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Analysis of the Monocyte Chemoattractant Protein 1 −2518 Promoter Polymorphism in Korean Patients with Alopecia Areata

Monocyte chemoattractant protein-1 (MCP-1) levels are increased in scalp lesions of patients with alopecia areata (AA), suggesting a role in the development of AA. Recently, a biallelic A/G polymorphism in the MCP-1 promoter at position −2518 has been found, influencing the level of MCP-1 expression in response to an inflammatory stimulus. We investigated whether the presence of these polymorphisms were associated with AA in Korean population. 145 Korean patients with AA, 246 healthy subjects without clinical evidence of AA were screened for genotype with a PCR-based assay. In the AA patients the frequency of the A and G alleles was 40.3 and 59.7%, respectively and the distribution of the A/A, A/G and G/G genotypes was 19.3, 42.1 and 38.6%, respectively. Amongst the controls the frequency of the A and G alleles was 39.8 and 60.2%, and the distribution of the A/A, A/G, G/G genotypes in the same group was 17.5, 44.7 and 37.8%, respectively. There was no significant difference in the allele frequencies and genotype distributions between the patients and the controls (p=0.889, p=0.848, respectively). Our data indicates that no association exists between the −2518A/G polymorphism of the *MCP-1* gene and susceptibility to alopecia areata.

Key Words : *Alopecia Areata; Chemokines; MCP 1 Protein, human; Polymorphism, Genetic*

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INTRODUCTION

Alopecia areata (AA) is a disfiguring disease characterized by nonscarring hair loss. It is a chronic inflammatory disorder affecting both the hair follicles and the nail apparatus, with T-lymphocyte infiltration and chemokine expression being characteristic in involved tissue (1). The prevalence rates of AA is 0.1% in the world population (2) and 0.9-6.9% in Korea (3). The association of AA with autoimmune diseases including thyroid disorders, pernicious anemia and vitiligo is well established and alopecia areata itself is conventionally regarded as an autoimmune disease (4). The importance of immune-mediated mechanism, but non necessarily autoimmunity, in the pathogenesis of alopecia areata is suggested by the therapeutic efficacy of immunosuppressive drugs such as cyclosporin A, tacrolimus and corticosteroids.

It is well known that locally infiltrating lymphocytes have been implicated as effector cells being responsible for the reversible alteration of the hair follicles and the resulting hair loss (5, 6). In general, the directional recruitment of leukocytes is regulated by chemokines and their counteracting chemokine receptors (7). Recently, in human models of AA it was shown that the increased expression of chemokines including monocyte chemoattractant protein-1 (MCP-1), monokine induced by interferon-γ (MIG) might play a pivotal role in the attraction of monocytes around the hair bulb (1).

MCP-1 is a β-chemokine that is thought to be responsible for monocyte and lymphocytes T recruitment in acute inflammatory conditions and may be an important mediator in chronic inflammation. In fact, it has been proposed that MCP-1 may be responsible for tissue inflammation in autoimmune diseases because its tissue expression in human and experimental autoimmune models (8-11). Thus, genetic polymorphism in the regulatory regions of the *MCP-1* gene could be implicated in the susceptibility or progression of autoimmune disease. A biallelic A/G polymorphism at position −2518 of the *MCP-1* gene has been described. The polymorphism proved functionally important, as peripheral blood mononuclear cells of individuals with G/G and A/G genotype produced significantly more MCP-1 after stimulation with IL-1β than those with Caucasian wild-type A/A (12, 13). An association of the presence of G at position −2518 with the presence of cutaneous vasculitis could be shown in patients with systemic lupus erythematosus (14) as well as an association with development of coronary artery aneurysms after acute Kawasaki disease (13). Furthermore, the G/G genotype was identified as a genetic risk factor for severe coronary artery disease (15) and a correlation between the incidence

and severity of asthma and the G allele at position –2518 has been shown. These findings suggest an important role for the MCP-1 and the A/G polymorphism in its regulatory region in inflammatory processes.

The *MCP-1* gene polymorphism in AA patients has not been reported yet. In this study, we performed a case-control study in 145 alopecia areata patients and 246 matched Korean controls to analyze the possible influence of the polymorphism (A/G) at position –2518 of the *MCP-1* gene in the susceptibility of AA.

MATERIALS AND METHODS

Patients and controls

A total of 145 Korean patients with AA (70 men and 75 women) were examined at the Dermatology Clinic at the Kyung Hee University Medical Center. A total of 246 healthy subjects (80 men and 166 women), who had no clinical evidence of AA, were recruited as controls (Table 1). This study was approved by the ethics review committee of the Medical Research Institute, Kyung Hee University Medical Center.

The clinical diagnosis of AA was based on the presence of initially patchy alopecia with exclamation mark hairs and the exclusion of other causes of alopecia. Detailed clinical information was obtained from each patient, including age at onset of first episode, family history, type of disease (patchy alopecia, alopecia totalis [AT] and alopecia universalis [AU]), duration of disease, presence of other autoimmune disease, nail involvement, and other body sites hair loss. Clinical information was updated at follow-up examinations. We divided the AA patients into two subgroups according to disease severity. Subgroup 1 contained patients with patchy disease (patchy AA) and subgroup 2 patients with more extensive hair loss (AT and AU) (16). Patchy AA can involve the scalp or other body sites such as the beard area. In patchy AA (subgroup 1) there are well demarcated, mainly circular areas of hair loss typically 2-6 cm in diameter. In subgroup 2, hair loss progresses until all the hair on the scalp has been lost (AT); or to complete loss of the entire body hair (AU). We also divided the AA patients into two subgroups on the basis of age at onset of disease (17). The division of these subgroups with a boundary at age 30 yr was in keeping with the distribution of age at onset in our patients, which was bimodal with peaks at approximately age 20-30 and 30-40 yr.

Table 1. Clinical characteristics of the 145 Korean alopecia areata patients and healthy controls

	Alopecia areata (n=145)	Healthy controls (n=246)
Average age (yr, mean±SD)	28.90±13.22	51.0±12.60
No. of male/female	70/75	80/166
Onset age (yr, mean±SD)	27.43±13.82	
Subgroups according to disease severity (patchy AA/AT or AU)	(128/17)	
Subgroups according to age at onset (Onset age ≤30/ Onset age >30 yr)	(105/40)	

AA, alopecia areata; AT, alopecia totalis; AU, alopecia universalis.

MCP-1 genotyping

Genomic DNA was prepared from heparinized venous blood samples using a NucleoSpin DNA isolation kit (MACHE-REY-NAGEL GmbH & Co., Duren, Germany). The identification of the polymorphism was carried out using PCR, followed by a restriction fragment length polymorphism (RFLP) assay, using a *Pvu*II site, which is introduced by the presence of the G nucleotide. The regulatory region of the *MCP-1* gene (from –2746 at –1817) was amplified by polymerase chain-reaction (PCR) using the forward primer 5′CCGAGATGTTCCCAGCACAG3′ and the reverse primer 5′CTGCTTTGCTTGTGCCTCTT3′. PCR was performed using buffer 10× (10 mM Tris-HCl pH 9, 2.0 mM $MgCl_2$, 50 mM KCl), 200 μm dNTPs, 2.5 pmoles of each primer, 0.5 μL of DNA, 0.5 U *Taq* polymerase (Pharmacia) and ddH_2O up to a final volume of 10 μL. The following thermal profiles were run: 94°C for 1 min, 55°C for 1 min, and 72°C for 1.5 min. After a final extension of 10 min at 72°C, 7 μL of the PCR products were resolved in 2% agarose gels stained with ethidium bromide previous dilution in blue juice buffer to check the expected 930-bp band. After checking, 8 μL of the PCR products were digested with 10 U of *Pvu*II in 10× buffer and H_2O up to a final volume of 20 μL at 37°C for 2 hr 30 min.

The resulting products were separated by gel-electrophoresis in 1.5% agarose gels, containing ethidium bromide in a final concentration of 0.5 μg/mL. Samples showing only a 930 bp band were assigned as A/A, samples showing two bands of 708 and 222 bp were considered G/G and samples showing three bands at 930, 708 and 222 bp were typed A/G.

Statistical analysis

The distribution of the *MCP-1* A/G genotypes for patients and control subjects were compared by the chi-square (χ^2) test. p value less than 0.05 were considered as statistically significant.

RESULTS

In the case-control study, genotype distributions and allele frequencies of the MCP genetic marker in both AA and the control population was analyzed (Table 2). In the AA patients the frequency of the A and G alleles was 40.3 and 59.7%,

respectively and the distribution of the A/A, A/G and G/G genotypes was 19.3, 42.1 and 38.6%, respectively. Amongst the controls the frequency of the A and G alleles was 39.8 and 60.2%, and the distribution of the A/A, A/G, G/G genotypes in the same group was 17.5, 44.7 and 37.8%, respectively. The observed genotype frequencies of the AA patient (p=0.129) and the control population (p=0.574) did not show significant difference predicted by the Hardy-Weinberg equation. When the observed control and patient genotype frequencies were compared with expected values using 3×2 contingency table in a standard chi-square test (Table 2), there was no significant difference (p=0.848). Also, there was no significant difference in the allele frequencies between the patients and the controls (p=0.889).

We then divided the patients into two subgroups (subgroup 1 [patchy AA] and subgroup 2 [alopecia totalis and alopecia universalis]) according to disease severity and also into two further subgroups on age at onset of disease (subgroup a [onset age ≤30] and subgroup b [onset age >30]). Comparison of the genotype distributions and the allele frequencies between each subgroup and the control population revealed no significant difference (Table 3A, B).

DISCUSSION

Although MCP-1 has been proposed as the main chemokine responsible for initiating autoimmune tissue damage (8, 11, 18, 19), the association of polymorphisms in the regulatory regions of the *MCP-1* gene with alopecia areata has not been studied until now. Recently, in human models of AA it was shown that the increased expression of MCP-1 might play a pivotal role in the attraction of monocytes around the hair bulb (1). In addition, the *MCP-1* –2518G allele was found to increase the MCP-1 expression (12). Therefore, we initially hypothesized that the development of AA might have been related to G allele in the *MCP-1* gene polymorphism in AA patients. The results of this study, however, did not support this assumption. In this case-control study, the genotype distribution and allelic frequencies showed no significant

Table 2. Distribution of alleles and genotypes for the A/G SNP in *MCP-1* in alopecia areata and control population

	Observed allele frequency (%)			Observed genotype counts (%)			Expected genotype count*			*p*-value
	No.	A	G	A/A	A/G	G/G	A/A	A/G	G/G	
Control	492	196 (39.8%)	296 (60.2%)	43 (17.5%)	110 (44.7%)	93 (37.8%)	39	117	89	0.574*
Patients	290	117 (40.3%)	173 (59.7%)	28 (19.3%)	61 (42.1%)	56 (38.6%)	23	69	51	0.129*
p-value		0.889†			0.848‡					
Odds ratio (95%CI)		0.979 (0.728-1.316)								

*Observed vs. expected according to the Hardy-Weinberg equation, †Controls vs. patients using the chi-square test with 2×2 contingency table, ‡Controls vs. patients using the chi-square test with 3×2 contingency table.

Table 3A. Comparison of genotype distributions between each subgroup and the control population

	Observed genotype counts (%)			*p*-value
	A/A	A/G	G/G	
Control	43 (17.5%)	110 (44.7%)	93 (37.8%)	
Patchy AA (subgroup 1)	1 (5.8%)	8 (47.1%)	8 (47.1%)	0.438*
AT/AU (subgroup 2)	27 (21.1%)	53 (41.4%)	48 (37.5%)	0.670*
Onset age ≤30 yr (subgroup a)	24 (22.9%)	41 (39.0%)	40 (38.1%)	0.435*
Onset age >30 yr (subgroup b)	4 (10.0%)	20 (50.0%)	16 (40.0%)	0.490*

*Controls vs. patients using the chi-square test with 3×2 contingency table.

Table 3B. Comparison of allele frequencies between each subgroup and the control population

	Observed allele frequencies (%)				*p*-value
	No.	A	G	Odds ratio (95% CI)	
Control	492	196 (39.8%)	296 (60.2%)		
Patchy AA (subgroup 1)	256	107 (41.8%)	149 (58.2%)	0.922 (0.678-1.253)	0.604*
AT/AU (subgroup 2)	34	10 (29.4%)	24 (70.6%)	1.589 (0.744-3.396)	0.228*
Onset age ≤30 yr (subgroup a)	210	89 (42.4%)	121 (57.6%)	0.900 (0.649-1.250)	0.530*
Onset age >30 yr (subgroup b)	80	28 (35.0%)	52 (65.0%)	1.230 (0.751-2.015)	0.411*

*Controls vs. patients using the chi-square test with 2×2 contingency table.

difference between the patients population and the controls (p=0.848, p=0.889, respectively).

There are several potential reasons why there was no association between the –2518A/G polymorphism and alopecia areata. It is noteworthy that there are considerable differences in the frequencies of these polymorphisms among the different races. Indeed, it is well known that ethnicity and population structure may strongly influence the role of genetic risk factors in chronic inflammatory diseases including various autoimmune diseases. The distribution of several gene variations may be greatly different between various countries, as well as among different area in the same countries. The dominance of the G-type allele in Koreans, which were observed in this present study, is contrasting to what has been reported from Caucasians and AfroAmericans, where the proportion of G allele was 29 and 22%, respectively (12). Such reversed ratio was also observed in Japanese (13), suggesting that the genotypic profile of *MCP-1* –2518 polymorphism varies greatly among ethnic groups. And, the –2518A/G polymorphism may not be associated with alopecia areata. Previously, it was shown that allelic frequency of –2518 polymorphism among patients with other autoimmune diseases with increased serum MCP-1 level, such as rheumatoid arthritis, systemic lupus erythematosus and adult-onset Still's disease, is also similar to that of normal controls (20). In addition, in study about the chemokine expression pattern of AA, Benoit et al. have demonstrated that MCP-1 expression does not appear to be a particular feature of AA due to showing MCP-1 expression by keratinocytes of the inner root sheath but only weak expression around the hair follicles. And chemokine expression patterns did not appear to correlate with clinical severity such as extent of hair loss, number of AA episodes, or presence of associated diseases (1). These findings argue against a role of *MCP-1* –2518A/G polymorphism for susceptibility to alopecia areata.

In conclusion, this study found no association of –2518A/G polymorphism in the distal regulatory region of the *MCP-1* with alopecia areata susceptibility or severity in a Korean population. However, it is noteworthy that there are considerable differences in the frequencies of these polymorphisms among the different races. This variation may contribute to the pathogenesis of alopecia areata and must be considered in gene-association studies in different ethnic populations.

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