Case Report

Vitamin D-Dependent Rickets Type II: Report of a Novel Mutation in the Vitamin D Receptor Gene

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Hereditary vitamin D-resistant rickets type or vitamin D-dependent rickets type II is a genetically determined and rare autosomal recessive disorder, most often caused by mutations in the vitamin D receptor gene. It usually presents with rachitic changes not responsive to vitamin D treatment and the circulating levels of 1,25 $(OH)_2$ vitamin D-3 are elevated, differentiating it from vitamin D-dependent rickets type I. Alopecia capitis or alopecia totalis is seen in some families with vitamin D-dependent rickets type II. This is usually associated with a more severe phenotype. In this report, we present the clinical findings on a family which exhibited the typical clinical features of hereditary vitamin D-resistant rickets in two siblings. In addition, molecular analysis of the vitamin D receptor gene was performed by sequencing all coding exons. The cardinal findings in the index patient were alopecia totalis, renal tubular acidosis, mild generalized aminoaciduria, refractory rickets, high alkaline phosphatase, and hyperparathyroidism. Other routine biochemical tests were within normal limits, but 1+ glycine was detected in his urine. Skin biopsy results were compatible with alopecia areata. A previous child with similar phenotype was reported to be deceased at the age of 32 months. Mutation analysis of the vitamin D receptor gene by direct sequencing analysis of all coding exons showed a homozygous c.122G→A(p.Cys41Tyr) variant in exon 2 with several arguments pointing to a pathogenic effect. We should be aware of this very rare disease whenever we see a patient with refractory rickets and alopecia.

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Introduction

Hereditary vitamin D (Vit D)-dependent rickets type II is an autosomal recessive disorder resulting from end-organ resistance to 1,25$(OH)_2$ Vit D3.[1] It is caused by a defect in the Vit D receptor (VDR) gene. The defect leads to an increase in the circulating ligand, 1,25$(OH)_2$ Vit D3, the major regulator of calcium homeostasis which is considered to be a true steroid hormone acting via intranuclear VDR.[2] VDR is a member of the steroid-thyroid-retinoid gene superfamily of nuclear transcription factors that regulates gene transcription.[3] The VDR structure consists of an N-terminal DNA- binding domain (DBD) that enables interaction with Vit D response elements located in the promoter regions of target genes and a C-terminal ligand-binding domain (LBD) that binds to 1,25$(OH)_2$ Vit D3. Initiation of gene transcription by the VDR, requires binding as a heterodimer with the retinoid X receptor (RXR), and the recruitment of coactivator proteins. The coactivator proteins modify DNA and link nuclear receptors to the general transcription machinery and RNA polymerase II.[4]

Hereditary Vit D-resistant rickets (HVDRR), also known as Vit D-dependent rickets type II (VDDRII) is a rare genetic disorder caused by

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mutations in the VDR gene.[5] Patients with HVDRR exhibit early onset rickets, hypocalcemia, and secondary hyperparathyroidism. The significantly elevated serum levels of $1,25(OH)_2$ Vit D3 distinguishes this disorder from 1-α-hydroxylase (type I) deficiency which is associated with low levels of $1,25(OH)_2$ Vit D3. In most cases of type II disease, patients exhibit total alopecia. Consanguinity is often a predisposing factor in the transmission of this autosomal recessive disease. A growing number of genetic mutations have been reported in the VDR gene, including missense and nonsense mutations, splice site mutations, and partial deletions of VDR gene.[5] VDDRII with normal VDR is an unusual form of rickets due to abnormal expression of a hormone response element binding protein that interferes with the normal function of the VDR. Molecular analysis has revealed no mutations in the VDR coding region.[6]

Case Report

This 18-month-old Iranian boy, the proband, is the third offspring of consanguineous parents (Figure 1). The pregnancy, labor, and delivery were uneventful, and he was delivered by cesarean section. He was healthy at birth with birth weight of 3300 g. He was breast- fed in the first 20 days, and then received formula. The patient was born with normal hair, but on day 20, he developed progressive scalp hair loss, that transformed to total alopecia. X-rays were performed, and elevated serum concentration of alkaline phosphatase was noted, suggesting early- onset rickets. He did not respond to high doses and repeated administration of Vit D (Figure 2).

At six months, his wrist X-ray revealed rickets again; serum alkaline phosphatase was also high. At one year, he had a serum calcium of 9.4 mg/dL, phosphorus of 2.9 mg/dL, alkaline phosphatase of 729 IU, and PTH of 502 (normal: 7.0 – 82.0) pg/mL. Generalized aminoaciduria, and cystinuria were also detected (Figure 3).

Sections of skin biopsy specimen showed no significant changes of the epidermis except for a slight acanthosis and follicular plugging of the epidermis. There was a reduced number of follicles and increased number of small miniaturized follicles throughout the mid-dermis. Mild peri-

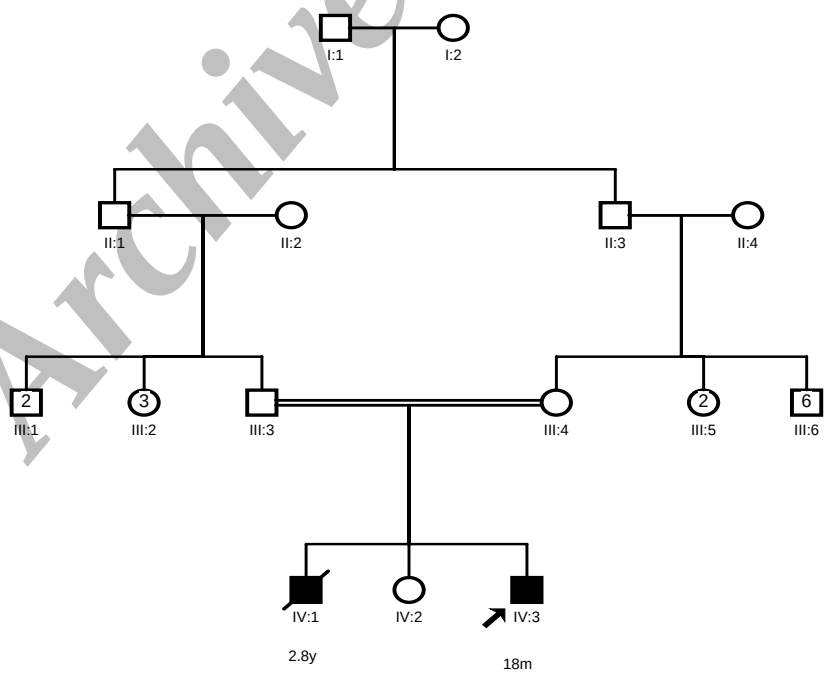

Figure 1. Pedigree of the family.

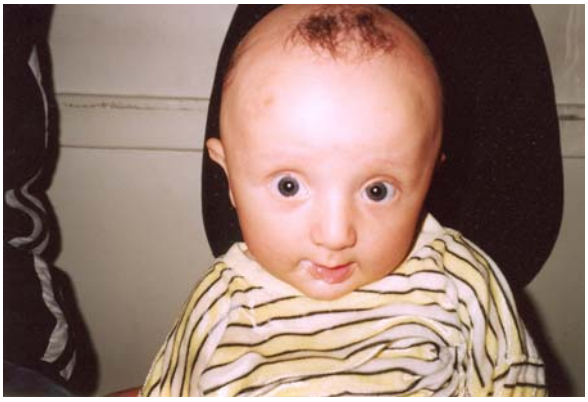

Figure 2. The proband (A) and his deceased elder brother (B), with apparent alopecia totalis.

bulbar fibrosis admixed with sparse lymphocytic infiltration was observed throughout the mid-dermis. The diagnosis of alopecia areata was made by the dermatopathologist (Figure 4).

The parents had two older children. The firstborn was a boy with similar signs and symptoms, refractory rickets, renal tubular acidosis (RTA), and alopecia, and had died at the age of two years and eight months. The second child was a healthy five-year-old girl. The mother had been under specialized prenatal care during pregnancy and avoided drugs. High-resolution ultrasonographies during pregnancy were normal.

Based on clinical findings, high alkaline phosphatase, elevated PTH level, and history of the similar disorder in his elder brother, the diagnosis of hereditary Vit D-dependent rickets type II (HVDDR) was suggested.

The proband was treated with high-dose intramuscular Vit D3, and oral $1,25(OH)_2$ Vit D3 (Rocartrol), and calcium. The treatment was somehow effective, but it had no effect on the alopecia. At the present time, the patient's general condition is good and he can stand with support.

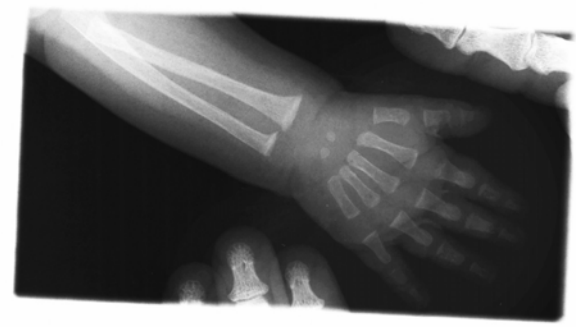

Figure 3. Wrist roentgenogram of the proband with rachitic changes.

His recent laboratory report showed an alkaline phosphatase of 1,125 IU, 25(OH) Vit D of 47.4 (normal: 7.6 – 75) ng/mL, and PTH of 509 (normal: 7 – 82) pg/mL. Unfortunately, measurement of 1, $25(OH)_2$ Vit D was not available.

Molecular analysis

Mutation analysis of the VDR gene was performed to confirm the clinical diagnosis of HVDRR II. Sequence analysis of all coding exons revealed a homozygous c.122G→A substitution in exon 2 resulting in a p.Cys41Tyr variant at the protein level. Both parents were found heterozygous carriers of p.Cys41Tyr.

Discussion

Vit D-deficient rickets is a common nutritional disease in infants and children, and can easily be diagnosed and treated by oral or parenteral Vit D. Vit D-resistant rickets, on the other hand, is a very rare disorder characterized by resistance to traditional therapeutic regimens that appears in several different phenotypes, heterogeneous pathogenesis, and clinical features. VDDRII is extremely rare and is mostly accompanied by alopecia. The disease is caused by target organ resistance to $1,25(OH)_2$ Vit D3, the biologically active form of Vit D.[7] It is diagnosed through the finding of normal or elevated circulating levels of $1,25(OH)_2$ Vit D3, which differentiate it from Vit

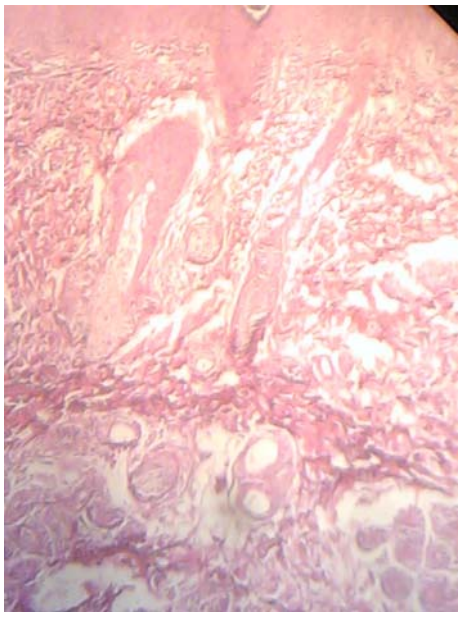

Figure 4. Section of the scalp biopsy specimen from the proband showing abortive development of the hair follicles (see text for details).

D-dependent rickets type I (VDDR I). The latter is caused by defective 1-α-hydroxylation of 25(OH) Vit D3 in the kidneys, resulting in low serum levels of $1,25(OH)_2$ Vit D3.[8,9] In our proband, 25 (OH) Vit D3 was 47.4 (normal: 7.6 – 75) ng/mL; however, we could not measure $1,25(OH)_2$ Vit D3. For the confirmation of the diagnosis, molecular testing for mutations in the VDR gene can be performed.

A wide range of clinical features exists in VDDR type II. These include the variable response to therapy with Vit D derivatives and the presence or absence of alopecia.[7,8,10]

In cases of HVDDR II, there seems to be several types of abnormality in the VDR complex.[11] The most common defect is undetectable binding of $1,25(OH)_2$ Vit D3 to the receptor either because of an absent VDR or a defective steroid- binding domain of the VDR.[11] Mutations causing premature termination of the VDR protein,[5,12] and mutations in the VDR DBD that affect VDR-DNA interactions[13] result in complete hormone resistance. Mutations in the VDR LBD affect ligand-binding heterodimerization with RXR, or cofactor binding. These mutations result in partial or total hormone unresponsiveness.[12,14,15] A relationship between alopecia and marked resistance to $1,25(OH)_2$ Vit D3 has been recognized.[7,16] In our index patient, we identified a homozygous c.122G→A (p.Cys41Tyr) variant, which has not been described yet. This substitution affects one of the two zinc modules located in the DBD. Moreover, the cysteine at position 41 is highly conserved in the VDR of mammals, birds, and amphibians and in other nuclear hormone receptors. We did not find the c.122G→A variant in our control population (100 chromosomes). All these arguments point to a high likelihood of the pathogenic effect of this substitution.

The alopecia can be present at birth, but usually starts in the first few months of life and becomes complete in early childhood.[9] It can be associated with decreased hair in other body parts, such as the eyebrows and eyelashes, or body hair, and is usually unresponsive to VitD treatment,[7] as was also the case in our patient. The development of alopecia is felt by some investigators to be associated with a more profound $1,25(OH)_2$ Vit D3 resistance.[17] This was confirmed by Marx et al. when they reviewed and analyzed the relationship between alopecia and resistance to $1,25(OH)_2$ Vit D3 in 22 cases from 30 kindreds in whom they noticed that alopecia was associated with the severest grades of resistance to $1,25(OH)_2$ Vit D3.[17]

The VDR is found in several tissues not thought to play a role in mineral ion homeostasis. It has been shown to be expressed in the outer root sheath of keratinocytes and in the dermal papillae of hair follicle.[18] Its precise function in these tissues as well as its developmental role remains unclear. There is paucity of information on the findings of scalp biopsies. Our patient showed no significant changes of the epidermis, but reduced number of follicles was reported and even one showed total absence of hair follicles.[19] One report revealed normal numbers and normal light microscopic features of the hair and hair follicles, which supported the authors' opinion that an ultrastructural or biochemical defect is responsible for the hair loss. In two reports by Marx et al., [2,17,19] patients' biopsies revealed features typical of alopecia totalis with no lymphocyte infiltration, and one showed normal morphological features of the epidermis, lack of hair shafts in the hair follicles, and no lymphocyte infiltration. Li et al.[20] developed an animal model of HVDDRII, generated by targeted ablation of DNA encoding the second zinc finger of the DBD of the VDR. The resultant rats were phenotypically normal at birth but developed hypocalcemia, hyperparathyroidism, and alopecia within the first month of life. This suggests that the VDR plays a key role in the hair cycle rather than in primary hair growth.

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