

Vitamin D deficient rickets: Not always nutritional

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ABSTRACT

Rickets in pediatrics due to vitamin D deficiency is still considering a major problem even in sunny and tropical countries. The prevalence of vitamin D deficiency and insufficiency in children between 6 and 15 years of age in the Kingdom of Saudi Arabia is 95.4%. Vitamin D requires two steps of hydroxylation to be functionally active. The first hydroxylation step occur in the liver by 25-hydroxylase encoded by CYP2R1 gene (11p15.2) to produce 25(OH)D3 and the second step of hydroxylation occur in proximal convoluted tubules in kidney by 1 α -hydroxylase encoded by CYP27B1 gene (12q13.1) to produce hormonally active 1,25-dihydroxyvitamin D3 (1 α ,25(OH)2D3). Vitamin D-dependent rickets type 1B (VDDR1B) is a form of rickets due to mutation in CYP2R1 gene. Until today, only five mutations were found to affect CYP2R1 gene which lead to abnormal structure and function of 25-hydroxylase enzyme. Presence of symptoms of vitamin D deficiency in good dietary history with poor response or depending on regular to high dose of vitamin D2 or D3 to maintain 25(OH)D3 level should raise the suspicion of genetic causes of CYP2R1 mutations. These mutations are inherited as autosomal recessive; the severity and response to medication depend on number of allele affected. Some of the homogenous mutation patients showed some improvement in using Calcitriol. Bypassing the first step of hydroxylation (25-hydroxylase) in liver using 25-OH-D3 (Calcifediol) as treatment lead to dramatic improvement of biochemical and radiological finding in seven recent reported cases which needs further study.

Introduction

Classical vitamin D deficiency remains the major cause of rickets among children worldwide. A combination of poor nutritional, social, and climatic conditions explains much of this deficiency. Even in sunshine countries such as Saudi Arabia, rickets remains prevalent in different age groups¹. The prevalence of vitamin D deficiency (25-hydroxyvitamin D3 [25(OH)D3] is \leq 25 nmol/L) and vitamin D insufficiency (25(OH)D3 level is 25-50 nmol/L) in children and adolescents between 6 and 15 years of age in the Kingdom of Saudi Arabia is 45.5% and 49.9% respectively, over all 95.4%².

An understanding of the physiology of vitamin D metabolism and its action is important to know the pathology and genetic causes of vitamin D deficiency. The source of vitamin D in the body is through the conversion of 7-dehydrocholesterol to vitamin D₃ in the skin upon exposure to sunlight ultraviolet-B and through ingestion of foods containing either cholecalciferol (vitamin D₃) or ergocalciferol (vitamin D₂) or through supplements of these

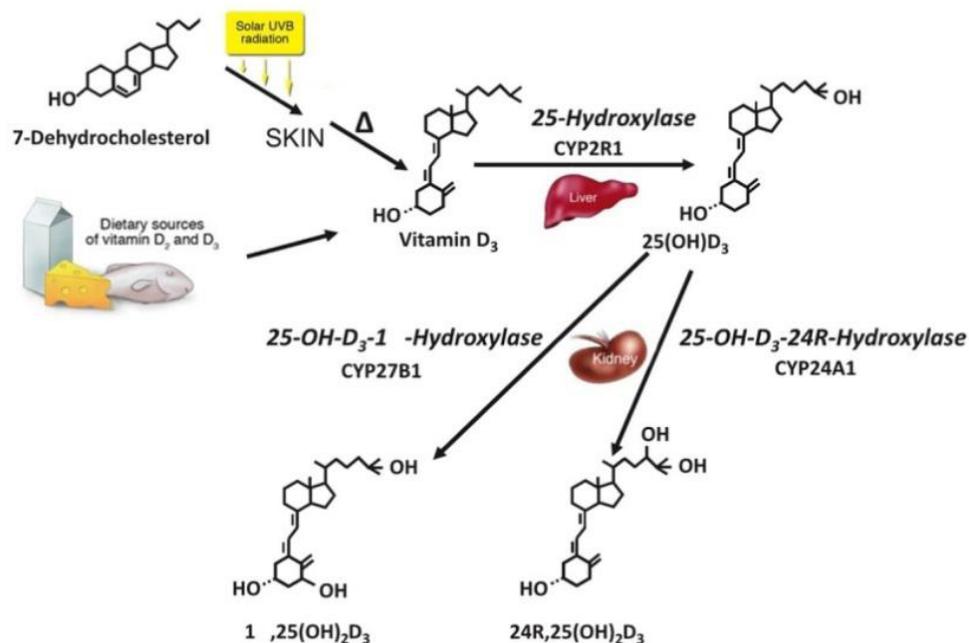


Figure 1. Metabolism of vitamin D₃ in the skin, liver and kidney. 7-Dehydrocholesterol in the skin is converted in two steps and beginning with the exposure to ultraviolet light to vitamin D₃, which is transported in the blood (mostly protein-bound) to the liver where the side chain is hydroxylated at the C25-position. The resulting 25(OH)D₃ is the major circulating form of vitamin D. In the kidney, 25(OH)D₃ is hydroxylated at either the C1- or the C24 position to form either 1 α ,25(OH)₂D₃ or 24R,25(OH)₂D₃. The cytochrome P450 (CYP) dependent enzymes responsible for these transformations are, respectively, the 25-hydroxylase (CYP2R1), the 1 α -hydroxylase (CYP27B1) and the 24R-hydroxylase (CYP24A1 or CYP24). *Helen L. Henry copyright 2011. Modified with permission*
Helen L. Henry. Regulation of vitamin D metabolism. *Best Practice & Research Clinical Endocrinology & Metabolism*, BPRCEM. 2011; 25:531–541.

substances. Once produced in the skin or absorbed from the intestine, vitamin D (including D₂ and D₃) requires two steps of hydroxylation to be in active and physiologic form. The first hydroxylation step occurs in the liver by 25-hydroxylase (CYP2R1, 11p15.2) converting vitamin D₃ to 25(OH)D₃ and the second step of hydroxylation occur in proximal convoluted tubules in kidney by 1 α -hydroxylase (CYP27B1, 12q13.1) to convert 25(OH)D₃ to hormonally active 1,25-dihydroxyvitamin D₃ (1 α ,25(OH)₂D₃) (figure 1). The active form of vitamin D has important roles in maintaining normal calcium and phosphate level by enhancing absorption of these elements from kidney and intestine which are important in bone mineralization. Defect in any of these steps will lead to effect on calcium and phosphate hemostasis and bone mineralization that will lead to rickets in children and osteomalacia in adults^{3,4}.

Defect in 1 α -hydroxylase enzyme due to mutation in CYP27B1 gene resulting in defect the synthesis of active form of vitamin D (1 α ,25(OH)₂D₃), this condition called Vitamin D-dependent rickets type 1A (VDDR1A; MIM 264700). Vitamin D-dependent rickets type 1B (VDDR1B; MIM 600081) is a form of rickets due to mutation in CYP2R1 gene encoding 25-hydroxylase. Vitamin D-dependent rickets type 2A (VDDR2A; 277440) is caused by end-organ unresponsiveness of active vitamin D due to mutation

in the gene encoding the vitamin D receptor. Vitamin D-dependent rickets type 2B (VDDR2B; MIM 600785) is an unusual form of end-organ unresponsiveness to active vitamin D due to an abnormal protein that interferes with the function of the VDR⁵.

Hepatic 25-hydroxylase is mitochondrial and microsomal enzyme that has six cytochrome P450 enzymes, three of them found in the animals (CYP2C11 in rat, CYP2D25 in pig, and CYP2J3 in rat) and the other three found in the human (CYP3A4, CYP27A1, and CYP2R1). CYP3A4 has activity to hydroxylate D₂ but not D₃ while CYP27A1 has activity to hydroxylate D₃ but not D₂⁶. CYP2R1 has high affinity to hydroxylate both D₂ and D₃ with hydroxylation activity toward D₃ equal to 26-fold higher than CYP27A1, suggesting that CYP2R1 plays the major role in vitamin D hydroxylation in humans^{6,7}.

Experimental studies

The second hydroxylation step of vitamin D is clearly to be carried out by a single enzyme, 1 α -hydroxylase coded by CYP27B1. However, it is not clear if the first hydroxylation step carried out by single or multiple enzymes responsible for 25-hydroxylation. An experimental study was done by Jing G. Zhu et al. to evaluate the physiological role of CYP2R1 in the vitamin D activation pathway. They

have created Cyp2r1^{-/-} and Cyp2r1^{-/-}/Cyp27a1^{-/-} Mice (double knockout mouse models). They found that both types of mice had more than 50% reduction in 25(OH)D₃ level but still measurable with normal and unchanged in 1,25(OH)₂D₃ concentration as compared with wild-type mice⁸. This finding coincides with the observation of that patients who are carrying CYP2R1 mutation had an abnormally low 25(OH)D₃ measurable level with a normal 1,25(OH)₂D₃ concentration^{11,12,13,16}. Also 25(OH)D₃ is reduced in heterozygous but the level is in between the wild-type and homozygous levels. On the other hand, the mice with Cyp27a1^{-/-} showed two- to threefold increase in 25(OH)D₃ and this finding is identical to the previous reports^{8,9}. CYP27A1 is responsible enzyme for cholesterol metabolism and bile acid synthesis but has a minimal effect on vitamin D metabolism. Mutation in CYP27A1 leads to defect in bile acid synthesis and this condition seen in patient with cerebrotendinous xanthomatosis^{9,10}.

These results observed by Jinge G. Zhu et al. who supported the idea of CYP2R1 as the major and most important enzyme responsible for the first step of vitamin D hydroxylation at C-25, but not exclusive as there is another enzyme contributed to this 25 hydroxylation but to a lesser extent⁸.

Reported cases

From Nigeria

The first mutation reported in CYP2R1 gene was announced by S.J. Casella et al. study in 1994 in Nigerian boy and his brother. Both had typical symptoms of rickets with no history of malabsorption. 25(OH)D level was low which improved on a high dose of vitamin D (4000 IU/day) and back to a low level with modest dose of vitamin D (1000 IU/day). Genetic test done and showed the first mutation reported in CYP2R1 gene which is c.296T > C (L99P)¹¹.

Cohort study was conducted by Tom D. Thacher et al. about the prevalence of CYP2R1 mutation in Nigerian children. They found a new novel mutation in addition to the previously known reported mutation of CYP2R1 in two out of twelve families. One mutation was c.296T > C (L99P) found in 8 members of 2 generations of both families. The other mutation was c.726A > C (K242N) found in heterozygous mother of the index case in the second family¹². These mutations effect CYP2R1 structure and function. L99P amino acid replacement will effect on CYP2R1 folding while K242N amino acid change will affect interaction surface of CYP2R1 with vitamin D^{13,14}.

The first family had L99P mutation of CYP2R1 in form of homozygous (the index case, his brother and father) and heterozygous (2 sisters and mother) with more severe rickets seen in homozygous members. In homogenous patients, index case and his brother were presented at

age of 12.5 and 11 years respectively. Both were had legs deformity, bone pain, difficulty in walking, signs of rickets, and history of low calcium rich diet associated with severe biochemical and radiological picture of rickets. In heterozygous members, only one sister had a history of mild genu varum that resolved spontaneously, others were normal. The second family had heterozygous L99P mutation of CYP2R1 in index case, her sister and father. Both sisters were had typical clinical, biochemical, and radiological pictures of rickets¹².

As seen in experimental and Al sagheir A. et al studies, 25(OH)D₃ level was low in heterozygous patients but in between the homozygous and normal^{8,12,16}. Homozygous patients (L99P) showed no response to the treatment with 50000 IU of vitamin D compared with heterozygous patients (L99P and K242N). They demonstrated that the incremental levels of 25(OH)D₂ and 25(OH)D₃ at day three (the peak) after supplementing D₂ and D₃ were blunted in homozygous while partial increments in heterozygous compared with control (significant P value) group. In L99P homozygous subjects, the 1,25(OH)2D was relatively low and increased with supplement of D₂ and D₃.¹² On the other hand, the improvement in rickets clinically and radiologically were seen after using very high dose of vitamin D, 600,000 IU IM, indicating CYP2R1 mutation can be overcome by high dose of vitamin D as seen in the previous experimental study which showed low but measurable 25(OH)D₃ in Cyp2r1^{-/-} mice^{8,12}. This observation supporting that there might be a role of other P450 enzymes in 25 hydroxylation.

From Saudi Arabia

Two new mutations in CYP2R1 were described by Al Mutair A. N. et al. in two siblings of Saudi family. The index case was 13 years old girl first presented to clinic at age of 10 years as short stature. The second sibling was the older brother 14.7 years old who had symptoms at age of 5 years. Both siblings had bow legs, bone pain, and decrease activity in addition to difficulty in walking and other signs of rickets seen in older brother. They had normal development, nutritional, antenatal and postnatal history in addition to absent of malabsorption symptoms. Both were on vitamin D supplement at different doses and the brother used additionally calcium supplement. Initial investigation showed classical biochemical picture of vitamin D deficiency in presence of normal 1,25(OH)₂D₃ (table 1)¹⁵.

Both siblings were treated with Cholecalciferol (5000 IU/day for the girl and 10,000 IU/day for the boy). As seen in table 1, the abnormal biochemical results improved with treatment except 25(OH)D₃ level, which was increased but remain low in spite of high dose treatment, coincides with experimental study in mice^{8,15}. Serum level of 1,25(OH)₂D₃ remained normal with treatment reflecting normal one alfa hydroxylase enzyme activity that is under parathyroid

Table 1. Biochemical results (baseline and after therapy)

	Serum PTH (pmol/liter)	Serum total calcium (mmol/liter)	Serum phosphate (mmol/liter)	Serum alkaline phosphatase (U/liter)	Serum 25-OH-D (nmol/liter)	Serum 1,25(OH) ₂ D (pmol/liter)
Normal value	1.6–7.2	2.20–2.70	0.7–1.52	<500 prepubertal, 40–150 in adults	50–125 (20)	41–127
Baseline results						
Patient 1	46	1.4	1.4	445	<10	48
Patient 2	28	1.6	1.61	1145	<10	44
Results after treatment						
Patient 1	5.7	2.3	1.58	288	23	50
Patient 2	16.7	1.8	1.6	293	28	46

All values were measured in serum.

Angham N. Al Mutair, Ghada H. Nasrat, and David W. Russell. Mutation of the CYP2R1 Vitamin D 25-Hydroxylase in a Saudi Arabian Family with Severe Vitamin D Deficiency. The Journal of Clinical Endocrinology & Metabolism, JCEM. 2012; 97(10):E2022–E2025

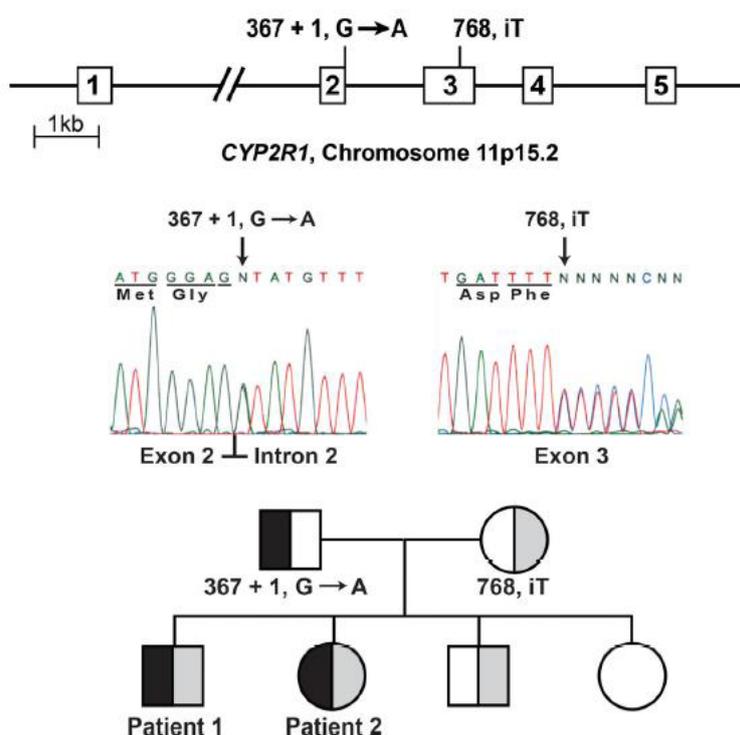


Figure 2. DNA sequence of CYP2R1 in patients 1 and 2 and pedigree of family. Affected individuals inherited one CYP2R1 allele with a G to A transition in the splice donor sequence of intron 2 and a second allele with a T insertion in exon 3. The inheritance of these alleles in the family is shown.

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hormone control. The unaffected sibling had normal serum 25(OH)D₃ level without vitamin D supplementation. Their mother had vitamin D deficiency by history and on regular supplement of vitamin D to maintain 25(OH)D₃ in normal level. Father refused to be tested. These findings raised suspicion of selective 25-hydroxyvitamin D₃ deficiency¹⁵.

Molecular genetic testing was done for the family and showed new not previously reported compound heterozygous mutations (figure 2) in both siblings. One mutation was inherited from the father with transition

of G to A in the first nucleotide of the highly conserved splice donor sequence of intron 2 (c.367+1,G>A). This mutation will cause abnormal splice in mRNA that will cause truncated CYP2R1 protein. The second mutation was inherited from the mother with T insertion in exon 3 (c.768,insT) that alters the normal translational reading frame. This mutation will cause again truncated CYP2R1 protein. Both truncated CYP2R1 proteins lack the domains required for normal function of 25-hydroxylase enzyme. These mutations have autosomal recessive inheritance pattern (figure 2)¹⁵.

A second study done by Al sagheir A. et al. reported the clinical and molecular characterization of 25-Hydroxylase deficiency in 30 Saudi patients who presented with classical symptoms of vitamin D deficiency and low 25(OH)D₃ (<50 nmol/L), who poorly responded to the usual treatment doses of vitamin D, and had no other medical problems including nutrition disorder¹⁶. Two mutations were found similar to the mutations reported by Al Mutair A. N. et al: c.367+1,G>A (44.4%) and c.768,insT (55.6%), 18 patients were had homozygous mutations, and heterozygous mutations were found in 9 patients from two families with rickets (familial rickets). There were no mutation detected in 3 patients who had no family history of rickets (non familial rickets)^{15,16}.

Homozygous patients had more severe symptoms and lower 25(OH)D₃ as compared to heterozygous patients, as seen in the experimental study and the previous Saudi siblings reports^{8,15,16}. The most clinical presentations were bone pain, limitation in activity, bone deformities and hypocalcaemia with percentage 86.2%, 58.6%, 31%, and 17.2% respectively¹⁶. All heterozygous patients and most of homozygous patients (72.2%) were responded to a high dose of cholecalciferol (50,000-100,000 IU/week). The remaining of homozygous patients (27.8%) were responded to Calcitriol¹⁶.

From France

Arnaud Molin et al. discovered a new mutation in CYP2R1 that is not previously reported (c.124_138delinsCGG [p.Gly42_Leu46delinsArg]) in one family. Interestingly, a selective 25-hydroxy vitamin D therapy (Calcifediol) was tried as treatment in difficult diagnosed patients. They described 7 patients from two unrelated families, one from France and the other from Moroccan extraction. The proband case of France family was 4 years old boy, on Cholecalciferol 1000-1200 IU/day till age of 18 months. He developed leg deformity, bone pain and hypotonia. Biochemical and radiological investigation showed typical picture of vitamin D deficiency. Initially diagnosed as VDDR1A and given Alfa calcidol which lead to partial improvement of biochemical parameters except parathyroid hormone (PTH) and alkaline phosphatase (ALP) but later he developed hypercalcaemia and hypercalciuria as complications of treatment. Once diagnosed as VDDR1B with homozygous mutation, shifted to Calcifediol (25-OH-D₃) which showed marked improvement in all biochemical parameters. The proband case of Moroccan family was 9 years old boy had difficulty in walking at age of 5 years. He was initially on Cholecalciferol 800 IU/day till the age of 18 months then he was put on 100,000 IU vitamin D yearly. At 7 years of age he presented with proximal muscle weakness and diagnosed as myopathy with normal muscle biopsy. Later he was found to have homozygous mutation in CYP2R1. Both patients had low serum 25(OH)D₃ level

and high 1,25(OH)₂D₃. Another 5 members from Moroccan family were had homozygous mutations in CYP2R1 with very low serum 25(OH)D₃ level that normalized after using Calcifediol at different doses¹⁷. Genetic study showed a new mutation in CYP2R1 gene in family 1 (c.124_138delinsCGG [p.Gly42_Leu46delinsArg]), confirmed in vitro as a loss of function mutation¹⁷.

This study explore the difficulty in diagnosing patients with VDDR1B mutations and this is the first study used Calcifediol (25-OH-D₃) as treatment; bypass the first step of hydroxylation (25-hydroxylase) in liver, which lead to dramatic improvement of biochemical and radiological finding in all 7 cases with both homozygous mutations (c.124_138delinsCGG [p.Gly42_Leu46delinsArg] and c.296T>C [p.Leu99Pro])¹⁷.

Conclusion

Rickets due to vitamin D deficiency is still considered as a major problem in pediatrics even in sunny and tropical countries.¹ This problem will be more prevalent if there are multiple risk factors present together like poor nutritional, social, and climatic conditions, and it is rarely due to genetic causes. One of the genetic defects is related to 25 hydroxylase enzyme found in liver which is mainly coded by CYP2R1 gene located at chromosome 11p15.2; other genes are involved in 25-hydroxylation but to a lesser extent. CYP2R1 is responsible for normal structure and function of 25 hydroxylase enzyme^{3,4,6,7}.

Until today, only five mutations were found to affect CYP2R1 gene. Two mutations were reported from Nigeria (c.296T > C [L99P] and c.726A > C [K242N]), another 2 were from Saudi Arabia (c.367+1,G>A and c.768,insT), and the recent one from France (c.124_138delinsCGG)^{11,12,15,17}.

As observed from the previous studies, symptoms of vitamin D deficiency with normal dietary history and poor response of 25(OH)D₃ level to D2 or D3 treatment especially with strong family history of vitamin D deficiency should raise the suspicion of genetic causes for CYP2R1 mutations (VDDR1B). Patients with CYP2R1 mutations have normal or high 1,25(OH)₂D₃ (if received vitamin D) and they need supra-physiological doses of Cholecalciferol to see full or partial improvement^{11,12,15,16,17}.

The phenotype appearance in CYP2R1 mutations (VDDR1B) seem to be more complicated and depend on the number of allele affected as compared with autosomal recessive VDDR1A¹². Patient with one allele affect has modest low of 25(OH)D₃ and will respond to regular vitamin D supplement compared with two alleles affect patient with severe low of 25(OH)D₃ who may respond to higher dose of cholecalciferol or Calcitriol^{12,15,16}. Using Calcifediol (25-OH-D₃) as treatment; will bypass the first step of hydroxylation (25-hydroxylase) in liver, which lead to dramatic improvement of biochemical and radiological

finding in seven cases from a single report that may need further studies¹⁷.

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