Association of DOCK8, IL17RA, and KLK12 Polymorphisms with Atopic Dermatitis in Koreans

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Background: Early-onset and severe atopic dermatitis (AD) in patients increase the probability of the development of allergic rhinitis or asthma. Treatment and prevention strategies in infants and young children with AD are targeted toward treating the symptoms, restoring skin barrier functions, and reducing the absorption of environmental allergens in an attempt to attenuate or block the onset of asthma and food allergy. Objective: Given that the initiating events in AD remain poorly understood, identifying those at risk and implementing strategies to prevent AD is necessary. Methods: Whole-exome sequencing (WES) was performed in a 43 control group and a disease group with 20 AD patients without atopic march (AM) and 20 with AM. Sanger sequencing was carried out to validate found variants in cohorts. Results: DOCK8, IL17RA, and KLK12 single-nucleotide polymorphisms were identified by WES as missense mutations: c.1289C > A, p.P97T (rs529208); c.1685C > A, p.P562G (rs12484684); and c.457 + 27 > C, rs3745540, respectively. A case-control study showed that total immunoglobulin E (IgE) level was significantly increased in the AA genotype of DOCK8 compared to the CA genotype in allergic patients. The rs12484684 of IL17RA increased risk of adult-onset AD (odds ratio: 1.63) compared to the control for (A) allele frequency. AD and AM patients with the IL17RA CA genotype also had elevated IgE levels. rs3745540 of KLK12 was associated with AD in dominant model (odds ratio: 2.86). Conclusion: DOCK8 (rs529208), IL17RA (rs12484684), and KLK12 (rs3745540), were identified using a new WES filtering method. The result suggests that polymorphism of DOCK8 and IL17RA might be related to increase the total IgE level. (Ann Dermatol 32(3) 197 ~ 205, 2020)

Keywords: Atopic dermatitis, DOCK8, Exome sequencing, IL17RA, KLK12, Sanger sequencing

INTRODUCTION

The pathophysiology of atopic dermatitis (AD) is complex and multifactorial, involving elements of alterations in cell-mediated immune responses, immunoglobulin E (IgE)-mediated hypersensitivity, environmental factors, and barrier dysfunction including increased transepidermal water loss (TEWL), pH alterations, dehydration, and filaggrin (FLG) gene mutations. Early-onset and severe AD in patients increase the probability of the development of allergic rhinitis or asthma, a phenomenon known as the ‘atopic march’ (AM). Treatment and prevention strategies in infants and young children with AD are targeted toward treating the symptoms, restoring skin barrier functions, and reducing the absorption of environmental allergens in an attempt to attenuate or block the onset of asthma and food allergy. Given that the initiating events in AD remain poorly understood, identifying those at risk and implementing strategies to prevent AD is impractical. A genome-wide association study (GWAS) and epigenetic studies found chromosomal susceptibility loci and the epigenetic modulation associated with AD. Filaggrin degradation produces natural moisturizing factors (NMF) and...
flattens keratinocytes to maintain skin homeostasis. Loss-of-function \textit{FLG} mutations have been reported as a major risk factor in skin barrier damage and are known to elevate AD severity\textsuperscript{4,5}, allowing the prediction of early-onset AD from such genetic information or family history\textsuperscript{6}. Hence, the genetic diversity of \textit{FLG} mutations can be mapped in East Asian countries, and visualized using phylogenetic trees\textsuperscript{7}, and the search for new genetic candidate markers can continue. Identification of genetic variants for early diagnosis will prevent AD development and being a major part of personalized therapy. Meanwhile, whole-exome sequencing (WES) for clinical diagnostics has increased steadily to identify causal variants in Mendelian and rare diseases, including cancer\textsuperscript{8}. Eighty five percent disease-causing mutations are detected in coding regions (exome), which is comprised of about 180,000 exons\textsuperscript{9}. WES is also a preferred analysis method with a better filtering system for identifying common and rare variants in a large cohort study than next-generation sequencing\textsuperscript{10,11}. In an attempt to eliminate certain variables and identify potential causative variants of AD, we divided three study groups as follows; (1) an adult-AD group that has not developed allergic rhinitis or allergic asthma, (2) an AM group, and (3) the Korean Personal Genome Project (KPGP) control group.

In evolutionary biology, it is known that there is a strong probability that conserved amino acid sites are likely to have a significant effect on protein function\textsuperscript{12}. Thus, candidate risk variants, extracted from WES, were validated in a population-based case-control study, using Sanger sequencing. This study suggests that \textit{IL17RA}, \textit{DOCK8}, and \textit{KLK12} polymorphisms are novel candidate variants associated with allergic diseases.

**MATERIALS AND METHODS**

**Patients**

All patients with AD were given a diagnosis according to the revised criteria of Williams et al.\textsuperscript{13} The AM group was previously diagnosed with allergic rhinitis, asthma and allergic conjunctivitis along with AD. This study included a KPGP control group consisting of 43 patients and a disease group with 20 AD patients without AM and 20 with AM. Adult-onset (age \(\geq 18\) years) of AD were recruited. KPGP is the Korean reference genome mapping project using the Illumina HiSeq platform\textsuperscript{14}. Adult-AD means the development of AD in an adulthood, not a children, and the onset of childhood has a weakness to be dependent on memory. The development of AD in adulthood significantly reduces the incidence of asthma and allergic rhinitis compared to early-onset AD in infancy\textsuperscript{15}. AM group were recruited both adults and children group who were progressed to allergic asthma and rhinitis. The clinical phenotype of the AM patients included 19 with AD plus allergic rhinitis and one with AD plus asthma (Table 1). Eighty one healthy control, 87 adult AD and 72 AM patients were enrolled, and the characteristics of the study cohorts are presented in Table 2. Sanger sequencing was performed to validate the risk of AD for \textit{DOCK8}, \textit{IL17RA}, and \textit{KLK12} polymorphisms. Peripheral whole blood samples were obtained from all subjects.

The present study protocol was reviewed and approved by the Chung-Ang University Hospital Institutional Review Board, IRB No. C20152538 (1716). Informed consent was obtained from all subjects when they were enrolled.

**Whole-exome sequencing**

Whole blood samples were collected in ethylenediaminetetraacetic acid tubes from all subjects and the QIAamp DNA Mini Kit (Qiagen Inc., Valencia, CA, USA) was used to isolate genomic DNA. A Qubit fluorometer (Life Technologies, Grand Island, NY, USA) and Nanodrop spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA) were used to assess DNA concentration and purity. According to the manufacturer’s standard protocol, WES was performed using SureSelect Human All Exon V4+UTR 71 Mb (Agilent, Santa Clara, CA, USA), and DNA samples were mechanically sheared using Covaris (Covaris, Woburn, MA, USA). A paired-end (PE) and deep sequencing DNA library was prepared through classic protocol steps (shearing, end-repair, A-tailing, peak detection, PE adaptor ligation, and amplification). After the library was hybridized with bait sequences for 24 hours, the captured library was purified and amplified with an index barcode tag. Then, library quality and quantity were measured. Sequencing of

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**Table 1. Clinical information of the patients in whole-exome sequencing**

| Variable          | Control (KPGP) | AD  |
|-------------------|----------------|-----|
| Total             | 43             | 40  |
| Age (yr)          | -              | 26.0 ± 7.4 |
| Male/female       | -              | 25/15 |
| Total immunoglobulin E (KU/L) | - | 2,336 ± 2,062 |
| Eosinophils (mm\(^3\)) | - | 438.7 ± 369.7 |
| Clinical phenotype | -              | 19  |
| AD                | 20             |     |
| AD + AR           | 19             |     |
| AD + AS           | 1              |     |

Values are presented as number only or mean ± standard deviation. KPGP: Korean Personal Genome Project, AD: atopic dermatitis, AR: allergic rhinitis, AS: asthma, -: not available.
the exome library was carried out using the 100-bp PE mode of the HiSeq SBS kit.

**WES processing and alignment**

Millions of base sequence reads in FASTQ format were mapped to UCSC hg19 of the human assembly using the Burrows-Wheeler Aligner (BWA, v0.7.7)\(^6\) with "mem" and seed value parameters "-k 45" to create SAM files with correct mate-pair information for the genome. Picard (v1.92) was then used to convert the sequence alignment map (SAM) files to compressed binary alignment map (BAM) files, and then sort the BAM files by chromosome coordinates. The Genome Analysis Toolkit (v2.3.9Lite)\(^7\) was used for local realignment of the BAM files at intervals, corresponding to potential insertion and deletion alignment errors. Single-nucleotide polymorphisms (SNPs) and indels were annotated using snpEff (v3.6c; http://snpeff.sourceforge.net/index.html)\(^8\), which classified the variants.

**Annotation**

The dbNSFP is an integrated database of functional alteration predictions from multiple algorithms (scale invariant feature transform [SIFT], Polyphen2, PhyloP, PhastCons, 1000 Genome).

Filter 1: SnpEff annotates types of variants and predicts their effects on genomic regions (e.g., synonymous, non-synonymous, nonsense, missense, stop-gain, frameshift, point mutations, and indels).

Filter 2: A simple assessment of putative variant impact was done using SnpEff. (High: Frame_Shift, Start_Lost, Splice_Site, Stop_Gained; Moderate: Non_Synonymous_coding, Codon_Insertion and _Deletion, Low: Start_gained, etc.).

Filter 3: SIFT and Polyphen2

The probability that an amino acid substitution will affect protein function (SIFT score) was predicted based on the degree of amino acid conservation derived from related sequences through PSI-BLAST. The SIFT score ranges from 0 to 1; a value lower than 0.05 or not significant is considered detrimental, otherwise, it is considered tolerable.

The Polyphen2 HDIV score was based on HumDiv, i.e., hdiv_pred ranged from 0 to 1, and categorized as "probably damaging, 0.957 ∼ 1", "possibly damaging, 0.453 ∼ 0.956", and "benign, 0 ∼ 0.452."

Filter 4: The PhyloP of dbNSFP reflects the score of evolutionary conservation sites under negative or positive selection over branches of the phylogenetic tree. A higher score indicates a more conserved site (http://varianttools.sourceforge.net/Annotation/DbNSFP). PhastCons scores contain multiple alignments of 99 vertebrate genomes to the human genome and predict conserved elements. A high score (>0.2) represents a functionally important genomic region.

Filter 5: The 1000 Genome Project was design to select variants with minor allele frequencies (MAFs, less than 0.01 or unknown) in the global population.

Filter 6: The in-house Korean variation database at the Theragen Etex Bio Institute was used to select variants (MAFs, less than 0.02 or unknown).

**Sanger sequencing**

Polymerase chain reaction (PCR) amplification conditions were as follows: 95°C for 10 minutes, followed by 35 cycles at 95°C for 30 seconds, 55°C ∼ 58°C for 30 seconds, and 72°C for 40 seconds, with a final extension at 72°C for 1 minutes 30 seconds. The PCR mix (50 μl) contained 25 μl of 2× EF-Taq premix (SolGent, Seoul, Korea), 18 μl of distilled water, 2.5 μl of oligonucleotide primer (10 pmol/μl), and 2 μl of template containing 20 ng genomic

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**Table 2. Clinical characteristic of subjects in Sanger sequencing**

| Variable                  | Control          | AD   | AM   | p-value* |
|---------------------------|------------------|------|------|----------|
| Total                     | 81               | 87   | 72   |          |
| Age (yr)                  | 27.2 ± 12.6      | 28.8 ± 9.2 | 24.9 ± 9.6 |          |
| Male/female               | 52/29            | 47/40| 47/25|          |
| Total immunoglobulin E (KU/L) | -               | 1,430 ± 1,670 | 1,833 ± 1,888 | 0.1620\(^1\) |
| Eosinophils (mm\(^3\))   | -                | 459.4 ± 377.8 | 449.4 ± 376.1 | 0.8701\(^1\) |
| Clinical phenotype        | AD               | 87   | 59   |          |
| AD + AR                   | -                | 2    | 6    |          |
| AD + AS                   | -                | 2    | 2    |          |

Values are presented as number only or mean ± standard deviation. AD: atopic dermatitis, AM: atopic march, AR: allergic rhinitis, AS: asthma, AC: allergic conjunctivitis, -: not available. *By student's t-test between AD and AM, \(^1\)DOCK8, IL17RA, and KLK12 polymorphisms in atopic dermatitis.
DNA. The PCR products were purified, using a PCR purification kit (Favorgen, Pingtung, Taiwan), and sequenced on an Applied Biosystems 3500 DNA sequencer (Applied Biosystems, Foster City, CA, USA), according to the manufacturers’ instructions.

**Statistical analysis**

Using logistic regression analysis, crude odds ratios (ORs), 95% confidence intervals (CIs), and associations between AD and the SNPs under investigation, were calculated. Associations between polymorphisms (rs529208, rs12484684, and rs3745540) and AM were assessed using Fisher’s exact test. To compare total IgE and eosinophil count among AD and the SNPs under investigation, were calculated. The significance level was set at p-value < 0.05.

**RESULTS**

**Whole-exome sequencing in 40 AD**

WES was conducted to identify candidate variants that are processed to AM. The control KPGP group data were compared to AD at the Theragen Etex Bi institute. KPGP is a reference group, and there is no clinical information for AD (Table 1). To overcome this limitation, we carried out a large-cohort study to validate extracted variants, using Sanger sequencing (Table 2).

After DNA shearing, variant type identification excluding meaningless variants proceeded through the six-stage filtering process (Supplementary Fig. 1). To identify susceptible variants for AD and AM, fast and convenient filters for dominant, recessive, and compound hetero models were used. Several variants were filtered out, and conserved and functional variants remained in filters 5 to 6. All variants were counted after each filtering step (Supplementary Table 1).

We compared raw exome sequencing data with the difference between 40 AD cases and 43 KPGP control group. Considerable numbers of missense SNPs that influence a codon via amino acid substitution were observed in filter 1 (Supplementary Table 1). We focused on common functional variants (MAF greater than 1%) in filter 4, rare variants (MAF lower than 1%) for global population in filter 5 and rare variants (MAF lower than 2%) for Koreans in filter 6. Among these filtered candidate genes, functions related to allergic skin disease were inferred through a literature search. DOCK8, IL17RA, and KLK12 SNPs were identified as missense mutations: c.1289C>G, A, p.P97T (rs529208); c.1685C>A, p.P562G (rs12484684); and c.457+27>C, rs3745540, respectively. DOCK8 (rs529208) was located in chromosome 9, and IL17RA (rs12484684) was identified in chromosome 22. KLK12 (rs3745540), a splice donor variant that is located in RNA splicing site in intron region, was located in chromosome 19. Low SIFT scores of less than DOCK8 (0.05) and IL17RA (0.03) variants predicted high protein damage via amino acid substitution. The polyphen2 scores close to 1 in the DOCK8 (0.996) and IL17RA (1.0) variants predicted harmful effects on protein structure due to substitution. The Phastcons scores of the DOCK8 (3.149) and IL17RA (4.583) variants indicated that two sites were evolutionarily conserved in multiple alignments of 99 vertebrate genomes to the human genome. The phastcons scores of the DOCK8 (0.975) and IL17RA (0.03) variants showed strong conservation in evolutionary events. KLK12 (rs3745540) is splice donor variant that is located in RNA splicing site and predicted to be fatal impact in filter 2. There are no SIFT and Polyphen2 scores because it is an intron lesion. About 53% to 75% (DOCK8), 11% to 33% (IL17RA), and 53% to 65% (KLK12) MAF were identified in 1,000 global, 286 East Asians, and 800 Koreans, respectively (Table 3).

**Sanger sequencing in 81 control, 87 AD, and 72 AM**

A case-control study was performed to determine whether DOCK8 (rs529208), IL17RA (rs12484684), and KLK12 (rs3745540) are candidate risk factors for only AD and AM. Eighty-seven patients aged greater 18 years with adult-onset AD, without an early-onset, and 81 control subjects, 72 atopic march patients were enrolled. A total IgE level of 1,430±1,670 KU/L, and a total eosinophil level of 459.4±377.8 mm³ for AD patients, and 1,833±1,888 KU/L, and 449.4±376.1 mm³ for AM patients were recruited in this study (Table 2).

Sanger sequencing was carried out, and the genotype and allele frequencies of Koreans were determined by direct variant counting using the BioEdit software. The A minor alleles of IL17RA (rs12484684) increased from 26% to 36% in AD compared to the control group. The genotype (GG + AG) of KLK12 (rs3745540) increased from 83% to 93% in AD compared to the control group. The minor allele frequency of DOCK8 (rs529208) showed no significant differences among AD, AM and control groups (Table 4).

ORs with 95% CI were estimated for individual risk alleles. The rs529208 of DOCK8 showed no correlation with AD and AM in any of the allele and genotype (dominant and recessive) models (Table 5).

The rs12484684 of IL17RA increased risk of adult-onset AD (OR, 1.63; 95% CI, 1.02–2.62; p = 0.041) compared to the control for the A allele frequency. The AA + CA genotype was also associated with a trend towards an increased risk of AD with an OR of 1.77 (95% CI, 0.95–3.29; p = 0.067; Table 6).
Table 3. Effects of DOCK8, IL17RA, and KLK12 SNPs and protein function prediction scores

| Gene   | RS#       | Chr | POS   | AAC*       | Type     | Poly-phen2† | PhyloP‡ | Phast-Cons§ | Global¶ | East Asian | Korean++ |
|--------|-----------|-----|-------|------------|----------|-------------|----------|-------------|---------|------------|----------|
| DOCK8  | rs529208  | chr9| 286593| P97T       | cSNP     | 0.05        | 0.996    | 3.149       | 0.975   | 0.529      | 0.721    | 0.746     |
| IL17RA | rs12484684| chr22| 17589794| P562G | cSNP     | 0.03        | 1.0      | 4.583       | 1       | 0.113      | 0.298    | 0.337     |
| KLK12  | rs3745540 | chr19| 51535130| -       | Splice donor variant | -         | 0.937    | 0.78        | 0.534   | 0.637      | 0.655    |           |

SNP: single-nucleotide polymorphisms, RS: reference SNP, Chr: chromosome, POS: position, AAC: amino acid changes, cSNP: SNP in coding regions, SIFT: scale invariant feature transform, †: not available. *Single-letter codes for amino acids. †Prediction scores for amino acid substitutions that affect protein function (damaging < 0.05, tolerance > 0.05; scores range from 0 to 1). ‡Prediction of the possible impact of amino acid substitutions (0.957 < probably damaging < 1, 0.453 < possibly damaging < 0.956, 0 < benign < 0.452; scores range from 0 to 1). §Prediction of conserved sites across species; a higher score indicates a more conserved site (values > 0). ¶Global frequency, variants with minor allele frequencies as low as 1% or with an unknown frequency (value < 0.01). **Korean frequency, variants with MAFs as low as 2% or in unknown genes (value > 0.02).

Table 4. Allele and genotype frequency of DOCK8, IL17RA, and KLK12 polymorphisms*

| Gene   | SNP       | Allele & genotype | Control | AD | AM |
|--------|-----------|-------------------|---------|----|----|
| DOCK8  | rs529208  | c.1289C>A         | C       | 44 (28) | 47 (28) | 38 (27) |
|        |           |                   | A       | 114 (72) | 121 (72) | 102 (73) |
|        |           |                   | Genotype | CC | 3 (4) | 6 (7) | 4 (6) |
|        |           |                   |         | CA | 38 (48) | 35 (42) | 30 (43) |
|        |           |                   |         | AA | 38 (48) | 43 (51) | 36 (51) |
| IL17RA | rs12484684| c.1685C>A         | C       | 119 (74) | 110 (64) | 99 (69) |
|        |           |                   | A       | 41 (26) | 62 (36) | 45 (31) |
|        |           |                   | Genotype | CC | 43 (54) | 34 (40) | 32 (44) |
|        |           |                   |         | CA | 33 (41) | 42 (49) | 35 (49) |
|        |           |                   |         | AA | 4 (5) | 10 (12) | 5 (7) |
| KLK12  | rs3745540 | g.51031874A>G     | A       | 62 (38) | 52 (30) | 57 (40) |
|        |           |                   | G       | 100 (62) | 122 (70) | 87 (60) |
|        |           |                   | Genotype | AA | 14 (17) | 6 (7) | 12 (17) |
|        |           |                   |         | AG | 34 (42) | 40 (46) | 33 (46) |
|        |           |                   |         | GG | 33 (41) | 41 (47) | 27 (38) |

Values are presented as number (%). SNP: single-nucleotide polymorphisms, AD: atopic dermatitis, AM: atopic march. *81 healthy controls, 87 adult-AD, and 72 AM patients.

rs3745540 of KLK12 was associated with AD in dominant model (Table 7). The GG+AG genotype showed a significantly increased risk of AD development (OR, 2.86; 95% CI, 1.04 ∼ 7.83, p = 0.041). However, there was no significant correlation when using a recessive model. The relationship between the genotype of three SNPs (rs529208, rs12484684, rs3745540) and the clinical characteristics (total IgE level and eosinophil count) of AD+ AM patients has been compared. Total IgE level was significantly increased in the AA genotype of DOCK8 compared to the CA genotype (p = 0.045). There was no association between genotypes and eosinophil count (Fig. 1). AD and AM Patients with the IL17RA CA genotype also had elevated IgE levels (Fig. 2) and were not associated with the total eosinophil count. The KLK12 variant was not related to the clinical characteristics of AD (Fig. 3).
**Table 5.** Odds ratios (ORs) and 95% confidence intervals (CIs) for AD associated with DOCK8 polymorphisms in an allele model

| DOCK8 rs529208 | Allele | Genotype (dominant) | Genotype (recessive) |
|----------------|--------|---------------------|---------------------|
|                | Minor allele | Major allele | OR (95% CI) | p-value | Case | Control | OR (95% CI) | p-value | Case | Control | OR (95% CI) | p-value |
| Con vs. AD     | A      | C              | 0.99 (0.61~1.61) | 0.97 | AA+CA | CC | 0.51 (0.12~2.13) | 0.36 | AA | CA+CC | 1.13 (0.61~2.09) | 0.7532 |
| Con vs. AM     | A      | C              | 1.03 (0.62~1.72) | 0.89 | AA+CA | CC | 0.65 (0.14~3.01) | 0.58 | AA | CA+CC | 1.14 (0.60~2.17) | 0.68 |
| Con vs. AD+AM  | A      | C              | 1.01 (0.66~1.55) | 0.95 | AA+CA | CC | 0.57 (0.15~2.13) | 0.4 | AA | CA+CC | 1.13 (0.66~1.95) | 0.64 |
| AD vs. AM      | A      | C              | 1.04 (0.63~1.72) | 0.87 | AA+CA | CC | 1.04 (0.63~1.72) | 0.87 | AA | CA+CC | 1.00 (0.53~1.90) | 0.98 |

Con: control, AD: atopic dermatitis, AM: atopic march.

**Table 6.** Odds ratios (ORs) and 95% confidence intervals (CIs) for AD associated with IL17RA polymorphisms in an allele model

| IL17RA rs12484684 | Allele | Genotype (dominant) | Genotype (recessive) |
|-------------------|--------|---------------------|---------------------|
|                   | Minor allele | Major allele | OR (95% CI) | p-value | Case | Control | OR (95% CI) | p-value | Case | Control | OR (95% CI) | p-value |
| Con vs. AD        | A      | C              | 1.63 (1.02~2.62) | 0.041* | AA+CA | CC | 1.77 (0.95~3.29) | 0.067 | AA | CA+CC | 2.5 (0.75~8.31) | 0.135 |
| Con vs. AM        | A      | C              | 1.31 (0.8~2.17) | 0.277 | AA+CA | CC | 1.43 (0.76~2.75) | 0.25 | AA | CA+CC | 1.41 (0.36~5.49) | 0.613 |
| Con vs. AD+AM     | A      | C              | 1.48 (0.97~2.27) | 0.067 | AA+CA | CC | 1.62 (0.94~2.78) | 0.08 | AA | CA+CC | 1.99 (0.64~6.21) | 0.23 |
| AD vs. AM         | A      | C              | 0.80 (0.50~1.29) | 0.369 | AA+CA | CC | 0.81 (0.43~1.54) | 0.32 | AA | CA+CC | 0.57 (0.18~1.74) | 0.32 |

Con: control, AD: atopic dermatitis, AM: atopic march. *Statistically significant (p<0.05).

**Table 7.** Odds ratios (ORs) and 95% confidence intervals (CIs) for AD associated with KLK12 polymorphisms in an allele model

| KLK12 rs3745540 | Allele | Genotype (dominant) | Genotype (recessive) |
|-----------------|--------|---------------------|---------------------|
|                 | Minor allele | Major allele | OR (95% CI) | p-value | Case | Control | OR (95% CI) | p-value | Case | Control | OR (95% CI) | p-value |
| Con vs. AD      | G      | A              | 1.45 (0.92~2.28) | 0.1 | GG+AG | AA | 2.86 (1.04~7.83) | 0.041* | GG | AG+AA | 1.32 (0.72~2.44) | 0.36 |
| Con vs. AM      | G      | A              | 0.95 (0.6~1.5) | 0.81 | GG+AG | AA | 1.04 (0.45~2.43) | 0.92 | GG | AG+AA | 0.87 (0.46~1.67) | 0.682 |
| Con vs. AD+AM   | G      | A              | 1.2 (0.81~1.77) | 0.36 | GG+AG | AA | 1.64 (0.77~3.51) | 0.195 | GG | AG+AA | 1.10 (0.64~1.90) | 0.723 |
| AD vs. AM       | G      | A              | 0.64 (0.40~1.02) | 0.06 | GG+AG | AA | 0.36 (0.13~1.03) | 0.056 | GG | AG+AA | 0.66 (0.34~1.24) | 0.194 |

Con: control, AD: atopic dermatitis, AM: atopic march.

**DISCUSSION**

FLG protein has an essential role in normal skin barrier function and epidermal homeostasis. Loss-of-function genetic mutation and copy-number variant in the FLG gene disrupts the integrity of the epidermal barrier that helps the development of AD. FLG mutation is a higher risk factor for AD. There are several FLG genetic studies in various ethnic groups. Our target was to find other genetic mutations that can affect skin barrier dysfunction and the immune system.

Several susceptibility loci have been identified in GWAS\(^1^9\), so finding a novel genetic variant for AD using exome sequencing is essential. AD is a common and complex disease involving environmental factors, thus, finding a rare variant that directly relates to disease is difficult. The com-
Fig. 1. The relationship of the DOCK8 polymorphism with total immunoglobulin E (IgE) and eosinophil counts in atopic dermatitis + atopic march patients. Dot-plot graphs showing total IgE levels (A) and eosinophil counts (B) for the CC, CA, and AA alleles. Values are presented as mean ± standard deviation. *p < 0.05 by Mann-Whitney U-test for statistical significance. NS: not significant.

Fig. 2. The relationship of the IL17RA polymorphism with total immunoglobulin E (IgE) and eosinophil counts in atopic dermatitis + atopic march patients. Dot-plot graphs showing total IgE levels (A) and eosinophil counts (B) for the CC, CA, and AA alleles. Values are presented as mean ± standard deviation. *p < 0.05 by Mann-Whitney U-test for statistical significance. NS: not significant.

Fig. 3. The relationship of the KLK12 polymorphism with total immunoglobulin E (IgE) and eosinophil counts in atopic dermatitis + atopic march patients. Dot-plot graphs showing total IgE levels (A) and eosinophil counts (B) for the AA, AG, and GG alleles. Values are presented as mean ± standard deviation. *p < 0.05 by Mann-Whitney U-test for statistical significance. NS: not significant.
common disease-common variant (CD-CV) hypothesis in the field of genetics was the first dominant theory. We focused on the common variants and genes related to skin immunity through a literature search. To detect candidate variants associated with AD and AM, allele frequency among groups and protein prediction scores were used through six whole-exome sequencing filtering steps before the population-based study. Functional sequences can be predicted by confirming evolutionary conserved elements across species. Our WES data suggest that rs529208 and rs12484684 are strongly conserved protein coding regions among 100 vertebrate species. The DOCK8 gene is a dedicator of cytokinesis 8 located in 9p24.3. The protein is an activator of small G protein involved in intracellular signaling networks. It was reported that DOCK8 deficiency associated with hyper-IgE syndrome impairs T cell activation. Increased Th2 and low Th1 signals, along with increased IgE levels, are found as early as in the cord blood of newborns with AD. Recent studies suggest an association between atopic phenotypes and serum IgE levels. Children with severe AD and high IgE levels are at risk for sensitization to food allergens and aeroallergens. Our study shows that a high IgE level was induced at the AA homo genotype of rs529208 compared to the CA hetero genotype in AD+AM patients. This represents the possibility of causing hyper-IgE in patients with the rs529208 genotype (AA) of DOCK8. The IL17RA gene is an interleukin-17 receptor A located in 22q11.1 and a key regulator of type 2 inflammatory responses, and it protects the skin barrier against allergic inflammation. IL17RA-deficiency mice have a defective skin barrier with altered Flg and increased TSLP expression aberrant skin inflammation. IL17RA-deficiency mice crossed with Flg mutation mice induce severe skin inflammation. Our data show that total IgE level was further increased in the CA hetero genotype than in the CC homo genotype IL17RA (rs12484684). Thus, this variant may affect exacerbation of AD. A more functional genetic study is necessary to validate its influence.

KLK12 is a kallikrein-related peptidase 12 protein located in 19q13.4 and a subgroup of serine proteases. Co-expression of various KLKs and SPINK5 messenger RNA is suspected to affect serine protease activities in the stratum corneum. KLK12 messenger RNAs in both normal human epidermal keratinocyte cells and skin tissue were not amplified by RT-PCR. The functional role of the KLK12 gene is limited in AD. However, it might be worth using variation as a tool for early-onset diagnosis of AD. Eosinophil counts of all three variants were not associated with AD and AM. Our data was limited as there was no clinical data for the 43 KPGP control group individuals. To overcome small sample size, the frequency of DOCK8, IL17RA, and KLK12 polymorphisms in Sanger sequencing was also compared with the frequency of 1,000 global, 286 East Asian, and 800 Korean subjects without clinical information. The 72% to 74% MAF of DOCK8 (rs529208) in the 286 East Asian and the 800 Koreans surveyed almost identical that of the 82 health controls (72% MAF) in Sanger sequencing. Other variants (IL17RA and KLK12) also nearly matched as well (Supplementary Table 2). These data demonstrate the credibility of the data despite the small study size.

Taken together, three novel AD candidate variants, DOCK8 (rs529208), IL17RA (rs12484684), and KLK12 (rs3745540), were identified using a new WES filtering method. The high PolyPhen and evolutionary conservation PhyloP scores of these two SNPs predict deleterious protein function. Although the relationship with AM was not discovered in this study, our case-control study suggests that polymorphism of DOCK8 and IL17RA might be related to increase the total IgE level. The A minor allele of IL17RA allele and the (GG+AA) KLK12 genotype seem to enhance the risk of AD. In conclusion, three novel AD candidate variants, DOCK8 (rs529208), IL17RA (rs12484684), and KLK12 (rs3745540), were identified using a new WES filtering method. Although the relationship with AM was not discovered in this study, the result suggests that polymorphism of DOCK8 and IL17RA might be related to increase the total IgE level.

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SUPPLEMENTARY MATERIALS

Supplementary data can be found via http://anndermatol.org/src/sm/ad-32-197-s001.pdf.

CONFLICTS OF INTEREST

The authors have nothing to disclose.
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