

Genetic Disorders and Defects in Vitamin D Action

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KEYWORDS

- 1 α -Hydroxylase deficiency
- Hereditary vitamin D-resistant rickets
- Vitamin D receptor • Mutations • Rickets • Alopecia

Vitamin D is an inactive precursor, requiring 2 hydroxylation steps, first in the liver and then the kidney, to be converted to 1 α ,25-dihydroxyvitamin D₃ (calcitriol), the active hormone. The details of this metabolic conversion are described in other articles in this issue. Calcitriol then binds to the vitamin D receptor (VDR) to mediate the actions of the hormone. As described in other articles in this issue, calcitriol/VDR complexes regulate multiple target genes throughout the body. Most obvious are genes that regulate calcium and phosphate metabolism and that are responsible for normal mineralization of bone. In the absence of either the active hormone (calcitriol) or a functional receptor (VDR), calcium absorption is impaired and bones become inadequately mineralized. When this occurs in children, the disease rickets develops, when it happens in adults, osteomalacia develops. The most common cause of rickets and osteomalacia is vitamin D deficiency as described in articles by Thandrayen and Pettifor; and Bhan and colleagues elsewhere this issue for children and adults.

However, 2 rare genetic diseases can also cause rickets in children. These diseases, and the knockout mouse models of the 2 human diseases, have provided exceptional insight into the metabolism and mechanism of action of calcitriol. The critical enzyme to synthesize calcitriol from 25-hydroxyvitamin D [25(OH)D], the circulating hormone precursor, is 25-hydroxyvitamin D-1 α -hydroxylase (1 α -hydroxylase). When this enzyme is defective and calcitriol can no longer be synthesized, the disease 1 α -hydroxylase deficiency develops. The disease is also known as vitamin D-dependent rickets type 1 (VDDR-1) or pseudovitamin D deficiency rickets. When the VDR is defective, the disease hereditary vitamin D-resistant rickets (HVDRR), also known as vitamin D-dependent rickets type 2 (VDDR-2), develops. Both diseases are rare autosomal

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recessive disorders characterized by hypocalcemia, secondary hyperparathyroidism, and early onset severe rickets. As discussed later in more detail, a crucial difference between the 2 diseases is that 1α -hydroxylase deficiency is characterized by extremely low to absent serum calcitriol levels, whereas HVDRR, characteristic of a target organ resistance disease, is distinguished by exceedingly high levels of calcitriol. In this article, these 2 genetic childhood diseases, which present similarly with hypocalcemia and rickets in infancy, are discussed and compared. However, the focus is on HVDRR, a more complex and serious disease, because affected children do not usually respond to treatment with calcitriol, whereas 1α -hydroxylase deficiency does.

1α -HYDROXYLASE DEFICIENCY IN CHILDREN

In 1973 Fraser and colleagues¹ postulated that VDDR-1 was an inborn error of vitamin D metabolism involving defective conversion of 25(OH)D to $1\alpha,25(\text{OH})_2\text{D}_3$. Approximately 25 years later in 1997 the gene (CYP27B1) encoding the 1α -hydroxylase enzyme was cloned by several groups²⁻⁶ and it was immediately demonstrated that VDDR-1 was caused by mutations in the CYP27B1 gene.²

Children with 1α -hydroxylase deficiency present with a clinical picture of joint pain and deformity, hypotonia, muscle weakness, growth failure, and sometimes hypocalcemic seizures or fractures in early infancy.⁷⁻⁹ Laboratory analysis reveals hypocalcemia, secondary hyperparathyroidism, increased alkaline phosphatase activity and low or undetectable calcitriol in the presence of adequate 25(OH)D levels (**Table 1**). Radiographic findings show characteristic changes of rickets. The children have no clinical response to high doses of cholecalciferol but respond to physiologic doses of calcitriol or 1α -hydroxyvitamin D.¹⁰ Although it is a rare inborn error, 1α -hydroxylase deficiency is more frequently found in the French Canadian population.¹¹

MUTATIONS IN THE CYP27B1 GENE AS THE MOLECULAR BASIS FOR 1α -HYDROXYLASE DEFICIENCY

VDDR-1 is caused by heterogeneous mutations in the CYP27B1 gene that abolish or reduce 1α -hydroxylase enzymatic activity. A recent review by the Miller and Portale group¹² catalogs the many different mutations in the CYP27B1 gene that have been described including missense mutations, deletions, duplications, and splice site changes. To date, 48 patients have been described with mutations in the CYP27B1 gene including both genders of children from multiple ethnic backgrounds often in the setting of consanguinity.

The 1α -hydroxylase is a type 1 mitochondrial P450 enzyme that functions as an oxidase. The reaction uses electrons from reduced nicotinamide adenine dinucleotide

Table 1
Comparison of genetic defect in vitamin D action

	1α-Hydroxylase Deficiency	HVDRR
Gene	CYP27B1	VDR
$1,25(\text{OH})_2\text{D}_3$	Low	High
Parathyroid hormone	High	High
Calcium	Low	Low
Phosphate	Low	Low
Alopecia	No	Yes
Response to calcitriol	Yes	No

phosphate and molecular oxygen. The P450 moiety binds the 25(OH)D substrate, receives electrons and molecular oxygen, and catalyzes the reaction using the heme group iron to coordinate oxygen.^{12,13} Deletions, premature termination as a result of splice site mutations and insertions, are the commonest defects that disrupt or eliminate the heme-binding domain and thereby inactivate the enzyme.¹² In some cases of 1α -hydroxylase deficiency, missense mutations have been identified in the CYP27B1 gene that disrupt α helical structures, the meander sequence, the cysteine pocket that binds the heme group and the substrate recognition sites (**Fig. 1**).

The major site in the body of 1α -hydroxylase activity is the kidney. 1α -Hydroxylase is also expressed in many tissues of the body including breast, prostate, colon, placenta, and macrophages.¹⁴ These regions of local production are hypothesized to be of considerable importance for the expanded role of calcitriol in reducing cancer risk, fighting infection, and mediating antiinflammatory actions, and so forth. However, it is the renal enzyme that determines the circulating $1\alpha,25(\text{OH})_2\text{D}_3$ concentration. The renal enzyme is under careful regulation by parathyroid hormone (PTH), calcium status, and calcitriol levels so that calcium homeostasis is tightly controlled by complex feedback loops.

Children with 1α -hydroxylase deficiency caused by mutations in the CYP27B1 gene present for medical attention with metabolic abnormalities in early infancy usually before 2 years of age. In utero development is considered to be normal as a result of maternal normocalcemia. When the infants come to medical attention they characteristically show growth retardation, bone and joint deformities, and bone pain. Teeth may show marked hypoplasia of the enamel. Serum levels show hypocalcemia, hypophosphatemia, secondary hyperparathyroidism, and increased alkaline phosphatase activity. Radiographs show the classic features of rickets. Since the availability of 1α -hydroxylated vitamin D metabolites such as calcitriol or 1α -hydroxyvitamin D, therapeutic responses and complete remission of the disease can be routinely achieved. Dosages of calcitriol of 1 to 3 $\mu\text{g}/\text{d}$ are adequate to heal the rickets and return serum chemistries to normal. Calcium levels improve within days and radiologic improvement in bones can be seen within 2 to 3 months. Healing of rickets has been reported in 9 to 10 months.¹⁰

MOUSE MODELS OF 1α -HYDROXYLASE DEFICIENCY

Several groups have developed mouse models of 1α -hydroxylase deficiency using targeted disruption of the CYP27B1 gene.^{15,16} The targeted region of the gene has been the hormone-binding and heme-binding domains of the protein. After weaning, the 1α -hydroxylase null mice develop the classic features of human 1α -hydroxylase deficiency with hypocalcemia, secondary hyperparathyroidism, retarded growth, and skeletal changes of rickets. Many of the nonskeletal changes seen, including reproductive and immune function abnormalities, were probably at least partly caused by severe hypocalcemia. It was subsequently discovered that feeding the mice a rescue diet high in calcium, phosphorus, and lactose could normalize the hypocalcemia present in these mice as well as the VDR null mice and seemed to heal the rickets.^{17,18} The Goltzman group went on to compare findings in 1α -hydroxylase null mice with VDR null mice and the double mutant that combines disruption of both 1α -hydroxylase and VDR.^{19,20} In addition, the investigators studied the effects of normalizing calcium homeostasis with the rescue diet and treatment with calcitriol. They concluded that normalization of calcium cannot entirely substitute for $1,25(\text{OH})_2\text{D}_3$ action in skeletal and mineral homeostasis and that the 2 agents have discrete and overlapping functions. Both are required to maintain normal osteoclastic

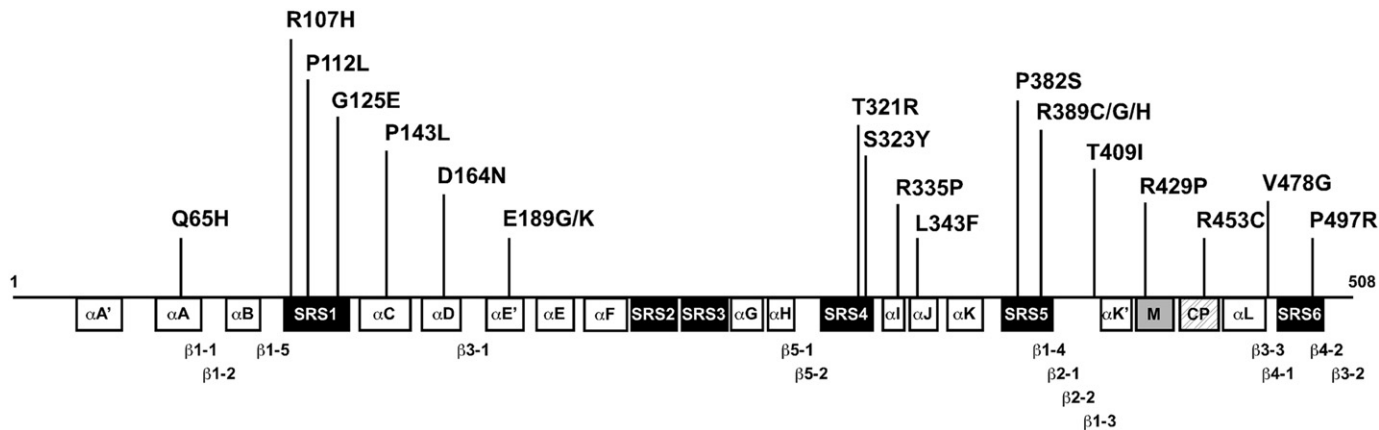


Fig. 1. Missense mutations identified in patients with 1 α -hydroxylase deficiency. The human 1 α -hydroxylase enzyme is composed of 508 amino acids. The locations of the missense mutations are shown above the line. The names of the α helices (open boxes), the meander sequence (M, gray box), the cysteine pocket (CP, hatched box), substrate recognition sites (SRS, black boxes), and the β turns are shown below the line.

bone resorption and osteoblastic bone formation. The widened cartilaginous growth plates characteristic of rickets could only be completely normalized by a combination of calcium and $1,25(\text{OH})_2\text{D}_3$. These issues are discussed further in the sections on HVDRR and VDR mutations.

HVDRR IN CHILDREN

Children with HVDRR develop hypocalcemia and severe rickets usually within months of birth. Affected children have bone pain, muscle weakness, hypotonia, and occasionally have convulsions caused by the hypocalcemia. They are often growth retarded and in some cases develop severe dental caries or hypoplasia of the teeth.^{21,22} The laboratory findings include low serum concentrations of calcium and phosphate and increased serum alkaline phosphatase activity. The children have secondary hyperparathyroidism with markedly increased PTH levels. Serum $25(\text{OH})\text{D}$ values are usually normal and $1,25(\text{OH})_2\text{D}$ levels are substantially increased. This clinical finding distinguishes HVDRR from 1α -hydroxylase deficiency in which serum $1,25(\text{OH})_2\text{D}$ values are low or absent (see **Table 1**). Many children with HVDRR also have sparse body hair and some have total scalp and body alopecia including eyebrows and in some cases eyelashes. This feature also helps distinguish HVDRR from 1α -hydroxylase deficiency. Most affected children are resistant to therapy with supraphysiologic doses of all forms of vitamin D including calcitriol.

HVDRR is an autosomal recessive disease with males and females equally affected. The parents of patients, who are heterozygous carriers of the genetic trait, usually show no symptoms of the disease and have normal bone development. These findings indicate that a single defective allele is not sufficient to cause disease. In most cases, consanguinity is associated with the disease with each parent contributing a defective gene.

MUTATIONS IN THE VDR GENE AS THE MOLECULAR BASIS FOR HVDRR

More than 100 cases of HVDRR have been recorded and many of these have been analyzed at the biochemical and molecular level.^{21–23} Presently, 34 heterogeneous mutations have been identified in the VDR gene as the cause of HVDRR (**Fig. 2**). Mutations in the DNA-binding domain (DBD) prevent the VDR from binding to DNA causing total $1,25(\text{OH})_2\text{D}_3$ resistance even though $1,25(\text{OH})_2\text{D}_3$ binding to the VDR is normal. On the other hand, mutations in the ligand-binding domain (LBD) may disrupt ligand binding, or heterodimerization with retinoid X receptor (RXR), or prevent coactivators from binding to the VDR and cause partial or total hormone resistance. Other mutations that have been identified in the VDR that cause $1,25(\text{OH})_2\text{D}_3$ resistance include nonsense mutations, insertions/substitutions, insertions/duplications, deletions, and splice site mutations. Some of these VDR mutations are described in this article.

To date, 9 missense mutations have been identified in the VDR DBD as the cause of HVDRR (**Fig. 2A**). These mutant VDRs have normal ligand binding but defective DNA binding.^{21,22} A common feature of patients with mutations in the DBD is that they all have alopecia. The heterozygotic parents with a single mutant allele are asymptomatic.

Several nonsense mutations have been identified in the VDR gene that truncate the VDR protein.^{21,22} Fibroblasts from patients with VDR nonsense mutations exhibit no ligand binding and often the truncated VDR protein cannot be detected by immunoblotting. One particular mutation, a single base change in exon 8 that introduced a premature termination codon (Y295X), was identified in several families in a large kindred where consanguineous marriages were common.²⁴ The VDR mRNA was

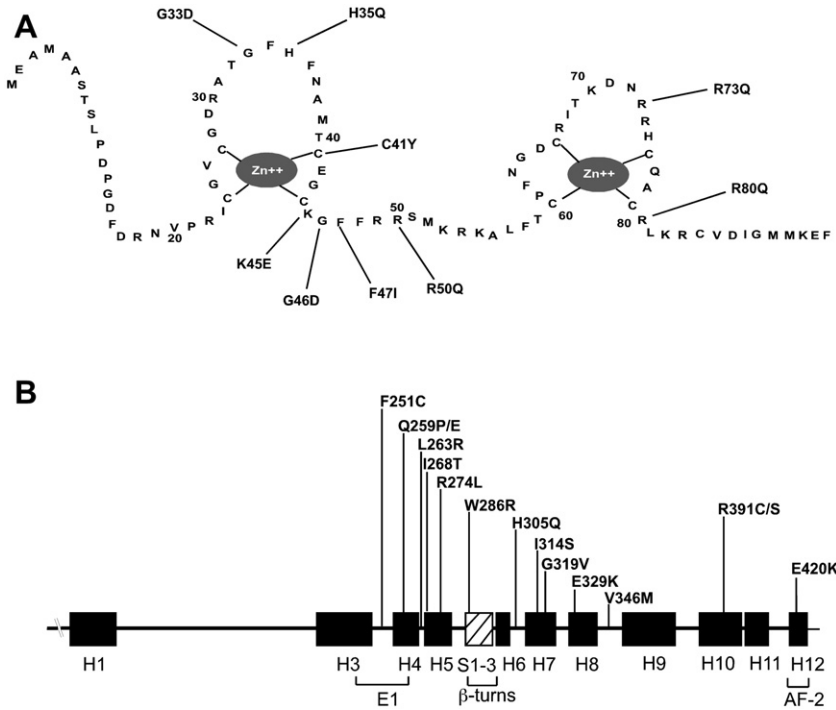


Fig. 2. Missense mutations identified in patients with HVDRR. (A) The locations of missense mutations in the VDR DNA-binding domain depicted as a 2-zinc finger structure. (B) The locations of the missense mutations in the VDR LBD are shown above the line. The filled rectangles represent α helices (H1–H12) and the hatched box represents the β sheet structure. The location of the E1 and AF-2 domains are also indicated.

undetectable by Northern blot analysis indicating that the Y295X mutation led to nonsense-mediated mRNA decay. In 1 family with an R30X mutation, the parents of the affected child had somewhat increased serum $1,25(\text{OH})_2\text{D}_3$ levels indicating some resistance to $1,25(\text{OH})_2\text{D}_3$.²⁵ All of the affected children with nonsense mutations had alopecia.

Splice site mutations in the VDR gene have also been identified as the cause of HVDRR. These mutations usually cause a frameshift and eventually introduce a downstream premature stop signal resulting in a nonfunctional VDR. Splice site mutations in the VDR gene cause exons to be skipped^{26,27} or cause incorporation of an intron into the VDR mRNA.²⁸ In 1 case, a cryptic 5' donor splice site was generated in exon 6 that deleted 56 nucleotide bases and led to a frameshift in exon 7.²⁹

To date, 15 missense mutations have been identified in the VDR LBD (see Fig. 2).^{30,31} One patient had an R274L mutation that altered the contact point for the 1α -hydroxyl group of $1,25(\text{OH})_2\text{D}_3$.^{30,32} The mutation lowered the binding affinity of the VDR for $1,25(\text{OH})_2\text{D}_3$. A second patient had an H305Q mutation that altered the contact point for the 25-hydroxyl group of $1,25(\text{OH})_2\text{D}_3$.^{32,33} This mutation also lowered the binding affinity of the VDR for $1,25(\text{OH})_2\text{D}_3$. These cases illustrate the importance of critical amino acids as contact points for $1,25(\text{OH})_2\text{D}_3$ and demonstrate that mutations of these residues can be the basis for HVDRR.

Mutations have also been identified in the VDR that disrupt VDR:RXR protein interaction as a cause of HVDRR. For example, an R391C mutation in the VDR LBD had no

affect on ligand binding but reduced its transactivation activity. R391 is located in helix H10 where the RXR dimer interface is formed from helix H9 and helix H10 and the interhelical loops between H7-H8 and H8-H9 in VDR.³² R391 was also mutated to serine (R391S).³⁴ Several other mutations have been identified in the VDR that affect RXR heterodimerization including Q259P and F251C.^{29,35} Q259 was also mutated to glutamic acid (Q259E).³⁶ A V346M mutation was identified in a patient with HVDRR and may be important in RXR heterodimerization.³⁷ All of the patients with defects in RXR heterodimerization had alopecia.

A mutation has also been identified in the VDR that prevented coactivator recruitment that is critical for transcriptional activity.³⁸ An E420K mutation located in helix H12 had no effect on ligand binding, VDR-RXR heterodimerization, or DNA binding. However, the E420K mutation abolished binding by the coactivators SRC-1 and DRIP205. The child with the E420K mutation did not have alopecia³⁸ suggesting that ligand-mediated transactivation and coactivator recruitment by the VDR is not required for hair growth.

Several compound heterozygous mutations in the VDR gene have been identified in children with HVDRR. In these cases each heterozygotic parent harbored a different mutant VDR and consanguinity was not involved. One patient was heterozygous for an E329K mutation and had a second mutation on the other allele that deleted a single nucleotide 366 (366delC) in exon 4.³⁹ The single base deletion resulted in a frameshift creating a premature termination signal that truncated most of the LBD. The E329K mutation in helix H8, which is important in heterodimerization with RXR, likely disrupts this activity. L263R and R391S compound heterozygous mutations were also identified in the VDR gene in a child with HVDRR and early childhood-onset type 1 diabetes.³⁴ The mutant VDRs in this case had differential effects on 24-hydroxylase and RelB promoters. The 24-hydroxylase responses were abolished in the L263R mutant but only partially altered in the R391S mutant. On the other hand, RelB responses were normal for the L263R mutant but the R391S mutant was defective in this response.³⁴ The reason for the differential activities of these VDR mutants is unknown. Compound heterozygous mutations were also found in the VDR gene in a patient with HVDRR and alopecia.⁴⁰ The patient was heterozygous for a nonsense mutation R30X and a 3-bp deletion in exon 6 that deleted the codon for lysine at amino acid 246 (Δ K246). The Δ K246 mutation did not affect ligand binding but abolished heterodimerization with RXR and binding to coactivators.⁴⁰ All of the patients with compound heterozygous mutations had alopecia.

Two cases of insertions/duplications in the VDR gene causing HVDRR have been reported. In 1 case a unique 5-bp deletion/8-bp insertion was found in the VDR gene.⁴¹ The mutation deleted amino acids H141 and Y142 and inserted 3 amino acids (L141, W142, and A143). Only the laboratory engineered A143 insertion into the wild-type (WT) VDR disrupted transactivation to the same extent as the natural mutation. The patient with this mutation did not have alopecia. In the second case, a 102-bp insertion/duplication was found in the VDR gene that introduced a premature stop (Y401X) and deleted helix H12.⁴² The truncated VDR was able to heterodimerize with RXR, bind to DNA, and interact with the corepressor hairless (HR) but failed to bind coactivators and was transactivation defective. The patient with this mutation had patchy alopecia.

There is only a single reported case where investigators failed to detect a mutation in the VDR as the basis of HVDRR.⁴³ In this case the investigators speculated that the resistance to the action of $1,25(\text{OH})_2\text{D}_3$ was caused by abnormal expression of hormone response element-binding proteins belonging to the hnRNP family that

prevented the VDR-RXR complex from binding to vitamin D response elements in target genes.⁴⁴

MOUSE MODELS OF HVDRR

Mouse models of HVDRR have been created by targeted ablation of the VDR.^{45,46} The targeted region has been the DBD domain. The VDR null mice (VDRKO) recapitulate the findings in children with HVDRR. The VDRKO mice appear normal at birth and become hypocalcemic and their parathyroid hormone (PTH) levels increase sometime after weaning. Bone mineralization is severely impaired and the changes of rickets develop over time. The VDRKO mice have normal hair at birth but develop progressive alopecia, thickened skin, enlarged sebaceous glands and epidermal cysts.^{45,46} In 1 VDRKO mouse model, uterine hypoplasia with impaired folliculogenesis was found in female reproductive organs.⁴⁶ When the VDRKO mice are fed a rescue diet, their calcium can be normalized and the rickets reversed or prevented as was described for children with HVDRR successfully treated with intravenous or oral calcium. Many nonskeletal abnormalities seen in the hypocalcemic mice are prevented by the rescue diet indicating the abnormalities resulted from the hypocalcemia and were not directly caused by the absence of a functional VDR. However, again as in children with HVDRR, the alopecia is not reversed or prevented by normalization of calcium homeostasis.

THERAPY FOR HVDRR

Mutations in the VDR that cause HVDRR result in partial or total resistance to $1,25(\text{OH})_2\text{D}_3$ action. The patients become hypocalcemic predominately because of a lack of VDR signaling in the intestine to promote calcium absorption. The hypocalcemia leads to a decrease in bone mineralization and causes rickets. Some patients with HVDRR improved clinically and radiologically when treated with pharmacologic doses of vitamin D ranging from 5000 to 40,000 IU/d, 20 to 200 $\mu\text{g}/\text{d}$ of $25(\text{OH})\text{D}_3$, and 17 to 20 $\mu\text{g}/\text{d}$ of $1,25(\text{OH})_2\text{D}_3$.^{21,22} Some patients also responded to $1\alpha(\text{OH})\text{D}_3$.^{21,22} The patient with the H305Q mutation, a contact point for the 25-hydroxyl group of $1,25(\text{OH})_2\text{D}_3$, showed improvement with 12.5 $\mu\text{g}/\text{d}$ calcitriol treatment.^{33,47} On the other hand, the patient with the R274L mutation, a contact point for the 1α -hydroxyl group of $1,25(\text{OH})_2\text{D}_3$, was unresponsive to treatment with 600,000 IU vitamin D, up to 24 $\mu\text{g}/\text{d}$ of $1,25(\text{OH})_2\text{D}_3$, or 12 $\mu\text{g}/\text{d}$ $1\alpha(\text{OH})\text{D}_3$.⁴⁸

When patients fail to respond to vitamin D or $1,25(\text{OH})_2\text{D}_3$, intensive calcium therapy is used. Oral calcium can be absorbed in the intestine by vitamin D-dependent and vitamin D-independent pathways. In children with nonfunctional VDR, the vitamin D-independent pathway becomes critical. When oral calcium therapy is successful, the calcium levels in the gut have been increased enough so that passive diffusion or other non-vitamin D-dependent absorption is adequate to maintain normocalcemia. Intravenous (IV) calcium infusions are used to treat children with HVDRR who fail previous treatments with large doses of vitamin D derivatives and/or oral calcium.⁴⁹⁻⁵³ IV calcium therapy bypasses the calcium absorption defect in the intestine caused by the lack of action of the mutant VDR. However, in affected children receiving IV calcium, when the IV therapy is discontinued the syndrome recurs slowly over time. Some children have been managed with intermittent IV calcium regimens using oral calcium in the intervals.²⁷ Once the child is older, perhaps when the skeleton has finished major growth, oral calcium often suffices to maintain normocalcemia and the IV calcium regimen can be discontinued.⁵² Oral calcium alone has sometimes

been used successfully as a therapy for patients with HVDRR.⁵⁴ Spontaneous healing of rickets has been observed in some patients with HVDRR as they get older and sometimes all therapy can be discontinued.⁵⁵⁻⁵⁷ In all of the cases regardless of the therapy, if alopecia is present, it is unchanged by the treatment despite normalization of calcium and healing of rickets.

A most unexpected finding is that raising the serum calcium level to normal by IV or oral calcium administration reversed all aspects of HVDRR including hypocalcemia, hypophosphatemia, secondary hyperparathyroidism, rickets, increased alkaline phosphatase activity, and so forth, except for alopecia (discussed later). Correcting the hypocalcemia often corrects the hypophosphatemia without the need for phosphate supplements. This finding indicates that the low phosphate level was caused by the secondary hyperparathyroidism, which normalizes with correction of the hypocalcemia even in the absence of VDR action. The inescapable conclusion is that the most important actions of $1,25(\text{OH})_2\text{D}_3$ on calcium and bone homeostasis occur in the intestine on calcium absorption and not in the bone. The ability of the rachitic bone abnormality to normalize in the absence of VDR-mediated vitamin D action was surprising. The data are incomplete in patients about whether the bones are entirely normal and Panda and colleagues,²⁰ using VDR knockout mice, has data suggesting that subtle defects remain in the bones of VDR null mice whose serum calcium level had been corrected by a rescue diet. However, the reversal of all clinical aspects of HVDRR with IV calcium does indicate that healing of bone and reversal of secondary hyperparathyroidism and hypophosphatemia can take place without normal VDR-mediated vitamin D action. There is no doubt that vitamin D has important actions on bone and parathyroid cells. However, these actions can apparently be compensated for in vivo if the calcium level is normalized.

In recent years there have been many new actions attributed to vitamin D that mediate important and widespread effects that are unrelated to calcium and bone homeostasis.^{58,59} These include actions to reduce the risk of cancer, autoimmune disease, infection, neurodegeneration, and so forth. At this time, the authors have not detected a trend toward an increased risk for any of these potential problems in children with HVDRR. However, there are very few cases of HVDRR and most of the cases are detected in infants and young children so that it may be too early in their life to detect an increased tendency toward any of these potential health problems.

ALOPECIA

The molecular analysis of the VDR from patients with HVDRR with and without alopecia has provided several clues to the functions of the VDR that are important for hair growth. For example, patients with premature stop mutations and VDR knockout mice have alopecia indicating that the intact VDR protein is critical for renewed hair growth after birth.^{45,46} Expression of the WT VDR in keratinocytes of VDR knockout mice prevented alopecia, a finding that further supports a role for the VDR in regulating hair growth.⁶⁰ Patients with DBD mutations also have alopecia indicating that VDR binding to DNA is critical to prevent alopecia. Patients with VDR mutations that inhibit RXR heterodimerization have alopecia indicating an essential role for VDR-RXR heterodimers in hair growth.^{29,31,35} Inactivation of RXR α in keratinocytes in mice also caused alopecia clearly demonstrating a role for RXR in hair growth.⁶¹

On the other hand, patients with VDR mutations that abolish ligand binding or patients with 1α -hydroxylase deficiency and other forms of vitamin D deficiency do not have alopecia suggesting that a ligand-independent action of the VDR is critical to regulate the normal hair cycle.^{38,62-64} The patient with the E420K mutation that

abolished coactivator binding (but not ligand binding or RXR heterodimerization) did not have alopecia indicating that VDR actions to regulate hair growth were independent of coactivator interactions.³⁸ Also, when ligand-binding defective or coactivator-binding defective mutant VDRs were specifically expressed in keratinocytes in VDR knockout mice that have alopecia, hair growth was fully or partially restored.⁶⁴

The alopecia associated with HVDRR is clinically and pathologically indistinguishable from the generalized disease atrichia with papular lesions (APL) found in patients with mutations in the *hairless* (*hr*) gene.^{39,65,66} The *hr* gene product, HR acts as a corepressor and directly interacts with the VDR and suppresses 1,25(OH)₂D₃-mediated transactivation.^{62,66,67} It has been hypothesized that the role of the VDR in the hair cycle is to repress the expression of a gene(s) in a ligand-independent manner.^{38,62,64,66} The ligand-independent activity requires that the VDR heterodimerize with RXR and bind to DNA.^{38,63} The corepressor actions of HR may also be required in order for the unliganded VDR to repress gene transcription during the hair cycle. Mutations in the VDR that disrupt the ability of the unliganded VDR to suppress gene transcription are hypothesized to lead to the derepression of a gene(s) whose product, when expressed inappropriately, disrupts the hair cycle that ultimately leads to alopecia.^{38,62,64,66} Inhibitors of the Wnt signaling pathway are possible candidates.^{68,69} Thus far, there have been no reports of mutations in the VDR that affect interactions with HR. The role of HR in regulating the unliganded action of the VDR during the hair cycle remains to be discovered.

SUMMARY

The biochemical and genetic analysis of the VDR in patients with HVDRR has yielded important insights into the structure and function of the receptor in mediating 1,25(OH)₂D₃ action. Similarly, study of children affected by HVDRR continues to provide a more complete understanding of the biologic role of 1,25(OH)₂D₃ in vivo. A concerted investigative approach to HVDRR at the clinical, cellular, and molecular levels has proved valuable in gaining knowledge of the functions of the domains of the VDR and elucidating the detailed mechanism of action of 1,25(OH)₂D₃. These studies have been essential to promote the well-being of the families with HVDRR and in improving the diagnostic and clinical management of this rare genetic disease.

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