Analysis of Vitamin D Receptor (VDR) Gene Polymorphisms in Alopecia Areata

Alopesi Areata'da Vitamin D Reseptör (VDR) Polimorfizminin Analizi

VDR Polymorphism in Alopecia Areata

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Özet

Amaç: Alopesi areata (AA), vücudun herhangi bir bölgesindeki saçlı deride meydana gelen saç kaybıyla karakterize edilmekte olup, genel populasyonun yaklaşık %1-2'sini etkileyen bir hastalıktır. Hastalığın etiyopatogenezi bilinmemekle birlikte, genetik, enfeksiyon, fizyolojik ve otoimmun faktörlerin rol oynadığı bilinmektedir. Vitamin D'nin immun sistem üzerinde düzenleyici etkisi olduğu ve bu etkinin vitamin D reseptörüne (VDR) bağlı olduğu düşünülmektedir. Bu otoimmun hastalıklar tarafından paylaşılan otoimmun bileşen göz önüne alınarak bu çalışmada AA gelişiminde VDR gen polimorfizmlerinin rolü araştırıldı. Gereç ve Yöntem: Çalışma grubu 198 AA'lı birey ve 167 AA'sız kontrol bireyinden oluşturuldu. Genomik DNA, DNA izolasyon kiti kullanılarak kan örneklerinden elde edildi. VDR gen polimorfizmlerine ait genotip ve alleller, Polimeraz Zincir Reaksiyonu ve Restriksiyon Parça Uzunluk Polimorfizmi yöntemleri kullanılarak belirlendi. Bulgular: Elde edilen sonuçların istatistiksel analizininde VDR geni BsmI (rs1544410), ApaI (rs7975232) ve TaqI (rs731236) polimorfizmleri ile AA arasında anlamlı bir bağlantı olmadığı bulundu ((p=0.8891, 0.7309, 0.6761, sırasıyla). Tartışma: Çalışma bulguları AA'ya genetik yatkınlığın belirlenmesinde VDR polimorfizmlerinin rolünün olamayacağını göstermektedir.

Anahtar Kelimeler

Alopesi Areata; Polimorfizm; VDR Geni

Abstract

Aim: Alopecia areata (AA) is a disease characterized with hair loss on the hair skin any region of the body. This disease affects approximately 1–2% of the general population. The etiopathogenesis of this disease is unclear but infections, genetic, psychological and autoimmune factors is known play to role. Vitamin D is thought to be a regulator of the immune system and the action of it is dependent on the vitamin D receptor (VDR). Given the autoimmune component shared by this autoimmune diseases. In this study investigated the role of VDR gene polymorphisms in the development of AA. Material and Method: The study group included 198 patients with AA and 167 control. Genomic DNA was extracted from blood samples using DNA isolation kit. The frequency of VDR gene polymorphisms genotypes and allelic variants were analyzed by using Polymerase Chain Reaction (PCR) and Restriction Fragment Length Polymorphisms (RFLP) method. Results: Statistical evaluation of data results showed a not significant association for genotypic frequency distribution between the VDR gene BsmI (rs1544410) and ApaI (rs7975232), TaqI (rs731236) polymorphisms and AA (p=0.8891, 0.7309, 0.6761, respectively). Discussion: Our study reflects that VDR gene polymorphisms could not play a role in determining genetic susceptibility to AA.

Keywords

Alopecia Areata; Polymorphism; VDR Gene

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Introduction

Alopecia areata (AA) is a common, chronic and inflammatory disease characterized by the skin in which hair is lost from certain or all areas of the body [1-3] Also, AA is a autoimmune disorder that affects approximately 1–2% of the worldwide [1,4]. There are in forms called alopecia totalis ([AT]; loses all of the hair on the entire scalp) and alopecia universalis ([AU]; the entire body hair is lost) in diseases [5,6]. This disease can occur in any ages, especially among people aged 4-5 and 15-40, both gender and all ethnic groups [1]. Various studies have reported that peak incidence of the disease occurs is between 20 and 25 years of age [7,8]. Etiology of AA are not fully understood but genetic factors, environmental factors, and the contribution of autoimmune responses can play important role in the development of disease [6-8]. This genetic basis of AA is strongly supported concordance rate in identical twins studies, genetic linkage analysis and environmental triggers [9]. This condition is suggested by the occurrence for AA incidence 10-42% in among first-degree relatives and incidence 55% in monozygotic twins [7,10]. It is also well known that AA is related to other autoimmune disorders, ie vitiligo, lupus erythematosus, thyroid, myasthenia gravis, scleroderma, ulcerative colitis, Type I diabetes, allergic diseases, thyroiditis and rheumatoid arthritis [8,11].

In recent years has found an association between vitamin D and the development of many autoimmune diseases [12]. Vitamin D, mediate its effect by binding to vitamin D receptor (VDR), which is located on a great number of immune cells [12,13]. It can also modulated immune cells activity. In addition, it is able to trigger both innate and adaptive immune responses. Thus, VDR gene polymorphisms can consider to associated with autoimmune disorders [12].

The human VDR gene is located on chromosome 12q12–q14. More than 60 polymorphisms in introns or regulatory areas other than exons have been reported in the gene. Three common polymorphisms have been identified –BsmI (rs1544410) and ApaI (rs7975232), both in intron 8; TaqI (rs731236), in exon 9- [14-17]. These three polymorphisms are located near the 3end of the VDR gene [18]. But the functional effects of the polymorphisms, are unclear. Some studies suggest that the three polymorphisms may alter polyadenylation of the VDR mRNA transcript and thus affect mRNA stability [19].

In this study, we investigated the possible role of VDR gene polymorphisms, BsmI, ApaI and TaqI, associated in genetic susceptibility to AA patients with healthy controls in a Turkish population.

Material and Method

Subjects: The study group consisted of 198 patients with AA 77 female and 121 male; mean age 32.62 ± 9.621 standard deviation [SD] years), and 167 healthy subjects 66 female and 101 male; mean age 31.56 ± 11.319 [SD] years) as the control group. All AA patients and control groups were collected by Department of Dermatology of Gaziosmanpasa University, School of Medicine, Tokat, Turkey. The control subjects matched for age, sex and geographic area. The study protocol was approved by the ethics committee of Gaziosmanpasa University, Faculty of Medicine and written informed consent was obtained from the study participants.

Genotyping: Genomic DNA was extracted from peripheral blood samples using an Invitrogen DNA isolation kit according to the manufacturer's directive (Invitrogen Life Technologies, Carlsbad, CA, USA). Polymorphisms of VDR gene were genotyped with of a polymerase chain reaction- based restriction fragment length polymorphism (PCR-RFLP) method.

BsmI PCR and restriction digestion;

Genomic DNA was amplified by PCR using specific primers as previously described: for BsmI site of the VDR gene were as follows: 5'-CAA CCA AGA CTA CAA GTA CCG CGT CAG TGA-3'and 5'-AAC CAG CGG GAA GAG GTC AAG GG-3'. The PCR analysis was also performed using a modification of a previously described study [20]. PCR was carried out in a total volume of 25 µl reaction contained, 2.5 mM 10X reaction Buffer, 1.5 mM MgCl2, 0.3 mM dNTP and, 0.8 mol/L of each primer, 1 U of Taq polymerase and approximately 50 ng of genomic DNA. The PCR conditions were 5 min at 94°C for initial denaturation; followed by 30 cycles of 30 sec at 94 oC, 30 sec at 65°C and 30 sec at 72 oC; and a final elongation step of 5 min at 72 oC. Specific PCR products for BsmI polymorphism of the VDR gene were obtained 825 bp. PCR products were digested with the restriction en-zymes (BsmI) according to the manufacturer's in¬structions, and Restriction fragments of BsmI polymorphisms in VDR gene were electrophoresed on an 2% agarose and 1% nusieve agarose gel stained with ethidium bromide, and the genotypes were determined under ultraviolet (UV) illumination. BsmI genotypes: BB (825 bp), Bb (825, 650, and 125 bp), and bb (650 and 125 bp) [13].

ApaI and TaqI PCR and restriction digestion

ApaI and TaqI sites of the VDR gene were as follows: 5'-CAG AGC ATG GAC AGG GAG CAA G-3'and 5'-GCA ACT CCT CAT GGG CTG AGG TCT CA-3'. The PCR analysis was also performed using a modification of a previously described study [20]. PCR was carried out in a total volume of 25 µl reaction contained, 2.5 mM 10X reaction Buffer, 1.5 mM MgCl2, 0.3 mM dNTP and, 0.8 mol/L of each primer, 1 U of Taq polymerase and approximately 50 ng of genomic DNA. The PCR conditions were 5 min at 94°C for initial denaturation; followed by 30 cycles of 30 sec at 94 oC, 30 sec at 60°C and 30 sec at 72 oC; and a final elongation step of 5 min at 72 oC. Specific PCR products were obtained 740 bp for ApaI-TaqI polymorphisms of the VDR gene. PCR products were digested with the restriction en¬zymes (ApaI, TaqI) according to the manufacturer's in¬structions, and restriction fragments of ApaI and TaqI polymorphisms in VDR gene were electrophoresed on an 2% agarose and 1% nusieve agarose gel stained with ethidium bro-mide, and the genotypes were determined under ultraviolet (UV) illumination. ApaI genotypes AA (740 bp), Aa (740, 520, and 220 bp), and aa (520 and 220 bp) and TaqI genotypes: TT (495 and 245 bp), Tt (495, 290, 245, and 205 bp), and tt (290, 245, and 205 bp) [20].

Statistical analysis

Statistical analysis was performed by using PEPI 3.0 (available at:http://www.usdinc.com/pepi.html). Statistical analysis of VDR gene polymorphisms between the in Alopecia areata patients and the healthy controls were compared by using the

x2 or Fisher's exact test. The p values smaller than 0.05 were considered significant.

The x2 test was used to evaluate the Hardy–Weinberg equilibrium for the distribution of the genotypes of the patients and the controls. The correlation of the mean age with groups was analyzed using the t-test for independent samples.

Results

Table 1 shows the demographic characteristics of AA patients and healthy controls. There were no significant differences between the patient and controls for the mean age or gender distribution. Allelic and genotypic distributions of the studied polymorphisms are shown in Table 2.

The frequencies of BB, Bb and bb genotypes of VDR gene BsmI polymorphism in the patients were 28, 44 and 28 % and in the controls were 26, 44 and 30 %, respectively. B and b allele frequencies BsmI polymorphism were 50 and 50 % in patient group, and 48 and 32 % in control group, respectively. The frequencies of AA, Aa and aa genotypes of VDR gene ApaI polymorphism in the patients were 35, 53 and 12 % and in the controls were 38, 49 and 13 %, respectively. A and a allele frequencies VDR gene ApaI polymorphism were 62 and 38 % in patient group, and 63 and 37 % in control group, respectively. The frequencies of TT, Tt and tt genotypes of VDR gene TaqI polymorphism in the patients were 42, 50 and 8 % and in the controls were 38, 52 and 10 %, respectively. T and t allele frequencies TaqI polymorphism were 68 and 32 % in patient group, and 64 and 36 % in control group, respectively. Statistical analysis of this results showed not significant association between either genotype and/or allelic frequencies for VDR gene BsmI, ApaI and TaqI polymorphisms and AA. (p= 0.8891, p= 0.315, BsmI; p=0.7309 and p=0.396; ApaI; p=0.6761 and p=0.137, TaqI) (Table 2).

Table 1. The demographical characteristics of AA patients and healthy controls

	AA patients n=198	Healthy controls n=167
Gender, no. of Female	77 [39%]	66 [40%]
Male	121 [61%]	101 [60%]
Age (years) mean ± SD	36.62±9.6	37.38±11.31

Table 2. Genotype distributions and allele frequencies of Bsm I, Apa I and Taq I polymorphisms of VDR gene in AA patients and controls

VDR gene polymorphism	Patient Group n=198	Control Group n=167	P
VDR Bsm I Genotypes			0.8891
BB	55 [28%]	44 [26%]	
Bb	88 [44%]	73 [44%]	
bb	55 [28%]	50 [30%]	
Allele frequency			0.315
B	198 [50%]	161[48%]	
b	198 [50%]	173[52%]	
VDR Apa I Genotypes			0.7309
AA	70 [35%]	64[38%]	
Aa	104[53%]	81[49%]	
aa	24 [12%]	22[13%]	
Allele frequency			0.396
A	244 [62%]	209[63%]	
a	152 [38%]	125[37%]	
VDR Taq I Genotypes			0.6761
TT	83 [42%]	64[38%]	
Tt	99 [50%]	86[52%]	
tt	16 [8%]	17[10%]	
Allele frequency			0.137
T	269[68%]	214[64%]	
t	127[32%]	120[36%]	

Discussion

Alopecia areata (AA) is a common immune-mediated disorder, but the exact cause of the disease is still largely unknown [10]. However, recent and timely research has made much progress in our understanding of the disease mechanism. It is thought that AA might be an inflammation-driven disease, with autoimmune origin or strong genetic contribution [10,21,22]. Epidemiological and laboratory investigations have shown that vitamin D deficiency is associated with several common diseases, including autoimmune diseases [23].

Vitamin D's has a variety tasks, such as interleukin (IL) -2 inhibition, antibody production and lymphocyte proliferation. Therefore, vitamin D is considered to can be a regulator of the immune system. The task of vitamin D is dependent on vitamin D receptor (VDR) [24]. This gene is located on chromosome 12q13.11 and in the gene have been identified VDR polymorphisms has come from analysis of only limited researches, such as ApaI, EcoRV, BsmI, TaqI, and Tru9I polymorphisms [24,25]. In the present study, we examined the distribution of genotype and allelic frequencies of three functional polymorphisms of the VDR gene, BsmI, ApaI and TaqI. The three functional polymorphisms genotypic frequency was not different as compared with AA patients than healthy controls (p= 0.7722, p=0.5807, p=0.8292, respectively).

Akar et al. [13] examined 32 patients with AA and 27 healthy control subjects the distribution of genotype and allelic frequencies of BsmI, ApaI and TaqI polymorphisms of the VDR gene in case–control populations. Akar et al findings correlated with our results. Number of studies that investigated the relationship between VDR gene polymorphisms and AA is limited. Therefore, effect on disease of the gene was explained together with possible related between VDR gene polymorphisms and other autoimmune disease. For example; Type 1 diabetes mellitus (T1DM) is an autoimmune disorder. Zhang et al. [15], explored the association between polymorphisms in the VDR gene and T1DM by meta-analysis. In this meta-analysis included total of 57 case–control studies. This results shows that the BsmI polymorphism is associated with increased risk of T1DM (BB + Bb vs. bb: OR = 1.30, 95% CI = 1.03–1.63). But there are not associated the FokI, ApaI and TaqI polymorphisms and disease [15]. The autoimmune disease the other in is Rheumatoid arthritis (RA). Song et al., with meta-analysis research the association between polymorphisms in the VDR gene and RA. Meta-analysis revealed no association between RA and the BsmI B allele and TaqI T allele in study subjects (OR =1.065, 95% CI =0.911–1.245, p=0.427; OR =1.065, 95% CI =0.834–1.361, p=0.613, respectively) [17]. Psoriasis is most common autoimmune disorder the other. Lee et al., in meta-analysis were included nine relevant studies with VDR polymorphisms and pso-

riasis. This study suggests that the VDR ApaI (p = 0.041) and FokI (FF and ff genotypes of the FokI polymorphism p = 0.009; p = 0.002, respectively) polymorphisms confers susceptibility to psoriasis in the Turkish population and between the BsmI (p= 0.041) polymorphism and susceptibility to psoriasis in Asians [24].

As a result, between autoimmune disease and VDR gene polymorphism contradictory results have been reported. These controversial results could be due to heterogeneity between populations and the small number of samples used in the studies. Not forgetting the small sample size of our study, the present data suggests that VDR polymorphisms do not play a role in AA susceptibility in a group of Turkish population. Further work is required to confirm these findings in different study groups.

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Competing interests

The authors declare that they have no competing interests.

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