Published in final edited form as:

Mol Genet Metab. 2010 January ; 99(1): 72–79. doi:10.1016/j.ymgme.2009.09.004.

Hereditary 1,25-dihydroxyvitamin D-resistant rickets with alopecia resulting from a novel missense mutation in the DNA-binding domain of the vitamin D receptor

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Abstract

The rare genetic recessive disease, hereditary vitamin D resistant rickets (HVDRR), is caused by mutations in the vitamin D receptor (VDR) that result in resistance to the active hormone 1,25-dihydroxyvitamin D₃ (1,25-(OH)₂D₃ or calcitriol). In this study, we examined the VDR from a young boy with clinical features of HVDRR including severe rickets, hypocalcemia, hypophosphatemia and partial alopecia. The pattern of alopecia was very unusual with areas of total baldness, adjacent to normal hair and regions of scant hair. The child failed to improve on oral calcium and vitamin D therapy but his abnormal chemistries and his bone x-rays normalized with intravenous calcium therapy. We found that the child was homozygous for a unique missense mutation in the VDR gene that converted valine to methionine at amino acid 26 (V26M) in the VDR DNA-binding domain (DBD). The mutant VDR was studied in the patient's cultured skin fibroblasts and found to exhibit normal [³H]1,25-(OH)₂D₃ binding and protein expression. However, the fibroblasts were unresponsive to treatment with high concentrations of 1,25(OH)₂D₃ as demonstrated by their failure to induce CYP24A1 gene expression, a marker of 1,25(OH)₂D₃ responsiveness. We recreated the V26M mutation in the WT VDR and showed that in transfected COS-7 cells the mutation abolished 1,25(OH)₂D₃-mediated transactivation. The mutant VDR exhibited normal ligand-induced binding to RXRα and to the coactivator DRIP205. However, the V26M mutation inhibited VDR binding to a consensus vitamin D response element (VDRE).

In summary, we have identified a novel V26M mutation in the VDR DBD as the molecular defect in a patient with HVDRR and an unusual pattern of alopecia.

Keywords

calcitriol; resistance; calcium; intravenous; hairless; retinoid X receptor

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INTRODUCTION

The hormonally active form of vitamin D, $1\alpha,25$ -dihydroxyvitamin D_3 ($1,25$ -(OH) $_2D_3$ or calcitriol) is critically important for the normal regulation of calcium and phosphate metabolism and in bone and mineral homeostasis [1]. These calcitriol actions determine the quality of bone mineralization and prevent rickets in children and osteomalacia in adults as well as play a role in the prevention of osteoporosis. The biological actions of calcitriol are mediated by the vitamin D receptor (VDR), a member of the steroid-thyroid-retinoid receptor gene superfamily of nuclear transcription factors. In humans, heterogeneous mutations in the VDR gene cause the rare recessive genetic disease known as hereditary vitamin D resistant rickets (HVDRR) also known as vitamin D dependent rickets type II. A number of mutations have been identified in the VDR and shown to be the molecular basis of HVDRR [2,3]. Patients with HVDRR develop early onset rickets and have hypocalcemia, hypophosphatemia, elevated serum calcitriol levels and secondary hyperparathyroidism. Some HVDRR patients also have total body alopecia and may develop skin lesions or dermal cysts [2,3]. These latter findings are similar to the disease atrichia with papular lesions (APL) caused by mutations in the hairless gene (*hr*). The HR protein is a corepressor of the VDR, as well as thyroid hormone receptor (TR) and retinoid-related orphan receptor (ROR) (6–8). In general children with VDR defects that cause alopecia are unresponsive to oral calcium and vitamin D or calcitriol therapy and require intravenous calcium infusions to bypass the defective VDR that prevents normal calcium absorption in the intestine.

The VDR is composed of an N-terminal DNA binding domain (DBD) that mediates binding and specificity of VDR-DNA interactions and a C-terminal ligand-binding domain (LBD) that binds the ligand calcitriol. The LBD is composed of 12 α -helices and 3 β -sheets and contains binding sites for coactivator and corepressor proteins. The VDR heterodimerizes with the retinoid X receptor (RXR) the complex then binds to vitamin D response elements (VDREs) in target gene promoters. Calcitriol binding to the VDR causes the repositioning of helix H12, that contains an activation function 2 (AF-2) domain, allowing for the recruitment of coactivators. Mutations have been identified in the VDR that disrupt ligand binding, DNA binding, RXR heterodimerization and coactivator binding [2,3]. In this report, we describe a novel mutation in the VDR DBD that disrupts DNA binding in a patient with HVDRR and partial alopecia.

MATERIALS AND METHODS

Informed consent laboratory testing and cultured fibroblasts

Informed consent was given by The Department of Social and Rehabilitation Services under a Stanford University IRB approved protocol. Laboratory tests were performed by routine methods using a Vitros chemistry analyzer (Ortho-Clinical Diagnostics, Rochester, NY) in the Children Mercy Hospital clinical lab. 25 -(OH) D (combination of D_2 and D_3) was measured by Liquid Chromatography Tandem Mass Spectrometry and $1,25$ -(OH) $_2D$ was measured by radioimmunoassay method at Quest Diagnostics (San Juan Capistrano, CA). PTH was measured by chemiluminescent Immulite immunoassay (Siemens, Los Angeles, CA). Dermal fibroblasts were cultured from an interscapular skin biopsy of the patient as previously described [4].

DNA isolation and sequencing

Genomic DNA was extracted from peripheral blood using the QIAamp DNA blood mini kit following the manufacturers instructions (Qiagen, Valencia, CA). Exons 2-9 of the VDR gene were amplified by PCR and directly sequenced at the Stanford protein and nucleic acid facility.

[³H]1,25(OH)₂D₃ binding and immunoblotting

Ligand binding assays with [³H]1,25(OH)₂D₃ were performed as previously described [5]. Hydroxyapatite was used to separate bound and free hormone. VDR antibody D-6 (Santa Cruz Biotechnology, Santa Cruz, CA) was used to detect VDR by immunoblotting as previously described [6]. The blots were developed using ECL Plus western blotting detection system (GE Healthcare, Piscataway, NJ). Protein concentrations were determined by the Bradford method [7].

CYP24A1 (24-Hydroxylase) gene induction

Fibroblasts were treated with and without 1,25(OH)₂D₃ (in ethanol) for 6 hr in medium containing 1% calf serum. RNA was isolated from the patient's fibroblasts using RNeasy spin columns (Qiagen). cDNA was prepared by reverse transcription using superscript III cDNA synthesis kit (Invitrogen, Carlsbad, CA). CYP24A1 (upper primer 5'-AGTATGGCAAGATTTTCCGC and lower primer 5'-CACTTCCCCTGGTTTCATTAG) and TATA binding protein (TBP) (upper primer 5'-TGCTGAGAAGAGTGTGCTGGAG and lower primer 5'-TCTGAATAGGCTGTGGGGTC) genes were then amplified from the cDNA using SYBR-green qPCR kit (New England Biolabs, Ipswich, MA) and semi-quantified using real time PCR as previously described [6]. Experiments were performed in triplicate with duplicate determinations.

Site-Directed Mutagenesis

Site-directed mutagenesis of the WT VDR cDNA in pSG5 (Stratagene, La Jolla, CA) was performed using the QuickChange II XL site-directed mutagenesis kit (Stratagene). Clones were verified by sequencing.

GST-pull down assays

WT and V26M mutant VDR proteins were synthesized using the TNT Quick-coupled in vitro transcription/translation system (Promega, Madison, WI) and then incubated with GST-RXR α and GST-DRIP205 with vehicle or increasing concentrations of 1,25(OH)₂D₃. After washing, the bound proteins were eluted with 2X sample buffer and subjected to immunoblotting with the anti-VDR D-6 antibody [6].

Co-immunoprecipitation

HR and VDRs were expressed in COS-7 cells. Co-immunoprecipitation was performed as previously described [8].

Transactivation Assays

COS-7 cells were grown in DMEM containing 10% bovine growth serum (Hyclone, Logan, UT) in 12-well tissue culture plates. Cells were transfected in triplicate with 62.5 ng/well WT or mutant VDR expression plasmid and 125 ng/well rat 24-hydroxylase promoter VDRE-luciferase plasmid using 5 μ l/well Polyfect transfection reagent (Qiagen). A renilla-luciferase plasmid, pRLnull (5 ng/well) that served as an internal control for transfection efficiency was included in each assay. Following a 16 hr transfection, the cells were incubated in DMEM containing 1% bovine growth serum with vehicle (ethanol) or 1,25(OH)₂D₃ for 24 hrs. The cells were then lysed in 250 μ l of reporter lysis buffer reagent (Promega) and assayed for luciferase activity using the Dual Luciferase Assay (Promega) and a Turner Design luminometer (Turner Design, Sunnyvale, CA). VDR protein expression in the transfected cell extracts was assayed by immunoblotting.

RESULTS

Case report

A male child was brought to the hospital at 13 months of age for evaluation of neglect, failure to thrive, weight loss, constipation and abdominal distention. He was born to a 22 year-old mother with severe mental retardation and cerebral palsy secondary to a perinatal event. His birth weight was 3062 g and length 46 cm. At birth, he was noted to have a large umbilical hernia and bilateral postaxial polydactyl of both hands and one foot which were surgically ligated. The child is suspected of being the product of a half-brother and half-sister union. The family history was negative for birth defects or other known genetic diseases except for postaxial polydactyl in both mother and maternal grandmother. At 10 months of age, the child had been seen in Neurology clinic for a seizure-like event. His total serum calcium at the time was 6.9 mg/dl. His neurological examination had shown global developmental delay and macrocephaly, with mild decrease in white matter and mild ventriculomegaly on MRI scan.

At presentation to our service, the child was co-operative but was not active during his examination. He was small for his age, weight 8.025 kg, length 65 cm and head circumference 51 cm. His head appeared disproportionately large for his size with large open anterior fontanel (2.5×2.5 cm). The lateral and posterior fontanels were closed. The cranial sutures were normal. There was mild frontal bossing. The distribution of hair was abnormal. He had patches of scalp that were devoid of hair, while other regions had sparse hair, and still other areas had full hair (Fig. 1A). Eyebrows were very sparse and some eyelashes were present. He had a rachitic rosary over his chest, metaphyseal widening of his wrist and ankles, and bowing of his upper and lower extremities. He had a wide nasal bridge and ear pits on the posterior lobe. The rest of the examination was normal except for the pertinent findings of delayed dentition, a large umbilical hernia and generalized developmental delay without a focal neurological deficit.

Evaluation at the time of his presentation included vision and hearing assessment that were normal. The karyotype was normal 46, XY. An evaluation for an inborn error of metabolism showed normal urinary amino acids, organic acids and oligosaccharides, and normal serum lactate, ammonia, acylcarnitine, TSH, cortisol and normal AST and ALT. The enzymatic studies for mucopolysaccharidoses on skin biopsy were normal.

At presentation, his serum chemistry panel showed normal serum electrolytes, albumin, magnesium, and renal function. The evaluation of his calcium-bone status was extremely abnormal with hypocalcemia and secondary hyperparathyroidism. His serum chemistry panel revealed calcium (Ca) 7.4 mg/dL (iCa 0.96 mmol/L), phosphorus (P) 2.4 mg/dL, parathyroid hormone (PTH) 1103 pg/ml and alkaline phosphatase (ALP) 893 U/L. His 25(OH)D was 15 ng/ml and 1,25(OH)₂D₃ 200 pg/ml (see Table 1). Urine cAMP was elevated at 9.4 (normal 1.3–3.7 nmol/dL GFR) suggesting a normal response of renal tubules to elevated PTH. The skeletal survey showed generalized osteopenia with advanced features of rickets. There was cupping and fraying at the metaphyseal ends of long bones of upper and lower extremities and widening of growth plates (Fig. 1B). There was no evidence of nephrocalcinosis on abdominal ultrasound.

The initial biochemical presentation was suggestive of nutritional rickets but the elevated 1,25(OH)₂D₃ levels and the severity of rickets both clinically and on x-rays had raised the possibility of a diagnosis of non-nutritional rickets. He was initially treated as nutritional rickets with ergocalciferol, calcium and calcitriol. We found that even after adequate correction of the 25(OH)D level, there was no evidence of healing of rickets on x-rays or improvement in his serum Ca, P, ALP and PTH levels. An attempt was made to treat with supra-physiological doses of calcitriol and calcium, but the child's chemistry panel continued to show unresponsiveness (see Table 1, Fig. 1B). During this 6 months period of oral therapy, his 25

(OH)D levels ranged from 35–117 ng/ml, 1,25(OH)₂D rose to 200–669 pg/ml, and iCa 0.92–1.13 mmol/L. At this time, it became clear that the child was resistant to calcitriol and his DNA was evaluated for a mutation in the VDR gene.

Once a VDR mutation was confirmed (see below), and the child had failed therapy with oral supra-physiological doses of calcitriol and calcium, it was decided to embark on intravenous (IV) calcium therapy and placement of a central port. With the family's agreement, IV calcium therapy was initiated. IV calcium gluconate was administered at a dose of 550 mg of elemental calcium given over a period of 10 hours, and was subsequently titrated based on his biochemical response. The infusion was first increased to 600 mg over 10 hours, then decreased to 450 mg over 15 hours, then to 450 mg over 20 hours and then to 400 mg over 20 hours. He responded to IV calcium therapy with a slow correction of his serum Ca, P, ALP and PTH, and radiological healing of his rickets (Table 1, Fig. 1B). The IV calcium therapy was complicated by multiple central line infections with various organisms including *Serratia*, *Citrobacter*, *Enterobacter*, *Enterococcus* and *Staphylococcus* species that failed to respond to conventional antibiotic therapy necessitating central line removal. He underwent placement and removal of three central ports and two Hickman lines during this 8 month period. To evaluate an immunologic deficit as a cause of recurrent infection testing including blood count, T- and B-cell enumeration, serological response to childhood immunization, serum immunoglobulins, dihydrorhodamine oxidation, nitroblue tetrazolium reduction test, and mutation analysis for chronic granulomatous disease were all normal other than a low superoxide production. The comprehensive immunological work up failed to detect a defect that could explain his marked predisposition to central line infections. Except for line infections, his infection history has been otherwise unremarkable.

The family requested that we discontinue IV calcium therapy when his x-rays and mineral metabolism showed improvement. At the time that the IVs were discontinued, the child showed normal chemistries and improved x-rays, but no change in alopecia. On no therapy other than 400 units of ergocalciferol that he received with his multivitamin preparation, we observed a subtle increasing trend in his ALP and PTH despite normal serum calcium levels (Table 1). We then initiated high dose oral calcium therapy that seemed to stabilize the ALP and PTH at elevated levels. Our plan is to reinstitute IV calcium therapy when the early changes of hypocalcemia and rickets begin to appear on serum chemistries or x-rays. On his last follow up examination at 36 months of age he had normal serum electrolytes, albumin and renal function. His blood work showed serum Ca 8.8 mg/dL, P 4.0 mg/dL, PTH 218 pg/ml, ALP 379 U/L and 25(OH)D 27 ng/ml (Table 1, Fig 1B).

Molecular analyses of VDR gene

Exons 2-9 of the patient's VDR gene were amplified by PCR from DNA isolated from blood and directly sequenced. A single G to A missense mutation was identified in exon 2 that changed the codon for valine to methionine at amino acid 26 (V26M) (Fig. 2). The patient was homozygous for the mutation with both alleles affected. No other mutations were found. Sequence analyses of exon 2 also showed that the patient was homozygous f/f for the *FokI* polymorphism that occurs in the translation initiation start site (data not shown) [9].

Analyses of the VDR in the patient's fibroblasts

The patient's VDR was analyzed using [³H]1,25(OH)₂D₃ binding assays and immunoblotting. [³H]1,25(OH)₂D₃ binding in cell extracts from the patient's fibroblast had a mean specific binding of approximately 22 fmol/mg protein (data not shown) that is within the normal range [10]. Immunoblotting demonstrated that the patient expressed the VDR V26M mutant at similar levels to WT VDR expressed in normal control fibroblasts (Fig. 3A). To determine whether the patient's cells were resistant to 1,25(OH)₂D₃, we examined CYP24A1 induction

using real-time RT-PCR. The patient's fibroblasts and normal control fibroblasts were treated with $1,25(\text{OH})_2\text{D}_3$ for 6 hr. As shown in Fig. 3B, the patient's fibroblasts failed to induce CYP24A1 in response to treatment with 1000 nM $1,25(\text{OH})_2\text{D}_3$ while the normal fibroblasts exhibited an approximately 200-fold induction of CYP24A1 transcription in response to treatment with 10 nM $1,25(\text{OH})_2\text{D}_3$. These results demonstrated that the patient's fibroblasts were resistant even to exceptionally high concentrations of $1,25(\text{OH})_2\text{D}_3$.

Transactivation

We recreated the V26M mutation in the VDR cDNA and examined the effects of the mutation on VDR transactivation in COS-7 cells. As shown in Fig. 4A, WT VDR exhibited a dose-dependent increase in luciferase activity in response to $1,25(\text{OH})_2\text{D}_3$ treatment. In contrast, the V26M mutant VDR was unresponsive when treated with up to 1000 nM $1,25(\text{OH})_2\text{D}_3$. Immunoblotting showed that both WT and mutant VDRs were expressed at similar levels (Fig. 4B). These results demonstrated that the V26M mutation abolishes transactivation by the mutant VDR.

RXR heterodimerization and DNA binding

VDR transactivation involves heterodimerization with RXR and DNA binding. We first determined whether the V26M mutation affected RXR heterodimerization. Using GST pull-down assays (Fig. 5A), we showed that the VDR V26M mutant was bound to GST-RXR in the absence of $1,25(\text{OH})_2\text{D}_3$. Addition of $1,25(\text{OH})_2\text{D}_3$ increased the binding in a dose-dependent manner similar to the WT VDR indicating normal RXR dimerization (Fig. 5A). The V26M mutation was expected to interfere with VDR-DNA interactions. We examined VDR-DNA binding *in vitro* using gel shift assays. As shown in Fig. 5B, a band shift was generated by the WT VDR in the presence of 500 nM $1,25(\text{OH})_2\text{D}_3$. On the other hand, the V26M mutant VDR failed to generate a band shift when treated with 500 nM $1,25(\text{OH})_2\text{D}_3$ demonstrating that the mutation disrupts DNA binding. Together these results demonstrated that V26M mutation inhibited DNA binding and not VDR-RXR α heterodimerization.

Coactivator-corepressor interactions

We also examined whether the V26M mutation affected VDR interactions with the coactivator DRIP205. As expected, using GST pull-down assays both the WT VDR and the VDR V26M mutant exhibited a dose-dependent increase in binding to GST-DRIP205 when treated with $1,25(\text{OH})_2\text{D}_3$ (Fig. 6A). These results demonstrated that V26M mutation does not affect VDR interactions with coactivators. The data further demonstrate that DNA binding is not required for either RXR dimerization or coactivator binding.

The child in this study also had patchy alopecia thus, we were interested in determining the effects of the V26M mutation on VDR interactions with HR. In co-IP assays, the V26M mutant VDR was immunoprecipitated with FLAG-HR similar to WT VDR (Fig. 6B, lanes 4 and 6). In the absence of transfected VDR, a small amount of endogenous VDR was immunoprecipitated with the FLAG-HR from the COS-7 cell extract (Fig. 6B, lane 2). These data indicate that the mutant VDR interacts with HR and suggests that the alopecia was not caused by a disruption in protein-protein interaction.

DISCUSSION

The patient described in this report exhibited the classical clinical pattern of HVDRR [2,3]. Despite a VDR mutation that caused the VDR to be totally unresponsive to even high concentrations of $1,25(\text{OH})_2\text{D}_3$, the patient's serum chemistries and bone abnormalities were reversed by high doses of IV infused calcium. Normalization of chemistries and x-rays took approximately 26 weeks of IV therapy. As previously noted [2,3,11,12], phosphorous

supplementation was not necessary and phosphate was normalized along with suppression of hyperparathyroidism with calcium supplementation alone. The findings again indicate that the major defect in HVDRR is an inability to absorb calcium in the GI tract. Bypassing this step using IV calcium to restore normocalcemia results in healing of the rickets and reversal of secondary hyperparathyroidism and hypophosphatemia [2,3,11,12]. Although we know from the work of many investigators that 1,25(OH)₂D₃ has many actions to regulate multiple important target genes in bone [1], the radiologic healing of rickets can be accomplished merely by normalizing the calcium concentration. Careful biopsy studies in VDR knockout mice do demonstrate some subtle abnormalities in mice treated with a “rescue” diet that corrects the serum chemistries and x-ray findings of rickets [13,14]. The resolution of the rachitic changes on x-rays has been sustained in this child for at least 9 months on the last evaluation, although minor biochemical changes have started to be apparent by 3 months following discontinuation of IV calcium. Presumably the actions of vitamin D in bone *in vivo* are redundant and alternative regulatory pathways can mostly compensate for the absence of 1,25(OH)₂D₃ action. Another possibility is that non-genomic actions of the mutant VDR remain intact since the defect in this case is limited to the DBD [15]. However, in other HVDRR cases with absent VDR due to premature termination mutations, healing with IV calcium is also routinely seen [16]. The data also show that the secondary hyperparathyroidism can be suppressed to normal by restoring the serum calcium concentration to normal even in the absence of vitamin D action. At present we are attempting to maintain normal serum calcium in the child without marked elevation in serum PTH using treatment with high dose oral calcium (200 mg/kg/day) as recently shown by Ma et al [12].

Several important clinical concepts that were learned while caring for this complicated child include the following three points. (1) Short periods of IV calcium infusion (10–12 hours) only suppress PTH transiently. The PTH is again elevated prior to the next infusion. In addition, there is marked increase in urinary calcium at the end of the infusion, which could increase the risk for nephrocalcinosis. We believe the optimal infusion time is 18–20 hours to allow at least a few hours in the day for childhood activities. The calcium dose has to be titrated based on serum Ca, P and PTH values, and urine Ca and P excretion. (2) We also treated the child with a thiazide diuretic to decrease urinary calcium and to help prevent development of nephrocalcinosis, which may be a complication of IV calcium therapy in children with HVDRR. (3) During the early course of IV calcium therapy, the serum phosphorous is depressed and the urinary phosphorous excretion is low despite markedly elevated levels of PTH. Urinary and serum phosphorous start to increase without phosphate supplementation as secondary hyperparathyroidism resolves the bone begins to heal. As previously noted [2,3, 11,12], one does not need to provide oral phosphorus supplementation despite very low levels of serum phosphorous.

The child was prone to opportunistic gram negative bacterial infections (*Serratia*, *Citrobacter* and *Enterobacter*) with central ports that have no disruption in skin barrier, while the Hickman lines which are associated with disruption in skin barrier from an exit site wound were infected within one week of placement with *Staphylococcus* and *Enterococcus*. Vitamin D has been shown to be an important player in innate immunity [17,18]. Although the immunological workup related to adaptive immunity was normal, we have not evaluated his innate immunity that could be important in his marked predisposition to central line infections.

In this child, we identified a homozygous single base change in exon 2 that resulted in a V26M mutation as the molecular basis for HVDRR. The V26M mutation is located in the first zinc finger in the VDR DBD. Our functional analyses showed that V26M mutation abolished VDR-mediated regulation of gene transcription due to the inability of the mutant VDR to bind to DNA. The V26M mutation does not affect ligand binding, RXR heterodimerization or interactions with coactivators or corepressors.

A question that warrants discussion is why the parents who are heterozygous for this mutation have no apparent symptoms of the disease. Most heterozygotes are asymptomatic [2,3]. However, in this case, the V26M mutant VDR is still able to form heterodimers with RXR and interact with coactivators and therefore it can compete with the WT VDR for these critical VDR-associated factors. This would raise the possibility of the mutant VDR allele potentially being able to exhibit dominant negative activity squelching the WT VDR. One possible explanation for this not happening is that the VDR is limiting in the cells and RXR or the coactivators are not. Since RXR and coactivators are essential for the function of multiple receptors, they are likely to be present in excess compared to the VDR. A second potential explanation is that although the DBD mutant VDRs can form heterodimers with RXR and bind coactivators in response to ligand, they cannot bind to DNA and therefore do not compete with the WT VDR at VDREs in target gene promoters.

A second question that warrants discussion is the basis of the child's partial alopecia. The pattern of alopecia is unusual and some hair is present but clearly, the overall pattern is very abnormal. We have now seen scanty or sparse alopecia in several cases and in one recent case normal hair adjacent to total alopecia [12]. The VDR and HR are thought to repress specific gene(s) during the hair cycle and that mutations in VDR or HR cause alopecia by derepression of these critical gene(s) whose protein products then disrupt the hair cycle [6,19–21]. We showed that the VDR V26M mutant binds to HR and therefore the findings suggest that the alopecia in this child was not due to the failure of the mutant VDR to interact with HR. Since the major defect in this mutant VDR is defective DNA binding, the data also suggest that VDR binding to DNA is a critical function of the VDR in its regulation of the hair cycle. Although we [19–21] and others [22,23] have previously speculated that unliganded VDR, that is mutant VDR unable to bind hormone, may still be able to regulate the hair cycle, the current data suggest that VDR-HR activity to prevent alopecia is dependent on the VDR binding to DNA. Whether this action requires ligand binding is not clear from the current findings. A possible explanation for the regions of normal or sparse hair in this child is that the hair was present at birth and has not undergone its first catagen. Most newborns lose their hair within the first 3–6 months thus it will be interesting to see if this child becomes alopecic as it gets older. To date there has been no visible change in his alopecia for over two years.

In conclusion, we have identified a novel missense mutation in the VDR gene in a patient with HVDRR and an unusual pattern of partial alopecia. The mutation substitutes methionine for a highly conserved valine at amino acid 26 in the first zinc-finger module in the VDR DBD and disrupts VDR-DNA interactions.

Acknowledgments

Supported by NIH Grant DK42482 (to D.F.)

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A. **B.** **Fig 1.**

Fig. 1A. Photo of child with partial alopecia. The child has an unusual pattern of scalp alopecia with areas of baldness, areas of scanty hair and areas with fuller hair. He has reduced hair in the eyebrows and lashes.

Fig. 1B. The figure shows sequential x-rays of wrist and knee. [A] The baseline x-rays at age 13 months show changes consistent with rickets. [B] The findings remain essentially unchanged at age 19 months despite adequate supplementation of vitamin D and supra-physiological doses of elemental calcium and calcitriol. The x-rays show progressive healing of rickets at 22 months [C], 24 months [D] and 27 months [E] while the child was receiving intravenous calcium therapy. [F] The last follow up x-ray at 36 months, 9 months after discontinuation of intravenous calcium therapy, shows no reversal in rachitic features.

Fig. 2.

A missense mutation was identified in exon 2 of the VDR gene. A unique missense (G to A) was identified in exon 2 that changed the codon for valine to methionine at amino acid 26. A, Exon 2 of the VDR gene was amplified by PCR and sequenced directly. The patient's sequence is shown in upper panel. The wild-type sequence is shown in the lower panel. B, Schematic diagram of the VDR DNA-binding domain and zinc-finger structure. Arrow indicates the location of the V26M mutation. Shaded circles represent conserved amino acid residues.

Fig. 3.

The patient's fibroblasts express the V26M mutant VDR but are resistant to 1,25(OH)₂D₃. A, VDR in cell extracts from the patient's fibroblasts were analyzed by immunoblotting. B, Induction of CYP24A1 gene expression by 1,25(OH)₂D₃ in the patient's fibroblasts was analyzed by real time RT-PCR. Cells were treated with vehicle or 100 nM 1,25(OH)₂D₃ for 6 hr. Values were normalized to TBP expression.

Fig. 4.

The recreated V26M mutant VDR is transcriptionally inactive. A, Functional activity analyzed by transactivation assays. The WT and V26M mutant VDRs in pSG5 or the pSG5 vector were transfected individually into COS-7 cells along with the CYP24A1 promoter luciferase reporter. The cells were treated with graded concentrations of 1,25(OH)₂D₃ for 24 hr and luciferase activity determined. The mutant VDR showed no stimulation of the CYP24A1 promoter as compared to the WT VDR that exhibited a dose-dependent stimulation of the promoter activity. A small but significant amount of luciferase activity due to endogenous VDR was observed in the pSG5 vector control. B, Immunoblot of VDRs expressed in COS-7 cells.

Fig. 5.

The V26M mutation disrupts DNA binding but not RXR heterodimerization. A, RXR heterodimerization was analyzed by GST-pull down. In vitro synthesized VDRs were incubated with GST-RXR in the presence of vehicle or graded concentrations of 1,25 (OH)₂D₃. The samples were subjected to GST-pull down assays and VDRs detected by immunoblotting. B, DNA binding analyzed by gel shift. Cell extracts of COS-7 cells transfected with WT VDR and VDR V26M cDNA expression vectors were incubated with a [³²P]-labeled VDRE consensus sequence with and without 500 nM 1,25(OH)₂D₃. The grey arrowhead indicates non-specific bands. The black arrowhead indicates the VDR-VDRE complex.

Fig. 6.

The V26M mutation does not disrupt coactivator or corepressor interactions with VDR. A, Coactivator interactions were analyzed by GST-pull down. In vitro synthesized VDRs were incubated with GST-DRIP205 in the presence of vehicle or graded concentrations of 1,25 (OH)₂D₃. The samples were subjected to GST-pull down assays and VDRs detected by immunoblotting. Both the WT and V26M mutant VDRs exhibited a ligand-dependent interaction with GST-RXR and GST-DRIP205. B, Corepressor interactions were analyzed by coIP. WT and mutant VDRs were co-expressed with FLAG-tagged human HR in COS-7 cells. Cell extracts were prepared and proteins immunoprecipitated with anti-FLAG or non-specific IgG antibodies. Co-immunoprecipitating VDR was detected by immunoblotting. HR expression was detected using HR specific antibodies. No bands were observed with the non-specific IgG control. In, input; α-VDR, immunoprecipitating antibody; IgG, non-specific control immunoprecipitating antibody.

Table 1

Clinical course of child with HVDRR

The table shows the therapy administered to the child with calcium, ergocalciferol and calcitriol over a 2 year period. Representative changes in serum levels for calcium (Ca), phosphorous (P), alkaline phosphatase (ALP) and parathyroid hormone (PTH) are also shown.

Age (months)	Calcium Intake* (mg/day)	Ergocalciferol (units/day)	Calcitriol (µg/day)	Serum Ca (mg/dL)	Serum P (mg/dl)	Serum ALP (U/L)	Serum PTH (pg/ml)
Reference Range				8.6–10.5	3.5–6.8	110–320	7–75
Prior to Intravenous Calcium Therapy							
13	0	0	0	7.4	2.4	893	1103
15	800	8000	0.4	8.4	2.8	1165	641
17	1600	800	1	8.6	2	920	1055
18	2400	800	1.5	7.3	2.1	529	1147
18	4000	800	6	8.6	2.2	725	642
19	8000	800	6	7.8	2.9	485	895
During Intravenous Calcium Therapy							
20	550 mg over 10 hrs	400	0	7.8	2.9	851	799
21	600 mg over 10 hrs	400	0	8.3	2.2	1039	360
22	450 mg over 15 hrs	400	0	8.2	2.8	805	158
23	400 mg over 20 hrs	400	0	8.5	5.0	532	193
24	400 mg over 20 hrs	400	0	9.0	4.0	348	99
25	400 mg over 20 hrs	400	0	9.2	6.6	383	94
26	400 mg over 20 hrs	400	0	8.8	4.9	332	136
27	400 mg over 20 hrs	400	0	9.1	5.9	294	115
Following Intravenous Calcium Therapy							
28	None	400	0	8.9	4.9	256	59
30	None	400	0	8.7	4.5	277	172
32	None	400	0	9.3	5.5	392	239
33	1800	400	0	9.0	4.9	382	92
34	2400	400	0	9.1	5.4	370	134
36	3000	400	0	8.8	4.0	379	218

* Calcium intake is reported as elemental calcium component of calcium carbonate.