Genetic and clinical characteristics of the patients with Vitamin D Dependent Rickets Type 1A

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Abstract

Objective: Vitamin D dependent rickets type 1A (VDDR1A) is an autosomal recessive disorder caused by mutations in the 25OHD 1 α -hydroxylase gene (*CYP27B1*). As it may be confused with nutritional rickets and hypophosphatemic rickets, genetic analysis is important for making a correct diagnosis.

Methods: We analysed genomic DNA from 11 patients from 8 different Turkish families. The patients were recruited for our studies if they presented with diagnosis of vitamin D dependent rickets.

Results: The mean age at diagnosis was 13.1 $\pm$ 7.4 months. Seven patients had mild hypocalcemia at presentation while 4 patients had normal calcium levels. All patients underwent *CYP27B1* gene analysis; The most prevalent mutation was the c.195 + 2T>G splice donor site mutation, affecting 5 out of 11 patients with VDDR1A. Two patients from the fourth family was compound heterozygous for c.195 +2T>G and c.195 +2 T>A in intron 1. Two patients from different families were homozygous for a previously reported duplication mutation in exon 8 (1319_1325dupCCCACCC, Phe443Profs*24). One patient had homozygous splice site mutation in intron 7 (c.1215+2 T>A). And one patient had homozygous mutation in exon 9 (c.1474 C>T).

Conclusion: Intron 1 mutation was the most common mutation as in the previous studies, and all patients carrying that mutation were from same city of origin suggesting a “founder” or a “common ancestor” effect. VDDR1A should be definitely considered when a patient with signs of rickets has a normal 25-OH level or when there is unresponsiveness to vitamin D treatment.

What is already known on this topic?

Although vitamin D dependent rickets type 1A (VDDR1A) is a rare disease, it is relatively more common in Turkey. Intron-1 mutations have been reported only from Turkey so far. Intron-1 mutations have been reported to be associated with milder clinical findings. Clinical and laboratory findings can overlap with other types of rickets. Serum 1, 25-dihydroxyvitamin D (-OH₂D) levels are usually known to be low with VDDR1A.

What this study adds?

In this study, we have observed that the patients with intron-1 mutations can present with clinical findings of variable severity. We have also emphasized that the concentrations of 1, 25-OH₂D levels could be in inappropriately normal ranges in patients with VDDR1A and can lead to diagnostic confusion.

Introduction

Vitamin D (calciferol) comprises two biologically inactive, fat-soluble pro-hormones: ergocalciferol (vitamin D₂), derived from ergosterol after UV light exposure; and cholecalciferol (vitamin D₃), derived from animal tissues and 7-dehydrocholesterol, which is formed in human skin by the action of UV rays in sunlight (1).

Both forms need a two-step hydroxylation at 25th and 1st carbons for activation. The first step occurs in the liver, where vitamin D is hydroxylated to 25-hydroxyvitamin D (25-OHD) by the hepatic 25-hydroxylase. The second step occurs mainly in the kidney, where 25-OHD is hydroxylated by the mitochondrial vitamin D 1 α -hydroxylase to the biologically active hormone 1, 25- dihydroxyvitamin D (1, 25-OH₂D), which binds to its nuclear receptor and exerts its biological activities (1, 2, 3). The biologically active 1, 25-OH₂D plays a central role in calcium homeostasis and bone metabolism, and also has a significant influence on cell proliferation and

differentiation of a variety of tissues (1, 3, 4). The renal synthesis of 1, 25-OH₂D from its precursor 25-OHD is a rate-limiting step and is tightly regulated by serum 1, 25-OH₂D, parathyroid hormone (PTH), fibroblast growth factor 23 (FGF23), calcium and phosphate, with renal 1 α -hydroxylase being stimulated by PTH, hypophosphatemia, or hypocalcaemia, and inhibited by FGF23 (4).

Four rare genetic errors of vitamin D metabolism that can cause rickets have been described. The first one involves 1 α -hydroxylase deficiency, which is also described as vitamin D dependent rickets type IA (VDDR1A). A selective mutation in *CYP27B1* gene, which leads to 25-hydroxylase deficiency, called type 1B (VDDR1B). The second type involves a defective vitamin D receptor (VDR), resulting in vitamin D resistant rickets (VDDR), also known as vitamin D dependent rickets type 2A (VDDR2A). VDDR2B is an unusual form of rickets due to abnormal expression of a hormone response element-binding protein that interferes with normal function of vitamin D receptor (5-8).

VDDR1A is an autosomal recessive disorder caused by mutations in the 25-OHD 1 α -hydroxylase gene (*CYP27B1*). *CYP27B1* is composed of 9 exons and is approximately 5 Mb in size. The gene has been mapped to the chromosomal region 12q14.1 (9-12). Clinically, VDDR1A is characterized by hypotonia, muscle weakness, inability to walk, growth failure, and radiographic findings of rickets. Typical laboratory findings are hypocalcaemia, elevated serum levels of alkaline phosphatase (ALP) and parathyroid hormone (PTH) with low or normal levels of 1, 25-OH₂D despite normal or increased concentrations of 25-OHD (9, 13). Patients with VDDR1 may present with aminoaciduria and hyperchloremic acidosis (3).

To date, over 100 patients with 78 mutations have been identified in the *CYP27B1* gene in patients from multiple ethnic groups. These mutations span all exons of the gene and mostly include missense and nonsense changes, along with splice site changes, insertions, deletions, and duplications (14, hgmd, <http://www.hgmd.cf.ac.uk/ac/index.php>). Mutations in *CYP27B1* lead to a loss of the 1 α -hydroxylase activity and require treatment with calcitriol to normalize the clinical and laboratory abnormalities (15).

In the present study, we report 11 patients with VDDR1A from 8 unrelated Turkish families. The most prevalent mutation was the c.195 + 2T>G splice donor site mutation, affecting 5 out of 11 patients with VDDR1A. Clinical findings of patients were examined in detail and genotype-phenotype correlations were evaluated.

Methods

We analyzed genomic DNA of 11 patients from 8 different Turkish families. In five of these families parents were related. The study was approved by the University of Health Science Umraniye Training and Research Hospital Clinical Research Ethical Committee (approved number: 19/01/2018- 2926), and informed consent was obtained from patients/ families.

Eleven patients had the clinical findings of rickets including; X-bain deformity or bowed leg, chest rosary, harrison's groove, frontal bossing, widening of the wrist, growth retardation, hypotonia and inability to walk, hypocalcaemic seizure. Furthermore, the patients had biochemical features suggestive of rickets such as hypophosphatemia, hypo- or normocalcemia, elevated PTH and ALP, normal or high 25-OHD levels, and low or normal 1, 25-OH₂D levels. Wrist and knee radiographs of all patients demonstrated widened physes and metaphyseal cupping and fraying. Differentiation of nutritional rickets and VDDR1A was made by: normal/high 25-OHD levels, low/inappropriately normal 1, 25-OH₂D levels and recovery of clinical, biochemical and radiological findings of rickets after replacement of calcitriol. All patients received calcitriol and patients with hypocalcaemia received calcium replacement. Calcitriol was started at a dose of 1-1.5 mcg/day, twice daily. Subsequently the calcitriol dose was titrated 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.

Targeted second generation sequence analysis

DNA was isolated from a 200 μ l peripheral blood sample using QIAamp DNA Blood Mini QIAcube Kit and QIAcube device (QIAGEN, Hilden, Germany). Then, the exons of the *CYP27B1* gene were amplified for targeted sequencing. Amplification was controlled with agarose gel electrophoresis technique. Sequencing was carried out using Illumina MiSeq NGS

System (Illumina Inc., San Diego, CA, USA) MiSeq Reagent Kit V3 (600 cycles). The readings were aligned with hg19 genomic sequence and compared.

Sanger Sequencing

10 ml venous blood sample in EDTA tube was taken from each patient. DNA isolation was performed using the QIAamp DNA Mini QIAcube Kit from the peripheral blood. The Primer design was made to include *CYP27B1* gene exons and close introns (Table 1). The products of PCR reaction [94 ° C -5 min, (95 ° C 30 sec -60 ° C 30 sec -72 ° C 30 sec) x 34, 72 ° C 5 min] with the primers, which were also shown in Table 1, were checked on a 2% agarose gel. After the amplification of correct gene regions, purification of PCR products was made by keeping 15 min at 37 ° C (enzyme activation temperature) and 15 min at 80 ° C (enzyme inactivation temperature) in the thermal cycler using ExoSAP enzyme. After purification, the primer and the cleaned template DNA were added to the PCR solution, which is called "The Big Dye Ready Reaction Mix", and the PCR reaction was

started. The purification process was repeated after the PCR sequencing for the removal of uncoupled ddNTPs in the solution. Sanger sequencing of the purified samples was performed on the ABI 3130 XL (Applied Biosystems) capillary sequencing device. The obtained data were analysed by SeqScape analysis program.

Data analysis

Sequenced data was analyzed with the Genomize Variant Analysis Program and IGV (Integrative Genomics Viewer). The homozygote or compound heterozygote variants in the databases such as NCBI, HGMD, and Clinvar were primarily selected for data filtering. The effects of mutations on protein structures were tested with various in silico prediction tools, particularly Mutation Taster (16), Polyphen-2 (17), and SIFT (18).

Statistical Analysis

Statistical analysis was performed using IBM SPSS 21.0 for windows statistical software. The data were presented as mean $\pm$ SD (ranges).

Results

Among patients diagnosed with VDDR1A, 6 were male and 5 female, making up a total of 11 patients from 8 families. Clinical presentation and laboratory findings of the patients were summarized on Table 2. The mean age at diagnosis was 13.1 ± 7.4 months. Seven patients had mild hypocalcemia at presentation while 4 patients had normal calcium levels. Five of eight families had consanguineous marriages. The two families that were not related were from the same city. All patients had clinical and laboratory features of rickets at the time of diagnosis. All patients had low levels of phosphorus whereas quite high levels of PTH and ALP levels. Five patients had fairly high levels of 25 OHD due to being formerly diagnosed with nutritional rickets and treated with vitamin D. Levels of 1, 25-OH₂D, on the other hand, were normal in 3 patients. One patient was previously followed for hypophosphatemic rickets, and treated with calcitriol and phosphate. When he was diagnosed with VDDR1A, he had elevated PTH levels and typical radiological findings of rickets (Figure-1).

After the definite diagnosis all patients received calcitriol treatment. The duration of treatment with calcitriol ranged between 6 months and 7 years. Biochemical improvement with treatment occurred in a period ranging between 4 months and 12 months.

All patients underwent *CYP27B1* gene analysis (Table 3); The most prevalent mutation was the c.195 + 2T>G splice donor site homozygous mutation, affecting 5 out of 11 patients with VDDR1A. Two patients from family-4 had compound heterozygous mutation for c.195 + 2T>G and c.195 + 2 T>A in intron 1. Two patients from different families had homozygous duplication mutation in exon 8 (1319_1325dupCCCACCC, Phe443Profs*24), which was previously reported (Figure 2). A homozygous c.1215 + 2T>A mutation in the splice donor site of intron-7 was found in one patient. And one patient was found to have a homozygous mutation in exon 9 (c.1474 C>T).

Discussion

In the present study, we report the clinical, biochemical, and genetic analysis of 11 patients with VDDR1A. We identified five previously reported mutations. The most prevalent mutation was the c.195 + 2T>G splice donor site mutation. Five patients from two different families had this mutation as homozygous and two patients from the same family had it as hemizygous as a part of compound heterozygous mutation. Durmaz E, et al (19) reported this mutation for the first time in a Turkish patient, and c.195+2T>G homozygous mutation in intron 1 is present in a total of 20 patients including ours so far, all reported from Turkey (4, 9, 19). These patients were homozygous for the previously described splice donor site mutation c.195+2T>G, where a thiamine is substituted for a guanine in the second nucleotide of intron 1. Since this mutation is common in Turkish patients and have not been reported in other ethnic groups, it may be unique representing a 'founder' or "common ancestor" effect, given the high rates of consanguinity. Although it has not been reported in other publications, all patients in the study by Tahir S, et al (9) were living in Diyarbakır or neighbouring provinces; all of our patients carrying that mutation were from Batman, which is geographically very close to Diyarbakır. While Tahir S, et al (9) reported that patients with intron 1 mutation had a milder clinical presentation, Demir K, et al (4) reported that the most severe form of the disease occurred in a patient with intron 1 mutation. We could not identify any relationship between genotype and phenotype. All patients in the literature who had an intron 1 mutation had delayed walking and bowed legs at admission. While four of our patients also had the same complaint, another patient presented with hypotonia. Although 4 of 5 patients with intron 1 mutation had a height below -2 SD, patients with other mutations also had short stature.

We had only one patient presented with hypocalcaemic convulsion at the age of 11 months. Hypocalcaemic convulsion has also been reported rarely by other studies from Turkey. Tahir S, et al (9) reported hypocalcaemic convulsion in 5 of 22 patients; Demir K, et al (4) in 4 of 8 patients; and Durmaz E, et al (19) in 2 of 7 patients. Kim JC, et al (20) reported that 4 of 10 patients presented with hypocalcaemic convulsion. Edouard T, et al (21) reported that the admission symptom was hypocalcaemic convulsion in 4 of 21 pediatric patients. As these

patients had calcium levels that are in the lower limit of normal, hypocalcemic convulsion is not frequently encountered.

The clinical presentations of patients with VDDR1A could lead to a misdiagnosis of nutritional rickets or hypophosphatemic rickets. It can be differentiated from hypophosphatemic rickets by a high PTH level and nutritional rickets by a normal 25-OH₂D level. The hypophosphatemia in VDDR1A is a result of elevated PTH and renal excretion of phosphate. Clinical and laboratory features of VDDR1A are very similar to nutritional rickets; its differential diagnosis can be made by a low or inappropriately normal 1, 25-OH₂D level and unresponsiveness to vitamin D treatment. In our study, 6 patients also had been treated with vitamin D for a long time with the diagnosis of nutritional rickets, and they had extremely high 25-OH₂D levels. Four patients had normal calcium levels, and one of them had been followed with hypophosphatemic rickets. There were a few patients with normal 1, 25-OH₂D levels diagnosed with VDDR1A in the literature (4, 8). In fact, the expected 1, 25-OH₂D levels in 1 α -hydroxylase deficiency are low, inappropriately normal 1, 25-OH₂D levels also indicate that the enzyme activity is insufficient. Recently, Nishikawa N, et al (22) reported that liver mitochondrial CYP27A1 can catalyze 1 α -hydroxylation of 25-OHD. A small increase in serum 1, 25-OH₂D level has been observed in CYP27B1 knockout mice after given high dietary vitamin D, suggesting a conversion from 25-OHD to 1, 25-OH₂D by a non-CYP27B1 enzyme. Three of eleven patients of our study had normal 1, 25-OH₂D level. There was a history of high dose vitamin D intake in two of these three patients with normal 1, 25-OH₂D. In these patients, conversion from 25-OHD to 1, 25-OH₂D by a non-CYP27B1 enzyme may contribute to the normal 1, 25-OH₂D.

Maternal 1, 25-OH₂D does not cross the fetoplacental barrier (21, 23). 1, 25-OH₂D is increasing 2-3 fold in the first weeks of pregnancy where as maternal 25-OHD crosses the placental barrier. The rise in circulating 1, 25-OH₂D concentrations in the mother facilitates optimal in utero bone development by attaining a positive calcium balance (24). Edouard T, et al (21) reported that, unlike patients with severe vitamin D deficiency who can present within the first 6 months of age, none of the VDDR1A patients were symptomatic before the age of 6 months. Indeed, the infant who was diagnosed with VDDR1A at the age of 1 month had a low serum level of 1, 25-OH₂D and a positive *CYP27B1* sequencing result but did not have any clinical or radiological signs of rickets (21). This indicates that 1,25-OH₂D is not critical for mineral ion homeostasis and growth plate mineralization in first months of life owing to in utero positive calcium balance in these patients. All patients in this study group were 6-month or older at admission.

Generally, a good response to treatment with alfacalcidol or calcitriol (10- 400 ng/kg/day) is expected in cases with VDDR1A (4, 21). Calcitriol dose was tailored based on biochemical and clinical findings. Edouard T, et al (21) indicated short and long-term outcomes of calcitriol treatment in their patients. They started calcitriol treatment at a dose of 1.0 μ g/day, given in two doses of 0.5 μ g. Treatment with calcitriol resulted in the normalization of biochemical parameters within 3 months. The aims of the treatment were to achieve normocalcemia, to maintain PTH levels within normal limits, and to avoid hypercalciuria. Our patients did not reach their final height, and their treatment duration ranged between 6 months and 7 years. Improvement of biochemical parameters occurred at a somewhat later period, between 4 months and 12 months.

Study Limitations

The main limitation of our study is the relatively small number of patients.

Conclusion

Although VDDR1A is a rare disease, it is more common in Turkey where autosomal recessive disorders are common. In this study, we evaluated the genetic and clinical features of 11 patients with the diagnosis of VDDR1A. Intron 1 mutation was the most common mutation as in the previous studies, and all patients carrying that mutation were from same city of origin suggesting a “founder” or a “common ancestor” effect. As it may be confused with nutritional rickets and hypophosphatemic rickets, genetic analysis is important for making a correct diagnosis. VDDR1A should be considered when a patient with signs of rickets has a normal 25-OH level or when there is unresponsiveness to vitamin D treatment. We have also emphasized that the concentrations of 1, 25-OH₂D levels could be in normal ranges in patients with VDDR1A and can lead to diagnostic confusion.

Conflict of interest

The author declares that there is no conflict of interest.

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Authorship Contributions

Concept: FD, BH, Design: FD, HK, EK, Data collection or processing: FD, EK, GÖ, BH, Analysis or interpretation: FD, BH, HK, EK, GÖ, Literature Search: FD, BH, HK, EK, GÖ, Writing: FD, HK, EK, BH, GÖ

References

1. Holick MF. Vitamin D deficiency. *N Engl J Med* 2007; 357: 266- 281.
2. Alzahrani AS, Zou M, Baitei EY, Alsshaikh OM, Al-Rijjal RA, Meyer BF, Shi Y. A novel G102E mutation of CYP27B1 in a large family with vitamin D-dependent rickets type 1. *J Clin Endocrinol Metab* 2010; 95(9): 4176- 4183.

3. Miller WL, Portale AA. Vitamin D biosynthesis and vitamin D 1 α -hydroxylase deficiency. *Endocr Dev* 2003; 6:156- 174.
4. Demir K, KattanWE, Zou M, Durmaz E, BinEssa H, Nalbantoğlu Ö, Al-Rijjal RA, Meyer B, Özkan B, Shi Y. Novel CYB27B1 Gene Mutations in Patients with Vitamin D-Dependent Rickets Type 1A. *PloS One* 2015 doi: 10.1371/0131376.
5. Malloy PJ, Feldman D. Genetic disorders and defects in vitamin D action. *Rheum Dis Clin North Am* 2012; 38: 93- 106.
6. Cheng JB, Levine MA, Bell NH, Mangelsdorf DJ, Russel DW. Genetic evidence that the human CYP2R1 enzyme is a key vitamin D 25- hydroxylase. *Proc Natl Acad Sci* 2004; 101(20): 7711- 7715.
7. Babiker AM, Al Gadi I, Al-Jurayyan NA, Al Nemri AM, Al Haboob AA, Al Boukai AA, Al Zahrani A, Habib HA. A novel pathogenic mutation of the CYP27B1 gene in a patient with vitamin D- dependent rickets type 1: a case report. *BMC Res Notes* 2014; 7: 783.
8. Acar S, Demir K, Shi Y. Genetic causes of rickets. *J Clin Res Pediatr Endocrinol* 2017; 9:88-105.
9. Tahir S, Demirbilek H, Ozbek MN, Baran RT Tanriverdi S, Hussain K. Genotype and Phenotype Characteristics in 22 Patients with Vitamin D-Dependent Rickets Type I. *Horm Res Paediatr* 2016; 85: 309-317.
10. Fu GK, Lin D, Zhang MY, Bikle DD, Shackleton CH, Miller WL, Portale AA. Cloning of human 25-hydroxyvitamin D- 1 alpha-hydroxylase and mutations causing vitamin D-dependent rickets type I. *Mol Endocrinol* 1997; 11: 1961- 1970.
11. St-arnaud R, Messerlian S, Moir JM, Omdahl JL, Glorieux FH. The 25-hydroxyvitamin D1 α hydroxylase gene maps to the pseudovitamin D- deficiency rickets (PDDR) disease locus. *J Bone Miner Res* 1997; 12: 1552- 59.
12. Monkowa T, Yoshida T, Wakino S, Anazawa H, Deluca HF, Suda T, Hayashi M, Saruta T. Molecular cloning of cDNA and genomic DNA for human 25-hydroxyvitamin D3 1 α hydroxylase. *BiochemBiophys Res Commun* 1997; 239: 527- 533.
13. Kim CJ. Vitamin D dependent rickets type I. *Korean J Pediatr* 2011; 54:51- 54.
14. Stenson PD, Ball EV, Mort M, Phillips AD, Shiel JA, Thomas NS, Abeyasinghe S, Krawczak M, Cooper DN: Human Gene Mutation Database (HGMD):2003 update. *Hum Mut* 2003;21: 577- 581.
15. Shaw NJ. Vitamin D deficiency rickets. *Endocr Dev* 2003; 6:93- 104.
16. Schwarz JM, Rödelberger C, Schuelke M, Seelow D. mutationTaster evaluates disease-causing potential of sequence alterations. *Nat Methods* 2010; 7: 575-576.
17. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, Kondrashov AS, Sunyaev SR. a method and server for predicting damaging missense mutations. *Nat Methods* 2010; 7: 248-249.
18. Kumar P, Henikoff S, Ng PC. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. *Nat Protoc* 2009; 4: 1073-1081.
19. Durmaz E, Zou M, Al-Rijjal RA, Bircan I, Akcurin S, Meyer B, Shi Y. Clinical and genetic analysis of patients with vitamin D-dependent rickets type 1A. *Clin Endocrinol (Oxf)* 2012; 77(3): 363- 369.
20. Kim JC, Kaplan LE, Perward F, Huang N, Sharma A, Choi Y, Miller WL, Portale AA. Vitamin D 1 alpha-hydroxylase gene mutations in patients with 1alpha-hydroxylase deficiency. *J Clin Endocrinol Metab* 2007; 92(8):3177- 3182.
21. Edouard, T, Alos, N, Chabot, G, Roughley, P, Glorieux, FH, Rauch, F. Short-and long-term outcome of patients with pseudo-vitamin D deficiency rickets treated with calcitriol. *J Clin Endocrinol Metab* 2011; 96(1): 82-89.
22. Nishikawa M, Yasuda K, Takamatsu M, Abe K, Nakagawa K, Tsugawa N, Hirota Y, Tanaka K, Yamashita S, Ikushiro S, Suda T, Okano T, Sakaki T. Generation of 1,25-dihydroxyvitamin D₃ in cyp27b1 knockout mice by treatment with 25-hydroxyvitamin D₃ rescued their rachitic phenotypes. *J Steroid Biochem Mol Biol* 2018 doi: 10.1016/j.jsbmb. 2018.07.012.
23. Kovacs CS. Vitamin D in pregnancy and lactation: maternal, fetal, and neonatal outcomes from human and animal studies. *J Clin Nutr* 2008; 88: 520S-528S.
24. Karras SN, Wagner CL, Castracane VD. Understanding vitamin D metabolism in pregnancy: from physiology to pathophysiology and clinical outcomes. *Metabolism* 2017 doi: 10.1016/j.metabol.2017.10.001.

Figure 1: X-rays of this patient before (A) and at the 6th month of calcitriol treatment (B). A: abnormal cupping, widening and fraying of the metaphyses consistent with rickets. B: recovery of cupping and fraying, and a provisional calcification zone suggesting healing rickets.

Figure 2: A. Wild type sequence of exon 8 in CYP27B1 gene. B. Sequencing analysis of the CYP27B1 gene exon 8 showing the homozygous mutation (1319_1325dupCCCACCC, Phe443Profs*24).

Table 1: List of primers used for polymerase chain reaction amplification of the nine coding exons of CYP27B1 gene.

Primer Name	Primer sequence
CYP27B1_1F:	GTCATCACCTCACCCAAAGG

CYP27B1 1R:	TCTGACGCTGTCAAAACCAG
CYP27B1 2F:	GAAGCTCCCTATTCCCAAGC
CYP27B1 2R:	CATGCCCCCAGATTGATAGT
CYP27B1 3-4 F:	CTCCTTCACTGCAGCCAGTC
CYP27B1 3-4 R:	GTGGGTAGAAGGCACGTGAA
CYP27B1 5 F:	GCATTTGGTAAGGCACAGGT
CYP27B1 5 R:	CATAATGGATCCCCTGCAAC
CYP27B1 6-7 F:	CCATAATCTGCACCCTCTGC
CYP27B1 6-7 R:	GGGCCCAAGATAGTGAGGA
CYP27B1 8 F:	TCTTCATGCCTGCCCTATTC
CYP27B1 8 R:	CAGGGGAAAGAGCTCACAAC
CYP27B1 9 F:	CACCCAATCATTGACCATTC
CYP27B1 9 R:	CATACTTCACACATTGGTCAGG

Table 2: Clinical and laboratory findings of 11 patients with VDDR1 from 8 families

Subjects	Age (Month)	Presenting symptoms	Height SD	Ca mg/dL N:9- 11.5	P mg/dL N:4- 6.5	PTH pg/mL N:11- 67	ALP IU/L N<455	25- OHD ng/mL N>20	1,25- OH₂D pg/mL N: 20-153
1.1	18	Bowed legs, growth retardation	-2	8.0	2.3	441	2120	26	15
1.2	36	Bowed legs, growth retardation	-2.5	7.1	3.1	784	3100	64	10
1.3	18	Bowed legs, growth retardation	-2.1	6.2	2.8	980	1940	120	12
2	9	O-bine deformity, failure to thrive	-3.1	7.8	3.0	625	1445	45	6
3	14	Hypotonia	-4.7	8.4	2.2	972	3111	194	38
4.1	6	History of VDDR sibling	0.28	8.0	2.9	546	1325	50	11
4.2	7	Inability to work	-1.06	8.9	2.1	423	1400	132	45
5	14	Growth retardation	-2.2	5.91	3.4	925	2531	41	<1.3
6	11	Hypocalcaemic seizure	-0.55	6.7	3.6	467	651	89	15.4

7	24	Inability to walk	-2.1	8.7	2.3	1397	4479	18.7	21.5
8	24	Elevated ALP, inability to walk	-3.1	8.2	2	571	1001	135	5.5

The patients were indicated with respective family number and the number of individual in that family. Patients 1.1, 1.2, 1.3. and 4.1 and 4.2 were siblings. SD: standart deviation, Ca: calcium, P: phosphate, PTH: parathyroid hormone, ALP: alkaline phosphatase, N: normal range

Table 3: Characteristics of the mutations detected in 11 patients with VDDR1 from 8 families

Family number	Exon/Intron	DNA description	Zygosity	CF	MO/PO
1.1	Intron 1	c.195 + 2 T>G	HM	yes	Batman/Batman
1.2	Intron 1	c.195 + 2 T>G	HM	yes	Batman/Batman
1.3	Intron 1	c.195 + 2 T>G	HM	yes	Batman/Batman
2	Intron 1	c.195 + 2 T>G	HM	no	Batman/Batman
3	Intron 1	c.195 + 2 T>G	HM	no	Batman/Batman
4.1	Intron 1	c.195 + 2 T>G / c.1215 +2 T>A	CHT	no	Batman/Bitlis
4.2	Intron 1	c.195 + 2 T>G / c.1215 +2 T>A	CHT	no	Batman/Bitlis
5	Exon 8	p.Phe443Profs*24 (c.1319_1325dupCCCACCC)	HM	yes	Erzurum/Erzurum
6	Intron 7	c.1215+2T> A	HM	yes	Mardin/Mardin
7	Exon 9	c.1474C>T p.R492W	HM	yes	Mersin/Mersin
8	Exon 8	p.Phe443Profs*24 (c.1319_1325dupCCCACCC)	HM	yes	Elazig/Elazig

CF: consanguineous family, HM: homozygous, CHT: Compound Heterozygosity, MO:maternal origin / PO: paternal origin. Locations of origins: Batman, Bitlis, Mardin: South-eastern Anatolia. Erzurum, Elazig: Eastern Anatolia. Mersin:Mediterranean Region