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ORIGINAL ARTICLE

# Are Podoplanin Gene Polymorphisms Associated with Atopic Dermatitis in Koreans?

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**Background:** The histologic characteristics of atopic dermatitis (AD) include perivascular edema and dilated tortuous vessels in the papillary dermis. A single nucleotide polymorphism (SNP) of the fms-related tyrosine kinase 4 (FLT4) gene is associated with AD. **Objective:** To investigate the associations between podoplanin (PDPN) gene SNPs and AD. **Methods:** We genotyped 9 SNPs from 5 genes of 1,119 subjects (646 AD patients and 473 controls). We determined the promoter activity of 1 SNP (rs355022) by luciferase assay; this SNP was further investigated using 1,133 independent samples (441 AD patients and 692 controls). **Results:** The rs355022 and rs425187 SNPs and the C-A haplotype in the PDPN gene were significantly associated with intrinsic AD in the initial experiment. The rs355022 SNP significantly affected promoter activity in the luciferase assay. However, these results were not replicated in the replication study. **Conclusion:** Two SNPs and the C-A haplotype in the PDPN gene are significantly associated with intrinsic AD; although, the results were confirmed by luciferase assay, they could not be replicated with independent samples. Nevertheless, further replication experiments should be performed in future studies. **(Ann Dermatol 27(3) 275～282, 2015)**

**-Keywords-**
Atopic dermatitis, Luciferases, Podoplanin protein, Genetic polymorphisms

## INTRODUCTION

Atopic dermatitis (AD) is a genetically complex disease involving gene-gene and gene-environment interactions[1]. Genetic linkage analysis and association studies have identified several candidate genes associated with either epidermal barrier function or the immune system. Stress, bacterial, or viral infections, exposure to airborne or food allergens, and hygienic factors are thought to aggravate AD symptoms[1].

The K14-IL-4 transgenic mouse model of AD demonstrates that progressive dermal lymphatic growth is a prominent feature of AD; this is characterized by increased vessel number, vessel diameter, and the percent vascularized area[2]. This transgenic mouse model exhibits significantly increased dermal expression of podoplanin (PDPN), lymphatic vessel endothelial hyaluronan receptor 1 (LYVE-1), and fms-related tyrosine kinase 4 (FLT4, vascular endothelial growth factor receptor 3 [VEGFR 3]). The histological findings of human AD include dilated tortuous vessels within the papillary dermis, perivascular edema, mononuclear cell accumulation, and rare neutrophils and eosinophils[3]. However, the role of the dermal vasculature in AD pathogenesis remains poorly understood.

PDPN/T1a/aggrus/PA2.26 antigen, a transmembrane glycoprotein, is a well-known lymphatic endothelial marker. PDPN expression surrounding malignant tumors is a prognostic factor associated with lymphangiogenesis and distant metastasis[4]. Immunostaining with D2-40 mouse monoclonal antibody shows that PDPN is highly expressed in

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lymphatic endothelial cells and the basal cell layer of sebaceous glands but not in normal human interfollicular epidermis[5]. Marked PDPN expression is detected in the outer root sheath of hair follicles from the mid portion to the hair bulb excluding the bulge area[5]. Gröger et al.[6] report that PDPN and LYVE-1 are not only lineage markers for lymphatic endothelial cells, but also activation markers of blood endothelial cells. In normal skin, they found 2 types of vessels: vessels expressing high levels of PDPN (i.e., lymphatic vessels), and vessels negative for PDPN (i.e., blood vessels). However, within the papillary dermis in cases of eczema and psoriasis, they found a third type of vessel expressing low amounts of PDPN. Henno et al.[7] report that lymphatic vessels are expressed after blood vascular development in psoriasis.

We previously reported that single nucleotide polymorphisms (SNPs) and haplotypes of the FLT4 and VEGFA genes are associated with psoriasis[8] and that the rs10085109 SNP in the FLT4 gene is associated with AD susceptibility[9]. These results suggest FLT4 may increase dermal vasculature in Korean patients with AD and psoriasis. Therefore, in the current study, we performed a similar experiment comparing patients with AD with normal controls to determine the association between PDPN SNPs and AD in Korean.

## MATERIALS AND METHODS

### Subjects

This study included 1,119 samples from 646 patients with AD and 473 normal control (NR) subjects at the initial stage. The AD samples were collected from non-asthmatic patients with AD examined at Samsung Medical Center, Seoul, Korea. AD was diagnosed according to the criteria of Hanifin and Rajka, and classified as extrinsic or intrinsic (ADe and ADi, respectively) according to serum immunoglobulin E (IgE) level and/or the presence or absence of allergy following the CAP test and/or skin prick test. Details regarding AD diagnosis, the criteria for classifying ADe and ADi, and the blood and prick tests for allergens are described in our previous report[9]. All patients with AD (357 men and 289 women, mean age: 13.58±9.62 years) met our previously reported inclusion/ exclusion criteria[10,11]. Among patients with AD, 433 (257 men and 176 women, mean age: 15.7±9.47 years) had ADe, and 213 (100 men and 113 women, mean: 9.26±9.74 years) had ADi. The 473 NR subjects included medical students and volunteers (253 men and 220 women, mean age: 23.23±2.24 years) with no history of AD skin lesions. For the replication study, 1,133 samples (227 ADe, 214 ADi, and 692 NR) independent of the initial study were included; the samples were obtained from a cohort from Jeju Island, Korea[12]. The demographic characteristics of the study participants are summarized in Table 1.

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 (IRB: 2008-09-044-003).

### Marker selection

The SNP information was retrieved from the dbSNP (build 141, http://www.ncbi.nlm.nih.gov/SNP; accessed 12 Sep 2014). We selected 37 SNPs from 5 kbp upstream to 5 kbp downstream of the PDPN gene. The selected SNPs were genotyped from 48 independent samples from the general Korean population (data not shown). On the basis of these genotype results, we selected SNPs by using the linkage disequilibrium bin approach in the Tagger program (http://www.broad.mit.edu/mpg/tagger). This approach

**Table 1.** Demographic characteristics of the initial and replication study samples

| Variable | Initial group | | | Replication group | | |
|---|---|---|---|---|---|---|
| | ADe | ADi | NR | ADe | ADi | NR |
| No. of subjects (F/M) | 433 (176/257) | 213 (113/100) | 473 (220/253) | 227 (92/153) | 214 (105/109) | 692 (366/326) |
| Age (yr) | 15.7±9.47 | 9.26±9.74 | 23.23±2.24 | 15.08±10.03 | 9.4±9.58 | 14.58±1.43 |
| Immunoglobulin E (U/ml) | 1,933.43±3,315.06 | 52.08±46.22 | 240.53±416.01 | 1,638.64±1,821.32 | 89.26±478.86 | - |
| Eosinophil count | 583.84±641.08 | 380.54±364.41 | - | 680.89±702.01 | 352.19±289.60 | - |
| Eosinophilic cationic protein (ng/ml) | 72.70±256.3 | 36.6±47.44 | - | 58.40±71.34 | 31.33±33.48 | - |
| Scoring of atopic dermatitis | 33.35±20.06 | 22.3±16.38 | - | - | - | - |

Values are presented as number only or mean±standard deviation. ADe: extrinsic type of atopic dermatitis, ADi: intrinsic type of atopic dermatitis, NR: normal control, F: female, M: male.

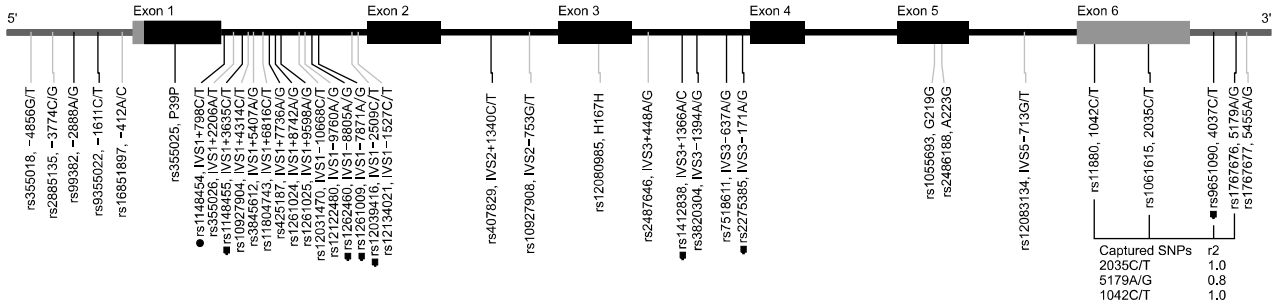


**Fig. 1.** Map of the podoplanin gene on chromosome 1p36.21 (41.7 kb). Black and grey blocks indicate coding exons, and the 5′ and 3′ untranslated regions, respectively. The first nucleotide of the translation start site is denoted as nucleotide +1. Genotyped polymorphisms are marked. Black and grey lines indicate minor allele frequencies of the polymorphisms in 48 samples >10% and <10%, respectively. Dots indicate polymorphisms genotyped in the larger population (n=1,119). Linkage disequilibrium between the tag single nucleotide polymorphisms (SNPs) and their tagged SNPs are presented as $r^2$.

defines 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[13]. We used an $r^2$ threshold of 0.8 and a minimum allele frequency of 0.1. Thus, a total of 9 SNPs from PDPN were selected as markers for the association study (Fig. 1).

### Genotyping with fluorescence polarization detection

We extracted genomic DNA from 5-ml whole blood samples by using a commercially available DNA isolation kit (Gentra Genomic DNA Purification Kit; Qiagen, Minneapolis, MIN, USA) in accordance with the manufacturer's protocol. Genotypes were identified with the ultra-high throughput GenomeLab SNPstream system[14], which uses multiplex polymerase chain reaction (PCR) in conjunction with tag array single-base extension genotyping technology (Beckman Coulter, Fullerton, CA, USA) and the SNPstream software suite[15]. PCR amplifications were performed in a PTC-225 Peltier Thermal Cycler (MJ Research, Waltham, MA, USA) with Taq Gold DNA polymerase (Applied Biosystems, Foster City, CA, USA). The sequences of the PCR primers and extension primers are available upon request. Multiplex PCR and genotyping were performed in homogeneous reactions, and the 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 processed for graphical review. All genotyping results were reviewed and confirmed manually by experienced researchers.

### Luciferase assay

Double-stranded oligonucleotides were synthesized with three concatenated copies of the T or C allele for a 21-bp region centered on the polymorphism with *Kpn*I and *Bgl*II at the 5′ and 3′ ends, respectively. The oligonucleotides were subsequently cloned into the pGL3-promoter vector (Promega, Madison, WI, USA), which has a simian virus 40 (SV40) promoter.

HEK293 cells were transfected with 1 $\mu$g reporter constructs and 0.1 $\mu$g pRL-TK *Renilla* luciferase vector (Promega) with Lipofectamine 2000 (Invitrogen; Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's protocol. The transfection efficiency was normalized to that of *Renilla* luciferase activity. The medium was changed to growth medium 18 hours after transfection. Cells were harvested 24 hours after the medium was changed, and the luciferase activity was measured with the Dual-Luciferase Reporter Assay System (Promega).

### Statistical analysis

The $\chi^2$ test was used to determine if individual variants were in Hardy-Weinberg equilibrium at each locus for normal samples. The allelic (i.e., additive) and genotypic effects of individual SNPs were tested using a logistic regression model adjusted for sex and age. The level of significance was set at $p<0.05$. Odds ratios (ORs) and 95% confidence intervals (CIs) were also estimated from the logistic regression model.

To detect the most significantly associated haplotype, the significance of overall haplotype effects was scanned for haplotypes of 2 to 4 SNPs by using a sliding-window approach. For the haplotype scan, the haplo.score function in the R package (http://www.r-project.org) was used; this function is a haplotype association test method that enables the simultaneous modeling of haplotype effects including various controlling covariates, and statistical significance is determined according to score test statistics[16]. Haplo.score provides global test statistics for a given haplotype locus in addition to results for individual haplotypes. For loci that had a significant effect in individual SNP tests, we obtained the combined effect by using the hap-

lo.glm function in the R package. ORs and the significance of effect differences with respect to the reference haplotype were calculated by haplo.glm adjusted for age and sex[17]. The most frequent haplotypes were used as the reference haplotype group in this analysis.

Total IgE levels were categorized as $<40$, 40 to 200, 200 to 500, 500 to 2,000, or $>2,000$ U/ml. Cumulative logistic regression analysis was subsequently conducted to examine the associations between genotype and total IgE levels. The genetic effects on blood eosinophil counts and eosinophilic cationic protein levels (both log-transformed) in patients with AD were tested using a linear regression model. The regression model was adjusted for age, sex, and the scoring of atopic dermatitis index.

Statistical analysis was performed with SAS ver. 9.1 (SAS Institute Inc., Cary, NC, USA) and the R statistical language (http://www.r-project.org; accessed 31 Mar 2009).

## RESULTS

Nine SNPs of the PDPN gene were genotyped in the 1,119 subjects at the initial stage. Information on the SNPs, including genomic function, chromosomal position, dbSNP ID, and minor allele frequency, is shown in Table 2. All SNPs were in Hardy-Weinberg equilibrium at a significance level of 0.01. The average genotyping success rate was 99.3%. We compared the distributions of the allelic and genotypic frequencies for the 9 SNPs between the AD and NR groups. Statistical significance was obtained in logistic regression analysis adjusted for age and sex. For further analysis, haplotype association tests were conducted using a sliding window approach.

### Associations of polymorphism gene single nucleotide polymorphisms with atopic dermatitis

Regarding the associations of the SNPs with AD, two SNPs (rs355022 and rs425187) had significantly different allelic or genotypic distributions between the ADi and NR groups (Table 3); rs355022 exhibited a greater difference between groups ($p=0.016$, OR=0.587, 95% CI: 0.38~0.907). The genotypic effect test showed a significant difference in the rs425187 SNP ($p=0.043$) with ORs of 0.616 (95% CI: 0.316~1.204) for A-A vs. A-G and 6.444 (95% CI: 1.021~40.678) for A-A vs. G-G (Table 3).

To calculate the combined effect size of the two loci, we used the haplo.glm function to estimate haplotypes simultaneously in a generalized linear model. The haplotype C-A of the two loci was significantly associated with ADi ($p=0.03$), and the OR vs. T-A was 0.603 (95% CI: 0.382~0.952). Although no other haplotypes showed significant associations, the C-G group, which has two risk alleles, tended to have an increased effect for ADi compared to individual SNPs (Table 4).

### rs355022 single nucleotide polymorphism luciferase assay

Because rs355022 is located in the promoter (−1611 C/T) region of the PDPN gene, its effect rs355022 on transcriptional activity was examined by luciferase reporter assay (Fig. 2). Luciferase gene constructs containing three concatenated copies of a 21-bp region of either the T or C allele centered on the SNP (i.e., p3X1562T and p3X1562C) were prepared. Transfecting p3X1562C into HEK293 cells decreased luciferase activity to a greater extent than p3X1562T ($0.44\pm0.25$ fold induction) (Fig. 2). This result suggests rs355022 affects the transcriptional activity of the PDPN gene.

### Replication of the single nucleotide polymorphism experiments

To confirm the effects of rs355022 and rs425187, we genotyped 1,133 independent samples from 441 patients with

**Table 2.** Genotyped SNP markers (dbSNP build 141)

| RS number | ID | Position | Function | MAF | HWE* | Success rate |
|---|---|---|---|---|---|---|
| rs355022 | 1611C/T | 13582195 | Promoter | 0.291 | 0.326 | 0.984 |
| rs1148454 | IVS1+798C/T | 13584898 | Intron 1 | 0.5 | 0.858 | 0.997 |
| rs1148455 | IVS1+3635C/T | 13587735 | Intron 1 | 0.357 | 0.536 | 0.988 |
| rs425187 | IVS1+7736A/G | 13591836 | Intron 1 | 0.118 | 0.288 | 0.999 |
| rs1261024 | IVS1+8742A/G | 13592842 | Intron 1 | 0.226 | 1 | 0.996 |
| rs1262460 | IVS1-8805A/G | 13598368 | Intron 1 | 0.363 | 0.835 | 0.999 |
| rs3820304 | IVS3-1394A/G | 13612293 | Intron 3 | 0.16 | 0.746 | 0.99 |
| rs7518611 | IVS3-637A/G | 13613050 | Intron 3 | 0.366 | 0.695 | 0.992 |
| rs2275385 | IVS3-171A/G | 13613516 | Intron 3 | 0.17 | 0.507 | 0.992 |

The minor allele frequency (MAF) and Hardy-Weinberg equilibrium (HWE) were calculated from 459 normal control samples. *$p$-values from 2 test for HWE.

**Table 3.** Allelic and genotypic frequencies of the 9 PDPN gene SNPs among the AD, AD subtype, and the normal control groups

| rs number (allele A/ allele B) | Group | Genotype frequency | | | MAF | Allele test | | Genotype test | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | AA | AB | BB | | *p*-value | OR (95% CI) | *p*-value* | OR (95% CI)† | OR (95% CI)‡ |
| rs355022 | NR | 239 (0.513) | 183 (0.393) | 44 (0.094) | 0.291 | | | | | |
| T/C | AD | 334 (0.525) | 266 (0.418) | 36 (0.057) | 0.266 | 0.06 | 0.805 (0.642~1.009) | 0.105 | 0.89 (0.662~1.198) | 0.544 (0.308~0.959) |
| | Ade | 220 (0.514) | 182 (0.425) | 26 (0.061) | 0.273 | 0.257 | 0.87 (0.684~1.107) | 0.322 | 0.97 (0.706~1.332) | 0.631 (0.346~1.153) |
| | ADi | 114 (0.548) | 84 (0.404) | 10 (0.048) | 0.25 | 0.016§ | 0.587 (0.38~0.907) | 0.058 | 0.627 (0.365~1.079) | 0.291 (0.085~0.993) |
| rs1148454 | NR | 119 (0.253) | 232 (0.494) | 119 (0.253) | 0.5 | | | | | |
| C/T | AD | 161 (0.249) | 344 (0.533) | 141 (0.218) | 0.485 | 0.643 | 0.954 (0.781~1.165) | 0.586 | 1.087 (0.772~1.53) | 0.907 (0.608~1.353) |
| | Ade | 102 (0.236) | 232 (0.536) | 99 (0.229) | 0.497 | 0.902 | 0.987 (0.795~1.224) | 0.439 | 1.201 (0.828~1.741) | 0.973 (0.631~1.502) |
| | ADi | 59 (0.277) | 112 (0.526) | 42 (0.197) | 0.46 | 0.351 | 0.846 (0.596~1.202) | 0.61 | 0.784 (0.437~1.407) | 0.726 (0.361~1.459) |
| rs1148455 | NR | 196 (0.42) | 208 (0.445) | 63 (0.135) | 0.358 | | | | | |
| C/T | AD | 282 (0.441) | 289 (0.452) | 68 (0.106) | 0.333 | 0.267 | 0.887 (0.718~1.096) | 0.523 | 0.913 (0.675~1.236) | 0.767 (0.482~1.222) |
| | Ade | 185 (0.434) | 192 (0.451) | 49 (0.115) | 0.34 | 0.551 | 0.933 (0.744~1.171) | 0.702 | 1.002 (0.724~1.388) | 0.817 (0.495~1.351) |
| | NR | 119 (0.253) | 232 (0.494) | 119 (0.253) | 0.317 | 0.072 | 0.704 (0.481~1.031) | 0.167 | 0.636 (0.369~1.095) | 0.55 (0.238,1~274) |
| rs425187 | NR | 366 (0.772) | 104 (0.219) | 4 (0.008) | 0.118 | | | | | |
| A/G | AD | 519 (0.805) | 117 (0.181) | 9 (0.014) | 0.105 | 0.701 | 0.94 (0.684~1.291) | 0.296 | 0.838 (0.59~1.189) | 2.224 (0.571~8.662) |
| | Ade | 347 (0.803) | 79 (0.183) | 6 (0.014) | 0.105 | 0.741 | 0.944 (0.671~1.329) | 0.787 | 0.901 (0.62~1.309) | 1.351 (0.304~5.996) |
| | ADi | 172 (0.808) | 38 (0.178) | 3 (0.014) | 0.103 | 0.611 | 0.858 (0.474~1.551) | 0.043§ | 0.616 (0.316~1.204) | 6.444 (1.021~40.678) |
| rs1261024 | NR | 282 (0.597) | 166 (0.352) | 24 (0.051) | 0.227 | | | 0.65 | 0.868 (0.642~1.173) | 0.918 (0.478~1.762) |
| A/G | AD | 399 (0.621) | 207 (0.322) | 36 (0.056) | 0.217 | 0.426 | 0.908 (0.715~1.152) | 0.743 | 0.887 (0.642~1.225) | 0.879 (0.44~1.752) |
| | Ade | 273 (0.636) | 131 (0.305) | 25 (0.058) | 0.211 | 0.466 | 0.91 (0.705~1.173) | 0.714 | 0.798 (0.459~1.387) | 0.83 (0.256,2.691) |
| | ADi | 126 (0.592) | 76 (0.357) | 11 (0.052) | 0.23 | 0.459 | 0.847 (0.545~1.315) | | | |
| rs1261009 | NR | 193 (0.409) | 216 (0.458) | 63 (0.133) | 0.362 | | | | | |
| A/G | AD | 277 (0.429) | 281 (0.435) | 88 (0.136) | 0.354 | 0.682 | 0.958 (0.78~1.177) | 0.919 | 0.961 (0.71~1.3) | 0.915 (0.586~1.429) |
| | Ade | 190 (0.439) | 176 (0.406) | 67 (0.155) | 0.358 | 0.819 | 0.975 (0.783~1.213) | 0.783 | 0.899 (0.65~1.244) | 1.007 (0.63~1.608) |
| | ADi | 87 (0.408) | 105 (0.493) | 21 (0.099) | 0.345 | 0.704 | 0.929 (0.634~1.36) | 0.368 | 1.212 (0.709~2.074) | 0.637 (0.252~1.611) |
| rs3820304 | NR | 272 (0.576) | 170 (0.36) | 30 (0.064) | 0.16 | | | | | |
| A/G | AD | 346 (0.541) | 242 (0.379) | 51 (0.08) | 0.169 | 0.106 | 1.254 (0.953~1.648) | 0.432 | 1.129 (0.836~1.524) | 1.4 (0.797~2.457) |
| | Ade | 228 (0.534) | 159 (0.372) | 40 (0.094) | 0.173 | 0.094 | 1.285 (0.959~1.722) | 0.383 | 1.093 (0.791~1.511) | 1.507 (0.835~2.72) |
| | ADi | 118 (0.557) | 83 (0.392) | 11 (0.052) | 0.161 | 0.581 | 1.151 (0.699~1.895) | 0.515 | 1.364 (0.804~2.313) | 1.106 (0.366~3.345) |
| rs7518611 | NR | 331 (0.703) | 129 (0.274) | 11 (0.023) | 0.366 | | | | | |
| A/G | AD | 439 (0.689) | 181 (0.284) | 17 (0.027) | 0.349 | 0.464 | 0.924 (0.748~1.142) | 0.254 | 1.218 (0.886~1.673) | 1.798 (0.712~4.539) |
| | Ade | 289 (0.678) | 127 (0.298) | 10 (0.023) | 0.363 | 0.558 | 0.934 (0.744~1.173) | 0.245 | 1.292 (0.92~1.814) | 1.612 (0.598~4.342) |
| | ADi | 150 (0.711) | 54 (0.256) | 7 (0.033) | 0.322 | 0.598 | 0.905 (0.623~1.313) | 0.497 | 0.998 (0.558~1.784) | 2.459 (0.547~11.05) |
| rs2275385 | NR | 187 (0.398) | 222 (0.472) | 61 (0.13) | 0.171 | | | | | |
| A/G | AD | 268 (0.419) | 297 (0.464) | 75 (0.117) | 0.169 | 0.376 | 0.886 (0.679~1.158) | 0.607 | 0.997 (0.735~1.353) | 0.803 (0.507~1.27) |
| | ADe | 167 (0.391) | 210 (0.492) | 50 (0.117) | 0.165 | 0.366 | 0.876 (0.658~1.167) | 0.618 | 1.028 (0.74~1.428) | 0.809 (0.493~1.328) |
| | ADi | 101 (0.474) | 87 (0.408) | 25 (0.117) | 0.178 | 0.79 | 0.936 (0.575~1.523) | 0.86 | 0.935 (0.546~1.603) | 0.795 (0.35,1~809) |

ORs and *p*-values were obtained from a logistic regression model adjusted for age and sex. PDPN: podoplanin, SNP: single nucleotide polymorphism, AD: atopic dermatitis, AA: homozygous genotype of A allele, AB: heterozygous genotype, BB: homozygous genotype of B allele, MAF: minor allele frequency, OR: odds ratio, CI: confidence interval, NR: normal control, ADe: extrinsic type of atopic dermatitis, ADi: intrinsic type of atopic dermatitis. *$p$-value of the type III effect of the genotype; †OR between individuals with AB and BB; ‡OR between individuals with AA and BB. §$p<0.05$.

**Table 4.** Analysis of haplotypes of the rs355022 and rs425187 loci to test for associations with AD and AD subtypes

| Haplotype | Frequency | AD vs. NR | | ADe vs. NR | | ADi vs. NR | |
|---|---|---|---|---|---|---|---|
| | | OR (95% CI) | *p*-value | OR (95% CI) | *p*-value | OR (95% CI) | *p*-value |
| T-G | 0.084 | 1.054 (0.712~1.562) | 0.792 | 1.054 (0.712~1.562) | 0.792 | 0.917 (0.474~1.775) | 0.797 |
| C-A | 0.25 | 0.85 (0.664~1.088) | 0.197 | 0.85 (0.664~1.088) | 0.197 | 0.603 (0.382~0.952) | 0.03 |
| C-G | 0.026 | 0.512 (0.232~1.131) | 0.098 | 0.512 (0.232~1.131) | 0.098 | 0.44 (0.131~1.479) | 0.185 |
| T-A | 0.64 | Reference | | Reference | | Reference | |

ORs and *p*-values were calculated by the haplo.glm function using age and sex as adjusting covariates. The most frequent haplotypes were used as the reference haplotype group. Bold type indicates $p<0.05$. AD: atopic dermatitis, NR: normal control, ADe: extrinsic type of atopic dermatitis, ADi: intrinsic type of atopic dermatitis, OR: odds ratio, CI: confidence interval.

AD and 692 controls (Table 1). However, rs425187 did not exhibit any association, while rs355022 exhibited a significant association in the replicate samples (data not shown). Thus, the direction of the effect was opposite to that of the initial study. Therefore, the association became non-significant for the merged dataset (Table 5).

## DISCUSSION

PDPN is a sensitive marker for identifying lymphatic vessels. Transgenic mice exhibit significantly elevated dermal expression of PDPN, LYVE-1, and VEGFR-3 (FLT4)[5], suggesting lymphatic vessels may be involved in AD pathogenesis in the animal model. VEGF and effector cells of skin inflammation (i.e., mast cells, basophils, eosinophils, macrophages lymphocytes, etc.) are major sources of the vast array of angiogenesis and lymphangiogenesis in AD. However, the role of lymphangiogenesis in AD is largely unknown[3].

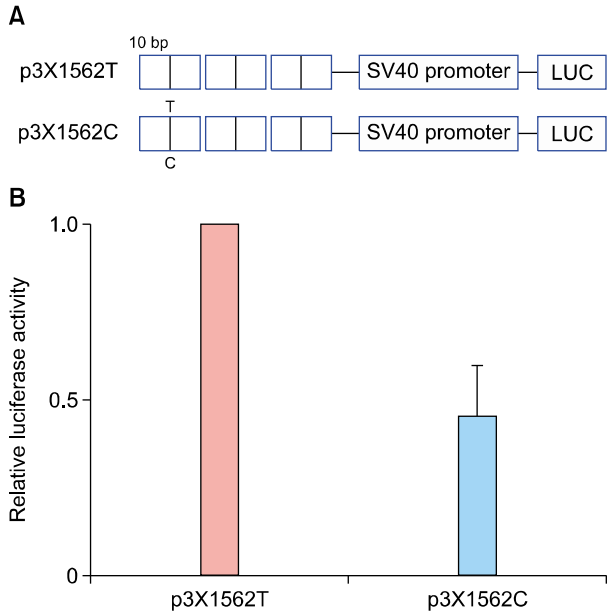


**Fig. 2.** Effect of the rs355022 single nucleotide polymorphism (SNP) of podoplanin on transcriptional activity. (A) Reporter gene constructs. Three concatenated oligonucleotides of the rs355022 T or C SNP allele were inserted into a pGL3-SV40 promoter vector. (B) The relative luciferase activity of the p3X1562C construct is presented as the ratio to that of the p3X1562T construct. The experiment was repeated three times in duplicate. HEK293 cells were used for transfection.

We previously reported that a SNP in the FLT4 gene is associated with AD susceptibility in Koreans[9]. On the basis of these reports, we hypothesized PDPN is associated with AD and therefore evaluated the association between the SNPs of PDPN and AD susceptibility. We initially identified two SNPs, rs355022 and rs425187, in the PDPN gene that were significantly associated with intrinsic AD in Koreans. As the rs355022 SNP is located in the promoter region of PDPN (−1611 C/T), we performed a luciferase assay; the results show that rs355022 can affect PDPN transcription, which indicates PDPN SNP T→C is associated with lower transcriptional activity of PDPN. To confirm these findings, we replicated the experiment with a different set of patients and controls. However, the replication study did not confirm the results of the initial experiment. The reason for the contrasting results of our two experiments is unknown. It is possible that the SNPs in PDPN gene weakly influence the whole-protein effect of this gene *in vivo*. This discrepancy highlights the importance of replication studies for SNPs.

The role of dermal vasculature in AD pathogenesis is controversial[3]. Our previous results suggest there might be differences between the K14-IL-4 transgenic mouse model and humans[5]. In the K14-IL-4 transgenic mouse model, interleukin (IL)-4-triggering macrophage recruitment has been suggested to be closely associated with lymphangiogenesis in AD. However, in comparison to K14-IL-4 transgenic mice, the active action time of IL-4 is not long enough in humans to sustain macrophage recruitment.

**Table 5.** Logistic regression analysis of the rs355022 SNP in the PDPN gene using combined data from the initial and replicate samples

| rs number (allele A/ allele B) | Group | Genotype frequency | | | MAF | Allele test | | Genotype test | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | AA | AB | BB | | *p*-value | OR (95% CI) | *p*-value* | OR (95% CI)† | OR (95% CI)‡ |
| rs355022 T/C | NR | 628 (0.544) | 443 (0.384) | 84 (0.073) | 0.265 | | | | | |
| | AD | 551 (0.52) | 439 (0.415) | 69 (0.065) | 0.272 | 0.911 | 1.008 (0.87,1.169) | 0.832 | 1.047 (0.864,1.269) | 0.949 (0.654,1.378) |
| | ADe | 334 (0.515) | 269 (0.414) | 46 (0.071) | 0.278 | 0.721 | 1.031 (0.873,1.217) | 0.883 | 1.057 (0.849,1.315) | 1.017 (0.673,1.539) |
| | ADi | 217 (0.529) | 170 (0.415) | 23 (0.056) | 0.263 | 0.49 | 0.92 (0.726,1.166) | 0.709 | 0.963 (0.712,1.302) | 0.766 (0.407,1.442) |

The model was adjusted for age, sex and sample source (i.e., initial or replicate). SNP: single nucleotide polymorphism, PDPN: podoplanin, AA: homozygous genotype of A allele, AB: heterozygous genotype, BB: homozygous genotype of B allele, MAF: minor allele frequency, OR: odds ratio, CI: confidence interval, NR: normal control, AD: atopic dermatitis, ADe: extrinsic type of atopic dermatitis, ADi: intrinsic type of atopic dermatitis. **p*-value for the type III effect of the genotype. †OR of G-C vs. C-C. ‡OR of G-G vs. C-C.

A substantial amount of research is performed worldwide to search for genetic factors in the etiology of AD; three main approaches are being used: candidate gene association, selecting genes for study based on a hypothesis of a known biological function, and genome-wide linkage screening. Efforts to identify candidate genes for AD through genome-wide linkage screening and DNA microarrays have identified at least 20 genes significantly associated with AD. However, only six of these genetic associations—IL-4, IL-4R, IL-13, mast cell chymase, serine protease inhibitor Kazal-type 5 (SPINK5), and filaggrin (FLG) genes—have been replicated in at least two independent studies[18]. At least four genome-wide association studies[19-22] have been performed since 2009. In each study, the authors identified 1 to 8 different candidate genes including FLG; however, most of the reported genes' functions have not yet been verified in AD. Regarding SNP studies in AD, there are many reports of different SNPs in several genes in different ethnicities. We previously reported associations between AD and SNPs in sphingomyelinase 2[23], IL-18[24], IL-5 & IL-5R[25], defensin 1[26], IL-12 & IL-12R[27], SPINK5[28], IL-9 & IL-9R[29], IL-4, IL-13 & IL-13R[30], and FLT4[9] in the Korean population. Other groups in Korea report associations of AD with SNPs in the FLG gene[31], FCεRI gene[32], and the haplotype of the IL-10 gene[33]. Among those reports, the FLT4 gene is the only gene that was studied in replication experiments with different samples.

In summary, we genotyped 9 SNPs from the PDPN gene in 1,119 samples and found two SNPs associated with ADi. In addition, the rs355022 SNP affects PDPN transcription. However, we could not replicate these results. Despite these conflicting results, replication experiments are critical for SNP studies.

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