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Identification and Subtype Analysis of Interleukin-17-related Diagnostic Biomarkers in Atopic Dermatitis Based on WGCNA and Machine Learning

Chunli Mei¹, Chunye Xu², Zhibin Yu¹, and Lihong Shen³

¹ Department of Dermatology, Deqing County People's Hospital, Deqing County, China

² Department of Endocrinology, Deqing County People's Hospital, Deqing County, China

³ Department of Orthopaedics, Deqing County People's Hospital, Deqing County, China

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ABSTRACT

Atopic dermatitis (AD) is a chronic inflammatory skin disease with heterogeneous immune dysregulation. Although interleukin-17 (IL-17) signaling is implicated in AD, IL-17-related diagnostic biomarkers and molecular subtypes remain unclear. This study aimed to identify IL-17-associated biomarkers, characterize immune infiltration and subtypes, and explore upstream regulatory mechanisms.

Gene expression datasets (GSE121212, GSE6012) were acquired from the Gene Expression Omnibus database, and an IL-17-related gene set was collected from the Gene Set Enrichment Analysis website (GSEA) (<http://www.gsea-msigdb.org/gsea/msigdb/search.jsp>). Differential expression (limma) and Weighted Gene Co-expression Network Analysis were integrated to identify candidate genes, followed by feature selection using Least Absolute Shrinkage and Selection Operator and random forest. We evaluated immune cell infiltration by applying the CIBERSORT algorithm alongside single-sample Gene Set Enrichment Analysis. GSEA was applied to investigate underlying biological processes. AD patients were clustered into subtypes relying on IL-17 scores using ssGSEA.

Our integrated analysis identified *IL4R* and *PRSS22* as key IL-17-related diagnostic biomarkers for AD, demonstrating excellent diagnostic accuracy across both training and validation cohorts. Immune-infiltration analyses revealed altered immune-cell composition and correlations observed between the identified biomarkers and specific immune cells. Two distinct AD subtypes were identified based on IL-17 scores, exhibiting immune infiltration patterns and enriched biological pathways. A ceRNA network highlighted potential regulatory mechanisms involving these biomarkers.

IL4R and *PRSS22* are robust IL-17-related diagnostic biomarkers for AD, with high predictive power across cohorts. Immune infiltration profiling and IL-17 score-based subtyping reveal AD heterogeneity. These findings provide a foundation for improved diagnosis, molecular stratification, and potential therapeutic targeting in AD.

Keywords: Atopic dermatitis; Diagnosis; Interleukin-17 related genes

Corresponding Author: Lihong Shen, MD, PhD;
Department of Orthopaedics, Deqing County People's Hospital,

Deqing County, China. Email: slh25051@163.com
*The first and second authors contributed equally to this study

INTRODUCTION

Atopic dermatitis (AD) is recognized as a chronic inflammatory skin disorder with a high global prevalence affecting millions worldwide, representing a significant global health burden.¹ With hallmark symptoms including intense itching, skin dryness, and eczematous manifestations, its debilitating nature profoundly impacts patients' quality of life, often leading to sleep disturbances, psychological comorbidities, and substantial socioeconomic strain.²⁻⁵ AD develops through a combination of intricate and diverse pathogenic processes, comprising a compromised epidermal barrier, immune dysregulation, genetic predispositions, and environmental triggers.⁶⁻⁸ While the helper T cell (T_H)2 immune response has traditionally been viewed as central to AD, recent research increasingly highlights the involvement of other immune pathways, including the interleukin-17 (IL-17) axis, especially within the framework of chronic and severe forms of the disease.^{9,10} Notably, accumulating evidence suggests that T_H2 and T_H17 pathways are not mutually exclusive but may act in concert to shape AD heterogeneity. T_H2 cytokines such as IL-4 and IL-13 drive barrier dysfunction and allergic inflammation, creating a permissive environment for T_H17 activation. In turn, IL-17 amplifies skin inflammation by inducing keratinocyte-derived cytokines and antimicrobial peptides, further exacerbating T_H2-driven pathology.¹¹ This interplay between T_H2- and T_H17-associated responses underscores the complexity of AD endotypes and highlights the need for biomarkers that can capture signals from both pathways.¹² This evolving understanding of AD's heterogeneous immune landscape underscores the critical need for identifying specific biomarkers to enhance diagnostic accuracy, predict disease trajectory, and facilitate the development of targeted, personalized therapeutic strategies.¹³⁻¹⁵

IL-17, mainly secreted by T_H17 cells, is essential for protecting the host against extracellular pathogens and is involved in the pathogenesis of several inflammatory and autoimmune disorders.^{16,17} Recent studies have revealed that, beyond its systemic immunological roles, IL-17 is increasingly recognized for its direct involvement in cutaneous immune responses, particularly in the pathophysiology of AD.¹⁸ IL-17 promotes inflammatory responses by stimulating keratinocytes and other skin cells to produce pro-

inflammatory cytokines, chemokines, and antimicrobial peptides, further exacerbating the inflammatory cascade in AD.^{19,20} Given the emerging significance of IL-17 in AD pathogenesis, the identification of IL-17-related biomarkers holds great promise for improving diagnostic accuracy, predicting disease severity, and guiding targeted therapeutic strategies.

In this investigation, we intended to uncover novel IL-17-associated diagnostic biomarkers for AD and to characterize AD subtypes based on IL-17 pathway activity. We employed a comprehensive bioinformatics pipeline, integrating Weighted Gene Co-expression Network Analysis (WGCNA), machine learning, immune infiltration profiling, and single-gene enrichment analysis. Our results offer an important understanding of the molecular pathways involved in AD and propose two feature genes as promising diagnostic biomarkers, potentially paving the way for improved diagnostic tools and targeted interventions for AD patients.

MATERIALS AND METHODS

Data Acquisition

Gene expression microarray datasets, GSE121212 and GSE6012, were downloaded from the Gene Expression Omnibus (GEO) database (<https://www.ncbi.nlm.nih.gov/geo/>). GSE121212, comprising 27 AD samples and 38 normal control samples, was designated as the training set. GSE6012, containing 10 AD samples and 10 normal control samples, served as the independent validation set. A comprehensive gene set of 390 IL-17-related genes was obtained from the Molecular Signatures Database (MSigDB) of the Gene Set Enrichment Analysis (GSEA) website (<http://www.gsea-msigdb.org/gsea/msigdb/search.jsp>).²¹

Differential Gene Expression Analysis

The limma R package was used to identify differentially expressed genes (DEGs) between AD and normal control groups in the GSE121212 training set. Genes with an adjusted *p* value (*p*.adj) < 0.05 and an absolute log₂ fold change (|log₂FC|) > 1 were considered statistically significant DEGs. The identified DEGs were then intersected with the 390 IL-17-related genes to obtain IL-17-related DEGs. The expression levels of these IL-17-related DEGs in both normal and AD samples were visualized using box plots. Furthermore,

the correlation among these DEGs was calculated and presented as a heatmap.

Weighted Gene Co-expression Network Analysis (WGCNA)

The WGCNA R package was employed to construct a co-expression network using the top 50% most variable genes from the GSE121212 training set. A hierarchical clustering approach was applied to group genes with similar expression patterns into distinct gene modules. The optimal soft-thresholding power (β) was determined to achieve a scale-free network topology. Pearson correlation analysis was used to calculate the correlation coefficients between each gene module and the sample phenotype. Hub genes within a module were identified based on two criteria: module membership (MM) > 0.8 and gene significance (GS) > 0.5 .

Identification and Validation of Diagnostic Feature Genes

The IL-17-related DEGs identified were intersected with the hub genes from the AD-associated WGCNA module to derive potential diagnostic feature genes. To further refine these candidates, machine learning algorithms, specifically Least Absolute Shrinkage and Selection Operator (LASSO) regression (using the *glmnet* R package) and Random Forest (using the *randomForest* R package), were applied to the training set. The intersection of genes selected by both LASSO and Random Forest was considered the final set of diagnostic feature genes.

The diagnostic performance of these feature genes was then validated using the independent GSE6012 dataset. Receiver operating characteristic (ROC) curves were generated, and the area under the curve (AUC) values were calculated to assess the diagnostic accuracy. Box plots were also generated to compare the expression levels of the feature genes between AD patients and normal controls in the validation set. To reduce the risk of overfitting, 10-fold cross-validation was applied during model construction in the training cohort (GSE121212), and diagnostic accuracy was further assessed in the external validation cohort (GSE6012). This 2-step strategy ensured both internal robustness and external generalizability.

Immune Infiltration Analysis

Immune cell infiltration levels in AD patients and normal control samples were estimated using the

CIBERSORT algorithm. The proportions of various immune cell types were calculated, and the differences in immune infiltration levels between the 2 groups were visualized using box plots. Additionally, single-sample GSEA (ssGSEA) using the *GSVA* R package was performed to quantify the enrichment of immune cell types and immune-related functions.

Single Gene Enrichment Analysis

To elucidate the potential biological functions and pathways associated with the identified diagnostic feature genes, single-gene GSEA was performed. Gene Ontology (GO) gene sets (“c5.go.v2024.1.Hs.symbol”), and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway gene sets (“c2.cp.kegg_legacy.v2024.1.Hs.symbols.gmt”) were downloaded from the MSigDB database (<https://www.gsea-msigdb.org/gsea/index.jsp>). The GSEA R package was utilized to perform enrichment analysis for each feature gene.

Subtype Analysis Based on IL-17 Pathway Activity

Based on the expression of the two IL-17-related differential genes identified, ssGSEA was performed using the *GSVA* R package to calculate an IL-17-related score for each sample. AD patients were then stratified into “high IL-17 score” and “low IL-17 score” subgroups based on the median IL-17 score. Subsequently, differential gene expression analysis ($|\log FC| > 0.585$, $p_{\text{adj}} < 0.05$) between these 2 subgroups was performed using the *limma* R package. GSEA, immune infiltration analysis, and functional enrichment analysis (GO and KEGG) were conducted to characterize the molecular and immunological differences between the high and low IL-17 score subgroups.

Construction of ceRNA Network

Experimentally validated microRNA (miR)-target gene interactions were obtained from the miRTarBase database (<https://mirtarbase.cuhk.edu.cn/>). Using the identified diagnostic feature genes as targets, corresponding miRs were identified. Subsequently, lncRNAs that regulate these miRs were identified. Finally, a comprehensive mRNA–miR–lncRNA competing endogenous RNA (ceRNA) regulatory network was constructed and visualized using Cytoscape software.

RESULTS

Differential Gene Expression Analysis

Analysis of DEGs between AD patients and control samples in the GSE121212 training set identified 413 upregulated genes and 506 downregulated genes (Figure 1A). The intersection of these AD-related DEGs ($|\log FC| > 1$, $p_{\text{adj}} < 0.05$) with the IL-17 related gene set yielded 11 IL-17 related DEGs (Figure 1B). A correlation heatmap revealed that the IL-17-related

DEGs exhibit intergene correlations (Figure 1C). Box plots demonstrating the gene expression profiles within normal and AD samples showed that *IL4R*, *NUP210*, *TMC5*, *PRSS22*, *SERPINB9*, and *CHAC1* were significantly upregulated in AD patients (Figure 1D). GO enrichment analysis performed on these genes indicated enrichment in biological processes such as fatty acid metabolic process, production of molecular mediators involved in inflammatory response, and mast cell-mediated immunity (Figure 1E).

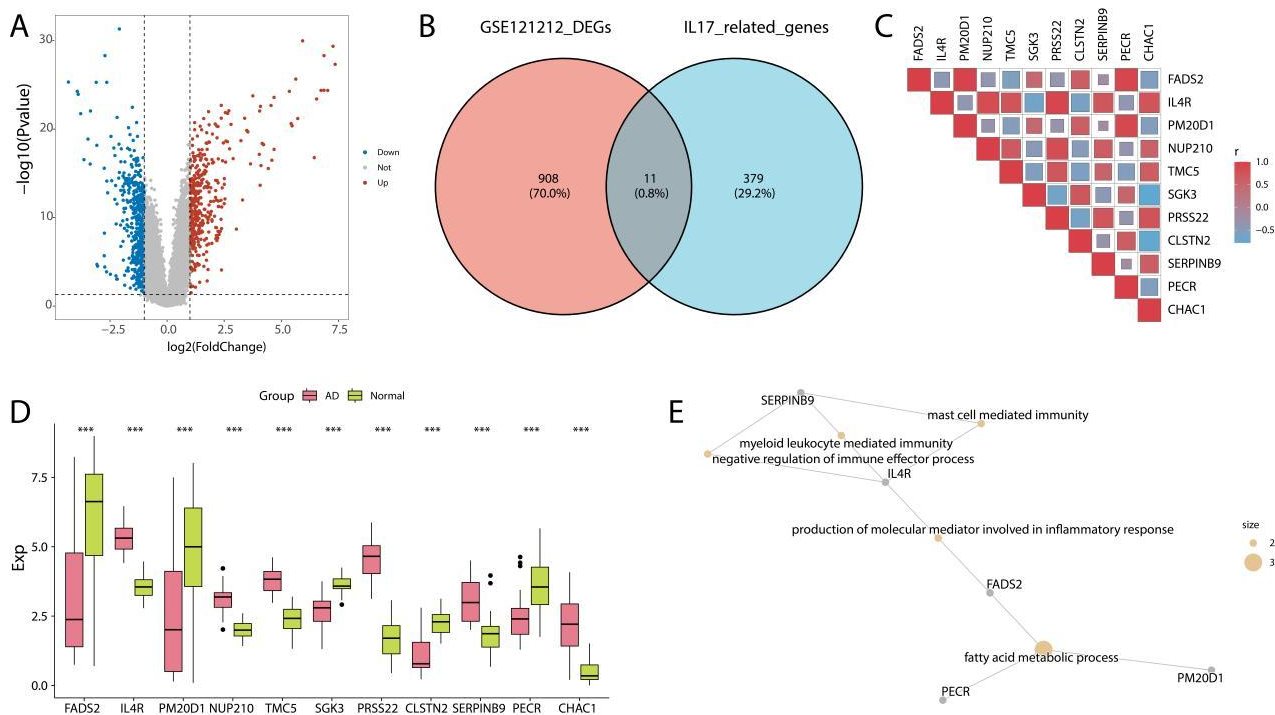


Figure 1. Differential gene expression analysis. A. Volcano plot showing DEGs between AD patients and normal samples. **B.** Venn diagram showing the intersection of AD-related DEGs and interleukin (IL)-17 related genes. **C.** Correlation heatmap of IL-17-related DEGs. **D.** Box plots illustrating the expression levels of IL-17-related DEGs in normal and AD samples. **E.** GO enrichment analysis of IL-17-related DEGs. AD: atopic dermatitis; DEGs: differentially expressed genes; GO: Gene Ontology.

Weighted Gene Co-expression Network Analysis (WGCNA)

We subsequently developed a WGCNA to identify key gene modules associated with AD. The soft threshold was set at 12 to establish the scale-free topology model ($R^2 > 0.85$), and the mean connectivity indicated that the network exhibited stable scale-free properties (Figure 2A). Module preservation analysis further demonstrated the robustness of the identified modules across datasets (Supplementary Figure S1). After merging highly correlated modules, 10 distinct gene modules were identified (Figure 2B). Among these,

the blue module exhibited the strongest positive correlation with AD ($\text{cor} = 0.85$) (Figure 2C). This blue module was therefore selected as the AD-associated hub module. From the 1390 genes within the blue module, 438 hub genes were further identified based on stringent criteria of module membership ($\text{MM} > 0.8$) and gene significance ($\text{GS} > 0.5$) (Figure 2D). The intersection of the 11 IL-17-related DEGs, and the 438 hub genes from the blue module is shown in Figure 2E.

Identification and Validation of Diagnostic Biomarkers

Further refinement using machine learning algorithms, specifically LASSO regression (Figure 3A) and Random Forest (Figure 3B), ultimately identified two robust diagnostic genes: *IL4R* and *PRSS22* (Figure 3C). The diagnostic performance of *IL4R* and *PRSS22* was evaluated using ROC curves. In the training set (GSE121212), *IL4R* and *PRSS22* demonstrated excellent diagnostic accuracy, with AUC values of

approximately 0.998 and 1.0, respectively. To validate these findings, we utilized the independent validation dataset (GSE6012). In this external cohort, *IL4R* exhibited an AUC of 0.98, and *PRSS22* showed an AUC of 0.91 (Figure 3D), confirming their strong diagnostic potential. Furthermore, the expression levels of both *IL4R* and *PRSS22* were significantly elevated in AD patients compared to normal controls in the independent validation set (Figure 3E), consistent with the training set findings.

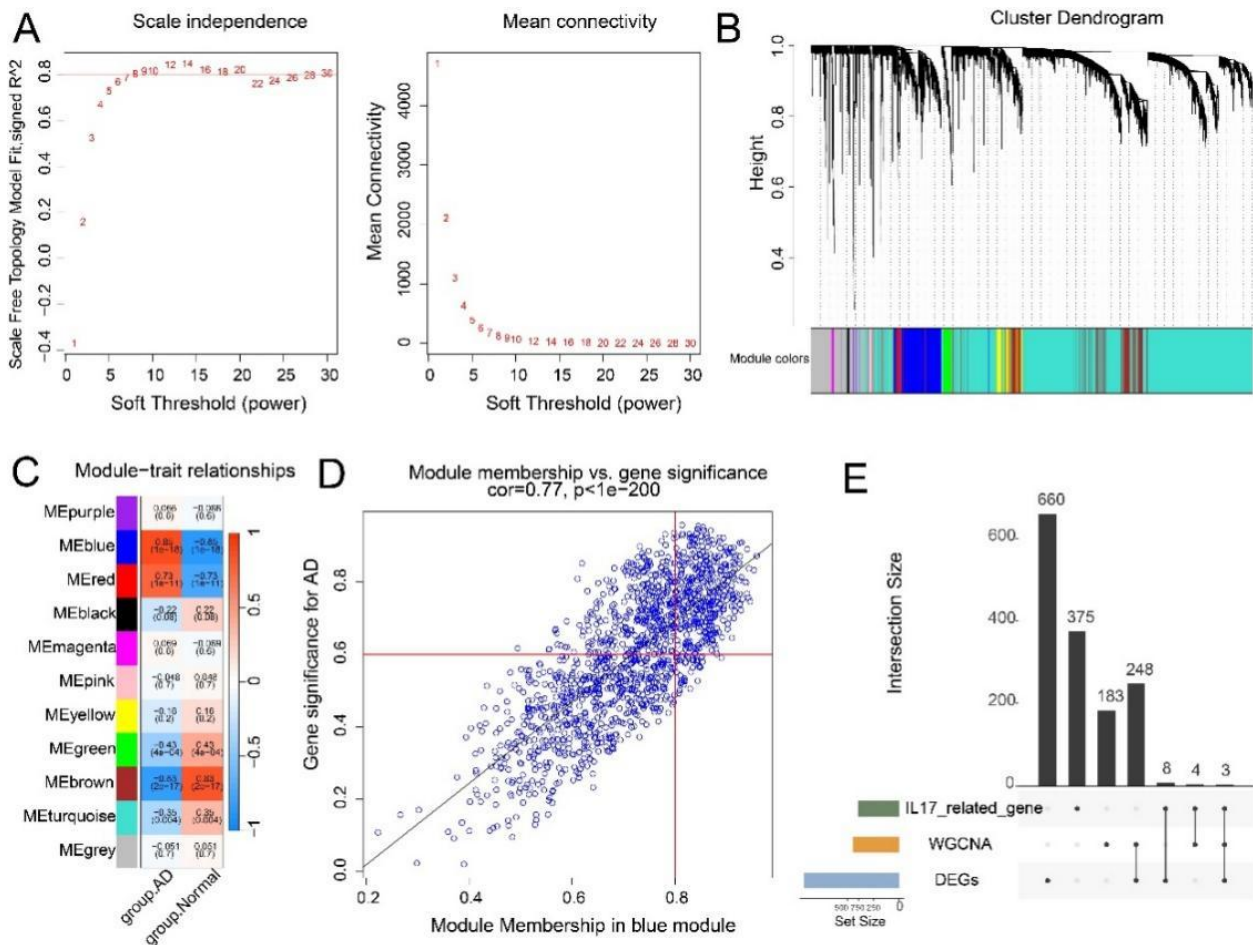


Figure 2. Weighted gene co-expression network analysis. **A.** Determination of soft-thresholding power $\beta=12$ for scale-free topology fit index. **B.** Hierarchical clustering tree showing gene modules under WGCNA. **C.** Heatmap illustrating module-trait relationships. **D.** Scatter plot of gene significance and module membership for the blue module. **E.** Venn diagram showing the intersection of IL-17-related DEGs and WGCNA hub genes. WGCNA: Weighted Gene Co-expression Network Analysis.

Immune Infiltration Analysis

To explore the involvement of immune cells in the progression of AD, we examined immune cell infiltration patterns within both AD patients and healthy controls utilizing the CIBERSORT algorithm (Figure 4A). Box

plots comparing immune cell infiltration between AD patients and normal controls revealed significant differences in several immune cell populations (Figure 4B). Specifically, resting mast cells, activated NK cells, and M0 macrophages showed significantly higher

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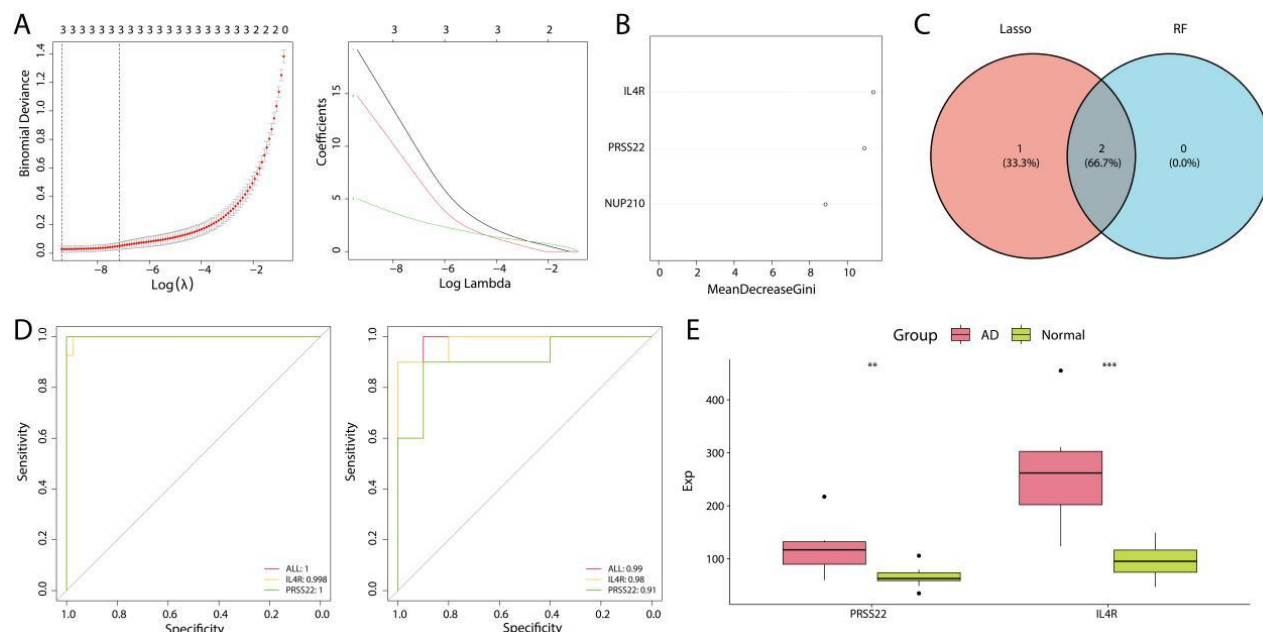
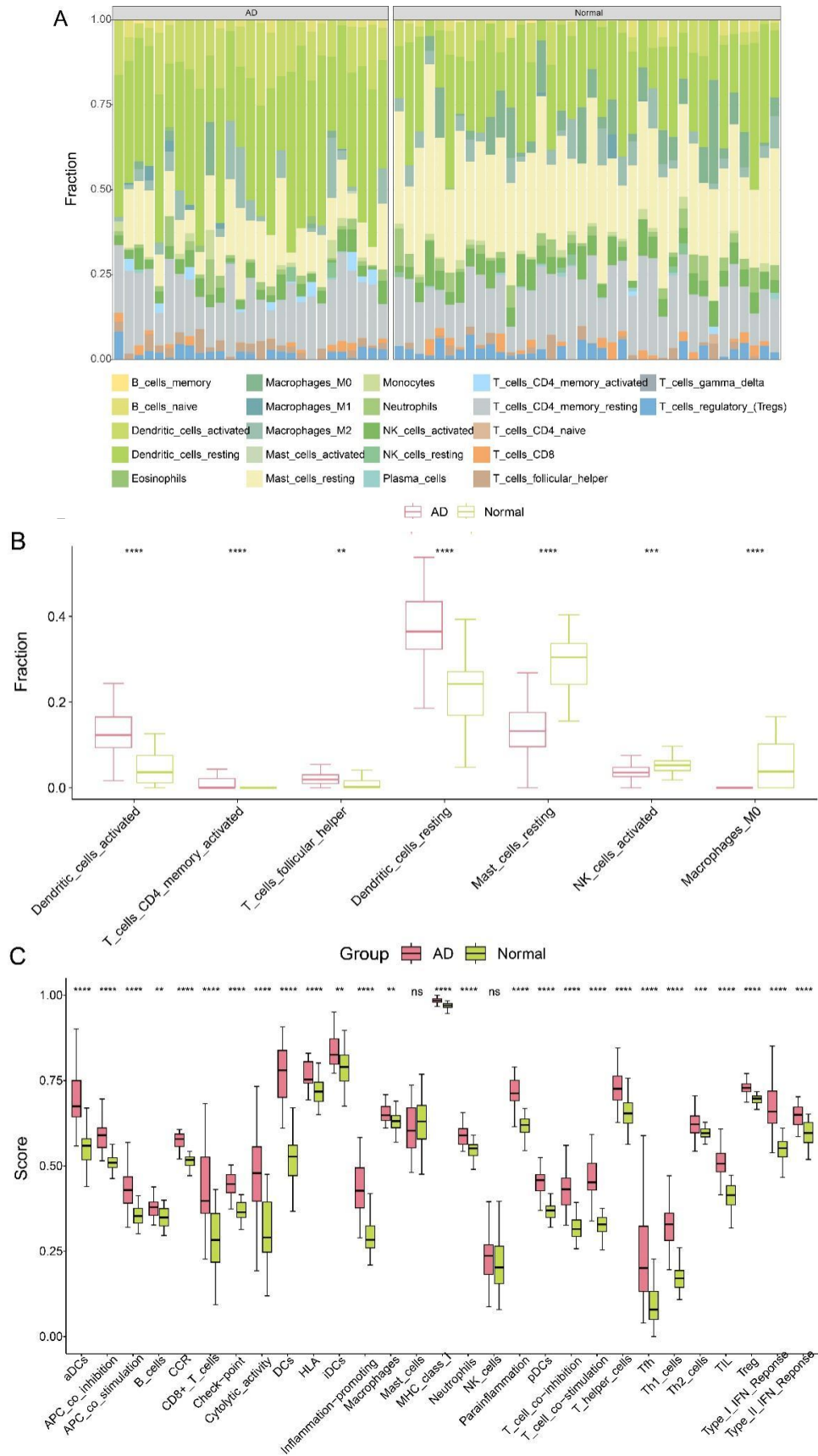


Figure 3. Identification and validation of diagnostic biomarkers. A. LASSO regression analysis for feature gene selection. B. Random Forest MeanDecreaseGini plot. C. Venn diagram showing feature variables selected by LASSO and Random Forest. D. ROC curves for *IL4R* and *PRSS22* in the training and validation sets. E. Box plots showing expression levels of feature genes in AD patients and normal samples in the independent validation set. ($p < 0.01$, *** $p < 0.001$). LASSO: Least Absolute Shrinkage and Selection Operator; ROC: receiver operating characteristic.**

IL-17-Related Biomarkers in Atopic Dermatitis



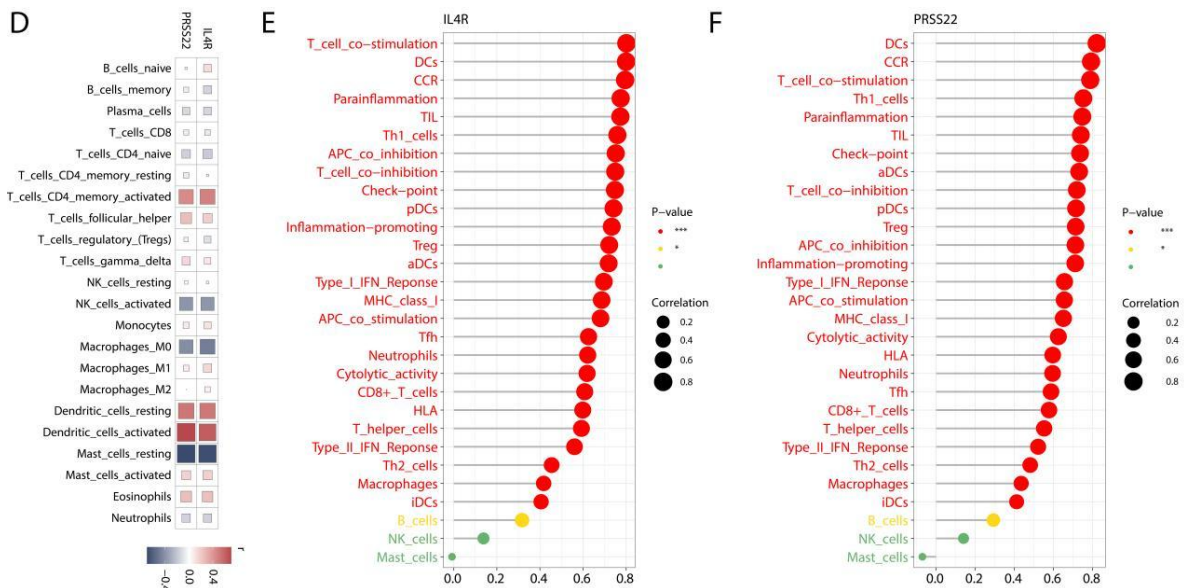


Figure 4. Immune infiltration analysis. A. Distribution of immune cell types in normal and AD samples. B. Boxplot of the differences in immune cell type distribution between AD patients and normal samples. C. Boxplot of the differences in immune infiltration levels between normal and AD patients. D. Correlation between feature genes and immune cell abundance. E. Correlation between *IL4R* and immune infiltration levels. F. Correlation between *PRSS22* and immune infiltration levels.

Single Gene Enrichment Analysis

To uncover potential biological functions, single-gene GSEA was applied to the selected diagnostic biomarkers, *PRSS22* and *IL4R*. For *PRSS22*, GO analysis indicated notable involvement in biological processes, including the regulation of immune effector functions, the regulation of adaptive immune response, and the regulation of immune effector process. In terms of cellular components, *PRSS22* was primarily enriched in the cytosolic large ribosomal subunit and T cell receptor complex (Figure 5A). For *IL4R*, GO enrichment analysis indicated significant enrichment in biological processes categories, including positive regulation of immune effector process, regulation of immune effector process, and regulation of leukocyte-mediated immunity. In cellular components, *IL4R* was enriched in the cytosolic large ribosomal subunit, cytosolic ribosome, and cytosolic small ribosomal subunit (Figure 5C). KEGG pathway analysis for both *PRSS22* and *IL4R* showed enrichment in pathways like chemokine signaling pathway, cytokine-cytokine receptor interaction, and hematopoietic cell lineage (Figure 5B and D).

Subtype Analysis Based on IL-17 Pathway Activity

Using the GSVA algorithm, IL-17-related scores were calculated for each sample. Relying on the median IL-17 score, AD patients were divided into two distinct

subgroups: a low IL-17 score group and a high IL-17 score group. Notably, AD patients exhibited significantly higher IL-17-related scores compared to normal controls (Figure 6A). Differential gene expression analysis between these two subgroups (using $|\log FC| > 0.585$, $p_{\text{adj}} < 0.05$) identified 19 upregulated genes and 2 downregulated genes (Figure 6B). Immune infiltration analysis of these two subgroups revealed that the high IL-17 score group displayed significantly higher levels of overall immune infiltration compared to the low IL-17 score group (Figure 6C). Functional enrichment analysis (GO) of the DEGs between the high and low IL-17 score subgroups revealed key biological processes. In terms of biological processes, enrichment was observed in pathways such as regulation of mast cell degranulation, regulation of leukocyte degranulation, and mast cell degranulation (Figure 6D). For molecular function, pathways like cytokine receptor binding, cytokine activity, and cytokine receptor activity were enriched (Figure 6E). In cell components, enriched terms included the external side of the plasma membrane, the early endosome, and the Golgi trans cisterna (Figure 6F). Additionally, to further validate the immune infiltration results, we cross-checked the CIBERSORT and ssGSEA analyses across independent comparisons, and consistent trends were observed (Supplementary Figure S2, S3).

IL-17–Related Biomarkers in Atopic Dermatitis

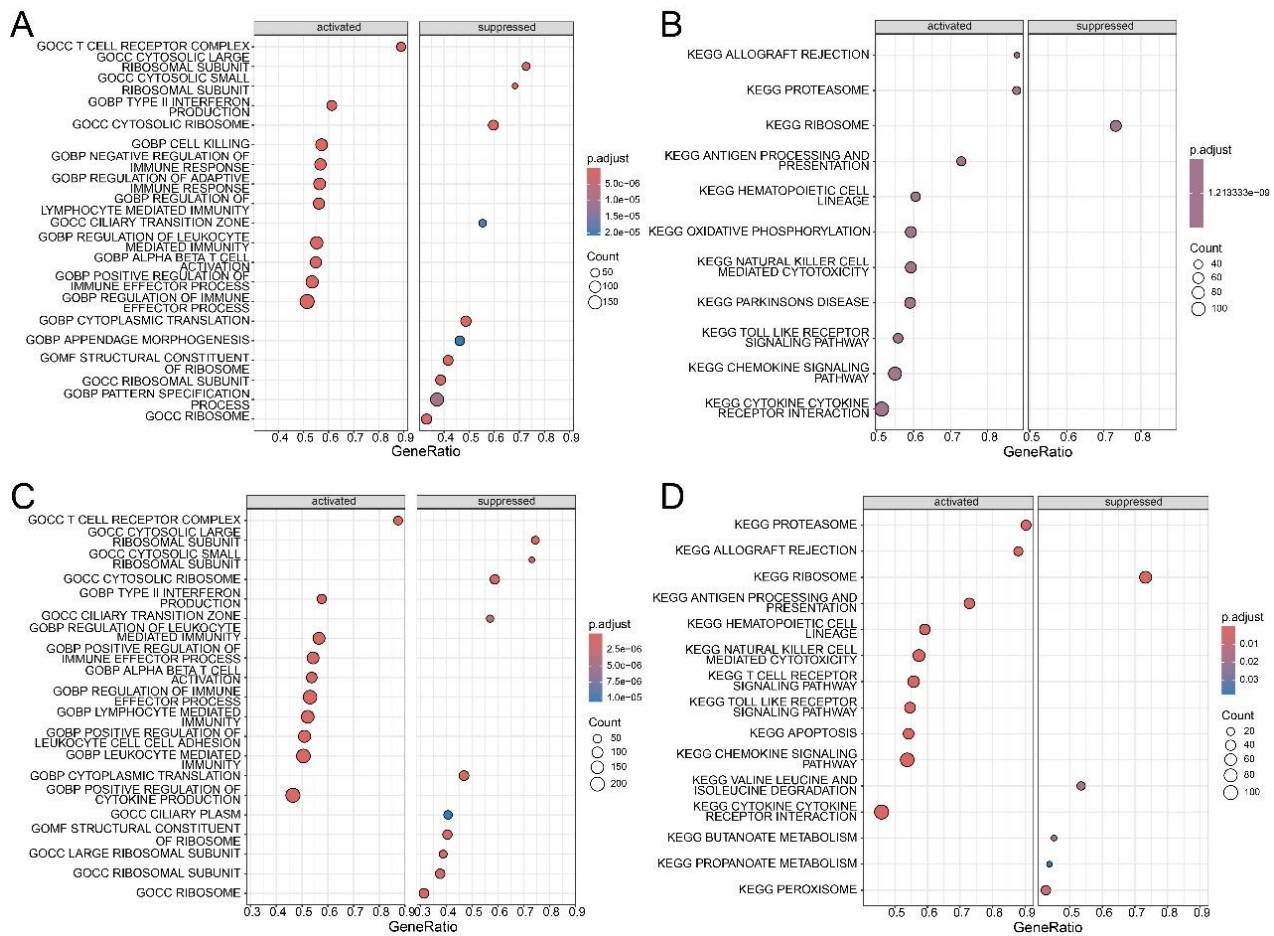
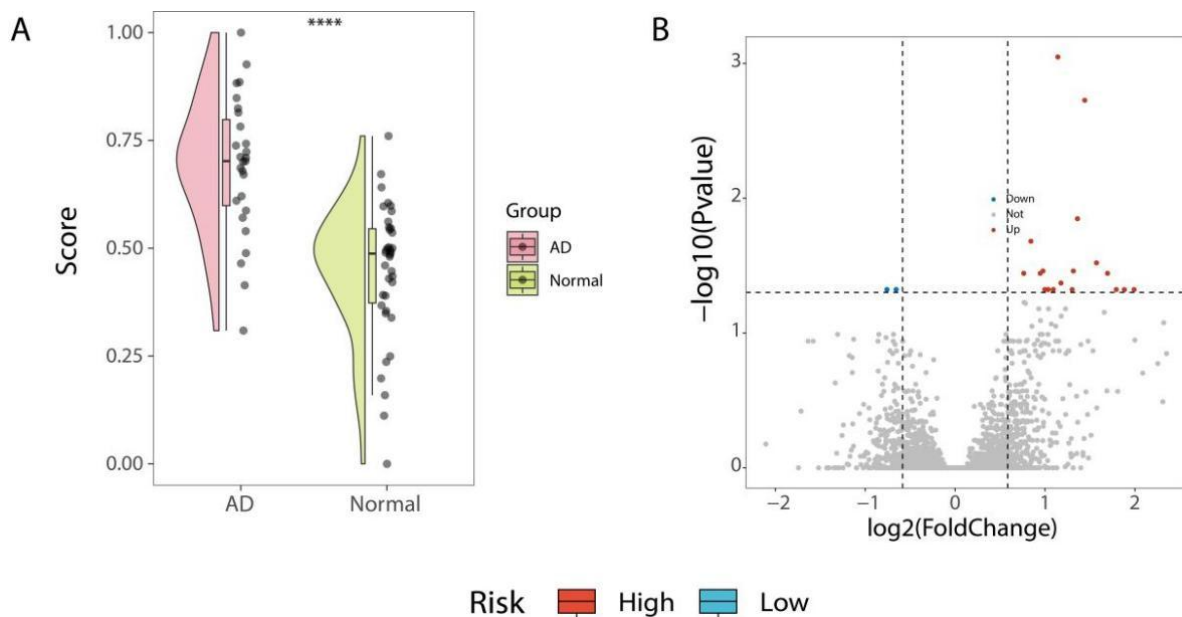


Figure 5. Single gene enrichment analysis. A–B. GO and KEGG enrichment for *PRSS22*. C–D. GO and KEGG enrichment for *IL4R*. KEGG: Kyoto Encyclopedia of Genes and Genomes; *PRSS22*: protease, serine 22.



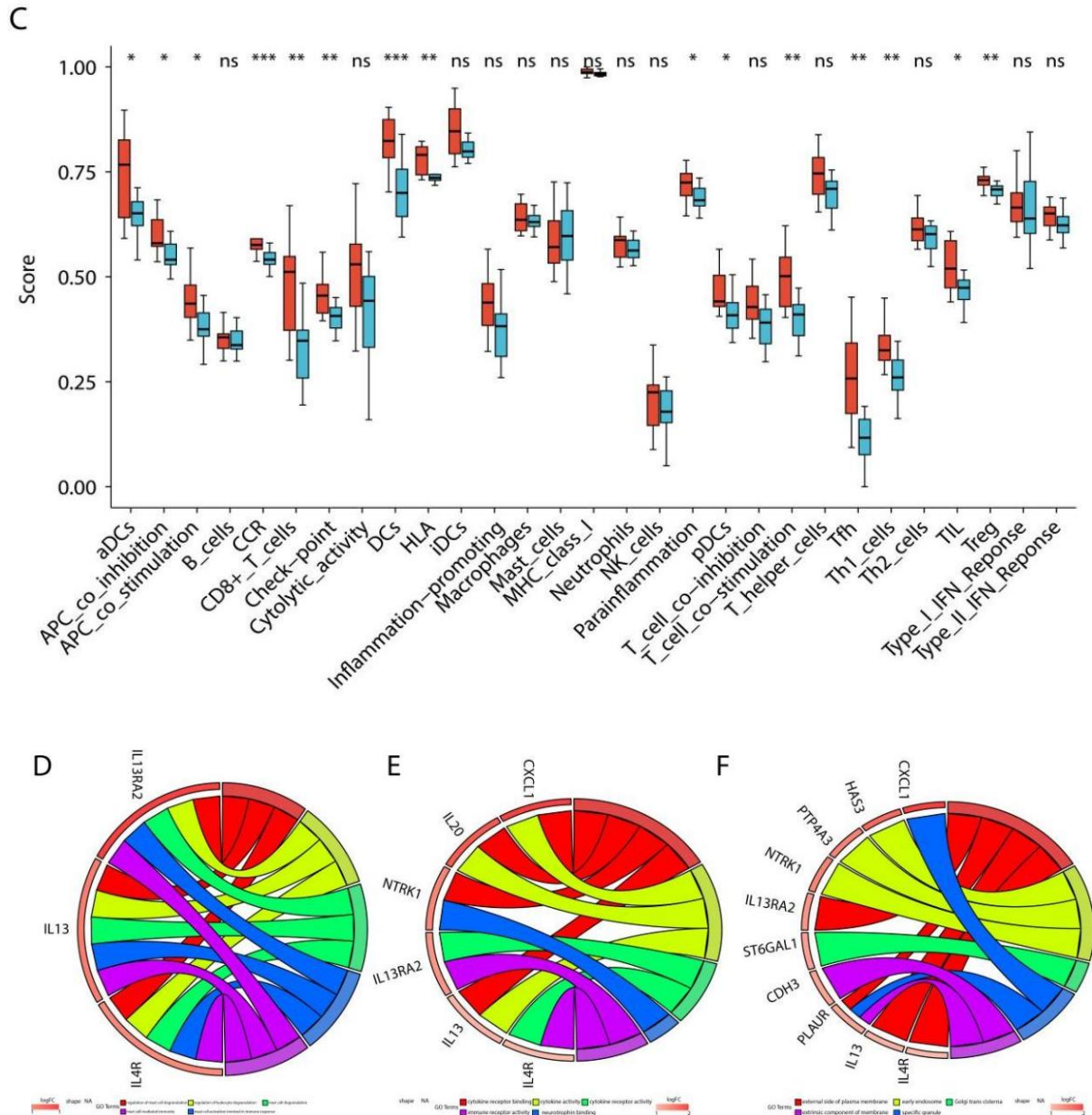


Figure 6. Subtype analysis based on IL-17 pathway activity. **A.** Boxplot of IL-17 scores in AD patients and normal samples. **B.** Volcano plot of DEGs between high and low IL-17 score groups. **C.** Boxplot of differences in immune infiltration levels between high and low IL-17 score groups. **D–E.** GO enrichment of DEGs in biological processes, molecular function (**E**), and cell components (**F**) between high and low IL-17 score groups.

Construction of ceRNA Network

A ceRNA regulatory network was constructed based on experimentally validated interactions from the miRTarBase database, using the two identified diagnostic feature genes (*IL4R* and *PRSS22*) as targets. This analysis identified 40 miRs that target *IL4R* and

PRSS22. Subsequently, 70 lncRNAs that regulate these miRs were identified. The resulting mRNA–miR–lncRNA ceRNA regulatory network, illustrating the complex interplay among these molecules, is presented in Figure 7A, offering insight into post-transcriptional regulation mechanisms in AD.

IL-17–Related Biomarkers in Atopic Dermatitis

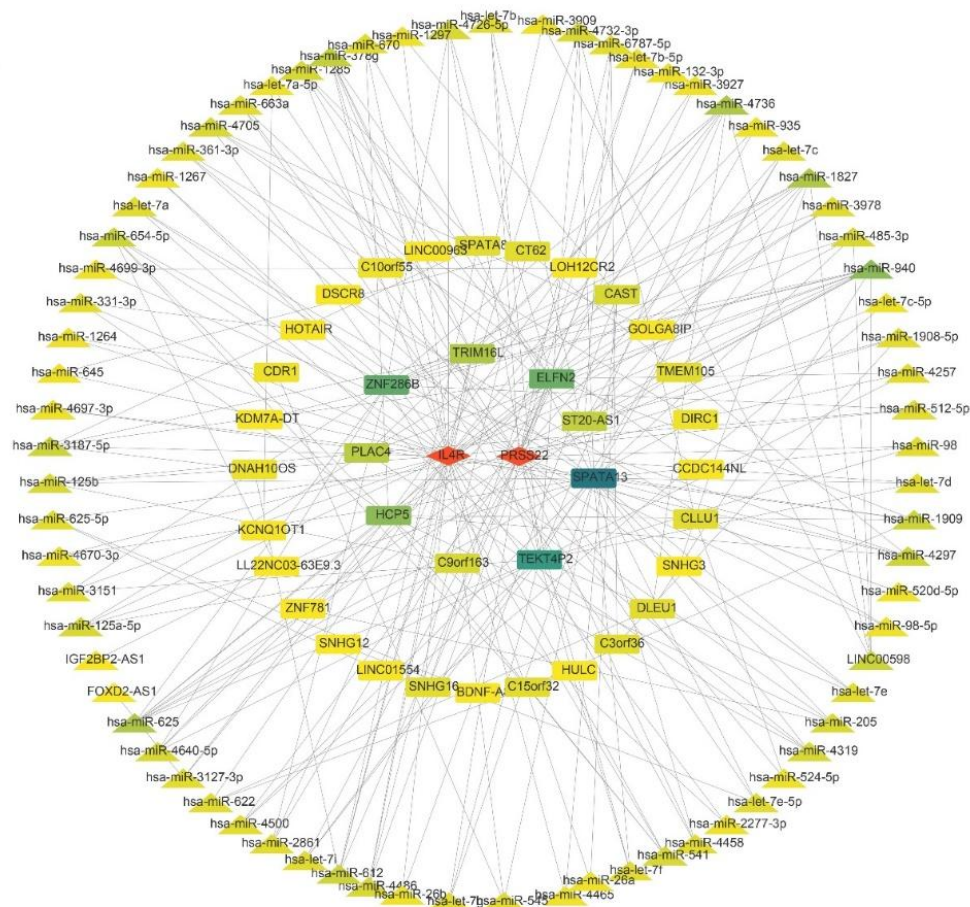
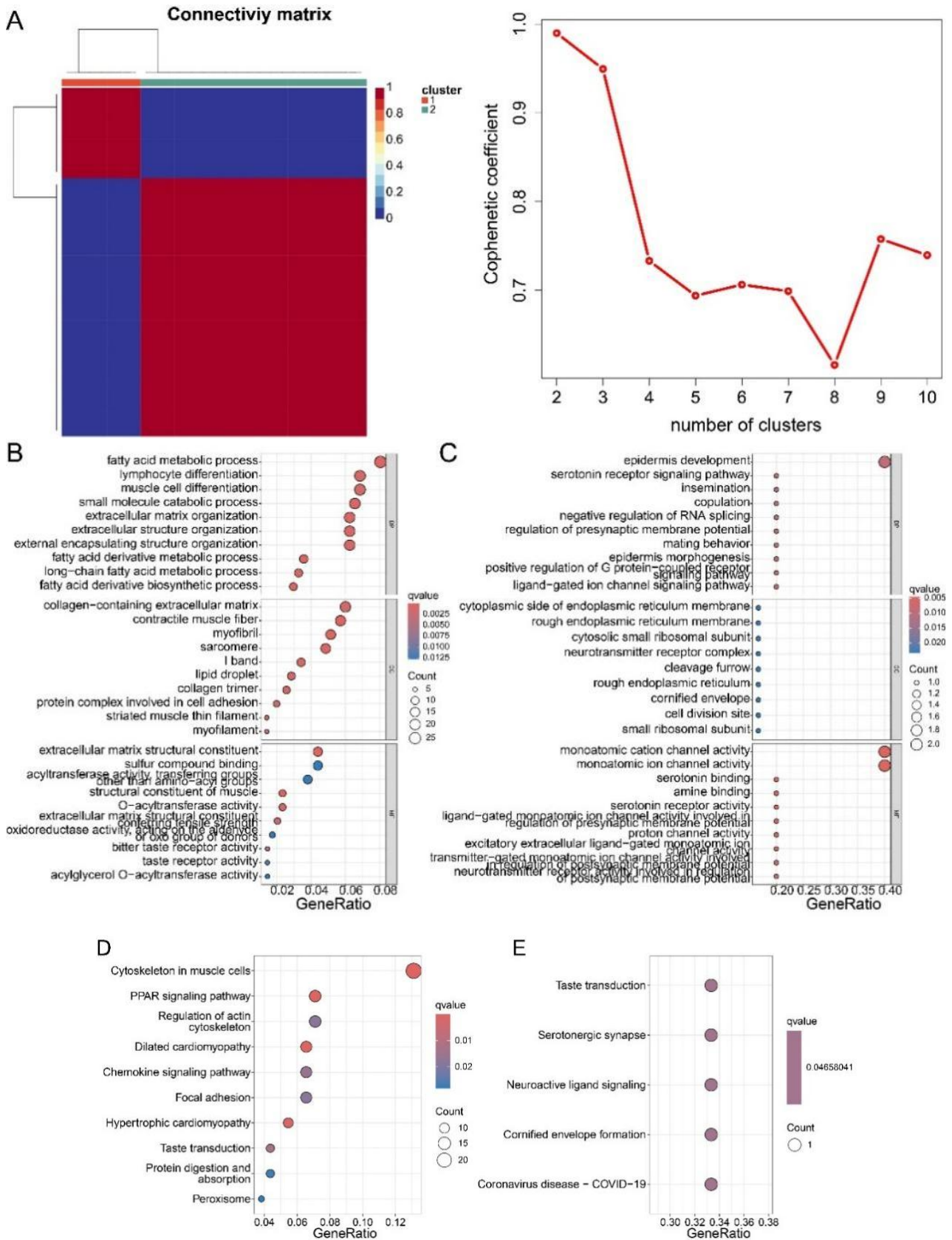


Figure 7. Construction of ceRNA network. mRNA-miRNA-lncRNA regulatory network based on feature genes. Different colors represent the number of nodes, with darker colors indicating a higher number of nodes.

Identification of IL-17-associated Molecular Subtypes in AD

To further validate the heterogeneity of IL-17 activity in AD, we performed an unsupervised clustering analysis based on IL-17-related gene expression. The cophenetic correlation coefficient supported an optimal classification into two clusters (Figure 8A). Functional enrichment analysis revealed marked biological differences between the two subtypes. Cluster 1 was enriched in metabolic and structural pathways, including fatty acid metabolism, extracellular matrix organization, and collagen-containing extracellular matrix (Figure 8B). In contrast, Cluster 2 was associated with epidermal and neural processes, such as epidermis development, monoatomic cation channel activity, and monoatomic ion channel activity (Figure 8C). KEGG pathway analysis further showed that Cluster 1 was enriched in the PPAR signaling pathway, cytoskeleton in muscle cells, and dilated cardiomyopathy (Figure 8D), whereas Cluster 2

was enriched in pathways such as serotonergic synapse, neuroactive ligand-receptor interaction, and cornified envelope formation (Figure 8E). Immune infiltration analysis also highlighted distinct immunological features between the two clusters. Cluster 2 exhibited higher proportions of naive B cells and neutrophil infiltration, while Cluster 1 was characterized by elevated resting dendritic cells (Figure 8F).



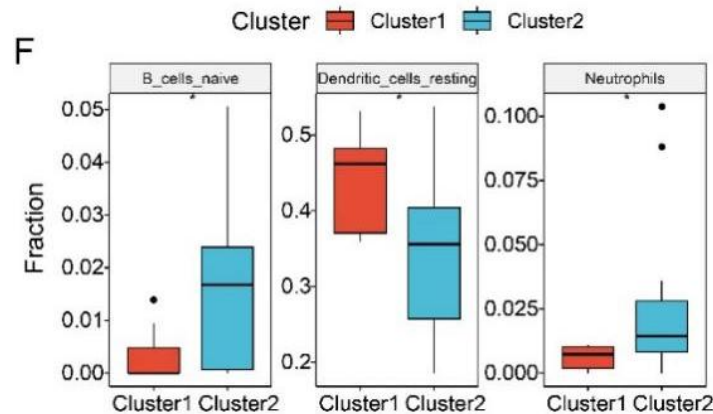


Figure 8. IL-17-based subtyping of AD patients. **A.** Consensus clustering heatmap and cophenetic correlation coefficient showing that $k=2$ yielded optimal stability. **B–C.** GO enrichment analyses of the two clusters. **D–E.** KEGG enrichment analyses of Cluster 1 and Cluster 2. **F.** Immune infiltration analysis comparing the two clusters.

DISCUSSION

AD is a persistent inflammatory skin disorder influenced by a multifaceted interaction of genetic, environmental, and immunological factors.²² While the T_H2 immune response has long been considered central to AD, the role of other immune axes, particularly the IL-17 pathway, is gaining increasing recognition, especially in certain AD endotypes or in patients with more severe or recalcitrant disease.²³ Identifying robust diagnostic biomarkers and understanding the underlying molecular mechanisms, especially those related to specific immune pathways like IL-17, is crucial for improving diagnosis, prognosis, and therapeutic strategies for AD. We used a comprehensive bioinformatics approach, integrating WGCNA and machine learning, to discover IL-17-related diagnostic biomarkers for AD and to explore distinct AD subtypes.

Our initial differential gene expression analysis identified 11 IL-17-related genes significantly altered in AD patients. Among these, *IL4R*, *NUP210*, *TMC5*, *PRSS22*, *SERPINB9*, and *CHAC1* were notably upregulated in AD samples, suggesting their potential roles in AD. Analysis of functional pathways enriched among IL-17-associated DEGs indicated enrichment in processes such as fatty acid metabolism, inflammatory response mediation, and mast cell-mediated immunity. This aligns with existing knowledge, as aberrant fatty acid metabolism has been implicated in skin barrier dysfunction in AD,²⁴ and mast cells are well-known contributors to AD pathogenesis through the release of various inflammatory mediators.²⁵

Subsequently, we identified *IL4R* and *PRSS22* as IL-17-related diagnostic biomarkers for AD through an integrated approach combining differential expression analysis, WGCNA, and machine learning. *IL4R*, interleukin-4 receptor α , a key component of the type I and type II IL-4/IL-13 receptor complex, is well-established in AD pathogenesis, mediating T_H2 -skewed inflammation and barrier dysfunction; blockade of IL-4R α has shown clinical benefit in AD patients and attenuates allergic skin inflammation in experimental models.^{26–28} *PRSS22*, otherwise known as tryptase epsilon, is classified within the serine protease family, and it has been documented that its expression is mainly localized in epithelial tissues, particularly in the airway and skin epithelium.²⁹ Serine proteases play diverse roles in skin barrier function, inflammation, and immune responses.³⁰ *PRSS22* is a less commonly studied gene in the context of AD; therefore, further investigation into the specific mechanisms by which *PRSS22* contributes to AD, particularly in the context of IL-17 signaling, is warranted. As a member of the serine protease family, *PRSS22* is theoretically druggable. Serine proteases have been implicated in skin inflammation through activation of protease-activated receptors (e.g., PAR2), and pharmacological strategies such as topical protease inhibitors, inhibitory peptides, or monoclonal antibodies have been explored in related contexts.^{30,31} Although no *PRSS22*-specific inhibitors are currently available, our findings highlight *PRSS22*-mediated pathways as potential therapeutic targets that merit future investigation. Besides, the outstanding diagnostic performance of *IL4R* and *PRSS22*, demonstrated by high

AUC values in both the training (GSE121212) and independent validation (GSE6012) sets, strongly supports their potential clinical utility. This rigorous validation across independent datasets enhances the reliability and generalizability of our findings, indicating that these two genes may function as effective diagnostic markers for AD.

Beyond their diagnostic value, *IL4R* and *PRSS22* may also hold translational potential for clinical management of AD. *IL4R* expression, which closely aligns with T_H2 -skewed inflammation and dendritic-cell activation, suggests that patients with high *IL4R* expression could particularly benefit from IL-4 α blockade (e.g., dupilumab).³² In contrast, *PRSS22* expression is linked to epithelial-immune regulation and IL-17-associated signatures, indicating a subset of patients who may respond more favorably to IL-17-targeted therapies.³³ When contextualized against established biomarkers such as TARC (CCL17), periostin, and filaggrin mutations, *IL4R* parallels classical T_H2 -associated markers but directly reflects the IL-4/IL-13 receptor axis that is therapeutically actionable,^{13,34} while *PRSS22* captures IL-17-related endotypes not represented by traditional markers. In addition, both genes may serve as longitudinal indicators of disease activity: dynamic changes in *IL4R* could track T_H2 -driven inflammation, whereas fluctuations in *PRSS22* may signal barrier disruption and IL-17-mediated flares. Although our current datasets are cross-sectional and lack treatment outcomes, these findings provide a hypothesis-generating framework and justify prospective validation of *IL4R/PRSS22* as companion biomarkers for biologic therapy, complementary to existing markers, and as candidates for predicting disease activity and flares. Taken together, these findings underscore the importance of integrating T_H2 - and T_H17 -related axes in AD biomarker research. Our identification of *IL4R* and *PRSS22* highlights the dual contribution of T_H2 - and T_H17 -related pathways in AD. *IL4R* reflects canonical T_H2 signaling, whereas *PRSS22* is linked to IL-17-driven epithelial-immune responses. The combination of these biomarkers therefore offers a novel framework to capture AD heterogeneity, bridging classical T_H2 inflammation with emerging T_H17 -associated endotypes.

The IL-17-related subtypes identified in our analysis may also correspond to distinct clinical phenotypes. Previous studies have linked heightened IL-17 activity to chronic, severe, and treatment-refractory AD,

suggesting that IL-17-driven subtypes may be associated with greater disease burden.³⁵ From a therapeutic perspective, while T_H2 -dominant patients generally respond well to IL-4 α blockade, IL-17-high subgroups may show weaker responses to T_H2 -targeted biologics but could benefit from IL-17-directed interventions. Moreover, stronger IL-17 activation observed in Asian and pediatric AD populations indicates potential demographic associations.³⁶ Collectively, these observations highlight the clinical utility of IL-17-based stratification in bridging molecular heterogeneity with personalized patient management.

Our immune infiltration analysis provided deeper insights into the altered immune landscape in AD. Consistent with previous studies, we observed significant changes in various immune cell populations. The increased infiltration of activated dendritic cells, CD4 memory activated T cells, and follicular helper T cells aligns with the chronic inflammatory nature of AD and the involvement of adaptive immunity.³⁷ *IL4R* and *PRSS22* showed positive correlations with dendritic cells, supporting roles in antigen presentation and T-cell activation.^{38,39} In contrast, their negative association with resting mast cells may indicate altered mast cell activity or complex immune crosstalk.⁴⁰ The robust correlation of our diagnostic biomarkers with T cell co-stimulation, CCR, and DCs pathways highlights their potential as indicators of overall immune activation and dysregulation in AD. Importantly, these immunological associations of *IL4R* and *PRSS22* expression are consistent with classical histopathological features of AD. *IL4R* correlated with dendritic-cell activation and T-cell co-stimulation, in line with the prominent dermal dendritic-cell and lymphocytic infiltrates observed in lesional skin.⁴¹ *PRSS22*, as an epithelial serine protease enriched in IL-17-related programs, may reflect keratinocyte activation, barrier disruption, and hyperkeratosis—hallmarks of AD pathology.⁴² Although the GEO datasets do not provide paired histological data, these findings suggest that *IL4R* and *PRSS22* may serve as molecular surrogates of characteristic histopathological changes. Prospective validation using immunohistochemistry or multiplex imaging in lesional versus non-lesional skin is warranted.

To further elucidate the functional implications of *IL4R* and *PRSS22*, we interpreted the GO and KEGG enrichment results in the context of AD pathogenesis. In

terms of functional enrichment, several pathways identified were directly related to immune and IL-17–associated processes, including chemokine signaling, cytokine–cytokine receptor interaction, hematopoietic cell lineage, and antigen processing and presentation. These results strongly support the role of *IL4R* and *PRSS22* in regulating immune cell activation and cytokine-mediated inflammatory responses in AD. In addition, enrichment in pathways such as fatty acid metabolism, ribosome, proteasome, and oxidative phosphorylation may at first appear less directly linked to IL-17 signaling. However, these pathways likely reflect the metabolic reprogramming and protein synthesis demands that accompany immune cell activation and chronic inflammation. Disturbances in lipid metabolism have also been implicated in epidermal barrier dysfunction and inflammatory amplification in AD, suggesting that these “background” pathways provide an important metabolic and structural context for *IL4R*- and *PRSS22*-mediated immune dysregulation.

Finally, the construction of a ceRNA network involving *IL4R*, *PRSS22*, their target miRs, and regulatory lncRNAs provides a comprehensive view of the post-transcriptional regulatory mechanisms that might govern their expression. Dysregulation of ceRNA networks has been implicated in various inflammatory diseases, including AD.⁴³ This network offers novel targets for therapeutic intervention by manipulating the expression or activity of specific lncRNAs or miRs to modulate *IL4R* and *PRSS22* expression, thereby impacting AD pathology. In particular, *IL4R* was predicted to interact with miRs such as hsa-miR-125b and hsa-miR-7, both previously associated with immune regulation and skin inflammation.⁴⁴ Similarly, hsa-miR-132 has been repeatedly reported to be dysregulated in AD studies and may regulate gene programs relevant to immune activation and barrier stress.⁴³ Moreover, lncRNAs including *HOTAIR* and *LINC00963* emerged as central upstream regulators within the network, consistent with their reported roles in inflammatory signaling.⁴⁵ By focusing on these key regulatory loops, the ceRNA network highlights specific axes that may fine-tune *IL4R* and *PRSS22* expression and contribute to AD heterogeneity, thereby offering a more biologically interpretable framework for therapeutic exploration.

In summary, through an integrated bioinformatics approach combining differential expression analysis, WGCNA, and machine learning, we identified *IL4R* and *PRSS22* as novel diagnostic biomarkers of atopic

dermatitis. These genes not only demonstrated strong diagnostic performance in both training and independent validation cohorts but were also functionally linked to immune regulation, IL-17–associated pathways, and ceRNA regulatory networks, highlighting their potential translational value. Nevertheless, our study has several limitations. The GEO datasets analyzed (GSE121212 and GSE6012) lacked demographic information such as age and ethnicity, which may influence gene expression profiles and AD endotypes; thus, we were unable to account for these potential confounding factors. While the independent validation in GSE6012 provides supportive evidence of robustness, we emphasized that further validation in larger, multi-center, and multi-ethnic cohorts will be essential to confirm the clinical applicability of *IL4R* and *PRSS22*. In addition, the sample sizes of the training and validation cohorts were relatively modest, and our analyses were based solely on transcriptomic data without experimental validation. Future studies incorporating larger, well-annotated, multi-ethnic cohorts and functional experiments are warranted to confirm the robustness and generalizability of our findings, and to further elucidate the mechanistic roles of *IL4R* and *PRSS22* in AD pathogenesis.

In brief, we observed *IL4R* and *PRSS22* in the role of robust IL-17-associated diagnostic biomarkers for AD, demonstrating excellent diagnostic performance and reflecting the disease’s immune heterogeneity. This study also revealed distinct AD subtypes, providing insights into personalized therapeutic strategies. Despite these significant findings, there are limitations to this study. Future studies should include experimental validation of the identified biomarkers in wet-lab settings and investigate their functional roles in AD pathogenesis. Furthermore, a deeper exploration of the identified AD subtypes, including their clinical characteristics and responses to different treatments, is warranted.

STATEMENT OF ETHICS

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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Not applicable.

DATA AVAILABILITY

The data and materials in the current study are available from the corresponding author on reasonable request.

AI ASSISTANCE DISCLOSURE

The authors declare that no artificial intelligence (AI) tools were used in the preparation of this manuscript.

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