Impact of vitamin D receptor gene polymorphisms on vitiligo susceptibility and clinical features in a Southeastern European Caucasian population

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Abstract. An association of vitamin D receptor (*VDR*) polymorphisms and vitiligo has been suggested. However, previous studies have reported contradictory results while including limited data among Caucasians. The aim of this single-center study was to evaluate the effect of three common *VDR* gene polymorphisms (*Fok*I, *Taq*I and *Bsm*I) on susceptibility and clinical aspects of vitiligo in a Southeastern European Caucasian population. A total of 110 unrelated vitiligo cases and 509 general population controls were enrolled from October 2018 to November 2019. Genomic DNA was extracted from whole blood after de-identification and anonymization of the samples and genotyped for the selected *VDR* polymorphisms by the qPCR (melting curve analysis). Subgroup analysis by clinical features among subsets of patients indicated that, compared to subjects with the *Fok*I TT genotype or T allele, carriers of the *Fok*I CC genotype or C allele exhibited significantly decreased risk of developing vitiligo before the age of 30 [TT vs. CC: odds ratio (OR)=0.286, 95% confidence interval (CI): 0.083-0.984, P=0.041; T vs. C: OR=0.545, 95% CI: 0.313-0.948, P=0.031]. Intra-patient analysis also revealed that, compared to T allele, the presence of *Taq*I C allele was adversely associated with the incidence of concurrent leukotrichia (T vs. C: OR=1.874, 95% CI: 1.018-3.451, P=0.042). Comparisons between the case and control groups showed no evidence to support an association between susceptibility to vitiligo and the *VDR Bsm*I, *Taq*I, and *Fok*I polymorphisms in this cohort. Thus, the studied *VDR* polymorphisms might indirectly impact the clinical course and treatment decision-making despite their lack of association with vitiligo *per se*. Further research with larger sample sizes, especially across Caucasian individuals, should be performed to confirm these findings.

Introduction

Vitiligo is the most common pigmentary skin disorder affecting 0.5-1% of the population worldwide. It is characterized by selective loss of epidermal melanocytes resulting in the occurrence of patchy depigmentation (1). Though not entirely clear, the pathogenesis of vitiligo is widely believed to reflect a complex interplay between genetic, immunologic,

and environmental factors interacting to promote a model of melanocyte-directed autoimmunity (1-3). The current thought is that vitiligo is a multifactorial, polygenic disorder, suggesting that different genes may be involved in the onset and evolution of the disease (4).

Vitamin D has been shown to be involved in multiple biologic processes and pathways. Its role in human carcinogenesis, as well as in osteoporosis is well known (5,6). Given the effects of vitamin D against autoimmunity (7), a potential link between vitamin D deficiency and immune-mediated dermatoses, such as psoriasis and atopic dermatitis, has already been suggested (8,9). In this context, due to the stimulatory and protective action of $1,25(OH)_2D_3$ on melanocytes, as well as its antioxidant and immunomodulatory properties, topical vitamin D and its analogs have been used as repigmentation agents in vitiligo, either alone or combined with other modalities, but reported outcomes have been conflicting (10,11).

Currently, great attention has also been focused on gene-disease associations, opening new perspectives for precision medicine (12,13). As the nuclear vitamin D receptor (VDR) mediates most of the genomic effects of $1,25(OH)_2D_3$, VDR might represent a susceptibility gene for vitiligo (14).

The *VDR* gene, located on chromosome 12q13.11, has been found to contain more than 200 single-nucleotide polymorphisms (SNPs) (15). Among these, the rs2228570 (*Fok*I), rs1544410 (*Bsm*I), and rs731236 (*Taq*I) have been widely studied. The *Fok*I polymorphism, located at exon 2 initiation codon, might result in two forms of the VDR protein, i.e., a long (allele f) and a short (allele F) version, with diverse transcriptional capacity. The *Bsm*I (in intron 8) and *Taq*I (in exon 9) alleles occur in the 3'-untranslated region (3'-UTR) of the gene. Although allelic variations within or near the *VDR* locus could modify the *VDR* gene expression and protein function, their clinical relevance remains largely unknown (16,17).

So far, implication of *VDR* in vitiligo has not been thoroughly explored among Caucasians (16,18), although an association between *VDR* SNPs and immune-mediated skin diseases, i.e., psoriasis and atopic dermatitis, has been supported (19,20). Moreover, the majority of studies evaluating the influence of *VDR* polymorphisms on vitiligo susceptibility in certain population settings have reported inconsistent results (16), indicating that ethnicity might be a potential source of heterogeneity.

The current study investigated whether three common SNPs in the *VDR* gene (*Fok*I, *Bsm*I, and *Taq*I) may confer susceptibility to vitiligo and influence its main clinical features in a Southeastern European Caucasian (SEC) population.

Patients and methods

Patients and setting. In this single-center, case-control study, a total of 110 unrelated vitiligo patients (42 males, 68 females) with mean ± standard deviation (SD) age of 45.1±13.4 years (age range 18-70 years) were recruited as a case group at the Vitiligo Outpatient Unit of 'A. Sygros' Hospital, Athens, Greece, from October 2018 to November 2019. Inclusion criteria were: i) age ≥18 years; ii) clinically diagnosed vitiligo; and iii) SEC ancestry. First- to third-degree blood relatives

Table I. Demographic and clinical data of vitiligo group (n=110).

Characteristics	Total, n=110
Sex, n (%)	
Male	42 (38.2)
Female	68 (61.8)
Age (years), mean ± SD (range)	45.1±13.4 (18-70)
Family History (yes), n (%)	38 (34.5)
Age at vitiligo onset (years), mean ± SD (range)	33.5±15.1 (5-66)
Early onset vitiligo, n (%)	52 (47.3)
Clinical type, n (%)	
Vulgaris	84 (76.4)
Acrofacial	16 (14.5)
Focal	7 (6.4)
Universal	3 (2.7)
BSA (% of body), n (%)	
≤5	74 (67.3)
5-20	25 (22.7)
>20	11 (10.0)
Koebner phenomenon, n (%)	45 (40.9)
Leukotrichia, n (%)	36 (32.7)
Sutton nevi, n (%)	12 (10.9)
Stable disease, n (%)	69 (62.7)
Comorbidities (yes), n (%)	77 (70.0)
Most common	
Thyroid disease, n (%)	50 (45.5)
Skin disease[a], n (%)	13 (11.8)

[a]Presence of psoriasis, atopic dermatitis, and/or urticaria. BSA, body surface area; SD, standard deviation.

were considered ineligible. A group of 509 SEC individuals from the general population (220 males, 289 females) with mean ± SD age of 40.5±11.3 years (age range 18-70 years) served as controls.

The study was conducted according to the principles outlined in the Declaration of Helsinki after obtaining ethics approval from the Institutional Review Board (protocol no. 3044/6-9-2018). All participants provided written informed consent for using their genetic data after de-identification and anonymization of the DNA samples, following the European Medicines Agency guidelines (EMEA/CPMP/3070/01).

Study assessments. Vitiligo was clinically diagnosed by experienced dermatologists after physical examination of the affected skin, often under the Wood's lamp. Clinical types of the disease were categorized as focal (one or few lesions in a non-dermatomal pattern), segmental (unilateral segmental distribution), acrofacial (limited to the face and/or distal extremities), generalized or vulgaris (scattered across the body), and universal (over 90% depigmentation), based on the latest criteria of classification (21). Stable vitiligo was defined

Table II. Genotype and allele frequencies of *VDR FokI* polymorphism in vitiligo cases according to age at disease onset.

	Onset >30 years (n=58), n (%)	Onset ≤30 years (n=52), n (%)	OR (95% CI)	P-value
FokI (rs2228570)				
TT	5 (8.62)	10 (19.23)	1.0 (reference)	
CT	25 (43.10)	26 (50.00)	0.520 (0.156-1.736)	0.283
CC	28 (48.28)	16 (30.77)	0.286 (0.083-0.984)	0.041
CT + CC	53 (91.38)	42 (80.77)	0.396 (0.126-1.248)	0.105
CC	28 (48.28)	16 (30.77)	1.0 (reference)	
CT + TT	30 (51.72)	36 (69.23)	2.100 (0.960-4.592)	0.061
CT	25 (43.10)	26 (50.00)	1.0 (reference)	
TT + CC	33 (56.90)	26 (50.00)	0.758 (0.357-1.607)	0.469
T allele frequency	30.17	44.23	1.0 (reference)	
C allele frequency	69.83	55.77	0.545 (0.313-0.948)	0.031

VDR, vitamin D receptor; CI, confidence interval; OR, odds ratio.

Table III. Genotype and allele frequencies of *VDR TaqI* polymorphism in vitiligo cases according to presence of leukotrichia.

	With leukotrichia (n=36), n (%)	Without leukotrichia (n=74), n (%)	OR (95% CI)	P-value
TaqI (rs731236)				
TT	19 (52.78)	26 (35.14)	1.0 (reference)	
CT	14 (38.89)	34 (45.95)	1.775 (0.752-4.188)	0.189
CC	3 (8.33)	14 (18.92)	3.410 (0.858-13.557)	0.071
CT + CC	17 (47.22)	48 (64.86)	2.063 (0.918-4.638)	0.077
CC	3 (8.33)	14 (18.92)	1.0 (reference)	
CT + TT	33 (91.67)	60 (81.08)	0.390 (0.104-1.455)	0.150
CT	14 (38.89)	34 (45.95)	1.0 (reference)	
TT + CC	22 (61.11)	40 (54.05)	0.749 (0.333-1.685)	0.484
T allele frequency	72.22	58.11	1.0 (reference)	
C allele frequency	27.78	41.89	1.874 (1.018-3.451)	0.042

VDR, vitamin D receptor; CI, confidence interval; OR, odds ratio.

as no appearance of new and/or progression of existing lesions for at least 1 year before inclusion in the study. Patients were considered to have i) early-onset vitiligo if the age at disease onset was prior or equal to 30 years, and ii) family history if they reported one or more first- to third-degree relatives affected by the condition.

Demographic and clinical disease-specific characteristics (i.e., age, sex, nationality, clinical type, age of onset, family history, duration of vitiligo, disease activity, presence of leukotrichia, Koebner phenomenon, and Sutton nevi, body surface area (BSA), and associated diseases) were retrieved for each patient via a predesigned questionnaire. Peripheral blood samples (5 ml) of all patients were collected in tubes containing ethylenediaminetetraacetic acid (EDTA).

DNA isolation, sample storage and genotyping. Whole blood samples were collected in 5 ml EDTA collection tubes followed by DNA isolation, using PureLink® Genomic DNA Mini kit (Thermo Fisher Scientific, Inc), according to the manufacturer's instructions. All DNA samples were stored in -20°C until genotyping.

Three polymorphisms of *VDR* [*FokI* (rs2228570), *BsmI* (rs1544410), and *TaqI* (rs731236)] were analyzed both in patients and controls. Genotyping was performed by qPCR (Light Cycler 480; Roche) using simple probes for each SNP (LightSnip Assays; TIBMOLBIOL) and melting curve analysis.

Statistical analysis. Descriptive statistics are presented as mean ± SD, or frequencies (numbers) with percentages (%), as appropriate. Distributions in genotype and allele frequencies in vitiligo cases versus controls were evaluated using the Chi-squared and Fisher's exact tests. The Hardy-Weinberg equilibrium (HWE) for each SNP was calculated among controls. ORs and 95% CIs, were used to investigate the association of the selected polymorphisms with clinical aspects and risk of vitiligo under five genetic models (allele, dominant, recessive, homozygous and heterozygous). All statistical tests

Table IV. Genotype and allele frequencies of selected *VDR* polymorphisms among cases and controls and their association with vitiligo risk.

	Cases (n=110), n (%)	Controls (n=509), n (%)	OR (95% CI)	P-value
*Fok*I (rs2228570)				
TT	15 (13.64)	51 (10.02)	1.0 (reference)	
CT	51 (46.36)	222 (43.61)	1.280 (0.668-2.455)	0.456
CC	44 (40.00)	236 (46.37)	1.589 (0.816-3.051)	0.173
CT + CC	95 (86.36)	458 (89.98)	1.418 (0.765-2.627)	0.265
CC	44 (40.00)	236 (46.37)	1.0 (reference)	
CT + TT	66 (60.00)	273 (53.63)	0.771 (0.507-1.173)	0.224
CT	51 (46.36)	222 (43.61)	1.0 (reference)	
TT + CC	59 (53.64)	287 (56.39)	1.117 (0.739-1.690)	0.599
T allele frequency	36.82	31.83	1.0 (reference)	
C allele frequency	63.18	68.17	1.248 (0.921-1.692)	0.152
*Bsm*I (rs1544410)				
GG	39 (35.45)	186 (36.54)	1.0 (reference)	
AG	49 (44.55)	243 (47.74)	1.040 (0.655-1.650)	0.868
AA	22 (20.00)	80 (15.72)	0.762 (0.425-1.368)	0.362
AG + AA	71 (64.55)	323 (63.46)	0.954 (0.620-1.467)	0.830
AA	22 (20.00)	80 (15.72)	1.0 (reference)	
AG + GG	88 (80.00)	429 (84.28)	1.341 (0.793-2.265)	0.272
AG	49 (44.55)	243 (47.74)	1.0 (reference)	
GG + AA	61 (55.45)	266 (52.26)	0.879 (0.581-1.331)	0.543
G allele frequency	57.73	60.41	1.0 (reference)	
A allele frequency	42.27	39.59	0.895 (0.666-1.203)	0.461
*Taq*I (rs731236)				
TT	45 (40.91)	197 (38.70)	1.0 (reference)	
CT	48 (43.64)	239 (46.95)	1.1137(0.726-1.781)	0.573
CC	17 (15.45)	73 (14.34)	0.981 (0.528-1.822)	0.951
CT + CC	65 (59.09)	312 (61.30)	1.096 (0.721-1.669)	0.667
CC	17 (15.45)	73 (14.34)	1.0 (reference)	
CT + TT	93 (84.55)	436 (85.66)	1.092 (0.615-1.937)	0.764
CT	48 (43.64)	239 (46.95)	1.0 (reference)	
TT + CC	62 (53.36)	270 (53.05)	0.875 (0.577-1.325)	0.527
T allele frequency	62.73	62.18	1.0 (reference)	
C allele frequency	37.27	37.82	1.024 (0.757-1.383)	0.879

VDR, vitamin D receptor; CI, confidence interval; OR, odds ratio.

were carried out using IBM SPSS Statistics version 22.0 (IBM Corp.). The significance level was set to $P<0.05$.

Results

Demographic and clinical data. The demographic and clinical disease-specific data of the case group are presented in Table I. Vitiligo vulgaris was the most common clinical form (n=84; 76.4%), followed by acrofacial (n=16; 14.5%), focal (n=7; 6.4%) and universal (n=3; 2.7%) patterns. The mean ± SD age at disease onset was 33.5±15.1 (age range 5-66 years), while vitiligo onset before 30 years of age was reported in 52 (47.3%) cases. Thirty-eight (34.5%) patients with family history of vitiligo were recorded. Koebner

phenomenon, leukotrichia, and Sutton nevi were present in 45 (40.9%), 36 (32.7%), and 12 (10.9%) cases, respectively. Stable vitiligo was reported by 69 (62.7%) patients. Regarding comorbidities, thyroid disease was the most common associated disorder (n=50; 45.5%).

Associations between VDR SNPs and vitiligo phenotypes. Subgroup analyses of subsets of patients based on clinical features indicated a significant correlation between the *VDR Fok*I and age at vitiligo onset (Table II). Both the CC genotype and C allele of *Fok*I SNP were overpresented in cases with vitiligo onset after the age of 30 compared to those with earlier disease onset (48.28 vs. 30.77%, P=0.041, respectively; 69.83 vs. 55.77%, P=0.031, respectively), conferring protec-
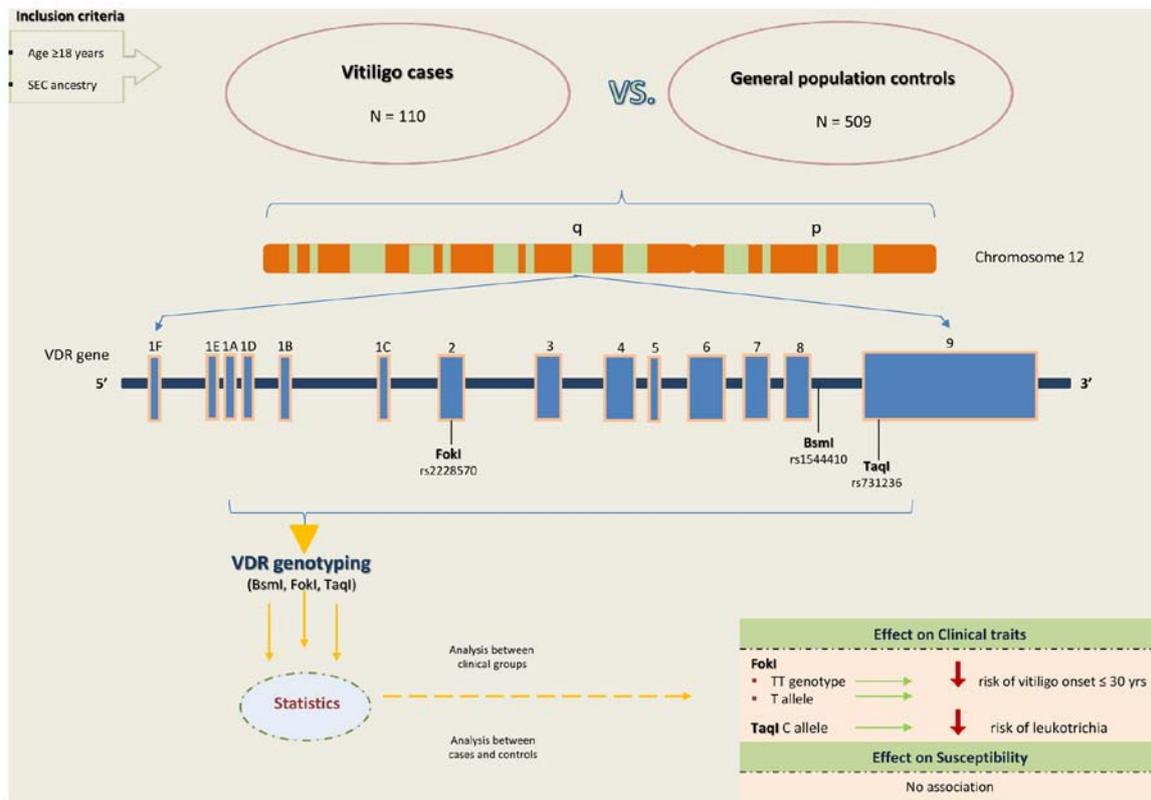
Figure 1. Schematic diagram of study design and summary of results. VDR, vitamin D receptor.

tion against early-onset vitiligo. Patients carrying the *Fok*I CC genotype or C allele may thus be at lower risk for development of vitiligo up to 30 years of age compared to carriers of the *Fok*I TT genotype or T allele, respectively (TT vs. CC: OR=0.286, 95% CI: 0.083-0.984, P=0.041; T vs. C: OR=0.545, 95% CI: 0.313-0.948, P=0.031).

For *Taq*I polymorphism, the variant C allele frequency was significantly higher in vitiligo cases devoid of leukotrichia compared to cases with concurrent leukotrichia (41.89 vs. 27.78%, P=0.042). Using the T allele as reference, the C allele was found to adversely affect the risk for occurrence of leukotrichia; *Taq*I C allele carriers were ~1.9 times less prone to develop leukotrichia compared to T allele carriers (T vs. C: OR=1.874, 95% CI: 1.018-3.451, P=0.042) (Table III). Either *Fok*I, *Bsm*I, or *Taq*I loci had no obvious correlation with other vitiligo-related clinical variables in our sample (data not shown).

Genotypic and allelic distributions of VDR polymorphisms. The genotype and allele frequencies of the selected *VDR* SNPs among cases and general population controls, as well as their associations with vitiligo susceptibility are summarized in Table IV. The relevant genotype frequencies were in accordance with the HWE equilibrium among the controls (P=0.909 for *Fok*I; P=0.966 for *Bsm*I; and P=0.970 for *Taq*I).

In overall analysis, no statistically significant differences between vitiligo cases and general population controls were observed for the *VDR Fok*I, *Bsm*I, and *Taq*I polymorphisms in any of the genetic models used, indicating a lack of association between the studied SNPs and susceptibility to vitiligo in this cohort.

A schematic diagram of study design and summary of results is provided in Fig. 1.

Discussion

This study investigated associations of the *Fok*I, *Bsm*I, and *Taq*I SNPs in the *VDR* gene with the risk of vitiligo and its clinical features. The results showed that the *VDR Fok*I SNPs (CC genotype and C allele) seemed to reduce the risk of vitiligo onset until the age of 30, while the *Taq*I C allele may confer a lower risk for leukotrichia. Our data also showed that either *Fok*I, *Bsm*I, or *Taq*I loci are not involved in the occurrence and development of vitiligo.

To date, genetic studies have identified over 50 vitiligo susceptibility loci, emphasizing the importance of genetic factors in the onset and evolution of the depigmentation process (2,4). In this regard, a limited number of studies covering mostly Latin American, African, and Asian populations have previously examined the role of *VDR* gene polymorphisms in vitiligo (16,18,22-27), but the reported findings have been controversial and inconclusive (Table V). In addition, studies investigating the *Bsm*I, *Taq*I, and *Fok*I SNPs in vitiligo patients are fewer compared to those conducted on *Apa*I (18), making it more difficult to yield meaningful results, especially among Caucasian subjects.

With respect to *VDR* SNPs effect on vitiligo phenotypes, two studies investigating the relationship of the *VDR Fok*I, *Bsm*I, and *Taq*I with clinical characteristics of vitiligo failed to demonstrate a link between the studied *VDR* SNPs and age at disease onset (22,26). In contrast, our intra-patient analysis showed that the *VDR Fok*I SNPs could have a protective effect

Table V. Characteristics of main studies on the effect of *VDR* gene polymorphisms (*Bsm*I, *Fok*I, and *Taq*I) on vitiligo risk.

First author, year	Population	Participants (n)		Sex F/M (%)		Age (years), mean (SD)		Effect on vitiligo risk			Refs.
		Cases	Controls	Cases	Controls	Cases	Controls	*Fok*I	*Bsm*I	*Taq*I	
Hassan, 2019	South Asian	100	100 (age/sex-matched)	61/39	60/40	28.7 (11.98)	-	No relation	No relation	No relation	(24)
Ochoa-Ramírez, 2019	Latin American	173	184 (age/sex-matched)	53.2/46.8	-	-	-	-	No relation	No relation	(26)
Sobeih, 2016	African	75	75 (age/sex-matched)	-	-	31.5 (13.5)	-	No relation	-	CC genotype ↑ CT genotype ↓	(27)
Aydıngöz, 2012	Asian	98	216 (age/sex-matched)	46.9/53.1	56.5/48.2	39 (12.05)	37.1 (9.8)	No relation	No relation	C allele ↑ CC genotype ↑	(22)
Li, 2012	East Asian	749	763 (age/sex-matched)	44.7/55.3	45.9/54.1	24.7 (13.6)	26.4 (13.3)	No relation	A allele ↓ GA genotype ↓	C allele ↓ CT genotype ↓	(25)
Birlea, 2006	Caucasian	31	33	67.7/32.3	-	53 (17.1)	-	No relation	-	No relation	(23)
Zhang, 2018[a]		7[b]		-	-	-	-	No relation	No relation	No relation	(18)
Li, 2015[a]		4[b]		-	-	-	-	-	GG genotype ↑ (in East Asians)	-	(16)
This study	Caucasian	110	509 (general population)	68/42	289/220	45.1 (13.4)	40.5	No relation	No relation	No relation	

[a]Meta-analyses. [b]No. of studies. VDR, vitamin D receptor; F, female; M, male; SD, standard deviation.
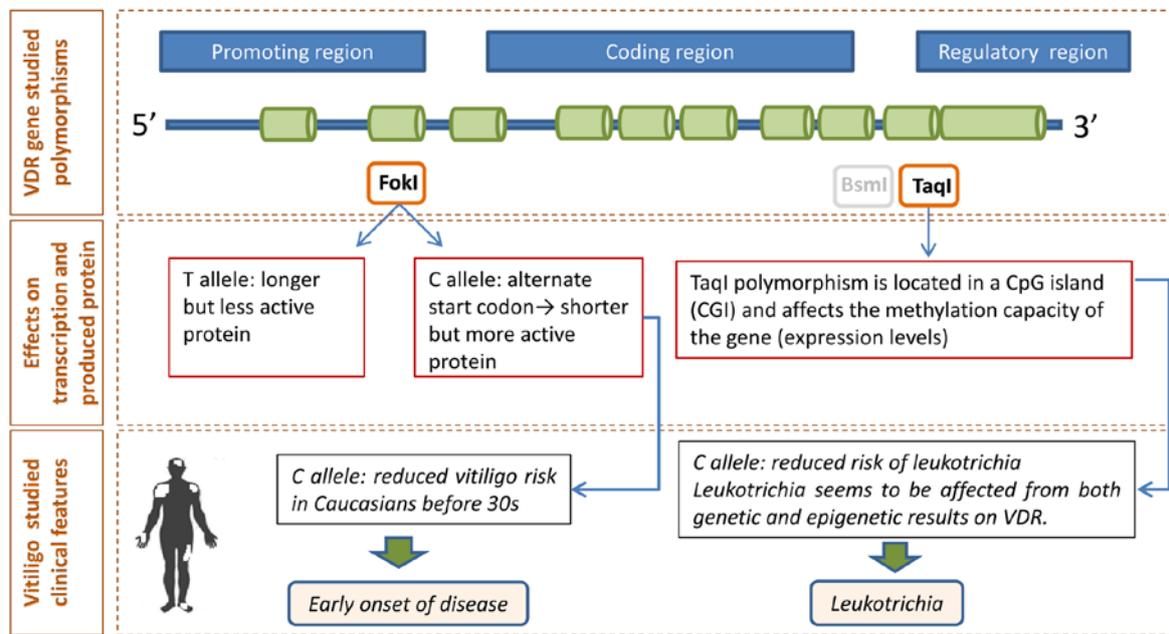
Figure 2. Subgroup analyses of patients based on vitiligo clinical features showed two statistically significant findings: (A) *Fok*I C allele was overpresented in cases with vitiligo onset after the age of 30 (possible protective effect on vitiligo development) and (B) *Taq*I C allele carriers were ~1.9 times less prone to develop leukotrichia compared to T allele carriers. There was no association between *Bsm*I polymorphism and vitiligo clinical features. VDR, vitamin D receptor.

against early-onset vitiligo, as carriers of the CC genotype or C allele of *Fok*I exhibited a 71.4% and a 45.5% decreased risk of developing vitiligo before the age of 30 compared to patients carrying the *Fok*I TT genotype or T allele, respectively. Although not related to vitiligo *per se*, VDR *Fok*I appears to delay the onset of vitiligo until the age of 30 and may thus be a potential biomarker providing prognostic clues for early detection of this condition.

In addition, using the T allele as reference, we observed a protective effect of the *VDR Taq*I C allele against leukotrichia. This finding contrasts with previous data demonstrating no association between the *VDR Fok*I/*Bsm*I/*Taq*I polymorphisms and leukotrichia (22). Given that the active melanocytes located into the black hair follicles can serve as a main source of perifollicular repigmentation, the presence of leukotrichia is known to predict a poor response to traditional treatments, pointing towards a surgical solution (28-30). In this sense, it seems possible that the *VDR Taq*I might indirectly be implicated in treatment decision making despite its lack of association with vitiligo *per se*. Moreover, since the *Taq*I polymorphism is located in the gene's regulatory region (CpG site), leukotrichia also appears as a clinical feature that can be influenced not only by genetic regulation but also by epigenetic factors that may affect VDR expression levels (Fig. 2) (31).

Contrary to Ochoa-Ramírez *et al* (26), no correlation between the *VDR Bsm*I and Koebner phenomenon was observed in our sample. Although, similar to prior studies (22,26), we could not reveal further associations between the studied *VDR* polymorphisms and other clinical manifestations of vitiligo, our findings underline the need for analyzing *VDR* variants according to disease-related clinical features in order to identify genetically-based subsets of patients that may benefit from personalized approaches to vitiligo diagnosis or treatment.

This study presents some limitations to consider. Apart from the relatively small sample size (110 cases), our analyses included data only from SEC subjects, thus making the results applicable only to this ethnotic group, especially considering that genetic variability differs within European populations (32-34). Moreover, investigations on other common *VDR* SNPs, such as the *Apa*I, as well as haplotype analysis was not conducted, thus limiting the acquisition of further genetic information. Regarding other clinical aspects, i.e., Sutton nevi, the limited data available did not allow us to analyze each feature separately, which might have influenced the results.

In conclusion, the current study provided evidence to support a potential implication of the studied *VDR* SNPs in the clinical course and treatment decision making of vitiligo. The *Fok*I CC genotype and C allele seemed to play a protective role in early onset of vitiligo (≤30 years), while the *Taq*I C allele may reduce the risk for future development of leukotrichia. Moreover, no evidence was found to support an association between the *VDR Bsm*I, *Taq*I, and *Fok*I loci and vitiligo susceptibility in our cohort.

Even with limitations, this study will enrich the evolving field of vitiligo genetics, especially among SEC subjects, providing a reference for subsequent investigations in order to translate all genetically derived data into clinical applications for early diagnosis and individualized, precision treatment of vitiligo. Further research with larger sample sizes is needed to validate our results and elucidate whether the same associations are also eligible in vitiligo patients among other Caucasian populations or diverse ethnotic groups.

Acknowledgements

Availability of data and materials

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

Authors' contributions

MSK, ND, EN conceived the presented idea, while DI and PS developed the whole theory. MSK designed the experiments and DI, ML, PS prepared the samples and performed the experiments. PS, EN, SM, MS, SG, AT and DR contributed to clinical data and sample collection. KX made the data entry and CD performed the statistical analyses. DI, MSK, PS, ND interpreted the results and designed the figures. PS, DI, AN and LK wrote the manuscript under the supervision of ND and EN, and DAS contributed to the final editing. All the authors discussed the results and contributed to the final manuscript.

Ethics approval and consent to participate

Written informed consent for participation in the study and use of their genetic data was obtained from all participants.

Patient consent for publication

Written informed consent for publication of any associated data was obtained from all participants.

Competing interests

DAS is the Editor-in-Chief for the journal, but had no personal involvement in the reviewing process, or any influence in terms of adjudicating on the final decision, for this article. The other authors declare that they have no competing interests.

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