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Bioinformatics insights into transcriptomic biomarkers for atopic dermatitis

Wirawan ADIKUSUMA [1,2] *, Eko MUGIYANTO [3], Lalu Muhammad IRHAM [4], Firdayani FIRDAYANI [5], Shelvi LISTIANA [5], Muthia Rahayu IRESHA [5], Ayu MASYITA [5], Maulida MAZAYA [1], Riza ALFIAN [6]

1 Research Center for Computing, Research Organization for Electronics and Informatics, National Research and Innovation Agency (BRIN), Cibinong 16911, Indonesia.
2 Department of Pharmacy, University of Muhammadiyah Mataram, Mataram 83127, Indonesia.
3 Department of Pharmacy, Faculty of Health Science, University of Muhammadiyah Pekajangan Pekalongan, Pekalongan 51172, Indonesia
4 Faculty of Pharmacy, Universitas Ahmad Dahlan, Yogyakarta 55164, Indonesia.
5 Research Center for Vaccine and Drugs, Research Organization for Health, National Research and Innovation Agency (BRIN), South Tangerang 15314, Indonesia.
6 Department of Pharmacy, School of Health Sciences ISFI, Banjarmasin 113099, Indonesia.

*Corresponding Author. E-mail: adikusuma28@gmail.com (W.A.)

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ABSTRACT: Atopic dermatitis (AD) is a long-term inflammatory skin condition characterized by a complex interplay of genetic and molecular factors. Understanding the underlying transcriptomic changes can aid in identifying biomarkers for diagnosis and therapeutic targets. This study aimed to discover and characterize transcriptomic biomarkers in AD using bioinformatics tools and techniques. Two pre-existing datasets, GSE6012 and GSE16161, were analyzed using the R limma package to identify differentially expressed genes (DEGs). Gene Ontology (GO) and REACTOME pathway enrichment analyses were conducted using WebGestalt 2019 to explore the biological properties and pathways associated with the identified genes. A protein-protein interaction (PPI) network was constructed using STRING and Cytoscape, with MCODE and CytoHubba plugins used to identify significant gene clusters and hub genes. The analysis identified 352 DEGs (158 upregulated, 194 downregulated) in GSE6012 and 5451 DEGs (2962 upregulated, 2489 downregulated) in GSE16161, with 226 overlapping genes. GO enrichment analysis revealed significant roles in cell proliferation, epidermis development, and immune response. REACTOME pathway analysis highlighted significant modifications in pathways related to skin structure and immune defense, including cornified envelope formation and antimicrobial peptides. The PPI network analysis identified three primary subclusters and pinpointed APOE and STAT1 as key hub genes. This research offers an understanding of the transcriptomic biomarkers of AD. The identified DEGs, enriched biological functions, pathways, and key hub genes offer valuable information for understanding AD's molecular mechanisms and potential therapeutic targets.

KEYWORDS: Atopic dermatitis; bioinformatics; biomarkers; transcriptomics

1. INTRODUCTION

Atopic Dermatitis (AD), often known as atopic eczema, is a widespread chronic skin condition characterized by recurring inflammation and distinct episodes of pruritic (itchy) eczematous lesions alongside dry skin [1, 2]. It is notably prevalent among children, affecting approximately 15% to 20% of this population, while its occurrence among adults ranges from 1% to 3% [1]. Furthermore, the impact of AD is enduring, as roughly 80% of pediatric cases persist into adulthood, often presenting with persistent lesions notably in regions like the flexures, head, and neck [3]. The complex pathogenesis of AD implicates a combination of genetic predispositions and environmental triggers [4, 5]. Genetic factors play a significant role, with certain genetic variations predisposing individuals to heightened susceptibility to the condition. Moreover, environmental factors such as allergens, irritants, and microbial exposures further exacerbate the inflammatory response, contributing to the development and persistence of AD symptoms [6].

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Understanding these intricate interplays between genetic predisposition and environmental influences is crucial for elucidating the underlying mechanisms driving the onset and progression of AD.

Functional and computational investigations have identified numerous genetic risk factors or causal genes associated with AD [7, 8]. A multi-ancestry genome-wide association study (GWAS) identified genetic variants linked to filaggrin (FLG), ovo-like transcriptional repressor 1 (OVOL1), and interleukin 6 receptor (IL6R) as potential risk loci for AD (9). Furthermore, additional functional or clinical studies have implicated interleukin-4 (IL-4), interleukin-13 (IL-13), toll-like receptor 2 (TLR2), matrix metalloproteinase 9 (MMP9), and MMP10 as susceptibility genes for AD [8, 9]. Despite these findings, the precise mechanisms underlying the pathogenesis of AD remain to be fully elucidated.

Advances in experimental and computational biology have accelerated the availability of genomic and biological information, resulting in the creation of multiple databases. These databases contain a variety of data sources, including transcriptome data with gene expression profiles from human patients, animal models of human diseases, and small molecule treatments, as well as other molecular profiling techniques. Publicly available databases offer unique opportunities to improve rational drug design by utilizing the concept of pharmacological targets within networks and the effectiveness of phenotypic screening [10, 11].

Transcriptome data holds particular promise for identifying and prioritizing biomarkers with potential therapeutic applications [12]. By combining transcriptomics and bioinformatics data, novel insights into the etiology of AD can be gained by identifying similar transcriptional characteristics. The use of bioinformatics tools to identify transcriptome biomarkers linked with AD has become increasingly popular due to the complexity of AD pathogenesis and the availability of transcriptomic data [13]. This research endeavor seeks to deepen comprehension of the discovery and characterization process of transcriptome biomarkers in AD through the use of bioinformatics tools and techniques. By combining transcriptome data with computational analyses, new biomarkers could be developed that may serve as prognostic markers, therapeutic targets, or diagnostic indicators for AD. An interdisciplinary approach is being used to increase our understanding of AD pathophysiology and develop personalized treatment solutions for this debilitating disease.

2. RESULTS

2.1 Detection of DEGs for AD

The study was based on two pre-existing datasets, GSE6012 and GSE16161, which were examined with the R limma package to detect DEGs. The selection of these datasets was influenced by several factors, including the availability of complete data with ethical approval for research, the inclusion of human individuals, and the inclusion of both diseased and healthy tissue samples from patients instead of just certain cell types. Figure 1A shows volcano plots indicating that the GSE6012 dataset has 352 DEGs, with 158 upregulated and 194 downregulated genes. Similarly, Figure 1B shows volcano plots indicating that the GSE16161 dataset has 5,451 DEGs, with 2,962 upregulated and 2,489 downregulated genes, comparing nine AD and nine normal skin tissues. To improve the precision of identifying risk genes, we focused on the intersection of DEGs from both datasets. This resulted in 226 overlapping genes, as shown in Figure 1C and Supplementary Table S1.

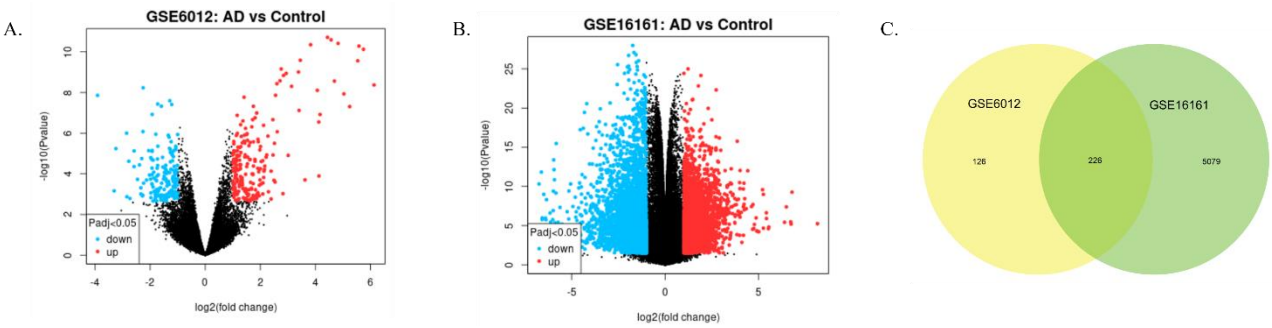

Figure 1. Identification of differentially expressed genes (DEGs) in Atopic Dermatitis. (A) A volcano plot for dataset GSE6012. (B) A volcano plot depicting DEGs in dataset GSE16161. (C) Venn diagrams illustrating the overlap of DEGs between GSE6012 and GSE16161 datasets.

2.2 Gene ontology and REACTOME pathway analysis

The biological properties of the discovered genes were explored using the WebGestalt 2019 web tools through GO enrichment analysis. The analysis comprised three main components: biological processes (BP), cellular components (CC), and molecular functions (MF). Enriched functions with statistical significance were identified by applying a significance threshold of q-value (FDR) < 0.05 to each study. A total of 289 functions were found to be significantly enriched through BP analysis. The top-ranked outcomes were strongly correlated with "cell proliferation," "epidermis development," and "response to biotic stimulus," suggesting these genes play pivotal roles in cellular growth and response mechanisms, as well as skin development and immune responses. The CC analysis revealed 14 significantly enriched functions, including "cytoplasmic vesicle lumen," "vesicle lumen," and "secretory granule lumen," offering insights into the cellular compartments where these genes exert their effects, likely influencing processes like intracellular transport and secretion. The analysis of MF uncovered 29 functions that were significantly enriched, such as "serine-type endopeptidase activity," "serine-type peptidase activity," and "serine hydrolase activity," underscoring the chemical and catalytic roles the encoded proteins play within biological pathways, particularly in protein processing and metabolic regulation. Figure 2 shows the results of the GO enrichment analyses (BP, MF, and CC), providing an overview of the enriched functions in each category and highlighting the important BP, CC, and MF connected to the discovered genes. A complete summary of the GO enrichment analysis results can be found in Supplementary Table S2.

Subsequently, we conducted a REACTOME enrichment pathway analysis using WebGestalt 2019 to investigate the possible engagement of pathways associated with the identified gene candidates. This approach aims to offer a thorough comprehension of the functional implications of these genes by identifying the specific biological pathways in which they are involved. The REACTOME analysis revealed significant modifications in four pathways, as indicated by a q-value threshold below 0.05 (refer to Figure 3). The top-ranking categories within these pathways included "Formation of the cornified envelope," "Metal sequestration by antimicrobial proteins," "Keratinization," and "Antimicrobial peptides." These results are noteworthy, suggesting a strong link between the identified genes and key biological processes such as immunological response, skin structure and function, and potentially, defense mechanisms against infections. The full findings of the REACTOME pathway enrichment analysis are detailed in Supplementary Table S3.

2.3 Construction of PPI network and the analysis of DEGs

The analysis of the PPI network was conducted utilizing STRING and Cytoscape to identify significant gene clusters. This network comprised 218 nodes and 536 edges, reflecting the protein interactions within the studied biological system. To pinpoint potential biomarkers within the PPI network, we utilized the MCODE and CytoHubba plugins in Cytoscape. MCODE was instrumental in identifying gene clusters that could serve as potential biomarkers. It segmented the PPI network into three primary subclusters: Cluster 1, with 11 nodes and 53 edges, scored 10.600; Cluster 2, including 41 nodes and 47 edges with a score of 7.231; and Cluster 3, comprising 8 nodes and 24 edges, scored 6.857, as shown in Figure 4.

Subsequent analysis with CytoHubba pinpointed key genes within the PPI network, employing three algorithms including: degree, closeness, and MNC to rank each node by its significance within the network. This analysis identified the hub genes, with *APOE* and *STAT1* emerging as the two most prominent based on the consistency across the three algorithms. The detailed outcomes of this analysis are captured in Supplementary Table S4.

This comprehensive PPI network analysis elucidates gene clusters and hub genes, revealing the intricate structural and functional network of protein interactions in the system under scrutiny. The subclusters defined by MCODE suggest distinct protein groups potentially engaged in specific biological processes. Furthermore, the hub genes identified by CytoHubba, particularly *APOE* and *STAT1*, underscore their vital roles in the network. Such insights not only augment our understanding of the network dynamics but also foster further investigation into the biological relevance and potential as biomarkers of these proteins.

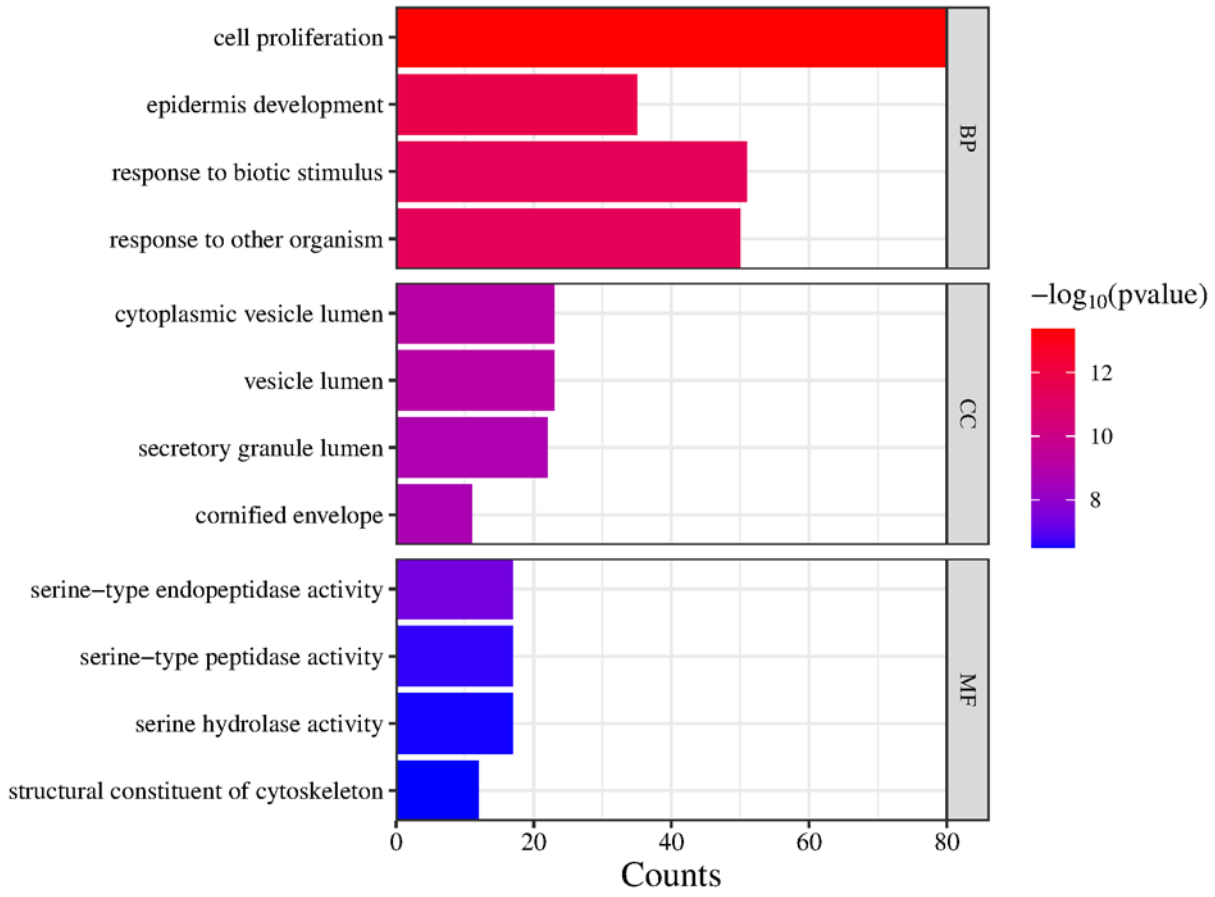

Figure 2. Gene ontology enrichment analysis of atopic dermatitis risk genes using WebGestalt 2019.

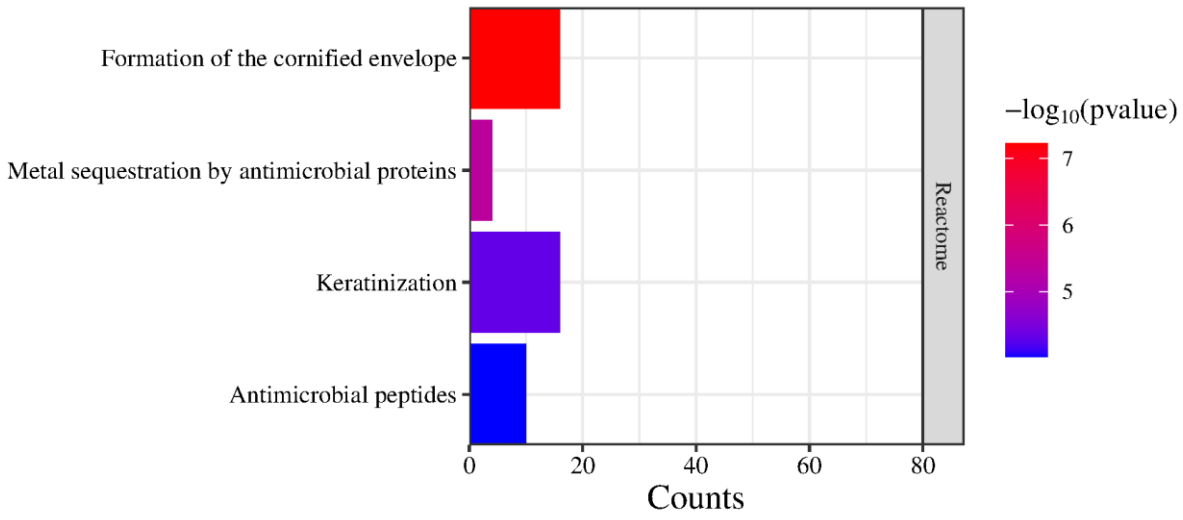

Figure 3. REACTOME pathway enrichment analysis of atopic dermatitis risk genes using WebGestalt 2019.

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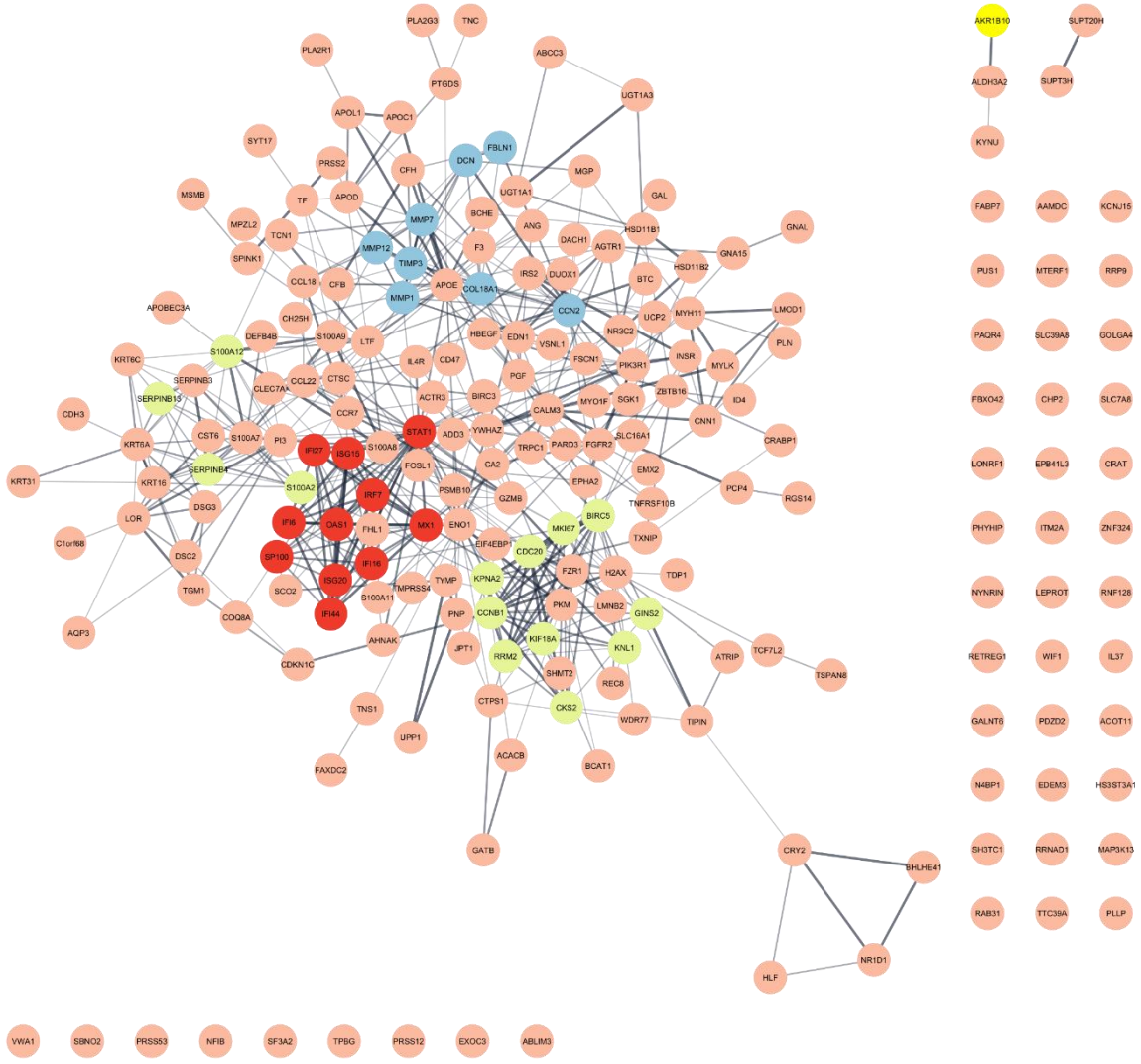

Figure 4. A protein–protein interaction (PPI) network of atopic dermatitis risk genes was created using STRING and Cytoscape. The network consisted of 218 nodes and 536 edges. Additionally, three main modules were identified using MCODE in Cytoscape, with the following cluster scores: Cluster 1 (red, score = 10.6), Cluster 2 (green, score = 7.231), and Cluster 3 (blue, score = 6.857).

3. DISCUSSION

AD is a prevalent chronic inflammatory skin condition impacting individuals globally with varying severity levels [14, 15]. Its pathogenesis is intricately linked to immune dysregulation [16]. Consequently, the pursuit of identifying novel DEGs holds promise in unraveling the molecular intricacies of AD. Leveraging bioinformatics methodologies offers an opportunity to refine the process of discovering and characterizing transcriptome biomarkers, potentially serving as prognostic markers, therapeutic targets, or diagnostic indicators, thereby fostering improved patient care. This study delves into two sets of gene expression profiles, GSE6012 and GSE16161, related to AD, aiming to elucidate potential biomarkers for diagnosis and treatment. The discovery of new biomarkers facilitates patient stratification, therapeutic response prediction, and prognosis assessment [17]. In our work, two hub genes, *APOE* and *STAT1*, emerge as the most promising biomarkers in AD, consistently across three algorithms, suggesting their potential utility in clinical applications.

The analysis of our top-ranked BP has yielded insightful results, particularly concerning the response to biotic stimuli. AD is notably associated with the abnormal presence of pathogens like Staphylococcus aureus on the skin [18, 19]. This condition is exacerbated by the fact that commensal bacteria,

which typically induce antimicrobial peptides (AMPs) to inhibit S. aureus, are compromised [20]. AMPs like Human Beta-Defensin 3 (HBD-3) and LL-37 are usually highly expressed following various exposures in normal, healthy skin, playing a crucial role in the skin's defense mechanisms [21, 22]. However, in AD, Th2 cytokines are known to down-regulate the expression of these AMPs, leading to recurrent microbial infections that could potentially disrupt the skin's pH balance [23]. Understanding these interactions and the cascading effects they have on skin health is vital. It highlights the importance of maintaining the balance of skin flora and the potential therapeutic targets for preventing or treating conditions like AD.

The vesicle lumen is of significant importance in the pathophysiology of AD. Recent research has highlighted the importance of extracellular vesicles (EVs) in the advancement of the disease [24]. These vesicles, which are lipid bilayer-delimited particles discharged by cells, have been found to carry proteins, RNA, and other molecules that can influence the immune response and contribute to the inflammatory processes characteristic of AD [25]. For instance, EVs from Staphylococcus aureus, a bacterium often associated with skin infections, can exacerbate the condition by disrupting the skin barrier and promoting the release of pro-inflammatory cytokines. Similarly, EVs from the skin fungus Malassezia sympodialis harbor allergens that may lead to allergic sensitization through the skin. Moreover, mast cell-derived EVs have been shown to stimulate different immune cells, potentially dampening the allergic response [26]. These findings, in line with our results, suggest that the vesicle lumen is a key player in mediating the complex interactions between skin cells and the immune system in AD.

Recent studies have elucidated the significant role of serine-type endopeptidases in the development of AD, highlighting the molecular functions of these enzymes as critical factors in the pathogenesis of the condition. Molecular function analysis has revealed that serine-type endopeptidases, such as kallikrein-related peptidases, are involved in the degradation of cell adhesion molecules within the epidermis, which is a pivotal process in maintaining skin integrity [27]. The aberrant activity of these proteases has been linked to the disruption of the epidermal barrier, a characteristic feature of AD. This disruption facilitates the entry of allergens and pathogens, exacerbating the inflammatory response [28]. Furthermore, the connection between heightened serine protease activity and raised levels of serum immunoglobulin E (IgE), as well as other biomarkers like thymus and activation-regulated chemokine (TARC) and eosinophil counts, underscores the multifaceted impact of these enzymes on AD [28].

Analyses have revealed a notable variation in the expression of the *APOE* gene, with both upregulation and downregulation observed. This gene, pivotal in lipid metabolism, has been the subject of extensive study due to its implications in various physiological processes and diseases [29]. Notably, the *APOEε4* allele has been associated with increased levels of vitamin D and adiponectin, both renowned for their anti-inflammatory and immunomodulatory properties. These findings suggest a complex interplay between *APOE ε4* and these beneficial compounds, potentially shedding light on the mechanisms by which *APOE ε4* influences disease processes and outcomes. The upregulation of *APOE* may be indicative of an increased demand for lipid transport or a response to cellular stress, while downregulation could signal a reduced need or dysfunction in lipid handling [30]. This association is particularly intriguing as it suggests a potential protective mechanism against inflammatory processes. In studies involving ApoE knockout mice, the inhibition of serine palmitoyltransferase (SPT) has been shown to lead to improved lipid profiles [31]. Furthermore, the enzyme Serine Palmitoyltransferase Long Chain Base Subunit 2 (SPTLC2), which is involved in the synthesis of sphingolipids, has been observed to increase in conditions with epidermal barrier abnormalities [32]. This finding opens new avenues for understanding the complex interplay between lipid metabolism and inflammation in AD.

The signal transducer and activator of transcription (*STAT*) pathway plays a pivotal role in modulating the complex immune responses implicated in the immunopathogenesis of AD. This pathway, particularly through the Janus kinase (*JAK*)–*STAT* signal transduction, orchestrates the activity of Th2 cytokines such as interleukin (*IL*)-*4, IL-5, IL-13, IL-31*, and thymic stromal lymphopoietin [33]. These cytokines are instrumental in the manifestation of chronic inflammation and pruritus, hallmark symptoms of AD. The *JAK–STAT* pathway also plays a significant role in maintaining the integrity of the epidermal barrier and in the modulation of peripheral nerves, which are crucial for the sensation of pruritus [34]. By targeting the *JAK–STAT* pathway, there is potential to dampen these inflammatory signals and achieve clinical improvement by suppressing the diverse immune pathways that contribute to the pathology of AD [35]. However, it is important to consider the limitations of our study. Our analysis is primarily based on computational approaches and relies on existing databases and literature, which may introduce biases and curation constraints. Furthermore, experimental validation is needed to confirm the functional relevance of

the identified biomarkers. Future studies should address these limitations by exploring the potential underlying mechanisms of *APOE* and *STAT1* in AD pathogenesis and treatment response through pre-clinical experimental investigations.

4. CONCLUSION

This study offers a thorough bioinformatics analysis of transcriptomic biomarkers associated with AD, utilizing datasets GSE6012 and GSE16161 to identify significant DEGs. The intersection of these datasets revealed 226 overlapping DEGs, highlighting critical roles in cell proliferation, epidermis development, and immune response, as shown by GO enrichment analysis. REACTOME pathway analysis further emphasized pathways related to skin structure and immune defense, such as cornified envelope formation and antimicrobial peptides. PPI network analysis identified three primary subclusters and key hub genes, notably *APOE* and *STAT1*, demonstrating their central roles in the molecular network. These comprehensive insights into the DEGs, enriched biological functions, pathways, and hub genes deepen our understanding of AD's molecular mechanisms and identify potential biomarkers for diagnosis and therapeutic targets. This provides a valuable foundation for future research and clinical applications in managing AD.

5. MATERIALS AND METHODS

5.1 Data sources

The publicly available NCBI-GEO database was used to find microarray datasets associated with AD. The GEO database (https://www.ncbi.nlm.nih.gov/geo/) provided two gene expression profile datasets (GSE6012 [36] and GSE16161 [37]) related to AD. To find DEGs in AD compared to non-AD subjects, relevant literature was reviewed using the search terms “human [organism] AND Atopic Dermatitis.” Eligible datasets had to meet two requirements: they had to compare AD patients with healthy (non-AD) controls, and they had to come from the same tissue type - skin, in this case.

5.2 Identification of Differential Expressed Genes (DEGs)

The GSE6012 expression profile was analyzed using the Affymetrix Human Genome U133A Array, which included 10 tissues from AD and 10 from normal skin. The Affymetrix Human Genome U133 Plus 2.0 Array was used to examine the expression profile of nine AD and nine normal skin tissues in GSE16161. The data, which had already been normalized, were used to identify DEGs with the R package limma. The criteria used to select DEGs were a p-value of 0.05 and a log fold change (log FC) greater than 1. The datasets were ensured to have received ethical approval and to contain all necessary data for analysis. Finally, a Venn diagram was used to represent the DEGs that intersected GSE6012 and GSE16161.

5.3 Analysis of DEGs at functional enrichment analysis

To explore the biological implications of the identified genes within AD tissues, we conducted a comprehensive analysis of GO functions and REACTOME pathways. The aim of this analysis was to reveal the functional categories and molecular pathways significantly enriched among the DEGs associated with AD. The GO Consortium's online resource (http://www.geneontology.org) was used for the GO function enrichment analysis [38]. This allowed us to investigate the functional annotations of the genes and identify the specific overrepresented BP, CC, and MF associated with the DEGs linked to AD. In addition, we conducted REACTOME pathway enrichment analysis, which provides a wide range of normal and disease-related biological processes (https://reactome.org/) [39]. By examining the enrichment of genes in specific REACTOME pathways, we aimed to gain insights into the main signaling cascades, metabolic pathways, and cellular processes involved in AD pathogenesis. These analyses were performed using the WEB-based GEne SeT AnaLysis Toolkit (WebGestalt 2019) (http://www.webgestalt.org/) [40]. The significance threshold was established at a false discovery rate (FDR) below 0.05 to ensure statistical significance and reduce the risk of false-positive results.

5.4 Construction of PPI network

The STRING database was used to investigate interactions among both known and predicted proteins. Its comprehensive collection of protein interactions was leveraged to study PPI networks and identify core regulatory genes [41]. To ensure reliability, protein interaction results with a confidence score exceeding 0.7 were specifically focused on. The PPI network was visualized using Cytoscape 3.10.1, a widely

adopted software for network analysis and visualization, to aid in identifying significant network properties and patterns [42]. Additionally, the Molecular Complex Detection (MCODE) application within Cytoscape 3.10.1 was employed to identify hub cluster modules within the PPI network. MCODE is adept at detecting densely connected regions, which are indicative of functional modules or protein complexes. Default parameter settings for MCODE were used, including a k-score of 2, degree cutoff of 2, node score cutoff of 0.2, and a maximum depth of 100 [43]. The aim of this PPI analysis was to reveal intricate protein interaction patterns and identify key hub clusters or modules within the network. This sheds light on underlying regulatory mechanisms and functional relationships among proteins relevant to our investigation.

5.5 Selection of hub genes

CytoHubba, a Cytoscape plugin, was utilized to identify key genes within the PPI network. The tool employs various algorithms to calculate key network characteristics and assess the network's architecture [44]. Three network analysis techniques were used in this study: degree, closeness and maximum neighborhood component (MNC). Higher degrees indicate greater connectivity. The degree represents the number of connections a gene has in the network, indicating how many interaction partners it has. By measuring the average shortest distance between a gene and every other gene in the network, closeness measures how central a gene is, with higher values corresponding to more central placements. The most comprehensive collection of connected nodes is highlighted by the MNC, which identifies the largest connected subset in the network. By combining the results of different approaches, we have identified genes that occupy critical positions within the network and named them hub genes. These genes are thought to play an important role in coordinating the biological processes regulated by the PPI network.

5.6 Statistical analyses

In our research, we employed RStudio version 4.2.1 as the primary tool for all analytical processes. To conduct over-representation analysis (ORA), which encompasses GO and REACTOME pathway enrichment, we utilized the WebGestalt 2019 package within R [40]. GO and REACTOME results were visualized using the ggplot2 package in R [45]. The Venn diagram, depicting the overlap between datasets GSE6012 [36] and GSE16161 [37], was generated with a specialized R package (R v4.2.1) [46]. Furthermore, for the construction and visualization of the PPI network, STRING and Cytoscape tools were instrumental [47].

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