Current Science
CS 5 (2), 1956-1970 (2025)
Received 15 Jan 2025 | **Revised 18 Feb** 2025 | **Accepted 21 March**. 2025 | **Online Available 25 April** 2025
https://doi.org/ 10.52845/CS/2025-5-2-55

Current Science

Current Science

OPEN ACCESS

ISSN(O)2795-8639

ORIGINAL ARTICLE

Identification of Shared Genes and Immune Pathways in COVID-19 and Alopecia Areata using Bioinformatics Analysis

Mingyang Lu[1,2], Lingling Jia[2#], Ruiyu Luo[1,2], Changjiang zhao[1,2], Yuan Zhu[1,2], Yufei Li[2]

[1]**Medical College, Tongji University, Shanghai, 200331, China**

[2]**Department of Plastic Surgery, Shanghai East Hospital, School of Medicine, Tongji University, Shanghai, 200120, China**

[*]**Corresponding Author: Yufei Li**

Abstract:

This study aimed to explore the shared molecular pathways and immune mechanisms between COVID-19 and alopecia areata (AA) using bioinformatics analysis. Transcriptomic datasets from COVID-19 (GSE171110) and AA (GSE45512) were analyzed to identify common differentially expressed genes (DEGs). Through comparative analysis, 71 overlapping DEGs were identified, with five core hub genes (HLA-DRA, GZMA, FCER1G, CD2, and CD8A) emerging as potential biomarkers. Functional enrichment analysis revealed significant involvement in immune response, antigen presentation, and T-cell activation. Immune cell infiltration analysis highlighted distinct dysregulation patterns, including macrophage and T-cell alterations in AA and COVID-19, respectively. ROC curve analysis demonstrated strong diagnostic potential for the identified hub genes. These findings suggest shared immune dysregulation pathways between COVID-19 and AA, providing insights into their co-pathogenesis and potential therapeutic targets. However, further experimental validation is required to confirm these computational predictions.

Background: Recent studies have consistently demonstrated a markedly increased incidence of COVID-19 among individuals diagnosed with alopecia areata (AA) relative to healthy controls. Despite these clinical observations, the pathophysiological basis for this association has not been fully characterized. The present research was designed to explore the molecular pathways involved in the pathogenesis of this disease interaction.

Methods: Transcriptomic datasets for COVID-19 (accession: GSE171110) and alopecia areata (accession: GSE45512) were acquired from the publicly available Gene Expression Omnibus repository. Through comparative analysis utilizing GEO2R analytical tools and Venn diagram visualization, shared differentially expressed genes between these conditions were identified. Subsequent bioinformatics analyses included functional enrichment assessment, protein interaction network mapping, modular cluster analysis, and key gene identification. Diagnostic performance evaluation was conducted through ROC curve analysis. To elucidate immunological aspects, we performed immune cell infiltration analysis to characterize immune dysregulation patterns in both disorders. Furthermore, regulatory network analyses were conducted, including TF-gene interactions and TF-miRNA coregulatory networks centered on the identified hub genes.

Results: Through comprehensive screening, we identified 71 overlapping differentially expressed genes (DEGs) for further investigation. Gene ontology analysis revealed significant enrichment in cellular components and biological processes associated with disease pathogenesis in both conditions. After identification, five core hub genes (HLA-DRA, GZMA, FCER1G, CD2, and CD8A) were found to be potential common exploratory biomarkers for the two diseases. Immune profiling analysis identified distinct cellular dysregulation patterns, with macrophage subtypes (M1 and M2) showing differential activation in alopecia areata, while specific T cell populations (CD4+ memory resting) and dendritic cell subsets (resting state) exhibited characteristic alterations in COVID-19.

Conclusions: Although existing literature has established a correlation between COVID-19 infection and

the initiation, progression, or relapse of alopecia areata, the shared molecular pathways underlying these conditions remain poorly characterized, particularly through computational biology methodologies. Our investigation represents the inaugural effort to delineate common molecular signatures and core regulatory networks between these disorders, providing valuable insights for mechanistic exploration and therapeutic development. Nevertheless, several constraints should be acknowledged. Primarily, our findings are derived exclusively from transcriptomic datasets obtained from public repositories, necessitating experimental confirmation. Additionally, the biological relevance of these computational predictions requires validation through in vitro and in vivo models, which will constitute a primary focus of our subsequent investigations.
Keywords: Alopecia areata, COVID-19, differentially expressed genes, hub genes, pathogenesis

Introduction

Alopecia areata (AA) is an autoimmune disorder characterized by non-permanent, non-scarring hair loss with preservation of hair follicles[1]. As the second most common form of non-cicatricial alopecia following androgenetic alopecia, AA affects approximately 2% of the general population during their lifetime[2]. The condition manifests in various clinical forms, ranging from localized patchy hair loss to more extensive diffuse patterns.

The pathogenesis of AA primarily involves the breakdown of immune privilege in hair follicles. Under normal conditions, hair follicles maintain immune privilege through low expression of MHC class I and II molecules and high expression of macrophage migration inhibitory factors, which collectively prevent lymphocyte infiltration[3]. In AA patients, the disruption of this immune privilege leads to perifollicular lymphocyte infiltration, particularly in the bulbar region and surrounding areas [4-5]. This immune response induces vacuolar degeneration of matrix cells, resulting in structurally compromised hair follicles that are prone to fracture during their emergence from the scalp[6]. Current evidence suggests that AA is a polygenic disorder with complex inheritance patterns, and several genetic susceptibility loci associated with signaling pathways crucial for hair follicle cycling and development have been identified.

Therapeutic strategies for AA primarily focus on immunomodulation and hair regrowth stimulation. First-line treatment options typically involve systemic immunosuppressants, particularly corticosteroids, combined with topical minoxidil application. However, the absence of a standardized therapeutic protocol remains a significant challenge in AA management[7].

A growing body of scientific evidence suggests that viral infections may trigger autoimmune responses in genetically susceptible individuals. Numerous studies have established specific viral-autoimmune disease associations: Coxsackievirus with type 1 diabetes mellitus[8], coronaviruses with rheumatoid arthritis[9], and Epstein-Barr virus with systemic autoimmune disorders[10]. Since the COVID-19 pandemic outbreak in 2020, global surveillance data from the World Health Organization (accessed December 15, 2024) recorded 777,074,803 confirmed cases worldwide (https://covid19.who.int/). Concurrently, there has been a marked increase in reported cases of autoimmune manifestations following COVID-19 infection.

Epidemiological investigations have provided compelling evidence regarding the autoimmune consequences of SARS-CoV-2 infection. A comprehensive retrospective cohort study revealed that COVID-19 patients exhibited significantly higher risks of both new-onset and exacerbated autoimmune conditions compared to non-infected controls, including systemic lupus erythematosus, inflammatory bowel disease, and rheumatoid arthritis[11]. Among these autoimmune sequelae, alopecia areata (AA) has been increasingly recognized as a potential post-COVID-19 complication, with documented cases of disease onset, progression, and recurrence following infection[12-13]. Supporting this observation, a large-scale South Korean cohort study demonstrated a substantially higher incidence rate of AA among COVID-19 patients (43.19 cases per 10,000 person-years [PY]) compared to uninfected controls (23.61 cases per 10,000 PY), with this trend consistent across all clinical subtypes[14].

Despite the established clinical association between COVID-19 and AA, the molecular

mechanisms underlying their potential pathogenic interplay remain poorly understood. Transcriptome analysis, a powerful genomic approach that comprehensively characterizes RNA molecules within cells or tissues, offers valuable insights into disease mechanisms. This technique employs high-throughput sequencing technologies to systematically analyze gene expression patterns, enabling researchers to elucidate regulatory networks, identify potential biomarkers, and investigate molecular pathways under various physiological and pathological conditions.

The investigation of shared transcriptional profiles between AA and COVID-19 may reveal novel mechanistic insights into their co-pathogenesis. This study was designed to identify critical molecular signatures associated with AA pathogenesis in the context of COVID-19 infection. Utilizing two independent gene expression datasets from the GEO database (GSE171110 and GSE45512), we performed comprehensive bioinformatics analyses, including differential expression profiling and functional enrichment analysis, to identify common molecular pathways. Through protein-protein interaction (PPI) network construction using STRING database and Cytoscape software (version 3.10.2), we conducted modular analysis and identified core hub genes. Our investigation revealed five significant hub genes, which were subsequently analyzed for their immune infiltration patterns and diagnostic potential.

The identification of these shared molecular signatures between COVID-19 and AA not only advances our understanding of their common biological pathways but also provides a foundation for developing targeted therapeutic interventions.

2. Materials and Methods

2.1 Datasets Preparation

The Gene Expression Omnibus (GEO; accessible at www.ncbi.nlm.nih.gov/geo) represents a comprehensive, publicly accessible repository of gene expression profiles across various disease states[15]. The GSE171110 dataset[16] comprises transcriptomic data obtained from peripheral blood samples, including 44 COVID-19 patients and 10 healthy controls, generated through high-throughput sequencing on the Illumina HiSeq 2500 platform. The GSE45512 dataset[17] contains gene expression profiles derived from scalp biopsies, comparing 5 AA patients with 5 healthy controls, utilizing the Affymetrix Human Genome U133 Plus 2.0 Array platform.

2.2 Identification of Shared Degs between COVID-19 and Alopecia Areata

GEO2R[18] (accessible at www.ncbi.nlm.nih.gov/geo/geo2r/) is an interactive web-based analytical tool designed for comparative gene expression analysis across different experimental groups. In the current investigation, we utilized GEO2R to identify differentially expressed genes (DEGs) in both GSE171110 and GSE45512 datasets. Following data normalization and log2 transformation, DEGs were visualized through volcano plot representation. The selection criteria for significant DEGs were established as an absolute log2-fold change ≥ 1.0 and an adjusted P-value (adj. P) < 0.05. Subsequently, shared DEGs between the two datasets were identified using the Venn Diagram package[19] implemented in R programming language.

2.3 Gene Ontology and KEGG Enrichment Analysis

Utilizing the identified gene sets, we conducted comprehensive functional enrichment analysis through the DAVID bioinformatics platform[20] (https://davidbioinformatics.nih.gov). The analysis encompassed Gene Ontology (GO) annotation across three domains: cellular components, biological processes, and molecular functions, complemented by Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis to elucidate metabolic pathways and systemic gene functions. Statistical significance was determined using a threshold of Adjusted P-value < 0.05 with an absolute fold change ≥ 1.5. The resulting enrichment data were subsequently visualized using the Bioinformatics online platform.

2.4 Construction of Protein–Protein Interaction Network and Module Analysis

The Search Tool for the Retrieval of Interacting Genes (STRING version 11.5; accessible at http://string-db.org)[21] represents a comprehensive protein interaction database encompassing over 14,000 species, 60 million proteins, and 20 billion interactions, including

both direct physical interactions and indirect functional associations. We constructed a protein-protein interaction (PPI) network for the common differentially expressed genes (DEGs) using STRING with a confidence threshold of > 0.4. Network visualization and analysis were performed using Cytoscape software (version 3.10.2)[22], with the Molecular Complex Detection (MCODE) plugin[23] employed to identify core functional modules. The MCODE parameters were configured as follows: degree cutoff = 2, max depth = 100, node score cutoff = 0.2, and K-core = 2. Subsequent functional characterization of module genes was conducted through KEGG pathway and GO term enrichment analyses using the Bioinformatics online platform (http://bioinformatics.com.cn/).

2.5 Identification and Analysis of Hub Genes

Hub gene identification was performed using cytoHubba[24], a Cytoscape plugin that employs multiple topological analysis algorithms, each with distinct computational principles and specific applications. We implemented seven independent algorithms (Closeness, MCC, Degree, MNC, Radiality, Stress, and EPC) to robustly identify core hub genes, with consensus candidates visualized through Venn diagram analysis. Subsequently, GeneMANIA[25] (accessible at http://genemania.org), a web-based platform for gene interaction prediction, was utilized to construct a comprehensive co-expression network for the identified hub genes.

2.6 Immune Infiltration Analysis

CIBERSORT is a proficient analytical instrument that evaluates the prevalence of specific immune cell subpopulations within tissue samples. The GSE45512 gene expression profile data was uploaded to CIBERSORT to examine the different immune cell types in AA, and GSE171110 was uploaded to examine the different immune cell types in COVID-19. The reference matrix file utilized for cell quantification was the LM22 Signature, which comprises 22 unique immune cell components. Pearson's correlation analysis was implemented to conduct a correlation analysis between genes and immune cells.

2.7 ROC Curves of Hub Genes

ROC curves were constructed, and the area under the ROC curve (AUC) was calculated to evaluate the diagnostic performance of the hub genes for COVID-19 and pSS using the R package "pROC"[26]. The pROC package, specifically designed for ROC analysis in R and S+, offers a range of statistical tests to compare ROC curves, including the analysis of partial areas under the curve, ensuring accurate interpretation of ROC results. It is available under the GNU General Public License at http://expasy.org/tools/pROC/ and is also distributed through the CRAN and CSAN public repositories, making installation straightforward.

3. Results

3.1 Identification of DEGs and Shared Genes between COVID-19 and Alopecia Areata

In the GSE171110 dataset, analysis identified 4007 differentially expressed genes (DEGs), including 2814 up-regulated and 1193 down-regulated genes (Figure 1a). Similarly, in the GSE45512 dataset, 414 DEGs were detected, with 169 up-regulated and 245 down-regulated genes (Figure 1b). Subsequently, an intersection analysis of the DEGs from both datasets revealed 71 shared differentially expressed genes, which were visualized using a Venn diagram (Figure 1c).

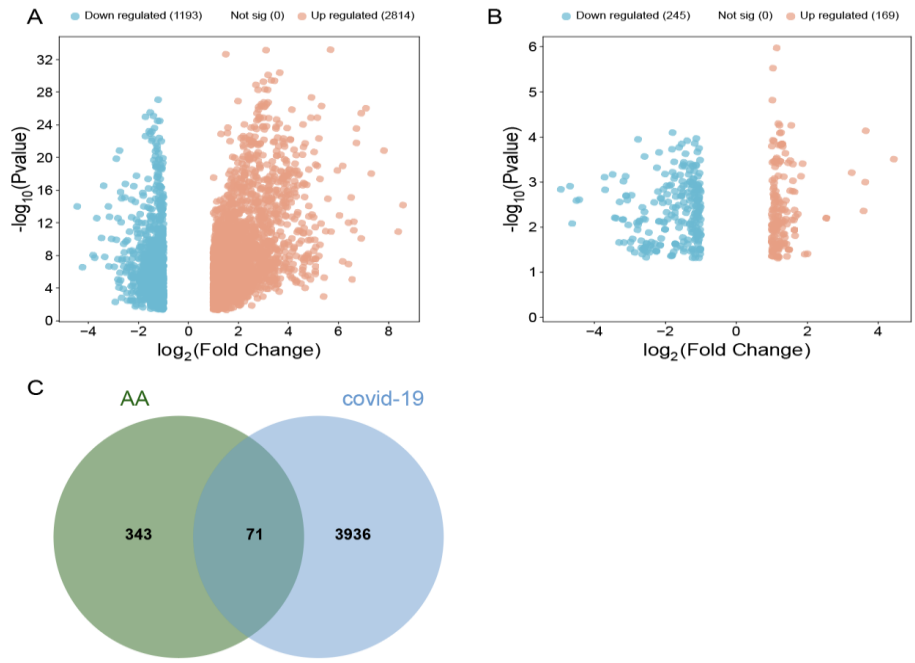

Figure 1: Volcano diagram and Venn diagram. (a) The volcano map of GSE171110. (b) The volcano map of GSE45512. Upregulated genes are colored in red; downregulated genes are colored in green. (c) The two datasets showed an overlap of 71 DEGs.

3.2 GO and KEGG Pathway Enrichment Analysis

Following the initial analysis, we conducted an enrichment analysis on the 71 intersecting genes. Gene Ontology (GO) analysis highlighted the five most significantly enriched terms: response to viral stimuli, nitric oxide transport, immune response, positive regulation of cell migration, and external plasma membrane (Figure 2a). Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis identified shared pathways associated with both diseases, including cell adhesion molecules, antigen processing and presentation, Th1 and Th2 cell differentiation, and Th17 cell differentiation (Figure 2b). These findings strongly suggest that cellular components and metabolic pathways play a pivotal role in the initiation and progression of these inflammatory conditions, with immune regulation being a key factor.

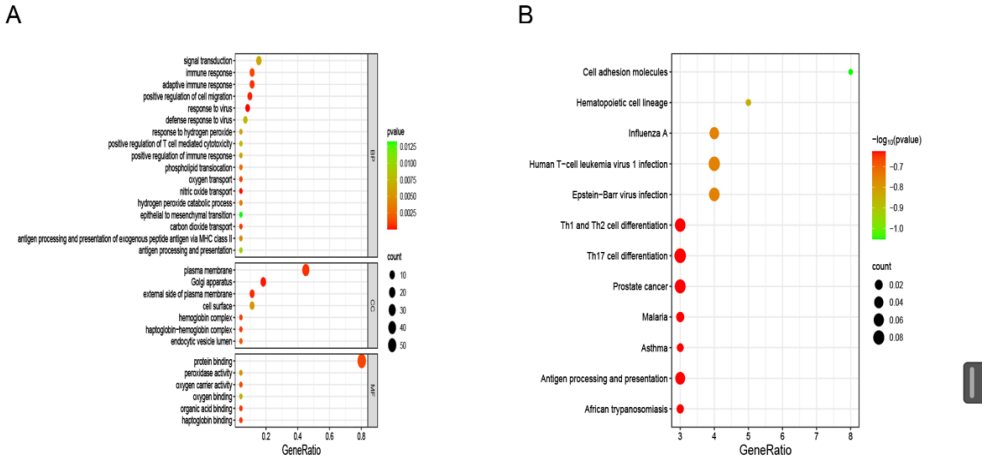

Figure 2: GO and KEGG enrichment analysis of common DEGs. (a)The enrichment analysis results of GO. (b)The enrichment analysis results of KEGG Pathway. Adjusted P-value < 0.05 was considered significant.

3.3 Protein–protein interaction network analysis and submodule analysis

Using the STRING database, we analyzed the protein-protein interaction (PPI) network of the shared DEGs, resulting in a network comprising 70 nodes and 135 edges, with a PPI enrichment p-value significantly less than 1.0e−16 (Figure 3). The PPI network was visualized using Cytoscape software, where the intensity of red coloration of a gene corresponds to its degree of connectivity. The top five genes with the highest connectivity were CD8A, CD2, GZMA, GZMK, and HLA-DRA.

Furthermore, by applying Cytoscape's MCODE plugin, we identified a critical gene module consisting of 13 shared DEGs: GZMA, XCL2, SELL, XCL1, HLA-DRA, MS4A4A, CD8A, HLA-DPA1, CD2, THEMIS, TRAT1, CD274, and FCER1G (Figure 4a). GO enrichment analysis of these genes revealed their primary involvement in neutrophil chemotaxis, cellular response to type II interferons, presentation of exogenous peptide antigens via MHC Class II pathways, antigen processing and presentation, and adaptive immune responses (Figure 4b). Additionally, KEGG enrichment analysis indicated that these genes are predominantly associated with cell adhesion molecules, antigen processing and presentation, and hematopoietic cell lineages (Figure 4c).

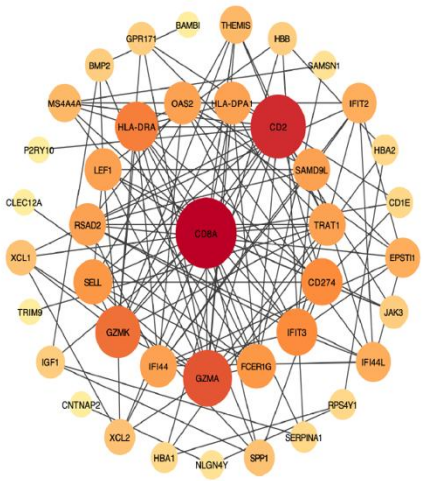

Figure 3: PPI network diagram. The redder the color of the gene in the network, the higher the connectivity of the gene with other genes

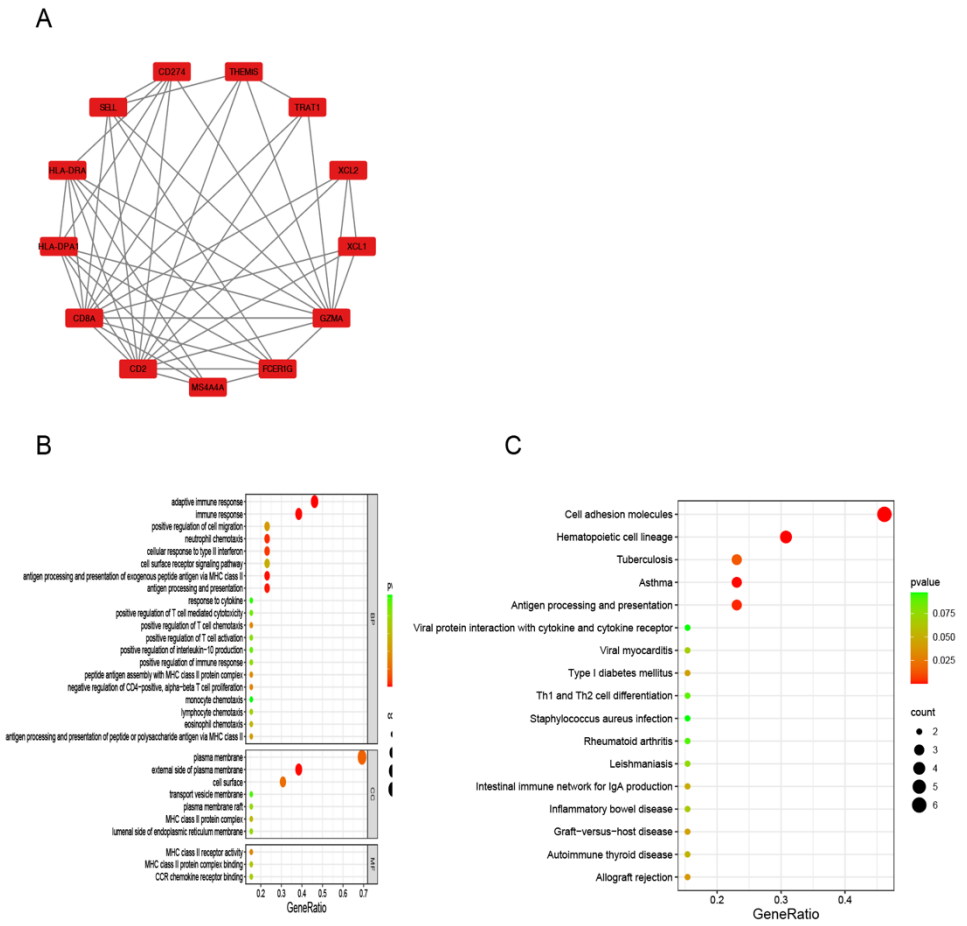

Figure 4 Significant gene module and enrichment analysis of the modular genes. (a) A significant gene clustering module. (b) GO enrichment analysis of the modular genes. (c) KEGG enrichment analysis of the modular genes.

3.4 Identification and Functional Analysis of Hub Genes

Through comprehensive analysis using seven independent algorithms in CytoHubba, we initially identified the top 15 candidate genes from each method. Intersection analysis of these gene sets revealed seven consensus hub genes: HLA-DRA, GZMA, FCER1G, GZMK, CD2, CD8A, and IFIT3 (Figure 5a). Further refinement through integration with MCODE-identified gene clusters yielded five core hub genes: HLA-DRA, GZMA, FCER1G, CD2, and CD8A.

To elucidate the functional relationships of these hub genes, we constructed an interaction network using the GeneMANIA database, which demonstrated the following interaction profiles: 77.64% physical interactions, 8.01% co-expression, 5.37% predicted interactions, and 3.63% co-localization (Figure 5b). Subsequent analysis identified 20 closely associated genes that primarily participate in critical biological processes, including lymphocyte differentiation, T cell maturation, antigen receptor-mediated signaling pathways, and MHC protein complex formation.

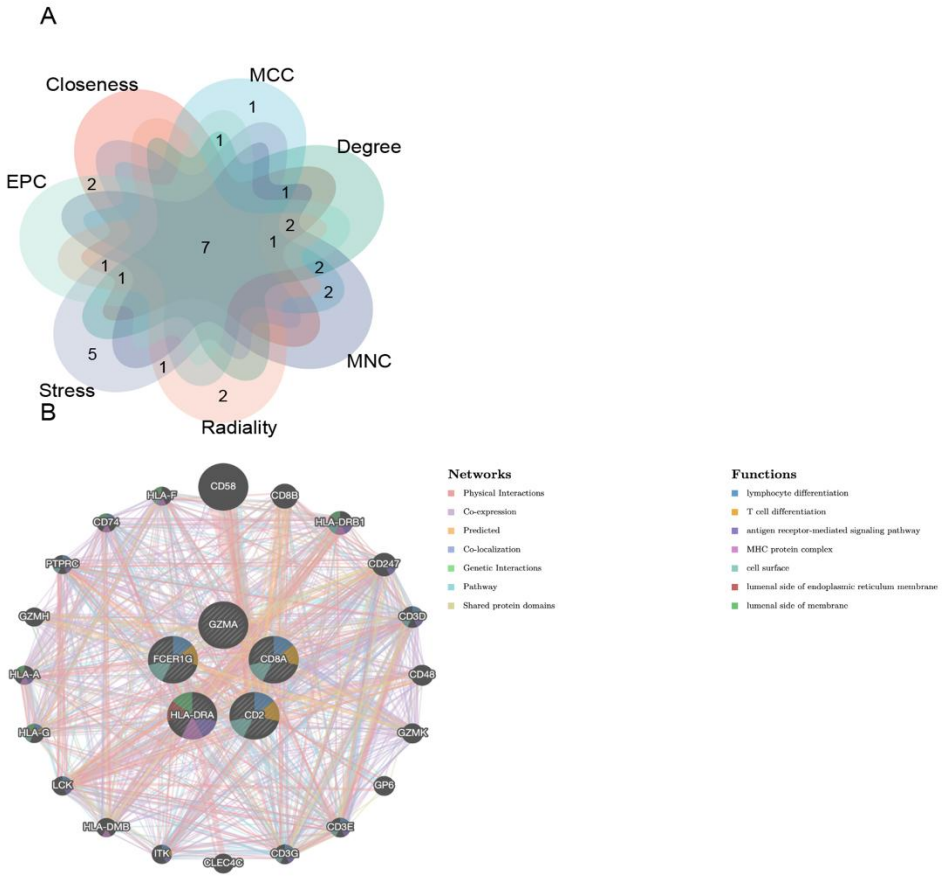

Figure 5: Venn diagram and co-expression network of hub genes. (a) The Venn diagram showed 7 overlapping hub genes screened by 7 algorithms. (b) Hub genes and their co-expression genes were analyzed via GeneMANIA.

3.5 Immune Cell Infiltration Analysis

Based on our analytical findings, it is clear that immune regulation plays a crucial role in both alopecia areata (AA) and COVID-19. Consequently, we conducted a detailed analysis of immune cell infiltration for these two conditions. The differences in the proportions of 22 distinct immune cell types between AA patients and healthy controls are illustrated in Figure AA-BAR. Notably, AA patients showed a decreased presence of resting CD4 memory T cells, follicular helper T cells, and activated dendritic cells, while an increased presence of M1 and M2 macrophages was observed compared to healthy controls (Figure 6). In the COVID-19 dataset, there was an increase in the proportion of memory B cells, plasma cells, naïve CD4 T cells, monocytes, M0 macrophages, activated dendritic cells, activated mast cells, and neutrophils in COVID-19 patients. Conversely, a decrease was noted in the proportion of naïve B cells, CD8 T cells, resting CD4 memory T cells, resting dendritic cells, and resting mast cells (Figure 7). Patients with AA and COVID-19 each exhibited distinct immune cell infiltration profiles. It is particularly noteworthy that certain immune cell types may serve as critical regulatory points in managing both diseases.

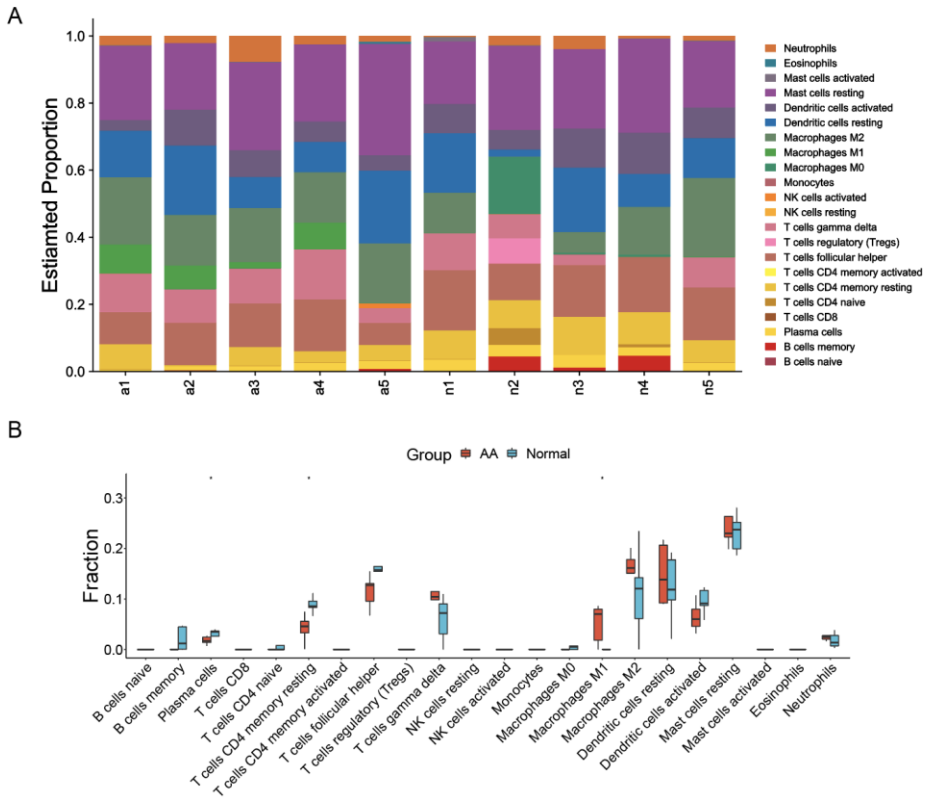

Figure 6: Identification of key immune cell types associated with AA. (a) The estimated percentage of immune cells. (B) The difference in immune infiltration between AA and controls. The control group was blue, and the AA group was red.

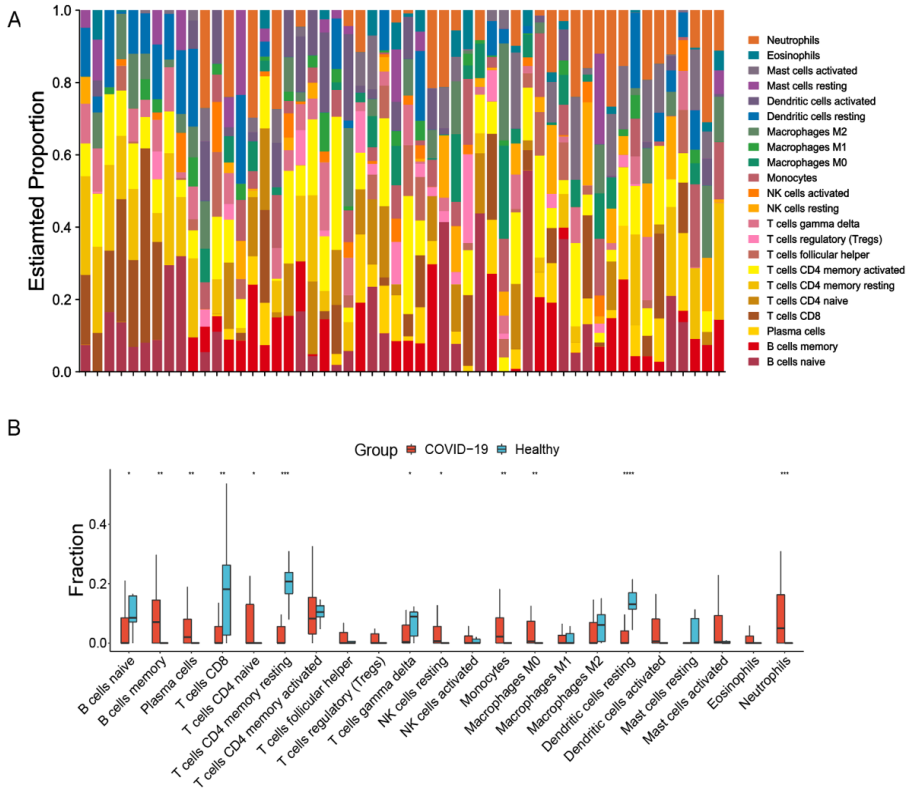

Figure 7:Identification of key immune cell types associated with COVID-19. (a) The estimated percentage of immune cells. (B) The difference in immune infiltration between COVID-19 and controls. The control group was blue, and the COVID-19 group was red.

3.6 ROC curves of Hub Genes

We utilized receiver operating characteristic (ROC) curves to assess the diagnostic efficacy of the five key hub genes. In the COVID-19 dataset, HLA-DRA (AUC: 0.980), GZMA (AUC: 0.905), FCER1G (AUC: 0.882), CD2 (AUC: 0.934), and CD8A (AUC: 0.936) exhibited high diagnostic accuracy in distinguishing COVID-19 patients from healthy controls (Figure 8a). Similarly, in the AA dataset, HLA-DRA (AUC: 1.000), GZMA (AUC: 0.960), FCER1G (AUC: 1.000), CD2 (AUC: 0.920), and CD8A (AUC: 0.960) also demonstrated strong diagnostic performance in differentiating AA patients from healthy controls (Figure 8b). The above results indicate that we have identified common exploratory biomarkers for the two diseases.

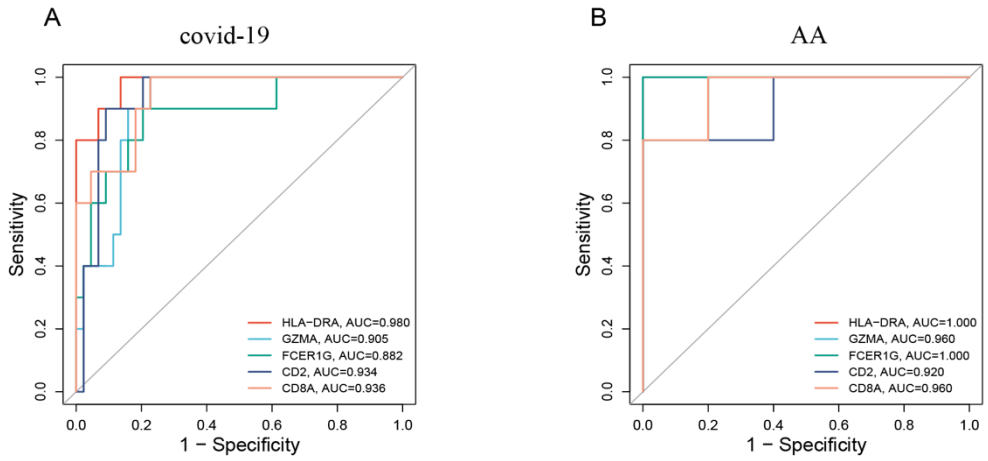

Figure 8: Validation of diagnostic shared biomarkers. (a) The ROC curve of the diagnostic efficacy verification in GSE171110. (b) The ROC curve of the diagnostic efficacy verification in GSE45512.

4. Discussion

Numerous studies have confirmed that viral infections, such as cytomegalovirus and Epstein-Barr virus, can alter the environmental factors of the human immune system, thereby triggering autoimmune diseases. In recent years, an increasing number of reports have highlighted the association between SARS-CoV-2 infection and the development or progression of autoimmune diseases. However, the exact mechanisms underlying this phenomenon remain incompletely understood.

Existing research has identified several mechanisms through which viral infections can induce autoimmune responses in the host, including molecular mimicry, epitope spreading, and bystander activation[27-29]. Based on these findings, it is plausible to hypothesize that SARS-CoV-2 infection may trigger the host's immune defense mechanisms, leading to the production of antibodies that structurally resemble self-antigens, thereby mediating the onset or exacerbation of autoimmune diseases.

Furthermore, it has been proposed that the autoimmune phenomena observed following COVID-19 may be related to transient immune suppression and inappropriate immune reconstitution in susceptible individuals[30]. Additionally, other potential mechanisms could explain the correlation between COVID-19 and autoimmune diseases. For instance, neutrophil extracellular traps (NETs) have been identified as a key pathological driver in COVID-19 and also play a crucial role in rheumatic diseases[31-32].

In summary, a growing body of evidence suggests a close association between COVID-19 and the onset, exacerbation, or relapse of alopecia areata (AA). However, research into the underlying mechanisms connecting the two conditions remains limited. Therefore, we conducted the first comprehensive bioinformatics analysis to explore the shared molecular biological functions and pathways between AA and COVID-19, as well as to identify key diagnostic genes common to both.

In this study, we identified 71 common differentially expressed genes (DEGs) between AA and COVID-19, ultimately narrowing down

to five core hub genes through further screening. These genes were found to play significant roles in immune activities.

HLA-DRA encodes the alpha chain of the Human Leukocyte Antigen (HLA) class II molecules, which are critical for immune system function. These molecules are primarily expressed on the surface of professional antigen-presenting cells (APCs), such as dendritic cells, macrophages, and B cells. A substantial body of research indicates that the expression of HLA-DRA protein is significantly decreased in patients with severe COVID-19[33-35]. In various cell types of patients with severe COVID-19, including myeloid cells, B cells, T cells, natural killer cells, and platelets in peripheral blood mononuclear cells (PBMCs) and bronchoalveolar lavage fluid (BALF), the downregulation of HLA-DRA gene expression suggests an overall immunosuppressive state in the blood and lungs at early stages. This may lead to impaired antigen presentation and T cell suppression, thereby promoting cytokine storms and excessive inflammation[36]. HLA-DRA has also been associated with various autoimmune diseases, such as rheumatoid arthritis, autoimmune thyroid disease, and Sjögren's syndrome[37-39]. Although contradictory evidence exists regarding the association of HLA with AA in different populations, a large-scale genome-wide association study suggests that HLA-DR is a key etiological driver of AA[40].

GZMA encodes granzyme A, a serine protease predominantly found within the cytotoxic granules of cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells. In cellular immunity, CTLs and NK cells induce apoptosis in target cells by releasing granules containing granzyme A[41]. In the context of COVID-19, as the disease progresses, there is a significant decrease in the absolute numbers of CD8+ T and NK cells; however, these cells become highly activated, with increased expression of perforin and granzymes, leading to a compensatory enhancement of cytotoxic potential[42]. The collapse of immune privilege in anagen hair follicles is a crucial prerequisite for the development of alopecia areata (AA), where affected follicles are rapidly infiltrated and damaged by NKG2D+ T cells and natural killer (NK) cells[43-44].

FCER1G encodes the Fc receptor γ chain, which is present in various types of immune cells. It aids in the removal of pathogens and antigens and promotes abnormal immune responses, such as IgE-dependent allergies, through interactions with the crystallizable fragments of immunoglobulins[45-47].

CD2, also known as LFA-2, is an adhesion molecule expressed on T cells and natural killer cells. It binds to CD58 on antigen-presenting cells (APCs), thereby facilitating the binding and signal transduction of the T cell receptor (TCR)[48]. CD2 is involved in the co-stimulatory signaling of T cells, working in conjunction with the primary T cell receptor (TCR) signals to promote full activation of T cells. It also plays a significant role in the cytotoxic function of NK cells[49-50]. Research indicates that anti-CD2 therapy, which blocks the CD2-CD58 co-stimulatory signals, may be a therapeutic strategy for suppressing autoimmune diseases. This is because the CD2-CD58 interaction enhances the synthesis of IL-2 and interferon-γ (IFN-γ), as well as cytotoxicity mediated by T and NK cells[51-52].

CD8A encodes the CD8a chain of the dimeric CD8 protein, which plays a crucial role in cell-mediated immune defense and T-cell development. CD8A has been extensively reported as a potential prognostic and diagnostic marker for various diseases, including chronic rhinosinusitis and rheumatoid arthritis[53-54].

A gene interaction network was constructed for the above five core hub genes, and the results showed that the functions of these genes and their interacting genes are primarily involved in immune processes such as lymphocyte differentiation, T cell differentiation, antigen receptor-mediated signaling pathways, and MHC protein complexes. This indicates that immune activity plays a significant role in the progression of both diseases.

Finally, we employed ROC curves to evaluate the diagnostic efficacy of the aforementioned five hub genes in AA and COVID-19. The AUC values for both diseases exceeded 0.850, indicating robust diagnostic potential. These findings suggest that these core hub genes hold promise as potential diagnostic biomarkers and therapeutic targets for both conditions in the future. However, it should be noted that the ROC curves of the hub genes in

AA (with AUC values of 1.0 for FCER1G and HLA-DRA) suggest the possibility of overfitting due to the small sample size (n=5 per group). Therefore, the above results require further experimental validation.

5. Conclusion

While previous studies have identified a link between COVID-19 and the incidence, exacerbation, or recurrence of alopecia areata (AA), there remains a significant gap in research exploring their common molecular mechanisms using bioinformatics. This study pioneers the identification of shared core hub genes between COVID-19 and AA, thereby paving the way for deeper inquiries into their pathogenic mechanisms and targeted therapeutic strategies. However, our study is not without limitations. First, our findings are derived exclusively from publicly available RNA sequencing data for AA and COVID-19, and lack experimental validation. Second, the functions of these hub genes need to be further confirmed through in vitro experiments, such as gene knockdown studies and Quantitative Real-Time PCR (qPCR). These limitations highlight the directions and priorities for our future work.

6. Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/supplementary material.

7. Conflict of Interest Statement

The authors declare no conflict of interest.

8. Funding Statement

No external funding received.

References

1. Pratt, C. H., King, L. E., Jr., Messenger, A. G., Christiano, A. M., & Sundberg, J. P. (2017). Alopecia areata. *Nat Rev Dis Primers*, *3*, 17011. https://doi.org/10.1038/nrdp.2017.11
2. Gip, L., Lodin, A., & Molin, L. (1969). Alopecia areata. A follow-up investigation of outpatient material. *Acta Derm Venereol*, *49*(2), 180-188.
3. Paus, R., Nickoloff, B. J., & Ito, T. (2005). A 'hairy' privilege. *Trends Immunol*, *26*(1), 32-40. https://doi.org/10.1016/j.it.2004.09.014
4. Meyer, K. C., Klatte, J. E., Dinh, H. V., Harries, M. J., Reithmayer, K., Meyer, W., Sinclair, R., & Paus, R. (2008). Evidence that the bulge region is a site of relative immune privilege in human hair follicles. *Br J Dermatol*, *159*(5), 1077-1085. https://doi.org/10. 1111/j.1365-2133.2008.08818.x
5. Paus, R., & Bertolini, M. (2013). The role of hair follicle immune privilege collapse in alopecia areata: status and perspectives. *J Investig Dermatol Symp Proc*, *16*(1), S25-27. https://doi.org/10.1038/jidsymp.2013.7
6. Messenger, A. G., & Bleehen, S. S. (1984). Alopecia areata: light and electron microscopic pathology of the regrowing white hair. *Br J Dermatol*, *110*(2), 155-162. https://doi.org/10.1111/j.1365-2133.1984.tb07461.x
7. Singh, R., Kumar, P., Kumar, D., Aggarwal, N., Chopra, H., & Kumar, V. (2024). Alopecia areata: review of epidemiology, pathophysiology, current treatments and nanoparticulate delivery system. *Ther Deliv*, *15*(3), 193-210. https://doi.org/10.4155/tde-2023-0071
8. Shih, W. L., Tung, Y. C., Chang, L. Y., Fang, C. T., & Tsai, W. Y. (2021). Increased Incidence of Pediatric Type 1 Diabetes With Novel Association With Coxsackievirus A Species in Young Children but Declined Incidence in Adolescents in Taiwan. *Diabetes Care*, *44*(7), 1579-1585. https://doi.org/10.2337/dc20-1092
9. Joo, Y. B., Lim, Y. H., Kim, K. J., Park, K. S., & Park, Y. J. (2019). Respiratory viral infections and the risk of rheumatoid arthritis. *Arthritis Res Ther*, *21*(1), 199. https://doi.org/10.1186/s13075-019-1977-9
10. Houen, G., & Trier, N. H. (2020). Epstein-Barr Virus and Systemic Autoimmune Diseases. *Front Immunol*, *11*, 587380. https://doi.org/10.3389/fimmu.2020.587380
11. Chang, R., Yen-Ting Chen, T., Wang, S. I., Hung, Y. M., Chen, H. Y., & Wei, C. J. (2023). Risk of autoimmune diseases in patients with COVID-19: A retrospective cohort study. *EClinicalMedicine*, *56*, 101783. https://doi.org/10.1016/j.eclinm.2022.101783
12. Christensen, R. E., & Jafferany, M. (2022). Association between alopecia areata and COVID-19: A systematic review. *JAAD Int*, *7*, 57-61. https://doi.org/10.1016/j.jdin.2022.02.002
13. Birkett, L., Singh, P., Mosahebi, A., & Dhar,

S. (2022). Possible Associations Between Alopecia Areata and COVID-19 Vaccination and Infection. *Aesthet Surg J*, *42*(11), Np699-np702. https://doi.org/10.1093/asj/sjac165
14. Kim, J. S., Lee, G. J., Jeong, C. Y., Yeom, S. W., Nam, K. H., Yun, S. K., & Park, J. (2024). Risk of Alopecia Areata After COVID-19. *JAMA Dermatol*, *160*(2), 232-235. https://doi.org/10.1001/jamadermatol.2023.5559
15. Barrett, T., Troup, D. B., Wilhite, S. E., Ledoux, P., Evangelista, C., Kim, I. F., Tomashevsky, M., Marshall, K. A., Phillippy, K. H., Sherman, P. M., Muertter, R. N., Holko, M., Ayanbule, O., Yefanov, A., & Soboleva, A. (2011). NCBI GEO: archive for functional genomics data sets--10 years on. *Nucleic Acids Res*, *39*(Database issue), D1005-1010. https://doi.org/10.1093/nar/gkq1184
16. Overmyer, K. A., Shishkova, E., Miller, I. J., Balnis, J., Bernstein, M. N., Peters-Clarke, T. M., Meyer, J. G., Quan, Q., Muehlbauer, L. K., Trujillo, E. A., He, Y., Chopra, A., Chieng, H. C., Tiwari, A., Judson, M. A., Paulson, B., Brademan, D. R., Zhu, Y., Serrano, L. R.,…Jaitovich, A. (2021). Large-Scale Multi-omic Analysis of COVID-19 Severity. *Cell Syst*, *12*(1), 23-40.e27. https://doi.org/10.1016/j. cels.2020.10.003
17. Horvath, S., Nazmul-Hossain, A. N., Pollard, R. P., Kroese, F. G., Vissink, A., Kallenberg, C. G., Spijkervet, F. K., Bootsma, H., Michie, S. A., Gorr, S. U., Peck, A. B., Cai, C., Zhou, H., & Wong, D. T. (2012). Systems analysis of primary Sjögren's syndrome pathogenesis in salivary glands identifies shared pathways in human and a mouse model. *Arthritis Res Ther*, *14*(6), R238. https://doi.org/10.1186/ar4081
18. Barrett, T., Wilhite, S. E., Ledoux, P., Evangelista, C., Kim, I. F., Tomashevsky, M., Marshall, K. A., Phillippy, K. H., Sherman, P. M., Holko, M., Yefanov, A., Lee, H., Zhang, N., Robertson, C. L., Serova, N., Davis, S., & Soboleva, A. (2013). NCBI GEO: archive for functional genomics data sets--update. *Nucleic Acids Res*, *41*(Database issue), D991-995. https://doi.org/10.1093/nar/gks1193
19. Chen, H., & Boutros, P. C. (2011). VennDiagram: a package for the generation of highly-customizable Venn and Euler diagrams in R. *BMC Bioinformatics*, *12*, 35. https://doi.org/10.1186/1471-2105-12-35
20. Sherman, B. T., Hao, M., Qiu, J., Jiao, X., Baseler, M. W., Lane, H. C., Imamichi, T., & Chang, W. (2022). DAVID: a web server for functional enrichment analysis and functional annotation of gene lists (2021 update). *Nucleic Acids Res*, *50*(W1), W216-w221. https://doi.org/10.1093/nar/gkac194
21. Szklarczyk, D., Gable, A. L., Lyon, D., Junge, A., Wyder, S., Huerta-Cepas, J., Simonovic, M., Doncheva, N. T., Morris, J. H., Bork, P., Jensen, L. J., & Mering, C. V. (2019). STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Res*, *47*(D1), D607-d613. https://doi.org/10.1093/nar/gky1131
22. Shannon, P., Markiel, A., Ozier, O., Baliga, N. S., Wang, J. T., Ramage, D., Amin, N., Schwikowski, B., & Ideker, T. (2003). Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res*, *13*(11), 2498-2504. https://doi.org/10.1101/gr.1239303
23. Bader, G. D., & Hogue, C. W. (2003). An automated method for finding molecular complexes in large protein interaction networks. *BMC Bioinformatics*, *4*, 2. https://doi.org/10.1186/1471-2105-4-2
24. Chin, C. H., Chen, S. H., Wu, H. H., Ho, C. W., Ko, M. T., & Lin, C. Y. (2014). cytoHubba: identifying hub objects and sub-networks from complex interactome. *BMC Syst Biol*, *8 Suppl 4*(Suppl 4), S11. https:// doi.org/10.1186/1752-0509-8-s4-s11
25. Franz, M., Rodriguez, H., Lopes, C., Zuberi, K., Montojo, J., Bader, G. D., & Morris, Q. (2018). GeneMANIA update 2018. *Nucleic Acids Res*, *46*(W1), W60-w64. https://doi.org/10.1093/nar/gky311
26. Robin, X., Turck, N., Hainard, A., Tiberti, N., Lisacek, F., Sanchez, J. C., & Müller, M. (2011). pROC: an open-source package for R and S+ to analyze and compare ROC curves. *BMC Bioinformatics*, *12*, 77. https://doi. org/10.1186/1471-2105-12-77
27. Kim, B., Kaistha, S. D., & Rouse, B. T. (2006). Viruses and autoimmunity. *Autoimmunity*, *39*(1), 71-77. https://doi.org/10.10 80/08 916930500484708
28. Getts, D. R., Chastain, E. M., Terry, R. L., & Miller, S. D. (2013). Virus infection, antiviral immunity, and autoimmunity. *Immunol Rev*,

255(1),197-209. https://doi.org/10.1111/imr.12091
29. Fujinami, R. S., von Herrath, M. G., Christen, U., & Whitton, J. L. (2006). Molecular mimicry, bystander activation, or viral persistence: infections and autoimmune disease. *Clin Microbiol Rev*, *19*(1), 80-94. https://doi.org/10.1128/cmr.19.1.80-94.2006
30. Cañas, C. A. (2020). The triggering of post-COVID-19 autoimmunity phenomena could be associated with both transient immunosuppression and an inappropriate form of immune reconstitution in susceptible individuals. *Med Hypotheses*, *145*, 110345. https://doi.org/10.1016/j.mehy.2020.110345
31. Narasaraju, T., Tang, B. M., Herrmann, M., Muller, S., Chow, V. T. K., & Radic, M. (2020). Neutrophilia and NETopathy as Key Pathologic Drivers of Progressive Lung Impairment in Patients With COVID-19. *Front Pharmacol*, *11*, 870. https://doi.org/10.3389/fphar.2020.00870
32. Apel, F., Zychlinsky, A., & Kenny, E. F. (2018). The role of neutrophil extracellular traps in rheumatic diseases. *Nat Rev Rheumatol*, *14*(8), 467-475. https://doi.org/10.1038/s41584-018-0039-z
33. Benlyamani, I., Venet, F., Coudereau, R., Gossez, M., & Monneret, G. (2020). Monocyte HLA-DR Measurement by Flow Cytometry in COVID-19 Patients: An Interim Review. *Cytometry A*, *97*(12), 1217-1221. https://doi.org/10.1002/cyto.a.24249
34. Moratto, D., Chiarini, M., Giustini, V., Serana, F., Magro, P., Roccaro, A. M., Imberti, L., Castelli, F., Notarangelo, L. D., & Quiros-Roldan, E. (2020). Flow Cytometry Identifies Risk Factors and Dynamic Changes in Patients with COVID-19. *J Clin Immunol*, *40*(7), 970-973. https://doi.org/10.1007/s10875-020-00806-6
35. Gatti, A., Radrizzani, D., Viganò, P., Mazzone, A., & Brando, B. (2020). Decrease of Non-Classical and Intermediate Monocyte Subsets in Severe Acute SARS-CoV-2 Infection. *Cytometry A*, *97*(9), 887-890. https://doi.org/10.1002/cyto.a.24188
36. Chan, K. R., Koh, C. W. T., Ng, D. H. L., Qin, S., Ooi, J. S. G., Ong, E. Z., Zhang, S. L. X., Sam, H., Kalimuddin, S., Low, J. G. H., & Ooi, E. E. (2023). Early peripheral blood MCEMP1 and HLA-DRA expression predicts COVID-19 prognosis. *EBioMedicine*, *89*, 104472. https://doi.org/10.1016/j. ebiom.2023.104472
37. Zhang, F., Wei, K., Slowikowski, K., Fonseka, C. Y., Rao, D. A., Kelly, S., Goodman, S. M., Tabechian, D., Hughes, L. B., Salomon-Escoto, K., Watts, G. F. M., Jonsson, A. H., Rangel-Moreno, J., Meednu, N., Rozo, C., Apruzzese, W., Eisenhaure, T. M., Lieb, D. J., Boyle, D. L.,…Raychaudhuri, S. (2019). Defining inflammatory cell states in rheumatoid arthritis joint synovial tissues by integrating single-cell transcriptomics and mass cytometry. *Nat Immunol*, *20*(7), 928-942. https://doi.org/10.1038/s41590-019-0378-1
38. Du, P., Zhu, J., Yao, Q., Cai, T., Xu, J., Fang, Y., Wu, Y., Zhang, W., & Zhang, J. A. (2022). HLA-DRA Gene Polymorphisms Are Associated with Graves' Disease as an Autoimmune Thyroid Disease. *Biomed Res Int*, *2022*, 6839634. https://doi.org/10.1155/2022/6839634
39. Rivière, E., Pascaud, J., Tchitchek, N., Boudaoud, S., Paoletti, A., Ly, B., Dupré, A., Chen, H., Thai, A., Allaire, N., Jagla, B., Mingueneau, M., Nocturne, G., & Mariette, X. (2020). Salivary gland epithelial cells from patients with Sjögren's syndrome induce B-lymphocyte survival and activation. *Ann Rheum Dis*, *79*(11), 1468-1477. https://doi.org/10.1136/annrheumdis-2019-216588
40. Betz, R. C., Petukhova, L., Ripke, S., Huang, H., Menelaou, A., Redler, S., Becker, T., Heilmann, S., Yamany, T., Duvic, M., Hordinsky, M., Norris, D., Price, V. H., Mackay-Wiggan, J., de Jong, A., DeStefano, G. M., Moebus, S., Böhm, M., Blume-Peytavi, U.,…Christiano, A. M. (2015). Genome-wide meta-analysis in alopecia areata resolves HLA associations and reveals two new susceptibility loci. *Nat Commun*, *6*, 5966. https://doi.org/10.1038/ncomms6966
41. Russell, J. H., & Ley, T. J. (2002). Lymphocyte-mediated cytotoxicity. *Annu Rev Immunol*, *20*, 323-370. https://doi.org/10.1146/annurev.immunol.20.100201.131730
42. Jiang, Y., Wei, X., Guan, J., Qin, S., Wang, Z., Lu, H., Qian, J., Wu, L., Chen, Y., Chen, Y., & Lin, X. (2020). COVID-19 pneumonia: CD8(+) T and NK cells are decreased in number but compensatory increased in cytotoxic potential. *Clin Immunol*, *218*,

108516. https://doi.org/10.1016/j.clim.2020.108516
43. Gilhar, A., Etzioni, A., & Paus, R. (2012). Alopecia areata. *N Engl J Med*, *366*(16), 1515-1525. https://doi.org/10.1056/NEJMra1103442
44. Bertolini, M., McElwee, K., Gilhar, A., Bulfone-Paus, S., & Paus, R. (2020). Hair follicle immune privilege and its collapse in alopecia areata. *Exp Dermatol*, *29*(8), 703-725. https://doi.org/10.1111/exd.14155
45. Küster, H., Thompson, H., & Kinet, J. P. (1990). Characterization and expression of the gene for the human Fc receptor gamma subunit. Definition of a new gene family. *J Biol Chem*, *265*(11), 6448-6452.
46. Fu, L., Cheng, Z., Dong, F., Quan, L., Cui, L., Liu, Y., Zeng, T., Huang, W., Chen, J., Pang, Y., Ye, X., Wu, G., Qian, T., Chen, Y., & Si, C. (2020). Enhanced expression of FCER1G predicts positive prognosis in multiple myeloma. *J Cancer*, *11*(5), 1182-1194. https://doi.org/10.7150/jca.37313
47. Amo, G., Cornejo-García, J. A., García-Menaya, J. M., Cordobes, C., Torres, M. J., Esguevillas, G., Mayorga, C., Martinez, C., Blanca-Lopez, N., Canto, G., Ramos, A., Blanca, M., Agúndez, J. A., & García-Martín, E. (2016). FCERI and Histamine Metabolism Gene Variability in Selective Responders to NSAIDS. *Front Pharmacol*, *7*, 353. https://doi.org/10.3389/fphar.2016.00353
48. Binder, C., Cvetkovski, F., Sellberg, F., Berg, S., Paternina Visbal, H., Sachs, D. H., Berglund, E., & Berglund, D. (2020). CD2 Immunobiology. *Front Immunol*, *11*, 1090. https://doi.org/10.3389/fimmu.2020.01090
49. Leitner, J., Herndler-Brandstetter, D., Zlabinger, G. J., Grubeck-Loebenstein, B., & Steinberger, P. (2015). CD58/CD2 Is the Primary Costimulatory Pathway in Human CD28-CD8+ T Cells. *J Immunol*, *195*(2), 477-487. https://doi.org/10.4049/jimmunol.1401917
50. Paul, S., & Lal, G. (2017). The Molecular Mechanism of Natural Killer Cells Function and Its Importance in Cancer Immunotherapy. *Front Immunol*, *8*, 1124. https://doi.org/10.3389/fimmu.2017.01124
51. Crawford, K., Stark, A., Kitchens, B., Sternheim, K., Pantazopoulos, V., Triantafellow, E., Wang, Z., Vasir, B., Larsen, C. E., Gabuzda, D., Reinherz, E., & Alper, C. A. (2003). CD2 engagement induces dendritic cell activation: implications for immune surveillance and T-cell activation. *Blood*, *102*(5), 1745-1752. https://doi.org/10.1182/blood-2002-07-2206
52. Erben, U., Pawlowski, N. N., Heimesaat, M. M., Loddenkemper, C., Doerfel, K., Spieckermann, S., Siegmund, B., & Kühl, A. A. (2015). Preventive Anti-CD2 Treatment does not Impair Parasite Control in a Murine Toxoplasmosis Model. *Eur J Microbiol Immunol (Bp)*, *5*(4), 306-315. https://doi.org/10.1 556/1886.2015.00040
53. Alromaih, S., Mfuna-Endam, L., Bosse, Y., Filali-Mouhim, A., & Desrosiers, M. (2013). CD8A gene polymorphisms predict severity factors in chronic rhinosinusitis. *Int Forum Allergy Rhinol*, *3*(8), 605-611. https://doi.org/10.1002/alr.21174
54. Zhou, S., Lu, H., & Xiong, M. (2021). Identifying Immune Cell Infiltration and Effective Diagnostic Biomarkers in Rheumatoid Arthritis by Bioinformatics Analysis. *Front Immunol*, *12*, 726747. https://doi.org/10.3389/fimmu.2021.726747