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Ten Macrophage-Specific Expressed Genes are Considered as Potential Biomarkers for the Diagnosis and Treatment of Asthma

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Asthma, Allergy, Airway inflammation, Macrophage, Diagnostic biomarker, Prognostic biomarker

ABSTRACT

The incidence of asthma is increasing every year, though prognosis is still poor. Studies have shown that macrophages play a crucial role in the pathogenesis of asthma, but the exact mechanism has not been fully elucidated. The microarray dataset GSE74986 was downloaded from the GeneExpressionOmnibus (GEO) database to identify differentially expressed genes (DEGs) in the airway epithelial cells in asthma patients. Protein-protein interaction (PPI) and hub gene networks using STRING database and Cytoscape software were studied. At the same time, macrophage related genes with co differential expression were screened in the dataset. DEGs related to macrophages (DEMRGs) were identified. Additionally mRNA-miRNA network was constructed and Gene Ontology (GO) labeling and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were performed on the target genes. Finally, GSE76262 was used to evaluate the expression of DEMRGs. As a result of that analysis seven potential DEMRGs (*CTSC, THBS1, CD36, MAPK9), ADAM9, TCP1, CX3CR1, MSR1, PTRC, IFNGR1*) were identified, which have good diagnostic value. The molecular functions (MFs) of DEMRGs mainly affect the biological processes (bp) of myeloid leukocyte activation. The phagosome pathway may be a key regulatory pathway for macrophages in asthma. Meanwhile, co expressed mRNA and miRNA were selected to construct an mRNA-miRNA interaction networks. In addition, based on the above 7 DEMRGs, we found 55 precisely targeted medications. This endeavor has identified ten DEMRGs as potential biomarkers for asthma diagnosis and treatment, providing insights into the genesis of asthma at the transcriptomic level.

INTRODUCTION

Asthma is a chronic inflammatory disease that afflicts the airways of the lungs, leading to airway inflammation, hyperresponsiveness, and obstruction. The prevalence of asthma has been increasing worldwide, with a significant burden on healthcare systems and individuals' quality of life (Agache *et al*., 2021). Despite advances in our understanding of asthma and its management, there is still a need for improved diagnosis and treatment strategies that can provide optimal control and reduce the risk of exacerbations (Lommatzsch *et al*., 2023). Understanding the underlying mechanisms of asthma pathogenesis is crucial for developing effective treatment strategies. The pathogenesis of asthma is a intricate process, involving various cellular and molecular mechanisms. Airway inflammation is a hallmark of asthma and is characterized by infiltration of inflammatory cells, such as eosinophils, neutrophils, and T cells, into the airway walls (Miller *et al*., 2021). These cells release a variety of mediators, including cytokines, chemokines, and growth factors, which contribute to the development and maintenance

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of the inflammatory response (Lambrecht *et al*., 2019). Recent studies have shown a significant link between the activity of macrophages and the development of asthma, indicating a vital role of these immune cells in the onset of the disease (Lechner *et al*., 2022; Zhang *et al*., 2022; Britt *et al*., 2023).

Macrophages are a class of immune cells that play an important role in immune response and tissue homeostasis. They exist in almost every tissue of the body. Macrophages originate from bone marrow progenitor cells and are recruited into tissues where they differentiate into tissue-specific macrophages (Lazarov *et al*., 2023). Macrophages play a crucial role in the immune response to infection. They release cytokines such as tumor necrosis factor (TNF) and interleukin-1 (IL-1), which can activate other immune cells and promote inflammation. Macrophages also release nitric oxide (NO) and reactive oxygen (ROS) that are toxic to microorganisms. Macrophages also promote tissue homeostasis by removing dead cells and debris and producing growth factors and cytokines that promote tissue repair and remodeling (Sheu and Hoffmann, 2022; Siebeler *et al*., 2023). However, macrophages can also promote the development of diseases such as cancer, atherosclerosis and neurodegeneration. In cancer, macrophages can promote tumor growth by releasing growth factors and cytokines that support tumor cell survival and proliferation (Zhang *et al*., 2023). In atherosclerosis, macrophages promote plaque formation by swallowing LDL particles and releasing inflammatory cytokines (Huang *et al*., 2023). In neurodegenerative diseases, macrophages help eliminate dead neurons and produce cytokines that promote inflammation and tissue damage (Bartels *et al*., 2020).

In asthma, macrophages respond to inhaled allergens or irritants by releasing cytokines and other inflammatory mediators. These mediators attract other immune cells to the lungs, resulting in airway inflammation and bronchospasm. One of the key functions of macrophages in asthma is their ability to phagocytose and remove inhaled particles, such as allergens or irritants. However, in asthmatic individuals, macrophages can become "primed" and overreact to these particles, leading to excessive inflammation and airway narrowing (Saradna *et al*., 2018). The interaction between macrophages and other immune cells in the lungs is also crucial in asthma. For example, macrophages can activate T-cells, which further contribute to the inflammatory response (Zhu *et al*., 2020). On the other hand, macrophages can also release anti-inflammatory mediators that can help to relieve asthmatic symptoms. The role of macrophages in asthma is further complicated by the presence of different subpopulations of macrophages with distinct functions. For example, resident macrophages in the lungs are important for maintaining lung homeostasis, while recruited macrophages during an asthma attack contribute to the inflammatory response (Tee *et al*., 2023).

In the past decade, high-throughput sequencing has rapidly developed, and the advancement of gene microarray expression analysis has greatly facilitated the exploration of key genes related to asthma incidence (Hecker *et al*., 2023). This study aims to investigate the relationship between macrophage-associated genes and induced sputum in asthmatic patients. In this investigation, we examined an asthma patient-derived sputum microarray data set from the gene expression omnibus (GEO) repository to identify differentially expressed genes (DEGs) in asthma-induced sputum. At the same time, the DEGs were intersectioned with macrophage-related genes. Then construing a correlated protein-protein interaction (PPI) network through the screened differentially expressed macrophage-related genes (DEMRGs). DEMRGs were further investigated through genetic ontology (GO) and Kyoto encyclopedia of genes and genomics (KEGG) analysis, followed by construction of mRNA-RNA networks, mRNA-transcription factor (TF) networks, and mRNA-drug networks to delve into the potential regulatory influences of RNA, TFs, and drugs on macrophage-related differentially expressed genes in asthma. Finally, we leveraged GSE76262 to verify macrophage-specific differentially expressed genes.

MATERIALS AND METHODS

Macrophage-associated gene set gathering

A total of 35 macromolecular-related gene ensembles were retrieved from the MSigDB database, encompassing a collective of 240 macromolecular-related genes (mags; http://www.gseamsigdb.org/groad/msigdb/index.jsp). For additional insights into the 35 gene sets and 240 macromolecular-related genes, consult Supplementary Table SI-II (Liu *et al*., 2022). mRNA expression profile data sets GSE74986 and GSE76262 were sourced from the esteemed GEO database (https://www.ncbi.nlm.nih.gov/geo/). The dataset GSE74986 is housed on the GPL6480 platform [Agilent-014850 Whole Human Genome Microarray 4 x 44KG4112F (ProbeNameversion)], while the dataset GSE76262 resides on the GPL13158 platform [HT_HG-U133 Plus PM Affymetrix HTHG-U133 + PM Array Plaza]. The GSE74986 dataset constitutes a sampling of airway epithelial cells, which we employed to identify DEG. GSE76262 represents an artificially manipulated sputum specimen employed to verify the expression of genes previously identified as potential markers of macrophage activity. After identity document

(ID) conversion, when multiple probes correlate with a single gene, the average expressive value is adopted as the gene expression value. The raw data is logarithmically transformed and the fractional digits are normalized before analysis. The specifics of the two datasets are exhibited in Table I, while the flowchart of the research design is depicted in Figure 1.

Table I. Details of GEO Ashma data.

Accession	Platform	Sample	Healthy	Asthma	Gene
GSE74986	GPL6480	Airway epithelial	12	68	*mRNA*
GSE76262	GPL13158	Sputum specimen	21	118	*mRNA*

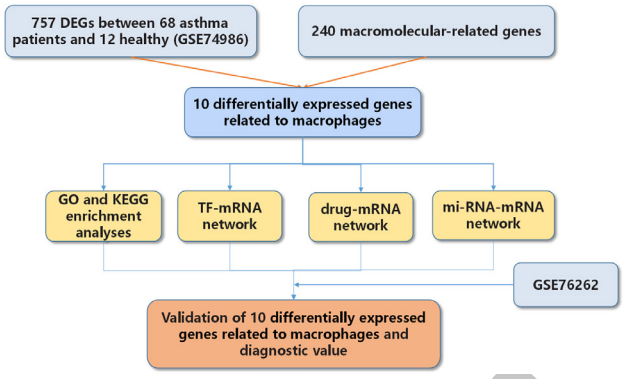

Fig. 1. The overall protocol of this study.

The analysis of differential gene expression

A standardized representation matrix of microarray data is elegantly downloading from two data sets and displayed in an ornate graph. The probes are subsequently imbued with annotations through the utilization of the annotation file in the dataset. Use the illustrious R package ggplot 2 to create a visually captivating principal component analysis (PCA) diagram to discern the grouping patterns between sample cohorts.

Using the limma package of the R software, a gene with an adjusted P value of less than 0.05 and an absolute multiple change (FC) of greater than 1 is deemed to be a DEG (Davis and Meltzer, 2007). Heat maps, volcano maps, and box line maps were crafted utilizing the R programming language (version 3.6.3) heat map and ggplot 2 package (version 3.3.3) (Gu *et al*., 2016).

PPI analysis and correlation analysis of DEG

DEG's PPI network were analyzed through the STRING database (https://string-db.org/) with immaculate precision.

The establishment of the mRNA-miRNA regulatory network

Interactions between differentially expressed microRNAs and differentially expressed messenger RNA were predicted utilizing the miRNet database (available at https://www.minet.ca/). Then, high-order MN-microRNA regulatory complexes were constructed to elucidate the interplay between high-order MNs and microRNAs as prospective therapeutic focuses in asthmatic airway epithelial cells. Cytoscape software serves as a visualization tool for regulatory networks.

GO and KEGG pathway enrichment analyses of genes

The ClusterProfiler suite in R was employed for comprehensive GO and KEGG pathway analysis of DEMRGs. This particular species is exclusive to the human race, and a modified P value of 0.05 is deemed statistically significant. GO terms encompass three essential criteria: molecular function (MF), cellular component (CC), and biological process (BP).

Validation of DEMRGs

Subject work characterization (ROC) curve analysis was performed using sophisticated HiPlot software (version 0.1.0) to ascertain the sensitivity and specificity of the target gene with precision. Multigenic ROC analysis was performed with poised elegance, basing its calculations on the predicted likelihood of numerous genes conspiring to produce the outcome within each sample, as deduced through binary logic regression utilizing the esteemed software package SPSS Version 22.0. Results yielded an area beneath the receiver operating characteristic (ROC) curve (AUC) that was quantified, and genes exhibiting an AUC > 0.6 were deemed diagnostic.

Potential therapeutic compounds prediction

Protein-drug interaction information gleaned from the DSigDB repository (http://tanlab.ucdenver.edu/DSigDB) were utilized to identify potential therapeutic compounds for the treatment of asthma, with those yielding an FDR of less than 0.05 and a holistic score exceeding 5000 serving as threshold values.

Animal model

Female Sprague Dawley (SD) rats (250 ± 20 g) were sourced from Guangdong medical experimental animal center. Rats were kept under a specific pathogen-free (SPF) condition with 40–70% humidity and 12-h light-controlled environment at 20–24°C. A 7-day acclimation with free access to water and food was allowed before experiments. Animals were divided into two groups (n=6 per group): (1) Naive rats: PBS-treated rats. (2) Asthma rats: OVA-sensitized rats. In brief, chicken egg white albumin

(ovalbumin (OVA); Sigma-Aldrich, St. Louis, MO) was mixed with aluminium hydroxide (Sigma Aldrich, St Louis, MO). Rat were injected intraperitoneally (IP) with the mixture containing 1000ug OVA and 20 mg aluminium hydroxide per rat at days 1, 8, and 15. On day 21, followed by a daily intranasal challenge with OVA diluted in sterile normal saline (300μg OVA/20μl per nostril) on days 22-28. The control animals received sterile saline instead of OVA. On day 28, the expression of target genes and proteins in lung tissues were examined through histological staining, and real-time qPCR (RT-qPCR).

RT-qPCR

The isolation of total RNA from lung tissue was conducted utilizing a kit from Takara Bio, Inc. cDNA synthesis was performed using the Bestar™ qPCR RT Kit (Takara Bio, Inc.). RT-qPCR was conducted on the Applied Biosystems 7500 RT PCR System with TB Green Premix Ex Taq II (cat. no. RR820A; Takara Bio, Inc.) as the detection reagent. The reaction involved an initial pre-denaturation at 95°C for 30 sec, followed by 40 cycles of denaturation at 95°C for 5 sec and annealing/extension at 60°C for 30 sec. The primer sequences for GAPDH, CD36, TCP1 and MAPK9 are in Table II. Besides, the $2^{-\Delta\Delta Cq}$ method was utilized to determine the relative expression of the target genes (Livak and Schmittgen, 2001).

Table II. Primer sequences of *TCP1, MAPK9, CD36* and *GAPDH*.

Gene	Species	Sequence 5`-3`
GAPDH	Rat	F= 5′-CAACGGGAAACCCATCACCA-3′ R= 5′-ACGCCAGTAGACTCCACGACAT-3′
TCP1	Rat	F= 5′-GCTGAAGGACAGCGAGAAGATC-3′ R= 5′-GGGTCCATTTTGGGCTTTTTCCG-3′
MAPK9	Rat	F= 5′-CCTCCTCTACCAGATGCTTTGT-3′ R= 5′-GTGCCAGGCCAAAGTCAAGG-3′
CD36	Rat	F= 5′-AACTCAGGACCCCAAGGACA-3′ R= 5′-TGCCACAGCCAGATTGAGAA-3′

IHC staining

Paraffin sections were deparaffinized, rehydrated, and subjected to antigen retrieval through autoclaving at 121 °C for 10 min. Sections were then incubated with serum-free protein block (Dako), followed by pretreatment with 100% methanol containing 3% hydrogen peroxide. Next, the sections were further incubated with the CD36 (ET1701-24, Hangzhou Huaan Biotechnology Co. Ltd.), TCP1 (FNab08563, Wuhan Fine Biotech Co., Ltd.) and MAPK9 (ET1610-11, Hangzhou Huaan Biotechnology Co. Ltd.) antibody. Primary-stained sections underwent incubation with secondary antibodies appropriately conjugated with peroxidase (GB23303; Servicebio) and diaminobenzidine substrate. The 3,3'-diaminobenzidine (DAB) reagent was used for staining each section, followed by counterstaining with hematoxylin.

Statistical analysis

We utilized GraphPad Prism 5 and R software version 3.6.3 for sophisticated data analysis. The data are exhibited as means ± standard deviations, and the unpaired student's t-test is employed for intergroup comparisons. A statistically significant P value of less than 0.05 is a notable indicator of a meaningful result.

RESULTS

Macrophage-associated genes in asthmatic tract epithelial cells

The expression matrix of data set GSE74986 has been properly normalized, revealing a consistent distribution trend across the box graph, which generally follows a linear pattern (Fig. 2A). In light of the need to validate the reproducibility of the data within the cohort, a principal component analysis of the GSE74986 dataset was conducted, yielding encouraging results indicating consistent replication (Fig. 2B).

After fine-tuning the filtering parameters to |log 2 (FC)| > 1 and P value < 0.05, a total of 757 genes (134 upregulated and 623 downregulated) were discovered within the GSE74986 dataset. The DEG volcano map in the GSE74986 data set is depicted in Figure 2C. A wealth of detailed information regarding DEG can be found in (https://cn.amegroups.cn/static/public/atm-22-1009-1.docx) Next, we crafted Venn diagrams (Fig. 2D) revealing that 10 genes are synchronously differentially expressed in the GSE74986 dataset and macrophage-related genes: Codon therapy and stability control (CTSC), thrombospondin type 1 mattrix Gla (THBS1), CD36, mitogen-activated protein kinase kinase 9 (MAPK9), ADAM9, tight junction protein complex (TCP1), chemokine (C-C Motif) receptor 1 (CX3CR1), matrix science research (MSR1), phosphatidylinositol 3-kinase substrate tropomyosin receptor (PTRC), interferon gamma receptor 1 (IFNGR1). The interaction of the above 10 genes was elegantly crafted into a PPI network through the utilization of the comprehensive STRING database (Fig. 2E).

GO/KEGG enrichment analysis of DEMRGs

Ten distinctly expressed macromolecule-related genes were investigated for GO and KEGG enrichment.

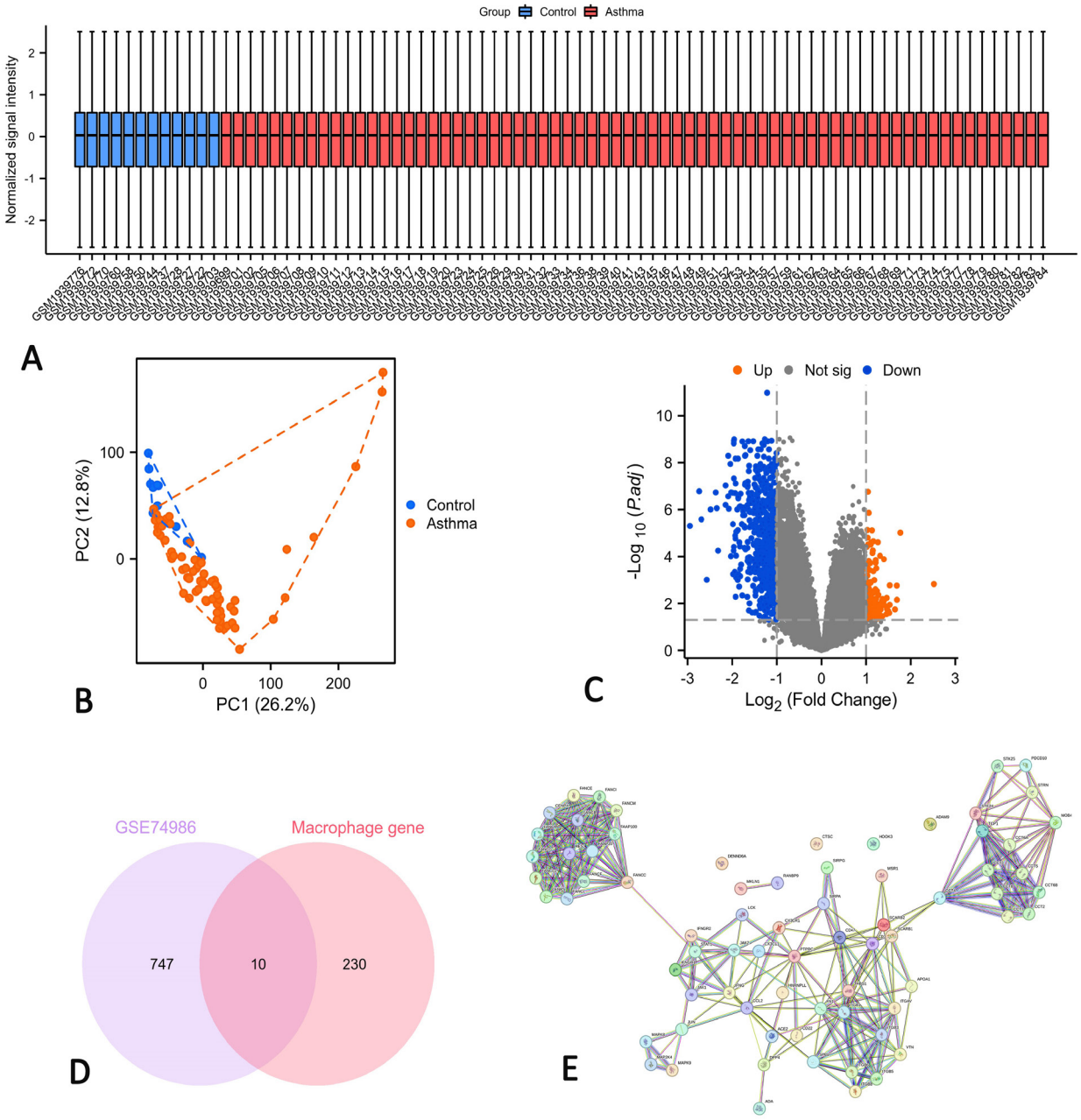

Fig. 2. Differentially expressed macrophage-related genes of the GSE74986 dataset.
A, Normalized expression matrices. B, PCA diagrams of the GSE74986 datasets. C, The volcano map of GSE74986. D, Differential genes in macrophage-related genes (DEMRGs) and GSE74986 datasets. E, Protein–protein interaction (PPI) network building of DEMRGs in string. PCA, principal component analysis.

The findings revealed that the most significant abundance of GO classes occurred during the process of cell pairing, particularly in the sequences corresponding to steps myeloid leukocyte activation, macrophage activation, external side of plasma membrane, platel*et al*pha granule, low-density lipoprotein particle binding and lipoprotein particle binding. In KEGG enrichment assays, DEG exhibits heightened involvement in metabolic processes including Phagosome, Th1 and Th2 cell differentiation, ECM-receptor interaction, Adipocytokine signaling pathway, Malaria (Fig. 3).

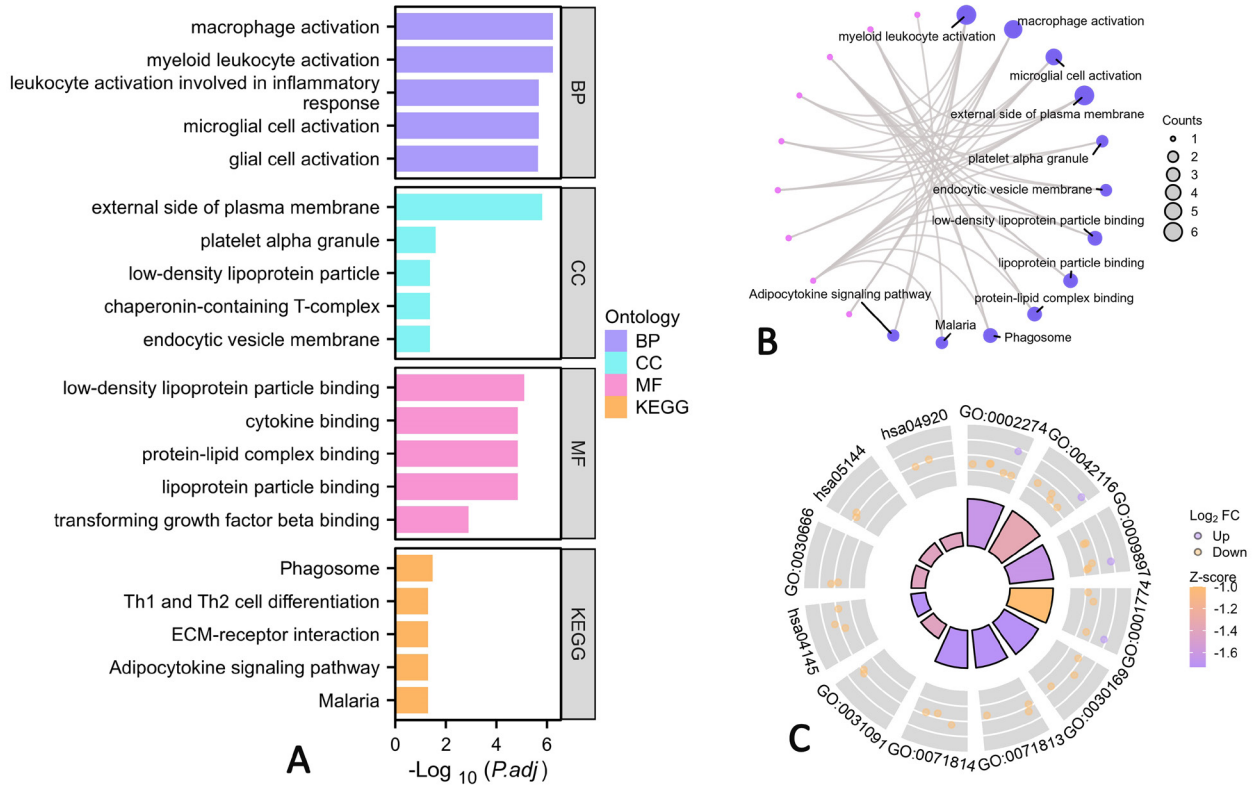

Fig. 3. GO and KEGG analyses of DEMRGs. A-C, GO and KEGG analyses of DEMRGs.

Construction of DEMRGs gene network

We employed the miRNet tool to forecast the designated microRNA of DEMRGs. Finally, we uncovered 426 sought-after miRNAs through DEMRGs and recognized 748 mRNA-miRNA associations. According to the anticipated outcomes, Cytoscape compiled a coexpression network of messenger RNA (mRNA) and microRNA (miRNA) composed of 426 nodes and 748 connections (Fig. 4). There were 231 microRNAs that governed THBS1,224 that moderated THBS1,974 that regulated TCP1,66 that controlled CTSC,47 that regulated MAPK9,38 that influenced IFNGR1,31 that modulated CD36,25 that directed PTRNA,7 that oversaw RNR1, and 5 that managed CX3CR1 (Supplementary Table SIII).

We leveraged the sophisticated miRNet tool to forecast the designated transcription factor for DEMRGs. Finally, we were able to identify 50 key transcription factors for DEMRGs. According to the predicted outcomes, Cytoscape erected a synchronous network of messenger RNA and transcription factors composed of 60 vertices and 93 edges (Fig. 5A). There were 4 TF adjustments (THBS1), 1 TF adjustment (TF 9), 8 TF adjustments (TCP1), 10 TF adjustments (CTSC), 11 TF adjustments (MAPK9), 6 TF adjustments (IFNGR1), 25 TF adjustments (CD36), 4 TF adjustments (PTPRC), 9 TF adjustments (MSR1), and 15 TF adjustments (CX3CR1) **(**Supplementary Table SIV**)**.

Targeted drug prediction

We leveraged the DGIdb database to prognosticate potential therapeutic drugs that might be targeted towards key genes involved in modulating macrophage immunity, with the goal of treating asthma. A total of 55 precisely targeted medications were definitively forecasted, and the respective focal genes were enumerated within the confines of (https://cn.amegroups.cn/static/public/atm-22-1009-3.xlsx) (Fig.5B).

GSE76262 underscores the utterance and diagnostic significance of DEMRGs

The expression of the screened target gene was evaluated through GSE76262 testing. The results demonstrated that the manifestation of CTSC, CD36, MAPK9, W59, TCP1, MSR1, PTPRC conformed to the forecast within 10 DEMRGs exhibiting differential expression between healthy individuals and asthma patients, whereas the appearance of THBS1, CX3CR1, IFNGR1 diverged from the predicted pattern (Fig. 6A-J).

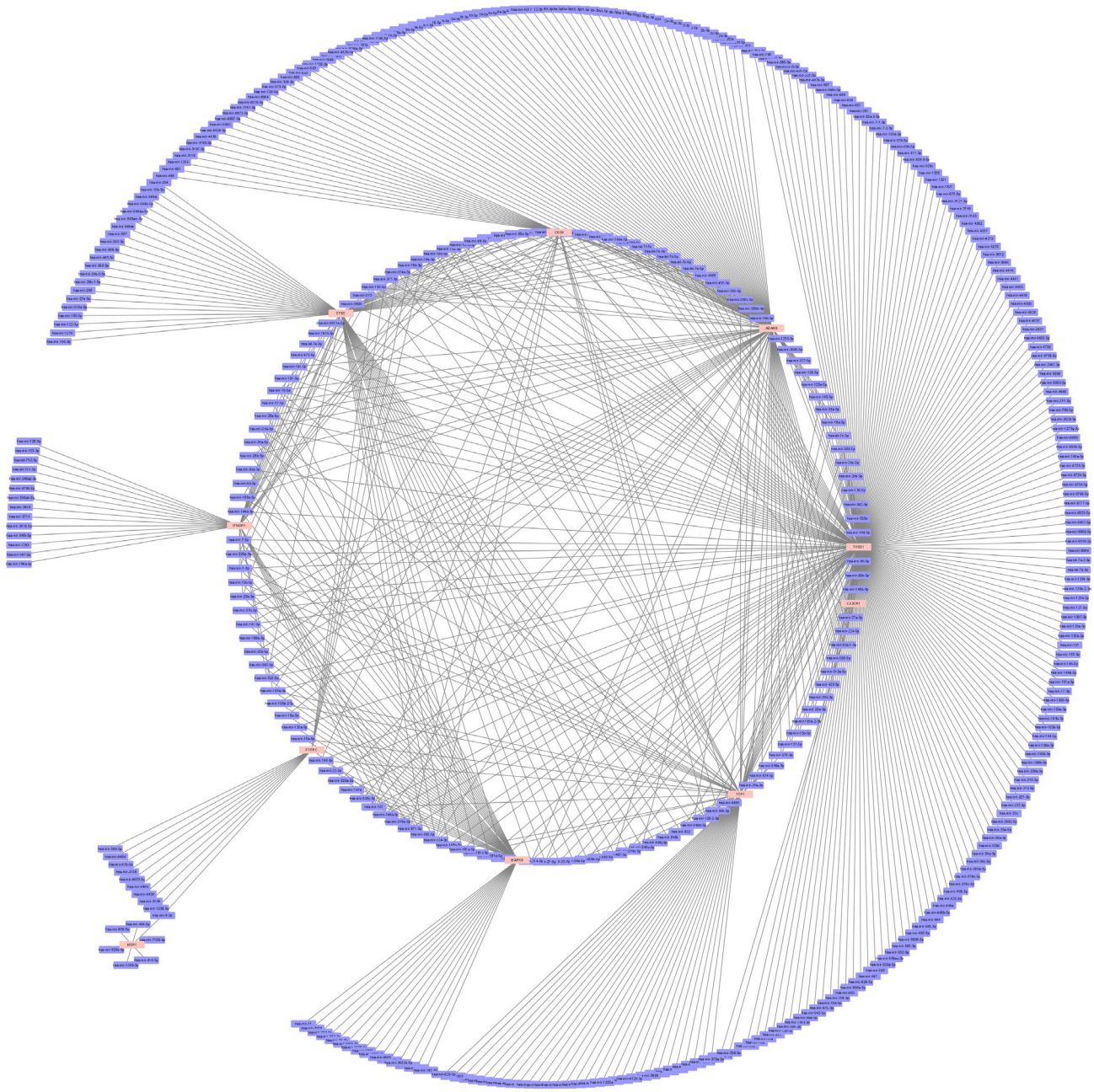

Fig. 4. mRNA-miRNA regulatory network.

We crafted ROC curves utilizing data from healthy individuals and those afflicted with asthma. Seven genetic markers proved pivotal in diagnosing asthma: AUC of CTSC 0.749 (95% CI: 0.647-0.852), AUC of THBS1 0.743 (95% CI: 0.643-0.843), AUC of CD36 0.745 (95% CI: 0.646-0.843), AUC of MAPK9 0.732 (95%CI: 0.604-0.861), AUC of TCP1 0.733 (95%CI: 0.613-0.852), AUC of MSR1 0.726 (95%CI: 0.624-0.827), and AUC of IFNGR1 0.771 (95%CI: 0.676-0.867) (Fig. 6K-T).

The expression of 10 DEMRGs in moderate asthma, severe asthma and healthy samples in GSE74986 dataset

The data from GSE74986 analysis of 10 DEMRGs in the expression of healthy individuals, moderate asthma, and severe asthma. The findings indicate that in comparison to the healthy individuals THBS1, CD36, MAPK9, ADAM9,

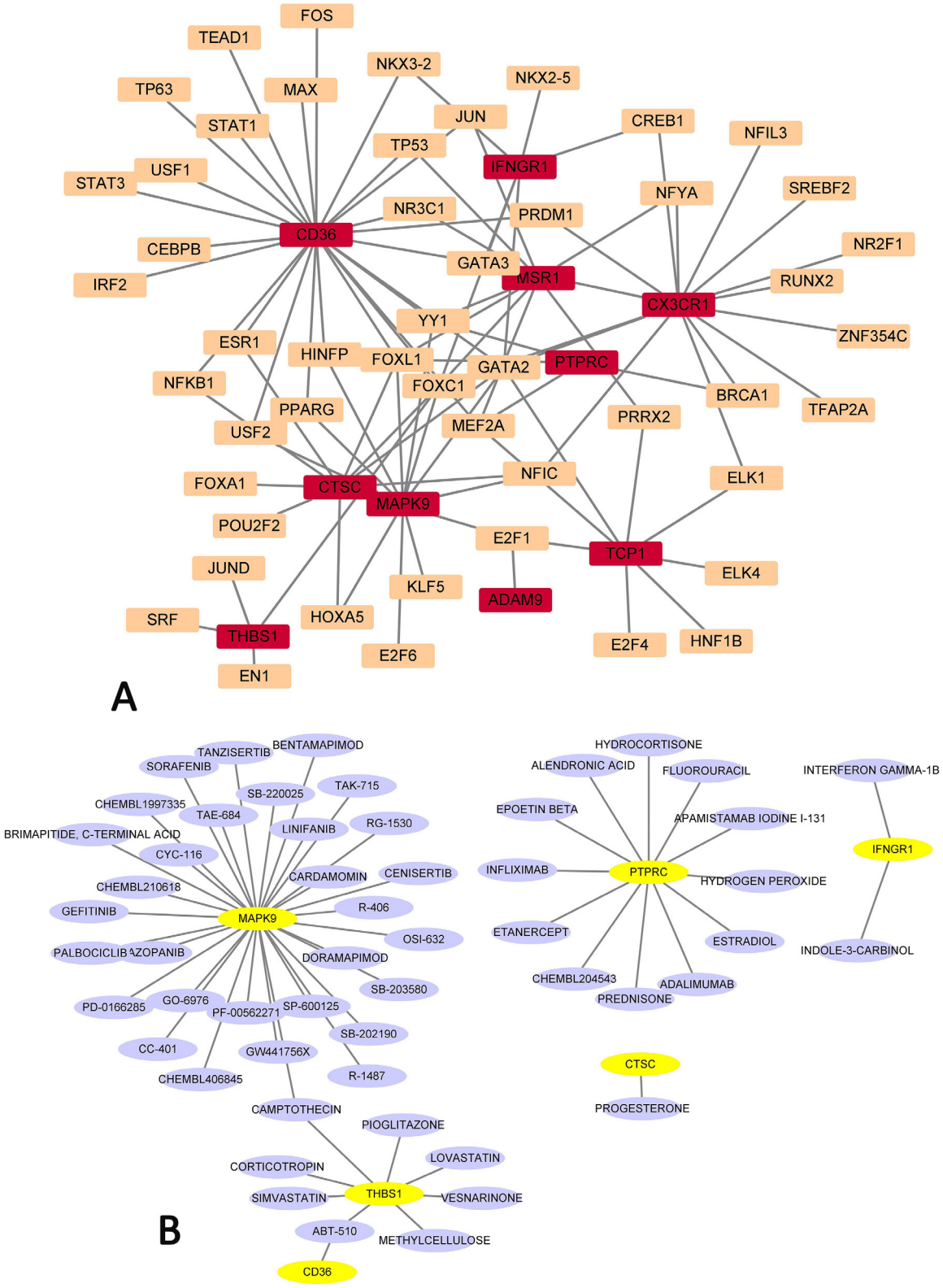

Fig. 5. mRNA-TF and mRNA-Drug regulatory network. A, mRNA-TF regulatory network. B, mRNA-Drug regulatory network.

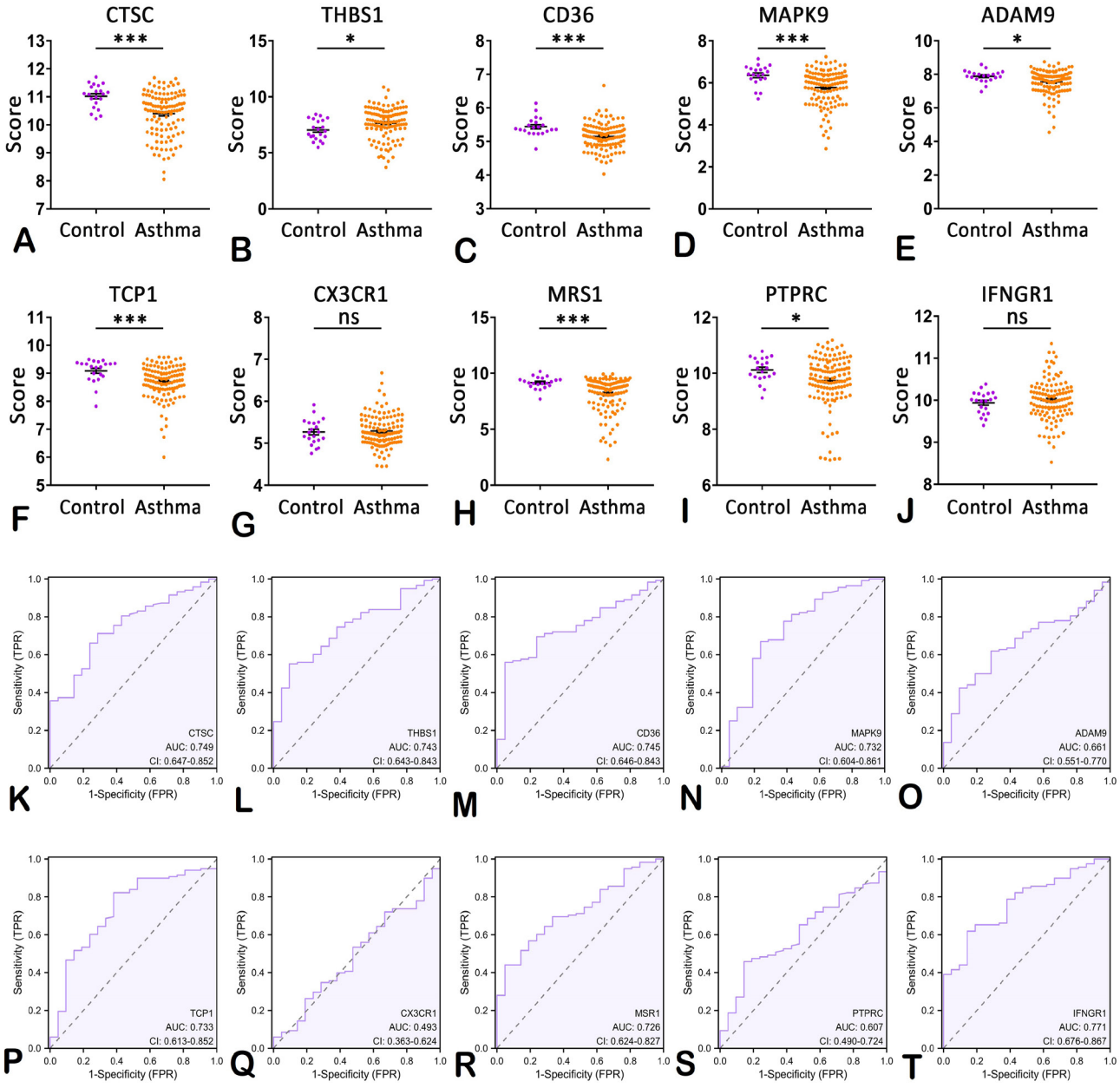

Fig. 6. Comparison of the expression and diagnostic ROC curves of 10 DEMRGs in GSE76262 dataset. A-J, Comparison of the expression of 10 DEMRGs in asthma and healthy samples. K-T, Diagnostic ROC curves of 10 DEMRGs in asthma and healthy samples. ***P<0.001; **P < 0.01; *P < 0.05; ns, not significant. ROC, receiver operating characteristic.

and TCP1, the expression of these genes is significantly diminished in the moderately asthmatic patients, while a notable reduction in THBS1, CD36, MAPK9, ADAM9, and TCP1 is observed in the severely asthmatic patients. Yet, CTSC, CX3CR1, MSR1, PTPRC, and IFNGR1 exhibit no differential expression among the moderate asthmatics and the healthy individuals (Fig. 7).

The expression of CD36, MAPK9 and TCP1 in rat lung tissue

IHC analysis confirmed lower CD36, MAPK9 and TCP1 protein levels in lung tissues of asthma rats than in lung tissues of naive rats (Fig. 8A-I). RT-qPCR analysis further validated reduced CD36, MAPK9 and TCP1 mRNA levels in lung tissues of asthma rats (Fig. 8J-L).

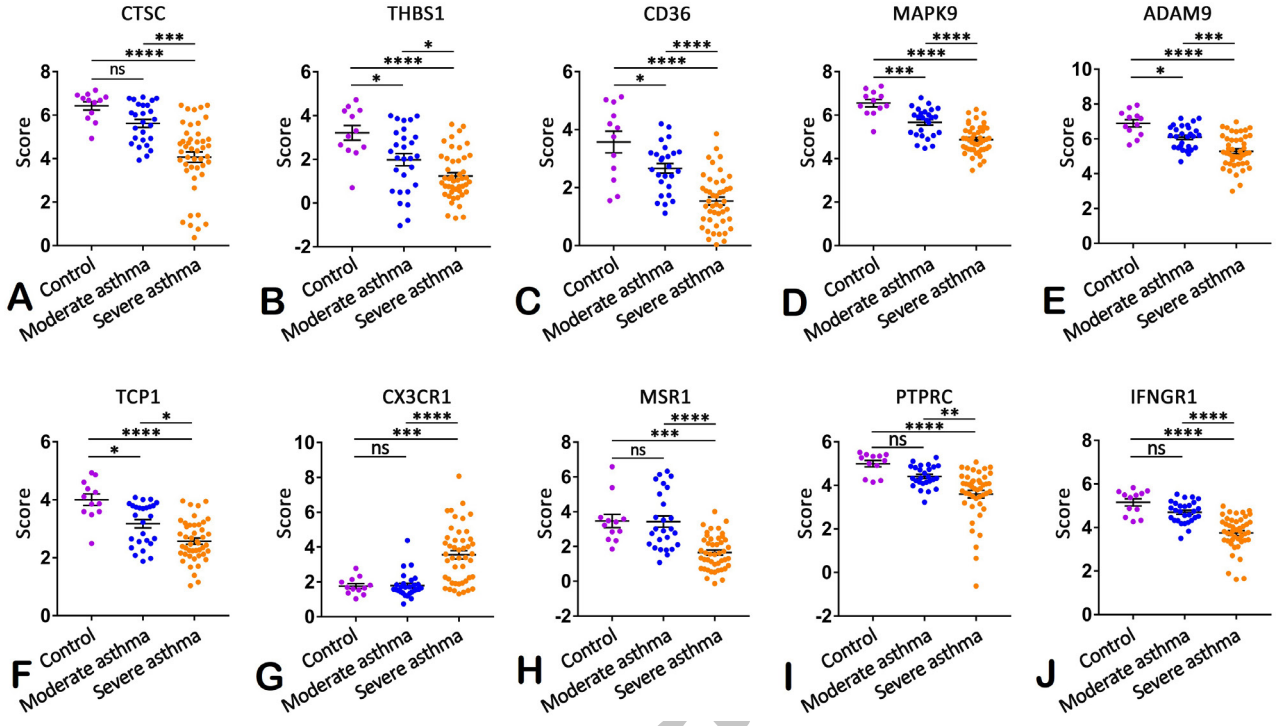

Fig. 7. Comparison of the expression of 10 DEMRGs in moderate asthma, severe asthma and healthy samples in GSE74986 dataset. A-J, Comparison of the expression of 10 DEMRGs in moderate asthma, severe asthma and healthy samples. ***P<0.001; **P < 0.01; *P < 0.05; ns, not significant.

DISCUSSION

The incidence of asthma is increasing year by year, and there are more and more studies on its diagnosis and treatment. However, due to the limited understanding of the pathogenesis of the disease, the prognosis of asthma patients is still very poor. In this study, we constructed the PPI network by analyzing the asthma airway epithelial cell microarray datasets (gse74986 and gse76262) in the geo database, and screened the DEGs in the datasets. At the same time, 10 differentially expressed genes related to macrophages were screened, including *CTSC, thbs1, CD36, MAPK9, ADAM9, TCP1, CX3CR1, MSR1, PTPRC, IFNgR1*. These 10 genes were further screened by mirnet tool, and the results of macrophage related differentially expressed genes were combined with miRNA interaction network, resulting in a total of 426 miRNAs and 748 pairs of mRNA miRNAs. The dgidb database was used to predict 55 potential targeted drugs related to macrophage related differentially expressed genes. Then, through go and KEGG pathway analysis, we found that the most enriched GO categories were myoid leukocyte activation, macrophage activation, external side of plasma membrane, platel*et al*pha granule, low density lipoprotein particle binding, lipoprotein particle binding. In KEGG enrichment analysis, DEG is mainly involved in metabolic processes, such as phagosome, Th1 and Th2 cell differentiation, ECM receptor interaction, adipocytokine signaling pathway, and malaria. After that, we validated 10 DEMRGs through the asthma induced sputum microarray dataset (GSE76262) in the geo database, in which the expression of *CTSC, CD36, mapk9, ADAM9, TCP1, MSR1, PTPRC* was consistent with the prediction and had good diagnostic value.

Among these 7 DEMRGs, the *CTSC* gene encodes for the enzyme cathepsin C, which is a protease involved in the regulation of immune responses and airway inflammation. Cathepsin C is expressed by immune cells such as neutrophils, macrophages, and dendritic cells, and it plays a crucial role in the activation and differentiation of immune cells (Xiao *et al*., 2021). Dai *et al* showed that cathepsin C could participate in the regulation of macrophage M1 polarization through p38/Mapk pathway in sudden cardiac death (Dai *et al*., 2021).

The *CD36* gene encodes a transmembrane glycoprotein that is involved in multiple physiological processes, including fatty acid metabolism and signal transduction. In patients with liver metastases, high expression of CD36 mediated metabolic crosstalk between

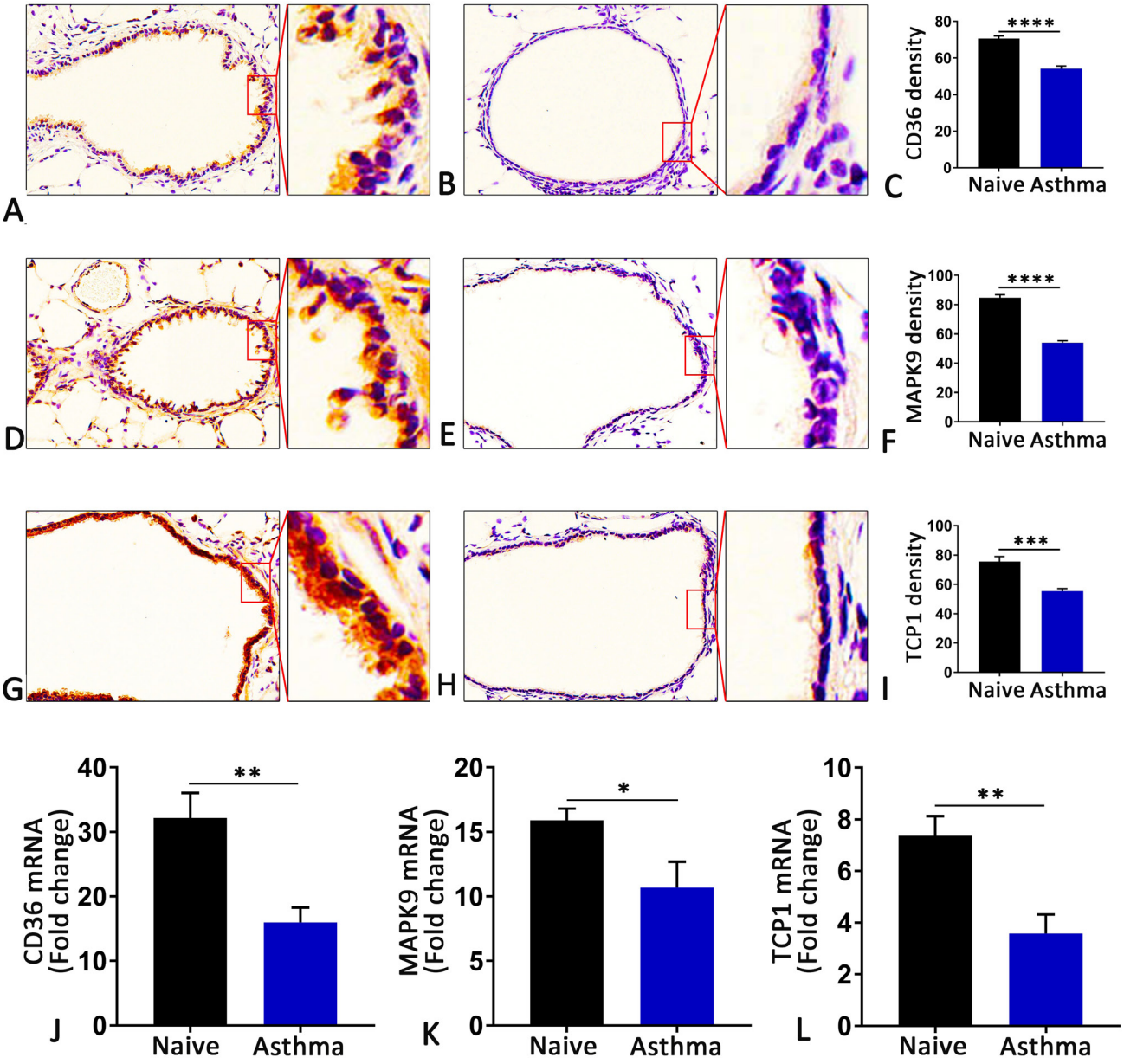

Fig. 8. The expression of CD36, TCP1 and MAPK9 in lung tissues of rat. A, Representative images showing immunohistochemica (IHC) staining results of CD36 in lung tissue of rats (scale bar, 20 μm). B, Semiquantitative analysis of CD36 IHC staining (n=6). C, Representative images showing IHC staining results of MAPK9 in lung tissue of rats (scale bar, 20 μm). D, Semiquantitative analysis of MAPK9 IHC staining (n=6). E, Representative images showing IHC staining results of TCP1 in lung tissue of rats (scale bar, 20 μm). F, Semiquantitative analysis of TCP1 IHC staining (n=6). G, The mRNA expression levels of CD36 in lung tissue of rats (n=6). H, The mRNA expression levels of MAPK9 in lung tissue of rats (n=6). I, The mRNA expression levels of TCP1 in lung tissue of rats (n=6). Data are presented as the mean ± SEM. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

tumor cells and macrophages, creating a highly immunosuppressive tumor microenvironment (Yang *et al*., 2022). CD36 has been implicated in various lung diseases, including asthma and pulmonary fibrosis (Gao *et al*., 2019; Kwak *et al*., 2023). CD36 mediates the uptake of oxidized low-density lipoproteins and thrombospondin-1, which are involved in airway remodeling and inflammation (Nergiz-Unal *et al*., 2011).

The mitogen-activated protein kinase (MAPK) signaling pathway is an essential signaling axis within

cells, involved in a multitude of biological processes. Among these, MAPK9 plays an indispensable role in the activation and polarization of macrophages as well as their functions (Oh *et al*., 2018; Pan *et al*., 2018).

ADAM9 (a disintegrin and a metalloprotease 9) is a membrane-anchored protein that participates in a variety of physiological functions. ADAM9 can impact the suction capacity of macrophages and the signalling pathways relevant to inflammation and immune responses. Moreover, ADAM9 can impact the polarization of macrophages (Wang *et al*., 2018). The studies reveal that ADAM9 can influence the activation and functions of immune cells such as macrophages and dendritic cells, thereby impacting the immunological response to asthma (Paulissen *et al*., 2006; Dreymueller *et al*., 2015).

The *TCP1* gene (also known as the T-complex protein 1) is a genomic entity discovered across various organisms, wherein it encodes a molecule known as the TCP1 circular complex (CCT), nomenclature also used for the TCP-1 circular complex. This complex plays a pivotal role in protein folding, particularly throughout cellular cycles and divisions. The aberrant expression and functional modifications of the *TCP1* gene are associated with certain varieties of cancer. For instance, in ovarian cancer, TCP1 regulated PI3K/AKT/mTOR signaling pathway to promote proliferation of cancer cells (Weng *et al*., 2021).

The MSR1 gene (macrophage scavenger receptor 1) is an esteemed genetic component within the immune system, particularly for the role of macrophages (Govaere *et al*., 2022; Sheng *et al*., 2022). MSR1 aids in the recognition and engulfment of cell fragments, oxidized lipids, and pathogens by macrophages, thereby ensuring the upkeep of tissue cleanliness and immune surveillance (Zhao *et al*., 2020; Gudgeon *et al*., 2022).

The PTPRC gene, also known as the *CD45* gene, plays a pivotal role in the immune system (Hermiston *et al*., 2003). CD45 influences the activity of the macrophage surface receptor, thereby regulating the activation and function of the macrophages. Furthermore, CD45 plays a pivotal role in the regulation of immune responses mediated by macrophages, encompassing responses to pathogens and the modulation of inflammation (Ahmad *et al*., 2020; Guilliams *et al*., 2022).

In recent years, an ever-increasing number of studies have revealed the pivotal role of miRNA (microRNA) in the pathogenesis of asthma. Several studies have indicated a correlation between the aberrant expression of miRNA and asthma vulnerability (Sharma *et al*., 2022; Soccio *et al*., 2023). For instance, the expression of miR-146a in the peripheral blood mononuclear cells of asthmatics was markedly reduced, which may be pertinent to the onset of asthma (Yan *et al*., 2021). Furthermore, the expression level of miRNA is related to the severity of asthma. For instance, the expression of miR-21 in the bronchial epithelial cells of asthma patients was significantly augmented, possibly linked to the airway hyperresponsiveness of asthma (Sun *et al*., 2022). MicroRNAs play a pivotal role in the differentiation of T-lymphocytes, particularly that of Th2 cell differentiation (Scherm and Daniel, 2020; Siddiqui *et al*., 2021). miRNA's role in asthma pathogenesis extends beyond its role in gene regulation; it also participates in the inflammatory response by influencing the secretion of inflammatory cytokines. For instance, miR-146a can curtail the expression of IL-17, thereby mitigating the aggregation of neutrophils and the inflammatory response in the airways (Srivastava *et al*., 2017). In this study, we have garnered a total of 426 decoy miRNAs for DEMRG and identified 748 miRNA-mRNA interactions. This denotes that miRNA plays a pivotal role in the pathogenesis of asthma by regulating the activity of macrophages. This furnishes new strategies for the precise treatment of asthma. However, the genesis of asthma involves a multitude of genes and environmental elements, necessitating additional investigation into the role of miRNA in this condition.

Research indicated that a multitude of transcription factors are involved in the genesis of asthma. Examples include the overexpression of transcription factors such as *GATA3, FOXP3,* and *STAT6* among asthma patients, which can lead to the excessive release of inflammatory markers and thereby exacerbate asthma symptoms (Williams *et al*., 2021; Marques *et al*., 2022; Tiwari *et al*., 2022). In this study, we have garnered a quintet of transcription factors from the DEMRGs, amounting to fifty in total. There exists a or multiple transcription factors involved in the regulation of DEMRGs. This denotes that a multitude of transcription factors might mediate the regulation of asthma pathogenesis by managing the function of macrophages.

Numerous studies have revealed the involvement of multiple signaling pathways in the onset of asthma. A number of studies have indicated that crucial components of various signaling pathways exhibit aberrant expression in patients with asthma. For instance, the JAK-STAT signaling pathway, the NF-κB signaling pathway, and the MAPK signaling pathway exhibit aberrant activation in patients with asthma, which may be pertinent to the onset of asthma (Howell *et al*., 2018; Tan *et al*., 2022). Our investigation reveals that DEMRGs predominantly cluster along the pathways of Phagosome, Th1 and Th2 cell differentiation, ECM receptor interaction, adipocyte cytokine signaling pathways, and malaria. This suggests that DEMRGs may primarily utilize these pathways to

influence the onset of asthma.

Asthma is a common chronic respiratory disease that significantly impacts the quality of life for patients. Despite the abundance of treatment options, a multitude of patients continue to struggle in effectively managing their symptoms. At present, the principal medications utilized in the treatment of asthma encompass anti-inflammatory agents (such as corticosteroids, leukotriene modifiers, and phosphodiesterase-4 inhibitors) (Huang and Mancini, 2006; Montuschi, 2010; van Boven and Chapman, 2022), anti-IgE antibodies (including omalizumab), and anti-IL-5 antibodies (including mepolizumab and reslizumab) (Charles *et al*., 2022; Akenroye *et al*., 2023). In this study, we anticipate the emergence of 55 potential targeted drugs related to critical genes, with these genes potentially treating asthma by modulating the immune response of macrophages. Among these, MAPK9 boasts potential targeted drugs in 31 varieties, PTPRC with 12 possibilities, and THBS1 with 8 options for potential targeting drugs. Therefore, these three genes might emerge as the nexus of drug therapy.

The limitations of this study lie in the necessity to conduct progenitor clinical trials and profound molecular biological experiments to further validate the mechanisms by which these seven DEMRGs contribute to the onset and progression of asthma.

DECLARATIONS

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IRB approval

This study protocol was reviewed and approved by the first affiliated hospital of Nanchang University, approval number CDYFY-IACUC-202302QR059.

Ethical statement

The study was approved by the Ethics Committee of The First Affiliated Hospital of Nanchang University, and all procedures were conducted in accordance with the experimental animal guidelines of The First Affiliated Hospital of Nanchang University, Nanchang, China.

Data availability statement

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Supplementary material

There is supplementary material associated with this article. Access the material online at: https://dx.doi.org/10.17582/journal.pjz/20250518045501

Generative AI and AI assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Statement of conflict of interest

The authors have declared no conflict of interest.

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