

Association of Single Nucleotide Polymorphisms in the VDR and CYP27B1 Genes with Risk of Developing Vitamin D3 Deficiency

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Abstract

This study was carried out to investigate the relationship between common variants in two vitamin D pathway genes (*VDR* and *CYP27B1*) and vitamin D3 serum levels. In this study, serum vitamin D metabolite levels were measured in the blood samples of 200 patients with alopecia areata. Then, single nucleotide polymorphisms (SNPs) in *VDR* and *CYP27B1* were analyzed using polymerase chain reaction (PCR)-sequencing. Sixty-three variations were observed in these genes (42 variations in *CYP27B1* and 21 variations in *VDR*). A significant difference in Rs1544410 (odds ratio: 7, $P < 0.0005$) and rs4646536 (odds ratio: 4.043, $P < 0.0005$) variants was found between the patients and controls. The study showed the relationship between the two polymorphisms, Rs1544410 (odds ratio: 7, 95% CI, 1–8) and rs4646536 (odds ratio: 4.043, 95% CI, 3–14.038) on the genes *VDR* and *CYP27B1*, respectively, with increased risk of developing vitamin D3 insufficiency in the Iranian population. Therefore, SNPs in the *VDR* and *CYP27B1* genes can be considered as prognostic biomarkers of the risk of developing vitamin D3 deficiency.

Keywords: VDR, CYP27B1, VITAMIN D3, RS 1544410, SNPs

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INTRODUCTION

Vitamin D is a group of biologically inactive, fat-soluble prohormones with known importance in maintaining body health. There are two forms of vitamin D (calciferol): vitamin D₂ (ergocalciferol) and vitamin D₃ (cholecalciferol). The accepted indicator of vitamin D₃ sufficiency in humans is serum 25-hydroxyvitamin D₃ (25(OH)D₃) levels (normal levels defined as 25(OH)D₃ serum levels from 30–100 ng/mL). Currently, there is ample evidence suggesting an association of vitamin D₃ deficiency (< 10 ng/mL) with various physiological disorders, especially immunological dysfunctions such as alopecia areata. The main causes of human vitamin D₃ insufficiency (defined as serum 25(OH)D₃ levels <30 ng/mL) include limited dietary sources, inadequate sun exposure, and presence of polymorphisms in several vitamin D genes (*VDR*, *VDBP*, *CYP24A1*, *CYP2R1*, *CYP27A1*, and *CYP27B1*)¹⁻⁴.

Several studies have reported on the biological role of the vitamin D receptor (*VDR*) on the immunomodulatory capabilities of 1,25-dihydroxy-vitamin D₃ (1,25(OH)₂D₃). The *VDR* gene is located in the human 12q12-q22 region. It has more than 470 single nucleotide polymorphisms (SNPs), some of which modulate 1,25(OH)₂D₃ uptake. In several experimental animal models, 1,25(OH)₂D₃ administration markedly prevented the development of autoimmune diseases. Therefore, it is possible that point mutations in the polymorphisms of *VDR* may have a pivotal role in the development of autoinflammatory and other autoimmune diseases⁵⁻⁸.

Studies have shown a significant association between SNPs in *CYP27B1* and serum 25(OH)D₃ levels (9, 10). *CYP27B1* is a mitochondrial cytochrome P450. Its gene encodes 25-hydroxyvitamin D-1 α -hydroxylase (1 α -hydroxylase), an enzyme that catalyzes the final step of vitamin D activation to calcitriol and the 1 α -hydroxylation of 25(OH)D₃ to produce 1,25(OH)₂D₃. Furthermore, 1,25(OH)₂D₃ plays a significant role in immune system regulation of the human body and also has prodifferentiative and antiproliferative properties on numerous cell types^{11,12}.

The main goal of this study was to investigate the association of common polymorphisms in the *VDR* and *CYP27B1* genes with vitamin D₃ serum levels in an Iranian population. The Consider of the examined polymorphisms as biomarkers for the exact diagnosis of vitamin D deficiency cannot be ignored.

MATERIALS AND METHODS

Subject selection and sampling

Archived blood samples from 2016 to 2019 of 800 patients with alopecia areata were obtained from the blood bank of the Iranian Association of Patients with Alopecia areata. The eligibility criteria of the patients included individuals aged 25 \pm 5 years living in central Tehran. In order to eliminate the effect of sunlight on the level of vitamin D₃ in the blood, each year (2016, 2017, 2018 and 2019) only the blood samples of patients collected between July and September were examined. Patients who traveled outside of Tehran during the study, pregnant or

Table 1. Demographic and serum vitamin D₃ variables in participant

Characteristics/variables	Baseline vitamin D ₃ status* (N=200)		
	Deficient(N=50) patients	Insufficient (N=50) patients	Sufficient (N=100) Case-control
25(OH)D ₃ (ng/mL)	X \leq 10	10 <X< 30	30 \leq X \leq 100
Gender			
F	84(64/12%)	37(28/24%)	10(7.64%)
M	5(7.24%)	47(68.12%)	3(24.64%)
BMI (Kg/m ²)	20<X<27	20<X<27	20<X<27
age	25 \pm 5 years	25 \pm 5 years	25 \pm 5 years

lactating women, and patients with a history of taking vitamin d supplements were excluded from the study.

The levels of 25(OH)D in frozen serum were measured using an ELISA kit (EIA Kit, IDS, UK 25-OH-D). The usual quality control method was used to ensure the accuracy and precision of the tests throughout the experimental investigation. The sensitivity range of the kit was 5 nmol/L or 1.5 ng/mL, the intra-test coefficient of variation was 4.3%, and the extracorporeal coefficient of variation was 8.7%.

Questionnaires that detailed demographic data, sex, age, history of various diseases, medications, and smoking were collected from the Data Bank of the Iranian Association of Patients with Alopecia areata. Based on this, 200 of the 800 studied cases were included in the study. One hundred subjects were assigned as the patient group (50 cases with vitamin D3 insufficiency and 50 cases with vitamin D3 deficiency) and 100 individuals as the normal group (normal levels defined as 25(OH)D3 serum levels from 30–100 ng/mL) through random selection (Table 1).

As previous studies have shown a significant association between SNPs in the *CYP27B1* and *VDR* genes and serum 25(OH)D3 levels, we investigated SNPs in these genes using the polymerase chain reaction (PCR)-sequencing

method. We also measured the association between these SNPs and changes in serum vitamin D3 metabolite levels in patients with alopecia areata.

DNA extraction and primer

Genomic DNA from the blood was isolated using the DNA extraction kit DNP (Sinaclon, Iran), and its quality and quantity were analyzed using agarose gel electrophoresis and a NanoDrop 2000 UV-Vis Spectrophotometer (Thermo Fisher Scientific), respectively.

Dedicated primers were designed using Primer 3 software to investigate important polymorphisms in the *VDR* (F: 5'-GTATGCCTAGACTCCACCTCC-3'. R: 5'-GGAAGAGGTCAAGGGTCAC-3') and *CYP27B1* (*CYP27B1*[A]: F: 5'-CCTATCCCAAGCCCAGTC-3'. R: 5'-GGAGAGGGATTGCGTCTG-3' and *CYP27B1*[B]: F: 5'-CTGACCCACTTCTGTTC-3', R: 5'-CAGTAGAAAGGGTGCATAGG-3') regions according to the sequencing-PCR method. These primers were synthesized by SinaClon.

Polymerase chain reaction (PCR)-sequencing

The final volume used in the PCR reactions was 25 mL, in which genomic DNA (205–250 ng) was amplified. The reaction used PCR Master Mix (2X) Sinaclon kit (0.08 units/ μ l Taq DNA polymerase in reaction buffer, 3 mM MgCl, 2 mM dATP, 0.4 mM dCTP, 0.4 mM dGTP, and 0.4 mM

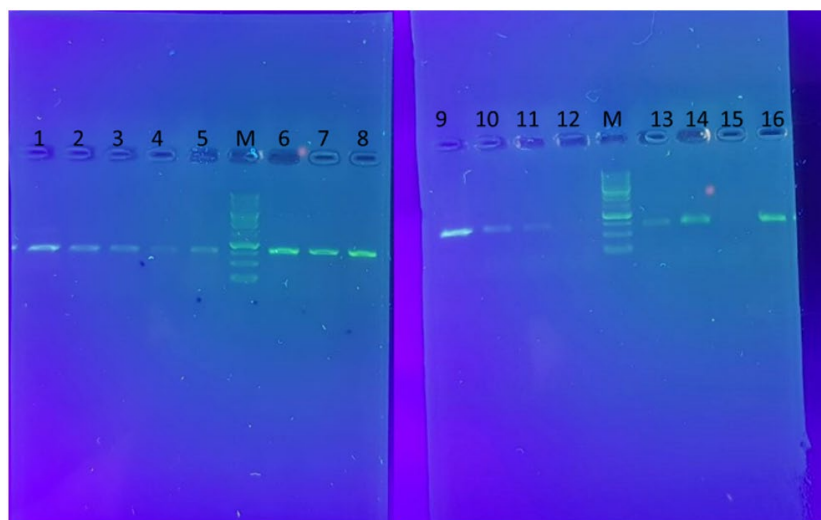


Fig. 1. The amplified sequences of *CYP27B1*(B) (1,2,3,4,5), *VDR* (6,7,8) and *CYP27B1*(A) (9,10,11,13,14,16) through the sequencing- primer PCR method. M; ladder 1KB. Also, 12 and 15 are negative control.

dTTP) (Cat. No.: MM2061). An advanced biometric thermocycler was used in this study. The protocol for PCR amplification is mentioned in Table 2.

The PCR products were analyzed by electrophoresis in 1% agarose gel alongside a Thermo Scientific Gene Ruler 100 bp DNA Ladder for reference (Fig. 1).

The PCR products were sent to Rooyan Zista Gene Company for sequencing in both the forward and reverse directions. The sequencing data were analyzed using Finch TV version 1.4.0. NCBI BLAST was used as reference (Fig. 2).

Statistical analysis

Fisher’s exact test was used to evaluate the association between variables and to study the deviation from the Hardy-Weinberg equilibrium. A nominal significance level of P < 0.05 was accepted in all tests. All statistical analyses were performed using SPSS version 22.

RESULTS

In the present study, 100 blood samples with serum 25(OH)D3 levels under 30ng/mL and 100 blood samples from the control group with serum 25(OH)D3 levels between 30 and 100 ng/mL were genotyped for *CYP27B1* and *VDR* region variations. The age range of all participating participants was 20 to 30 years old were reviewed. There were 69 males (24.64% normal and 75.36% patients) and 131 females (7.63% normal and

92.36% patients). The subsequent results showed 42 variations in *CYP27B1* and 21 variations in *VDR* (Table 3). Four of these have been previously reported (Rs531190881, Rs1544410, Rs6447, and Rs4646536). Other mutations in the patients were novel mutations (5993 C=G, 6004 G>C, 6004 G=C, 6439 G=A, 6492 G=A, 6106 G>A, 6106 G=A, 6093 C=G, 6423 G=A, 6469 T=A, 6070 C=T, 6316 G=A=C=T, 6357 C=G, 7645 T>G, 7447 T=G, 7447 T>G, 7717 G=A, 7741 G=A, 7609 A=G, 7623 A=C, 7533 DEL C, 7533 C=T, 7540 G>C, 7540 G=A, 7555 G=C, 7555 G=T=C, 7565 G=C=T, 7565 G=T, 7716 G=A, 7716 G=T, 7902 G>A, 7902 G=A, 7798 T=A, 7479 C=G, 7800 G=A, 7484 C=T, 7443 INS T, 7446 T=G, 7446 INS T, 7904 G=C=T, 7904 G=T, 7948 G=A, 7823 G=A, 7724 A=G, 7843 G=A, 7553 T=C, 7842 A=T, 7936 G=T, 7879 T=C, 7878 G=C, 7941 G=A, 7643 INS T, 63459 G=A, 63459 G>A, 63474 G=T, 93959 INS G, 63814 G=C, 64086 T>G, 63472 G>T, 63472 G=T, 63454 A>C, 64066 INS A, 64073 INS G, 63481 A=T, 64118 G>A, 63565 C>A, 63558 T=A=C, 63545 G=C, 63537 G>T=C, 64076 INS C) (Table 3). Most of the variations were single nucleotide substitutions. The significant associations between the two variants Rs4646536 (*CYP27B1*) and Rs1544410 (*VDR*) with risk of developing vitamin D3 insufficiency and deficiency (P < 0.05) are shown in Table 3.

Table 2. PCR reaction protocol

Gene	Primer	Primer		DNA (200-250 ng)	Master mix PCR	DEPC1 Water
		Forward	Reverse			
CYP27B1(A)	F	CCTATCCCAAGCCAGTC	0.5 µl	1 µl	10µl	13µl
	R	GGAGAGGGATTGCGTCTG	0.5 µl			
CYP27B1(B)	F	CTGACCACTTCCTGTTC	0.5 µl	1 µl	10µl	13µl
	R	CAGTAGAAAGGGTGCATAGG	0.5 µl			
VDR (V)	F	GTATGCCTAGACTCCACCTCC	0.7 µl	1 µl	10µl	12.6µl
	R	GGAAGAGGTCAAGGGTCAC	0.7 µl			

		first denaturation	Cycle:35			Final extension	Product size
		Denaturation	Annealing	Extension			
CYP27B1(A)	950C – 5 min	950C- 30 sec	58 0C- 45sec	720C- 30 sec	720C – 15 min	703pb	
CYP27B1(B)	950C – 5 min	950C- 30 sec	550C- 45 sec	720C- 30 sec	720C – 15 min	773pb	
VDR (V)	950C – 5 min	950C- 30 sec	580C- 45 sec	720C- 30 sec	720C – 15 min	774pb	

1Diethyl pyro carbonate

Table 3. frequency of SNPs in Case-control and patients

genotype	X < 10 patients	k	pvalue	OR	95% CI	10 < X < 30 patients	k	pvalue	OR	95% CI	X > 30 Case-control
5993 C=G (a)	14/28571	15.054a	0.000	1.00	1.074	0	-	-	-	-	0
6004 G>C	7/142857	7.000a	0.007	1.075	1.019	0	-	-	-	-	0
6004 G=C	7/142857	7.000a	0.007	1.075	1.019	0	-	-	-	-	0
6439 G=A	7/142857	7.000a	0.007	1.075	1.019	0	-	-	-	-	0
6492 G=A	7/142857	7.000a	0.007	0	0	0	22.000a	0.005	0	0	20
6106 G>A	7/142857	7.000a	0.007	1.075	1.019	0	-	-	-	-	0
6106 G=A	7/142857	7.000a	0.007	1.075	1.019	0	-	-	-	-	0
6093 C=G	7/142857	7.000a	0.007	1.075	1.019	0	-	-	-	-	0
6423 G=A	0	-	-	-	-	12/5	13.000a	0.004	1	1.065	0
6469 T=A	0	-	-	-	-	12/5	13.000a	0.005	1	1.065	0
6070 C=T	0	-	-	-	-	12/5	13.000a	0.005	1	1.065	0
6316 G=A=C=T	0	-	-	-	-	12/5	13.000a	0.005	1	1.065	0
6357 C=G	0	-	-	-	-	12/5	13.000a	0.005	1	1.065	0
7645 T>G (b)	5/882353	6.000a	0.013	1.064	1.012	0	-	-	-	-	0
7447 T=G	5/882353	6.000a	0.013	1.064	1.012	8/333333	8.000a	0.004	1.087	1.026	0
7447 T>G	0	-	-	-	-	8/333333	8.000a	0.004	1.087	1.026	0
7717 G=A	5/882353	6.000a	0.013	1.064	1.012	0	-	-	-	-	0
7741 G=A	5/882353	6.000a	0.013	1.064	1.012	0	-	-	-	-	0
7609 A=G	5/882353	6.000a	0.013	1.064	1.012	0	-	-	-	-	0
7623 A=C	5/882353	6.000a	0.013	1.064	1.012	0	-	-	-	-	0
7989 T=C	29/41176	11.000a	0.0001	3	1	8/333333	0.000a	0.0005	0.00	0.000	10
7989 T>C	5/882353	6.000a	0.013	1.064	1.012	16/66667	18.000a	0.004	1	1	0
7989 T=G	0	22.000a	0.0005	0	0	0	22.000a	0.005	0	0	20
7533 DEL C	0	-	-	-	-	8/333333	8.000a	-	-	1.026	0
7533 C=T	0	-	-	-	-	8/333333	8.000a	0.004	1.087	1.026	0
7540 G>C	0	-	-	-	-	8/333333	8.000a	0.004	1.087	1.026	0
7540 G=A	0	22.000a	0.0005	0	0	0	22.000a	0.005	0	0	20
7555 G=C	11/76471	12.000a	0.0005	1	1.057	0	-	-	-	-	0
7555 G=T=C	0	-	-	-	-	8/333333	8.000a	0.004	1.087	1.026	0
7565 G=C=T	0	-	-	-	-	8/333333	8.000a	0.004	1.087	1.026	0
7565 G=T	0	-	-	-	-	8/333333	8.000a	0.004	1.087	1.026	0
7716 G=A	0	-	-	-	-	8/333333	8.000a	0.004	1.087	1.026	0
7716 G=T	0	-	-	-	-	8/333333	8.000a	0.004	1.087	1.026	0

Table 3. Cont...

genotype	X < 10 patients	k	pvalue	OR	95% CI	10 < X < 30 patients	k	pvalue	OR	95% CI	X > 30 Case-control
7902 G>A	5/882353	6.000a	0.013	1.064	1.012 1.000	8/333333	8.000a	0.004	1.087	1.026 1.000	0
7902 G=A	0	-	-	-	-	8/333333	8.000a	0.004	1.087	1.026 1.000	0
7479 C=G	11/76471	12.000a	0.0005	1	1.057 1.000	8/333333	8.000a	0.004	1.087	1.026 1.000	0
7798 T=A	0	-	-	-	-	8/333333	8.000a	0.004	1.087	1.026 1.000	0
7800 G=A	0	-	-	-	-	8/333333	8.000a	0.004	1.087	1.026 1.000	0
7483 G=A	5/882353	6.000a	0.013	1.064	1.012 1.000	0	-	-	-	-	0
7484 C=T	5/882353	6.000a	0.013	1.064	1.012 1.000	0	-	-	-	-	0
7443 INST	5/882353	6.000a	0.013	1.064	1.012 1.000	0	-	-	-	-	0
7446 T=G	5/882353	6.000a	0.013	1.064	1.012 1.000	0	-	-	-	-	0
7446 INST	0	-	-	-	-	8/333333	8.000a	0.004	1.087	1.026 1.000	0
7904 G=C=T	0	-	-	-	-	8/333333	8.000a	0.004	1.087	1.026 1.000	0
7904 G=T	5/882353	6.000a	0.013	1.064	1.012 1.000	0	-	-	-	-	0
7948 G=A	5/882353	6.000a	0.013	1.064	1.012 1.000	0	-	-	-	-	0
7823 G=A	5/882353	6.000a	0.013	1.064	1.012 1.000	0	-	-	-	-	0
7724 A=G	5/882353	6.000a	0.013	1.064	1.012 1.000	0	-	-	-	-	0
7843 G=A	5/882353	6.000a	0.013	1.064	1.012 1.000	0	-	-	-	-	0
7553 T=C	5/882353	6.000a	0.013	1.064	1.012 1.000	0	-	-	-	-	0
7842 A=T	5/882353	6.000a	0.013	1.064	1.012 1.000	0	-	-	-	-	0
7936 G=T	11/76471	12.000a	0.0005	1	1.057 1.000	0	-	-	-	-	0
7879 T=C	5/882353	6.000a	0.013	1.064	1.012 1.000	0	-	-	-	-	0
7878 G=C	5/882353	6.000a	0.013	1.064	1.012 1.000	0	-	-	-	-	0
7941 G=A	5/882353	6.000a	0.013	1.064	1.012 1.000	0	-	-	-	-	0
7643 INST	0	-	-	-	-	8/333333	8.000a	0.004	1.087	1.026 1.000	0
63459 G=A (v)	7/692308	8.000a	0.004	1.087	1.026 1.000	0	-	-	-	-	0
63459 G>A	0	-	-	-	-	10	10.000a	0.001	1	1.041 1	0
63474 G=T	7/692308	8.000a	0.004	1.087	1.026 1.000	0	-	-	-	-	0
63980 G=A	15/38462	16.000a	0.0005	1	1.083 1.000	30	35.000a	0.005	1	1 1	0
63980 G>A	38/46154	7.000a	0.005	2	1.000 4.000	40	9.000a	0.002	2	1 5.020	20
63980 G>A=T	7/692308	8.000a	0.004	1.087	1.026 1.000	0	-	-	-	-	0
93959 INS G	7/692308	8.000a	0.004	1.087	1.026 1.000	0	-	-	-	-	0
63814 G=C	7/692308	8.000a	0.004	1.087	1.026 1.000	0	-	-	-	-	0
63959 INS G	7/692308	8.000a	0.004	1.087	1.026 1.000	0	-	-	-	-	0

Table 3. Cont...

genotype	X < 10 patients	k	pvalue	OR	95% CI	10 < X < 30 patients	k	pvalue	OR	95% CI	X > 30 Case-control
64069 INS G	7/692308	8.000a	0.004	1.087	1.026 1.000	0	-	-	-	-	0
64086 T>G	7/692308	8.000a	0.004	1.087	1.026 1.000	0	-	-	-	-	0
63472 G>T	7/692308	8.000a	0.004	1.087	1.026 1.000	0	-	-	-	-	0
63472 G=T	7/692308	8.000a	0.004	1.087	1.026 1.000	0	-	-	-	-	0
63471 G=T	7/692308	8.000a	0.004	1.087	1.026 1.000	0	-	-	-	-	0
63454 A>C	7/692308	8.000a	0.004	1.087	1.026 1.000	0	-	-	-	-	0
64066 INS A	7/692308	8.000a	0.004	1.087	1.026 1.000	0	-	-	-	-	0
64073 INS G	7/692308	8.000a	0.004	1.087	1.026 1.000	0	-	-	-	-	0
63481 A=T	7/692308	8.000a	0.004	1.087	1.026 1.000	0	-	-	-	-	0
64118 G>A	0	-	-	-	-	10	10.000a	0.001	1	1.041	0
63565 C>A	0	-	-	-	-	10	10.000a	0.001	1	1.041	0
63558 T=A=C	0	-	-	-	-	10	10.000a	0.001	1	1.041	0
63545 G=C	0	-	-	-	-	10	10.000a	0.001	1	1.041	0
63537 G>T=C	0	-	-	-	-	10	10.000a	0.001	1	1.041	0
64076 INS C	7/692308	8.000a	0.004	1.087	1.026 1.000	0	-	-	-	-	0

The P values were calculated from logistic regression analyses. Bold numbers mean significance association. The P_c values were calculated using Bonferroni's correction OR, odds ratio; CI, confidence interval.

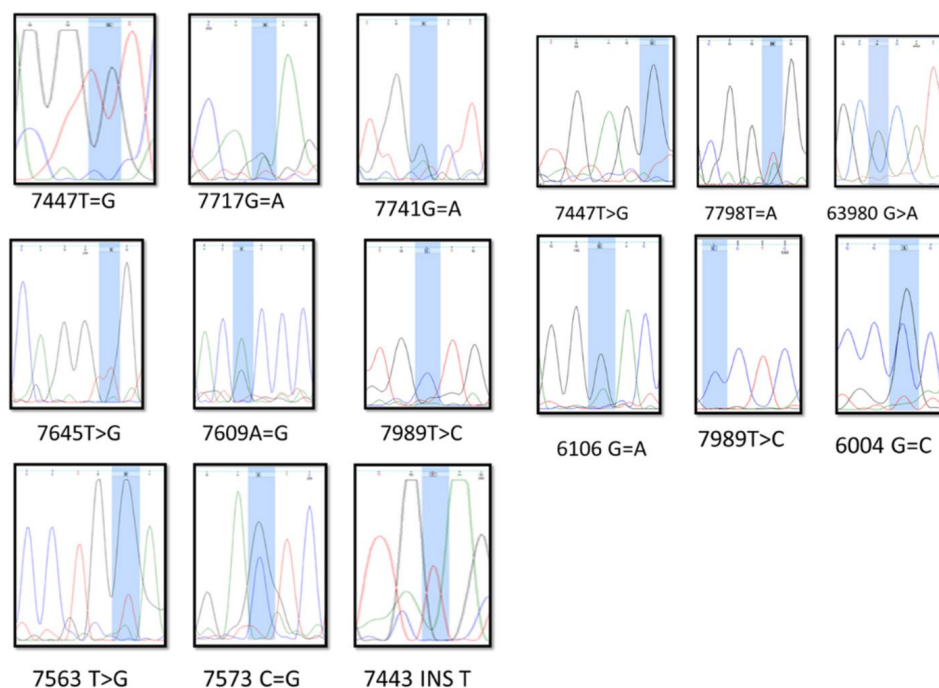


Fig.2. The result of the sequenced PCR products

DISCUSSION

Several studies have shown that there is a significant relationship between serum vitamin D3 levels and the development of several diseases^{7,8}. In addition, numerous investigations have indicated a marked association between some of the polymorphisms in *VDR* and *CYP27B1* genes and serum levels of vitamin D^{3,13}. Furthermore, several studies have shown that in viral diseases such as COVID-19, the viruses cause upper respiratory infection by using angiotensin inverted enzyme receptors that bind to heart and lung cells¹⁴. Vitamin D deficiency causes overexpression of angiotensin receptors on cell surfaces¹⁵. Hence, subjects with vitamin D deficiency may be more susceptible to viral invasion of the lungs. The present study is the first to assess the frequency of polymorphisms in the aforementioned genes in Iranian patients with alopecia areata living in Tehran.

Only 210 of the 800 studied blood samples showed 25(OH)D3 levels from 30–100 ng/mL, with 100 of them derived from patients living in Tehran. According to previous studies, vitamin D deficiency in the Iranian population is a serious

health problem, with a prevalence of 81.3% in the urban population¹⁶. Therefore, the high prevalence of vitamin D3 insufficiency and deficiency in patients with alopecia areata reflects the pivotal role of vitamin D3 in the development of alopecia areata. The first record of the inverse correlation between vitamin D and alopecia areata severity ($P < 0.001$; $r = -0.409$) was reported by Carman et al.¹⁷. Consistent with the previous findings, a negative association was reported between the severity of alopecia areata and vitamin D levels ($P = 0.00$; $r = -0.474$) as assessed using the SALT score used by Unal and Gonulalan¹⁸, Bhat et al.¹⁹, and Rehman et al.²⁰.

Given that 25(OH)D3 levels are known to be lower in the serum samples collected in winter and spring than in the samples collected in summer and fall², samples collected between July and September were included in succeeding studies to decrease the rate of error during the investigation. Vitamin D deficiency is much more common among Iranian women than men¹⁶. Although the results have shown that women are at greater risk of developing vitamin D deficiency than men, a significant association between

observed polymorphisms and gender was not found.

The BMI of all participants was between 20 and 27. Individuals outside this range were excluded. This is due to findings from previous research that have shown that people with a BMI >30 are at a higher risk of developing vitamin D deficiency than others²¹⁻²⁴.

The *CYP27B1* gene was evaluated by PCR sequencing in two regions located between 57764840 and 57764087 (exons 5–7) and between 57766295 and 57765610 (exon 2). These regions of the gene were selected because, according to previous studies, the most important polymorphisms related to vitamin D deficiency in these areas were rs118204007, rs118204009, rs118204010, rs118204011, rs28934606, rs28934607, rs4646536, rs1057520815, rs28934604, rs28934605, and rs387906260. In addition, regions 47846656–47845901 located on the *VDR* gene were investigated using the same method. Previous studies have shown that this area of the gene contains notable polymorphisms related to vitamin D3 deficiency, such as rs121909798, rs121909799, rs121909802, and rs1544410.

However, the study only illustrated 63 variations (42 in *CYP27B1* and 21 variations in *VDR*) between the two genes, and risk of developing vitamin D3 insufficiency and deficiency was reported to be significantly associated with them (Rs4646536 in *CYP27B1* and Rs1544410 in *VDR*) ($P < 0.05$). The *CYP27B1* rs4646536 variation may play a vital role in the development of islet autoimmunity and the preclinical phase of type-1 diabetes^{25,26}. In this investigation, the rs4646536 variation indicated a higher risk of developing vitamin D3 deficiency (odds ratio: 4, $P < 0.0005$). Furthermore, rs1544410 is known to be a BsmI polymorphism. Multiple studies analyzing bone mineral density (BMD) in women have reported a significant association between the A allele and increased risk of low BMD (odds ratio of A; A genotype: 2)²⁷⁻²⁹. In addition, a meta-analysis of several studies also identified a reduced risk of osteoporosis associated with the G; G genotype, with an odds ratio of 0.61, 95% CI, 0.40–0.92³⁰. Rs1544410 is an important polymorphism identified in this study and has been shown to

be most associated with risk of having vitamin D3 insufficiency (odds ratio of 9, 95% CI, 4–17, $P < 0.0005$) and deficiency (odds ratio of 6, 95% CI, 3–12, $P < 0.0005$).

CONCLUSION

In summary, our results showed a significant association between SNPs in the *CYP27B1* and *VDR* genes and serum 25(OH)D3 levels. We also found that the Rs1544410 variation was associated with an increased risk of having vitamin D3 deficiency. Unfortunately, vitamin D3 insufficiency has dramatically increased in recent years. The pivotal role of serum 25(OH)D3 levels in health has been confirmed by several studies. Therefore, diagnosing polymorphisms of genes as biomarkers of genetic predisposition to vitamin D3 deficiency might be a potential approach. Our data confirm previous observations that A-A genotype (Rs1544410) carriers have a higher risk of having vitamin D3 deficiency than other genotypes. This information might have serious implications in choosing personalized lifestyle management strategies to prevent vitamin D insufficiency in genetically susceptible individuals. Thus, it seems that the evaluation of *VDR* and *CYP27B1* polymorphisms could be considered a prognostic mechanism to identify subjects with high risk of developing vitamin D3 insufficiency. Future genetic studies in more extensive populations are needed to further evaluate the importance of these two genes in diseases related to vitamin D3 deficiency.

Limitations

This trial only tested participants with alopecia areata living in a specific area of Tehran. Since one of the important resources in the maintenance of vitamin D levels is sunlight, geographical regions may affect serum vitamin D levels. In addition, there was a time limitation in sample collection. Furthermore, there was a limitation in only selecting participants with specific BMIs, given that BMI could have an effect on serum vitamin D levels.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

SS and MH: conceived of the presented idea and they had participated in study design and performed the statistical analysis. HS, MK, AD and MV participated in laboratory and clinical data gathering and analysis. All authors discussed the results and contributed to the final manuscript. HS, MK, AD and MV experimented. SS wrote the manuscript with support from other authors. The sample was provided by SS. All authors give final approval of the version to be submitted and any revised version, as well as all authors, participate in drafting the article or revising it critically for important intellectual content.

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None.

DATA AVAILABILITY

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

ETHICS STATEMENT

This study was approved by the ethical committee of the Royesh gene, Karaj, Iran. The signed consent forms by participants in the research have been collected by the Iranian Association of Patients with Alopecia areata.

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