Clinical Case Reports

CASE REPORT

Case report: vitamin D-dependent rickets type 1 caused by a novel *CYP27B1* mutation

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Key Clinical Message

Vitamin D-dependent rickets type 1 VDDR-1 is a recessive inherited disorder with impaired activation of vitamin D, caused by mutations in *CYP27B1*. We present long-time follow-up of a case with a novel mutation including high-resolution peripheral quantitative computed tomography of the bone. Adequate treatment resulted in a normalized phenotype.

Keywords

Introduction

Vitamin D is one of the major hormones regulating blood levels of calcium and mineral homeostasis of the bone. It is an inactive prohormone that can be ingested or synthesized in the skin. The production of the active hormone, calcitriol or 1α,25-dihydroxyvitamin D_3 (1,25$(OH)_2$D), results from sequential hydroxylation by members of the cytochrome P-450 enzymes [1]. The regulated step is 1-hydroxylation (Fig. 1) by 1-α-hydroxylase (1α-OHase), primarily expressed in the kidney [2]. 1,25$(OH)_2$D exerts its biological function by binding to a nuclear receptor in target tissues, thereby altering transcription of various vitamin D-dependent proteins. In short, 1,25$(OH)_2$D facilitates bone mineralization by promoting osteoblast function and osteoclast differentiation while maintaining blood calcium and phosphate levels through enhanced gastrointestinal and renal absorption [3]. It also regulates its own activity by up-regulating 24-hydroxylase (which metabolizes 1,25$(OH)_2$D) and fibroblast growth factor-23 (FGF-23) and down-regulating parathyroid hormone (PTH) [2,3].

Vitamin D-dependent rickets type 1 (VDDR-1) [4], also known as pseudovitamin D deficiency rickets (PDDR)[5] is a rare autosomal recessive disease where 1α-OHase deficiency impedes the activation of vitamin D (MIM #264700). Symptoms usually present at an age of 6–12 months with growth retardation and irritability, presumably due to bone pain and muscle weakness. At this age, clinical rickets is evident with classical symptoms such as widening of ankles, wrists and costochondral junctions, frontal bossing, delay in closure of fontanelles, and eruption of teeth, [6–8]. Laboratory findings include hypocalcaemia, hypophosphatemia, and elevated serum alkaline phosphatase and PTH. Patients with VDDR-1 may present with secondary reduced urinary calcium excretion, aminoaciduria, and hyperchloremic acidosis [9]. Plasma levels of 25-hydroxyvitamin D are normal, but levels of 1,25$(OH)_2$D are low or undetectable [4].

CYP27B1 (MIM #609506), the only gene in which mutations cause VDDR-1, is located on chromosome 12q13.3 [10]. To date, more than 50 different mutations
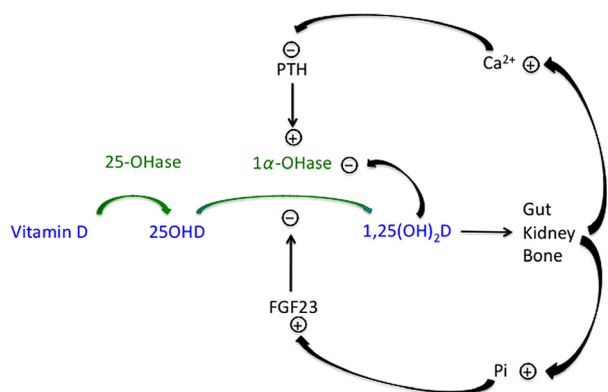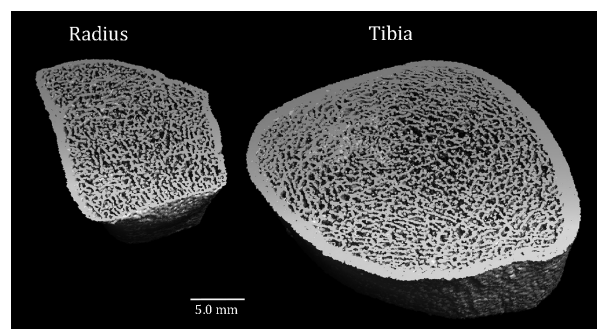
Figure 1. Regulation of Vitamin D hydroxylation. Vitamin D is sequentially hydroxylated by hepatic 25-OHase (*CYP2R1*) and renal 1α-OHase (*CYP27B1*) rendering the active hormone 1,25(OH)$_2$D. 1-hydroxylation is the rate limiting and regulated step. 1α-OHase is stimulated by parathyroid hormone (PTH) and inhibited by FGF-23 and by itself.

Figure 2. HR-pQCT scans of distal radius and tibia of patient with VDDR-1.

have been reported including missense or nonsense mutations, deletions, splicing mutations, and duplications [11–14]. The clinical symptoms regress and biochemical findings normalize within months of sufficient treatment with calcitriol, but the necessary dose varies between individuals, which can only in part be explained by molecular studies of the specific mutations [12,15]. Successful cessation of vitamin D treatment around puberty has been reported in a very limited number of cases of VDDR-1 [15–17].

Bone mineral density (BMD) assessed by dual-energy X-ray absorptiometry (DXA) was markedly reduced (median z-score −5.2) at diagnosis in five children with VDDR-1 and normalized within 3 months of treatment [18]. While DXA measures bone mass, high-resolution peripheral quantitative computed tomography (HR-pQCT) measures both bone mass, geometry and microarchitecture (Fig. 2). We report a novel mutation causing VDDR-1, and the clinical and skeletal phenotype assessed by use of DXA and HR-pQCT.

Clinical report

The male patient was born after an uneventful pregnancy by spontaneous delivery. The parents are nonconsanguineous healthy Caucasians, and the patient has two elder and one younger healthy siblings. Birth weight and length were 3430 g and 51 cm, respectively. He was fully breastfed the first 6 months and partly breastfed until age 10.5 months. Growth and development milestones were normal including the ability to sit at 6 months, crawl, and stand up at 8 months. At the age of 8 months, growth and weight stagnated, and he lost the

ability to crawl and stand. There was no history of seizures. The first clinical examination at 18 months revealed height −3 SD and weight −3.5 SD. Craniofacial features were not clearly dysmorphic, but a triangular mouth and low ears were noted. Musculoskeletal examination showed a generally atrophic body with muscle weakness and reduced subcutaneous fat, and signs of rickets with rachitic rosary, lack of tooth eruption, palpable widening of distal radius, and double malleoli. Biochemical tests (normal intervals in parenthesis) showed markedly decreased ionized serum calcium 1.08 mmol/L (1.20–1.35 mmol/L), serum phosphate 0.72 mmol/L (1.20–1.80 mmol/L) and 1,25(OH)$_2$D 24 pmol/L (adults 71–177pmol/L), normal serum chloride 107 mmol/L (102–112 mmol/L) and increased serum alkaline phosphatase 14020U/L (55–515U/L), PTH 57.0 pmol/L (1.1–6.9 pmol/L), and serum 25-(OH)D 96 nmol/L (adults: 37–120 pmol/L with supplements and 10–66 without). Twenty-four-hour urine showed signs of phosphate wasting with tubular reabsorption of 65% (normally above 80%), low calcium excretion, ketoaciduria, and hyperaminoaciduria. There was a tendency toward metabolic acidosis with negative base excess of −5. According to the medical record, radiologic findings included general rough halisteresis of all bones with severe cupping of metaphyses, poorly defined bone nuclei, and subperiosteal bleedings. There were fractures of both ulna and tibia with visible fracture lines and callus. Chest X-ray was described with rachitic rosary, and otherwise normal heart and lung findings.

On suspicion of hereditary hypophosphatemic rickets, treatment was initiated with vitamin D, calcium, and phosphate supplements, and later changed to alfacalcidol, as the diagnosis was corrected to VDDR-1. Within 5 months of treatment, 0.5 μg alfacalcidol daily sufficed to hold blood samples within normal ranges. Normal growth and development resumed, and normal bone age was reached at 9 years of age. Treatment was adjusted

according to the levels of serum calcium and phosphate, PTH, and urinary calcium/creatinine ratio. Minor nephrocalcinosis was observed on ultrasound, but renal function remained normal. On average, daily doses of alfacalcidol varied between 0.36 and 1.29 *µ*g, showing a tendency toward higher doses with increasing age.

At 18 years of age, the patient reported neither symptoms of rickets nor fractures after initiation of treatment. He reported having experienced bone pain and general malaise after a few days without medication. Physical examination revealed a height of 167.6 cm, corresponding to the expected height estimated from his parents, and weight of 58.4 kg. A prominent sternoclavicular joint and upper costosternal joints on the right side were observed. Chest X-ray showed no visible pathology (Fig. S1). Blood samples were within normal ranges and renal ultrasound was without nephrocalcinosis.

DXA showed Z-scores of −1.0 and +1.3 in the lumbar spine (L1-4) and total hip, respectively. HR-pQCT (Table 1 and Fig. 2) showed overall values for bone mineral density (BMD), trabecular and cortical BMD, trabecular number, thickness and spacing, and cortical thickness comparable to a cohort of 64 Danish males aged 20-29 [19], and was thus considered normal.

Materials and methods

DNA Sequencing

To identify the genetic mutations causing VDDR-1, genomic DNA was isolated from peripheral blood leucocytes of the patient and his parents after obtaining written informed consent. All exons and exon-intron boundaries of *CYP27B1* (RefSeq: NM_000785.3) were analyzed by bidirectional sequencing using the BigDye® Terminator v.3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA, USA) and an ABI3730XL capillary sequencer (Applied Biosystems).

Mutation prediction

We used the prediction programs PolyPhen-2, Mutationassessor, Mutationtaster, and SIFT to assess the effect of amino acid substitution for p.Arg432Ser, which had not been previously described. Clustal W (MegAlign; DNA STAR, Madison, WI, USA)) was used to test the evolutionary conservation of the substituted amino acid by alignment.

Results and Discussion

Direct sequencing of *CYP27B1* revealed two missense mutations. The exon 7, c.1166 G>A, p.Arg389His (CGT>CAT) mutation was inherited from the mother and has previously been identified as disease causing [15,17,20,21]. The second mutation, exon 8, c.1294 C>A p.Arg432Ser (CGC>AGC), has, to the best of our knowledge, not previously been described (Fig. S2). The latter mutation was not identified among the parents and may be a "de novo" mutation or due to gonadal mosaicism. A mutation located in the same codon c.1294 C>T p.Arg432Cys (CGC>TGC) was evaluated by Cui et al. [12] who performed site-directed mutagenesis and compared 1α-OHase activity of mutant to WT enzyme in

Table 1. HR-pQCT results from the patient compared to a group of 64 Danish men aged 20-35 years examined on the same machine as Hansen et al. [19].

	Radius		Tibia	
	Subject Result (SD from median)	Control group Mean ± SD	Subject Result (SD from median)	Control group Mean ± SD
Total area (mm^2)	292.7 (−1.1)	358 ± 61	734.2 (−1.1)	898 ± 144
Cortical area (mm^2)	54.7 (−1.7)	75 ± 12	159.5 (±0.0)	160 ± 32
Trabecular area (mm^2)	233 (−0.7)	278 ± 62	575.6 (−1.1)	737 ± 152
Total BMD (mg/cm^3)	330.3 (−0.4)	354 ± 53	368 (+0.5)	339 ± 54
Cortical BMD (mg/cm^3)	776.7 (−2.3)	873 ± 42	862.1 (−0.4)	876 ± 37
Trabecular BMD (mg/cm^3)	215.6 (+0.5)	199 ± 33	232 (+0.5)	218 ± 30
Trabecular BV/TV (%)	18 (+0.5)	16.5 ± 2.8	19.3 (+0.5)	18.1 ± 2.48
Trabecular number (1/mm)	2.44 (+1.7)	2.06 ± 0.22	2.36 (+0.6)	2.20 ± 0.26
Trabecular thickness (mm)	0.074 (−0.5)	0.080 ± 0.013	0.082 (−0.1)	0.083 ± 0.010
Trabecular spacing (mm)	0.336	0.406 [0.367–0.449][a]	0.342	0.362 [0.341–0.418][a]
1/SD Tb.N (mm)	0.119	0.163 [0.148–0.182][a]	0.135	0.153 [0.139–0.181][a]
Cortical thickness (mm)	0.78 (−1.4)	1.02 ± 0.17	1.51 (+0.8)	1.31 ± 0.25

[a]Median [p25–p75].

BMD, bone mineral density; SD, standard deviation; Tb.N, trabecular number.

Figure 3. Homology analysis. Alignment of vertebrate 1α-OHase corresponding to position 393–471 of human 1α-OHase. Identical amino acids are marked in green. R432 is boxed in red, blue box the consensus "meander" sequence; FXXGXRXCXG.

cell culture. They found a reduced activity to 6.9% of the WT enzyme.

The variant p.Arg432Ser was evaluated using the prediction softwares Polyphen2, SIFT, Mutationassessor, and Mutationtaster. All except SIFT predicted the substitution to be pathogenic. Homology analysis of p.Arg432Ser in different species found the residue to be highly conserved (Fig. 3).

Albeit having only about 20% sequence homology, CYP enzymes in both prokaryotes, and eukaryotes retain a remarkable conservation in secondary and tertiary structure [22], and thus it was possible to produce a 3D model of *CYP27B1* on the basis of the crystal structure of rabbit *CYP2C5* [23], which may be used to predict the functional consequences of mutated residues responsible for VDDR-1. The amino acid R432 in *CYP27B1* was determined to be part of a highly conserved triad of Glutamic Acid-Arginine-Arginine, the ERR-motif. Although the position of these amino acids in primary structure varies widely in different CYP enzymes, they form salt bridges and thus a highly conserved tertiary structure close to the heme-binding region. The motif was already proposed to be essential for core folding and heme stabilization by hydrogen binding with the meander region of all CYP enzymes in the first comparative studies of CYP crystal structures in 1995 and disruption of the motif renders the enzymes inactive [22]. It is thus tempting to speculate that exchange of a positively charged amino acid capable of forming salt bridges such as Arginine with an uncharged amino acid such as Serine (or Cystein [12]) may be detrimental. In 1999, a role of the ERR-motif in redox partner interaction has also been proposed [24].

Our results on BMD, measured by DXA confirmed earlier findings of a normal bone phenotype in well-treated patients with VDDR-1 [18]. In addition, the HR-pQCT showed normal geometry, bone volumetric density, and microarchitecture. These observations are probably explained by treatment with alfacalcidol and close monitoring, resulting in near-normal values of electrolytes and PTH.

In the present case study, we identified a novel *CYP27B1* mutation in exon 8 c.1294 C>A p.Arg432Ser (CGC>AGC) in a patient with clinical and biochemical manifest VDDR-1. The typical VDDR-1 phenotype of the untreated patient, as well as the documented VDDR-1 in an unrelated patient with a mutation in the same codon and the position of the mutation in an essential part of the enzyme, strongly suggests that this mutation is pathogenic. Results from homology analysis and prediction software also support this hypothesis. Definite experimental proof would require site-directed mutagenesis and functional analysis of mutant 1α-OHase protein.

Although long-term follow-up data on aged VDDR-1 patient is awaited, no skeletal abnormalities or increased fracture risk have been reported in well-treated patients. The normal bone microarchitecture and geometry in our adult patient supports that well-treated subjects may have normal bone morphology. In conclusion, we present a case study of a patient with a novel *CYP27B1* mutation and the follow-up after 18 years with adequate medical treatment. The skeletal phenotype was normalized and there were no clinical symptoms.

Conflicts of interest

The authors have on conflicts of interest to declare.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Radiographs. A. Posterior-anterior chest X-ray at age 18 years, described as normal. B. Left hand at age 14 years, described as normal bone age. C. Left clavicula at 18 years D. Right clavicula at 18 years.

Figure S2. Direct sequencing revealed the heterozygous mutation c.1166G>A, p.Arg389His inherited by the mother a presumable de novo mutation c.1294C>A; p.Arg432Ser.