https://doi.org/10.5021/ad.2019.31.4.489

Association between *EGF* and *EGFR* Gene Polymorphisms and Susceptibility to Alopecia Areata in the Korean Population

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Dear Editor:

The roles of epidermal growth factor (EGF) and epidermal growth factor receptor (EGFR) in the pathogenesis of alopecia areata (AA) are unknown. However, several reports have suggested an association between EGF signaling and AA. In mice, EGF blocked hair follicle induction by down-regulation of signaling pathways such as Wnt, Sonic hedgehog, and bone morphogenetic protein pathways[1]. In human hair follicle culture, EGF and EGFR showed a capacity for inhibiting hair shaft elongation and changing the morphology to catagen growth pattern by suppressing mitotic regulators including RCC2 and Stathmin1[2,3]. A previous study reported that the use of EGFR inhibitors can cause skin inflammation and exacerbation of autoimmune diseases, and that these immune-related effects of EGFR inhibitors are due to their direct effects on the expression of the major histocompatibility complex class I and/or class II molecules[4].

The role of single nucleotide polymorphisms (SNPs) of *EGF* and *EGFR* on the pathogenesis of AA has not yet been studied; however, our study suggested that *EGF* and *EGFR* could be associated with the pathogenesis of AA. The study aimed to determine whether variations in *EGF* and *EGFR* contribute to risk of AA in Korean population. Therefore, we investigated the significance of EGF and EGFR gene polymorphisms in the susceptibility to AA and to understand the pathogenesis of AA.

The study included patients who had AA and healthy control subjects and visited Kyung Hee University Hospital at Gangdong. The controls were recruited after they had been determined to be mentally and physically healthy in a general health check-up program. In this study, 231 patients with AA (105 males and 126 females, average age: 28.6±13.5 years) (Table 1) and 270 healthy controls (144 males and 126 females, average age: 35.7±11.8 years) were included. Informed consent was obtained from each subject, and the study was approved by the Institutional Review Board of Kyung Hee University Hospital at Gangdong (KHNMC IRB 2008-022). Genomic DNA was iso-

Table 1. Clinical characteristics of study groups

Characteristic	Alopecia areata	Control
Patient number	231	270
Male/female	105/126	144/126
Age (yr)	28.6±13.5 (3~70)	35.7±11.8 (11~48)
Age of onset		
<30 years	162	
≥30 years	69	
Family history		
(+)	18	
(−)	213	
Type		
Patch	197	
Totalis or universalis	34	

Values are presented as number only or mean±standard deviation (range).

Received May 9, 2018, Revised March 16, 2019, Accepted for publication March 23, 2019

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lated from peripheral blood using a genomic DNA isolation reagent kit (Core BioSystem, Seoul, Korea).

Five SNPs (rs11568835 [promoter], rs11568943 [exon], rs2237051 [exon], rs11569017 [exon], and rs3756261 [promoter]) for *EGF* and another five SNPs (rs17337023 [exon], rs1140475 [exon], rs2293347 [exon], rs1050171 [exon], and rs6965469 [promoter]) for *EGFR* with a heterozygosity greater than 0.3 among SNPs located in the promoter or exon (http://www.ncbi.nlm.nih.gov/SNP) were selected. All 10 selected SNPs were included using Hardy–Weinberg Equilibrium test (HWE, $p>0.05$). The genotypes were determined by direct sequencing. The samples were sequenced using an ABI Prism 3730XL Analyzer (PE Applied Biosystems, Foster City, CA, USA). Sequence data were analyzed using the SeqManII software (DNASTAR Inc., Madison, WI, USA).

The HWE for the two SNPs was assessed using SNPStats (http://bioinfo.iconcologia.net/index.php)[5]. Multiple logistic regression models with three alternative models (co-dominant, dominant, and recessive)[6] were calculated for the odds ratio, 95% confidence interval, and corresponding *p*-values, with control for gender as a covariable. SNPStats, HelixTree software (Golden Helix Inc., Bozeman, MT, USA), and SNPAnalyzer (ISTECH Inc., Goyang, Korea) were used.

Baseline characteristics of patients and controls are summarized in Table 1. Among five SNPs, one SNP (rs11569017) of *EGF* showed significant difference between the AA group and control group (Table 2). One SNP (rs6965469) of *EGFR* showed a significant difference between the AA group and control group (Table 2). There were no significant differences in expression of any of the SNPs of *EGF* and *EGFR* between early-onset AA and late-onset AA (data not shown). Moreover, none of the SNPs of *EGF* and

Table 2. Logistic analysis of *EGF*, *EGFR* polymorphisms in patients with alopecia areata, and in normal control subjects

Gene	SNP	Genotype	Case (*EGFR*, n=260; *EGF*, n=287)	Control (n=231)	Codominant		Dominant		Recessive	
					OR (95% CI)	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
EGFR	rs17337023				NA		NA		NA	
	rs1140475	C/C	230 (88.5)	203 (87.9)	0.89 (0.49~1.62)	0.15	0.98 (0.55~1.76)	0.96	NA (0.00~NA)	0.057
		C/T	30 (11.5)	25 (10.8)						
		T/T	0 (0.0)	2 (0.9)						
	rs2293347	A/A	26 (10.0)	27 (11.7)	0.96 (0.65~1.42)	0.87	0.99 (0.68~1.44)	0.96	1.15 (0.64~2.09)	0.63
		G/A	118 (45.4)	100 (43.3)						
		G/G	116 (44.6)	102 (44.2)						
	rs1050171	A/A	5 (1.9)	3 (1.3)	0.73 (0.45~1.17)	0.33	0.71 (0.45~1.13)	0.15	0.60 (0.13~2.70)	0.5
		G/A	57 (21.9)	39 (16.9)						
		G/G	198 (76.2)	187 (81.0)						
	rs6965469	C/C	158 (60.8)	157 (68.0)	0.77 (0.52~1.14)	0.048*	0.72 (0.49~1.07)	0.11	0.15 (0.02~1.25)	0.037*
		T/C	95 (36.5)	71 (30.7)						
		T/T	6 (2.3)	1 (0.4)						
EGF	rs11568835	A/A	9 (3.1)	6 (2.6)	1.20 (0.80~1.79)	0.6	1.15 (0.78~1.71)	0.47	0.74 (0.24~2.33)	0.61
		G/A	84 (29.3)	71 (30.7)						
		G/G	194 (67.6)	153 (66.2)						
	rs11568943	A/A	9 (3.1)	5 (2.2)	0.76 (0.50~1.24)	0.4	0.76 (0.51~1.13)	0.18	0.76 (0.24~2.42)	0.64
		G/A	86 (30.0)	58 (25.1)						
		G/G	192 (66.9)	167 (72.3)						
	rs2237051	A/A	130 (45.3)	105 (45.5)	0.84 (0.57~1.24)	0.1	0.97 (0.67~1.40)	0.85	1.80 (1.00~3.26)	0.05
		A/G	131 (45.6)	93 (40.3)						
		G/G	26 (9.1)	32 (13.9)						
	rs11569017	A/A	183 (63.8)	166 (71.9)	0.63 (0.42~0.95)	0.0008*	0.69 (0.47~1.03)	0.068	NA (0.00~NA)	0.0025*
		A/T	103 (35.9)	59 (25.5)						
		T/T	0 (0.0)	5 (2.2)						
	rs3756261	A/A	187 (65.2)	165 (71.4)	0.72 (0.48~1.09)	0.29	0.73 (0.49~1.09)	0.12	0.93 (0.34~2.56)	0.89
		A/G	89 (31.0)	58 (25.1)						
		G/G	11 (3.8)	7 (3.0)						

Values are presented as number (%) or OR (95% CI). SNP: single nucleotide polymorphism, OR: odds ratio, CI: confidence interval, NA: not applicable. *$p<0.05$.

EGFR showed significant differences between patients with and without familial history (data not shown). There was no significant difference associated with any of the SNPs of *EGF*. On the contrary, one SNP (rs17337023) of *EGFR* showed significant differences between patchy-type AA and alopecia totalis (AT) (data not shown).

The present study is, to the best of our knowledge, the first to investigate a potential influence of the *EGF* and *EGFR* polymorphisms in patients with AA. There are amount of studies to association between *EGF* and *EGFR* gene polymorphism and internal malignancies. However there are only a few studies about the relationship between *EGF* and *EGFR* polymorphisms and autoimmune disease. One study from Taiwan reported the association between *EGFR* and rheumatoid arthritis (RA)[7]. In this study, 188 patients with RA and 128 controls were enrolled. The study revealed new information on *EGFR* polymorphisms (rs17337023) with regard to the association between susceptibility to development of RA and polymorphisms. The rs17337023 SNP was also found to be associated with systemic lupus erythematosus (SLE)[8], endometriosis, leiomyomas[9], and malignant oral keratinocytes[10], in previous studies. In our study, the rs17337023 SNP also showed significant differences between patchy-type AA and AT, suggesting that rs17337023 is an important SNP that is extensively involved in the phenotype of AA as well as in other autoimmune diseases.

Polymorphism of *EGF* may cause compensatory expression of EGF. The elevation of EGF inhibits the induction of anagen phase and decreases hair shaft elongation. The polymorphism of *EGFR* may cause functional deceleration of protein-like EGFR inhibitor and thus, modulate the expression of immune molecules. In this manner, the SNPs of *EGF* and *EGFR* polymorphisms may be correlated with the pathogenesis of AA.

To our knowledge, this is the first study to demonstrate that *EGF* and *EGFR* polymorphisms are involved in the pathophysiology of AA or AA phenotypes. The genotype frequency of rs11569017 in *EGF* and rs6965469 in *EGFR* was significantly increased in patients with AA compared with the corresponding frequencies in healthy controls. In addition, rs17337023 polymorphism of *EGFR* may contribute to the clinical type of AA (patchy type or AT). Previous studies on RA and SLE have reported no significant clinical features according to different genotypes[7,8].

In conclusion, *EGF* and *EGFR* polymorphisms may contribute to the increased susceptibility to AA, and may be associated with the phenotype of AA in the Korean population. In particular, the rs17337023 SNP of *EGFR* is a notable SNP that is involved in the phenotype of AA as well as in other autoimmune diseases.

ACKNOWLEDGMENT

This work was supported by the National Research Foundation of Korea(NRF) grant funded by the Korea government(MSIT)(No. 2017R1C1B5017740).

CONFLICTS OF INTEREST

The authors have nothing to disclose.

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https://doi.org/10.5021/ad.2019.31.4.492

Comments on "Cutaneous Abscess as a Complication of Bisphosphonate-Related Osteonecrosis of the Jaw" by Yang et al.

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Dear Editor:

Yang et al.[1] recently reported an interesting case entitled, "Cutaneous Abscess as a Complication of Bisphosphonate-Related Osteonecrosis of the Jaw".

Bisphosphonate-related osteonecrosis of the jaw (BRONJ) represents a rare complication of bisphosphonate treatment. This condition is characterized by necrosis of the maxilla and mandible[2]. *Actinomyces* species (spp.) are gram-positive, non-acid fast, filamentous bacteria and mostly are facultative anaerobe. These species are commensals of the mucosa of mouth, colon and vagina. The key step in pathogenesis of actinomycosis is mucosal disruption by trauma, surgical procedures, or foreign bodies[3,4]. *Actinomyces* spp. are considered to be important agents involved in the pathogenesis of BRONJ. One of the clinical manifestations of actinomycosis is oral cervicofacial disease which can present as large abscess with or without mandibular osteomyelitis, ulcer or mass lesion. The diagnosing of cervicofacial actinomycosis is by histological examination and culture of abscess pus or suspected bone specimen, if osteomyelitis is considered. The microscopic study for visualization of gram positive, non-acid-fast, thin, branching filaments are helpful. Culture for isolation may take up to 2 to 4 weeks and this note should be considered. The demonstration of sulfur granules in pus or pathologic section of surgical specimens is also helpful and diagnostic. The treatment of choice is penicillin with or without surgical therapy especially for abscess drainage and resection of necrotic bone in cases of osteomyelitis and osteonecrosis. The agents that should be avoided for treatment are metronidazole, aminoglycosides, anti-staphylococcal penicillin such as cloxacillin and first generation of cephalosporin[5]. Hence; the diagnosis of cervicofacial actinomycosis should always be considered in any painless mass at jaw and also in the differential diagnosis of any lesion in neck and head for appropriate management.

Yang et al.[1] described bacterial culture of skin tissue revealed gram positive cocci (*Streptococcus constellatus*). Also they reported that fungal and mycobacterial cultures were both negative, but authors have not mentioned that study of the specimen in this case for detecting actinomyces had performed or not. The diagnosis of actino-

Received April 6, 2018, Revised August 4, 2018, Accepted for publication August 29, 2018

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