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Title: - Association of *Vitamin D binding protein BP rs2282679 Gene* polymorphism and Serum Levels of Vitamin D in Patients with Vitiligo

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[1*]Dr. Ali S. Shakir

[2]Alaa T. Hamza

[1]Lecturer, Department of Clinical Immunology,

College of Dentistry, University of

Al-Qadisiyah, Diwaniya, Iraq.

[2] Lecturer, Department of Biochemistry, College

of Dentistry, University of Al-

Qadisiyah, Diwaniya, Iraq .

Corresponding Author E-mail ID:- alihas@yahoo.com

Abstract

In order for vitamin D to have an impact, the vitamin D receptor (VDR) must be expressed and activated in the nucleus. The VDR has a number of known genetic variants. Biological impacts can be caused by changes in DNA sequences known as "polymorphisms" that are common in the population. Vitiligo is a disease that causes loss of skin color in patches, and it is a chronic (long-lasting) autoimmune disorder loss of color. The skin turns a milky white color because the skin cells that create pigment, called melanocytes, are attacked and destroyed. For comparison, the study included 80 samples total; 40 of them came from vitiligo sufferers (male and female) and 40 from healthy individuals. Under aseptic conditions, five milliliters of blood were collected by puncturing a vein with disposable syringes. The 2 ml of each sample was placed in an EDTA tube and kept at -20 C until the polymerase chain reaction amplification and detection of the vitamin D binding protein BP (rs2282679) gene (ARMS-PCR) method could be performed in order to avoid repetitive thawing and freezing. The remaining 3 ml were moved to a sterile gel tube, left to clot at room temperature, and then spun at 2500 rpm for 10 minutes to prevent the Vitamin D ELISA Kit (Mabtech USA) from undergoing repeated thawing and freezing. The serum that had been isolated was placed in Eppendorf tubes and quickly refrigerated at -20 C until it could be used again. With p=0.644 for gender and p=0.813 for age, the results did not show any significant differences between the groups. The current study showed (37.5%) of Vitiligo patients have a positive family history, 25(OH) D level decreased in inpatients compared to control, and showed that The most abundant gene is AA in vitiligo patients compared to controls, and this enhanced decreased result of 25(OH) D. Also suggested an association between Vitamin-D binding-protein BP *rs2282679* polymorphism and the levels of Vitamin-D in Vitiligo patient.

Keywords: Vitiligo, Polymorphism, Vitamin D, Genotype, Cholecalciferol.

1. Introduction

Spots of pigmentation on the skin, caused by the death of melanin cells in the cutaneous epidermis, are the hallmarks of vitiligo, an inherited pigmentary condition. Based on surveys conducted across large populations globally, the prevalence of this disease has been estimated to be 1-2%. Both sexes are equally affected by this disease, and it does not discriminate based on age or race[(1)]. Vitiligo prevalence estimates vary greatly, ranging from 0.004% to 2.28% globally[(2)]. Vitamin D is a substance found in food, and the body can also manufacture it. It is considered a hormone and is a type of fat-soluble vitamin (A, D, E, K). It works to absorb and preserve calcium and phosphorus, as they are considered important and necessary elements in building bones [(3)]. Research studies have found that it works to reduce bone cancer infection and inflammation. The mechanism of vitamin D formation occurs through the skin with the formation of cholecalciferol (vitamin D3), and this occurs by exposing the skin to ultraviolet rays. The liver converts cholecalciferol to calcidiol, and vitamin D is considered its basic form[(4)]. After that, the kidneys work to convert calcidiol into calcitdiol, and Vitamin D is considered in its active form, which ultimately binds to endogenous D receptors [(4,5)]. Its main function lies in maintaining the normal level of calcium and phosphorus in the blood by increasing the effectiveness of the small intestine to absorb these two minerals from food[(6)]. It also works to regulate parathyroid hormone and enhance calcium absorption by the kidneys. Calcitriol interacts with vitamin D receptors in the small intestine and thus stimulates the absorption of calcium and phosphorus[(7)]. Calcitriol also binds to vitamin-D receptor in bone cell to stimulate (RANK) receptor activator, which in turn interacts with (NFkB) on immature cells, and thus they become mature bone cells resorbing, as they work to get rid of calcium and phosphorus from the bones and thus maintain their levels in the bloodstream[(8)]. A circulating protein called vitamin D binding protein is produced by the liver and encoded by the group-specific component (GC) gene. It binds to 25(OH)D and carries it throughout the bloodstream, which impacts the bioavailability of active 25(OH)D[(9)]. With almost 200 single-nucleotide polymorphisms (SNPs), the VDR gene on chromosome 12q13.11 is particularly unique. *rs2228570*, *rs1544410*, and *rs731236* are three of the most studied of these. Possible variants of the VDR

protein with different transcriptional capacities could arise from the *rs2228570* polymorphism, which is positioned at the exon 2 start codon. These variants could be long (allele-f) or short (allele-F). This gene's 3'-untranslated region (3'-UTR) has the *rs1544410* (in intron 8) and *rs731236* (in exon 9). Despite the fact that changes in the VDR locus or nearby genes may affect the expression and function of protein, the clinical significance of these differences is yet unclear[10]. In this work, we investigate how vitiligo patients' vitamin D serum levels are correlated with the *rs2282679* polymorphism in the vitamin D binding protein BP.

2. Materials and Methods

During the months of March through August of 2023, a total of 40 patients, 14 men and twenty-six females, ranging in age from twenty-five to forty, were investigated. A control group of forty people, sixteen men and twenty-four women, were also included in the study. These people were clinically deemed to be healthy and had no history of systemic disease. People who had malignant tumours, kidney illness, high blood pressure, diabetes, infectious disease, positive HCV antibodies, or any other rheumatologic condition were not included in the study. Aseptically, five millilitres of blood were drawn by inserting a disposable syringe into a vein. For the purpose of the ARMS-PCR method, which involves amplification and detection of the VDBP *(rs2282679) gene* by polymerase chain reaction, 2 ml of each sample was placed into an EDTA tube and kept at -20 C until needed. For the Vitamin D ELISA Kit (Mabtech USA) test, which requires the sample to be frozen and thawed multiple times, the remaining 3 ml were moved to a sterile gel tube, let to clot at room temperature, and then spun at 2500 rpm for 10 minutes. The serum that had been isolated was placed in Eppendorf tubes and quickly refrigerated at -20 C until it could be used again. The study design is illustrated in Figure (1). The research adhered to the ethical rules of Al-Diwaniyah Teaching Hospital, and all patients verbally consented.

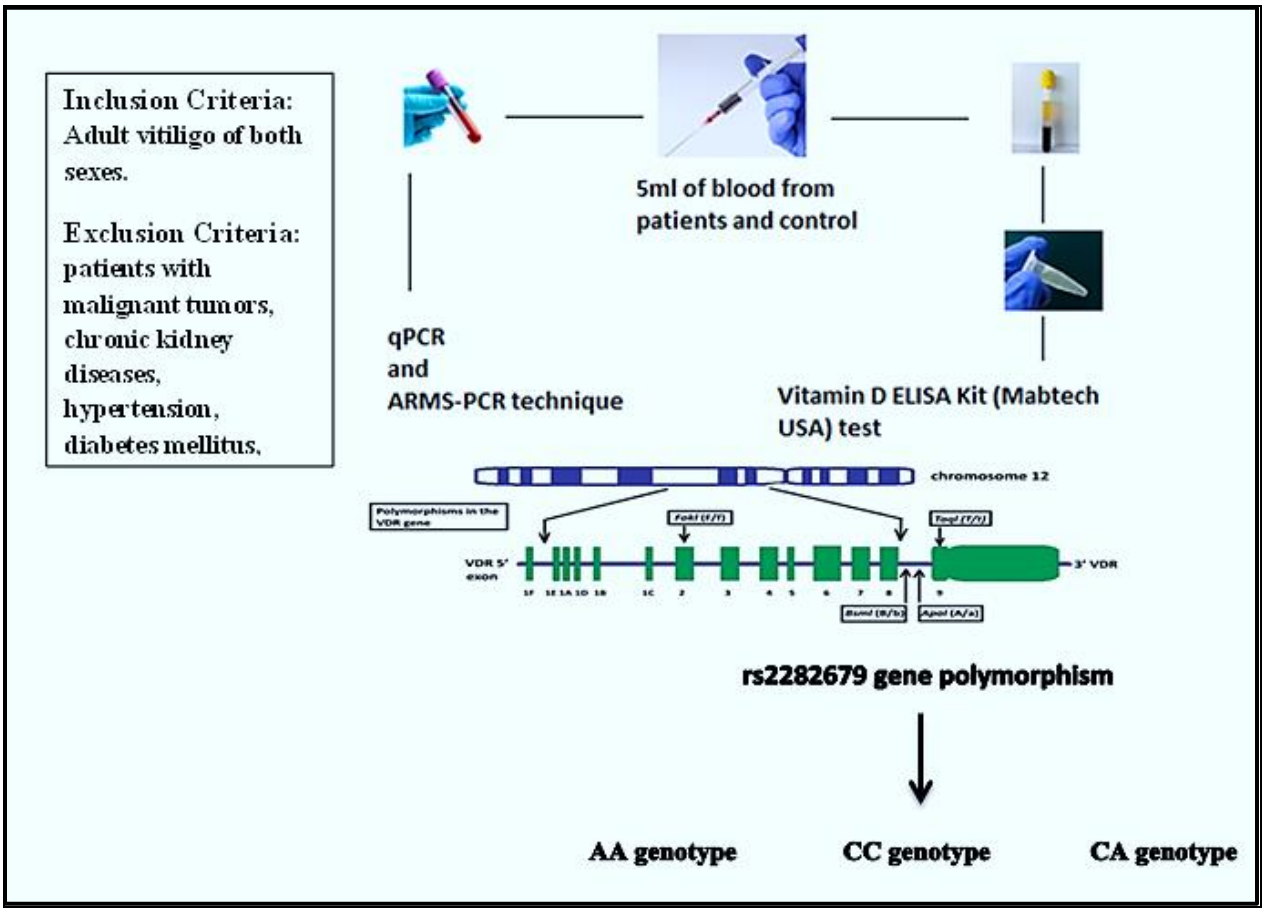

Figure (1): Study design of this study

3. Results

3.1. Characteristics of the study population

The present study enrolled 40 Vitiligo patient and 40 control subjects. The demographic characteristics of patients and control subjects are shown in table (1). According to age, the mean age of patients with Vitiligo was 33.23 ± 8.80 years old and that of control subjects was 32.32 ± 6.34 years old and there was no significant difference between both groups (P = 0.813). Regarding to gender, in overall, 30 (37.5%) male and 50 (62.5%) female were included. Patients with Vitiligo included 14 (35.0 %) cases were male gender and 26 (65.0 %) cases were female, while control subjects included 16 (40.0 %) cases were male gender and 24 (60.0%) cases were female and there was non-significant difference in the frequency distribution of patients and control subjects according to gender (P = 0.644). Regarding BMI, the present results show highly significant increase of BMI in patients with Vitiligo in compared with healthy control, 28.21 ± 3.21 versus 21.34 ± 1.39 respectively, (p < 0.001).

Table (1): Characteristics of patients with Vitiligo and healthy subjects

Characteristics	Vitiligo patient (n=40)	Healthy (n=40)	*P*
Age (years)	33.23 ± 8.80	32.32 ± 6.34	0.813
Gender			
Male	14 (35.0 %)	16 (40.0 %)	0.644
Female	26 (65.0 %)	24 (60.0%)	
BMI	28.21 ± 3.21	21.34 ± 1.39	< 0.001
25(OH) D level (ng/mL)	14.45 ± 4.19	38.11 ± 8.88	< 0.001

3.2.Family History of Hashimoto Thyroiditis.

The presence of family history is an important contributory factor in Vitiligo. This study showed 15 (37.5%) of Vitiligo patients have positive family history, and 25 (62.5%) of Vitiligo patients have negative family history, figure (2).

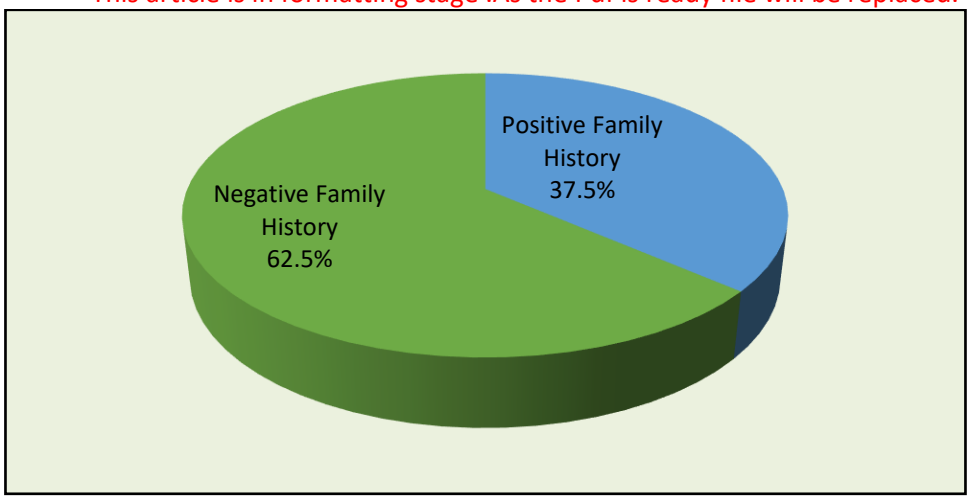

Figure (2): Distribution of patients with Vitiligo according to family history.

3.3. Measurements of Vitamin D parameters

Vitamin D concentrations in patients with Vitiligo were significantly lower than healthy control subjects (14.45 ± 4.19 ng/mL and 38.11 ± 8.88 ng/mL, respectively, P< 0.001), figure(3).

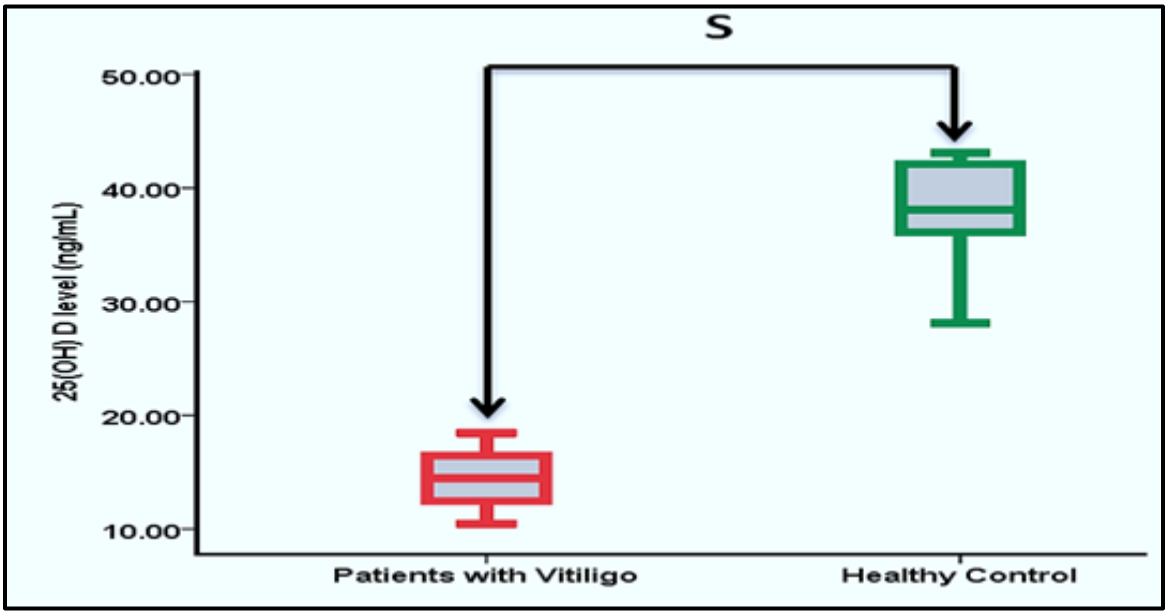

Figure (3): serum level of Vitamin D in patients with Vitiligo and healthy control subjects. S: statistically significant P < 0.05.

3.4. Detection of Genes Polymorphisms

Vitamin D-binding proteins and their distribution The BP rs2282679 gene polymorphism was discovered using the ARMS-PCR technology. At this locus, there are three genotypes: AA, AC, and CC. The dominant homozygote genotype yielded an amplified product size of 205 bp, indicating that only the C allele was present. The mutant type homozygote genotype showed exclusive A allele amplification at a product size of 205 kb. The A allele was amplified at 205 bp and the C allele at 125 bp in the heterozygote genotype, as

shown in Figure 4. Hardy-Weinberg equilibrium was maintained in the genotype distribution across all study groups. When compared to controls, the frequency of AA genotypes was much higher in vitiligo patients (P=0.005). Furthermore, at rs2282679, Vitiligo patients were more likely to possess the A allele of DBP compared to healthy individuals (P=0.001) Table (2). After a thorough comparison between DBP rs2282679 genotypes and vitamin D levels, the current results show that patients with the mutant AA genotype had higher mean serum vitamin D hormone levels than other groups, but this difference was not statistically significant (P= 0.231) Table (3).

Table (2) *DBP rs2282679* POLY genotype frequency in patients and healthy control.

Mode	*DBP rs2282679*	Patients *n* = 40	control *n* = 40	*P*	OR	95% CI
Co-dominant	AA	12 (30.0%)	3 (7.5 %)	0.005	6.22	1.53-29.9
	C/A	10 (25.0%)	9 (22.5 %)	0.317	1.72	0.58 -5.07
	CC	18 (45.0 %)	28 (70.0 %)	Reference		
Dominant	AA+ C/A	22 (55.0 %)	12 (30.0 %)	0.023 ¥ S	Reference	
	CC	18 (45.0 %)	28 (70.0 %)		0.350	0.139-0.88
Recessive	AA	12 (30.0%)	3 (7.5 %)	0.009 ¥ S	5.28	1.36-20.5
	C/A +CC	28 (70.0%)	37 (92.5%)		Reference	
Alleles	A	34 (42.5%)	15 (18.8%)	0.001 ¥ S	3.2	1.56-5.65
	C	46 (57.5%)	65 (81.2%)		Reference	

Table (3): The association between ARMS-PCR finding and Vitamin D levels in patients with Vitiligo

Hormones	ARMS-PCR finding			
	CC genotype n =18	*CA genotype* n = 10	*AA genotype* n =12	*P value*
Vitamin D levels				
Mean ± SD	**16.34 ± 4.32**	**15.42 ± 4.28**	**11.89± 4.16**	**0.231 † NS**
Range	12.31 – 24.14	9.41 – 23.18	8.0 – 22.48	

SD: standard division;

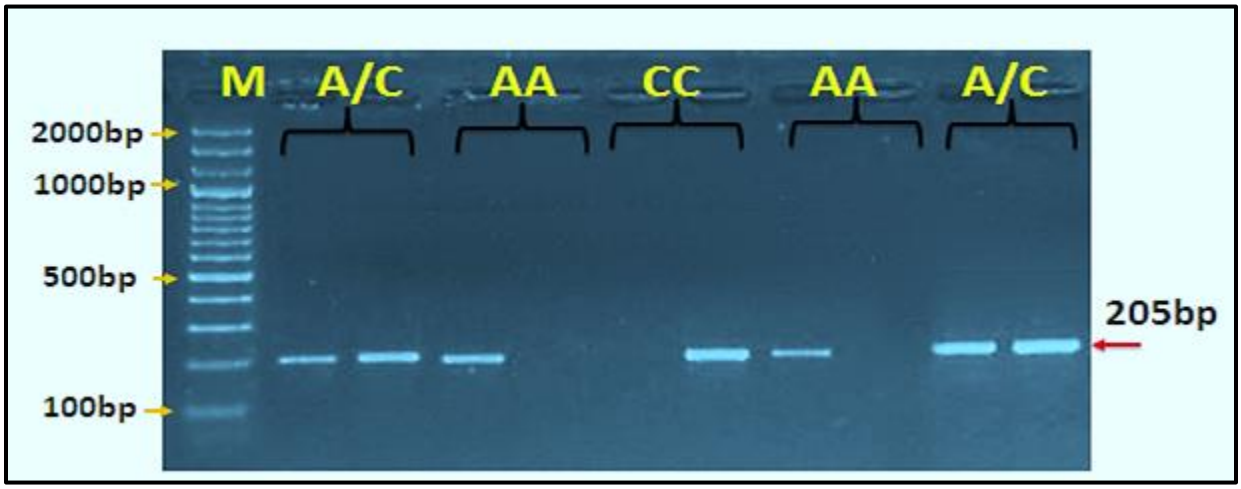

Figure 4: Genomic study of the vitamin D-binding protein (VDBP) (rs2282679) gene polymorphism using ARMS-PCR product analysis on an agarose gel electrophoresis. M stands for marker, which ranges from 2000 to 100 bases. Homozygotes of the wild type (AA) were only seen in the A allele, those of the mutant type (CC) were only seen in the C allele, and those of the (A/C) heterozygote were seen in both the A and C alleles. The presence of either the A or C allele was detected at a product size of 205 kb.

Discussion

There was no statistically significant difference in age or gender between the control group and the vitiligo patients, according to the results. Due to the matched nature of the groups, the study also failed to detect any statistically significant differences in terms of gender or age. There was no statistically significant difference between the sexes and age groups when compared to Mahmoud *et al.*,[(11)]. Variables differed between the healthy group (18 females and 12 males) and the vitiligo group (20 females and 10 males). The patient age groups were as follows: 20–40 (37.7 ± 11.6) and 20–38 (36.5 ± 11.4). Among those who had vitiligo, 37.5% had a favourable family history, according to the study. Despite the fact that 62.5% had never had the condition, the vitiligo group was not significantly different from the healthy group. Concerning vitiligo in the family tree. Vitamin D's immunomodulatory actions are still a mystery, but our knowledge is expanding. There is a dearth of evidence to support the concept that vitamin D helps prevent autoimmune illness in people, despite in vitro and animal evidence to the contrary [(12)]. Multiple sclerosis, inflammatory bowel disease, type I diabetes mellitus, rheumatoid arthritis, and multiple sclerosis are among autoimmune illnesses that have been linked to reduced vitamin D levels compared to healthy controls, according to a substantial body of research. However, these studies have not been able to definitively establish a causal relationship between the two. The effects of 1,25(OH)2D3 on dendritic cell maturation and differentiation, T-cell proliferation, and Th1 cytokine secretion have been demonstrated in multiple

prior investigations. Along with this, 1,25(OH)2D3 has the potential to boost IL-10 secretion and the quantity of regulatory T cells[13].
Despite numerous research trying to explain it, the significance of vitamin D in vitiligo is still not understood. According to Koizumi *et al.*,[14], vitamin D can suppress the production of vitiligo-causing proinflammatory and proapoptotic cytokines such IL-6, IL-8, TNF-α, and TNF-γ. Through the production of sphingosine-1-phosphate, Sauer *et al.*,[15] shown that 1,25(OH)2D3 inhibits the death of human melanocytes. There have also been efforts to treat vitiligo with topical vitamin D. One study suggests that calcipotriol can activate keratinocytes and melanocytes, which in turn stimulates melanin synthesis [16].

Vitamin D concentrations were found to be considerably lower in Vitiligo patients compared to healthy control subjects in the current study (14.45 ± 4.19 ng/mL vs. 38.11 ± 8.88 ng/mL, respectively, P<). Upala *et al.*,[17] found a significant connection between low 25(OH)D levels and vitiligo, which is consistent with these results. Kim *et al.*,[18] found no statistically significant difference in the mean serum levels of 25(OH)D in 172 vitiligo patients and the normal Korean population, however the current data contradict this. Also, I disagree with Khurrum *et al.*,[19] that said the vitamin D levels of the control subjects and vitiligo sufferers were the same. The study revealed that CC was the most common genotype in the control group (70% of cases), whereas AA was the most common genotype in the vitiligo group (30% of cases). This difference was statistically significant.

The study also showed that the degree of vitiligo was higher in AA genotype patients compared to CC and CA genotype patients, but without a significant statistical difference. A Romanian study by Perlea *et al.*,[20,21] showed that homozygous mutant CC version APa1 was significantly associated with vitiligo susceptibility, which is inconsistent with our results, this may be due to this study is working in a different allele. In comparison with the results of this study it showed that the that homozygous mutant AA was significantly associated with vitiligo susceptibility.

Conclusions

(OH) 25D, which is derived from both food and skin, is the most reliable measure of vitamin D intake.Multiple studies have linked autoimmune illnesses, such as vitiligo, to low vitamin D3 levels and shown that this nutrient has potent immunosuppressive effects. As a result, vitamin D may influence both the innate and adaptive immune responses via stem cell, macrophage, and T and B lymphocyte receptors. Because vitamin D stimulates melanin synthesis, the incidence of vitiligo may rise. The study confirmed that polymorphisms of the VDR gene were associated with Iraqi patients with vitiligo, as allelic variation in the VDR gene or other genes associated with an imbalance of this gene may lead to Development of vitiligo. This supports our findings, which are a lower level of 25(OH)D level in the serum of patients compared to the healthy group, and this can be linked genetically, as the study found that the AA gene was the most abundant compared to CC and CA, and also for the most prominent allele, A (42.5%) was in patient group with statistically significant differences. Conclude from this that the increase in the AA gene

in the serum of patients compared to healthy people is associated with a decrease 25(OH)D, which in turn causes a deficiency of melanin in the skin, which is the pigment that gives the skin its natural color, and this deficiency leads to the development of vitiligo, which leads to the appearance of white spots on the skin. In the end, we conclude that the higher the percentage of AA genotype in the patients' serum, the lower the level of Vitamin D, meaning that the relationship between them is inverse.

Appendix:

Abbreviation	Fullname
BP	Binding Protein
VDR	vitamin D receptor
DNA	deoxyribonucleic acid
EDTA	Ethylenediamine tetraacetic acid
ARMS	Amplification-refractory mutation system
PCR	Polymerase chain reaction
ELISA	Enzyme-linked immunosorbent assay
RANK	Receptor activator of nuclear factor k B
NFkB	Nuclear factor kappaB
SNPs	Single nucleotide polymorphisms

The ARMS-PCR primers with their sequence and amplicon size:

Primer	Sequence (5'-3')	Product size
Wild type Forward Primer	**TCTGTCTCTTAATTATCTCACGA**	**205 bp**
Mutant Forward Primer	**TCTGTCTCTTAATTATCTCACGC**	
Common Reverse Primer	**CACAGCCTCAGTTCCTATGT**	

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Conflict of Interest

The authors declare no conflict of interest.

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