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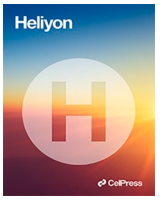

Research article

Establishment of a diagnostic model based on immune-related genes in children with asthma

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ABSTRACT

Objective: Allergic asthma is driven by an antigen-specific immune response. This study aimed to identify immune-related differentially expressed genes in childhood asthma and establish a classification diagnostic model based on these genes.
Methods: GSE65204 and GSE19187 were downloaded and served as training set and validation set. The immune cell composition was evaluated with ssGSEA algorithm based on the immune-related gene set. Modules that significantly related to the asthma were selected by WGCNA algorithm. The immune-related differentially expressed genes (DE-IRGs) were screened, the protein-protein interaction network and diagnostic model of DE-IRGs was constructed. The pathway and immune correlation analysis of hub DE-IRGs was analyzed.
Results: Eight immune cell types exhibited varying levels of abundance between the asthma and control groups. A total of 112 differentially expressed immune-related genes (DE-IRGs) was identified. Through the application of four ranking methods (MCC, MNC, DEGREE, and EPC), 17 hub DE-IRGs with overlapping significance were further selected. Subsequently, 8 optimized were identified using univariate logistic regression analysis and the LASSO regression algorithm, based on which a robust diagnostic model was constructed. Notably, TNF and CD40LG emerged as direct participants in asthma-related signaling pathways, displaying a positive correlation with the immune cell types of immature B cells, activated B cells, activated CD8 T cells, activated CD4 T cells, and myeloid-derived suppressor cells.
Conclusion: The diagnostic model constructed using the DE-IRGs (CCL5, CCR5, CD40LG, CD8A, IL2RB, PDCD1, TNF, and ZAP70) exhibited high and specific diagnostic value for childhood asthma. The diagnostic model may contribute to the diagnosis of childhood asthma.

1. Introduction

Asthma is the most prevalent chronic respiratory condition during childhood [1]. Manifestations of asthma encompass recurring wheezing, coughing, chest constriction, and breathlessness, often accompanied by nocturnal and early morning symptoms, which can significantly impact one's quality of life [2]. Recently, there has been a sharp increase in the overall prevalence of asthma among children worldwide, particularly among preschool-aged children [3]. This not only profoundly affects the physical and mental

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well-being of children but also imposes substantial emotional and economic burdens on families and society at large [4]. Asthma symptoms may manifest early in life, with approximately one-third of children experiencing wheezing within their first three years [5]. Although the majority of these children will cease to wheeze by the age of six, 40 % will continue to experience wheezing, either having already developed asthma or developing it later in life [6]. Depending on the methodology used for assessment, up to 10–15 % of children may exhibit asthma symptoms by school age [7]. Nevertheless, the underlying molecular mechanisms responsible for the occurrence and progression of childhood asthma remain elusive. Hence, elucidating the molecular mechanisms underlying childhood asthma could enhance early diagnosis, prognostic evaluation, and disease management.

With the advancement of bioinformatic methodologies, high-throughput analysis have become extensively utilized to furnish pivotal insights into the realm of molecular mechanisms [8]. Bioinformatic scrutiny can unveil an intricate biological progression that traverses diverse functional networks across distinct disease models [9]. Regrettably, in the realm of asthma-related investigations, only a handful of studies have delved into the gene expression profiles. For instance, gene expression analyses have identified disparate expression patterns of CXCR2, ALPL, CLC, CPA3, DNASEIL3, and IL1B in the pathogenesis of asthma [10,11]. Katayama et al. have discerned a distinctive gene module exclusive to acute wheezing in the blood samples of preschool wheezers [12]. This module exhibited associations with vitamin D levels and asthma medication. Shi et al. found that TNF and HLA-DPA1 were associated with immune responses in childhood atopic asthma [13]. Moreover, a mounting body of evidence suggests that innate immune cells play indispensable roles in the development of various asthma phenotypes [14]. Immune cells have been implicated in the pathogenic progression of asthma, which is characterized by granulocytic inflammation infiltrating the airways [15,16]. Nonetheless, the bioinformatics analysis investigating the correlation between infiltrating immune cells and pivotal genes in childhood asthma remains largely understudied.

In this paper, immunity was integrated into the whole genome analysis for genomics and predictive model of asthma in children. The distribution of immune cells in samples was evaluated, and then WGCNA algorithm was used, the hub genes related to immunity in children's asthma were obtained by constructing the network, and the candidate genes closely linked to immunity in childhood asthma were identified. Finally, the asthma classification diagnostic model was constructed based on the gene expression level. The results may help for early diagnosis and effective treatment of childhood asthma.

2. Materials and methods

2.1. GEO data download

GSE65204 and GSE19187 datasets were downloaded from NCBI Gene Expression Omnibus (GEO) [17] (http://www.ncbi.nlm.nih.gov/geo/) on March 25, 2023. The GSE65204 dataset [18] was used as the training set, encompassing 69 samples of nasal epithelial tissue from children, consisting of 33 normal control samples and 36 asthma samples. The detection platform was Agilent-028,004 SurePrint G3 Human Ge 8 × 60K Microarray. The GSE19187 dataset [19] consists of 38 samples of nasal epithelial tissue from children, of which 24 relevant samples (11 normal control tissues and 13 asthma disease samples) were selected as validation set. The detection platform was Affymetrix Human Gene 1.0 St Array.

2.2. Analysis of immune cell infiltration

To evaluate the type of immune infiltration of samples, the immunologic signature gene sets were downloaded from the Gene Set Enrichment Analysis (GSEA) database [20]. Subsequently, the samples in the GSE65204 dataset were assessed for immune infiltration types using the single sample gene set enrichment analysis (ssGSEA) algorithm [21], based on the GSVA package (Version 1.36.3) [22] (http://www.bioconductor.org/Packages/bioc/GSVA.html) in the R3.6.1 language. Next, the Kruskal-Wallis method was used to compare the differences in the proportions of immune cells between the control and asthma samples.

2.3. Weighted gene correlation Network Analysis

Weighed Gene Co-expression Network Analysis (WGCNA) is a bioinformatics algorithm utilized to construct co-expression networks, aiming to identify modules associated with diseases and select crucial pathological mechanisms or potential therapeutic targets [23]. Based on the gene expression levels in GSE65204, the R3.6.1 WGCNA package Version 1.61 [24] (https://cran.r-project.org/web/packages/wgcna/index.html) was used to screen for modules significantly correlated with sample grouping. The threshold for module was: at least 100 genes in the module set and cutheight = 0.995. Subsequently, the associations between each module and disease status, age, gender, and differentially expressed immune cells were calculated. Modules with p-values less than 0.05 and correlation coefficients exceeding 0.3 in absolute value were selected as modules associated with asthma. The genes within these modules that are significantly correlated with asthma were identified as candidate genes related to the disease and immune cells.

2.4. Screen of immune-related differentially expressed genes (DEGs)

First, immune-related genes (IRGs) were downloaded from The Immunology DataBase and Analysis Portal (Immport) database [25] (https://www.immport.org/home). The DEGs between control and asthma samples in GSE65204 were then screened using the R3.6.1 limma package Version 3.34.7 [26] (https://bioconductor.org/Packages/Release/BIOC/HTML/Limma.html) with screening thresholds of FDR less than 0.05 and log2 of absolute fold-change ($\log_2$FC) > 0.263. Two-directional hierarchical clustering [27] of

DEGs was performed on expression values based on Euclidean distance [28] by the pheatmap package (Version 1.0.8) [29] (https://cran.r-project.org/package=pheatmap). After that, the screened DEGs were compared with the IRGs, and the overlapping genes were preserved as DE-IRGs. Using DAVID version 6.8 [30] (https://david.ncifCrf.gov/), the GO function and KEGG signaling pathway enrichment analysis of DE-IRGs were performed with FDR less than 0.05 as screening threshold. Finally, the DE-IRGs were compared with genes obtained by WGCNA algorithm, the overlapped genes were retained as asthma-related DE-IRGs for the next analysis.

2.5. Construction of protein-protein interaction (PPI) network and identification of hub genes

Using the Retrieval of Interacting Genes (STRING) [31] (version: 11.0, http://string-db.org/) database, the interaction relationship between asthma-related DE-IRGs were searched to build an interaction network, and the network was visualized through Cytoscape Version 3.9.0 [32] (http://www.cytoscape.org/). Then, CytoHubba Version 0.1 [33] in Cytoscape3.9.0 was used to identify hub genes in PPI network based on the topology analysis algorithm of MCC, MNC, DEGREE, and EPC. The overlapping hub genes screened under 4 algorithms were as the final hub DE-IRGs.

2.6. Construction of diagnostic model

Based on the expression levels of the hub DE-IRGs in the GSE65204 set, an univariate logistic regression analysis was performed using rms version 6.3-0 (https://cran.r-project.org/web/packages/index.html) [34] in R3.6.1, and the genes with p-values less than 0.05 were retained for further analysis. Next, the optimized DE-IRGs were screened using the LASSO algorithm in the R3.6.1 language lars package Version 1.2 [35] (https://cran.r-project.org/web/packages/lars/index.html). The risk value of each sample was calculated based on the LASSO coefficients of the obtained optimized DE-IRGs and the Kruskal- Wallis was used to compare the risk values between disease and normal control samples.

In GSE65204, The support vector machine (SVM) approach was utilized to construct a disease diagnostic classifier (Core: Sigmoid Kernel; Cross: 100-Fold Cross Validation) using R3.6.1 e1071 version 1.6–8 [36] (https://cran.r-project.org/web/packages/e1071). The sensitivity and specificity of the receiver operating characteristic (ROC) curve calculated using R 3.6.1 pROC version 1.12.1 [37] (https://cran.r-project.org/web/packages/proc/index.html) were used to evaluate the performance of the diagnostic model in the GSE65204 and GSE19187 dataset.

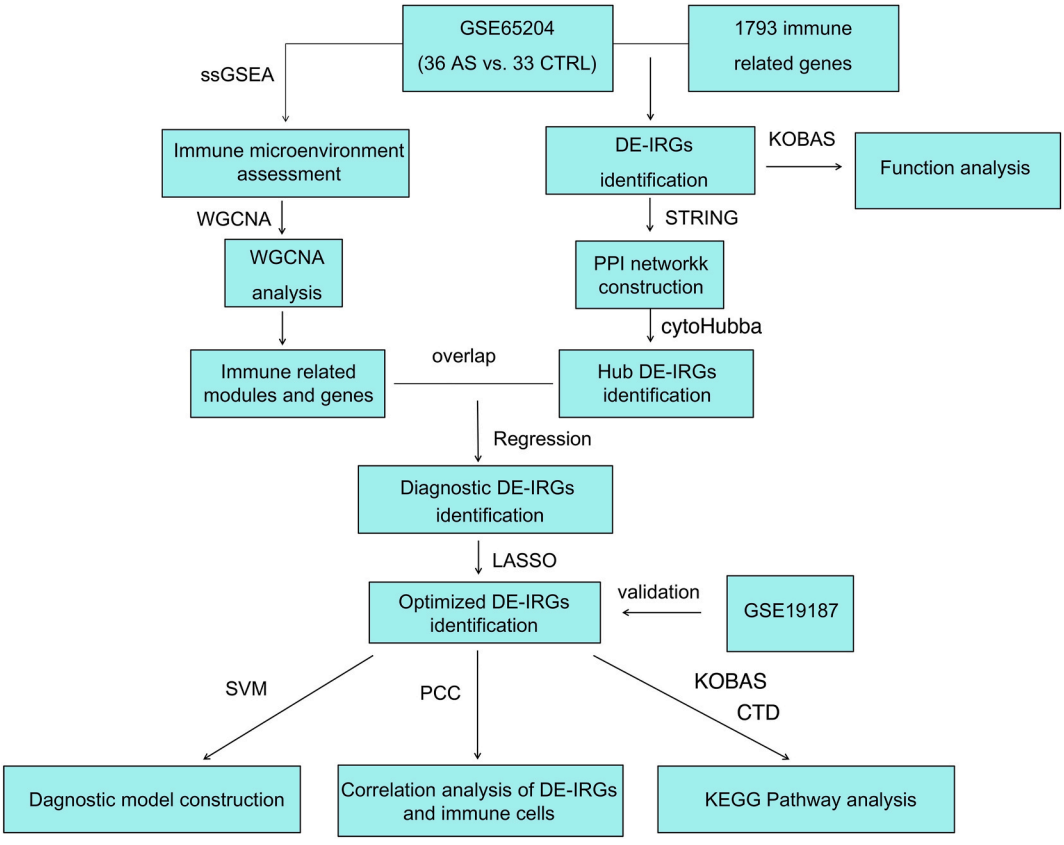

Fig. 1. The flow chart of the study.

2.7. Analysis of the pathway and immune correlation analysis of hub DE-IRGs

Based on the expression levels of hub DE-IRGs in GSE65204, the correlation between their expression levels and immune cells was calculated using the "cor" function in the R 3.6.1 language. Subsequently, the hub DE-IRGs were subjected to KEGG enrichment analysis using the KEGG Orthology Based Annotation System 3.0 (KOBAS 3.0) database [38] (http://kobas.cbi.pku.cn/index.php.). Furthermore, asthma-related KEGG signaling pathways were downloaded from the Comparative Toxicogenomics Database 2023 update database (http://ctd.mdibl.org/) and compared with the significantly correlated KEGG signaling pathways identified earlier among the hub DE-IRGs, retaining the overlapping portions. Finally, by integrating the immune cell-DE-IRGs-asthma related KEGG signaling pathway connections, a immune cell-DE-IRGs-asthma related KEGG signaling pathway relationship network was constructed.

2.8. Statistical analysis and workflow

All the statistical analysis carried out in this study were performed using R 3.6.1. A p value < 0.05 was considered statistically significant (*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$). The flow chart of the study was described in Fig. 1.

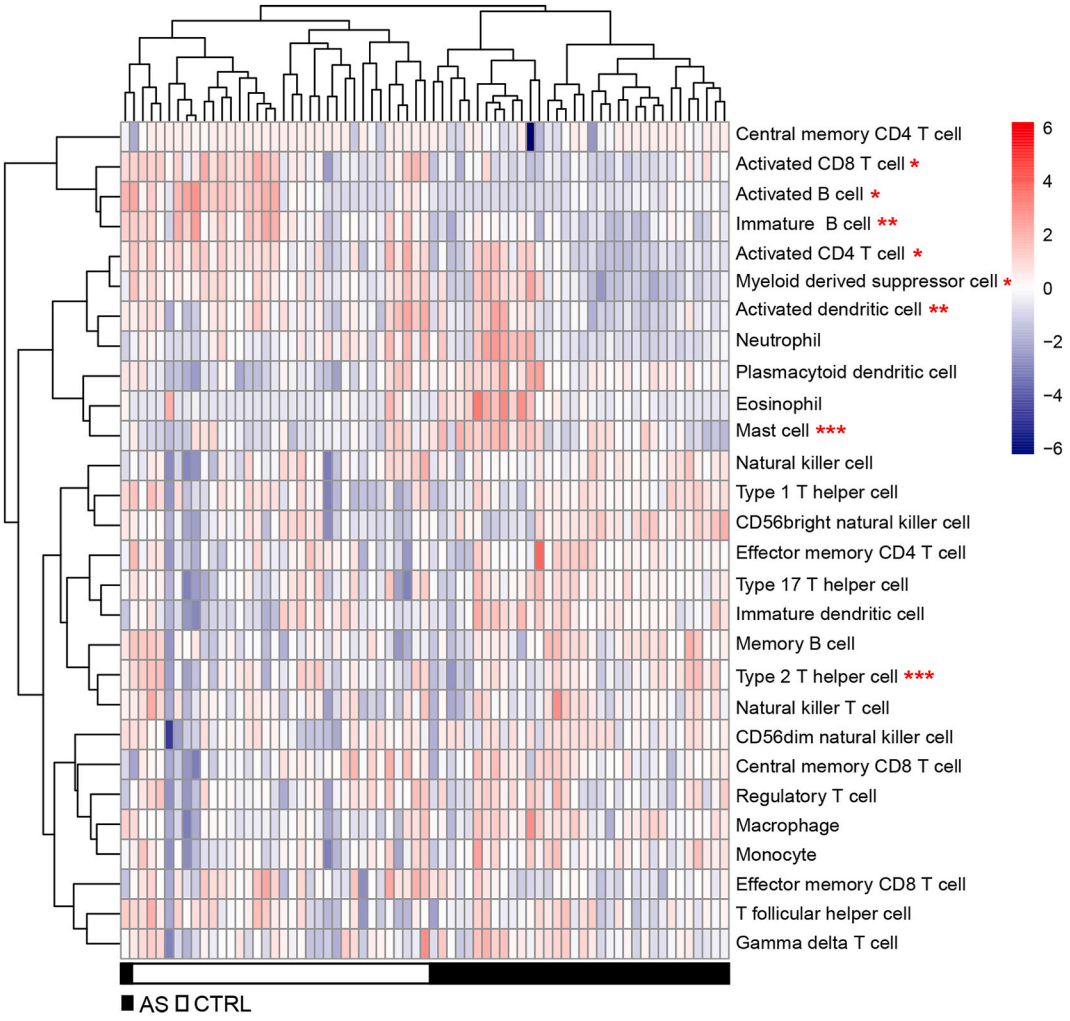

Fig. 2. Heat map showing the distribution of each type of immune cell in two groups. *: $p < 0.05$, **: $p < 0.01$, and ***: $p < 0.005$. AS: asthma; and CTRL: control.

3. Results

3.1. Evaluation of immune cell infiltration

The relative infiltration levels of 28 immune cells in the asthma group compared with control group were obtained by ssGSEA algorithm analysis (Fig. 2). The results showed that Type 2 T helper cell, Mast cell, Activated dendritic cell, Immature B cell, Activated CD8 T cell, Activated CD4 T cell, Myeloid derived suppressor cell and Activated B cell were significantly different between asthma and control groups.

3.2. Construction of weight gene co-expression network and identification of the key module

To elucidate the link between functional modules and immune cell infiltration in asthma patients, we performed WGCNA analysis. The results demonstrated that when squared correlation coefficients (r^2) = 0.9, the power value was 12, and the average node connectivity of the constructed co-expression network was 1 (Fig. 3A and B). Then, we set the minimum number of genes per module to 100, and Cutheight = 0.995, a total of 11 modules were obtained (Fig. 3C). Multi-dimensional scaling of expression data of all genes in these modules demonstrated that the genes contained in the various different modules tend to be distributed in the same regions

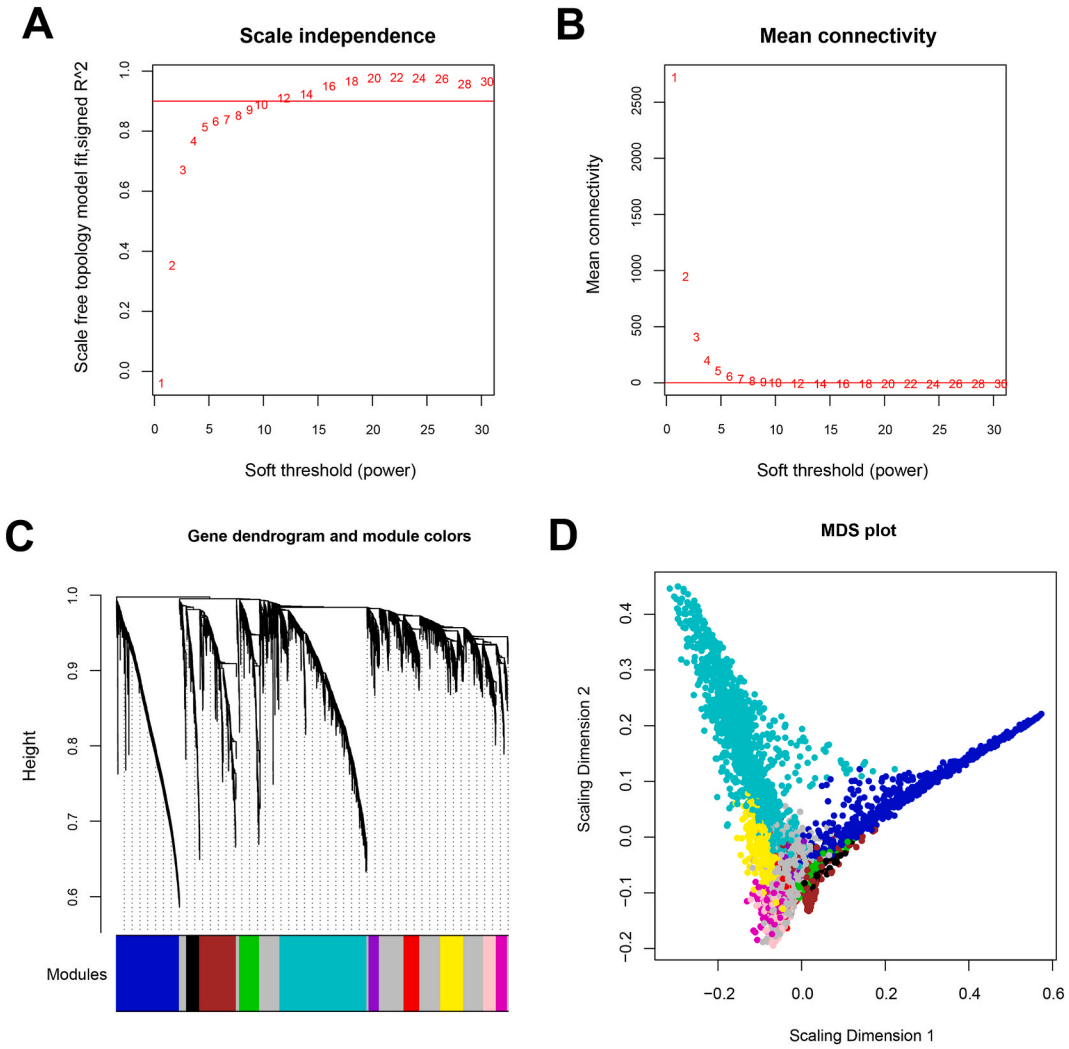

Fig. 3. A: The adjacency matrix of a weighted graph. The horizontal axis represents the weight parameter power, and the vertical axis represents the square of the correlation coefficient between log(k) and log(p(k)) in the network. The red line represents the standard line where the square of the correlation coefficient reaches 0.9. B: Schematic diagram of the average connectivity of genes under different power parameters. The red line represents the value of the average connectivity of network nodes under the power value of A, the average connectivity of network nodes was 1. C. Module division tree. Each color represents a different module. D. MDS map of genes in each module. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

(Fig. 3D). Correlation analysis between the eigengenes of each module and immune cell is presented in Fig. 4. Subsequently, four modules (brown, green, purple and red) were screened according to $p < 0.05$ and $|r|>3$, which contained 542, 293, 152, and 234 genes respectively. These genes are thought to be asthma-related genes.

3.3. Asthma-related DE-IRGs were obtained

In the GSE65204 dataset, a total of 657 DEGs were screened (329 down-regulated and 328 up-regulated), and the volcano plot was shown in Fig. 5A. The heat map showed that the expression values of DEGs can separate different types of samples, indicating that the DEGs had characterized expression features (Fig. 5B). Then, the DEGs were compared with the 1793 IRGs obtained from the ImmPort database, and 112 DE-IRGs were obtained. GO and KEGG pathway enrichment analysis identified 81 Biology processes, 23 Cellular components and 31 Molecular Funcrion, and 24 KEGG signaling pathways. The top 10 were displayed in Fig. S1. Finally, the 112 DE-IRGs were compared with the genes in the four modules, and a total of 59 asthma-related DE-IRGs were obtained.

3.4. The PPI network and hub DE-IRGs

The STRING data set was used to search for the interaction network of 59 asthma-related DE-IRGs product, 469 pairs of interaction connections with connection score higher than 0.4 were retained, and a PPI network was constructed (Fig. 6A).

To further explore functional relationships in the PPI networks, we applied the CytoHubba App to identify hub genes in the PPI networks. Four ranking methods (MCC, MNC, DEGREE and EPC) ranked top 20 genes from the network. By comparing the top 20 genes identified from these four topological methods, we identified 17 overlapping genes that used as important hub DE-IRGs and were selected for further analysis: C–C motif chemokine receptor 5 (*CCR5*), LCK Proto-Oncogene, Src family tyrosine kinase (*LCK*), interleukin 7 receptor (*IL7R*), CD247 molecule (*CD247*), CD19 molecule (*CD19*), CD244 molecule (*CD244*), C–C Motif chemokine ligand 5 (*CCL5*), CD40 ligand (*CD40*LG), Zeta chain of T cell receptor associated protein kinase 70 (*ZAP70*), CD48 molecule (*CD48*), interleukin 2 receptor subunit beta (*IL2RB*), Protein tyrosine phosphatase receptor type C (*PTPRC*), Programmed cell death 1 (*PDCD1*), FYN proto-oncogene, Src family tyrosine kinase (*FYN*), integrin subunit alpha L (*ITGAL*), CD8 subunit alpha (*CD8A*), and tumor necrosis factor (*TNF*) (Fig. 6B).

The diagnostic model based on DE-IRGs 17 diagnostic DE-IRGs were screened by univariate logistic regression analysis and $P < 0.05$ was used as cutoff (Fig. 7A). LASSO algorithm was performed on the 17 DE-IRGs, and a total of 8 optimized diagnostic DE-IRGs were obtained (Fig. 7B and C), including *CCL5*, *CCR5*, *CD40*LG, *CD8A*, *IL2RB*, *PDCD1*, *TNF* and *ZAP70*. Then, the risk value of each sample was calculated according to the LASSO coefficients, and the results showed that the risk score of the asthma group was significantly higher than that of the control group in the GSE65204 training set ($P < 0.05$, Fig. 8A). Subsequently, SVM method was used to construct a disease diagnosis model based on 8 optimized diagnostic DE-IRGs, which could distinguish between control and asthma

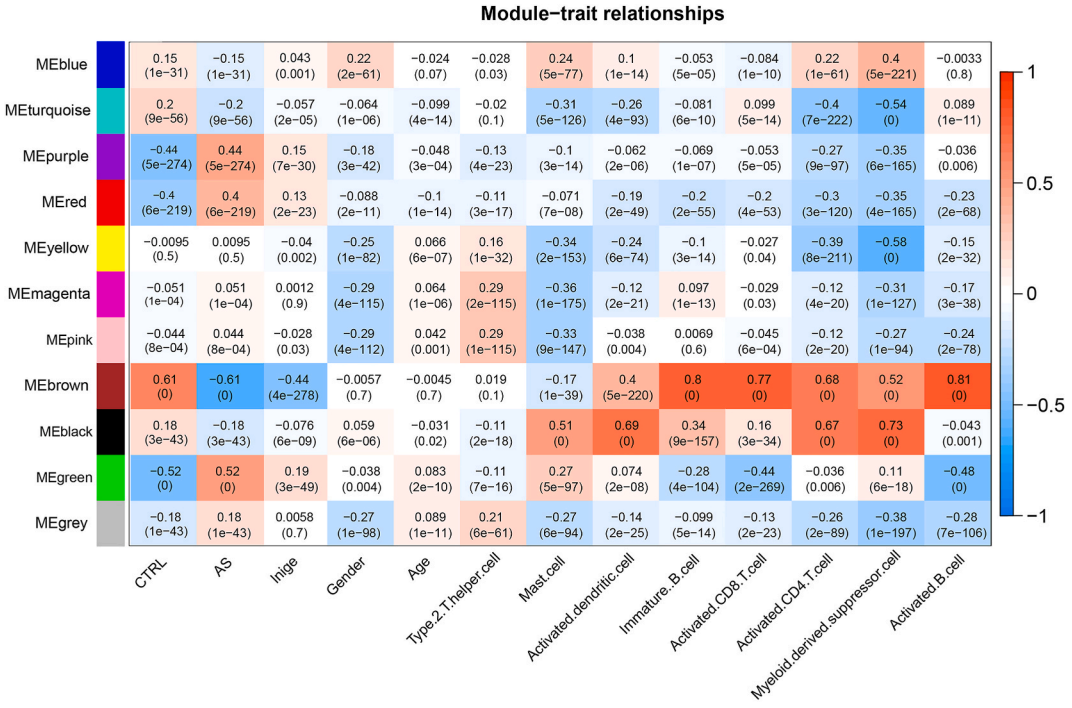

Fig. 4. The correlation heat map between the proportion of immune cells in the sample and the modules.

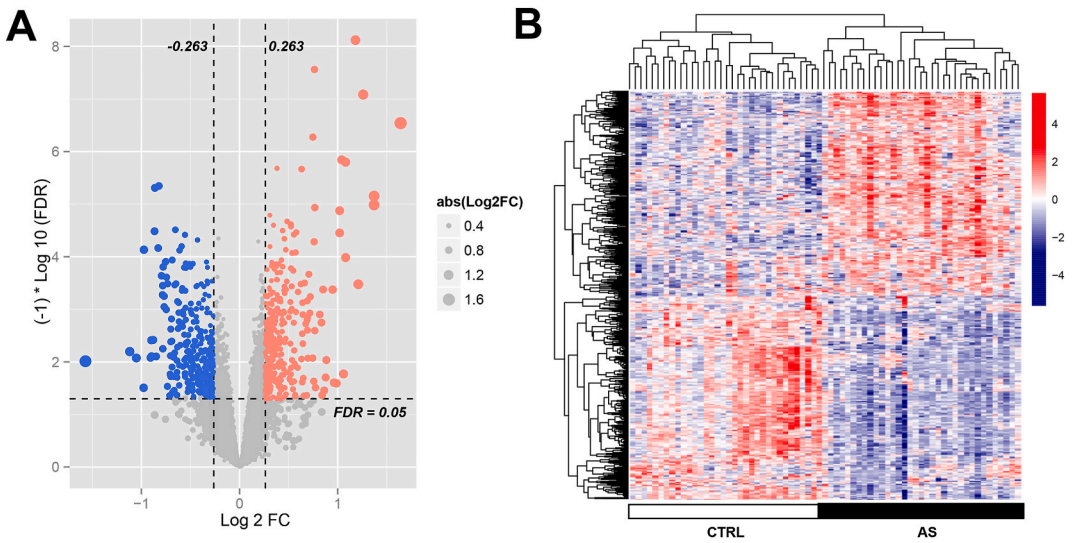

Fig. 5. A. The log_2FC-log10 (FDR, false discovery rate) volcano plot. Blue and red dots indicated lowly and highly expressed differentially expressed genes (DEGs) respectively, the black horizontal lines represent FDR <0.05, and two vertical line represent $|log_2FC$, fold change$| > 0.263$; B. Heat map based on DEGs expression level. The left black bar and the upper white bar represent asthma (AS) group and control (CTRL) control group, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

samples with an AUC of 0.888, according to the ROC curve (Fig. 8B). In addition, the expression levels of 8 optimized diagnostic DE-IRGs in control and asthma samples were evaluated, as shown in Fig. 8C. The results showed that the expression levels of all these 8 optimized diagnostic DE-IRGs were significantly lower in the asthma group than in the control group ($p < 0.05$). Finally, these results were verified in the validation set and the results were shown to be consistent with the training set (Fig. 8D–F).

3.5. The 8 optimized DE-IRGs were associated with pathways and immune cells

Considering the important role of multiple immune components in asthma diagnosis and pathological mechanism, we analyzed the correlation between 8 immune cells that were significantly different between the control and asthma groups and 8 optimized DE-IRG expressions. The results showed that immune cells of Activated B cell and Activated CD8 T cell were positively correlated with the expression of 8 optimized DE-IRGs, and Immature B cell, Activated CD4 T cell, and Myeloid derived suppressor cell showed significant positive correlation with the expression of 7 hub DE-IRGs except CD8A (Fig. 9 A).

Then, KOBAS 3.0 was used to perform KEGG enrichment analysis on 8 optimized DE-IRGs, and 16 significantly related KEGG signaling pathways were screened. Then the KEGG signaling pathways directly related to asthma were downloaded from the CTD database, and a total of 195 related KEGG signaling pathways were obtained, which were compared with the 16 KEGG signaling pathways involved in 8 optimized DE-IRGs. A total of 15 overlapping signaling pathways were obtained, including T cell receptor signaling pathway, Cytokine-cytokine receptor interaction, NF-kappa B signaling pathway, Cell adhesion molecules (CAMs), Asthma, Antigen processing and presentation, Rheumatoid arthritis, Th1 and Th2 cell differentiation, Toll-like receptor signaling pathway, Th17 cell differentiation, TNF signaling pathway, Natural killer cell mediated cytotoxicity, NOD-like receptor signaling pathway, Chemokine signaling pathway and Endocytosis. Subsequently, the immune cell-DE-IRGs-Asthma relationship network was constructed (Fig. 9B). The results showed that TNF and CD40LG were directly involved in the asthma-related signaling pathways, and they were positively correlated with immune cells of Immature B cell, Activated B cell, Activated CD8 T cell, Activated CD4 T cell, and Myeloid derived suppressor cells.

4. Discussion

Asthma is mainly caused by inflammatory disorder of the respiratory tract. Immune system activation containing innate and adaptive immunity is very important for asthma [39]. Based on the GSE65204 dataset, eight immune cell subsets with different abundances were identified between asthma and control groups. A total of 112 DE-IRGs were obtained, which were significantly enriched in various inflammatory and immune-related functions and pathways. Seventeen overlapping hub DE-IRGs were further screened out by applying four ranking methods (MCC, MNC, DEGREE, and EPC). Then, eight optimized DE-IRGs, including CCL5, CCR5, CD40LG, CD8A, IL2RB, PDCD1, TNF, and ZAP70, were finally selected based on univariate logistic regression analysis and the LASSO regression algorithm. Subsequently, a diagnostic model was constructed based on these eight optimized DE-IRGs. Moreover, TNF and CD40LG, which were involved in the pathway of asthma, were positively correlated with immune cells of immature B cell, activated B cell, activated CD8 T cell, activated CD4 T cell, and myeloid derived suppressor cell.

In this study, the results showed that Type 2 T helper cell, Mast cell, Activated dendritic cell, Immature B cell, Activated CD8 T cell,

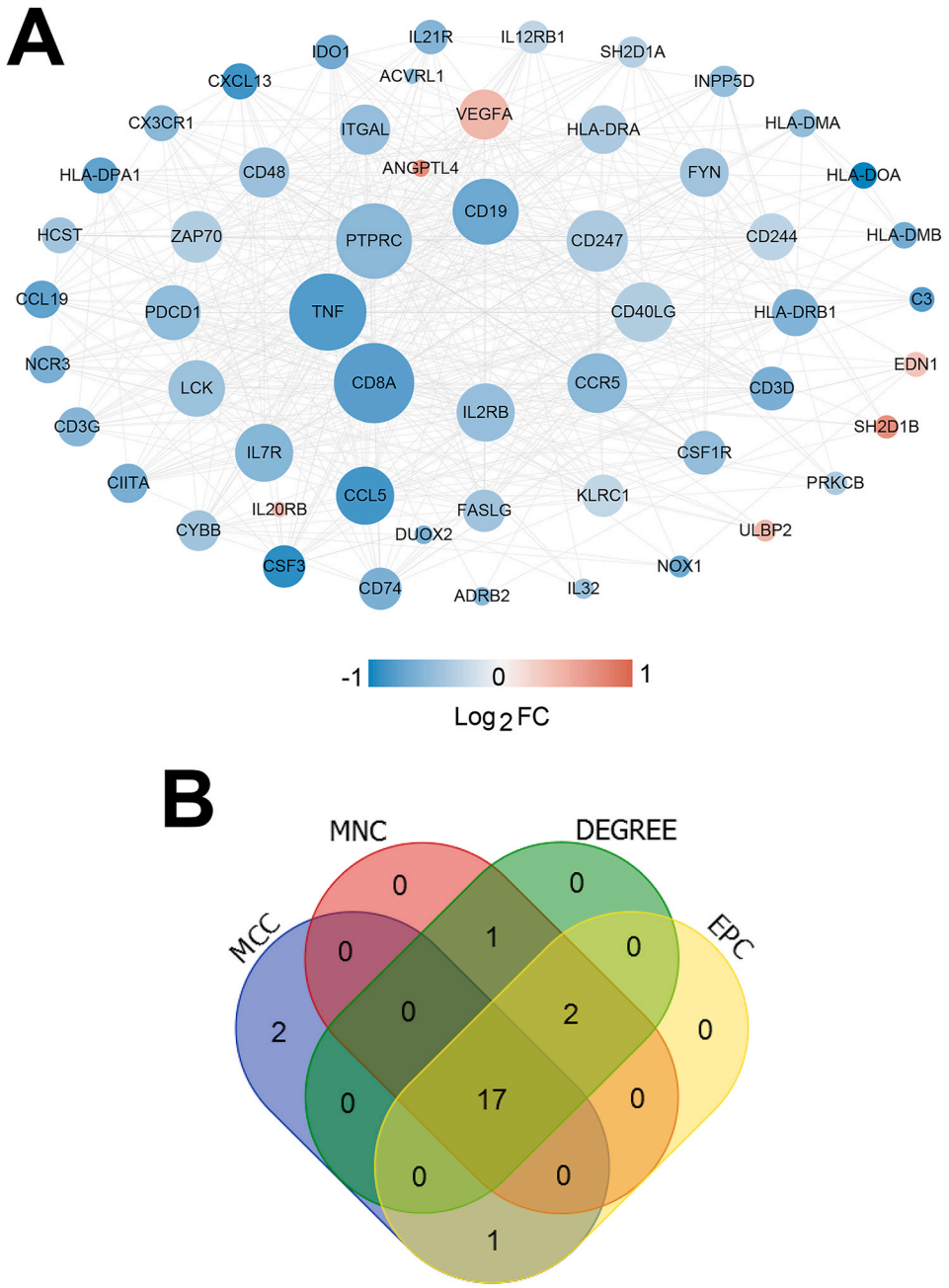

Fig. 6. A, the protein-protein interaction (PPI) network, the size of nodes represents the size of connectivity, and the color represents the significant difference. B. Venn diagram of the top20 candidate hub genes of MCC, MNC, DEGREE and EPC algorithms. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Activated CD4 T cell, Myeloid derived suppressor cell, and Activated B cell were significantly different between asthma and control groups. Asthma is the result of complex interactions between structural cells (e.g., epithelial cells) and immune cells, and studies have reported the potential of immunomodulators for the treatment of asthma [40,41]. The present study identified differential immune cells in asthma samples, which may provide new ideas for the treatment of asthma.

Eight optimized DE-IRGs associated with immune cells were identified as asthma diagnostic genes by univariate logistic regression analysis and the LASSO regression algorithm, including *CCL5*, *CCR5*, *CD40*LG, *CD8A*, *IL2RB*, *PDCD1*, *TNF* and *ZAP70*. A diagnostic model was then developed based on these 8 optimized DE-IRGs and showed strong predictive performance for asthma detection. In addition, expression of these 8 optimized DE-IRGs in childhood asthma and control groups was evaluated, and results showed that these 8 optimized DE-IRGs were significantly lower expressed in childhood asthma compared to control groups. *CCL5*, a chemokine

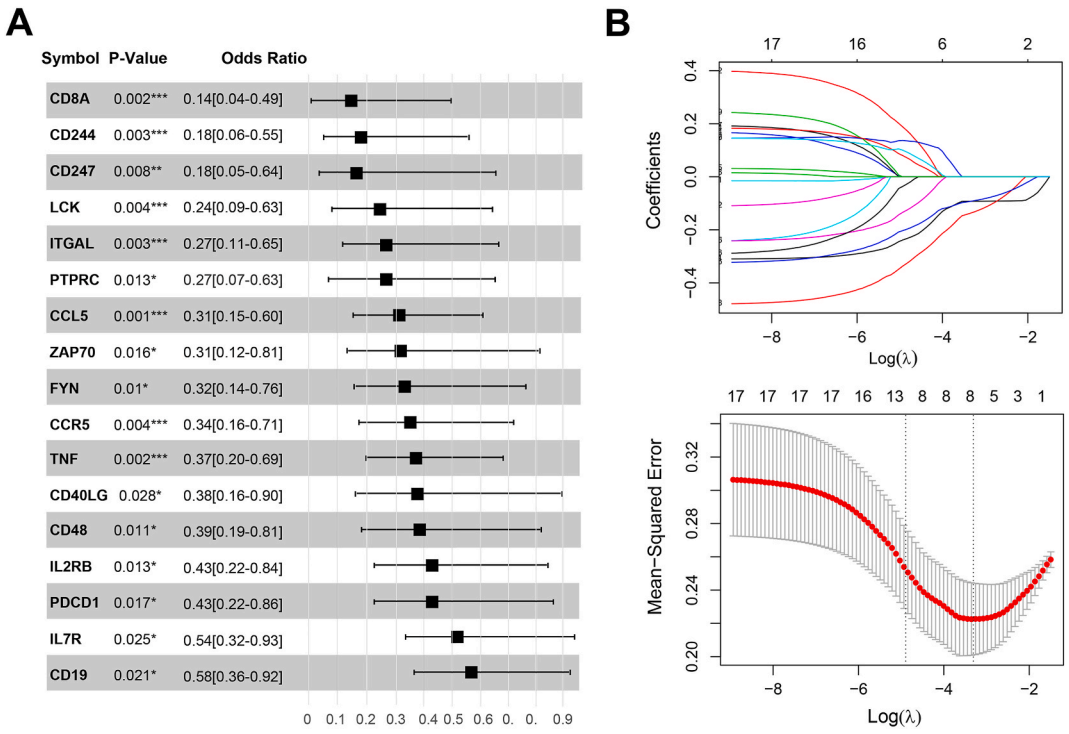

Fig. 7. A: The forest map of significantly differentially expressed (DE)- immune-related genes (IRGs). B and C: Parameters of the LASSO algorithm.

factor, is a member of the CC chemokine family and plays a crucial role in the inflammatory process [42]. Hamsten et al. discovered that the levels of *CCL5* were significantly lower in children with persistent asthma compared to the healthy control group [43]. Robroeks et al. found significantly higher levels of CCL5 in exhaled breath condensate in children with asthma compared to controls ($p < 0.05$) [44]. CCR5 is a seven-membrane G protein-coupled receptor (GPCR), which binds various ligands [45]. A deletion of 32 base pairs (delta32) in the CCR5 receptor gene results in loss of gene function and is associated with several chronic diseases due to altered immunity. Berce et al. have discovered a correlation between the CCR5-delta32 mutation and a diminished likelihood of nonatopic asthma in children from Slovenia [46]. CD40LG is a cytokine that binds to CD40, a type II transmembrane protein that belongs to the tumor necrosis factor (TNF) gene superfamily [47]. CD40LG has been reported to be associated with the development of asthma [48,49]. Lee et al. indicated that CD40LG which interacted with CD86 involved in the development of Allergic Asthma [50]. *CD40*LG mutation was also identified in Asthma patients [51]. CD8A is a member of the gene associated with the T cell toxicity pathway and is responsible for encoding the CD8 antigen, a cell surface glycoprotein that is present on most cytotoxic T cells [52]. Liu et al. found a downregulation of CD8A in samples from children with atopic asthma as compared to its expression in the healthy control group [53]. IL2RB gene is a cytokine signaling gene involved in immune responses triggered by T cells. Studies have reported that IL2RB is associated with the progression of a variety of diseases, such as inflammatory bowel disease [54], multiple sclerosis [55], and asthma [56]. Moffatt et al. undertook an extensive genome-wide association study in Caucasian populations, wherein they unveiled the association of IL2RB with asthma [57]. PDCD1 gene, is a transmembrane receptor protein in CD28/CTLA-4 subfamily and is broadly expressed in various hematopoietic cells and other tissues [58]. Dmitrieva-Zdorova found a significant association of the PD-1.3 polymorphism of PDCD1 with IgE-mediated bronchial asthma and total serum IgE level in the Russian population [59]. TNF, being a constituent of the TNF superfamily, encompasses a diverse array of transmembrane proteins possessing a homologous TNF domain. Numerous studies of TNF polymorphisms have revealed a correlation with asthma [60,61]. Smith-Norowitz found that C. pneumoniae-induced TNF-α responses in peripheral blood mononuclear cell were lower in asthma compared with non-asthma [62]. ZAP70 is a critical cytoplasmic tyrosine kinase that initiates a signal pathway downstream of an activated T cell receptor. ZAP70 is associated with various autoimmune diseases, including asthma [63,64]. Overall, previous studies support our results, suggesting that the eight DE-IRGs play an important role in children with asthma.

The role of different immune cell subsets in asthma pathogenesis and progression has been extensively studied [65,66]. Several studies have shown that the levels of CD8 T cells are elevated in the bronchoalveolar lavage fluid and airway biopsy specimens of patients with asthma exacerbations, and that they are associated with the decline in lung function [66,67]. Regulatory B cells have been demonstrated to control airway hyperresponsiveness and airway remodeling in a murine asthma model, as well as to exhibit differences in human B cell populations between asthma and controls, and between mild-moderate and severe asthma [68,69]. Myeloid-derived suppressor cells have been reported to participate in the inflammatory response in autoimmunity and cancer through regulating innate and adaptive immune responses [70]. In an asthmatic mouse model, myeloid-derived suppressor cells recruited Treg cells and suppressed T-cell response [71]. Xue et al. showed that asthmatic mice had increased levels of myeloid-derived suppressor

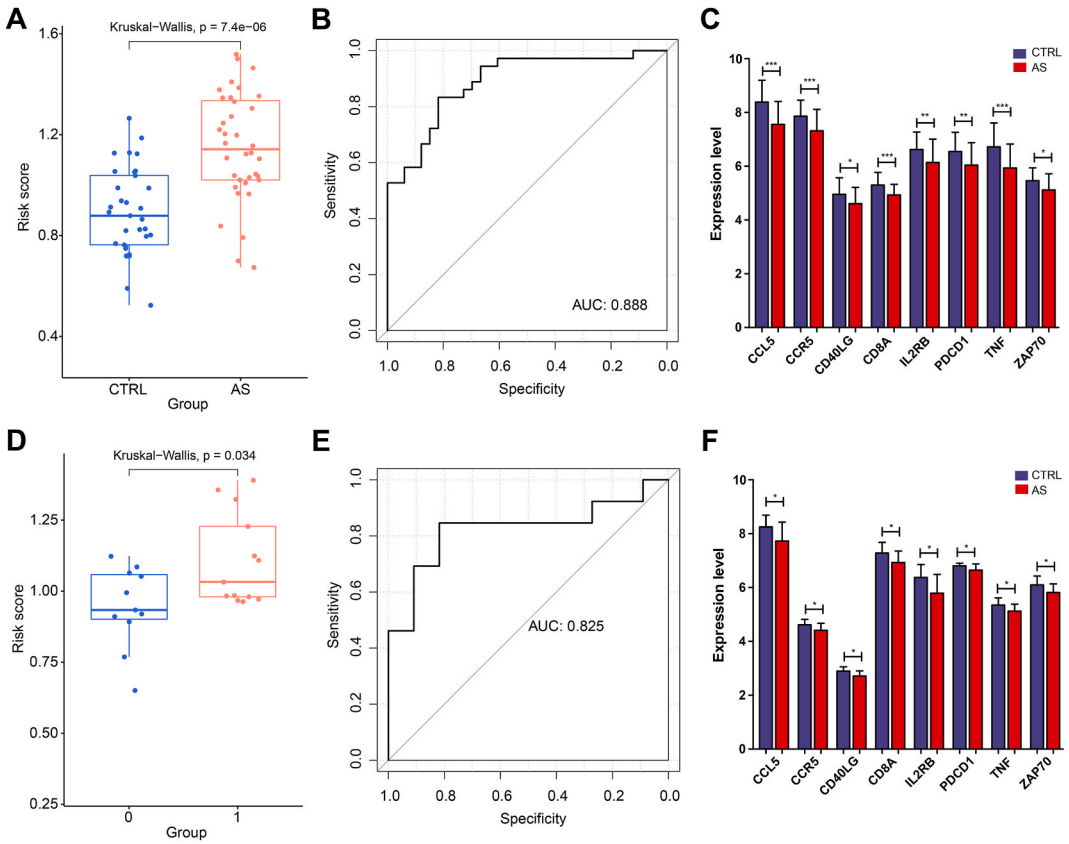

Fig. 8. The Risk-value distribution (A and D), ROC curve (B and E) and expressive level display (C and F) of 8 DE-IRGs genes of the diagnostic models in GSE65204 training data set (A–C) and the GSE19187 validation data set (D–F).

cells in splenocytes and lungs [72]. These findings suggest that CD8 T cells, regulatory B cells, and myeloid-derived suppressor cells are involved in the complex immunological network of asthma, and that they may have potential implications for future diagnostic and preventive strategies. In this study, we that Activated B cell and Activated CD8 T cell were significantly positively correlated with the expression of 8 important DE-IRGs. Meanwhile, Immature B cell, Activated CD4 T cell, Myeloid derived suppressor cell were also significantly positively correlated with the expression of *CCL5*, *CCR5*, *CD40*LG, *IL2RB*, *PDCD1*, *TNF* and *ZAP70*.

This study also has some limitations. First, the data we analyzed were obtained from public databases and the sample size of the validation set was small. Second, although the AUC of the model indicated good diagnostic ability, the model performance could be improved. For instance, the current diagnostic model could not provide relevant information on the severity and subtype of asthma. Moreover, no further in vivo experiments were conducted to validate these results (hub genes, immune infiltration cells and pivotal molecular pathways).

In conclusion, we found that CCL5, CCR5, CD40LG, CD8A, IL2RB, PDCD1, TNF and ZAP70 can be used as diagnostic markers for childhood asthma. Additionally, the involvement of immature B cells, activated B cells, activated CD8 T cells, activated CD4 T cells, and myeloid-derived suppressor cells is likely pivotal in the development and advancement of childhood asthma. Further exploration into these immune cell populations holds promise for identifying therapeutic targets in the realm of asthma immunotherapy, thereby facilitating the provision of immunomodulatory treatment to children afflicted with this condition.

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Availability of data and materials

The data set supporting the conclusions of this article are included within the article.

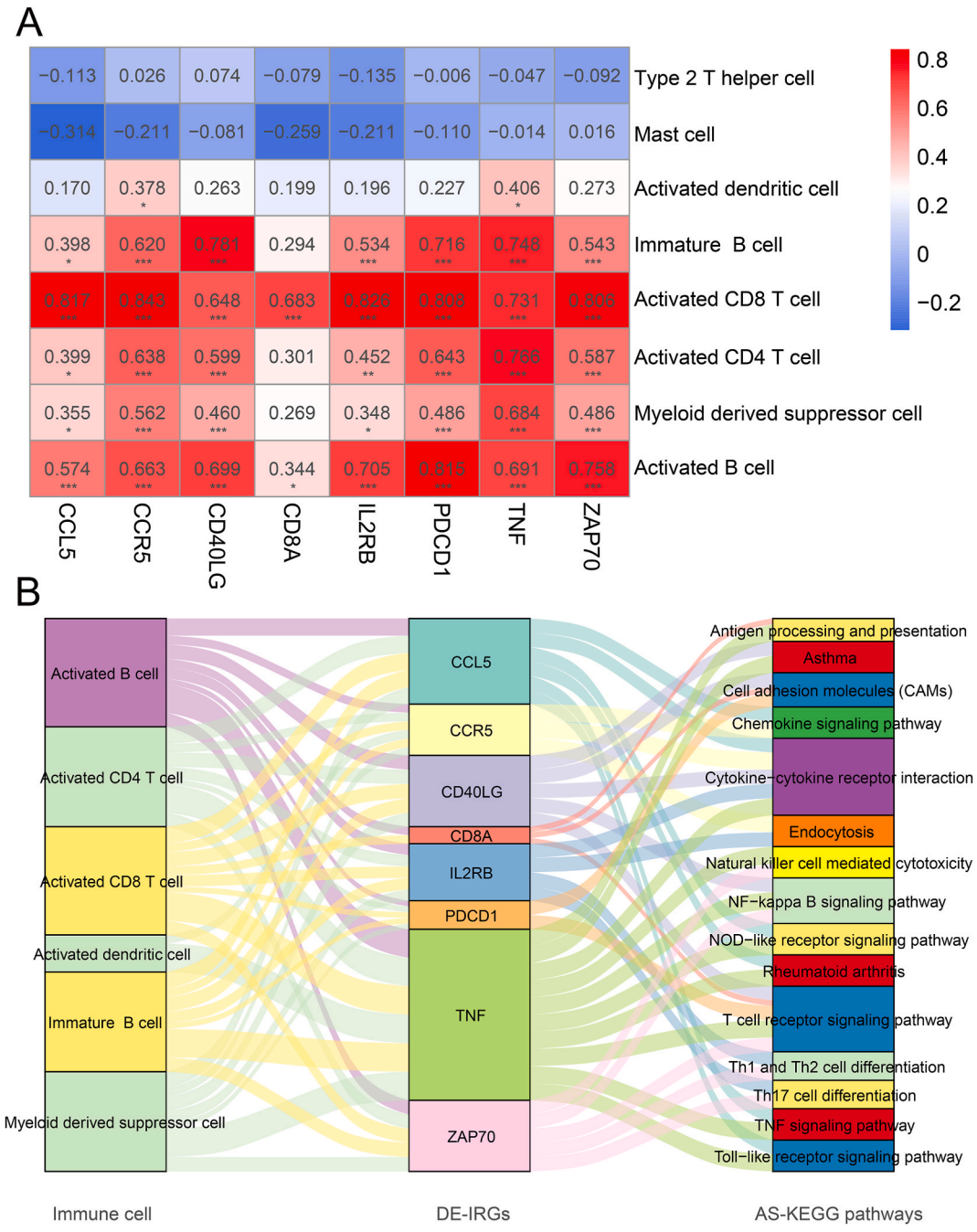

Fig. 9. The correlation among 8 optimized DE-IRGs, immune cells (A and B) and pathways (B).

CRediT authorship contribution statement

Yuyun Yuan: Writing – original draft, Formal analysis, Conceptualization. **Honghua Zhu:** Writing – original draft, Data curation, Conceptualization. **Sihong Huang:** Writing – review & editing, Resources, Methodology. **Yantao Zhang:** Writing – review & editing, Methodology, Investigation. **Yiyun Shen:** Writing – review & editing, Resources, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e25735.

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