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Polymorphisms in the promoter regions of the *CXCL1* and *CXCL2* genes contribute to increased risk of alopecia areata in the Korean population

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ABSTRACT. Alopecia areata (AA) is a common disease, which causes hair loss in humans. AA has a genetically complex inheritance. This study investigated the possible correlations between single nucleotide polymorphisms (SNPs) in the promoter regions of the chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha) (*CXCL1*) and chemokine (C-X-C motif) ligand 2 (*CXCL2*) genes and the development of AA in the Korean population. Two hundred and thirty-five AA patients and 240 control subjects were recruited. The specific SNPs occurring in the promoter regions of the *CXCL1* and *CXCL2* genes (rs3117604, -429C/T and rs3806792, -264T/C, respectively) were

genotyped. All data obtained was evaluated using the SNPStats, SPSS 18.0, and the Haploview v.4.2 software platforms. The Odd's ratios (OR), 95% confidence intervals (CI), and P values were calculated using multiple logistic regression models. Analyses of the genetic sequences obtained revealed a significant correlation between the two SNPs and the development of AA (rs3117604, P = 0.0009 in co-dominant model 1, P = 0.01 in co-dominant model 2, P = 0.004 in the dominant model, P = 0.005 in the log-additive model, P = 0.012 in allele distribution; rs3806792, P = 0.036 in co-dominant model 2, P = 0.0046 in the log-additive model). The TT and CC haplotypes were also observed to show a significant association with increased risk of AA (TT haplotype, P = 0.0018; CC haplotype, P = 0.0349). Our data suggests that the *CXCL1* and *CXCL2* genes may be associated with AA susceptibility.

Key words: *CXCL1; CXCL2;* Polymorphism; Alopecia areata; Case-control study

INTRODUCTION

Alopecia areata (AA) is a complex, non-scarring hair loss disease that affects approximately 1-2% of the general population and 0.9-6.9% of the Korean population (Hong et al., 2006; Dudda-Subramanya et al., 2007). Despite considerable research efforts being focused on AA, its pathogenesis remains to be fully explained. Multiple genetic backgrounds and environmental factors have been suggested as likely causes of the abnormal regulation of immune system, which leads to the development of AA (Alexis et al., 2004; Dudda-Subramanya et al., 2007). AA is characterized by a follicular infiltrate comprised of CD4+ and CD8+ T lymphocytes, macrophages, and Langerhans cells (Todes-Taylor et al., 1984). Previous studies have reported a 42% concordance rate of AA in identical twins, and a 10% concordance rate in dizygotic twins. Rapid progress in molecular genetics over the past few decades has led to the identification of many candidate genes in humans that are associated AA susceptibility. Alzolibani et al. (2012) have suggested a correlation between the histocompatibility locus antigen (*HLA*) gene and the non-HLA genes (including cytokine, chemokine, and autoimmune regulatory genes, and other genes) and AA.

Chemokines are members of a subfamily of homologous (8-10 kDa) proteins. They are structurally related molecules that regulate the trafficking of various types of leukocytes through chemical interactions. Chemokines play important roles in the development, homeostasis, and function of the immune system, as well as angiogenesis (Zlotnik and Yoshie, 2000; Park et al., 2013). Chemokines are classified into four subfamilies, based on their primary amino acid sequences. Chemokines can be divided into two major sub-families, CXC and CC, based on the arrangement of their conserved cysteine residues (Wang et al., 1998). They are known to act on neutrophils, monocytes, lymphocytes, and eosinophils, and play an important role in host defense mechanisms. CXC chemokines are located in chromosome 4q12-13 (Wang et al., 1998). Chemokines play an important role in the recruitment and activation of leukocytes, balancing angiogenesis and angiostasis, and modulating the roles played by T-lymphocytes; therefore, these are essential for the pathogenesis of AA (Zainodini et al., 2013).

In this study, two promoter single nucleotide polymorphisms (SNPs) in the chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha) (*CXCL1*) and chemokine (C-X-C motif) ligand 2 (*CXCL2*) genes were investigated for their associations with AA in the Korean population.

MATERIAL AND METHODS

Patients and controls

AA patients were recruited from the Kyung Hee University Hospital at Gang-Dong (Kyung Hee East West Neo Medical Center). The demographic and clinical characteristics of the selected AA patients are summarized in Table 1. A diagnosis of AA was made by a certified dermatologist based on the results of an anemia study, a venereal disease research laboratory test, a test for the presence of anti-nuclear antibodies, thyroid function test, and the presence of androgenic hormones, including testosterone, estradiol, luteinizing hormone (LH), and follicle stimulating hormone (FSH). The familial history of each patient was recorded; this included information regarding the general health of the concerned individual, such as a previous occurrence of AA, the presence of triggering factors, presence or absence of autoimmune diseases (e.g., atopy), and a family history of AA (Kim et al., 2014).

Table 1. Demographic and clinical characteristics of alopecia areata (AA) patients and the control subjects.

	Alopecia areata	Control
No. of subjects	235	240
Male/female	107/128	105/135
Age (mean ± SD)	28.7 ± 13.4	32.3 ± 8.5
First onset age [N (%)]		
<30 years	150 (68.2)	
≥30 years	70 (31.8)	
Type [n(%)]		
Patch	197 (84.2)	
Totalis or universalis	37 (15.8)	
Involvement of nail [N (%)]		
Presence	34 (14.5)	
Absence	200 (85.5)	
Involvement of body hair [N (%)]		
Presence	34 (14.5)	
Absence	200 (85.5)	
Scalp hair loss [N (%)]		
<25%	142 (63.1)	
26-50%	33 (14.7)	
51-75%	7 (3.1)	
76-99%	10 (4.4)	
100%	33 (14.7)	

AA = alopecia areata; N = number of subjects; SD = standard deviation.

The control subjects were recruited from among individuals enrolled in a general health checkup program, who did not have any severe diseases or symptoms. Informed consent was obtained from each participating individual. This study was approved by the Institutional Review Board of the Kyung Hee University Hospital at Gang-Dong.

SNP selection and genotyping

The promoter SNPs occurring in the *CXCL1* and *CXCL2* genes were selected based on the results of an extensive search conducted using the SNP database in the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/SNP, BUILD 137) website.

Genomic DNAs were extracted from peripheral blood using the Roche DNA Extraction kit (Roche, Indianapolis, IN, USA), and SNP genotyping was performed by direct sequencing. The sequenced samples were subjected to a polymerase chain reaction (PCR) using primers specific for the two promoter SNPs of interest: rs3117604 (-429C/T); sense primer, 5'-ACATTCTTCTCTGGAATCTGA-3'; anti-sense primer, 5'- CAGCTCCTTCTCCGTTCCCAG-3'; rs3806792 (-264T/C); sense primer, 5'-AAACACCAGGAAGGAGACAAAA-3', anti-sense primer, 5'-CCAACTGTGGGATGTTCTCTTT-3'. The PCR products were sequenced using an ABI PRISM 3730XL analyzer (Applied Biosystems, Foster City, CA, USA). The obtained sequencing data was analyzed using the SeqManII software (DNASTAR, Madison, WI, USA).

Statistical analysis

The SNPStats (http://bioinfo.iconcologia.net/index.php) and SPSS 18.0 (SPSS Inc., Chicago, IL, USA) software programs were used to analyze all genetic data obtained. The Hardy-Weinberg equilibrium (HWE) was calculated in the control group using the SNPStats software. Linkage disequilibrium (LD) block and haplotypes were calculated using the Haploview v.4.2 software platform (Daly Lab Inc., Cambridge, MA, USA). Multiple logistic regression models were created based on the genetic models described in a previous study (Lewis, 2002), and were used to obtain the Odds ratios (OR), 95% confidence intervals (CIs), and P values. P values < 0.05 were considered to be statistically significant. Bonferroni's correction was applied to the multiple test.

RESULTS

Two hundred and thirty five AA patients and 240 healthy control subjects were analyzed to identify any possible correlations between promoter polymorphisms in the *CXCL1* and *CXCL2* genes and AA. The genotype frequencies of the two promoter SNPs in the control group demonstrated Hardy-Weinberg equilibrium (rs3117604, P = 0.09 and rs3806792, P = 0.08, data not shown). Table 2 displays the genotypic and allelic distributions of two promoter SNPs in the AA and control groups. The data obtained genetic for the AA and control subjects was subjected to a logistic regression analysis, after adjusting for the age and gender. The expression of the SNP rs3117604 (-429C/T) in the *CXCL1* genes was significantly different between the AA and control groups [co-dominant model 1 (C/C versus C/T), OR = 0.44, 95%CI = 0.28-0.69, P = 0.0005; co-dominant model 2 (C/C versus T/T), OR = 0.47, 95%CI = 0.27-0.80 P = 0.005; dominant model (C/C and C/T versus T/T), OR = 0.45, 95%CI = 0.29-0.68, P = 0.0002; and the log-additive model (C/C versus C/T versus T/T), OR = 0.67, 95%CI = 0.51-0.87, P = 0.0025]. Allele distribution analysis revealed a correlation between the allele of the rs3117604 SNP and AA (OR = 0.67, 95%CI = 0.54-0.90, P = 0.006) (Table 2). In addi-

tion, a significant correlation was observed between the rs3806792 SNP (-264T/C) and the AA and control groups [co-dominant model 2 (T/T versus C/C), OR = 2.11, 95%CI = 1.14-3.92, P = 0.018; recessive model (T/T versus T/C and C/C), OR = 1.87, 95%CI = 1.05-3.34, P = 0.03; and the log-additive model (T/T versus T/C versus C/C), OR = 1.38, 95%CI = 1.04-1.83, P = 0.046] (Table 2). The allele of the rs3806792 SNP was also associated with AA (OR = 1.32, 95%CI = 1.01-1.72, P = 0.039). These significant associations remained even after the application of Bonferroni's correction (rs3117604, P = 0.0009 in co-dominant model 1; P = 0.01 in co-dominant model 2; P = 0.0004 in dominant model; P = 0.005 in log-additive model; allele analysis, P = 0.012; rs3806792, P = 0.036 in co-dominant model 2; and P = 0.046 in the log-additive model) (Table 2).

Table 2. Frequency of occurrence of the genotype and specific alleles of the single nucleotide polymorphisms (SNPs) occuring in the promoter regions of the *CXCL1* and *CXCL2* genes in the control subjects and patients with alopecia areata (AA).

SNP	Type	Control N (%)	AA N (%)	Model	OR (95%CI)	P	Bonferroni's correction P*
rs3117604	C/C	50 (20.9)	83 (35.3)	Codominant1	0.44 (0.28-0.69)	**0.0005**	**0.00094**
CXCL1	C/T	133 (55.6)	105 (44.7)	Codominant2	0.47 (0.27-0.80)	**0.005**	**0.01**
promoter	T/T	56 (23.4)	47 (20.0)	Dominant	0.45 (0.29-0.68)	**0.0002**	**0.0004**
-429C/T				Recessive	0.79 (0.51-1.24)	0.30	0.60
				Log-additive	0.67 (0.51-0.87)	**0.0025**	**0.005**
	C	233 (48.7)	271 (57.7)		1		
	T	245 (51.3)	199 (42.3)		0.67 (0.54-0.90)	**0.006**	**0.012**
rs3806792	T/T	99 (41.2)	82 (34.9)	Codominant1	1.23 (0.83-1.83)	0.27	0.54
CXCL2	T/C	120 (50.0)	117 (49.8)	Codominant2	2.11 (1.14-3.92)	**0.018**	**0.036**
promoter	C/C	21 (8.8)	36 (15.3)	Dominant	1.37 (0.94-2.00)	0.10	0.20
-264T/C				Recessive	1.87 (1.05-3.34)	**0.03**	0.06
				Log-additive	1.38 (1.04-1.83)	**0.023**	**0.046**
	T	318 (66.2)	281 (59.8)		1		
	C	162 (33.8)	189 (40.2)		1.32 (1.01-1.72)	**0.039**	0.08

AA = alopecia areata; SNP = singe nucleotide polymorphism; OR = odds ratio; CI = confidence interval; N = number of subjects. P values were calculated using logistic regression analyses, adjusting for the gender and age. Numbers in bold font indicate significant associations. *P values were calculated using Bonferroni's correction.

The two SNPs (rs3117604 in *CXCL1* and rs3806792 in *CXCL2*) were analyzed using the Haploview v.4.2 software platform. Four haplotypes were observed in the LD block (haplotype TT, frequency = 0.334; CT, frequency = 0.297; CC, frequency = 0.235; TC, frequency = 0.135) (Table 3). The distributions of these haplotypes (TT and CC) were associated with the development of AA (haplotype TT, chi square = 9.78, P = 0.0018; haplotype CC, chi square = 4.452, P = 0.0349) (Table 3).

Table 3. Frequencies of haplotypes in alopecia areata (AA) and control subjects.

Haplotype	Frequency	Control		AA		Chi square	P value
		+	-	+	-		
TT	0.334	182.9	297.1	134.8	337.2	9.78	**0.0018**
CT	0.297	135.1	344.9	147.2	324.8	1.065	0.3022
CC	0.235	99	381	124.8	347.2	4.452	**0.0349**
TC	0.135	63	417	65.2	406.8	0.101	0.7503

Haplotypes of the rs3117604 and rs3806792 single nucleotide polymorphisms. Numbers in bold fontindicate significant correlations.

Although the relationship between the SNPs occurring in the promoter regions of the *CXCL1* and *CXCL2* genes and the clinico-pathological features of AA (age at onset, family history, type of AA, involvement of nail, and involvement of body hair) were analyzed, no significant correlations were observed between the promoter SNPs of the *CXCL1* and the *CXCL2* genes ($P > 0.05$, data not shown).

DISCUSSION

The cause of AA has been broadly explained as a result of the numerous attempts conducted to evaluate the genetic factors of AA; this has resulted in the identification of an increasing number of genetic risk factors related to AA. Several HLA genes and putative susceptibility loci have been associated with an increased risk of AA (Entz et al., 2006; Martinez-Mir et al., 2007; Petukhova et al., 2010).

Previous studies have reported an association between the SNPs occurring in chemokine genes and autoimmune diseases, including systemic lupus erythematosus (SLE) (Brown et al., 2007; Im et al., 2014), Sjögren syndrome (Kahlmann et al., 2007), Kawasaki disease (KD) (Breunis et al., 2007; Jhang et al., 2009), and rheumatoid arthritis (RA) (Zapico et al., 2000; Teng et al., 2012). Brown et al. (2007) revealed that the G allele of the -2518A/G SNP in the *CCL2* gene significantly increased the risk of SLE among Caucasians, but not among African Americans ($P < 0.0001$). Jhang et al. (2009) reported a significant difference between the allele frequencies of the *CCR5* gene (-2135C/T) polymorphism in children with congenital heart disease and those with KD (-2135C/T, 16.75% versus 30.05%, OR = 2.14, 95%CI = 1.31-3.51). Teng et al. (2012) also identified the *CCR6* gene polymorphism (rs3093024) to be a risk factor of RA among females, but a protective factor among males. A number of studies have identified correlations between SNPs in chemokines and autoimmune diseases; however, few studies have investigated the SNPs occurring in chemokines in individuals suffering from AA. This is the first study detailing the association between the polymorphisms in the promoter region of the *CXCL1* and *CXCL2* genes and AA, in the Korean population. The two tested promoter SNPs (rs3117604; -429C/T in the *CXCL1* gene and rs3806792; -264 T/C in the *CXCL2* gene) were observed to be associated with susceptibility to AA. A statistically significant difference in genotype distributions was observed between the AA patients and normal subjects. The frequency of occurrence of the T allele of the rs3117604 SNP and the C allele of the rs3806792 SNP was significantly different between the AA patients and normal subjects ($P < 0.05$). The T allele of the rs3117604 SNP and the C allele of the rs3806792 SNP are both protective and risk factors for AA.

The CXC chemokines are generally divided into two subgroups, based on the presence or absence of a conserved amino acid sequence. CXCL1, the representative member of ELR-positive CXC chemokines displays angiogenic properties, whereas CXCL9 and CXCL10, the representative members of the ELR-negative CXC chemokines display anti-angiogenic (or angiogenesis inhibiting) effects (Zainodini et al., 2013). ELR-positive chemokines are known to act on neutrophils. However, ELR-negative chemokines serve as T lymphocyte recruiters, and their activation is a common event in autoimmune disorders (Strieter et al., 2004; Zainodini et al., 2013). Zainodini et al. (2013) showed a significant decrease in the serum CXCL1 levels in AA patients, compared to those in the control ($P < 0.001$).

In summary, this study suggests that two promoter SNPs (s3117604 in the *CXCL1*

gene and rs3806792 in the *CXCL2* gene) may function as risk factors for the development of AA in the Korean population. This result indicates that the promoter SNPs in the *CXCL1* and *CXCL2* genes may affect the development of AA. Our results must be further confirmed by conducting additional studies with larger cases or multiple populations.

Conflicts of interest

The authors declare no conflict of interest.

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