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The association of TNFα -238 G/A gene polymorphism with alopecia areata

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Abstract

Background/Aim: Alopecia areata (AA), which is characterized by hair loss, is an inflammatory autoimmune disease. Tumor necrosis factor-alpha (TNFα) is a potent proinflammatory cytokine that has a highly significant role in inflammatory and immune responses. The aim of this study is to evaluate whether there is a relationship between TNFα -238 G/A gene polymorphism and AA in the Turkish population.

Methods: In this case-control study, the frequency of TNFα-238 G/A gene polymorphism and its relationship with some clinical parameters of AA patients were investigated. Seventy-eight AA patients and 78 healthy individuals were included in our study. TNFα -238 G/A polymorphism was evaluated by the PCR-RFLP method.

Results: The distribution of TNFα -238 G/A genotypes was significantly different between patients and control subjects (P<0.001). Frequency of genotypes GG and AA in AA patients (53.8 and 6.4%, respectively) were evidently lower compared to the controls (59 and 25.6%, respectively). Individuals with AA genotype had a lower risk of AA disease (odds ratio (OR)=0.27; 95% CI=0.09-0.79; relative risk (RR)=0.65 (0.49-0.86); P=0.013). GA genotype was significantly higher in patients with AA (39.7%) compared to the control group (15.4%) and an increased risk of patchy AA was observed (OR=2.82, 95% CI=1.28-6.21; RR=1.87 (1.11-3.15); P=0.008).

Conclusion: These results suggest that the TNFα -238 G/A gene polymorphism is associated with AA and individuals with GA genotype may have an increased risk of AA.

Keywords: TNFα, Gene polymorphism, Alopecia areata

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Introduction

Alopecia areata (AA) is an inflammatory autoimmune disorder characterized by non-scarring hair loss [1, 2]. The most affected area is the scalp; nevertheless, there are more serious clinical forms like alopecia universalis (AU) that induces hair loss throughout the whole body and alopecia totalis (AT), including all hair loss on the scalp [2, 3]. Currently, the etiopathogenesis of AA is yet unknown. A strong relationship exists between AA and autoimmune disorders, especially autoimmune thyroiditis, type 1 diabetes, vitiligo, and pernicious anemia. Nearly 20% of the affected individuals have a positive family history, indicating a genetic predisposition. Thus, AA is regarded as a genetically determined immune-mediated disease [3].

The TNFα is a potent proinflammatory cytokine that plays a significant role in inflammatory and immune responses [4]. It is located on chromosome 6 in the class III region of the human leukocyte antigen (HLA). Studies have identified a number of single nucleotide polymorphisms in the promoter region [3, 4]. TNFα -238 G/A polymorphism is one of the few polymorphisms in the TNFα gene that alters the transcription of TNFα and regulates its production [5]. In the literature, TNFα -238 G/A gene polymorphism is reported to be related to diseases such as acne vulgaris, psoriasis, systemic lupus erythematosus, and rheumatoid arthritis [5-7].

Many polymorphisms associated with AA have been identified [8]. There is very limited literature on the correlation between TNFα gene polymorphism and AA patients. Therefore, the aim of the present study was to analyze the association between TNFα -238 G/A gene polymorphisms and Turkish AA patients to clarify if these polymorphisms influenced disease occurrence or led to increased disease risk.

Materials and methods

Subjects

This was a case-control study conducted on patients with patch AA and healthy individuals. The study was conducted with the approval of the Ethics Committee of Clinical Research at Kütahya University of Health Sciences (approval number: 2019/01-4). The sample size to be used in the study was calculated by Power analysis. The alpha error value was 0.05, and the power of the test was 0.95. As a result of the power analysis performed under these conditions, the total sample size was calculated as 156.

In our study, 78 patch AA patients (43 females, 35 males; mean age: 30.1 (1.16) years) were recruited from the dermatology outpatient clinic, Kutahya Health Sciences University. For the patients with patchy AA diagnosis (S1-S2), the AA investigational assessment guidelines were considered [9]. Demographic data, family history, duration of disease, nail dystrophy, autoimmune disease, and AA severity were recorded in all patients.

The control group comprised 30 females and 48 males with a mean age of 36.1 (1.74) years. They were healthy individuals who lived in the same geographical area, had no family history of AA and were free of any dermatological or autoimmune disease.

Genotyping

Blood samples were obtained from all subjects. DNA was isolated from blood by conducting the standard Phenol/chloroform extraction method. To analyze the TNFα -238 G/A gene polymorphisms assay, PCR based restriction fragment length polymorphism (RFLP) method was employed as described previously [7]. Three minutes of initial denaturation was performed for amplification at 94°C, which was followed by 35 cycles of 30 seconds at 94°C, 30 seconds at 60°C, and 1 minute at 72°C. Final extension involved 5 minutes at 72°C. The primer pairs used were 5′- ATC TGG AGG AAG CGG TAG TG-3′ and 5′- AGA AGA CCC CCC TCG GAA CC -3′. The 152 bp PCR product was digested with MspI (New England Biolabs, Ipswich, MA, USA) at 37 °C overnight after the amplification. Restriction fragments were separated on a 3% agarose gel stained with ethidium bromide, and under the ultraviolet (UV) illumination, the genotypes were identified. Wild-type (GG) was identified by the 133/19 bp fragment, heterozygotes (GA) by both the 133/19 bp and 152 bp, and the homozygote variant (AA) by only a 152 bp fragment. Due to its small size, the 19 bp fragment did not appear on the gel.

Statistical analysis

Statistical analysis was performed with the Statistical Package for the Social Sciences (SPSS 16). The distribution of the genotypes was evaluated with the $\chi 2$ test to analyze the Hardy–Weinberg equilibrium. The chi-square test was utilized to detect the correlation between TNFα -238 G/A gene polymorphisms and the clinical and demographic features. To evaluate the risk factors, the odds ratio (OR), and 95% confidence interval (CI) were used. All p values were 2-tailed, and confidence intervals were set at 95%. *P*-values of less than 0.05 indicated significance.

Results

Both clinical and demographic features of the AA patients are shown in Table 1. Age, gender, family history, duration of the disease, alopecia severity, nail dystrophy, and localization, and the presence of another autoimmune disease were analyzed. In our study, no relationship was found between the clinical and demographic characteristics of AA patients and TNFα -238 genotypes. However, genotype GA was found in 4 (12.9%) of AA patients with other autoimmune diseases (P=0.046) (Table 1).

The frequency of TNFα -238 G/A polymorphism genotypes in AA did not show an important discrepancy from the Hardy-Weinberg equilibrium (P=0.820), but there was a significant deviation from the controls (P<0.001) (Table 2).

The genotype frequency of TNFα -238 polymorphism was significantly different in the control and patient groups. It was found that 42 (53.8%) cases and 46 (59%) controls had GG genotype, while 31 (39.7%) cases and 12 (15.4%) controls had GA genotype, and 5 (6.4%) cases and 20 (25.6%) controls had AA genotype ($\chi 2$=17.5, df=2, P<0.001) (Table 3). As a reference, evaluation of the GG genotype revealed that the GA genotype was related with a higher risk of AA patients (OR=2.82; 95% CI=1.28-6.21; RR=1.87 (1.11-3.15); P=0.008), while the AA genotype was significantly linked with reduced

risk of AA disease (OR=0.27; 95% CI=0.09-0.79; RR=0.65 (0.49-0.86); *P*=0.013).

Table 1: Baseline clinical and demographical features of the study patients with AA stratified according to TNFα -238 G/A gene polymorphisms

Characteristic	Total n = 78	GG	GA	AA	*P*-value
Gender, male/female, n (%)	43/35 (55.1/44.9)	23/19 (54.8/45.2)	17/14 (54.8/45.2)	3/2 (60/40)	0.975
Age (years)	30.1 (1.16)	29.3 (1.60)	30.6 (1.85)	34.0 (4.77)	0.560
Disease duration (months)	8.66 (1.88)	10.7 (3.30)	6.84 (1.62)	3.0 (1.04)	0.503
Family history, n (%)	15 (19.2)	9 (21.4)	5 (16.1)	1 (20)	0.850
Nail dystrophy, n (%)	12 (15.4)	7 /16.7)	4 (12.9)	1 (20)	0.869
Alopecia severity					
<25%	62 (79.5)	33 (78.6)	24 (77.4)	5 (100)	0.498
25-50%	16 (20.5)	9 (21.4)	7 (22.6)	0 (0)	
Alopecia localization					
Scalp	52 (66.7)	27 (64.3)	22 (71)	3 (60)	0.778
Beard/Mustache	13 (16.7)	9 (21.4)	3 (9.7)	1 (20)	
Hair/Beard	12 (15.4)	5 (11.9)	6 (19.4)	1 (20)	
Body	1 (1.3)	1 (2.4)	0 (0)	0 (0)	
Other autoimmune disease	4 (5.1)	0 (0)	4 (12.9)	0 (0)	0.046*

Data were analyzed by analysis of variance and $\chi 2$ test. Mean plus standard error of the mean values are presented for age, disease duration. AA: alopecia areata, TNFα: tumor necrosis factor alpha, * *P*-value ≤0.05 is significant.

Table 2: Allele/Genotype frequencies and test of Hardy-Weinberg (HW) equilibrium

	Controls		Alopecia Areata	
	O	E	O	E
GG	46	34.6	42	42.3
GA	12	34.6	31	30.2
AA	20	8.6	5	5.3
	χ^2=33.3, df=2, *P*<0.001		χ^2=0.05, df=2, *P*=0.820	
f(G)	0.6667		0.7372	
f(A)	0.3333		0.2628	

f: observed frequency of each allele (G or A), O: observed genotype numbers, E: expected genotype numbers under a Hardy-Weinberg (HW) equilibrium assumption, $\chi 2$: Chi-square values, *P:* probability of difference

Table 3: Representation of genotype and allele frequencies of TNFα -238 G/A polymorphisms for patients and control groups

TNFα -238	AA patients n = 78 (%)	Controls n = 78 (%)	OR (95%CI)	RR (95% CI)	*P*-value
Genotypes					
GG	42 (53.8)	46 (59)	1	1	<0.001
GA	31 (39.7)	12 (15.4)	2.82 (1.28 - 6.21)	1.87 (1.11 - 3.15)	0.008
AA	5 (6.4)	20 (25.6)	0.27 (0.09 - 0.79)	0.65 (0.49 - 0.86)	0.013
	$\chi 2$=17.5, df=2, *P*<0.001				
G	115 (73.7)	104 (66.7)	-	-	
A	41 (26.3)	52 (33.3)	0.71 (0.43 - 1.16)	0.84 (0.67 - 1.06)	0.173
	$\chi 2$=1.85, df=1, *P*=0.173				

TNFα: tumor necrosis factor alpha, AA: alopecia areata, OR: odds ratio, 95% CI: 95% confidence interval, RR: relative risk. The statistically significant results are shown in bold.

The groups were similar in terms of allele frequency ($\chi 2$=1.85, df=1, *P*=0.173). G allele frequencies were found in 115 (73.7%) cases and 104 (66.7%) controls. Similarly, A allele frequencies were observed in 41 (26.3%) and 52 (33.3%) in cases and controls, respectively (OR=0.71, 95% CI=0.43-1.16; RR= 0.84 (0.67-1.06); *P*=0.173) (Table 3).

Discussion

In our study, the data of 156 individuals, including 78 AA patients and 78 control subjects, were collected from the dermatology outpatient clinic, Kutahya Health Sciences University, Turkey. We analyzed the function of the TNFα -238 G/A polymorphism in the development of patchy AA in the Turkish population and found a significant link between TNFα -238 G/A gene polymorphism and AA patients.

As far as we know, this study is the first to report the relationship between TNFα -238 G/A gene polymorphism and AA in the Turkish population. Therefore, it is not possible to compare the results of this study with others. Contrarily, Tan et al. [10] reported no association of alopecia areata with the polymorphisms of TNFα -238 G/A and TNFα -308 G/A. However, the relationship of TNFα -238 G/A gene polymorphism with other patient groups in other ethnic populations have also been reported. In the study performed by Aisha et al. [6], the authors found that the AA genotypes of TNFα -238 were related to an elevated risk of acne vulgaris. Rajesh et al. [7] reported that the TNFα -238 AA genotype was detected only in patients with moderate to severe psoriasis vulgaris. Another study suggested that TNFα -238 AA genotype significantly increased, while the GG genotype decreased in psoriasis, compared to the control group [11]. In various studies, the relationship between TNFα -238 G/A polymorphism and gastric disease risk were studied. In a study in the Chinese population, there was no significant relationship between the TNFα -238 GA genotype and gastric cancer, while another study reported that the TNFα -238 GA polymorphism is significantly related to the increased gastric cancer risk in Asians [12, 13]. It has been reported that TNFα -238 GA genotype was related to swollen joint count <5 as compared to the TNFα -238 GG genotype [14]. A recent study by Brinkman et al. [15] investigated TNFα -238 GA genotype and found it was associated with decreased radiologically detectable disease course. In another study, they reported that the TNFα -238 GG genotype was related to severe and unresponsive forms of rheumatoid arthritis in the Iranian population [16]. In our study, there was no significant difference in the allele frequencies of TNFα -238 G/A between AA patients and the control group. Szabó et al. [17] reported that there was no significant difference in genotype or allele frequencies between acne and control group in terms of TNFα -238 G/A gene polymorphism. Wang et al. [18] showed that the TNFα -238 A allele is related to elevated susceptibility to acne in Asians but not in European populations. Schmeling et al. [19] found that the TNFα -238 A allele was more prevalent in the psoriatic arthritis and juvenile idiopathic arthritis subgroup than the control group as well as in the non-psoriatic juvenile idiopathic arthritis patients. Aisha et al. [6] reported that patients with acne vulgaris had significantly higher TNFα -238 mutant A allele than healthy controls. In another study, TNFα -238 A allele was reported to raise the risk of psoriasis vulgaris in the Indian population [7]. In a study in the Tunisian population, they reported that the TNFα -238 A allele was significantly higher in non-small cell lung cancer patients than in the controls and that TNFα -238 G/A may be associated with elevated susceptibility to lung cancer [20].

Further analysis was performed to investigate the association between clinical and demographic features and TNFα -238 G/A genotypes of patients. According to these analyses, there was no relationship between TNFα -238 G/A genotypes and sex, age, disease duration, family history, nail dystrophy, and severity of the disease. Interestingly, however, there was a relationship between AA patients with another autoimmune disease and TNFα -238 GA genotype [4 (12.9%); *P*=0.046]. This result shows that the relationship between TNFα -238 G/A gene polymorphism and autoimmune diseases have a critical role in the development of AA.

AA pathogenesis remains incompletely understood. The cytokines may have a role in AA. In *in vitro* studies, TNFα inhibited hair follicle growth, indicating that it may play a significant role in AA [21]. Gohary et al. [22] showed that skin

TNFα levels were elevated in AA patients, which may indicate a significant role of TNFα in AA patients. Abdel Halim et al. [23] showed that both serum and tissue levels of TNFα in patients with AA were significantly higher than in the control group before and after treatment.

Limitations

To the best of our knowledge, this is the first study to investigate the relationship between TNFα -238 G/A and AA risk in the Turkish population. However, there were several limitations in our study: First, all subjects were Turkish, therefore extrapolation of our results to other ethnic groups is not possible. Second, although we did not have a restriction on the severity of the disease in our study, the participants were mild to moderate AA patients. This may stem from the limited number of patients and rarity of severe disease, such that alopecia totalis or alopecia universalis occur only in 2% of all AA cases [1]. Third, since serum TNFα levels of the participants were not measured, the relationship between TNFα -238 G/A gene polymorphism and serum TNFα level could not be demonstrated.

Conclusion

The findings of our present study proved an association between TNFα -238 G/A gene polymorphism and the susceptibility to patchy AA in the Turkish population. Our results show that TNFα -238 G/A gene polymorphism might have a role in patchy AA and the GA genotype may confer an increased risk of disease while the AA genotype has a lower risk for patchy AA in the Turkish population. It is not obvious if the relationship with TNFα -238 G/A gene polymorphism is dependent on the disease type. Hence, the data is not precise. Larger-scale studies on different ethnic groups, including other Alopecia clinical forms, are needed to further clarify their role in disease development. As AA is a multifactorial disease, their interaction with disease triggering factors, environmental factors, and other genetic studies, including gene-gene and gene-environment interactions, are needed for more clarification of disease pathogenesis.

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