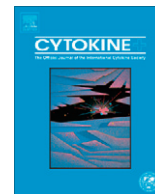




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Single nucleotide polymorphism in the FLT4 gene is associated with atopic dermatitis in Koreans

Jung-Hyun Namkung^{a,b,1}, Jong-Eun Lee^{c,1}, Eugene Kim^a, Ik-Soo Huh^d, Taesung Park^d, Eun-Soon Shin^c, Eun-Young Cho^c, Jun-Mo Yang^{a,*}

^a Department of Dermatology, Sungkyunkwan University School of Medicine, Samsung Medical Center, Seoul 135-710, Republic of Korea

^b Case Western Reserve University School of Medicine, Cleveland, OH, USA

^c DNA Link Inc., Seoul 120-110, Republic of Korea

^d Department of Statistics, Seoul National University, Seoul, Republic of Korea

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ABSTRACT

Background: The histology of atopic dermatitis includes dilated, tortuous vessels within the papillary dermis and perivascular edema.

Method: This study included 1120 case-control samples (646 AD patients and 474 normal controls), for which we genotyped 34 SNPs from four VEGF family genes and the FLT4 gene. For the rs11607007 SNP in the VEGFB gene and three SNPs (rs10085109, rs3736062, and rs11949194) in the FLT4 gene, which had significant *p*-values in the initial stage, were further investigated using 1132 independent samples (440 AD patients and 692 normal controls).

Result: Of the four SNPs, rs10085109 in the FLT4 gene was only significantly associated with the AD phenotype in both initial and replication samples. Although no SNPs in the VEGFA gene were significantly associated with AD, the rs2010963 SNP had a marginally significant effect on log-eosinophil counts.

Conclusion: The rs10085109 SNP in the FLT4 gene were associated with susceptibility to AD.

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1. Introduction

Atopic dermatitis (AD) is characterized by severe itching, a chronic relapsing clinical course, and typically distributed lesions with dry skin, excoriations, and lichenification. The histological findings of AD include dilated, tortuous vessels within the papillary dermis, as well as perivascular edema, collections of mononuclear cells, and rare neutrophils and eosinophils. The number of mast cells is either normal or elevated, depending on the stage of AD. However, histamine is released from mast cells in AD skin lesions, and histamine is believed to be a major vasoactive mediator in AD. Patients with AD often have elevated histamine concentrations in the plasma and skin [1,2], which may cause skin lesions by increasing vascular permeability, leading to erythema and an itching sensation. However, antihistamine therapy is less effective in the treatment of AD, and the amount of VEGFA is higher in the lesional scales of patients with AD [3], so it is important to determine the relationship between the VEGF gene family and AD. In human skin,

VEGF is expressed and secreted by keratinocytes, fibroblasts, smooth muscle cells, platelets, neutrophils, and macrophages. The role of dermal vasculature in AD pathogenesis is poorly understood; we hypothesized that VEGF and/or FLT4 affects the onset and development of AD symptoms.

Vascular endothelial growth factors (VEGFs) are potent stimulators of angiogenesis that affect endothelial cell proliferation, motility, and vascular permeability. They are crucial regulators of vascular development during embryogenesis, and they also regulate blood vessel formation in adults. VEGFs are secreted, dimeric glycoproteins of approximately 40 kDa. In mammals, the VEGF family consists of VEGFA, VEGFB, VEGFC, VEGFD, and placenta growth factor (PLGF) [4]. VEGFs have specific sites of expression and show distinct binding patterns to the different VEGF receptors (VEGFRs), which indicates complex biological signaling [2–6]. VEGFRs seem to be unique in their ability to transduce signals and to regulate vascular permeability. VEGFRs are also distinguished by an extracellular domain containing seven immunoglobulin (Ig) homology domains. To date, the VEGFR family is known to include VEGFR-1 (FLT 1), VEGFR-2 (KDR/FLK-1), and VEGFR-3 (FLT 4). VEGFR-1 is a positive regulator of monocyte and macrophage migration and a positive and negative regulator of VEGFR-2 signaling capacity. VEGFR-2 influences all aspects of normal and pathological vascular endothelial cell biology, and VEGFR 3 is important

* Corresponding author. Address: Department of Dermatology, Sungkyunkwan University School of Medicine, Samsung Medical Center, 50 Ilwon-Dong, Gangnam-Gu, Seoul 135-710, Republic of Korea. Tel.: +82 2 3410 3541; fax: +82 2 3410 3869.

E-mail address: jmyang@smc.samsung.co.kr (J.-M. Yang).

¹ The authors wish it to be known that, in their opinion, the first two authors should be regarded as joint First Authors.

for lymphatic-endothelial cell development and function. VEGFA, VEGFB, and PLGF bind to VEGFR-1; VEGFA binds to VEGFR-2; and VEGFC and VEGFD bind to FLT4 [4].

It has been reported that the mRNA expression of VEGFA is up-regulated in the lesional epidermis of patients with psoriasis or contact dermatitis [7,8]. VEGFB is the closest structural homolog of VEGFA. Because VEGFB competes for two of the VEGFA receptors (VEGFR-1 and neuropilin 1), and because it forms a stable heterodimeric complex with VEGFA [9], the main function of VEGFB may be to regulate the bioactivity of VEGFA.

We previously reported the association of single nucleotide polymorphism (SNP) and haplotype in the FLT4 and VEGFA genes in patients with psoriasis [10]. In the current study, we performed a similar experiment in patients with AD and normal controls to determine the association between these genes and the AD phenotype.

2. Methods and materials

2.1. Subjects

This study included 1120 case-control samples from 646 patients with AD and 474 normal control participants (NR) at the initial stage. The AD samples were collected from non-asthmatic atopic patients who were examined at Samsung Medical Center, Seoul, Korea. The diagnosis of AD, the criteria for the classification of AD into ADe and ADi, and the blood test and prick test list for allergens were the same as those in our previous report [11]. In addition, all patients with AD (M:F = 357:289, 13.58 ± 10.02 years) met the inclusion/exclusion criteria of our previous reports [12,13]. Among the patients with AD, 433 (M:F = 257:176, 15.70 ± 9.47 years) were classified as ADe and 213 (M:F = 100:113, 9.26 ± 9.74 years) were classified as ADi. The NR subjects included medical students and volunteers (M:F = 254:220, 23.23 ± 2.24 years) with no history of AD skin lesions. For the replication stage, 1132 samples were used. Normal control samples were obtained from a cohort where participants were recruited from a rural area in Korea [14]. Table 1 summarizes the demographic characteristics of the study participants.

This study was conducted in accordance with the principles of the Declaration of Helsinki, and written informed consent was obtained from all participants. The Samsung Medical Center Ethics Committee approved this study.

2.2. Marker selection

SNP information was retrieved from the dbSNP (build 119, <http://www.ncbi.nlm.nih.gov/SNP>). We selected 192 SNPs in the region between 5Kb upstream and 5kb downstream of five genes: VEGFA, VEGFB, VEGFC, FIGF, and FLT4. The selected SNPs were genotyped using 48 independent samples from the general Korean population (data not shown). On the basis of these genotype

results, we chose SNPs using the linkage disequilibrium (LD) bin approach in the Tagger program (<http://www.broad.mit.edu/mpg/tagger>). The LD bin approach defines the bins of SNPs that are in very strong linkage disequilibrium with a specified r^2 threshold; one SNP is then selected to represent the remaining SNPs in a bin [15]. We used an r^2 threshold of 0.8 and a minimum allele frequency of 0.1. A total of 34 SNPs from the five genes (6 in VEGFA, 3 in VEGFB, 3 in VEGFC, 2 in FIGF, and 20 in FLT4) were selected as the markers for our study (Fig. 1, and Table 1).

2.3. Genotyping with fluorescence polarization detection

We extracted genomic DNA from 5 ml of whole blood using a commercially available DNA isolation kit (Gentra Genomic DNA purification kit, Minneapolis, USA) in accordance with the manufacturer's protocol.

Genotype identification was performed with an ultra-high throughput system, the GenomeLab SNPstream system [16], which uses multiplexed PCR in conjunction with tag array single base extension genotyping technology (Beckman Coulter, Fullerton, CA, USA) and the SNPstream software suite [17]. Polymerase chain reaction (PCR) amplifications were performed in a PTC-225 Peltier Thermal Cycler (MJ Research, Waltham, MA) with Taq Gold DNA polymerase. Sequences of the PCR primers and extension primers are available upon request.

Multiplexed PCR and genotyping were performed in homogeneous reactions, and assay results were read by direct two-color fluorescence on the SNPstream Ultra-High Throughput Array Imager. Individual genotypes were generated on the basis of the relative fluorescent intensities for each SNP and were processed for graphical review. All genotyping results were reviewed and confirmed manually by experienced researchers.

2.4. Statistical analysis

Chi-square tests were used to determine whether individual SNPs were in Hardy-Weinberg Equilibrium (HWE) for the control samples. The additive allelic effects and the genotypic effects of individual SNPs on the risk of AD were determined with logistic regression models with gender and age as adjusting covariates. The overall significance of the genotypic effect was presented as the type III effect for the genotypes. *P*-values less than 0.05 were considered statistically significant. Odds ratios were estimated from the logistic regression analysis.

We identified LD blocks in each gene using Gabriel's method and HaploView software [18]. For each identified block, we conducted haplotype association analysis in accordance with the methods of Lake et al. using a program called "haplo.glm" in R language [19]. The program performs regression analysis of a trait on haplotype effects, and it allows for ambiguous haplotypes. We used age and gender as controlling covariates in the regression models. We determined the effects of individual haplotypes relative to a

Table 1
Demographic characteristics of the two sample groups: (a) initial and (b) replication sets. The number of subjects, the mean and standard deviation for age, the IgE level, the eosinophil count, ECP, and the SCORAD index are shown.

Group	Number of subjects (F/M)	Age	IgE (U/ml)	Eosinophil count	ECP (ng/ml)	SCORAD
(a)						
ADe	433 (176/257)	15.7 (± 9.47)	1933.43 (± 3315.06)	583.84 (± 641.08)	72.70 (± 256.3)	33.35 (± 20.06)
ADi	213 (113/100)	9.26 (± 9.74)	52.08 (± 46.22)	380.54 (± 364.41)	36.6 (± 47.44)	22.3 (± 16.38)
NR	474 (220/254)	23.23 (± 2.24)	240.53 (± 416.01)			
(b)						
ADe	227 (92/135)	15.08 (± 10.04)	675.53 (± 702.6)	1821.32 (± 1638.64)	58.41 (± 71.34)	
ADi	213 (104/109)	9.39 (± 9.60)	352.19 (± 289.6)	89.26 (± 478.86)	31.33 (± 33.48)	
NR	692 (366/326)	14.58 (± 1.43)	107.98 (± 201.63)			

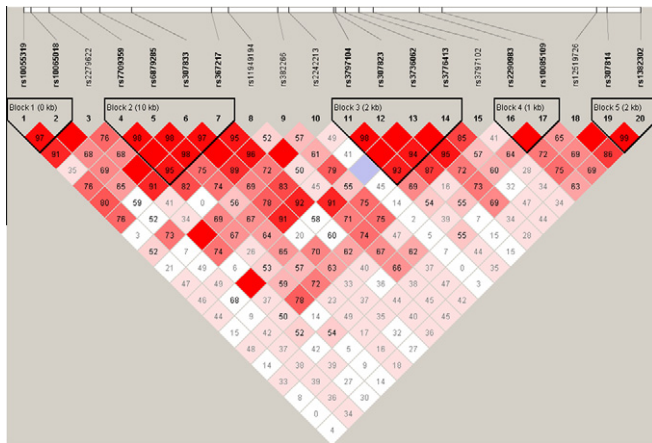


Fig. 1. Haplotype structure of FLT4. Haplotype blocks were constructed with 474 NR samples in Haploview v3.32 in accordance with the methods of Gabriel et al. D' values are shown in the square.

reference haplotype (i.e., the most common haplotype). Haplotypes with allele frequencies below 0.02 were pooled into a “rare allele haplotype” in the analysis.

We tested for an association between the SNPs and the total IgE level, the eosinophil count, and the ECP level using regression analysis and data from the AD patient samples. The total IgE levels were categorized into five groups: <40 U ml $^{-1}$, 40–200 U ml $^{-1}$, 200–500 U ml $^{-1}$, 500–2000 U ml $^{-1}$, and ≥ 2000 U ml $^{-1}$. Cumulative logistic regression analysis was used to determine the association between the genotypes and total IgE levels. We used a linear regression model to assess the genetic effect on the blood eosinophil counts and Eosinophilic Cationic Protein (ECP) levels in patients with AD. The log-transformed eosinophil count and ECP levels were used in the regression analysis. Age, gender, and the SCORAD index (an index of the severity of AD) were used as adjusting covariates in the regression models. Statistical analyses were performed with SAS 9.1 (SAS Institute Inc., Cary, NC, USA) and R statistical language (<http://www.r-project.org>).

3. Results

A total of 34 SNPs from five genes (VEGFA, VEGFB, VEGFC, FIGF and FLT4) were genotyped for 1120 subjects. Table 2 depicts the characteristics of the SNPs in VEGFB, including genomic function, chromosomal position, dbSNP id, and minor allele frequency. The information of SNPs from VEGFA, VEGFC, FIGF and FLT4 are presented in our previous report [20]. With the exception of rs2290983, all genotyped SNPs were in HWE at a significance level of 0.01. The average genotyping success rate was 99.2%.

We compared the distributions of allele and genotype frequencies for the 34 SNPs between the AD and control groups (data not shown). The statistical significance of the results was determined with logistic regression analysis with age and gender as adjusting covariates. In order to determine which subtype of AD was associ-

ated with the allelic or genotypic distribution of SNPs, we also compared each of the two AD subtypes with the control group. As a result, a few SNPs in FLT4 and VEGFB were significantly associated with AD; however, there were no significant results for the SNPs in the VEGFA, VEGFC, and FIGF genes. In addition, we used a regression model to analyze the relationship between the SNPs and the total IgE level, the eosinophil count, and the ECP level. Because quantitative trait values were available for the patients with AD, these were the only patients included in this analysis. Age, gender, SCORAD index, and subtype group were used as covariates in the regression analysis.

3.1. Association of polymorphisms in the FLT4, and VEGFB genes with AD

For the FLT4 gene, the following three SNPs had a significantly different allelic or genotypic distribution between controls and patients with AD: rs10085109 (IVS1-2026G/C), rs3736062 (Y448Y), and rs11949194 (P1066P). The rs3736062 and rs11949194 SNPs, which cause a synonymous change in the amino acid, were significantly associated with AD for both the allele and genotype tests (Table 3). The odds ratio of the T allele for the rs3736062 SNP was 0.682 (95% CI: 0.503–0.925, p -value = 0.014), which suggests a protective effect relative to the C allele with regards to susceptibility to AD. The odds ratios of the genotypes for the rs11949194 SNP were 0.734 (95% CI: 0.519–1.038) for CT vs. CC and 0.299 (95% CI: 0.092–0.969) for TT vs. CC. When we tested for an association with the two subtypes of AD, the rs3736062 SNP was significantly associated with the extrinsic type of AD (ADe), and the odds ratio for the T allele was 0.663 (95% CI: 0.475–0.924, p -value = 0.015). The genotype test for the SNP showed a marginally significant association with ADe (p -value = 0.049).

The odds ratio of the T allele for the rs11949194 SNP was 1.284 (95% CI: 1.009–1.634, p -value = 0.042), suggesting a causal effect on AD susceptibility relative to the C allele. The odds ratios of the genotypes for the rs11949194 SNP were 1.449 (95% CI: 1.069–1.965) for CT vs. CC and 1.231 (95% CI: 0.638–2.376) for TT vs. CC. In the AD subtype analysis, the rs11949194 SNP was significantly associated with the intrinsic type of AD (ADi). The odds ratio of the T allele was 1.658 (95% CI: 1.099–2.5, p -value = 0.016), and the genotype of the SNP showed a marginally significant association with ADi (p -value = 0.052).

The rs10085109 (IVS1-2026G/C) SNP in FLT4 was significantly associated with AD for the genotype test only (p -value = 0.026). The odds ratios of the SNP were 1.45 (95% CI: 1.074–1.956) for CG vs. CC and 0.879 (95% CI: 0.531–1.456) for GG vs. CC.

In addition, we tested for an association between the haplotypes of FLT4 and AD. We identified three haplotype blocks in which the SNPs were in high linkage disequilibrium (Fig. 1). One of the four-loci haplotypes in FLT4 (rs3776413, rs3736062, rs307823, and rs3797104) was significantly associated with AD and ADe. The GTGA haplotype, which accounted for about 12% of our sample, was associated with a lower susceptibility to ADe and had an odds ratio of 0.672 (95% CI: 0.472–0.956, p -value = 0.027) relative to the reference haplotype, GCAA. Because the haplotype

Table 2
The SNP markers genotyped for the case-control samples (dbSNP build 126).

Gene	ID	rs number	Chr	Position	Function	MAF ^a	HWE ^b	Success rate
VEGFB	D136D	12366035	chr11	63761268	exon 5	0.13	0.42	0.995
VEGFB	1811T/C	594942	chr11	63762868	3'UTR	0.31	0.75	0.994
VEGFB	3495C/T	11607007	chr11	63764552	3'UTR	0.18	0.65	0.984

P -values from a chi-square test for Hardy–Weinberg equilibrium.

MAF and HWE were calculated from 474 normal control samples.

^a MAF: Minor Allele Frequency.

^b HWE was tested with an all female group.

Table 3
Comparison of the allelic and genotypic frequencies of the rs10085109, rs3736062, and rs7709359 SNPs in the FLT4 gene and the rs12366035 SNP in the VEGFB gene between patients with AD and controls in the initial stage samples. Age and gender were included as adjusting covariates in the analysis models.

rs number (allele A/allele B)	Group	Genotype frequency			MAF	Allele test			Genotype test	
		AA	AB	BB		p value	OR (95% CI)	p value ^a	OR ^b (95% CI)	OR ^c (95% CI)
VEGFB rs11607007 (C/T)	NR	307 (0.66)	144 (0.31)	14 (0.03)	0.185					
	AD	393 (0.617)	210 (0.33)	34 (0.053)	0.218	0.047	1.291 (1.003, 1.661)	0.076	1.161 (0.853, 1.579)	2.322 (1.087, 4.963)
	ADe	264 (0.623)	137 (0.323)	23 (0.054)	0.216	0.087	1.265 (0.967, 1.656)	0.09	1.103 (0.792, 1.535)	2.413 (1.092, 5.336)
	ADi	129 (0.606)	73 (0.343)	11 (0.052)	0.223	0.372	1.222 (0.787, 1.899)	0.665	1.199 (0.698, 2.062)	1.577 (0.448, 5.555)
FLT4 rs10085109 (C/G)	NR	253 (0.536)	171 (0.362)	48 (0.102)	0.283					
	AD	301 (0.473)	279 (0.438)	57 (0.089)	0.308	0.349	1.108 (0.894, 1.374)	0.026	1.45 (1.074, 1.956)	0.879 (0.531, 1.456)
	ADe	199 (0.469)	186 (0.439)	39 (0.092)	0.311	0.172	1.173 (0.933, 1.473)	0.05	1.482 (1.075, 2.044)	1.046 (0.613, 1.785)
	ADi	102 (0.479)	93 (0.437)	18 (0.085)	0.303	0.544	0.887 (0.603, 1.306)	0.121	1.295 (0.764, 2.196)	0.43 (0.15, 1.23)
FLT4 rs3736062 (C/T)	NR	360 (0.759)	103 (0.217)	11 (0.023)	0.132					
	AD	502 (0.783)	131 (0.204)	8 (0.012)	0.115	0.014	0.682 (0.503, 0.925)	0.036	0.734 (0.519, 1.038)	0.299 (0.092, 0.969)
	ADe	336 (0.783)	89 (0.207)	4 (0.009)	0.113	0.015	0.663 (0.475, 0.924)	0.049	0.701 (0.481, 1.021)	0.311 (0.083, 1.163)
	ADi	166 (0.783)	42 (0.198)	4 (0.019)	0.118	0.478	0.822 (0.479, 1.412)	0.586	0.924 (0.504, 1.692)	0.299 (0.029, 3.078)
FLT4 rs11949194 (C/T)	NR	295 (0.633)	147 (0.315)	24 (0.052)	0.209					
	AD	371 (0.582)	238 (0.373)	29 (0.045)	0.232	0.042	1.284 (1.009, 1.634)	0.056	1.449 (1.069, 1.965)	1.231 (0.638, 2.376)
	ADe	254 (0.593)	157 (0.367)	17 (0.04)	0.223	0.265	1.158 (0.895, 1.499)	0.208	1.33 (0.961, 1.841)	0.951 (0.463, 1.954)
	ADi	117 (0.557)	81 (0.386)	12 (0.057)	0.25	0.016	1.658 (1.099, 2.5)	0.052	1.805 (1.052, 3.099)	2.362 (0.823, 6.778)

Significant p-values at the level of 0.05 are written in bold.

^a p-value for the type III effect of the genotype.

^b OR: odds ratio of GC vs. CC.

^c OR: odds ratio of GG vs. CC.

Table 4
Regression analysis of the log-transformed eosinophil count as a function of the genotypes of the rs2010963 SNP in VEGFA for the patients with AD. Means of the log-eosinophil count, the standard errors of the mean, and the p-values from the regression analysis for the allelic effect are shown.

Genotype	Mean (±SD)	Log-transformed (±SD)	Number of samples	Estimate	SE	p-value
GG	461.6 (±570.3)	5.76 (±0.90)	237 (0.321)			
GC	537.4 (±548.1)	5.94 (±0.89)	360 (0.487)	0.101	0.044	0.02
CC	557.9 (±636.2)	6.00 (±0.84)	142 (0.192)			

Significant p-values at the level of 0.05 are written in bold.

associated with AD had a similar odds ratio as rs3736062, the haplotype effect only reflected the single variant effect.

Three SNPs were genotyped from the VEGFB gene, and the rs11607007 showed a marginally significant association with AD for the allelic test (p -value = 0.047; Table 3). In the haplotype analysis, two-loci haplotypes in VEGFB (rs594942 and rs11607007) were significantly associated with AD. The TT haplotype had an estimated frequency of 20%, suggesting a causative effect on susceptibility to AD, and an odds ratio of 1.303 (95% CI: 1.011–1.679, p -value = 0.041) relative to the reference haplotype, CC.

A polymorphism in FLT4, rs10065018, showed significant effect (p -value = 0.018) in the generalized logistic regression of IgE levels, while age, gender, and scored were included as adjusting covariates. Increase of the number of minor allele of this SNP has negative effect on the total IgE levels.

3.2. Association of polymorphisms in the VEGFA gene with eosinophil counts

The regression analysis of log-transformed eosinophil counts revealed that the rs2010963 SNP in VEGFA was significantly associated with log-eosinophil counts. Table 4 depicts the mean of the log-eosinophil count, the standard error of the mean, and the p -value from the regression analysis for the allelic effect. As the G allele increased, the mean log-eosinophil counts increased by 0.101. In the haplotype analysis, a three-locus haplotype consisted of rs9381249, rs2010963, and rs2146323 also showed significant association with log-eosinophil counts and three haplotypes containing one rare allele at one of the three loci increased log-eosinophil count by the similar extent (0.229, 0.224, and 0.236 respectively) compared to the reference haplotype group. The reference haplotype is consisted of common alleles for all the three loci.

3.3. Replication of the association of rs10085109 in the FLT4 gene

In the replication study, one SNP from VEGFB and three SNPs from FLT4 were genotyped for the 1132 samples. Average success rate was 98.7% and all the SNPs were in HWE. Of the four SNPs, only rs10085109 had significant p -values in the association tests for the replication samples. The rs10085109 SNP had p -value of 0.048 at the AD and normal group comparison of genotypic distribution. The odds ratios of the SNP were 1.275 (95% CI: 1.05, 1.549) for CG vs. CC and 1.134 (0.815, 1.578) for GG vs. CC.

4. Discussion

In the initial stage study, we identified three SNPs (rs10085109, rs3736062 and rs11949194) in the FLT4 gene and one SNP (rs11607007) in the VEGFB gene that were significantly associated with the AD phenotype among Koreans. The role of dermal vasculature in AD pathogenesis is controversial. Although several studies have identified positive correlations between VEGFA and AD in the mRNA levels from the lesional skin of patients with AD [3,21,22], and one SNP (-1154G/A) in VEGFA is associated with AD [23], we could not find any correlations between SNPs in the VEGFA gene and AD susceptibility in our experiment. Instead, we found that the rs2010963 SNP in the VEGFA gene was significantly associated with log-eosinophil counts in patients with AD (Table 4). VEGFA + eosinophils are associated with a number of diseases, including asthma, urticarial, angioedema, and allergen-induced skin inflammation in atopic subjects [24]. We did not include the -1154G/A SNP in our experiments; however, the -1154G/A SNP is located between -4186C/T (rs9381249) and -634C/G (rs2010963), and this region comprised an LD block in our samples (data not

Table 5

Result of logistic regression analysis of the rs10085109 SNP in the FLT4 gene using combined data of the initial and replicate samples. Age, gender and sample source (initial or replicate) were included as adjusting covariates in the model.

Case group	Allele test		Genotype test		
	p value	OR (95% CI)	p value ^a	OR ^b (95% CI)	OR ^c (95% CI)
AD	0.072	1.143 (0.993,1.315)	0.048	1.275 (1.05,1.549)	1.134 (0.815,1.578)
ADe	0.107	1.113 (0.949,1.305)	0.23	1.213 (0.972,1.512)	1.11 (0.761,1.619)
ADi	0.124	1.132 (0.911,1.407)	0.172	1.333 (0.984,1.806)	1.052 (0.626,1.767)

Significant p-values at the level of 0.05 are written in bold.

^a p-value for the type III effect of the genotype.

^b OR: odds ratio of GC vs. CC.

^c OR: odds ratio of GG vs. CC.

shown). Thus, the association of rs2010963 with -1154G/A may explain why previous studies found a relationship between -1154G/A SNP and AD.

The human FLT4 gene contains 31 exons and is located in the chromosome 5q33–35. This gene is essential for blood vascular development during embryogenesis [4]. In adults, it is found almost exclusively in the lymphatic endothelium [5]. FLT4 expression has also been detected in certain blood capillaries associated with benign and malignant vascular tumors [25] and wound granulation tissue [26]. Mutations of this gene have been reported in patients with hereditary lymphedema [27,28], and FLT4 protein levels are higher in patients with various malignancies [6]. In a previous experiment, we found that two SNPs and a haplotype in the FLT4 gene were significantly associated with psoriasis; we also found that serum FLT4 levels in psoriasis patients were higher than those of controls [10]. In the current study, we found that three SNPs in the FLT4 gene were significantly associated with AD, but we could not find any difference in serum FLT4 levels between patients with AD and controls (data not shown). Of the four SNPs, only rs10085109 had significant p-values in the association tests for the replication samples.

In summary, we genotyped 34 SNPs from five genes in 1120 case-control samples in the initial stage and found that one SNP in the VEGFB gene and three SNPs in the FLT4 gene were selected for the further investigation with 1132 replication samples. As a result, the association of rs10085109 in FLT4 with susceptibility to AD was successfully replicated. This result suggests that FLT4 may increase dermal vasculature in patients with AD (Table 5).

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