

Medical genetics

Identification of candidate genes in atopic dermatitis based on bioinformatic methods

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Abstract

Background Atopic dermatitis (AD) is a chronic or relapsing inflammatory disorder of the skin that frequently precedes asthma and allergic disorders. This study aimed to identify candidate genes related to AD using bioinformatic methods.

Methods The microarray data of GSE32924, including 12 nonlesional AD (ANL) and 13 lesional AD (AL) skin samples obtained from 14 patients with AD as well as eight other normal human skin samples, was downloaded from the Gene Expression Omnibus database. Differentially expressed genes (DEGs) were screened in ANL and AL skin samples compared with normal controls, followed by gene ontology (GO) and pathway enrichment analyses. Furthermore, the selected overlapping DEGs were analyzed to identify co-expressed genes, and a co-expression network was established. GeneCodis database was selected for functional annotation of the differentially co-expressed genes and a regulatory network was constructed.

Results Compared with normal controls, 438 DEGs were identified in ANL skin samples and were mainly enriched in two pathways and the GO terms associated with epidermis development. Besides, 779 DEGs were identified in AL skin samples and were mainly enriched in four pathways and GO terms associated with immune response. Stimulated by retinoic acid 13 (*STRA13*), presenilin enhancer gamma secretase subunit (*PSENEN*), and nucleosome assembly protein 1-like 2 (*NAP1L2*) were significantly enriched by integration analysis of the co-expression and regulatory network.

Conclusion Genes *STRA13*, *PSENEN*, and *NAP1L2* were presumed to play critical roles in AD and they may serve as potential targets for the treatment of AD.

Highlights

1 *STRA13* may be essential for the progression of atopic dermatitis by interacting with hsa-miR-148b and hsa-miR-152.

2 *PSENEN* may play a crucial role in the progression of atopic dermatitis by participating in the Notch signaling pathway.

3 *NAP1L2* may play an important role in the development and progression of atopic dermatitis.

Introduction

Atopic dermatitis (AD) is an inflammatory, pruritic, and chronically relapsing skin disease with a wide range of severity that often occurs in families with atopic diseases.¹ It affects approximately 10–20% of children and 1–3% of adults, and its prevalence has been reported to

have an increase particularly in the industrialized countries,² which may in part be explained by an increased allergen exposure.³ There is an increasing body of evidence suggesting that use of modern fuels and higher levels of traffic-related particles may contribute to the increase of exposure to allergens.⁴ AD places significant impairment in the patients' life quality and a heavy economic burden on society. However, the current diagnosis of AD is most based on a constellation of clinical findings such as pruritus, eczema, and chronicity of the dermatitis.⁵ The regimens of AD therapy are still awaiting validation.⁶ Therefore, getting further insights into the understanding of the mechanisms underlying AD and developing more targeted therapy are crucial for the development of more effective treatment strategies.

The pathogenesis of AD has been ascribed to a complex interaction of the environment and host susceptibility genes, skin barrier dysfunctions, immune system or allergy,

and pruritus.^{1,7} Recently, Kabashima had demonstrated that skin barrier abnormality, allergy/immunological dysregulation, and pruritus were a highly complex interplay as a trinity and created a positive feedback loop in the development of AD.⁸ Moreover, Wollenberg and Bieber revealed that the normal-looking, nonlesional AD (ANL) skin was not normal and was characterized by clinically meaningful barrier dysfunction and a subclinical eczematous skin reaction.⁹ To date, a number of candidate gene studies using traditional approaches such as candidate gene association studies or the new genome-wide approach have been used to identify genes associated with AD.¹⁰ Palmer *et al.* found that common loss-of-function mutations of the epidermal barrier protein filaggrin (*FLG*) were a major predisposing factor for AD.¹¹ Additionally, Jensen *et al.* revealed that the proliferation marker (keratin 16) in association with altered expression of terminal differentiation proteins such as *FLG* had increased expression in ANL skin.¹² MicroRNAs (miRNAs), ~22 nt noncoding RNAs, which can regulate target gene expression at their post-transcriptional level through translational repression and/or degradation, act as key regulators in a wide variety of biological processes.¹³ Moreover, Sonkoly *et al.* found that miR-155 was significantly overexpressed in patients with AD and might contribute to chronic skin inflammation through decreasing cytotoxic T-lymphocyte-associated antigen 4 (*CTLA-4*) expression to increase the proliferative response of T-helper cells.¹⁴ However, studies have yielded few useful candidate genes, and our understanding about the regulatory relationships underlying the altered expression of genes remains largely unknown. Thus, further investigations are needed.

Recently, Suárez-Fariñas *et al.* discovered a high correlation in expression of immune genes in ANL skin with the disease extent and severity in patients with AD and demonstrated that systemic immune activation might play a significant role in the alteration of the normal epidermal phenotype in patients with AD.¹⁵ In the present study, we performed microarray analysis to identify the differentially expressed genes (DEGs) in lesional AD (AL) skin and ANL skin compared with the normal human controls using the same microarray data as Suárez-Fariñas *et al.* Furthermore, functional and pathway enrichment analyses upon the DEGs were performed, and a co-expression network as well as a regulatory network based on the overlapping DEGs identified in both AL and ANL skin samples was constructed to find the critical candidate genes in AD. We sought to identify candidate genes that might be involved in the pathogenesis of AD based on functional plausibility and their potential role in the development and progression of AD.

Materials and methods

Microarray data

The microarray data of GSE32924 deposited by Suárez-Fariñas *et al.*¹⁵ was downloaded from the National Center of Biotechnology Information Gene Expression Omnibus database (<http://www.ncbi.nlm.nih.gov/geo/>). The platform is GPL570 (HG-U133_Plus_2) Affymetrix Human Genome U133 Plus 2.0 Array. A total of 33 samples were applied, including 12 ANL and 13 AL skin samples obtained from 14 patients with AD (paired samples; for some patients, only one sample was available), and eight other normal skin samples from eight healthy volunteers. As described in the original study,¹⁵ the samples were 4–6 mm skin biopsies that were collected from patients with AD or healthy volunteers. Besides, the ANL skin samples were obtained from at least 10 cm away from any active lesion in patients with AD.

Data preprocessing and differentially expressed genes screening

Data preprocessing is a process that allows transformation of the raw fluorescence signal identified by microarray staining into a signal normalized for reducing experimental errors and thus improves both sensitivity and specificity of the microarray analysis.¹⁶ The main steps of preprocessing included background subtraction, experiment normalization, and transcript intensity summarization.¹⁶ The affy package in R language can be used for the storage, management, and analysis of the data at the Affymetrix probe level.¹⁷ Separate processing steps in the affy package consist of background correction, normalization, and summary expression value computation.¹⁷

In this study, the raw data (CEL files) retrieved from the Gene Expression Omnibus database were preprocessed using the affy package.¹⁷ After preprocessing, the gene expression matrix of specimens as an $m \times n$ data matrix was received, with columns corresponding to gene list and rows corresponding to sample list. DEGs were screened in ANL skin group and AL skin group compared with normal controls using the *t*-test¹⁸ in limma package¹⁹ in R, respectively. The raw *P* values were adjusted by Bonferroni method²⁰ for multiple testing correction. On the other hand, the fold change (FC) is also used to analyze whether the identified genes were differentially expressed. The FC is expressed as a $\log_2$ value that is in keeping with the microarray output format.²¹ To complete the statistical significance of a gene's differential expression evaluation, we attempted to get reliable lists of useful DEGs by simultaneously filtering our results on corrected *P* value and a cutoff of absolute $\log_2$ FC. The selection of cutoff criteria is based upon the usefulness of the data in hand and the results obtained for the following analysis. In this study, thresholds for DEGs were set as corrected $P < 0.05$ and $|\log_2 \text{FC}| > 1$. The overlapping

DEGs that were found in both the ANL and AL skin groups were identified.

Enrichment analysis of differentially expressed genes

Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses for DEGs identified in the ANL and AL skin groups were performed using the Database for Annotation, Visualization, and Integrated Discovery online tool (<http://david.abcc.ncifcrf.gov/>).²² For GO enrichment analysis, the false discovery rate (FDR) was applied using the Benjamini–Hochberg method²³ to circumvent the multiple testing problem that might induce too many false positive results. The selection of FDR was based on the magnitude of observed GO terms. In this study, we found that GO terms were initially enriched at a large number, and FDR <0.05 was useful to achieve more accurate results. In total, the GO terms that enriched by more than 10 genes and at FDR <0.05 were considered significant. However, we found that a relatively low number of KEGG pathways were enriched. After the repeated test, $P < 0.05$ was chosen as the cut-off criterion for KEGG enrichment analysis.

Co-expression network construction

To optimize the DEGs, the overlapping DEGs found in both the ANL and AL skin groups were applied to identify the differentially co-expressed genes in those skin groups. The DCGL package (<http://cran.r-project.org/web/packages/DCGL/index.html>) is a robust statistical method, which is released as an R package that included five differential co-expression analysis methods, two gene filtering functions, and three link filtering functions.²⁴ The gene co-expression analysis weights links with the correlation coefficient (cor) value, which ranges from -1 to 1 , indicating the contribution of negative and positive connectivity. The higher the $|cor|$ is, the more likely to prove the co-expression relationship of genes. In our study, the differentially co-expressed genes in both the ANL and AL skin groups were identified using the DCGL package. To get the most robust or reliable lists of differentially co-expressed genes based on the data in hand and the results obtained, the pairs of differentially co-expressed genes with $|cor| > 0.5$ were considered significant. All the differentially co-expressed gene pairs were imported into the Cytoscape plugin²⁵ to create co-expression network visualizations.

Construction of regulatory network

GeneCoDis (<http://genecodis2.dacya.ucm.es/>) is used for the functional analysis of gene lists and allows the integrated annotation analysis from different sources (for example, Swiss-Prot keywords, KEGG pathways, InterPro motifs, and GO) and can generate statistical rank scores for one annotation or their combinations.²⁶ In our study, function enrichment analysis for the differentially co-expressed genes was performed using GeneCoDis.²⁶ The significance (P value) of the enrichment was

calculated using the hypergeometric distribution, and the multitest problem was corrected via controlling the FDR using the Benjamini–Hochberg method.²³ A corresponding regulatory network was constructed with the differentially co-expressed genes and the enriched miRNAs as well as transcriptional factors identified by function enrichment analysis.

Candidate genes and regulatory factors screening

All the node degrees in the co-expression network and the regulatory network were calculated. The genes were ranked by the product of the node degree in two networks. The DEGs that had a higher node degree product were considered more significant. Moreover, corresponding regulatory factors of the DEGs were deemed important in the AD development and progression.

Results

Differentially expressed genes screening

A schematic flow diagram outlining the strategy for the screening of candidate genes in the mechanism of AD in this study is shown in Figure 1. The results of DEG screening are shown in Figure 2. A total of 438 DEGs were identified in the ANL skin sample group compared with their normal human skin controls, including 161 upregulated genes and 277 downregulated genes. A total of 779 DEGs were identified in the AL skin sample group compared with their normal human skin controls, including 395 upregulated genes and 384 downregulated genes. In total, 314 overlapping DEGs were found in the two gene groups.

Gene ontology and pathway enrichment analysis

The DEGs in the ANL skin sample group and the AL skin sample group were assigned various GO terms from each of the cellular component, biological process, and molecular function ontologies. Seven GO terms were enriched for DEGs in the ANL skin sample group (Table 1). GO enrichment analysis revealed that the DEGs in the ANL skin were strongly correlated with epidermis development. Furthermore, 20 GO terms were enriched for DEGs in the AL skin sample group (Table 2). The results showed that DEGs in the AL skin were mainly associated with immune response, cell cycle, and cell differentiation. On the other hand, two KEGG pathways were significantly enriched for DEGs in the ANL skin sample group, including sphingolipid metabolism and arachidonic acid metabolism. Thereinto, the sphingolipid metabolism pathway was enriched by five DEGs, including sphingosine-1-phosphate phosphatase 1 (*SGPP1*), alkaline ceramidase 1 (*ACER1*), UDP-glucose ceramide glucosyltransferase (*UGCG*), UDP glycosyltransferase 8 (*UGT8*), delta (4)-desaturase, and sphin-

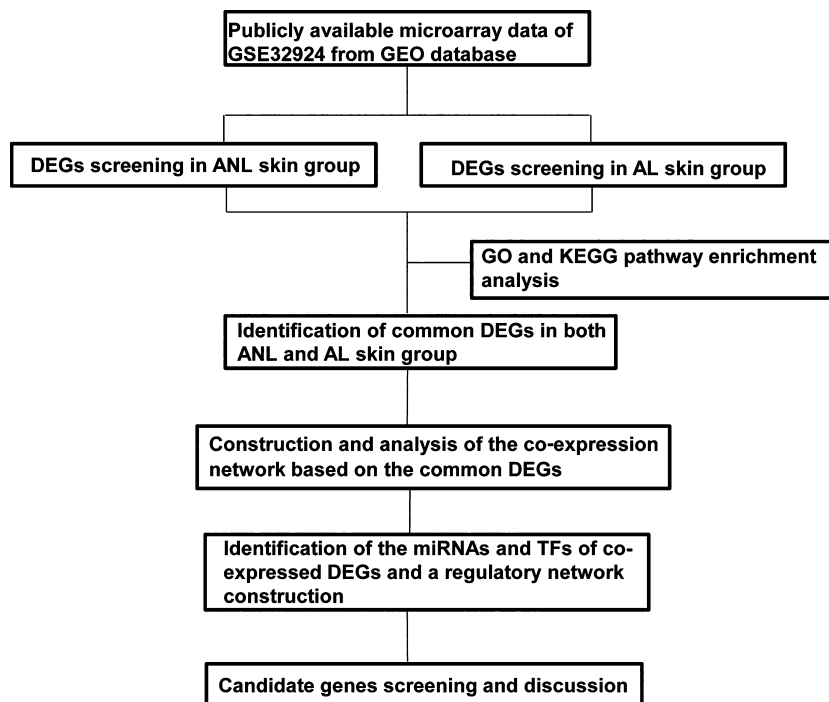


Figure 1 Flow diagram of gene expression profiling. AD, atopic dermatitis; AL, lesional AD; ANL, nonlesional AD; DEGs, differentially expressed genes; GEO, Gene Expression Omnibus; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; TFs, transcriptional factors

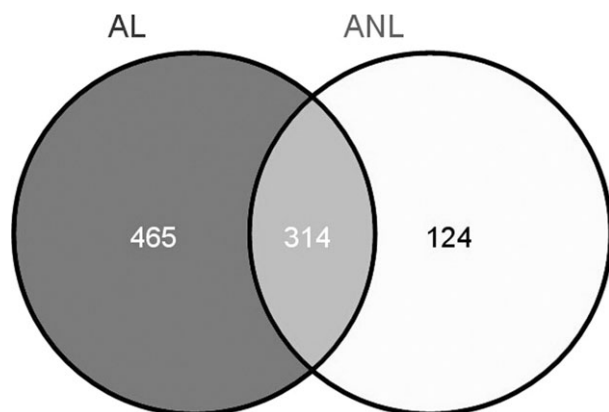


Figure 2 Identification of differentially co-expressed genes. AD, atopic dermatitis; AL, lesional AD; ANL, nonlesional AD

golipid 1 (*DEGS1*). Arachidonic acid metabolism was also enriched by five DEGs, including glutathione peroxidase 2 (*GPX2*), cytochrome P450, family 2, subfamily J, polypeptide 2 (*CYP2J2*), cytochrome P450, family 2, subfamily C, polypeptide 18 (*CYP2C18*), prostaglandin-endoperoxide synthase 1 (*PTGS1*), and hematopoietic prostaglandin D synthase (*HPGDS*). Moreover, four KEGG pathways were significantly enriched for DEGs in the AL skin sample group as shown in Table 3, including arachidonic acid metabolism, cell adhesion molecules, NOD-like receptor signaling pathway, and cytokine-cytokine receptor interaction.

Table 1 The enriched GO terms of differentially co-expressed genes in nonlesional atopic dermatitis skin samples

Category	GO ID	GO name	Gene no.	FDR
BP	GO:0008544	Epidermis development	26	3.63E-10
BP	GO:0007398	Ectoderm development	26	2.21E-09
BP	GO:0009913	Epidermal cell differentiation	13	7.26E-05
CC	GO:0005576	Extracellular region	81	3.45E-04
BP	GO:0030216	Keratinocyte differentiation	11	0.002474
BP	GO:0030855	Epithelial cell differentiation	15	0.002827
MF	GO:0030414	Peptidase inhibitor activity	14	0.024524

BP, biological process; CC, cellular component; FDR, false discovery rate; GO, gene ontology; MF, molecular function.

Differentially co-expressed gene identification and co-expression network construction

The differentially co-expressed genes and interactions between them were presented in the network as shown in Figure 3. The result showed that 76 differentially co-expressed genes were identified and there were 66 co-expressed gene pairs. Among them, chromosome 14 open reading frame 132 (*C14orf132*) (degree = 21) and

Stimulated By Retinoic Acid 13 (*STRA13*) (degree = 8) were identified as hub nodes in the co-expression network.

Regulatory network of construction and important genes or regulatory factors selection

Figure 4 represents the regulatory network. The result showed that there were 853 regulatory pairs made up by

Table 2 The enriched GO terms of differentially co-expressed genes in lesional atopic dermatitis skin samples

Category	GO ID	GO name	Gene no	FDR
BP	GO:0007051	Spindle organization	14	2.56E-05
CC	GO:0044421	Extracellular region part	78	2.42E-05
BP	GO:0000279	M phase	37	9.10E-05
BP	GO:0000280	Nuclear division	29	1.23E-04
BP	GO:0007067	Mitosis	29	1.23E-04
BP	GO:0000087	M phase of mitotic cell cycle	29	1.81E-04
BP	GO:0048285	Organelle fission	29	2.91E-04
BP	GO:0006955	Immune response	58	3.29E-04
BP	GO:0022403	Cell cycle phase	40	0.001321
CC	GO:0005576	Extracellular region	127	0.001268
BP	GO:0000278	Mitotic cell cycle	37	0.001661
BP	GO:0006952	Defense response	51	0.003084
BP	GO:0051301	Cell division	31	0.005539
BP	GO:0007049	Cell cycle	58	0.013528
CC	GO:0031012	Extracellular matrix	34	0.011353
CC	GO:0005615	Extracellular space	54	0.014252
BP	GO:0022402	Cell cycle process	45	0.038862
BP	GO:0000226	Microtubule cytoskeleton organization	19	0.045872
BP	GO:0009913	Epidermal cell differentiation	13	0.048796
CC	GO:0005578	Proteinaceous extracellular matrix	31	0.042639

BP, biological process; CC, cellular component; FDR, false discovery rate; GO, gene ontology.

182 miRNAs, nine transcriptional factors, and 60 other differentially co-expressed genes.

The node degree in the co-expression and the node degree in the regulatory network were calculated. The node degree product of differentially co-expressed genes that were found in both the two networks was ranked (data not shown). Genes with higher node degree product were considered more significant. We chose the top 19 gene nodes that had the node degree product of more than 20 as our potential targets (Table 4). Thereinto, *STRA13*, pseudouridylate synthase-like 1 (*PUSL1*), pre-nilin enhancer gamma secretase subunit (*PSENEN*), and nucleosome assembly protein 1-like 2 (*NAP1L2*) had the higher node degree. Furthermore, *STRA13* was regulated by 28 miRNAs in the regulatory network, such as hsa-miR-148b, hsa-miR-152, and hsa-miR-324.

Discussion

AD is increasingly common in developed countries and, nowadays, is a major cause of morbidity.²⁷ Identification of the genetic variants underlying AD may lead to new treatments for patients with AD. In this study, a total of 438 DEGs were identified in ANL skin samples compared with normal controls and they were mainly enriched in GO terms associated with epidermis development and two pathways, including sphingolipid metabolism and arachidonic acid metabolism. Moreover, a total of 779 DEGs were identified in AL skin samples compared with normal controls, and they were mainly enriched in GO terms associated with the immune response and four pathways, including arachidonic acid metabolism. The 314 overlapping DEGs were identified in both the ANL and AL skin samples. Genes *STRA13*, *PUSL1*, *PSENEN*, and *NAP1L2* were significantly enriched by integration analysis of the co-expression network and the regulatory network. Furthermore, *STRA13* was regulated by 28 miRNAs in the regulatory network, such as hsa-miR-148b, hsa-miR-152, and hsa-miR-324.

Table 3 The enriched pathways of differentially co-expressed genes in lesional atopic dermatitis skin samples

Pathway name	Gene no.	P value	Genes
Arachidonic acid metabolism	8	0.011301	AKR1C3, GPX2, CYP2J2, PTGS1, EPHX2, PLA2G3, HPGDS, ALOX12
Cell adhesion molecules	13	0.013778	ICAM3, CDH3, CLDN23, SDC2, CD34, PVRL3, CD274, CLDN1, CD2, CNTN1, NEGR1, SELE, SELPLG
NOD-like receptor signaling pathway	8	0.01916	CXCL1, HSP90AB1, HSP90B1, CCL2, CARD18, PYCARD, BIRC3, TAB 2
Cytokine-cytokine receptor interaction	19	0.042084	CXCL1, CCL2, TNFRSF12A, IL7, CCR1, CCL19, TNFRSF14, IL7R, CCL18, CCL17, CCL26, CXCL10, IL12RB2, TNFRSF1A, CCL22, CCR7, IL10RA, PDGFC, BMP7

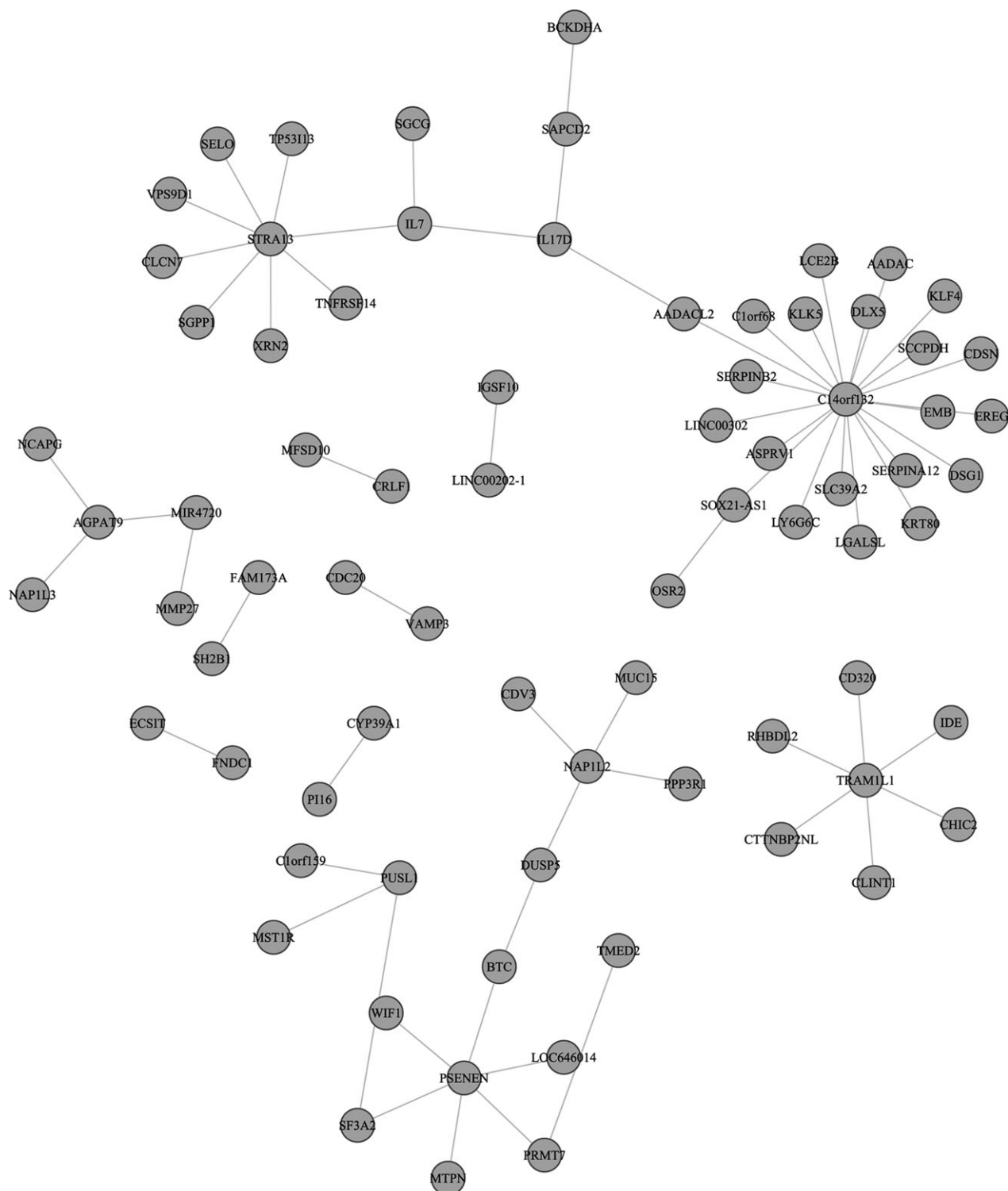


Figure 3 The co-expressed network for the differentially co-expressed genes

Mammalian skin is composed of two distinct layers, the underlying dermis and the overlying epidermis, which are divided by a basement membrane zone.²⁸ The

function of this skin barrier resides primarily in the extracellular lipid domains, and the metabolism of the unique sphingolipids such as glucosylceramide is reported to be

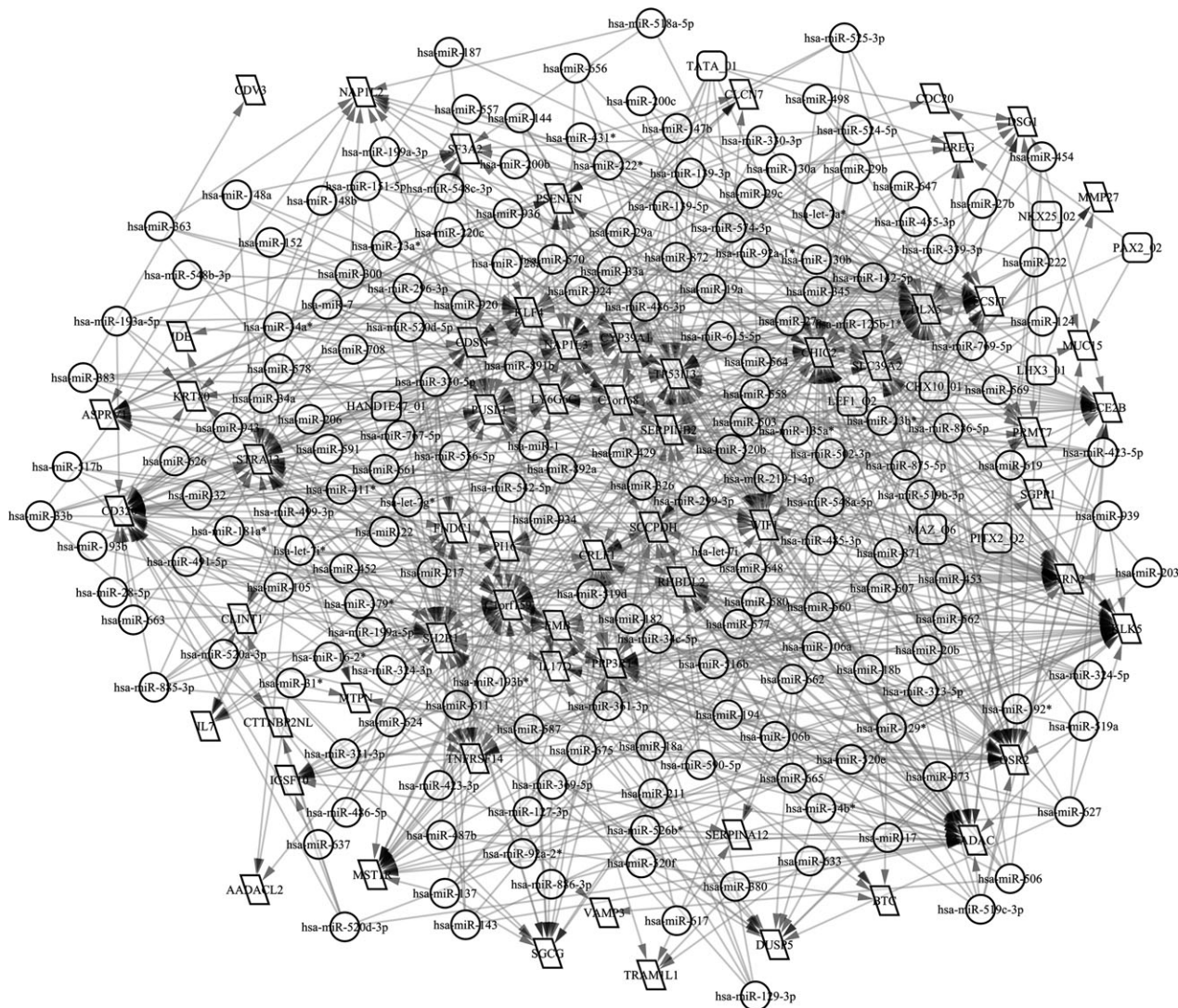


Figure 4 The regulatory network for the differentially co-expressed genes. Circular nodes represent miRNAs. Diamond nodes represent differentially co-expressed genes. Square nodes indicate transcription factors

Table 4 The genes with higher node degree product

Gene	Degree	Gene	Degree
STRA13	224	OSR2	30
PUSL1	66	XRN2	29
PSENEN	60	ECSIT	29
NAP1L2	44	TP53I13	28
AADAC	39	SH2B1	26
CD320	36	DUSP5	24
DLX5	36	TRAM1L1	24
KLK5	35	NAP1L3	22
CHIC2	34	IL17D	21
C1orf159	34		

associated with common skin disorders such as AD and psoriasis.²⁸ Hara *et al.* demonstrated that high expression of sphingomyelin deacylase was a crucial determinant of ceramide deficiency resulting in barrier disruption in AD.²⁹ On the other hand, as one of the body's essential fatty acids, arachidonic acid is required by most mammals.³⁰ The eicosanoids, the metabolites of arachidonic acid, comprise several lipid signaling mediators that play an essential role in cellular signaling cascades of pathophysiological and physiological relevance.³⁰ Furthermore, Weise *et al.* found that dietary docosahexaenoic acid in combination with arachidonic acid significantly ameliorated allergen-induced dermatitis in mice.³¹ In line with

the previous studies, our results revealed that pathways of DEGs identified in ANL and AL skin samples were also related to sphingolipid metabolism or arachidonic acid metabolism. In this context, we illustrated that sphingolipid metabolism and the arachidonic acid metabolism pathway might play crucial roles in the molecular mechanisms of AD.

STRA13 is a novel member of the basic helix–loop–helix protein family, and it is a putative transcription repressor when fused to a DNA-binding domain.³² Sun *et al.* had demonstrated that *STRA13* could act as a key regulator of lymphocyte activation, which was important for the maintenance of self-tolerance and for the constraint of immunity.³³ In addition, Boguniewicz and Leung revealed that AD was a disease of an abnormal skin barrier and immune dysregulation with activation of complex immunologic and inflammatory pathways.¹ Besides, Liu *et al.* had demonstrated that miRNA-148/152 could act as a fine-tuner in regulating the innate response and modulating antigen-presenting capacity of dendritic cells, which might be conducive to the immune homeostasis and regulation.³⁴ Furthermore, our findings show that *STRA13* was a hub gene by using the combination analysis of the co-expression and regulatory networks, and *STRA13* could be regulated by hsa-miR-148b and hsa-miR-152 in the regulatory network (Fig. 3). Therefore, we suggested that *STRA13* might play an essential role in the development and progression of AD through interactions with hsa-miR-148b and hsa-miR-152.

PUSL1 shares homology with the pseudouridylate synthase genes such as pseudouridylate synthase 1 (*PUS1*), which can modify RNA through converting uridine to pseudouridine, a glycosylated form of uracil.³⁵ However, there is rare research on *PUSL1* or *PUS1* associated with AD or related functions. In the present study, we identified that *PUSL1* had a higher degree based on the calculation of node degrees in the co-expression network and regulatory network. It is tempting to speculate that *PUSL1* may play a role in the development and progression of AD, while further validation is warranted.

PSENEN encodes an essential subunit of the γ -secretase complex, which is needed for intramembranous processing of several type I transmembrane proteins, such as the beta-amyloid precursor protein and Notch proteins.³⁶ Rasul *et al.* showed that Notch signaling was initiated by interactions between the cell surface ligands and plasma-embedded Notch heterodimer receptors and resulted in a conformational change in Notch, which was required for recognition and cleavage by the γ -secretase complex.³⁷ Furthermore, Dumortier *et al.* found that AD-like disease arose from loss of Notch signaling in the murine skin.³⁸ In agreement with previous studies, we found that

PSENEN was another hub gene by using the combination analysis of the co-expression and regulatory networks (Table 4). Taken together, we suggested that *PSENEN* might play a crucial role in the development and progression of AD via participation in the Notch signaling pathway.

NAP1L2 encodes a member of the nucleosome assembly protein family.³⁹ Studies had showed that the interaction of proliferating cell nuclear antigen (*PCNA*) and CCR4-associated factor 1 (*CAF1*) was at the heart of the nucleosome assembly.⁴⁰ Moreover, the study by Kawahira identified that anti-PCNA staining was used as a prognostic marker in a comparison between malignant skin diseases and nonmalignant skin diseases, such as resistant AD.⁴¹ In our study, we found that *NAP1L2* was a hub gene by using integration analysis of the co-expression and regulatory networks. Thus, we speculated that *NAP1L2* might play an important role in the development and progression of AD.

However, our study has several limitations. First, further investigations with a larger sample size are needed to determine our findings due to the small sample size in our study. Second, the lack of experimental verification and cross-check were the limitation of our study. Meta-analysis or statistical validation using other data sets may be used to cross-check our findings, and we intend to perform experimental verification in our further studies to confirm these results using different approaches such as the quantitative real-time reverse transcription–polymerase chain reaction, Western blot analysis, and immunohistochemistry as the other studies used.^{42,43} Third, there may be expression differences in different localizations of the skin considering the samples obtained in comparison with healthy skin.

In conclusion, our studies identified that several candidate genes (*STRA13*, *PSENEN*, and *NAP1L2*) were involved in the pathogenesis of AD. *STRA13* may play an essential role in the development and progression of AD through interactions with hsa-miR-148b and hsa-miR-152. Furthermore, *PSENEN* may play a crucial role in the development and progression of AD by participating in the Notch signaling pathway. Additionally, *NAP1L2* was identified to be another candidate gene that might play an important role in the development and progression of AD. Insights into the candidate genes should lead to novel early intervention strategies and more targeted therapy for AD.

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