Novel Insights from Clinical Practice

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# A Girl with a Novel Splice Site Mutation in *VDR* Supports the Role of a Ligand-Independent VDR Function on Hair Cycling

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**Established Facts**

- Majority of the hereditary vitamin D resistant rickets (HVDRR) patients have partial or total alopecia.
- Studies in an animal model suggested that alopecia in HVDRR might be due to the absence of a ligand-independent VDR function, but clinical data to support these findings are lacking.

**Novel Insights**

- We described a HVDRR patient with only partial alopecia, who was homozygous for a novel splice site mutation, expected to result in VDR with no ligand-binding domain.
- The phenotype and genotype of this patient supports that VDR function on hair cycling is ligand independent.

**Key Words**
Rickets · Alopecia · Vitamin D resistant · Vitamin D receptor · Mutation

**Abstract**
Mutations in vitamin D receptor (*VDR*) cause hereditary vitamin D resistant rickets (HVDRR). We reported a Thai girl with HVDRR, presenting with an early onset of rickets and partial alopecia. She was a product of a consanguineous couple. Mutation analysis showed that she was homozygous for a novel splice site mutation of the *VDR* gene, 462 + 1 G → C, resulting in incorporation of the whole 254 bp of the intron 4 into its mRNA. The mutated protein is expected to contain no ligand-binding domain. The fact that she did not develop total alopecia despite of no VDR ligand-binding domain supports that VDR function on hair cycling is ligand independent.


## Introduction

The loss-of-function mutations of vitamin D receptor (*VDR*), leading to target organ resistance to active vitamin D, are the main pathophysiologic defect in a rare

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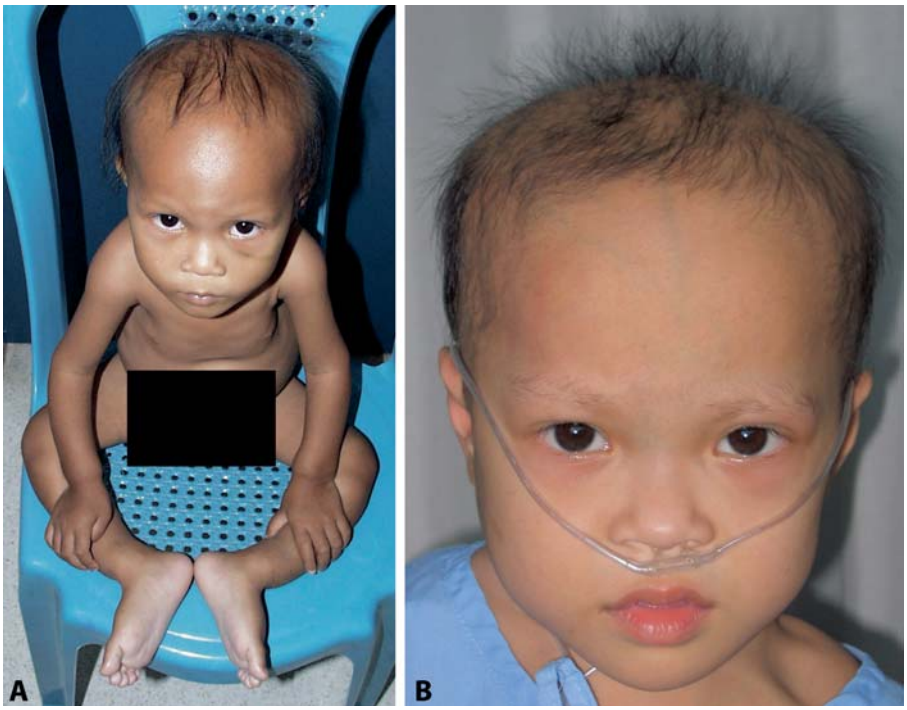


**Fig. 1.** The patient at the ages of **A** 34 months and **B** 52 months. Note partial alopecia.

autosomal recessive disease called hereditary vitamin D resistant rickets (HVDRR) or vitamin D dependent rickets type II (VDDR II) (MIM #277440: VDDR IIA and #277420: VDDR IIB) [1–3]. Patients with HVDRR typically present with an early onset of rickets, hypocalcemia and secondary hyperparathyroidism. Majority of the HVDRR patients also have partial or total alopecia. They usually are born with hair and eventually shed it within first days to first months of life [4]. The mechanism of alopecia in HVDRR is uncertain. Currently, the VDR is thought to play a key role in normal hair cycling especially in the anagen initiation. Recent studies in experimental animals suggested that alopecia in HVDRR might be due to the absence of ligand-independent VDR function [5, 6]. Nevertheless, there are no clinical data to support these experimental observations.

At least 24 mutations of the *VDR* gene in HVDRR patients have been reported. Most of them are missense and nonsense mutations in the ligand-binding or DNA binding domain [2, 7–10]. However, only two splice site mutations of the *VDR* gene have been reported [11, 12]. In the present study, we reported the third splice site mutation of the *VDR* gene in a Thai girl with HVDRR, which may provide empirical support to the role of ligand-independent VDR function on hair cycling.

## Case Report

A 34-month-old Thai girl who was the offspring of consanguineous parents was referred to us with an early onset of rickets and partial alopecia (fig. 1A). Her serum ionized calcium level was 1.11 mmol/l (reference value 1.15–1.30), serum phosphate was 2.7 mg/dl (reference value 4–6), serum alkaline phosphatase was 3490 U/l (reference value 100–320) and parathyroid hormone was 836 pg/ml (reference value 15–65). The diagnosis of HVDRR was made based on clinical features of rickets and alopecia. Although vitamin D level was not available, vitamin D resistance in this patient was evident from a poor response to a high dose of active vitamin D treatment. At the age of 52 months, she still had hair (fig. 1B).

*Mutation Analysis*

After informed consent, 3 ml of the patient and her mother's blood were taken. Genomic DNA (gDNA) and RNA were extracted. The blood of the patient's father was not available. Total complementary DNA (cDNA) was prepared from RNA by reverse transcription, using ImProm-II, reverse transcriptase (Promega, Madison, Wisc., USA). The *VDR* cDNA was amplified by polymerase chain reaction (PCR) using primers 5′-GGG TCT GAA GTG TCT GTG AG-3′ and 5′-TGA GGA GGG CTG CTG AGT AG-3′ at the annealing temperature of 65°C. The PCR products were sent for sequencing at Macrogen Inc., South Korea.

The whole exon 4 and the 5′ end of intron 4 of the *VDR* gene was amplified from gDNA of the patient and her mother by PCR using primers 5′-AAA GCC CCT CCT ATC TTG GA-3′ and 5′-CTT CCT ACC TTG GCC CTG AT-3′ at the annealing temperature of 56°C. The PCR products were sent for sequencing and

were digested with the restriction endonuclease *Nci*I (New England Biolabs, Beverly, Mass., USA), according to the manufacturer's instructions. The digested products were analyzed by 3% agarose gel.

## Results

Direct sequencing analysis of the *VDR* cDNA PCR products of the patient revealed a homozygous unspliced intron 4, with a single nucleotide change at the position 462 + 1 in the 5′-end of the intron, converting the wild type, G to C (462 + 1 G → C) (fig. 2A). This mutation was also found homozygously in the patient's (data not shown) and heterozygously in her mother's gDNA (fig. 2B). They were confirmed by RFLP analysis (fig. 2C).

## Discussion

In this report, we identified a Thai girl with HVDRR and a novel splice site mutation at the 5′-end of intron 4 of the *VDR* gene. This mutation resulted in an abnormal mRNA with persistent of the 254-bp intron 4. According to the mRNA sequences, the mutated protein was expected to be truncated with 154 amino acids of the wild type sequence and additional 23 amino acids from the unspliced intron 4. This mutated protein would lack the ligand-binding domain and a portion of the hinge region. Therefore, the reported patient, who had homozygous mutations, would have no ligand-dependent VDR functions.

Alopecia is an interesting feature in HVDRR. It usually presents very early and has correlation with more resistance to treatment [1–3]. Alopecia is likely to be related to the loss of a VDR function rather than the loss of vitamin D actions since it is not associated with vitamin D deficiency and 1α-hydroxylase deficiency rickets. Moreover, alopecia remains unchanged in patients with HVDRR that showed skeletal response to high doses of vitamin D and calcium [13]. The HVDRR patients with alopecia tend to have mutations in the DNA-binding domain of *VDR*, while HVDRR patients without alopecia tend to have mutations in the ligand-binding domain [2]. However, many patients with mutations in the ligand-binding domain of *VDR* had alopecia [7, 9, 12–14].

Our patient had only partial, not total alopecia, suggesting that the hair cycling could be at least partly maintained in the absence of the ligand-binding domain of VDR. This observation supports the previous hypothesis that VDR function on hair cycling is ligand-independent [5, 6].

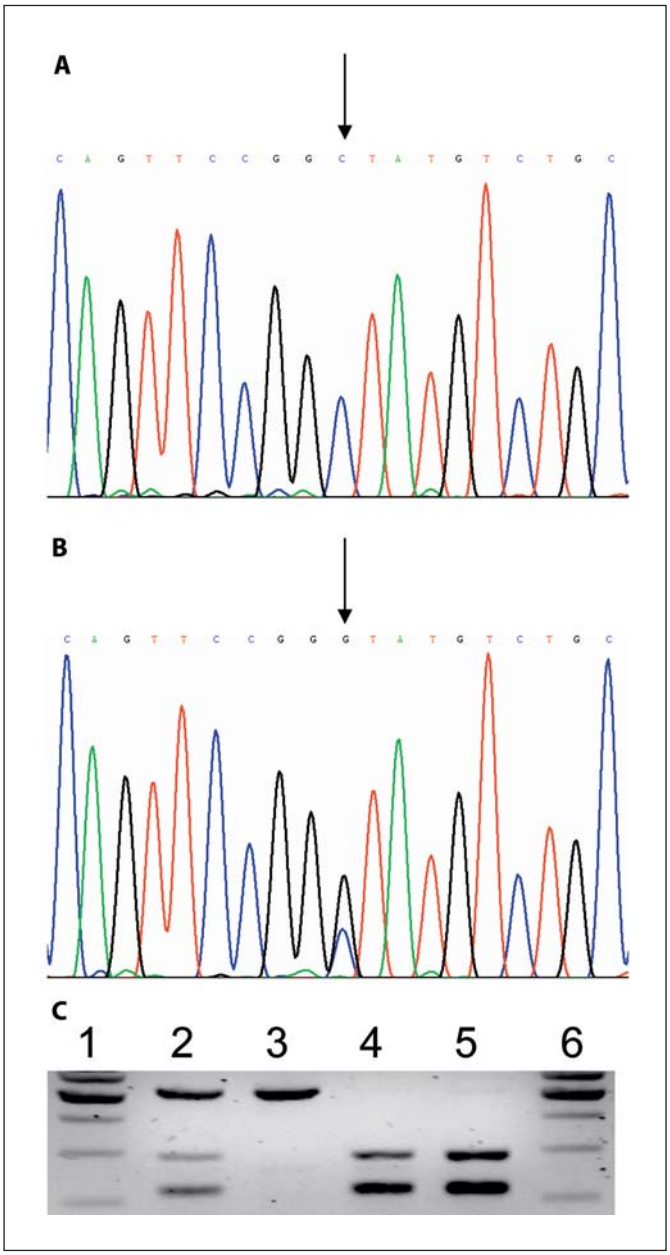


**Fig. 2.** Mutation analysis. **A** cDNA sequence of the patient shows a homozygous G to C mutation (arrow) at the nucleotide position 462 + 1. **B** gDNA sequence of her mother shows a heterozygous G to C mutation (arrow) at the same position. **C** PCR-RFLP analysis. Lanes 1 and 6, markers; lane 2, mother; lane 3, patient; lanes 4 and 5, controls. The 462 + 1 G to C mutation eliminates an *Nci*I restriction site. *Nci*I cut the wild type of gDNA PCR products of intron 4 into 2 fragments, 210 and 285 base pairs, while the mutated sequence was not cut by the enzyme and remained in a single fragment of 495 base pairs. The patient showed homozygous mutation while her mother showed heterozygous mutation.

Patients with mutations in the ligand-binding domain of *VDR*, who were reported to have total alopecia, had no detectable VDR protein and *VDR* mRNA [13]. On the contrary, our patient, who also had homozygous mutations in the ligand-binding domain of *VDR* but exhibited only partial alopecia, had detectable *VDR* mRNA. We hypothesized that the ligand-independent VDR function on hair cycling would be carried in the DNA binding domain of VDR. The mutations in the ligand-binding domain of *VDR* may cause alopecia by reduction of the mutated protein production, or by alteration of the conformational pattern of VDR molecules, thus the non-mutated DNA binding domain of VDR can not function properly.

## Conclusion

In conclusion, we described a HVDRR in a Thai girl with partial alopecia, who was homozygous for a novel splice site mutation, expected to result in VDR with no ligand-binding domain. The phenotype and genotype of this patient supports that alopecia in HVDRR would be due to the absence of the ligand-independent VDR function.


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