spine (in five patients) during the first 2 yr of treatment with calcitriol in young children with PDDR.

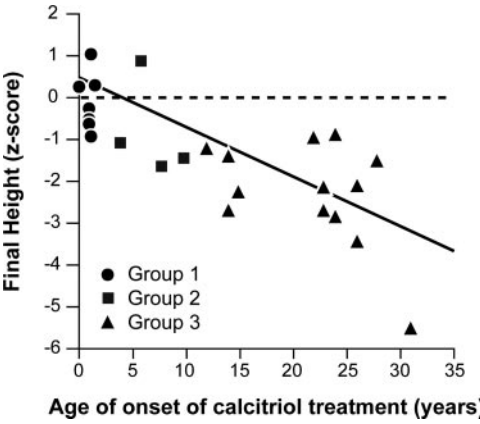


**FIG. 3.** Final height according to age of onset of calcitriol treatment in adult patients with PDDR. Group 1, Exclusively treated with calcitriol. Group 2, Initially treated with high doses of vitamin D but started calcitriol before the pubertal growth spurt. Group 3, Initially treated with high doses of vitamin D but started calcitriol after the pubertal growth spurt.

### Evolution of calcitriol doses from childhood to adulthood

The evolution of calcitriol doses from 4 to 17 yr of age was available in seven patients (one female, six males). After the initial adjustments described in the previous section, calcitriol doses were increased as needed to maintain PTH levels within normal limits. In the period from 4 to 9 yr of age, the median daily calcitriol dose increased from 0.25 to 0.50 $\mu$g. From 11 to 15 yr of age, median calcitriol doses increased from 0.50 to 0.75 $\mu$g/d.

### Long-term outcome in adults

During calcitriol treatment, 25OH D serum levels were normal in all 17 adult patients with available data (median of 101 nmol/liter, range 50–145 nmol/liter), and $1,25(OH)_2D$ levels were normal in 12 of 15 adult patients (median 101 pmol/liter, range 72–163 pmol/liter; normal range 65–134 pmol/liter) and slightly low in the three remaining patients (57, 58, and 61 pmol/liter).

The height of adult patients varied with treatment history and was significantly associated with the age at which calcitriol treatment was started ($R^2 = 0.57$, $P < 0.0001$) (Fig. 3). Patients who received calcitriol only after the pubertal growth spurt (group 3) were significantly shorter than patients who were treated with calcitriol since infancy (group 1) or patients who initially were treated with high doses of vitamin D but then started calcitriol treatment before the pubertal growth spurt (group 2, Table 3). In group 3, height was significantly lower than in the general population ($P < 0.001$), whereas average height in the other two groups was as expected for a healthy population. Lumbar spine areal BMD z-scores were normal in all adult patients, regardless of treatment history.

There was no evidence of increased bone fragility. Fracture history at the time of the last follow-up (available in 22 patients; median age 45.4 yr; range 18.4–063.9 yr) revealed that three patients had sustained fractures (wrist, humerus, and metatarsal bone) while receiving calcitriol, but these fractures occurred after significant trauma and healed normally.

Severe enamel hypoplasia was documented in 13 of 16 adult patients of groups 2 and 3 (81%), necessitating dental extraction in 10 patients. In group 1, two of five (40%) patients had some enamel hypoplasia but had not required dental extraction.

Screening for potential adverse events of hypercalcemia revealed that one patient (4% of the study population) had mild corneal calcium deposits, and four patients (16%) had mild nephrocalcinosis on renal ultrasound. The median urinary calcium to creatinine ratio of the 236 urine tests that were performed in the patients with nephrocalcinosis (0.37 mmol/mmol) was similar to the median urinary calcium to creatinine ratio of the 1068 urine test that were performed in the patients without nephrocalcinosis (0.34 mmol/mmol).

Except for moderate arterial hypertension in two patients, there was no history of other serious health disorders in the group of adult patients.

### Pregnancies in women with PDDR

Nine women with PDDR had a total of 19 documented pregnancies (Supplemental Table 2). During pregnancy, doses of calcitriol were adjusted according to the results of biochemical analyses (obtained every 4 wk) to maintain serum calcium levels within normal limits. Thus, calcitriol doses were increased during 12 of the 19 pregnancies. After delivery, calcitriol treatment was continued at prepregnancy doses. All these pregnancies were without complications. In particular, there was no case of intrauterine growth retardation and all newborns were normocalcemic at birth.

**TABLE 3.** Auxological, biochemical, and radiological data of adult patients with PDDR

| | Group 1 | | Group 2 | | Group 3 | | |
|---|---|---|---|---|---|---|---|
| | n | Value (range) | n | Value (range) | n | Value (range) | *P* |
| Number (female/male) | 7 | 7 (1/6) | 4 | 4 (4/0) | 14 | 14 (9/5) | |
| Age (yr) | 7 | 20.0 (17.0; 27.0) | 4 | 20.5 (18.0; 26.0) | 14 | 31.0 (24.0; 45.0)[a,b] | 0.0009 |
| Age of onset of calcitriol treatment (yr) | 7 | 1.0 (0.1; 1.6) | 4 | 7.0 (4.0; 10.0) | 14 | 23.0 (12.0; 31.0) | 0.0001 |
| Duration of calcitriol treatment (yr) | 7 | 19.1 (15.2–26.2) | 4 | 14.8 (11.2–18.2) | 14 | 11.0 (3.5–24.1)[a] | 0.0025 |
| Dose of calcitriol treatment ($\mu$g/d) | 7 | 0.75 (0.75; 1.00) | 3 | 1.25 (0.50; 1.50) | 14 | 0.5 (0.50; 1.00) | 0.24 |
| Height (z-score) | 7 | −0.3 (−0.9; 1.0) | 4 | −1.3 (−1.6; 0.9) | 14 | −2.2 (−5.5; −0.9)[a] | 0.0008 |
| Weight (z-score) | 7 | 0.5 (−1.4; 1.4) | 4 | 1.0 (0.43; 2.18) | 14 | −1.3 (−3.0; 1.4)[a,b] | 0.011 |
| Total calcium (mmol/liter) (Norm: 2.25–2.63) | 7 | 2.37 (2.29; 2.50) | 4 | 2.33 (2.26; 2.55) | 12 | 2.32 (2.10; 2.45) | 0.39 |
| Phosphate (mmol/liter) (Norm: 1.23–1.62) | 7 | 1.24 (0.87; 1.36) | 4 | 1.07 (0.75; 1.22) | 12 | 0.87 (0.74; 1.13)[a] | 0.008 |
| Alkaline phosphatase (UI/liter) (Norm: <300) | 7 | 86 (47; 130) | 4 | 85 (39; 158) | 12 | 61 (35; 103) | 0.36 |
| PTH (% normal maximal value) | 7 | 82 (42; 166) | 3 | 90 (50; 90) | 11 | 60 (50; 150) | 0.99 |
| Urinary calcium/creatinine ratio | 7 | 0.4 (0.1; 0.7) | 4 | 0.3 (0.1; 0.6) | 11 | 0.5 (0.1; 1.1) | 0.50 |
| LS volumetric BMD (Z score) | 7 | −1.1 (−1.9; 2.7) | 3 | 0.9 (0.0; 3.0) | 14 | 0.4 (−0.9; 2.3) | 0.42 |

*P* values were calculated using the Kruskal-Wallis test. Group 1 was exclusively treated with calcitriol. Group 2 was initially treated with high doses of vitamin D but started calcitriol before the pubertal growth spurt. Group 3 was initially treated with high doses of vitamin D but started calcitriol after the pubertal growth spurt. Norm, Normal.

[a] Significantly different between groups 1 and 3.

[b] Significantly different between groups 2 and 3.

## Discussion

Here we describe the largest cohort of PDDR patients that has been reported until now. Most patients were initially brought to medical attention as infants with neurological symptoms, especially delayed walking. This may be related to the importance of vitamin D for muscle strength and highlights the fact that the diagnosis of rickets should be considered in infants with developmental delay (19).

The distribution of presenting signs and symptoms seems to differ somewhat between PDDR and vitamin D deficiency rickets. Compared with the patients with vitamin D deficiency rickets reported by Ladhani *et al.* (20), our patients with PDDR presented more frequently with short stature (70% in patients with PDDR *vs.* 3% in patients with vitamin D deficiency rickets), motor delay (70 *vs.* 3%), and clinical signs of rickets (100 *vs.* 70%). In contrast, infants with PDDR seem to present with seizures less frequently than patients with vitamin D deficiency rickets (15 *vs.* 25%).

Contrary to patients with severe vitamin D deficiency who can present within the first 6 months of age (20), none of the PDDR patients described here were symptomatic before the age of 6 months. This is unlikely to be due to the presence of maternal $1,25(OH)_2D_3$ in the circulation of the infant because $1,25(OH)_2D_3$ does not cross the feto-placental barrier (21). Indeed, the infant who was diagnosed with PDDR at the age of 1 month had a serum low level of $1,25(OH)_2D$ and a positive *CYP27B1* sequencing result but did not have any clinical or radiological signs of rickets. This indicates that $1,25(OH)_2D$ is not critical for mineral ion homeostasis and growth plate mineralization in the first months of life. Similarly, 1$\alpha$-OHase deficient mice appear normal from birth until weaning (22).

After the start of calcitriol treatment, biochemical parameters quickly normalized, as previously described (11). A new observation is that lumbar spine areal BMD also normalized within 3 months. A similar increase in areal BMD has been reported in children and adults who were treated for vitamin D deficiency (23). The rapidity of the increase in areal BMD suggests that the treatment initially led to the mineralization of preexisting unmineralized osteoid rather than the production of new bone matrix. The height deficit persisted longer than the low areal BMD, but after 2 yr of calcitriol treatment, height was also normalized and remained so until adulthood. It is noteworthy that even those patients who received calcitriol only after puberty had normal areal BMD, even though their average height was low. It thus appears that having low levels of $1,25(OH)_2D_3$ during childhood is compatible with achieving a normal peak bone mass.

Although essentially expressed throughout the nephron, 1$\alpha$-OHase activity is also detected in chondrocytes and osteoblasts. It has been hypothesized that local production of $1,25(OH)_2D_3$ could play an important autocrine and paracrine role in endochondral ossification and chondrocyte development (24). Indeed, observations in mice suggest that chondrocyte-specific inactivation of Cyp27b1 or the vitamin D receptor leads to abnormalities in endochondral bone development (25, 26). These mouse data are somewhat at odds with the present observations in humans. It can be assumed that in our patients both the systemic and the local production of $1,25(OH)_2D$ were disrupted due to *CYP27B1* mutations but systemic treat-

ment with calcitriol nevertheless led to a normal growth rate and normal areal BMD. Despite careful follow-up over many years, no skeletal abnormalities were observed in patients with PDDR who had received calcitriol since infancy. It thus seems that in humans local production of $1,25(OH)_2D$ in bone or growth cartilage cells is not critically important for skeletal development and homeostasis, even though subclinical growth plate abnormalities could evidently not be excluded in our patients.

Female mice that are deficient in 1α-OHase (and that do not receive calcitriol) have uterine hypoplasia and absent corpora lutea and thus are infertile (22). The *1α-OHase* gene is expressed in human endometrial stromal cells with a significant increase in early pregnancy, suggesting a potential role of local production of $1,25(OH)_2D$ in pregnancy establishment or maintenance (27). In our study, female pubertal development seemed to be normal, and 19 pregnancies were documented in nine of the 13 women who were above 20 yr of age. It therefore appears that local production of $1,25(OH)_2D$ in female reproductive organs is not critical for fertility because systemic supplementation was sufficient to achieve fertility.

The pregnancies that occurred in the women of the present cohort were without any complications, and all newborns were normocalcemic at birth. This indicates that the approach of determining calcium levels every 4 wk during pregnancy and to increase calcitriol doses as needed to maintain normocalcemia was a successful strategy.

Treatment with calcitriol was well tolerated in our population, with a low rate of adverse events that might be attributable to hypercalcemia. In particular, mild nephrocalcinosis was observed in 16% of the patients described here, whereas about 80% of patients with hypophosphatemic rickets receiving calcitriol have been reported to develop nephrocalcinosis (28). The risk of nephrocalcinosis may be lower in patients with PDDR because phosphate excretion is normal, whereas phosphate excretion is elevated hypophosphatemic rickets.

### Conclusion

Treatment with calcitriol started in infancy results in short- and long-term correction of all clinical, biochemical, and radiological abnormalities related to PDDR, without serious adverse events.

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## References

1. **Bikle DD** 2010 Vitamin D: newly discovered actions require reconsideration of physiologic requirements. Trends Endocrinol Metab 21:375–384
2. **Fu GK, Lin D, Zhang MY, Bikle DD, Shackleton CH, Miller WL, Portale AA** 1997 Cloning of human 25-hydroxyvitamin D-1 α-hydroxylase and mutations causing vitamin D-dependent rickets type 1. Mol Endocrinol 11:1961–1970
3. **Fanconi A, Prader A** 1969 [Hereditary pseudo-vitamin D deficiency rickets]. Helv Paediatr Acta 24:423–447
4. **Scriver CR, Reade TM, DeLuca HF, Hamstra AJ** 1978 Serum 1,25-dihydroxyvitamin D levels in normal subjects and in patients with hereditary rickets or bone disease. N Engl J Med 299:976–979
5. **Wang X, Zhang MY, Miller WL, Portale AA** 2002 Novel gene mutations in patients with 1α-hydroxylase deficiency that confer partial enzyme activity *in vitro*. J Clin Endocrinol Metab 87:2424–2430
6. **Kim CJ, Kaplan LE, Perwad F, Huang N, Sharma A, Choi Y, Miller WL, Portale AA** 2007 Vitamin D 1α-hydroxylase gene mutations in patients with 1α-hydroxylase deficiency. J Clin Endocrinol Metab 92:3177–3182
7. **De Braekeleer M, Larochelle J** 1991 Population genetics of vitamin D-dependent rickets in northeastern Québec. Ann Hum Genet 55: 283–290
8. **Wang JT, Lin CJ, Burridge SM, Fu GK, Labuda M, Portale AA, Miller WL** 1998 Genetics of vitamin D 1α-hydroxylase deficiency in 17 families. Am J Hum Genet 63:1694–1702
9. **Arnaud C, Maijer R, Reade T, Scriver CR, Whelan DT** 1970 Vitamin D dependency: an inherited postnatal syndrome with secondary hyperparathyroidism. Pediatrics 46:871–880
10. **Balsan S, Garabedian M, Courtecuisse V, Gueris J, Dommergues JP, Creignou L, le Denuff MJ, Rivron J** 1977 Long-term therapy with 1α-hydroxyvitamin D3 in children with 'pseudo-deficiency' rickets. Clin Endocrinol (Oxf) 7(Suppl):225s–230s
11. **Delvin EE, Glorieux FH, Marie PJ, Pettifor JM** 1981 Vitamin D dependency: replacement therapy with calcitriol? J Pediatr 99: 26–34
12. **Reade TM, Scriver CR, Glorieux FH, Nogrady B, Delvin E, Poirier R, Holick F, DeLuca HF** 1975 Response to crystalline 1α-hydroxyvitamin D3 in vitamin D dependency. Pediatr Res 9:593–599
13. **Dardenne O, Prudhomme J, Hacking SA, Glorieux FH, St-Arnaud R** 2003 Rescue of the pseudo-vitamin D deficiency rickets phenotype of CYP27B1-deficient mice by treatment with 1,25-dihydroxyvitamin D3: biochemical, histomorphometric, and biomechanical analyses. J Bone Miner Res 18:637–643
14. **Ogden CL, Kuczmarski RJ, Flegal KM, Mei Z, Guo S, Wei R, Grummer-Strawn LM, Curtin LR, Roche AF, Johnson CL** 2002 Centers for Disease Control and Prevention 2000 growth charts for the United States: improvements to the 1977 National Center for Health Statistics version. Pediatrics 109:45–60
15. **D'Amour P, Labelle F, Lecavalier L, Plourde V, Harvey D** 1986 Influence of serum Ca concentration on circulating molecular forms of PTH in three species. Am J Physiol 251:E680–E687
16. **Faulkner RA, Bailey DA, Drinkwater DT, McKay HA, Arnold C,**

**Wilkinson AA** 1996 Bone densitometry in Canadian children 8–17 years of Age. Calcif Tissue Int 59:344–351
17. **Salle BL, Braillon P, Glorieux FH, Brunet J, Cavero E, Meunier PJ** 1992 Lumbar bone mineral content measured by dual energy X-ray absorptiometry in newborns and infants. Acta Paediatr 81:953–958
18. **Carter DR, Bouxsein ML, Marcus R** 1992 New approaches for interpreting projected bone densitometry data. J Bone Miner Res 7:137–145
19. **Ward KA, Das G, Berry JL, Roberts SA, Rawer R, Adams JE, Mughal Z** 2009 Vitamin D status and muscle function in post-menarchal adolescent girls. J Clin Endocrinol Metab 94:559–563
20. **Ladhani S, Srinivasan L, Buchanan C, Allgrove J** 2004 Presentation of vitamin D deficiency. Arch Dis Child 89:781–784
21. **Kovacs CS** 2008 Vitamin D in pregnancy and lactation: maternal, fetal, and neonatal outcomes from human and animal studies. Am J Clin Nutr 88:520S–528S
22. **Panda DK, Miao D, Tremblay ML, Sirois J, Farookhi R, Hendy GN, Goltzman D** 2001 Targeted ablation of the 25-hydroxyvitamin D 1$\alpha$ -hydroxylase enzyme: evidence for skeletal, reproductive, and immune dysfunction. Proc Natl Acad Sci USA 98: 7498–7503
23. **Adams JS, Kantorovich V, Wu C, Javanbakht M, Hollis BW** 1999 Resolution of vitamin D insufficiency in osteopenic patients results in rapid recovery of bone mineral density. J Clin Endocrinol Metab 84:2729–2730
24. **Anderson PH, Atkins GJ** 2008 The skeleton as an intracrine organ for vitamin D metabolism. Mol Aspects Med 29:397–406
25. **Naja RP, Dardenne O, Arabian A, St. Arnaud R** 2009 Chondrocyte-specific modulation of Cyp27b1 expression supports a role for local synthesis of 1,25-dihydroxyvitamin D3 in growth plate development. Endocrinology 150:4024–4032
26. **Masuyama R, Stockmans I, Torrekens S, Van Looveren R, Maes C, Carmeliet P, Bouillon R, Carmeliet G** 2006 Vitamin D receptor in chondrocytes promotes osteoclastogenesis and regulates FGF23 production in osteoblasts. J Clin Invest 116:3150–3159
27. **Viganò P, Lattuada D, Mangioni S, Ermellino L, Vignali M, Caporizzo E, Panina-Bordignon P, Besozzi M, Di Blasio AM** 2006 Cycling and early pregnant endometrium as a site of regulated expression of the vitamin D system. J Mol Endocrinol 36:415–424
28. **Verge CF, Lam A, Simpson JM, Cowell CT, Howard NJ, Silink M** 1991 Effects of therapy in X-linked hypophosphatemic rickets. N Engl J Med 325:1843–1848