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Genotype and phenotypic spectrum of vitamin D dependent rickets type 1A: our experience and systematic review

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Abstract

Background: Vitamin D dependent rickets type 1 (VDDR1) is a rare disease due to pathogenic variants in 1-α hydroxylase gene. We describe our experience with systematic review of world literature to describe phenotype and genotype.

Methods: Seven patients from six unrelated families with genetically proven VDDR1 from our cohort and 165 probands from systematic review were analyzed retrospectively. The clinical features, biochemistry, genetics, management, and long-term outcome were retrieved.

Results: In our cohort, the median age at presentation and diagnosis was 11(4–18) and 40(30–240) months. The delayed diagnoses were due to misdiagnoses as renal tubular acidosis and hypophosphatemic rickets. Four had hypocalcemic seizures in infancy whereas all had rickets by 2 years. All patients had biochemical response to calcitriol, however two patients diagnosed post-puberty had persistent deformity. Genetic analysis revealed two novel (p.Met260Arg, p.Arg453Leu) and a recurring variant (p.Phe443Profs*24). Systematic review showed that seizures as most common presentation in infancy, whereas delayed motor milestones and deformities after infancy. Diagnosis was delayed in 27 patients. Patients with unsatisfactory response despite compliance were >12 years at treatment initiation. Inappropriately normal 1,25(OH)2D may be present, however suppressed ratio of 1,25(OH)2 D/25(OH)D may provide a clue to diagnosis. Various region specific and hot-spot recurrent variants are described. Patients with truncating variants had higher daily calcitriol requirement and greatly suppressed ratio of 1,25(OH)2D/25(OH)D.

Conclusion: Delayed diagnosis may lead to permanent short stature and deformities. Truncating variants tend to have severe disease as compared to non-truncating variants. Diagnostic accuracy of 1,25(OH)2 D/25(OH)D ratio needs further validation.

Keywords: 1-alpha hydroxylase; calcitriol; VDDR1A.

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Introduction

Rickets is characterized by defective mineralization of growth plates leading to skeletal deformities. The most common cause of rickets is nutritional, which responds to calcium and/or vitamin D supplementation. Persistent rachitic changes despite adequate vitamin D replacement points towards vitamin D resistant rickets (VDDR) which can be broadly classified as calcipenic and phosphopenic rickets. Among the causes of calcipenic VDDR, genetic variations in enzymes involving vitamin D activation and vitamin D receptor defect are known as vitamin D-dependent rickets type 1 (VDDR1) and type 2 (VDDR2) respectively.

Vitamin D has two biologically inert prohormones, namely ergocalciferol (D_2) and cholecalciferol (D_3). These

must be hydroxylated first in the liver (25 hydroxylase) and subsequently in the kidney (1-α hydroxylase) to produce the active metabolite 1,25-dihydroxy vitamin D [1,25$(OH)_2$ D, calcitriol]. The latter is a rate-limiting step and is tightly regulated by parathyroid hormone (PTH), calcium, phosphorus, fibroblast growth factor 23(FGF23), and calcitriol itself. The defect in genes encoding 1-α hydroxylase (*CYP27B1*) and 25 hydroxylase (*CYP2R1*) are designated as VDDR1A and VDDR1B, respectively. *CYP27B1* gene, approximately five mb in size [1], is located on chromosome 12q13.3 [2]. It consists of nine exons with eight introns and encodes a 508 amino acid protein, 25-hydroxyvitamin D-1α-hydroxylase [3].

VDDR1, also called pseudo-VDDR, is a rare autosomal recessive disease. It can present within the first 12 months of age with seizures or at 6–24 months of age with rickets, dental enamel hypoplasia, delayed motor milestones, stunted growth, hypotonia, and weakness [4]. Biochemical investigations reveal hypocalcemia, hypophosphatemia, elevated alkaline phosphatase (ALP), elevated PTH level, normal 25-hydroxyvitamin D [25(OH)D] level, and low or inappropriately normal level of 1,25$(OH)_2$D. Patients usually show good biochemical and clinical response to treatment with calcitriol.

In an earlier study, genotype phenotype correlation was described in three common variants in VDDR1 [5]. However, there is no systematic review with detailed phenotypic description and its correlation with genotype across all the reported pathogenic variants in VDDR1. The data on genetics of VDDR1 is scarce from Indian subcontinent with only a single case report being described [6]. Hence, we describe the clinical features, biochemistry, genetics, molecular modeling, management, and long-term outcome of VDDR1 patients managed at a tertiary care center from Western India. We also conducted a systematic review of genetically diagnosed VDDR1 probands from world literature.

Methods

This retrospective study was conducted in a tertiary care center in Western India after approval from the institutional ethical committee, Seth G.S. medical college and KEM Hospital, Mumbai (EC/OA-154/2018), with waiver of consent. The records of patients with VDDR who had presented to our institute between 2005 and 2021 were reviewed and those with genetically proven pathogenic variants in *CYP27B1* gene were included in the study. Details regarding demographic profile, presenting complaints, biochemistry, imaging, treatment response, and genotypic characteristics were retrieved.

Serum levels of calcium, phosphorus, and ALP were measured with an automated analyzer (Bio-Rad AP, Hercules, CA). Serum PTH was measured with a second-generation assay (intact PTH, ADVIA CENTAUR CP; Siemens Healthcare Global, Erlangen, Germany) with inter-and intra-assay variabilities of ≤6% and ≤8%, respectively, and normal range of 11–69 pg/mL. Serum 25(OH)D (range: 30–100 ng/mL) and 1,25$(OH)_2$D (range: 19.9–79.3 pg/mL) were estimated using enzyme chemiluminescence immunoassay with inter- and intra-assay variability of ≤10%.

Molecular genetics analysis

Genomic DNA was isolated from peripheral blood leukocytes by standard techniques. Molecular screening of the CYP27B1 gene was performed by next-generation sequencing (NGS) at Molecular Endocrinology Laboratory (MEL) of CMC, Vellore [6]. The functional implication of variants was predicted using in-silico tools [Polyphen-2, Sort Intolerant from Tolerant (SIFT), and Mutation Taster]. The minor allele frequency (MAF) of the variants was checked in the databases like 1,000 genomes and gnomAD. Sequence variants were designated using the reference sequences of CYP27B1 [GenBank number NM_000785.4 (cDNA) and GenBank number NP_000776.1 (protein)].

Structural modeling of *CYP27B1*

The amino acid sequence of CYP27B1 was retrieved from the UniProtKB database (ID: O15528), and the three-dimensional structure was generated using the Swiss model tool [7]. Steepest descent method followed by a conjugate gradient was used to get the least energy near-global state conformation using Gromacs 2018.1 [7]. The quality check was performed by generating Ramachandran plot (only 0.5% residues were in the disallowed region) and PROSA (Z score: −9.19) [8]. The refined model was superimposed with the CYP2C5 (PDB ID: 1NR6), which shows similar secondary structure folding with RMSD of 1.2 Å; hence coordinates for cofactor heme were used from this structure. Substrate calcidiol was docked at a reported binding pocket using Autodock4.0 [9]. The parameters used for docking were taken from the earlier similar study [10]. Further we used Dynamut tool (http://biosig.unimelb.edu.au/dynamut/) to see the effect of missense variants on protein stability and flexibility. The high-resolution figures were generated using the Chimera 1.15 [11].

Systematic review of VDDR1 patients/case series with normal levels of 1,25 $(OH)_2$ D

We conducted a systematic review of world literature in July 2021 of probands with genetic diagnosis of VDDR1. The keywords "Vitamin D dependent rickets type 1", "pseudo-vitamin D deficiency rickets", VDDR1, "(VDDR) and (CYP27B1)", (VDDR1), and (CYP27B1), "1-apha hydroxylase and VDDR" were used to search PubMed database for English articles by two authors. An initial search of PubMed retrieved 531 articles of which eventually 36 were included (Figure 1). Total of 165 probands of genetically diagnosed VDDR1 probands were included for the analysis. Per patient data was tabulated to include demographic characteristics (gender, age at presentation and diagnosis, history of consanguinity, and geographical region of the origin (https://population.un.org/wpp/Definition Of Regions), clinical findings (presenting features and height SDS), serum/plasma biochemical parameters (calcium, phosphorus, alkaline phosphatase,

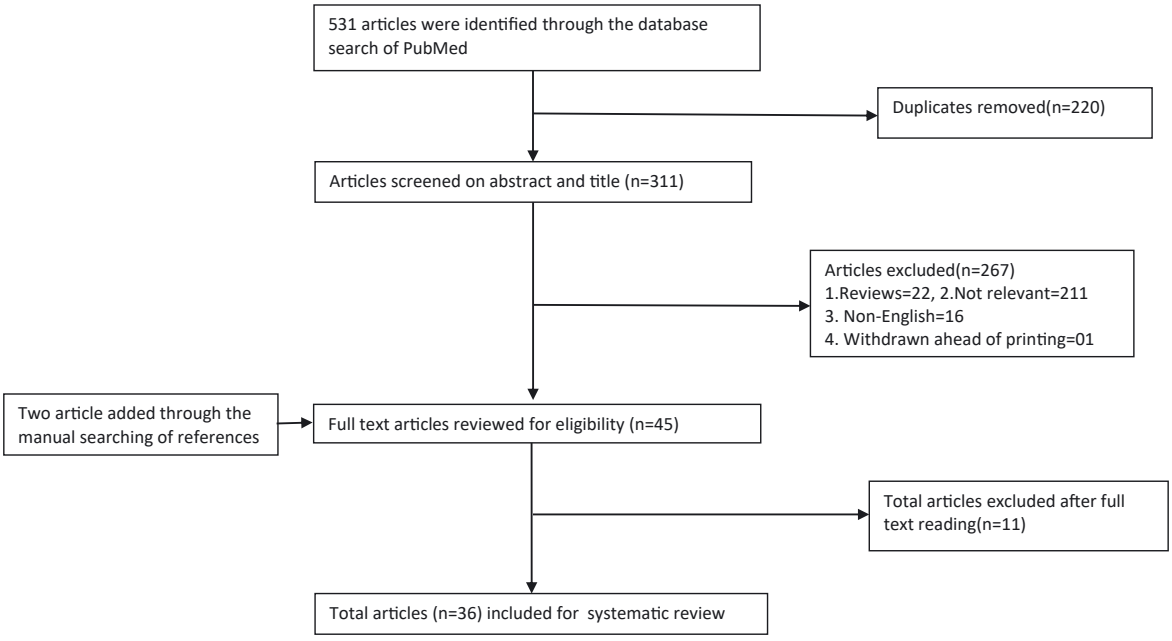

Figure 1: PRISMA flowchart for studies included in systematic review.

PTH, 25(OH)D, and 1,25$(OH)_2$D levels), treatment details (daily dose requirement of active vitamin D), and pathogenic variants in CYP27B1. Variants were designated using the reference sequences of CYP27B1 [GenBank number NM_000785.4 (cDNA) and GenBank number NP_000776.1 (protein)].

Statistical analysis

Categorical data were represented as actual numbers and percentages, whereas continuous data were represented as mean (standard deviation) or median (interquartile range). Differences between the two groups were determined by the chi-square test and Mann–Whitney U test for categorical and continuous data, respectively. In addition, ANOVA test was used to test the differences between means. p-value <0.05 was considered significant. All analyses were performed by using SPSS version 25.0 (IBM, Armonk, NY).

Results

Our study cohort

A total of seven patients (six proband) were diagnosed with VDDR1. The clinical and biochemical profile along with biochemical investigations are represented in Table 1. Five patients were females, and four (57.1%) had consanguinity. A family history of rickets was elicited in four patients (57.1%). The median (range) age at presentation and diagnosis were 11(4–18) months and 40(30–240) months, respectively with a delay of diagnosis by 29 (18–316) months. Most of the patients presented with seizure (4/7) followed by deformity (2/7) and tetany (1/7). Skeletal deformities and disproportionate short stature due to rickets were present in all patients, dental abnormalities in four and pathological greenstick fractures in three patients. The mean height SDS (standard deviation score) of our cohort at presentation was −4.56 ± 0.73. Three patients (P4a, P4b, and P6) had presented to our institute in postpubertal age.

Before referral to our institute, the patients were misdiagnosed as nutritional rickets (3/7), hypophosphatemic rickets (1/7), or rickets due to renal tubular acidosis (RTA) (3/7). These patients had persistent rickets despite high doses of cholecalciferol, phosphorus supplementation, and bicarbonate administration. Two patients (P1 and P3) had vitamin D toxicity [25(OH)D>160 ng/mL]. Hence we reevaluated all the patients after cessation of 25(OH)D for three months and calcitriol for one week (if patients were receiving it). All the patients referred to our institute had hypocalcemia, elevated ALP and PTH, normal/elevated 25(OH)D levels, and low levels of 1,25$(OH)_2$D along with X-ray suggestive of rachitic changes. However, a patient (P1) had inappropriately normal levels of 1,25 $(OH)_2$D. The median ratio of 1,25$(OH)_2$D (pg/mL) to 25(OH)D (ng/mL) was found to be 0.18(0.086–0.34). Five patients had non anion gap metabolic acidosis at the time of presentation to our institute.

Table 1: Clinical, biochemical and genetic profile of study cohort.

Sl. No	Sex	Age at diagnosis, months	Seizures onset, months	Rickets onset, months	Consanguinity/ family history	Height (SDS) (B/F)	Associated features	Serum biochemistry								Urine pH	Urine calcium/creatinine ratio (B)	Genetic variant
								Calcium, mg/dL (B/F)	Phosphorus, mg/dL (B/F)	Alkaline phosphatase, IU/L (B/F)	Parathyroid hormone, pg/mL (B/F)	25(OH) D, ng/mL (B)	$1,25(OH)_2D$, pg/mL (B)	$1,25(OH)_2D$/ 25(OH)D, pg/ml/ng/mL (B)	pH/HCO_3/potassium, mEq/L (B)			
P1	F	40	11	18	No/No	−4.28/ −3.2	Dental caries	7.1/9.8	4.8/5.9	4,176/286	270/9.1	85	33.2	0.39	7.4/19/4.3	–	0.05	c.1319_1325dupCCCACCC (p.Phe443Profster24)
P2	F	36	–	12	No/No	−4.28/0	Delayed motor milestones	7.1/10	2.9/4.3	1,081/171	1,093/16.6	26.7	8.2	0.3	7.28/15/3.5	6	0.02	c.779T>G (p.Met260Arg)[$]
P3	M	22	4	10	Yes/Yes	−4.33/ −2.2	Recurrent LRTI	5.5/9.3	3.2/4.4	1,227/408	267/20	54.6	10.3	0.18	7.4/20/3.1	–	0.11	c.1358G>T (p.Arg453Leu)[$#]
P4a	F	192	–	18	Yes/Yes	−5.4/ −5.4	Dental caries, fracture	7.6/8.6	1.6/2	951/108	167/100	31.9	5	0.15	7.4/28/3.8	3	0.2	c.1319_1325dupCCCACCC (p.Phe443Profster24)
P4b	M	324	8	18	Yes/Yes	−5.8/ −5.8	Dental caries, fracture	7.4/9.9	1.6/2.6	1,360/103	279/45.7	38.8	6.36	0.16	7.25/13/3.5	6.5	0.06	
P5	F	30	12	16	Yes/Yes	−3.85/	Tetany	6.5/-	2.5/-	1,605/-	269/-	45.9	15.9	0.34	7.25/15/3.5	6	–	c.1165C>T (p.Arg389Cys)
P6	F	240	11	24	No/Yes	−4/−4	Dental enamel hypoplasia, fracture	6.1/9.8	3.3/4.7	877/130	230/40	40	18	0.45	7.44/28/3.9	4	0.02	c.1319_1325dupCCCACCC (p.Phe443Profster24)

Deabbreviations: B-Baseline, F-last follow-up, LRTI-Lower respiratory tract infection. Lab Ranges: Calcium: 9–10.5 mg/dL, Phosphorus: Infants: 4.5–8.3, children: 3.5–7, adults: 2.5–5 in mg/dL, PTH: 11–69 pg/mL, +25(OH)D: 30–100 ng/mL, $1,25(OH)_2$ D: 19.9–79.3 pg/mL, spot urine calcium/creatinine ratio: <0.2, Normative ranges for ALP for individual patients – P1:294–783 IU/L, P2:265–849 IU/L, P3:311–884 IU/L, P4a, P4b and P6: <117 IU/L, P5:265–849 IU/L, [$]Novel variant, [#]Heterozygous variant.

Treatment and follow up

Calcitriol was initiated at 10–20 ng/kg/day along with elemental calcium replacement (30–75 mg/kg/day). The dose of calcitriol was titrated to maintain normal serum calcium, ALP, and PTH levels. Once the radiological resolution of lesions was documented, calcitriol was continued at 0.25–0.5 µg/day (Supplementary Figure 1). All patients showed clinical, biochemical, and radiological improvement with a physiological replacement dose (0.25–0.75 µg/day) of calcitriol. After a median follow-up of 33(24–66) months, all three pre-pubertal patients (P1, P2, and P3) had improvement in height from the baseline value (Δheight SDS: 2.42 ± 1.7). Hypocalcemia resolved within 3–6 months, whereas ALP and PTH normalized by 12–15 months. Acidosis resolved in all five patients, concomitant with PTH normalization. One patient had iatrogenic hypercalciuria (spot urinary calcium/creatinine ratio: 0.24) which resolved after reduction in the calcitriol dose. No patient developed nephrocalcinosis or renal calculi.

Genetic analysis

Four different pathogenic/likely pathogenic variants, including two novel variants (p.Met260Arg and p. Arg453Leu), were observed in six probands. Among these were three missense variants and one frameshift variant resulting from duplication of seven nucleotides in exon 8. All patients had homozygous variants except one (P3), who had a heterozygous missense variant p.Arg453Leu. Both novel variants were predicted to have a deleterious effect on protein function observed by the in-silico tools. These variants have not been reported in the 1,000 genome database, whereas p.Met260Arg has been reported in the gnomAD database with a MAF of 0.003% in the South Asian population.

Molecular modeling

Missense variants p.Arg389Cys, p.Arg453Leu, and p.Met260Arg are shown in the three-dimensional structure of CYP27B1 (Figure 2). We noted that the missense variants p.Arg389Cys and p.Arg453Leu are in the proximity to the catalytic site (Figure 2A).The native non bonded interactions (Figure 2B, shown in green) between heme with residues Arg389 and Arg453 are lost with change of amino acid to cysteine and leucine, respectively(show in red). Dynamut analysis predicts overall destabilizing effects for variant p.Arg389Cys (ΔΔG: –0.268 kcal/mol) and p.Met260Arg (ΔΔG: –0.991 kcal/mol), whereas p.Arg453Leu (ΔΔG: 0.256 kcal/mol) shows stabilizing effect. Notably, all three variants showed increased molecular flexibility at the catalytic site (Supplementary Figure S2).

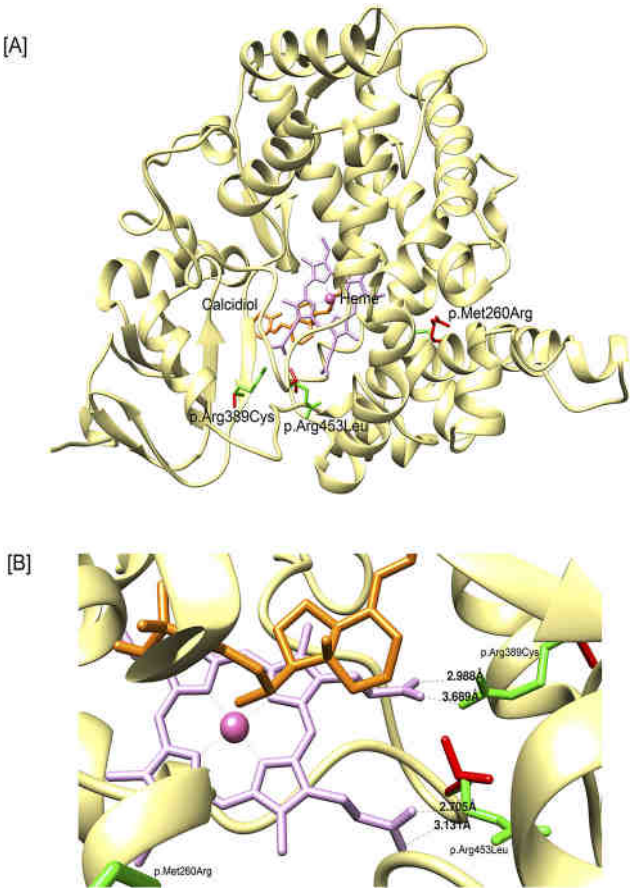

Figure 2: (A) The three-dimensional structure of CYP27B1 generated using Swiss model. The cofactor heme and substrate calcidiol is shown in plum and orange color respectively. The native residues are shown in green (stick form) and the variant residues are shown in red (stick form). (B) The magnified view represents non-bonded interaction of heme and the missense variants. The distance between Heme-Arg389 (2.988 Å and 3.689 Å) increased to Heme-Cys389 (8.443 Å and 8.169 Å) and the distance between Heme-Arg453 (2.705 Å and 3.131 Å) increased to Hem-Leu453 (3.815 Å and 4.583 Å).

Total cohort from a systematic review (including our cases)

A systematic review of total of 165 probands from world literature including the current cohort was performed. A complete clinical profile with biochemical characteristics have been represented in Table 2. The most common presentation was delayed motor milestones [58(38.4%)]

Table 2: Clinical profile and biochemical characteristics of VDDR1 patients by a systematic review.

Variables	Index patients(n=165)	Data available(n)
Sex, male/female	90(57.7%)/ 66(42.3%)	156
Consanguinity, yes/no	40(38.8%)/ 63(61.2%)	103
Age at first presentation, months	12(9–18)	108
Age at diagnosis, months	20(13–31)	144
Presenting manifestations[#]		
1. Delayed motor milestones	58(38.4%)	151
2. Delayed motor milestones + rickets	31(20.5%)	
3. Rickets	32(21.2%)	
4. Seizures	13(8.6%)	
5. Delayed motor milestones + rickets + seizures	8(5.3%)	
6. Delayed motor milestones + seizures	4(2.6%)	
Height SDS at presentation	−2.59 ± 1.58	78
Serum biochemistry*		
1. Calcium, mg/dL	7.4(6.4–8.4)	141
2. Phosphorus, mg/dL	2.9(2.2–3.7)	140
3. Alkaline phosphatase, IU/L	1,480(1,040–2,120)	134
4. Parathyroid hormone, pg/mL	425(228–644)	130
5. 25(OH) D, ng/mL	45(32–79)	127
6. $1,25(OH)_2D$, pg/mL	12(6.8–19)	113
7. Ratio[†](pg/mL)/(ng/mL)	0.25(0.13–0.53)	96
Misdiagnosis in patients where diagnosis was delayed by >1 year		
1. Nutritional rickets	6(22.2%)	27
2. Hypophosphatemic rickets	5(18.5%)	
3. Renal tubular acidosis	2(7.4%)	
5. Pseudohypoparathyroidism	1(3.7%)	
6. Not available	13(48.1%)	
Active vitamin D requirement		
Calcitriol, ng/kg/day	45(30–59)	37
Calcitriol, mcg/day	0.75(0.5–1)	21
Alfacalcidiol, mcg/day	1.5(1.2–1.5)	03

Data are represented as numbers(percentage), mean(±standard deviation), median(Interquartile range). *Normal lab ranges: Calcium: 9–10.5 mg/dL, Phosphorus: Infants: 4.5–8.3, children: 3.5–7, adults: 2.5–5 in mg/dL, Alkaline phosphatase-50-370 IU/L, PTH: 11–69 pg/mL, +25(OH)D: 30–100 ng/mL, 1,25(OH)2 D: 19.9–79.3 pg/mL, [#]Other presenting manifestations: deformity and seizures=01(0.6%), Excessive sweating with biochemistry showing raised ALP=01(0.6%), Stridor=01(0.6%) Dyspnea secondary to rib fracture=01(0.6%), hip dysplasia=01(0.6%). [†]Ratio of $1,25(OH)_2D/25(OH)$ D.

followed by rickets [32(21.2%)] and rickets with delayed motor milestones [31(20.5%)]. Hypocalcemic seizures were seen in 25(16.5%) patients. Mean height SDS at presentation was −2.59 ± 1.58. The median age at presentation and diagnosis was 12(9–18) and 20(13–31) months respectively. Daily requirement of calcitriol in the initial treatment of VDDR1 in overall cohort was 45(30–59) ng/kg/day irrespective of the genetic variants and ethnicity influence. Median age at initiation of therapy in patients with satisfactory response (attaining final adult height as per the MPH and resolution of deformities) was 22(16–31, n=08) months compared to 210(145–312, n=64) months in patients with no response.

Genetic analysis of whole cohort by a systematic review

Eighty-one pathogenic variants in the *CYP27B1* have been reported in patients with CYP27B1 deficiency (Supplementary data). Of these, there were 47 missense, 19 frameshift, eight nonsense, six splice-site variants, and one in-frame deletion. These variants have been distributed throughout the gene. Of 165 probands, 109 harbored homozygous variants, 54 harbored compound heterozygous variants and two had heterozygous variants. The recurring variants (≥3 probands) specific to geographical locations are p.Val88Trpfster71 (Europe); p.Gly57Val, p.Arg104LeufsTer225, p.Arg107His, and c.589 + 1G>A (East Asia); p.Leu58CysfsTer20, c.195 + 2T>G, p.Lys192-Glu, c.1215 + 2T>A, and p.Arg492Trp (West Asia, Turkey). Variant p.Phe443ProfsTer2 (7 bp duplication) is the most common variant reported worldwide (majorly from Asia and Europe). Two variants (p.Arg389His and p.Arg389-Cys) on the Arg389 position have been reported worldwide. Another recurrent variant p.Thr409Ile has been reported from Asia, Europe, and Latino.

Genotype-phenotype correlation

We compared truncating and non truncating variants. In truncating group, there was a trend towards higher PTH and ALP values, whereas ratio of $1,25(OH)_2$ D/25(OH) D was significantly suppressed {0.15(0.07–0.35) vs. 0.4(0.18–0.66), p=<0.001} in truncating variants compared to non truncating group. Calcitriol requirement (ng/kg/day) was

Table 3: Comparison between VDDR1 patients with truncating and non-truncating variants.

Variables	Truncating group(93)		Non-truncating group(71)		p-Value
Age at presentation, months	12(9–18)	n=63	12(9–19)	n=45	0.76
Age at diagnosis, months	18(12–25)	n=76	23.5(14.2–58)	n=68	**0.01**
Height SDS	−2.59 ± 1.71	n=53	−2.58 ± 1.3	n=25	0.9
Seizure	19(21.3%)	n=90	10(15.2%)	n=66	0.33
Serum biochemistry					
1. Calcium, mg/dL	7.3(6.6–8.4)	n=76	7.4(6.4–8.1)	n=65	0.24
2. Phosphorus, mg/dL	2.95 ± 1.1	n=76	3.1 ± 1.1	n=64	0.42
3. Alkaline phosphatase (IU/L)	1,652(1,049–2,139)	n=74	1,322(939–2030)	n=60	0.56
4. Parathyroid hormone, pg/mL	474(252–679)	n=73	363(203–537)	n=57	0.14
5. 25(OH) D (ng/mL)	47(35–110)	n=71	45(31–57)	n=56	0.12
6. $1,25(OH)_2D$ (pg/mL)	10(6–17.5)	n=61	13.5(8–23)	n=52	0.17
7. $1,25(OH)_2D/25(OH)$ D	0.15(0.07–0.35)	n=54	0.4(0.18–0.66)	n=47	**<0.001**
Calcitriol dose, ng/kg/day	50(33–67)	n=24	30(23–47.5)	n=13	**0.048**
Calcitriol dose, mcg/day	1.25(0.5–2.25)	n=06	0.75(0.5–1.0)	n=14	0.16

Data are represented as numbers(percentage), mean(±SD) and median(Interquartile range), SDS=Standard deviation score. 25(OH) D=25 hydroxy vitamin D, $1,25(OH)_2$ D=1,25 dihyroxy vitamin D. Bold values represent statistically significant difference (p-value <0.05).

higher in patients with truncating group than in non truncating group (50(33–67) vs. 30(23–47.5) p=0.048) suggesting severe disease in the former (Table 3).

Discussion

We present our experience of VDDR1 patients from a single center in western India with detailed clinical, biochemical, genetic profile, molecular modeling and follow up of genetically diagnosed cases of VDDR1. This study, the first series from India, highlights concerns of initial misdiagnosis and consequent short stature with irreversible bony deformities due to delay in appropriate management. It adds on two novel missense variants to the existing genetic spectrum of VDDR1. Further, we performed a systematic review of world literature of VDDR1 patients, and describe the phenotypic and genotypic spectrum.

The age of presentation and sex distribution in our cohort was similar to that obtained on systematic review. Four of six probands in our cohort had history of seizures in infancy. Similarly, in systematic review, seizures was the most common initial presentation (11/31) in those diagnosed during infancy (<1 year of age) whereas delayed motor milestones and skeletal deformities were common presenting features in those presented beyond infancy.

In our cohort, the median age at diagnosis and delay in diagnosis was greater than that observed in the literature review. On specifically analyzing the systematic review for patients with delayed diagnosis, 27 patients had diagnoses delayed for more than 1 year. Among these, nutritional rickets and hypophosphatemic rickets were the most common causes of misdiagnoses. VDDR1 should be suspected in patients with nutritional rickets who have poor response to vitamin D supplementation. Patients with hypophosphatemic rickets may have low levels of $1,25(OH)_2$ D due to functional suppression of 1α-hydroxylase by fibroblast growth factor 23. Further normal calcium levels may be present in patients with genetically proven VDDR1(51 patients in the current review had serum calcium >8 mg/dL) due to very high doses of cholecalciferol; this may have been an additional factor in misdiagnosis [12]. However, onset of symptoms in infancy, presence of delayed motor milestones, history of hypocalcemic seizures, and high PTH should warrant evaluation for VDDR1.

Patients with VDDR1 may present with transient non anion gap metabolic acidosis, which may be mistaken for renal tubular acidosis (RTA). The reason for acidosis could be attributed to direct effect of PTH on inhibiting proximal tubular bicarbonate resorption [13]. Further hypophosphatemia secondary to phosphaturic action of PTH may impair adenosine 5''-triphosphate (ATP) production and worsen bicarbonate resorption defect [14]. Additionally, the hallmark of VDDR1, that is, low 1,25 vitamin D levels may also be mimicked in patients with RTA due to acidosis mediated impairment of 1α-hydroxylase enzyme [15]. RTA may also be associated with mild derangements in PTH level, which could also contribute to missing diagnosis of VDDR1. Fourteen patients in our review had PTH levels <100 pg/mL; which may have been due to the labile nature of analyte (PTH). Nonetheless, PTH levels in VDDR1 are usually markedly elevated 425(228–644) pg/mL as described in our systematic review. Thus, clinical clues

like history of hypocalcemic seizures, presence of hypocalcemia, high PTH levels, and absence of hypokalemia or renal calculi should raise a suspicion for VDDR1. Notably, the acidosis was transient and improved with normalization of calcium and PTH in our cohort.

In a study by Bagga et al., PTH levels were markedly elevated in VDDR1 cases [350 (140–358) pg/mL] as compared to hypophosphatemic rickets [48(39.4–61) pg/mL], and RTA [91(67–95) pg/mL], indicating the significance of PTH levels for differentiating these conditions [16]. In such confusing case scenarios ratio of 1,25 $(OH)_2$ D to 25(OH) D may be useful. As VDDR1 involves a genetic defect in 1α-hydroxylation, we propose that the ratio could be much lower than other causes of functional decrease in hydroxylation (hypophosphatemic rickets, RTA). In the current review the ratio in VDDR1 cases was 0.25. However the cut offs and utility of this ratio warrants future studies.

The biochemical evaluation showed hypocalcemia, hypophosphatemia, elevated PTH and ALP, and low 1,25 $(OH)_2$D despite adequate 25(OH)D in all patients, except one patient with normal 1,25$(OH)_2$D. The latter patient had inappropriately normal 1,25$(OH)_2$D in the context of hypocalcemia. It could be due to higher levels of 25(OH)D in this patient. The plausible explanation for normal 1,25$(OH)_2$D could be presence of partial enzyme defect, exogenous consumption of active form (human and cow milk and fish), or 1α-hydroxylase activity of *CYP27A1*in liver. The latter is predominantly involved in 25 hydroxylation, however it has minimal 1α-hydroxylase activity in normal circumstances, which could be exaggerated in VDDR1 [17].

All patients had an excellent clinical and biochemical response to physiological dose of calcitriol (0.25–1 µg/day) as is reported in literature. However, patients who were diagnosed in post pubertal age group had no improvement in deformities or short stature due to fusion of growth plates and required corrective osteotomies. This reemphasizes the need for timely and accurate diagnosis. Among patients who did not show satisfactory response to treatment (resolution of deformity or catch up growth or attainment of adult height as per MPH) in systematic review, two patients (age at treatment 1 and 2 year) were non compliant to treatment, remaining were all greater than 12 years of age at treatment initiation. Whereas among those who responded, only one patient was 12 year 2 month old, rest all others were less than 9 years of age at treatment initiation. The former patient had catch up growth with treatment, but final adult height was not available.

In the present study, we have reported four different variants in six unrelated patients. The novel variants p.Met260Arg and p.Arg453Leu were found to be deleterious by in-silico tools, and were rarely reported in population databases. Computational modeling also showed altered flexibility at the catalytic sites. Further studies to assess the enzyme activity for these variants are warranted. The present systematic review revealed four most common pathogenic variants p.Phe443Profs*24, p.Val88Trpfs*71, c.195 + 2T>G, and p.Lys192Glu. The seven nucleotide duplication in exon 8 (c.1319_1325dupCCCACCC, p.Phe443Profs*24) is the most common variant reported in homozygous state in 46 patients from Turkey, China, Saudi Arabia, Canada, and Australia suggesting this duplication to be a hotspot, as it has repeat sequence [1, 18–20]. We also found this variant in four patients from three unrelated families. Region specifics recurring variants (p.Val88Trpfster71, p.Gly57Val, p.Arg104LeufsTer225, p.Arg107His, c.589 + 1G>A, p.Leu58CysfsTer20, c.195 + 2T>G, p.Lys192Glu, c.1215 + 2T>A, and p.Arg492Trp) are possibly due to founder effect in highly inbred populations. VDDR1 is an autosomal recessive disease, but patient (P3), was heterozygous for a known pathogenic variant p.Arg453Leu. This patient had classical clinical and biochemical diagnosis highly suggestive of VDDR1, and had therapeutic response to calcitriol. Similar patients with heterozygous variants have been reported. It is assumed that the other allele might have contained a variant in the non coding region or harbor a large deletion that was not identified by next generation sequencing. Multiplex ligation-dependent probe amplification (MLPA) to detect deletion at the other allele has been planned for our patient. In a previous study splice site truncating variant c.195 + 2T>G variant has been correlated with more severe disease (lower height SDS at presentation and requirement for higher calcitriol treatment doses) as compared to a missense nontruncating variant p.Lys192Glu [5]. Similarly this systematic review suggested, the disease was more severe in patients with truncating variants with greatly suppressed ratio of 1,25 $(OH)_2$ D/25(OH) D and higher calcitriol dose requirement compared to nontruncating variants.

The major limitations of our study are retrospective design with its inherent drawbacks and small sample size. Nonetheless, this is the first study from western India with detailed clinical, biochemical, genetic profile, and follow up and including a systematic review of world literature.

To conclude, VDDR1 though a rare disease, if suspected and diagnosed in time can be effectively treated with calcitriol. Delay in diagnosis may lead to permanent short stature and deformities. Inappropriately normal 1,25$(OH)_2$D may be present, however suppressed ratio of 1,25$(OH)_2$ D/25(OH) D may provide a clue to diagnosis. Various region specific and hot-spot recurrent variants are

described. Truncating variants tends to have severe disease as compared to nontruncating variants.

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