

Vitamin D Receptor Gene BSMI, FOKI, APAL, and TAQI Polymorphisms and the Risk of Atopic Dermatitis

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■ Abstract

Background and Objective: The association between vitamin D receptor (VDR) gene polymorphisms and the risk of skin diseases has been widely studied, yet there is only one study on atopic dermatitis. In this study, we aimed to investigate the association between 4 VDR polymorphisms and atopic dermatitis.

Patients and Methods: This cross-sectional case control study was performed between March 2013 and April 2014 at the University Hospital in Çanakkale, Turkey. Peripheral blood samples were collected in EDTA tubes. DNA extraction was performed using the spin column procedure. The VDR polymorphisms FokI (rs2228570), BsmI (rs1544410), Apal (rs7975232), and TaqI (rs731236) were determined by polymerase chain reaction-restriction fragment length polymorphism analysis in 42 atopic dermatitis patients and 96 healthy individuals from a Turkish population.

Results: The VDR rs1544410 polymorphism increased the risk of atopic dermatitis in our Turkish population [OR, 12.2; 95%CI, 0.44-336; P=.05]. The FokI, TaqI, and Apal polymorphisms were not significantly associated with atopic dermatitis susceptibility.

Conclusion: The VDR FokI, TaqI, and Apal gene polymorphisms were not associated with the risk of atopic dermatitis in the Turkish population but the BsmI polymorphism was found to increase risk.

Key words: FokI. rs2228570. BsmI. rs1544410. Apal. rs7975232. TaqI. rs731236.

■ Resumen

Introducción y Objetivo: Es frecuente la publicación de estudios que investiguen acerca de la asociación de diversas enfermedades cutáneas con los polimorfismos del gen que codifica el receptor de la vitamina D (VDR). Sin embargo, únicamente existe un estudio que lo haya hecho con la dermatitis atópica. Por lo tanto, el objetivo de este trabajo es investigar la asociación de los polimorfismos del VDR con el riesgo de padecer dermatitis atópica.

Pacientes y Métodos: Este estudio transversal, de casos y controles, se realizó entre marzo de 2013 y abril de 2014 en un Hospital Universitario de Çanakkale, Turquía. Se analizaron muestras de sangre periférica recogidas en tubos con EDTA. La extracción de ADN se realizó mediante columna de centrifugación. Los polimorfismos del VDR: FokI (rs2228570), BsmI (rs1544410), Apal (rs7975232) y TaqI (rs731236) se determinaron mediante reacción en cadena de la polimerasa de longitud de fragmentos de restricción (PCR-RFLP): Se estudiaron, en una población turca, pacientes con dermatitis atópica (n=42) e individuos sanos (n=96).

Resultados: El SNP rs1544410 del VDR se asoció con un aumento en el riesgo de dermatitis atópica [OR: 12,2; IC del 95%: 0,44 a 336; p: 0,05]. Sin embargo, los polimorfismos FokI, TaqI y Apal no se asociaron con la susceptibilidad de padecer dermatitis atópica en la población turca.

Conclusión: La presencia de polimorfismos del VDR FokI, TaqI y Apal no se asocia con la dermatitis atópica. En contraste, el polimorfismo BsmI aumenta el riesgo de padecer dermatitis atópica en la población turca.

Palabras clave: FokI. rs2228570. BsmI. rs1544410. Apal. rs7975232. TaqI. rs731236.

Introduction

Atopic dermatitis (AD) is a chronic skin disease with an unknown etiology and pathogenesis. It is a multifactorial, inflammatory, relapsing, noncontagious, and itchy skin disease that usually starts in infancy or early childhood [1-3]. In developed countries, 10% to 15% of all children have AD and the prevalence of the disease, like that of other allergic diseases, is rising progressively as a result of industrialization, urbanization, and climate change [4]. Interestingly, a history of atopy among parents, and mothers in particular, has been identified as a strong risk factor for the development of AD [5]. This suggests that AD patients have an as yet not understood genetic tendency and that the molecular mechanisms that regulate keratinocyte proliferation and differentiation may be involved in triggering the development of AD [6]. The major role of active vitamin D₃ is to regulate keratinocyte differentiation via suppression of keratinocyte proliferation [7]. Recent studies have shown that vitamin D has important effects on immune regulation, apoptosis, metastases, and angiogenesis [8], as well as on cell proliferation and differentiation. In addition, this vitamin plays a key role in calcium metabolism, bone development, and maintenance [9]. Vitamin D is a prohormone that is acquired mainly from the diet or through dermal synthesis following exposure to sunlight; it is enzymatically activated in the liver and kidneys. The active form of the vitamin (1.25(OH)₂D) freely passes into the cells through the membranes and binds with the nuclear vitamin D receptor (*VDR*) to form a heterodimer complex with retinoid X receptor (RXR), which then binds to vitamin D₃-responsive elements in the promoter region of the gene and modulates target gene transcription [10]. Thus vitamin D metabolism may directly affect gene expression and regulation. Several studies have investigated the association between *VDR* polymorphisms

and skin diseases (melanoma, basal cell carcinoma, vitiligo Behçet disease, psoriasis) [11-15]. The *VDR* gene is localized at chromosome 12q13.1 and numerous polymorphisms have been identified on the extensive promoter region of the gene (Figure 1). The alleles of FokI(T/C rs2228570), BsmI(G/A rs1544410)/ApaI(G/T rs7975232), and TaqI(T/C rs731236) are located in exon 2, intron 8, and exon 9, respectively. These *VDR* alleles are the most extensively studied alleles in this polymorphic region and their role has been investigated in several diseases. However, only Heine et al [16] have investigated the association between the BsmI, ApaI, and TaqI polymorphisms and severe AD in adults [16]. We performed a Turkish population-based study of the impact of 4 *VDR* polymorphisms—FokI(T/Crs2228570), BsmI(G/A rs1544410), ApaI(G/T rs7975232), and TaqI (T/C rs731236)—on the risk of AD. This is the first time that the DNA binding protein polymorphism FokI (T/C rs2228570) has been studied in patients with AD.

Patients and Methods

Participants

This hospital-based case-control study included 42 AD patients and 96 controls. All of the participants were recruited from the dermatology department at Çanakkale Onsekiz Mart University (ÇOMU) in Çanakkale, Turkey. The study was approved by the ethics committee at ÇOMU, and all the participants provided their informed consent to participate. The inclusion criteria were an age of above 18 years, Turkish ethnicity, and a diagnosis of AD according to the criteria of Hanifin and Rajka [17]. Patients with a known atopic condition were excluded from the control group.

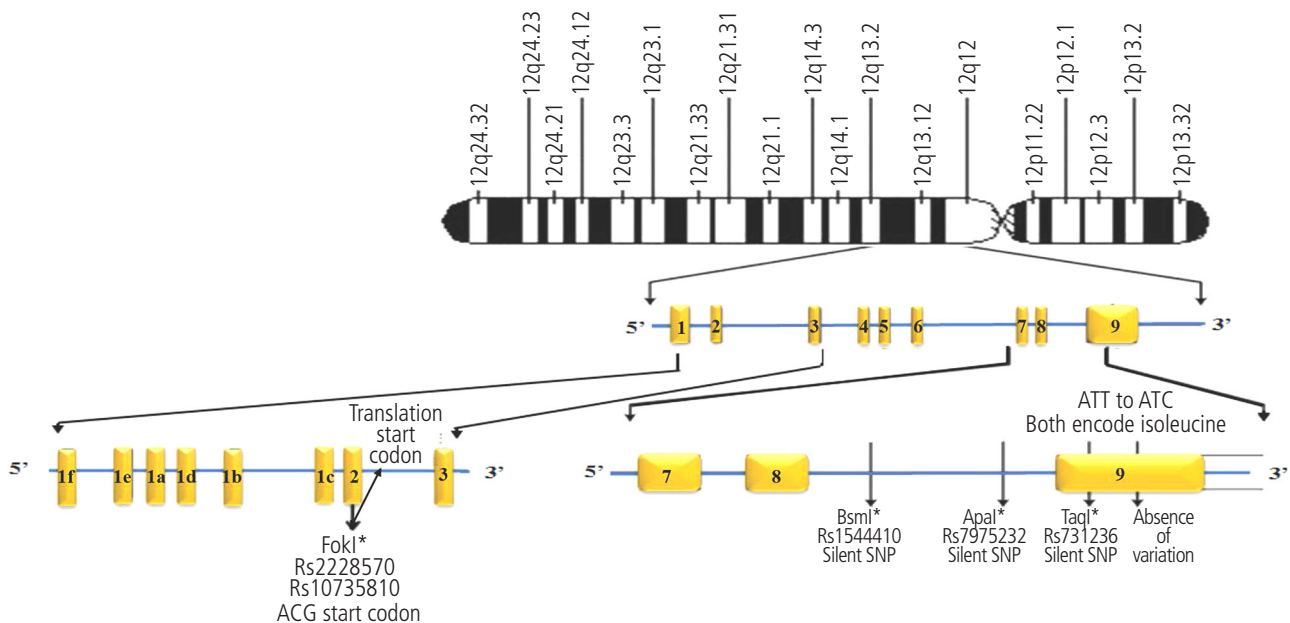


Figure. Polymorphic structure of the *VDR* gene. SNP indicates single nucleotide polymorphism.

Table 1. Primer Set and Specific Annealing Program for Polymerase Chain Reaction (PCR)

Locus	Primers	Allele	PCR Protocol
ApaI	Forward primer 5'-CAG AGC ATG GAC AGG GAG CAA G-3'	A / a	94°C for 30 sec, 72°C for 30 sec, 72°C for 30 sec for 30 cycles
	Reverse primer 5'-GCA ACT CCT CAT GGC TGA GGT CTC A-3'	T allele → G allele	
BsmI	Forward primer 5'AGTGTGCAGGCGATTCTAG3'	B / b	94°C for 30 sec, 58.5°C for 30 sec, 72°C for 30 sec for 30 cycles
	Reverse primer: 5'ATAGGCAGAACCATCTCTCAG3'	T allele → C allele	
FokI	Forward primer 5'-AGCTGG CCC TGG CAC TGA CTA TGC TCT-3'.	F / f	94°C for 30 sec, 61°C for 30 sec, 72°C for 30 sec for 30 cycles
	Reverse Primer: 5'-ATG GAA ACA CCT TGC TTC TTC TCC CTC-3'.	C allele → T allele	
TaqI	Forward primer 5'CAA CCA AGA CTA CAA GTA CCG CGT CAG TGA3'	T / t	94°C for 30 sec, 61°C for 30 sec, 72°C for 30 sec for 30 cycles
	Reverse Primer: 5'CAC TTC GAG CAC AAG GGG CGT TAG C3'	T allele → C allele	

Table 2. Distributions of Genotype and Carriage Rate of *VDR* Polymorphisms (FokI, BsmI, ApaI, and TaqI) in Patients and Controls

SNP	Hardy-Weinberg Equilibrium Test Results	
	Healthy Controls	Atopic Patients
ApaI	GG=25 (22.04)	GG=10 (10.25)
	GT=42 (47.92)	GT=21 (20.50)
	TT=29 (26.04)	TT=10 (10.25)
	f _{a1} =0.48 +/-0.038	f _{a1} =0.50 +/-0.055
	F̄=0.12	F̄=0.02
	P=.23 (Pearson)	P=0.88 (Pearson)
BsmI	BB=26 (38.76)	BB=6 (13.15)
	Bb=70 (44.48)	Bb=35 (20.70)
	bb=0 (12.76)	bb=1 (8.15)
	f _{a1} =0.36 +/-0.023	f _{a1} =0.44 +/-0.030
	F̄=0.58	F̄=0.69
	P=1.89-08 (Pearson)	P=7.61-06 (Pearson)
FokI	FF=53 (48.17)	FF=19 (19.34)
	Ff=30 (39.67)	Ff=19 (18.32)
	ff=13 (8.17)	ff=4 (4.34)
	f _{a1} =0.71 +/-0.037	f _{a1} =0.68 +/-0.050
	F̄=0.24	F̄=0.04
	P=.02 (Pearson)	P=0.81 (Pearson)
TaqI	TT=45 (43.34)	TT=22 (22.15)
	Tt=39 (42.33)	Tt=17 (16.70)
	tt=12 (10.34)	tt=3 (3.15)
	f _{a1} =0.67 +/-0.035	f _{a1} =0.73 +/-0.048
	F̄=0.08	F̄=0.02
	P=.44 (Pearson)	P=.91 (Pearson)
	P=.44 (Llr)	P=.91 (Llr)
	P=.49 (Exact)	P=1.00 (Exact)

Abbreviations: f_{a1}, frequency of allele 1 (SD); F̄, inbreeding coefficient; p (Pearson), Pearson χ^2 goodness-of-fit test; p (Llr), log likelihood ratio χ^2 (degrees of freedom = 1); p (Exact), exact test.

Genotyping of the *VDR* Gene

Peripheral blood samples of individuals were collected in EDTA-containing tubes, and genomic DNA was extracted using a commercially available spin-column method (GeneJet DNA Purification, Thermo Scientific). The FokI(T/Crs2228570), BsmI(G/A rs1544410), ApaI(G/T rs7975232), and TaqI(T/C rs731236) polymorphisms were analyzed by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP). Genomic DNA was amplified for 4 polymorphic sites on the *VDR* gene with the forward and reverse primers shown in Table 1. All the PCR reactions were carried out in a total volume of 50 μ L containing 10 μ g of genomic DNA, 25 pmol of each primer, 25 μ L of Dream Taq Green PCR Master Mix (include 2.5 units of Taq Polymerase), and 18 μ L of double distilled water. PCR was performed as follows: an initial denaturation cycle at 95°C for 3 minutes, annealing for 30 cycles at 95°C for 30 seconds, use of primer-specific temperatures for 30 seconds and a temperature of 72°C for 30 seconds, and a final one-step extension at 72°C for 5 minutes. Primer sequences and primer T_m values are shown in Table 2. The restriction endonucleases FokI, BsmI, ApaI, and TaqI were used to digest the polymorphic sites of the *VDR* gene. The enzyme-specific restriction reactions were performed at 37°C for 5 minutes for the BsmI, FokI, and ApaI enzymes, and at 65°C for 5 minutes for the TaqI enzyme. The sizes of the digested fragments were identified by 2% agarose gel electrophoresis, and the results were visualized under UV light and photographed. The size of the digested PCR products are shown in Table 2.

Statistical Analyses

All statistical analyses were performed using SPSS version 19. Allele and genotype frequencies were calculated by direct counting and the χ^2 test was used to compare frequencies between cases and controls. Hardy-Weinberg equilibrium was tested using the χ^2 goodness-of-fit test. The risk of AD associated with each allele and genotype was investigated

Table 3. Association Analyses of VDR Polymorphisms With Atopic Dermatitis

Polymorphism	Association Tests				
	Allele Frequency	Heterozygous	Homozygous	Allele Positivity	Cochran-Armitage Trend Test
BsmI	[A] ↔ [G]	[GG] ↔ [AG]	[AA] ↔ [GG]	[AA]+[AG] ↔ [GG]	Common OR
	OR=1.32 95% CI=0.82-2.31 $\chi^2=1.42$ P=.23 (P)	OR=2.17 95% CI=0.82-5.75 $\chi^2=2.49$ P=.12	OR=12.23 95% CI=0.45-336 $\chi^2=3.83$ P=.05	OR=2.23 95% CI=0.84-5.91 $\chi^2=2.69$ P=.10	OR=3.41 $\chi^2=3.57$ P=.06
ApaI	[G] ↔ [T]	[GG] ↔ [GT]	[GG+] ↔ [TT]	[GG] ↔ [GT+TT]	Common OR
	OR=0.920 95% CI=0.55-1.54 $\chi^2=0.10$ P=.75 (P)	OR=1.250 95% CI=0.51-.07 $\chi^2=0.24$ P=.63	OR=0.86 95% CI=0.31-2.41 $\chi^2=0.08$ P=.78	OR=1.092 95% CI=0.47-2.54 $\chi^2=0.04$ P=.84	OR=0.93 $\chi^2=0.09$ P=.76
FokI	[T] ↔ [C]	[TT] ↔ [TC]	[CC+] ↔ [22]	[TT] ↔ [TC+CC]	Common OR
	OR=1.15 95% CI=0.66-2.0 $\chi^2=0.25$ P=.62 (P)	OR=1.767 95% CI=0.83-3.85 $\chi^2=2.08$ P=.15	OR=0.86 95% CI=0.25-2.96 $\chi^2=0.06$ P=.81	OR=1.49 95% CI=0.72-3.09 $\chi^2=1.16$ P=.28	OR=1.05 $\chi^2=0.21$ P=.64
TaqI	[T] ↔ [C]	[TT] ↔ [TC]	[TT+] ↔ [CC]	[TT] ↔ [TC+CC]	Common OR
	OR=0.77 95% CI=0.438-1.360 $\chi^2=0.80$ P=.37 (P)	OR=0.89 95% CI=0.42-1.92 $\chi^2=0.09$ P=.77	OR=0.51 95% CI=0.13-2.0 $\chi^2=0.95$ P=.33	OR=0.80 95% CI=0.38-1.65 $\chi^2=0.35$ P=.55	OR=0.758 $\chi^2=0.76$ P=.38

Abbreviation: P, Pearson χ^2 goodness-of-fit test.

through calculation of ORs. The significance level was set at a *P* value of less than .05 for all statistical analyses.

Results

Population Characteristics

Forty-two AD patients with a mean (SD) age of 34.72 (12.28) years (range, 18-63 years) and 96 healthy controls with a mean (SD) age of 40.5 (10.44) years (range, 19-65 years) were enrolled in this study. Genotype distributions and allele positivity and frequency of FokI(T/Crs2228570), BsmI(G/A rs1544410), ApaI(G/T rs7975232), and TaqI (T/C rs731236) in the control and AD groups are summarized in Table 3.

Genotyping of rs2228570 (T→C) and rs731236 (T→C) showed no statistically significant differences for allele frequencies between the control group and the AD group. In addition, phenotype-genotype association analysis of the rs2228570 and rs731236 polymorphisms showed no significant associations between either group. The comparison of allele frequencies of the rs1544410 polymorphism in AD patients and controls also showed no significant differences (OR, 1.372; 95% CI, 0.82-2.31; *P*=.24). By contrast, homozygous mutant genotypes were observed to be associated with a 12.3-fold increased risk of AD compared with wild-type genotypes [OR, 12.23; 95% CI, 0.45-336; *P*=.05]. In the overall risk estimation, individuals with the BsmI polymorphic restriction site had a 3.4-increased risk of AD [OR, 3.41; χ^2 , 3.57; *P*=.06]. By contrast, no significant associations were observed between AD and genotype and allele frequencies of the ApaI polymorphism.

Discussion

VDR polymorphisms may have a role in skin diseases due to their impact on keratinocyte proliferation and differentiation. In this study, we investigated the association between the most widely studied VDR polymorphisms—FokI(T/Crs2228570), BsmI(G/A rs1544410), ApaI(G/T rs7975232) and TaqI (T/C rs731236)—and AD.

The FokI polymorphism leads to an alternative translation site 10 base pairs upstream from the promoter, resulting in longer VDR transcripts; this variant is known as the F allele. In the only study in the literature to investigate the association between VDR polymorphisms and AD, Heine et al [16] studied 3 polymorphisms: ApaI, BsmI, and TaqI. Our study is the first to investigate the additional association with FokI. Although this polymorphism results in an altered protein structure and causes a decrease in transcriptional activity, we found no significant association with AD.

The VDR TaqI polymorphism is located at codon 352 in exon 9 and causes a silent codon change for isoleucine. This polymorphism was not associated with an increased risk of AD in our Turkish population, coinciding with the observations of Heine et al [16], who found no significant association between the TaqI polymorphism and AD.

The BsmI and ApaI polymorphisms are located in intron 8 at the 3' end of the VDR gene. The BsmI polymorphism is a silent polymorphism yet it may decrease gene expression levels due to a reduction in mRNA stability. In our study, only a weak association was observed between the VDR BsmI BB genotype and AD, but it should be noted that our

sample was small. That said, our results were similar to those reported by Heine et al [16]. Finally, we found no significant association between the ApaI polymorphism and AD in the Turkish population.

In conclusion, the BsmI polymorphism was associated with an increased risk of AD in the Turkish population. However, more studies are needed to further investigate the association between the the FokI(T/Crs2228570), BsmI(G/A rs1544410)/ApaI(G/T rs7975232), and TaqI (T/C rs731236) polymorphisms and AD.

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Conflicts of Interest

The authors declare that they have no conflicting interests.

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