original article

Association of genetic variants of the vitamin D receptor gene with vitiligo in a tertiary care center in a Saudi population: a case-control study

Ghada Bin Saif,[a] Imran Ali Khan[b]

From the [a]Department of Dermatology, College of Medicine, King Saud University, Riyadh, Saudi Arabia; [b]Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, King Saud University, Riyadh, Saudi Arabia

Correspondence: Dr. Imran Ali Khan · Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, King Saud University, Riyadh 11433, Saudi Arabia imkhan@ksu.edu.sa · ORCID: https://orcid.org/0000-0002-9746-5300

Citation: Bin Saif G, Khan IA. Association of genetic variants of the vitamin D receptor gene with vitiligo in a tertiary care center in a Saudi population: a case-control study. Ann Saudi Med 2022; 42(2): 96-106. DOI: 10.5144/0256-4947.2022.96

Received: August 31, 2021

Accepted: November 2, 2021

Published: April 7, 2022

Funding: This research was funded by Researchers Supporting Project number (RSP-2021/409), King Saud University, Riyadh, Saudi Arabia x RSP-2021/409

BACKGROUND: Vitiligo is a common cutaneous disorder of the skin and hair caused by a systemic depigmentation disorder that affects 1% of the population or less due to its onset in early adulthood. Meta-analyses have documented a linkage between vitiligo and the vitamin D receptor (*VDR*) gene.

OBJECTIVE: Investigate the relationship between the ApaI, BsmI, FokI and TaqI genetic variants in the *VDR* gene with vitiligo in a Saudi population.

DESIGN: Case-control.

SETTING: Single tertiary care center.

PATIENT AND METHODS: The case-control study was carried out between January 2015- December 2015 in Saudi vitiligo patients and healthy controls. *VDR* genetic variants or polymorphisms (ApaI, BsmI, FokI and TaqI) were genotyped by polymerase chain reaction-restriction fragment length analysis followed by 3% agarose gel electrophoresis. Applicable statistical methods were used to assess relationships between vitiligo cases and controls.

MAIN OUTCOM MEASURE: Effect of genotype distribution among four single nucleotide polymorphisms.

SAMPLE SIZE: 152 vitiligo (median [IQR] 23 [19] years) patients and 159 healthy controls (45 [28.5] years).

RESULTS: We found an association of vitiligo with ApaI and BsmI polymorphisms ($P<.05$). However, a decreased risk was noted in vitiligo patients with FokI and TaqI polymorphisms and in the diplotype and haplotype analysis within males and females. A positive association with vitiligo was observed in ACAC and AC (adjusted by gender) haplotypes ($P<.05$). The strongest linkage disequilibrium was observed between rs79785232 (ApaI) and rs731236 (TaqI) polymorphisms ($r^2=.83$), followed by rs2228570 (FokI) and rs1544410 (BsmI) polymorphisms ($r^2=.53$).

CONCLUSIONS: Our results confirm an association of vitiligo with ApaI and BsmI polymorphisms and fail to show an association in TaqI and FokI polymorphism with vitiligo. Additional studies need to be carried out in different Arab populations to determine whether the polymorphisms are present.

LIMITATIONS: Controls not age matched, small sample size, lack of biochemical parameters.

CONFLICT OF INTEREST: None.

Vitiligo is defined as an acquired, idiopathic and hereditary skin disorder characterized by one or more patches of depigmented skin due to the loss of cutaneous melanocytes.[1] The global prevalence of vitiligo, primarily of the non-segmental form, is estimated to be between 0.5-2.0% of the diseased population.[2] The pathogenesis is not completely understood.[3] Although not life threatening, vitiligo often reduces quality of life.[4] Epidemiologically, vitiligo is connected with thyroid disorders, pernicious anemia, rheumatoid arthritis, type 1 diabetes, and lupus diseases.[5] To date, genome-wide association studies (GWAS) have identified several thousands of single nucleotide polymorphisms (SNPs) associated with human diseases and complex traits, and have been successful in elucidating the pathophysiological mechanism of underlying diseases with the genetic influence.[6] In addition to GWAS, case-control and family association studies have also identified many vitiligo susceptibility genes and SNPs.[7] Previous studies have confirmed the connection between vitiligo and the vitamin D receptor (VDR) gene, and vitamin analogs have been used for the treatment of the condition. Vitamin D analogs restrict the activation, proliferation, and migration of melanocytes, and can also regulate the activation of T-cells, which mediate the damage of melanocytes.[8] *VDR* is expressed in melanocytes and regulates melanin synthesis.[9] The biological function of *VDR* involves the conversion of vitamin D into 25-hydroxyvitamin D, followed by a 1α-hydroxylation step to yield 1,25-dihydroxyvitamin D3.[10] In humans, vitamin D is used to treat skin disorders, including psoriasis and vitiligo.[11] The *VDR* gene maps to chromosomes 12p13.[11] The gene contains numerous polymorphic sites in its untranslated region (which regulates the functional stability of the transcript), that can be assayed using the restriction enzymes for polymorphisms of the *VDR* gene. We selected four SNPs for this study: ApaI, BsmI, and TaqI, and there is a FokI variant in exon 2. The wild-type and mutant alleles of these variants are termed as A-a, B-b, T-t, and F-f according to the presence or absence of the respective restriction sites.[12,13] Previous case-control studies of *VDR* gene polymorphisms in vitiligo did not provide conclusive results because of a lack of ethnic variation, publication bias, sample size, and the types of vitiligo included. However, a meta-analysis of previous reports confirmed that the *VDR* gene contains potential biomarkers for early detection of vitiligo.[14,15] There are few genetic studies conducted in the Saudi population; therefore, we aimed to investigate the relationship between the ApaI, BsmI, FokI and TaqI genetic variants of the *VDR* gene and vitiligo in the Saudi population.

PATIENTS AND METHODS

This retrospective case-control study was carried out between January 2015 and December 2015 and included 152 unrelated patients of Arab descent from Saudi Arabia, who met strict clinical diagnostic criteria for vitiligo and 159 unrelated age and gender-matched healthy Saudis. Patients were consecutively recruited from the dermatology outpatient clinic of King Khalid University Hospital, Riyadh, Saudi Arabia. The clinical diagnosis of vitiligo was confirmed by two dermatologists according to the diagnostic criteria of the Vitiligo European Task Force.[16] All clinical diagnoses of vitiligo were agreed upon by both dermatologists. Demographic information and clinical information were collected from both the cases and controls, through a full clinical checkup. Vitiligo patients with any other skin disease, and non-Saudi nationalities were excluded from the study. Control subjects were healthy Saudi individuals with no clinical or family history of vitiligo or any other skin/autoimmune disorders. This study was approved by the Institutional Review Board, College of Medicine, King Saud University (08-649 and E-20-4773). All subjects provided written informed consent, and the study was performed in accordance with the ethical standards laid down in the Declaration of Helsinki.

Genetic analyses

Peripheral venous blood samples were collected and dispensed into 3-mL tubes containing 5.4 mg of ethylenediaminetetraacetic acid (EDTA). Genomic DNA was extracted from the leucocytes of all 311 participants using a commercial kit (Qiagen, United States). A NanoDrop spectrophotometer (ThermoFisher Scientific) was used to quantify the genomic DNA, which was then stored at -80°C. SNPs in the *VDR* gene were genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis using the ApaI, BsmI, TaqI and FokI restriction enzymes.The details of primers and relevant band sizes of specific SNPs are described in **Table 1**. Purified DNA containing the polymorphic regions was amplified by PCR in a total volume of 30 µL, including 20µL of the ready-made PCR master mix containing reaction buffer mix with magnesium chloride, 100 ng of genomic DNA, 0.5 µL of 100 picomoles of both sense and antisense primers, and 8.0 µL of distilled water.[17] Amplified products were digested for 16 hours with the specified restriction enzymes and separated by electrophoresis on 3% agarose gel. For restriction enzyme digestion, reactions contained 15 µL of PCR product, 2.0 µL of buffer, 1.0 µL of restriction enzyme, and 2.0 µL of distilled water. Quality control was performed for 16 (10%)

Table 1. Primers and restriction enzymes used in the polymerase chain reaction.

VDR single nucleotide polymorphisms	Forward primer	Reverse primer	PCR product	Restriction enzyme	Digested band sizes
ApaI (rs79785232)	AGAGCATGGA CAGGGAGCAAG	GCAACTCCTCATG GCTGAGGTCTCA	746bp	ApaI	A-746bp; C-529/217bp
BsmI (rs1544410)	CAACCAAGACTACA ACCGCGTCAGTGA	AACCAGCGGA AGAGGTCAAGGG	872bp	BsmI	A-872bp; G-701/171bp
TaqI (rs731236)	AGAGCATGGA CAGGGAGCAAG	GCAACTCCTCATG GCTGAGGTCTCA	746bp	TaqI	T-427/169/92/32/26bp; C-293/252/201bp
Fok1 (rs2228570)	AGCTGGCCCTGG CACTGACTCTGCTCT	ATGGAAACACCT TGCTTCTTCTCCCTC	267bp	FokI	T-193/70bp; C-267bp

samples selected randomly from cases and controls by repeating the genotyping procedure.

Statistical analysis

All analyses were performed using the statistical software IBM SPSS (Version 22). Data for controls and patients with vitiligo were analyzed separately, and values for the total population were also obtained. Data are presented as mean and standard deviation (SD) or median and interquartile range in the case of data not normally distributed. Frequency distribution analysis was performed. Genotype and allele frequencies were calculated. The significant differences between the case and control groups were determined using the chi-square test. Hardy–Weinberg equilibrium (HWE) was determined by the goodness-of-fit test. Frequencies of genotypes and alleles were compared between control and vitiligo patients and among different groups. Odds ratios and their 95% confidence intervals (CIs) were calculated. Probability values $P \leq .05$ were considered statistically significant. Differences between vitiligo cases and controls were evaluated with the help of the SNPstat software, using χ^2, and odds ratios and 95% CI calculation. Haploview (version 4.2) was used for the calculation of pairwise linkage disequilibrium (LD) coefficients (D') among the four SNPs.[18]

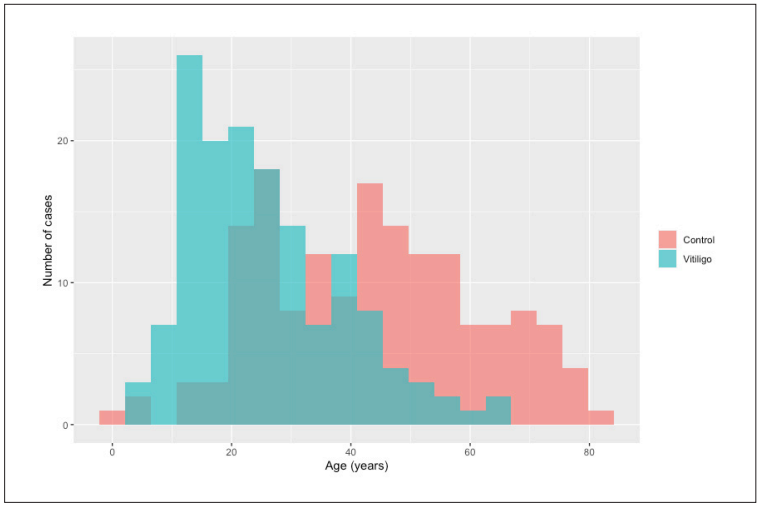

Figure 1. Age distribution of vitiligo patients and controls.

RESULTS

We identified 311 Saudi subjects from King Saud University and categorized them as 152 cases with vitiligo and 159 controls. All 152 cases were confirmed to have vitiligo. The median (IQR) ages in the groups were 23 (19) years in the vitiligo patients and 45 (28.5) in the controls ($P<.001$, Mann-Whitney U test) (**Figure 1**).

Among subjects with vitiligo 42.1% were male and 57.9% female, whereas, 42.1% of controls were male and 57.9% female, similar to the vitiligo cases ($P=.80$ and $P=.77$, respectively). Among vitiligo cases, 30.9% had a family history of the disease, and 20.4% of the vitiligo patients had consanguineous parents. Hardy-Weinberg equilibrium was calculated for cases versus controls. The TaqI and FokI polymorphisms were not in HWE ($P<.05$); however, the ApaI and BsmI polymorphisms were in equilibrium.

Genotype analysis of VDR genetic polymorphisms

The genotype and allele frequency distributions for the ApaI, BsmI, TaqI, and FokI *VDR* gene polymorphisms among vitiligo patients and controls are presented in **Table 2**. For the ApaI polymorphism, the genotypes AA, AC, and CC were identified in 48%, 52%, and 0% of cases and 53.4%, 40.9%, and 5.7% of controls. Comparison of the genotypic distribution of cases and controls indicated a statistically significant association under the dominant model (AC+CC vs. AA; OR=19.25, 95% CI [1.11–333.7]; $P=.04$). There was no statistically

Table 2. Genotype and allele frequencies for vitiligo cases and controls (including crude odds ratios for vitiligo risk).

	Vitiligo (n=152)	Controls (n=159)	Odds ratio	95% CI	*P* value
ApaI (rs79785232)					
AA	73 (48)	85 (53.4)			
AC	79 (52)	65 (40.9)	0.71	0.45-1.11	.1333
CC	0*	9 (5.7)	23.1	1.31-403.6	**.031**
Hardy-Weinberg equilibrium					
Chi-square	18.738	0.567			
P value	0.001	0.451			
AC+CC vs AA	152 (100)	150 (94.3)	19.25	1.11-333.7	**.04**
AC vs AA+CC	79 (52)	65 (40.9)	1.2	0.805-1.786	.37
CC vs AA+AC	0*	9 (5.7)	0.05	0.002-0.759	**.0317**
A	225 (0.74)	235 (0.739)			
C	79 (0.26)	83 (0.261)	0.994	0.694-1.422	.974
BsmI (rs1544410)					
AA	27 (17.8)	35 (22.1)			
AG	81 (53.3)	95 (59.7)	1.10	0.61-1.98	.73
GG	44 (28.9)	29 (18.2)	1.78	1.02-3.099	**.04**
Hardy-Weinberg equilibrium					
Chi-square	0.955	6.15			
P value	.328	.013			
AG+GG vs AA	125 (82.2)	124 (77.9)	1.18	0.803-1.74	.39
AG vs AA+GG	81 (53.3)	95 (59.7)	0.562	0.322-0.978	**.04**
GG vs AA+AG	44 (28.9)	29 (18.2)	1.5	0.885-2.55	.13
A	135 (0.44)	165 (0.52)			
G	169 (0.56)	153 (0.48)	1.35	0.984-1.85	.06
TaqI (rs731236)					
TT	91 (59.9)	60 (37.7)			
TC	61 (40.1)	93 (58.5)	0.43	0.27-0.68	**.0003**
CC	0*	6 (3.8)	0.05	0.002-0.919	**.04**
Hardy-Weinberg equilibrium					
Chi-square	9.578	16.519			
P value	.0019	0.001			
TC+CC vs TT	61* (40.1)	99 (62.3)	0.40	0.25-0.63	**.0001**
TC vs TT+CC	61 (40.1)	93 (58.5)	0.47	0.29-0.73	**.001**
CC vs TT+TC	0*	6 (3.8)	0.07	0.004-1.37	**.02**
T	243 (0.80)	213 (0.67)			
C	61 (0.20)	105 (0.33)	0.50	0.35-0.73	**.0003**

Table 2 (cont.).Genotype and allele frequencies for vitiligo cases and controls (including crude odds ratios for vitiligo risk).

	Vitiligo (n=152)	Controls (n=159)	Odds ratio	95% CI	*P* value
Fok1 (rs2228570)					
CC	110 (72.4)	114 (71.7)			
CT	42 (27.6)	45 (28.3)	0.97	0.58-1.58	.89
TT	0*	0*	1.07	0.02-55.17	.97
Hardy-Weinberg equilibrium					
Chi-square	3.90	4.32			
P value	.04	.037			
CT+TT vs CC	42 (27.6)	45 (28.3)	0.99	0.60-1.61	.97
CT vs CC+TT	42 (27.6)	45 (28.3)	0.93	0.018-48.13	.97
TT vs CT+CC	0*	0*	1.07	0.02-55.17	.97
C	262(0.86)	273 (0.85)			
T	42 (0.14)	45 (0.15)	0.97	0.618-1.53	.90

* Yates continuity correction.

significant association of allele or genotype frequencies, including the AC and CC genotypes, or when data were analyzed under the co-dominant or recessive models (C vs. A; OR=0.994, 95% CI [0.69–1.42]; *P*=.97).

Genotypes AA, AG, and GG are potentially detected using the BsmI polymorphism in the *VDR* gene. Heterozygotes were more common in controls (59.7%) than cases (53.3%); however, the variant genotype GG was more frequent in cases (28.9%) than in controls (18.2%). Based on these data, we identified a statistically significant association of the BsmI polymorphism with only the GG genotype (GG vs. AA; OR=1.78, 95% CI [1.02–3.10]; *P*=.04). No statistically significant associations with vitiligo subjects were observed under allelic, dominant, co-dominant, or recessive models (G vs. A; OR=1.35, 95% CI [0.98–1.85]; *P*=.06) and (AG+GG vs. AA, OR=1.18, 95% CI [0.80–1.74]; *P*=.39).

Genotype analysis indicated negative associations of vitiligo with both the TaqI and FokI *VDR* gene polymorphisms compared with that in controls, meaning that the frequency of the SNP was reduced in the vitiligo cases. For the TaqI polymorphism, heterozygote (TC) and variant (CC) genotypes were common in controls (TC=58.5% and CC=3.8%), whereas, among cases, only 40.1% of individuals carried the TC genotype and the CC genotype was completely absent. Statistically significant negative associations were detected under allelic, genotype, and other models (C vs. T, OR=0.50; 95% CI [0.35–0.73]; *P*=.0003); (CC vs. TT, OR=0.05, 95% CI [0.002–0.91]; *P*=.04) and (TC+CC vs. TT, OR=0.40, 95% CI [0.25–0.63]; P=.0001). A similar pattern was observed for heterozygous genotypes (CT) of the FokI polymorphism, which was carried by more controls (28.3%) than cases (27.6%). No TT genotypes were present in either cases or controls. There was no statistically significant association under any genetic model, even after Yates correction (T vs. C, OR=0.97, 95% CI [0.61–1.53]; *P*=.90); (TT vs. CC, OR=1.07, 95% CI [0.02–55.17]; *P*=.97), and (CT+TT vs. CC, OR=0.99, 95% CI [0.60–1.61]; *P*=.97).

Diplotype analysis

Diplotype analysis of combinations of the ApaI, BsmI, TaqI, and FokI *VDR* gene polymorphisms were estimated independently for vitiligo cases and controls (**Table 3**). Genotype comparisons between cases and controls found no statistically significant effects for any of the combinations of SNPs.

Haplotype analysis

Vitiligo cases and control haplotype data stratified by gender are presented in **Table 4**. No association was observed when we compared vitiligo cases and controls stratified by gender. No statistically significant association was detected. Ten haplotypes consisting of combinations of the four different polymorphisms in the *VDR* gene were observed in the study population (**Table 5**). After adjusting for the covariate gender, comparisons between cases and controls indicated an association with haplotype ACAC (OR=2.46, 95% CI [1.29–4.71]; *P*=.006). Haplotype interactions, adjusted for gen-

Table 3. Diplotype distribution of the VDR ApaI, BsmI, TaqI and FokI polymorphisms and estimated vitiligo risk.

ApaI/BsmI/TaqI/FokI	Vitiligo patients	Controls	Odds ratio	95% CI	*P* value
AC/GG/CC/TT	19	8	0.371	0.157–0.875	**.02**
AA/GG/CC/TT	3	5	0.620	0.146–2.64	.512
AC/AA/CC/TT	4	1	4.270	0.472–38.64	.161
AA/GG/TC/CT	1	0	–	–	–
AC/AG/TC/CT	4	10	0.403	0.124–1.313	.120
AA/AG/TC/CT	3	8	0.380	0.099–1.46	.144
AC/AA/TC/CT	0	1	–	–	–
AA/AA/TC/CT	5	6	0.867	0.259–2.903	.817
AC/GG/TT/CT	8	1	0.114	0.014–0.922	**.015**
AA/GG/TT/CT	2	4	0.517	0.093–2.86	.442
AC/AG/TT/CT	9	3	3.273	0.869–12.327	.065
AA/AG/TT/CT	8	5	1.711	0.547–5.35	.351
AC/AA/TT/CT	0	2	–	–	–
AA/AA/TT/CT	2	2	1.047	0.146–7.52	.964
AC/AG/CC/CC	0	1	–	–	–
AA/AG/CC/CC	0	4	–	–	–
AA/AA/CC/CC	0	1	–	–	–
AC/GG/TC/CC	2	6	0.340	0.068–1.711	.172
AA/GG/TC/CC	9	2	0.202	0.043–0.953	**.026**
AC/AG/TC/CC	17	20	0.875	0.44–1.74	.704
AA/AG/TC/CC	11	20	0.542	0.251–1.17	.116
AC/AA/TC/CC	0	3	–	–	–
AA/AA/TC/CC	9	16	0.563	0.241–1.315	.176
AC/AG/TT/CC	15	8	2.067	0.850–5.026	.101
AA/AG/TT/CC	14	11	1.365	0.599–3.109	.457
AA/AA/TT/CC	7	1	7.628	0.927–62.74	**.027**
Total	152	149	–	–	–

Table 4. Results of male and female comparative haplotype association analysis.

ApaI/BsmI/TaqI/FokI	Male					Female					Odds ratio	95% CI	*P* value
	Vitiligo patients	Controls	OR	95% CI	*P* value	Vitiligo patients	Controls	OR	95% CI	*P* value			
AC/AG/CC/TT	5	3	1.80	0.414–7.89	.424	10	5	2.231	0.731-6.811	.15	1.39	0.574–3.40	.455
AA/AG/CC/TT	8	5	1.77	0.547–5.732	.335	6	6	1.049	0.325-3.38	.936	0.648	0.286–1.47	.30
AC/AA/CC/TT	0	0	–	–	–	4	1	4.33	0.475-39.55	.158	–	–	–
AA/GG/TC/CT	0	0	–	–	–	1	0	-	-	-	–	–	–
AC/AG/TC/CT	2	6	0.328	0.064–1.684	.164	2	4	0.512	0.091-2.866	.438	0.530	0.179–1.566	.244
AA/AG/TC/CT	1	4	0.25	0.027–2.299	.188	2	4	0.512	0.091-2.866	.438	0.869	0.259–2.91	.82
AC/AA/TC/CT	0	1	–	–	–	0	0	-	-	-	–	–	–
AA/AA/TC/CT	2	2	1.048	0143–7.67	.96	3	4	0.776	0.169-3.573	.745	1.285	0.368–4.48	.694
AC/GG/TT/CT	4	1	4.4	0.478–40.47	.155	4	0	-	-	-	0.573	0.151–2.175	.408
AA/GG/TT/CT	1	4	0.25	0.027–2.299	.188	1	0	-	-	-	0.141	0.016–1.22	**.039**
AC/AG/TT/CT	3	1	3.24	0.329–32.04	.288	6	2	3.293	0.646-16.77	.131	1.477	0.435–5.012	.529
AA/AG/TT/CT	2	2	1.048	0.143–7.67	.96	6	3	2.171	0.526-8.96	.274	1.671	0.503–5.54	.397
AC/AA/TT/CT	0	1	–	–	–	0	1	-	-	-	0.726	0.045–11.71	.82
AA/AA/TT/CT	1	1	1.048	0.064–17.11	.97	1	1	1.046	0.064-16.98	.975	0.725	0.101–5.21	.75
AC/AG/CC/CC	0	0	–	–	–	0	1	-	-	-	–	–	–
AA/AG/CC/CC	0	3	–	–	–	0	1	-	-	-	0.238	0.025–2.31	.18
AA/AA/CC/CC	0	1	–	–	–	0	0	-	-	-	0.419	0.368–0.478	.24
AC/GG/TC/CC	0	3	–	–	–	2	3	0.69	0.113-4.231	.687	1.219	0.286–5.194	.789
AA/GG/TC/CC	3	2	1.598	0.258–9.894	.611	6	0	-	-	-	0.869	0.259–2.91	.82
AC/AG/TC/CC	8	6	1.45	0.474–4.45	.512	9	14	0.635	0.26-1.552	.316	1.224	0.604–2.48	.572
AA/AG/TC/CC	7	10	0.70	0.249–1.967	.497	4	10	0.39	0.118-1.295	.113	0.566	0.268–1.19	.131
AC/AA/TC/CC	0	0	–	–	–	0	3	-	-	-	–	–	–
AA/AA/TC/CC	3	3	1.049	0.204–5.399	.95	6	3	0.445	0.161-1.228	.11	2.459	0.954–6.33	**.048**
AC/GG/TT/CC	11	1	0.073	0.009–0.584	**.002**	8	7	0.824	0.286-2.375	.719	0.902	0.407–1.99	.799
AA/GG/TT/CC	0	2	–	–	–	3	3	1.047	0.206-5.332	.956	2.22	0.442–11.19	.32
AA/AA/TT/CC	3	0	–	–	–	4	1	4.33	0.475-39.55	.158	1.22	0.286–5.19	.787
Total	64	62	–	–	–	88	77	–	–	–	–	–	–

Table 5. Haplotype interaction analysis with the covariate gender.

	Haplotype association with response (n=311, adjusted by gender)						
	BSM1	**FOK1**	**APA1**	**TAQ1**	**Freq**	**Odds ratio (95% CI)**	***P* value**
1	G	C	A	T	0.2201	1.00	---
2	**A**	**C**	**A**	**C**	**0.1852**	**2.46 (1.29-4.71)**	**.0068**
3	A	C	A	T	0.1802	0.55 (0.24-1.22)	.14
4	G	C	C	T	0.1699	0.52 (0.22-1.25)	.15
5	G	C	A	C	0.0495	0.69 (0.16-2.94)	.62
6	A	T	A	T	0.0481	0.62 (0.18-2.12)	.44
7	A	C	C	T	0.0413	1.95 (0.61-6.19)	.26
8	G	T	A	T	0.0383	0.80 (0.20-3.16)	.75
9	G	T	C	T	0.0269	0.48 (0.09-2.69)	.41
10	A	T	A	C	0.0182	4.84 (0.06-389.27)	.48
Rare	*	*	*	*	0.0224	305 (305-305)	**<.0001**

*Combined genotypes. Global haplotype association *P* value=.00024

Table 6. Haplotype interaction analysis adjusted odds ratio (adjusted for gender).

	Haplotype association with response (n=311, crude analysis)			
APA1	**TAQ1**	**Freq**	**Odds ratio (95% CI)**	***P* value**
A	T	0.2204	1.00	-
A	**C**	**0.1852**	**2.50 (1.31-4.76)**	**.0058**
A	T	0.1806	0.55 (0.25-1.23)	.15
C	T	0.1687	0.52 (0.22-1.26)	.15
A	C	0.0491	0.60 (0.14-2.56)	.49
A	T	0.0487	0.60 (0.18-2.01)	.41
C	T	0.0413	1.96 (0.62-6.22)	.25
A	T	0.0379	0.85 (0.21-3.35)	.81
C	T	0.0275	0.46 (0.09-2.45)	.36
A	C	0.0177	5.71 (0.04-834.47)	.49
*	*	0.023	773 (773-773)	**<.0001**

*Combined genotypes. Global haplotype association *P* value=.00018

der, including odds ratios are presented in **Table 6**. Comparisons between cases and controls indicated an association with haplotype AC, consisting of a combination of the ApaI and TaqI polymorphisms (OR=2.50, 95% CI [1.31–4.76]; *P*=.006).

Linkage disequilibrium analysis

Linkage disequilibrium plot analysis generated delta coefficient (D') values for the four *VDR* SNPs, ApaI, BsmI, TaqI, and FokI, in vitiligo cases and controls. Strong linkage disequilibrium was observed among all four SNPs included in this study (**Figure 2**). The strongest linkage disequilibrium was observed between rs79785232 (ApaI) and rs731236 (TaqI) polymorphisms (r^2=.83), followed by rs2228570 (FokI) and rs1544410 (BsmI) polymorphisms (r^2=.53).

DISCUSSION

Vitiligo categorized as segmental vitiligo is unilaterally defined as an asymmetric distribution of macules

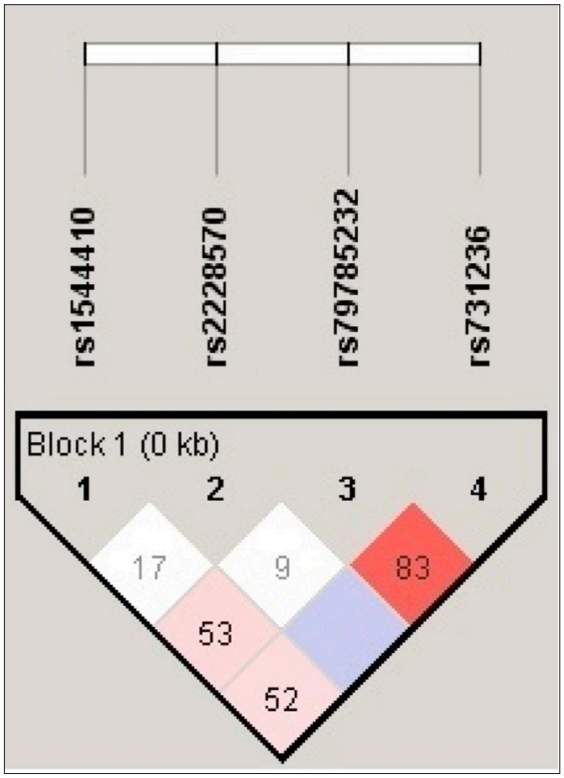

Figure 2. Linkage disequilibrium analysis performed for ApaI, BsmI, TaqI and FokI polymorphisms present in the *VDR* gene.

and patches along the midline. Vitiligo segmentation may be mono, bisegmental or multidisciplinary. Non-segmental vitiligo is a skin pigmentation-deficient dermatologic injury. The origin of the disease has been extensively examined and several theories put forth, but the autoimmune hypothesis is most commonly accepted.[19,20] Vitiligo is a polygenic, multifactorial and autoimmune disease that has deep skin and hair that is excessively distinguished by the loss of melanocytes. It also reflects the contributions of multiple genetic risk factors and environmental triggers.[21] Vitiligo cannot be explained by simple Mendelian genetics, and is characterized by incomplete penetrance, multiple susceptibility loci, and genetic heterogeneity.[22] Case-control studies are conducted to observe the prevalent factors associated with human diseases and to enable the investigation of multiple possible causes of the diseases. In this study, a case-control study was conducted in a Saudi population to investigate the association of *VDR* gene polymorphisms with susceptibility to vitiligo. Based on our knowledge, the present study was the first study implemented in the vitiligo subjects in the Saudi population. The results of this study indicate nominal associations with the ApaI and BsmI polymorphisms. The ApaI polymorphism was associated under the dominant model, whereas allele and genotype frequencies failed to exhibit associations under other genetic models. For the BsmI polymorphism, only the GG genotype was statistically significantly associated, based on comparisons between cases and controls. This variant was significantly more frequent among patients diagnosed with vitiligo (28.9%) than among controls (18.2%). Allele and genetic models failed to show positive or even nominal associations. The TaqI and FokI polymorphisms did not show any positive association of either allele or genotype frequencies, nor under the various genetic models. Therefore, only the ApaI and BsmI genetic polymorphisms exhibited associations with vitiligo. For the FokI polymorphism, alleles were equally distributed between cases and controls. Moreover, the CC genotype of the ApaI polymorphism was observed in 5.7% of controls, while it was completely absent from the cases. A similar pattern was observed for the TaqI polymorphism, with 3.8% of controls having the CC genotype, which was absent from vitiligo cases due to the age difference between cases and controls. As vitiligo develops in the mid-20s age range, our selection criterion for controls was a minimum age of 40 years without any disease. Therefore, the age difference between cases and controls may be a major limitation of our study. A similar age difference in *VDR* gene polymorphisms has been observed in studies of different diseases in the Saudi population.[23,24] Similar age groups were selected in a study of the *VDR* gene in a Saudi population with a different diseases,[25-27] and no controls were reported in another study of a Saudi population.[28]

The association between vitiligo and the *VDR* gene has been connected with a skin disorder, which is linked with vitiligo. Vitamin D is responsible for skin pigmentation, which increases the tyrosine activity of melanocytes.[11] Vitamin D levels are primarily determined by diet and exposure to sunlight, and it is an established environmentally modifiable factor.[29] Vitamin D is a lipophilic hormone that influences the nuclear *VDR*. The conversion and biological activation of vitamin D occur via 25-hydroxyvitamin D [25(OH)D], followed by 1α-hydroxylation to yield 1,25 dihydroxyvitamin D3 [1,25 (OH)2D3]. 25(OH)D levels precisely reflect the vitamin D levels in humans.[10]

Meta-analyses have become the method of choice for confirmation of the few case-control studies in the general population. Meta-analyses have become more relevant in the medical sciences in the context of analysis of specific SNPs, as such studies are designed as quantitative, epidemiological investigations consider-

ing previous studies. They help to derive overall conclusions resulting in the identification of diagnostic markers or risk factors for specific diseases.[30] Two meta-analyses studies documented an association between vitiligo and the *VDR* gene and results confirmed both ApaI and BsmI polymorphisms were strongly associated.[14,15] Consistent with these findings, our study also identified a statistically significant association with these SNPs. A study of *VDR* gene polymorphism in patients with vitiligo in an Egyptian population concluded that there were statistically significant associations with the ApaI and TaqI polymorphisms.[31] Various polymorphisms in the *VDR* gene have been investigated in vitiligo patients in East Asian and White populations, confirming both positive and negative associations.[8,19,32-34]

The strength of the current study was the inclusion of exclusively Saudi subjects. PCR-RFLP analysis was performed for accurate genetic characterization. Inaccurate age-matching of controls was a limitation of this study and the major reason for this was that subjects with vitiligo were younger (in their mid-20s), while we included standard controls, with a minimum age in the mid-40s. Missing biochemical parameters was one more limitation of our study. The other limitation of this study was limited sample size. The final limitation of our study was that we did not measure the serum levels in vitiligo patients.

In conclusion, our results confirmed the association of vitiligo with the *VDR* ApaI and BsmI polymorphisms; however, they failed to show positive associations with the TaqI and FokI polymorphisms. Future studies may be conducted in different Arab populations to determine any associations with other diseases. We also recommend studies of vitamin D serum levels and serological parameters involved with SNPs in the *VDR* gene.

REFERENCES

1. Basher NS, Malik A, Aldakheel F, Chaudhary AA, Rudayni HA, Alkholief M, et al. Deleterious effect of angiotensin-converting enzyme gene polymorphism in vitiligo patients. Saudi Journal of Biological Sciences. 2021;28(8):4478-83.
2. Ezzedine K, Silverberg N. A Practical Approach to the Diagnosis and Treatment of Vitiligo in Children. Pediatrics. 2016 Jul;138(1):e20154126. doi: 10.1542/peds.2015-4126. Epub 2016 Jun 21. PMID: 27328922.
3. Frisoli ML, Essien K, Harris JE. Vitiligo: Mechanisms of Pathogenesis and Treatment. Annu Rev Immunol. 2020 Apr 26;38:621–48.
4. El-Domyati M, El-Din WH, Rezk AF, Chervoneva I, Lee JB, Farber M, et al. Systemic CXCL10 is a predictive biomarker of vitiligo lesional skin infiltration, PUVA, NB-UVB and corticosteroid treatment response and outcome. Arch Dermatol Res. 2021. Epub 2021/04/19. doi: 10.1007/s00403-021-02228-9. PubMed PMID: 33866437.
5. Jin Y, Andersen G, Yorgov D, Ferrara TM, Ben S, Brownson KM, et al. Genome-wide association studies of autoimmune vitiligo identify 23 new risk loci and highlight key pathways and regulatory variants. Nature genetics. 2016.
6. Manolio TA. Bringing genome-wide association findings into clinical use. Nat Rev Genet. 2013 Aug;14(8):549–58.
7. Shen C, Gao J, Sheng Y, Dou J, Zhou F, Zheng X, et al. Genetic susceptibility to vitiligo: GWAS approaches for identifying vitiligo susceptibility genes and loci. Frontiers in genetics. 2016;7.[[AUTHOR: Reference incomplete]]
8. Aydingoz IE, Bingul I, Dogru-Abbasoglu S, Vural P, Uysal M. Analysis of vitamin D receptor gene polymorphisms in vitiligo. Dermatology (Basel, Switzerland). 2012;224(4):361-8. Epub 2012/06/29. doi: 10.1159/000339340. PubMed PMID: 22738935.
9. Doss RW, El-Rifaie AA, Gohary YM, Rashed LA. Vitamin D Receptor Expression in Vitiligo. Indian journal of dermatology. 2015;60(6):544-8. Epub 2015/12/18. doi: 10.4103/0019-5154.169123. PubMed PMID: 26677265; PubMed Central PMCID: Pmc4681190.
10. Li K, Shi Q, Yang L, Li X, Liu L, Wang L, et al. The association of vitamin D receptor gene polymorphisms and serum 25-hydroxyvitamin D levels with generalized vitiligo. The British journal of dermatology. 2012;167(4):815-21. Epub 2012/07/06. doi: 10.1111/j.1365-2133.2012.11132.x. PubMed PMID: 22762534.
11. Karagün E, Ergin C, Baysak S, Erden G, Aktaş H, Ekiz Ö. The role of serum vitamin D levels in vitiligo. Advances in Dermatology and Allergology/Post py Dermatologii i Alergologii. 2016;33(4):300.
12. Di Spigna G, Del Puente A, Covelli B, Abete E, Varriale E, Salzano S, et al. Vitamin D receptor polymorphisms as tool for early screening of severe bone loss in women patients with rheumatoid arthritis. European review for medical and pharmacological sciences. 2016;20(22):4664-9.
13. Wang Y, Cui Z-Q, Luo T-B, Liu L. Correlations of *VDR* and VDBP genetic polymorphisms with susceptibility to adolescent idiopathic scoliosis and efficacy of brace treatment. Genomics. 2016;108(5):194-200.
14. Zhang J-Z, Wang M, Ding Y, Gao F, Feng Y-Y, Yakeya B, et al. Vitamin D receptor gene polymorphism, serum 25-hydroxyvitamin D levels, and risk of vitiligo: A meta-analysis. Medicine. 2018;97(29).
15. Li L, Wu Y, Li L, Cai YF, Geng L, Gao XH, et al. Association of ApaI and BsmI polymorphisms with vitiligo risk: a meta-analysis. Clinical and experimental dermatology. 2015;40(7):794-803.
16. Taieb A, Picardo M. The definition and assessment of vitiligo: a consensus report of the Vitiligo European Task Force. Pigment cell research. 2007;20(1):27-35. Epub 2007/01/26. doi: 10.1111/j.1600-0749.2006.00355.x. PubMed PMID: 17250545.
17. Alharbi KK, Alnbaheen MS, Alharbi FK, Hasanato RM, Khan IA. Q192R polymorphism in the PON1 gene and familial hypercholesterolemia in a Saudi population. Ann Saudi Med. 2017;37(6):425-32. Epub 2017/12/13. doi: 10.5144/0256-4947.2017.425. PubMed PMID: 29229890; PubMed Central PMCID: PMC6074118.
18. Sowmya S, Sri Manjari K, Ramaiah A, Sunitha T, Nallari P, Jyothy A, et al. Interleukin 10 gene promoter polymorphisms in women with early-onset pre-eclampsia. Clinical and experimental immunology. 2014;178(2):334-41. Epub 2014/06/26. doi: 10.1111/cei.12402. PubMed PMID: 24962617; PubMed Central PMCID: Pmc4233382.
19. Saudi WM, Swelam MM, El–Barbary RA, Zakaria L, Gaber DA. Vitamin D receptor (VDR) gene polymorphism in Egyptian vitiligo patients. Journal of Cosmetic Dermatology. 2021;20(3):980-6.
20. Said-Fernandez SL, Sanchez-Domínguez CN, Salinas-Santander MA, Martinez-Rodriguez HG, Kubelis-Lopez DE, Zapata-Salazar NA, et al. Novel immunological and genetic factors associated with vitiligo: A review. Experimental and therapeutic medicine. 2021;21(4):1-.
21. Cai M, Yuan T, Huang H, Gui L, Zhang L, Meng Z, et al. Integrative Analysis of Omics Data Reveals Regulatory Network of CDK10 in Vitiligo Risk. Frontiers in Genetics. 2021;12:160.
22. Laddha NC, Dwivedi M, Gani AR, Mansuri MS, Begum R. Tumor Necrosis Factor B (TNFB) genetic variants and its increased expression are associated with vitiligo susceptibility. PloS one. 2013;8(11):e81736.
23. Al-Daghri NM, Al-Attas O, Alokail MS, Alkharfy KM, Draz HM, Agliardi C, et al. Vitamin D receptor gene polymorphisms and HLA DRB1*04 cosegregation in Saudi type 2 diabetes patients. Journal of immunology (Baltimore, Md : 1950). 2012;188(3):1325-32. Epub 2012/01/06. doi: 10.4049/jimmunol.1101954. PubMed PMID: 22219324.
24. Al-Daghri NM, Al-Attas OS, Alkharfy KM, Khan N, Mohammed AK, Vinodson B, et al. Association of VDR-gene variants with factors related to the metabolic syndrome, type 2 diabetes and vitamin D deficiency. Gene. 2014;542(2):129-33. Epub 2014/04/01. doi: 10.1016/j.gene.2014.03.044. PubMed PMID: 24680778.
25. Alkhayal KA, Awadalia ZH, Vaali-Mohammed MA, Al Obeed OA, Al Wesaimer A, Halwani R, et al. Association of Vitamin D Receptor Gene Polymorphisms with Colorectal Cancer in a Saudi Arabian Population. PLoS One. 2016;11(6):e0155236. Epub 2016/06/17. doi: 10.1371/journal.pone.0155236. PubMed PMID: 27309378; PubMed Central PMCID: Pmc4911096.
26. Mosaad YM, Hammad EM, Fawzy Z, Abdal Aal IA, Youssef HM, ElSaid TO, et al. Vitamin D receptor gene polymorphism as possible risk factor in rheumatoid arthritis and rheumatoid related osteoporosis. Human immunology. 2014;75(5):452-61. Epub 2014/02/18. doi: 10.1016/j.humimm.2014.02.009. PubMed PMID: 24530824.
27. Tayeb MT, Clark C, Haites NE, Sharp L, Murray GI, McLeod HL. Vitamin D receptor, HER-2 polymorphisms and risk of prostate cancer in men with benign prostate hyperplasia. Saudi medical journal. 2004;25(4):447-51. Epub 2004/04/15. PubMed PMID: 15083213.
28. Nemenqani DM, Karam RA, Amer MG, Abd El Rahman TM. Vitamin D receptor gene polymorphisms and steroid receptor status among Saudi women with breast cancer. Gene. 2015;558(2):215-9. Epub 2015/01/07. doi: 10.1016/j.gene.2014.12.065. PubMed PMID: 25560187.
29. Petersen MS, Bech S, Christiansen DH, Schmedes AV, Halling J. The role of vitamin D levels and vitamin D receptor polymorphism on Parkinson's disease in the Faroe Islands. Neuroscience letters. 2014;561:74-9.
30. Haidich A-B. Meta-analysis in medical research. Hippokratia. 2010;14(Suppl 1):29.
31. Sobeih S, Mashaly HM, Gawdat H, Amr K, Hamid MF, Shaalan E. Evaluation of the correlation between serum levels of vitamin D and vitamin D receptor gene polymorphisms in an Egyptian population. International journal of dermatology. 2016;55(12):1329-35. Epub 2016/11/05. doi: 10.1111/ijd.13363. PubMed PMID: 27420410.
32. Birlea S, Birlea M, Cimponeriu D, Apostol P, Cosgarea R, Gavrila L, et al. Autoimmune diseases and vitamin D receptor ApaI polymorphism are associated with vitiligo in a small inbred Romanian community. Acta dermato-venereologica. 2006;86(3):209-14. Epub 2006/05/20. doi: 10.2340/00015555-0093. PubMed PMID: 16710576.
33. Guo R, Hao Y, Wang J. Investigation on association of *VDR* gene polymorphism with Hui and Han ethnic vitiligo patients in NingXia. Chin J Lab Diagn. 2012;16:2223-6.
34. SUN Y, HAN J, WU R-q, XIE K-c, ZHU G-d, ZHENG J. Vitamin D Receptor Gene Apa I, BsmI, Fok I Polymorphism in Patients of Vitiligo [J]. The Chinese Journal of Dermatovenereology. 2009;11:005.