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ORIGINAL RESEARCH

Bioinformatics Analysis and Experimental Validation to Identify Key Glycosylation-Related Genes in Asthma

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Purpose: Asthma is a chronic inflammatory disease influenced by complex genetic and environmental factors. Despite extensive research, the intricate pathophysiology of asthma remains incompletely understood. Furthermore, the effects of glycosylation on asthma remain unclear. Considering that glycosylation-related genes have not been reported in patients with asthma, we aimed in this study to identify key glycosylation-related genes involved in asthma and their potential as therapeutic targets.

Material and Methods: In the GSE63142 microarray dataset, we performed weighted gene co-expression network, protein–protein interaction network, Gene Ontology, Kyoto Encyclopedia of Genes and Genomes pathway enrichment, and CIBERSORT analyses to identify glycosylation-related genes associated with asthma. Subsequently, these key genes were validated in the GSE67472 microarray dataset and BEAS-2B cells. Correlation analysis of key gene expression and clinical characteristics of asthma patients were performed using Spearman correlation analysis.

Results: Six key glycosylation-related genes related to asthma were identified: FUT5, FUT3, HCRT, B3GNT6, KDELR3, and SCGB1A1. Expression of FUT5, FUT3, B3GNT6, and KDELR3 was significantly upregulated and that of HCRT and SCGB1A1 significantly downregulated in BEAS-2B cells stimulated with IL-13/IL-4. Moreover, expression of key glycosylation-related genes in the peripheral blood of asthma patients correlated strongly with lung function and eosinophils.

Conclusion: Our findings have implications for identifying potential therapeutic targets and prognostic markers for asthma.

Keywords: asthma, glycosylation, gene, bioinformatics, biomarker

Introduction

Asthma, an inflammatory disease, is characterized by chronic airway inflammation and airway hyperresponsiveness, leading to recurrent symptoms such as wheezing, dyspnea, coughing, and chest tightness.[1,2] Its pathogenesis is believed to be related to genetics, the environment, and immunity.[3] However, the exact pathogenesis of asthma remains unclear, and thus, an ideal treatment method has yet to be established.

Glycosylation is the process of transferring glyco-groups to proteins under the action of glycosyltransferases, which results in the formation of glycosidic bonds with amino acid residues on proteins.[4] Glycosylation considerably improves proteome diversity and has important effects on protein function, stability, and subcellular localization.[5] Certain drugs, such as omalizumab and kaempferol-3-O-rhamnoside, have been demonstrated to reduce asthma attacks by altering glycosylation, offering innovative approaches for treatment of asthma.[6,7] Omalizumab, as the first targeted biological agent for the therapy of asthma, can extend the half-life of the drug to 26 days by virtue of its distinct glycosylation modification, thus improving the therapeutic effect. In a study, researchers used GSE134544 arrays and clinical data to analyze whole-blood transcriptomic expression profiling, thus identifying the biomarkers for the prediction of response to

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omalizumab. Researchers discovered that the glycoprotein CD3E could potentially act as a new predictive biomarker for assessing the response to omalizumab treatment in asthma patients, enabling the selection of more suitable asthma patients for this treatment.[8]

Weighted gene co-expression network analysis (WGCNA) serves as a data exploration tool and gene screening method for identifying potential disease-related molecular targets. In this study, we applied this method to first identify asthma-specific gene modules and then to identify glycosylation-related genes associated with asthma using gene set enrichment analysis (GSEA) data. Our core aim was to elucidate the molecular mechanisms underlying the involvement of glycosylation-related genes in asthma and to identify potential molecular targets that might improve early diagnosis of asthma and improve treatment.

Materials and Methods

Data Processing and Identification of Glycosylation-Related Genes

The total number of glycosylation-related genes was obtained from GSEA (https://www.gsea-msigdb.org/gsea/index.jsp). The National Center for Biotechnology Information Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo) is an open-access data platform. In this study, we utilized the GSE63142 (comprising fresh human bronchial epithelial cell data from 128 patients with asthma and 27 healthy participants), GSE67472 (comprising airway epithelial cell data from 62 participants with mild-to-moderate asthma and 43 healthy controls), and GSE142237 (comprising human bronchial epithelial cell data from eight patients with asthma and four healthy participants) microarray datasets. For each cohort, the data was normalized through the "sva" R package.

Identifying Differentially Expressed Genes

We screened differentially expressed genes (DEGs) across patients and controls in the GSE63142 dataset using the "limma" R package. adj.p < 0.05 and $|\log FC| \geq 0.5$ were set as the thresholds for DEG identification. The heatmap was drawn using the "pheatmap" R package. The volcano plot was drawn using the "ggplot2" R package.

Gene Ontology and Kyoto Encyclopedia of Genes and Genomes Enrichment Analyses

Gene Ontology (GO) enrichment analysis was performed to reveal regulatory associations between target genes and biological processes (BP), cellular components (CC), and molecular functions (MF). Furthermore, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis was performed to identify genes involved in disease pathways and annotate gene functions. GO and KEGG enrichment analysis were conducted based on enrichGO and enrichKEGG function from the "clusterProfiler" R package.

Co-Expression Network Analysis

The "WGCNA" R package was applied for these analyses. First, we sorted the data by standard deviation and selected the top 50% genes with more significant standard deviation for further analysis. Subsequently, a correlation adjacency matrix was constructed based on unsigned network. After determining a suitable soft threshold β for which the scale free topology fit R^2 exceeds 0.9, topological overlap measure (TOM) was calculated [TOM = TOM similarity (adjacency), dissTOM = 1 − TOM]. The modules were then divided based on the hierarchical clustering of genes, and the correlation coefficients and p-values of correlation tests between modules and disease traits were calculated. To recognize hub genes significantly correlated with asthma, genes in modules with a correlation coefficient > 0.4 or < -0.4 were selected for further study.

Protein–protein Interaction Network Analysis

Protein interactions play a pivotal role in revealing the molecular mechanisms governing protein functions. GeneMANIA (https://genemania.org/) was used to construct a protein–protein interaction network for DEGs to evaluate the functions of the key genes. The JASPAR database was accessed by the NetworkAnalyst (https://www.networkanalyst.ca/) to predict transcription factors (TFs).

RNA-Binding Proteins and Regulatory Network Construction

Proteins that bind to RNA, known as RNA-binding proteins (RBPs), have strong gene-regulatory activities. We used starBase v3.0 (https://rnasysu.com/encori/) to predict RBPs that might regulate key glycosylation-related genes. Cytoscape v3.8.2 was utilized to construct a RBP–key gene network.

miRNA–key Gene Regulatory Network Construction

Differentially expressed miRNAs were screened across patients and controls in GSE142237 using the "limma" R package. adj.p < 0.05 and |logFC| ≥ 1.0 were set as the thresholds for differentially expressed miRNA identification. The heatmap was drawn using the "pheatmap" R package. MiRNAs that regulate key glycosylation-related genes were predicted using miRWalk (http://mirwalk.umm.uni-heidelberg.de/). A miRNA–key gene regulatory network was then constructed using Cytoscape.

CIBERSORT Analysis

The CIBERSORT (http://cibersort.stanford.edu/) computational approach is a deconvolutional arithmetic method based on genetic expression that is used to assess variations in a gene group among other genes within specimens. We used CIBERSORT to determine associations between immune cells and expression of key genes in samples from participants with and without asthma. Our primary objective was to identify associations among immune cells.

Validation of the Diagnosis Roles and Differential Expression of Key Genes in Asthma

We used the "glmnet" and "pROC" R packages to construct receiver operating characteristic (ROC) curves for key glycosylation-related genes in the GSE63142 and GSE67472 datasets. The "ggplot2" and "ggpubr" R package were utilized to exhibited differential expression of key glycosylation-related genes in the GSE63142 and GSE67472 datasets.

Asthma Cell Model Construction

BEAS-2B cells, purchased from the Cell Bank of the Chinese Academy of Sciences (Beijing, China), were cultured in RPMI 1640 media (Gibco, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (Gibco) at 37 °C and 5% CO_2. IL-13 and IL-4 (Biosharp, Anhui, China) were used to mimic the asthma microenvironment. We used IL-13 (20 ng/mL) and IL-4 (10 ng/mL) to stimulate the cells for 48 hours. When the stimulation ended, the cell model was evaluated by detecting the expression of inflammatory marker (TSLP).

Patient Samples

This study was performed in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the First Affiliated Hospital of Zhengzhou University, China (approval no. 2019-KY-009). Fresh blood samples (37 patients with asthma) were collected. The selection criteria for asthma patients were as follows: 1) Diagnosed as bronchial asthma according to 2023 Global Initiative for Asthma guideline.[9] 2) The selected age range was 18–40 years, regardless of gender. 3) No upper/lower respiratory tract infections and no antibiotic treatment within 4 weeks. 4) Voluntarily participated in the study and signed informed consent forms. The exclusion criteria for asthma patients were as follows: 1) Patients with severe persistent and acute exacerbation of asthma requiring oral or intravenous corticosteroid treatment. 2) Patients with bronchiectasis, interstitial lung disease, active tuberculosis, pulmonary embolism, pleural effusion, or other lung diseases. 3) Patients with cardiovascular, cerebrovascular, digestive, endocrine, kidney, urinary, blood, and other systemic diseases or malignant tumors. 4) Patients who refused to sign informed consent forms.

Real-Time Quantitative PCR

Total cell RNA was extracted using RNA-easy isolation reagent (Vazyme, Nanjing, China). Total blood RNA was extracted with the RNAeasy™ blood RNA isolation kit with a spin column (Beyotime, Shanghai, China). Then, 1μg of total RNA was reverse-transcribed with a NanoDrop2000 (Thermo Fisher Scientific, USA) in a 20-μL reaction according to the manufacturer instructions. Real-time quantitative PCR (RT-qPCR) was performed with ChamQ Universal SYBR

Master Mix (Vazyme, Nanjing, China) in a 20-μL reaction containing 1μL of cDNA, and was run on an ABI Step One Plus Real-Time PCR system (Applied Biosystems). The PCR primer sequences directly were synthesized (Tsingke, Beijing, China) (Table S1). After being briefly mixed, the reaction mixture was at 95°C for 10 min, followed by 40 cycles at 95°C for 15s and 60°C for 1 min. β-Actin was used as an endogenous control to standardize the expression of each target gene, and the $2^{-\Delta\Delta\ CT}$ method was adopted to determine the relative target gene level.

Correlation Analysis of Key Gene Expression and Clinical Characteristics of Asthma Patients

Detailed clinical consultations, including age, gender, body mass index, were conducted for the asthma patients, and a comprehensive physical examination was performed. Lung function, complete blood count, fractional exhaled nitric oxide, and blood total immunoglobulin E tests were performed for all subjects. The clinical characteristics of the asthma patients are summarized in Table S2. Correlation analysis of key gene expression and clinical characteristics of asthma patients was performed using Spearman correlation analysis.

Statistical Analysis

Public gene expression data were analyzed using RStudio 3.1.3. Statistical analyses were performed using GraphPad Prism 8.0. All data were compared across the two groups, and a *t*-test was performed for comparison. Continuous variables are presented as the mean ± standard deviation. Differences were considered statistically significant at *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.

Results

Study Flowchart

We comprehensively analyzed GSE63142 to identify glycosylation-related genes associated with asthma. We predicted the TFs and RBPs that regulate key glycosylation-related genes. We downloaded and analyzed the GSE142237 dataset to construct a miRNA–key gene regulatory network. We further explored the relationship between expression of key glycosylation-related genes and immunocyte infiltration levels using the GSE63142 dataset, and we validated the key glycosylation-related genes using the GSE67472 datasets. In addition, key glycosylation-related genes were verified in BEAS-2B cells and samples from patients with asthma using RT–qPCR. Correlation analysis of key gene expression and clinical characteristics of asthma patients was performed using Spearman correlation analysis (Figure 1).

Identification of DEGs in Asthma and Enrichment Analyses

In our analyses of the GSE63142 dataset, we identified 132 DEGs, of which 72 were upregulated and 60 downregulated. These DEGs are shown as heatmap and volcano plot in Figure 2A and B. Information regarding all DEGs, including the logFC and adj.p values, are presented in Table S3. To obtain a deeper insight into the functions of these DEGs, GO and KEGG pathway enrichment analyses were employed. GO enrichment analysis indicated that these DEGs are mainly associated with cellular response to xenobiotic stimulus, apical part of cell, and glutathione transferase activity (Figure 2C). KEGG enrichment analysis indicated the DEGs to be mainly enriched in the metabolism of xenobiotics by cytochrome P450 and chemical carcinogenesis-DNA adducts signaling pathways (Figure 2D).

Identification of Key Modules in Asthma via WGCNA

Herein GSE63142 was utilized as the training dataset to identify key asthma-associated genes. This process involved the analysis of 155 samples and 15,608 genes, which were used to obtain a gene similarity matrix. After setting the soft-thresholding power to seven using the "pickSoftThreshold" function for scale-free network analysis, we converted the adjacency matrix to TOM (Figure 3A and B). The subsequent clustering of module eigengenes (MEs), which involved calculating their dissimilarity using the "mergeCloseModules" function, resulted in the identification of 31 distinct MEs (Figure 3C and D). Ultimately, these data were visualized in terms of module–trait relationships, determined by the Pearson correlation coefficient between MEs and diseases. Among these, the black and light yellow modules exhibited

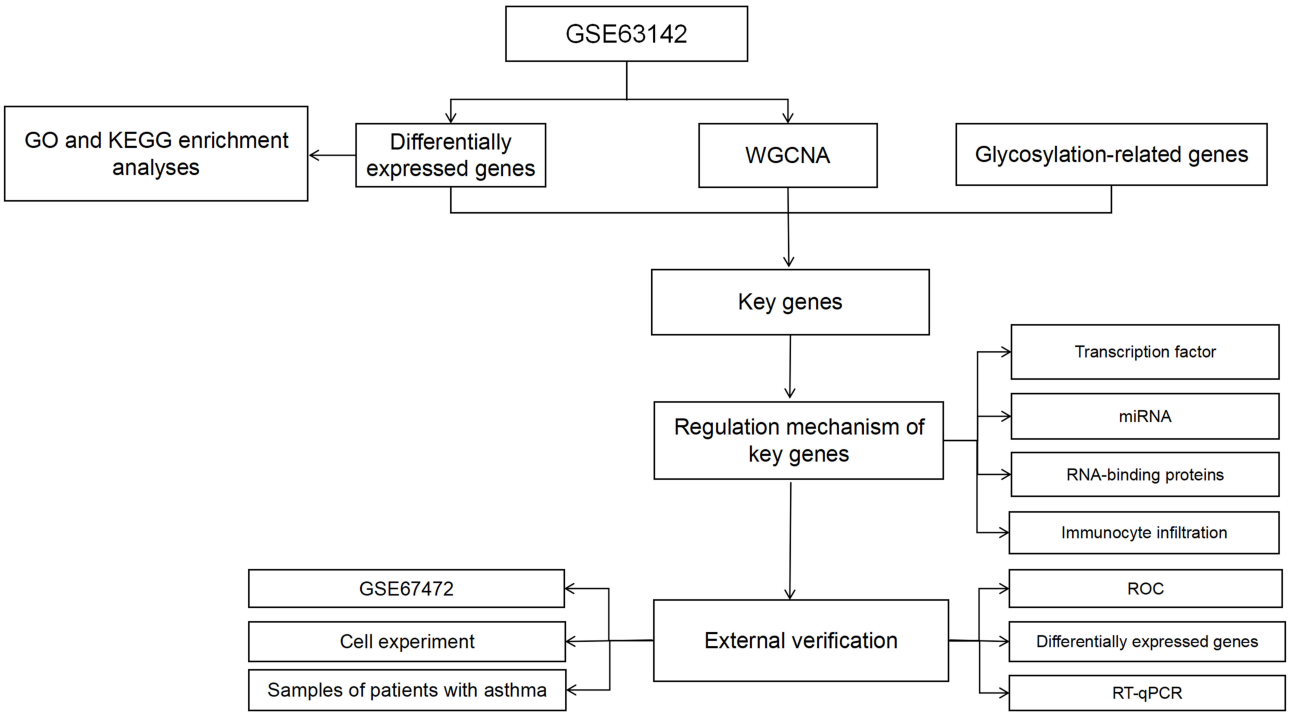

Figure 1 Study flowchart. Workflow of searching key glycosylation-related genes in asthma.

the strongest positive association with asthma, the blue, darkolivegreen, and dark turquoise modules exhibited the strongest negative association with asthma (Figure 3E). Thus, 2380 key module genes related to asthma were obtained for subsequent analysis (Figure S1A–E and Table S4).

Key Gene Determination

Six common genes (FUT5, FUT3, HCRT, B3GNT6, KDELR3, and SCGB1A1) were identified from the intersection of glycosylation-related genes, DEGs from GSE63142, and hub genes obtained through WGCNA (Figure 4A). Predictions of the interacting proteins regulated by these key glycosylation-related genes were achieved using GeneMANIA (Figure 4B). TFs that regulate these key glycosylation-related genes were predicted using the NetworkAnalyst database. Using the NetworkAnalyst database, 31 TFs were finally obtained, among which, there were 14 TFs with degree≥2, and they were PPARG, FOXC1, GATA2, TP63, FOXA1, USF2, TEAD1, FOXL1, TFAP2C, YY1, TP53, SREBF1, HNF4A, FOXF2 (Figure 4C and Table S5). Furthermore, we used starBase v3.0 to predict RBPs that might regulate the key genes, and Cytoscape was used to construct an RBP–key gene network. We identified 79 target RBPs of the key genes. FUT5 is regulated by 6 RBPs, FUT3 is regulated by 9 RBPs, HCRT is regulated by 53 RBPs, B3GNT6 is regulated by 19 RBPs, KDELR3 is regulated by 32 RBPs, SCGB1A1 is regulated by 1 RBPs respectively (Figure 4D).

miRNA–Key Gene Regulatory Network Construction

To construct a miRNA–mRNA regulatory network, we downloaded and analyzed the GSE142237 dataset from GEO. We identified 164 differentially expressed miRNAs in asthma, of which 67 were upregulated and 97 down-regulated (Figure 5A). MiRWalk was used to predict miRNAs that regulate key genes, and Cytoscape was used to construct a miRNA–key gene regulatory network. We identified 94 target miRNAs of the key genes. FUT5 is regulated by 38 miRNAs, FUT3 is regulated by 47 miRNAs, HCRT is regulated by 6 miRNAs, B3GNT6 is regulated by 58 miRNAs, KDELR3 is regulated by 19 miRNAs, SCGB1A1 is regulated by 5 miRNAs respectively (Figure 5B).

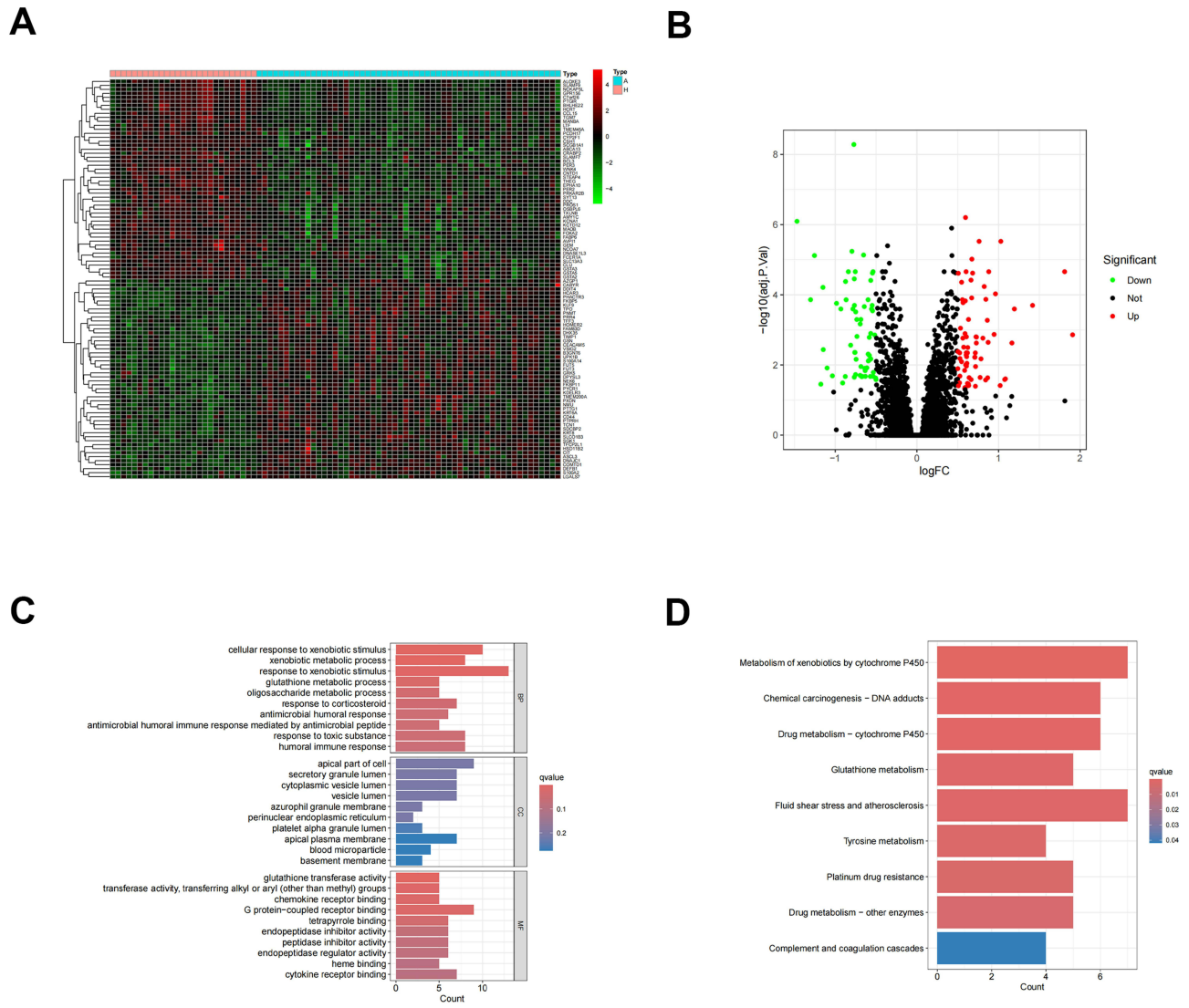

Figure 2 DEG identification in the GSE63142 dataset. (**A**) The heatmap of DEGs in GSE63142. (**B**) The volcano plot of DEGs in GSE63142. (**C**) The GO outcomes are displayed with a bar plot. (**D**) A bar plot was constructed to illustrate the KEGG outcomes.

Abbreviations: DEG, differentially expressed gene; BP, biological process; CC, cellular component; MF, molecular function; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

Key Glycosylation-Related Genes Were Correlated to Immunocyte Infiltration Levels

Studies had shown a strong connection between asthma and the immune microenvironment.[10,11] Consequently, we next used the GSE63142 database to predict the proportion of immune infiltrating cells in the immune microenvironment of asthma. The results are shown in Figure 6 and Table S6. Using the GSE63142 dataset, we further explored the relationship between expression of key glycosylation-related genes and immunocyte infiltration levels. We assessed immunocyte characteristics using CIBERSORT and examined the relationship between key gene expression and immunocyte infiltration levels. FUT5, FUT3, B3GNT6, and KDELR3 were found to correlate with type 17 T helper cells (Figure 6A, B, D and E), HCRT correlated with activated B cells (Figure 6C), and SCGB1A1 correlated with immature dendritic cells (Figure 6F). Our findings suggest that these key genes participate in asthma progression by regulating these immune cells.

Validation of the Differential Expression of Key Genes in Asthma

We created ROC curves for these key genes. ROC curves were plotted for key genes in GSE63142 (Figure 7A), and GSE67472 (Figure 8A).The results showed that these key genes had good diagnostic roles in asthma. We analyzed the

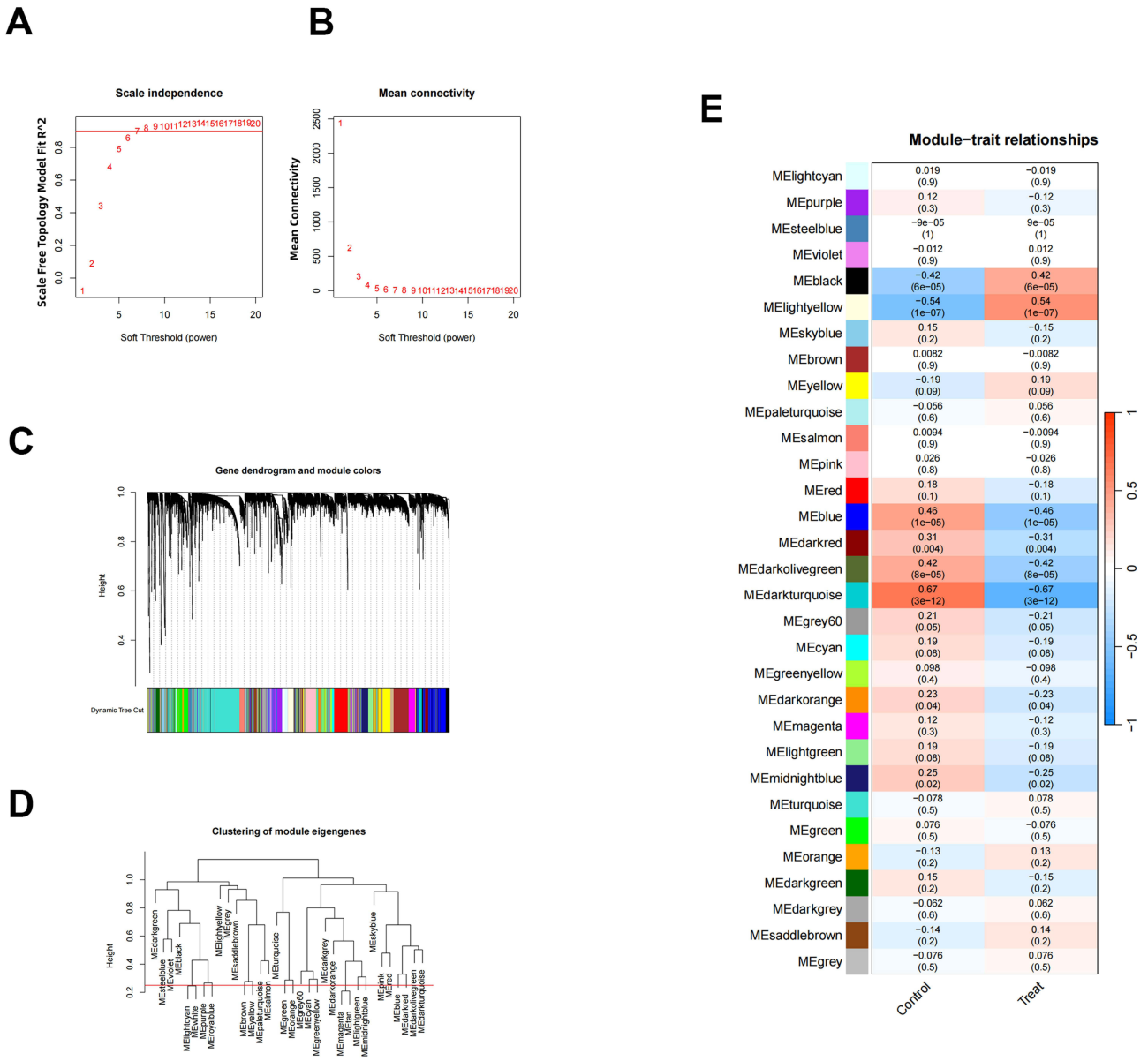

Figure 3 Detection of weighted gene co-expression networks and modules. (**A**) Scale-free topological indices at various soft-thresholding powers. (**B**) Correlation between soft-thresholding power and mean connectivity of the network. (**C**) Dendrogram of all genes clustered based on a dissimilarity measure (1 − TOM). Each branch represents a gene, and each color represents a co-expression module. (**D**) Clustering of module eigengenes. (**E**) Correlation between gene modules and asthma.
Abbreviation: TOM, topological overlap measure.

expression of key genes with the GSE63142 dataset (Figure 7B–G). We verified the expression of key genes with the GSE67472 dataset. The results showed that the expression of the key genes were consistent with the GSE63142 dataset (Figure 8B–G).

Preliminary Laboratory Validation of Key Glycosylation-Related Genes

We simulated a microenvironment of asthma by stimulating BEAS-2B cells with IL-13 or IL-4. Compared with that in the control group, expression of inflammatory marker (TSLP) was significantly upregulated in the experimental group (Figure 9A). The expression of TSLP was significantly upregulated, suggesting that we successfully constructed the asthma cell model. Compared with that in the control group, expression of key glycosylation-related genes was significantly up- or downregulated in the experimental group (Figure 9B-G).

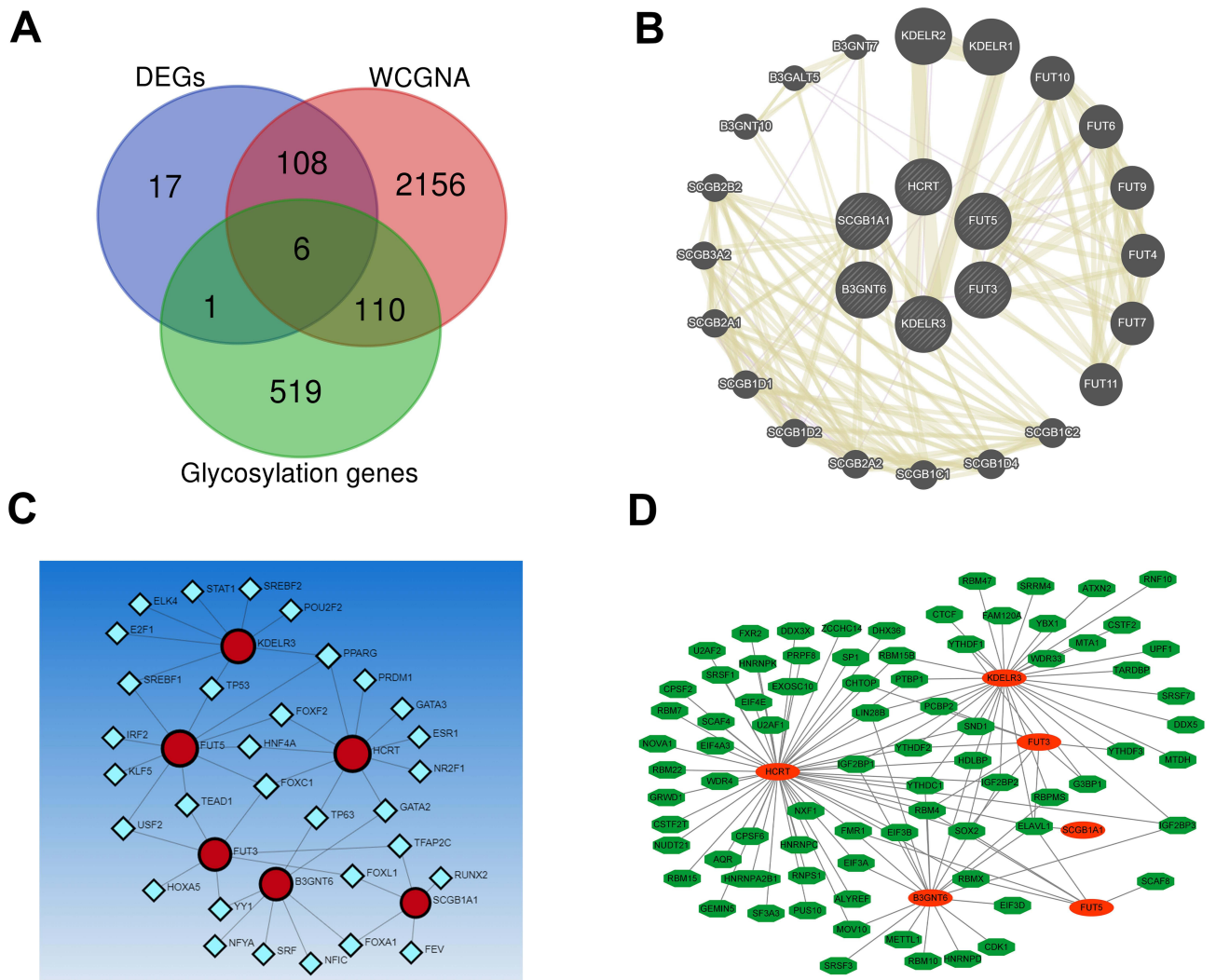

Figure 4 Construction of the protein–protein interaction network of key genes. (**A**) Venn diagram shows common genes in the three gene sets. (**B**) Interaction networks of key genes and proteins. (**C**) Interaction network of key genes and TFs regulating the key genes. (**D**) RBP–key gene regulatory network.
Abbreviations: TF, transcription factor; RBP, RNA-binding protein.

Preliminary Laboratory Validation of Key Glycosylation-Related Genes in Patient Samples

This study involved 37 patients with asthma, whose clinical characteristics are shown in Table S2. The correlation analysis of key gene expression and clinical characteristics of the asthma patients is summarized in Table 1. The expressions of FUT3, B3GNT6, and KDELR3 were negatively correlated with FEV1. The expression of SCGB1A1 was positively correlated with FEV1% predicted. The expression of FUT3 was negatively correlated with FVC. The expression of SCGB1A1 was positively correlated with FEV1/FVC and FEV1/FVC percent predicted. The expression of HCRT was negatively correlated with Eosinophils.

Discussion

Asthma is a heterogeneous disease characterized by chronic airway inflammation influenced by both environmental and genetic factors.[12] Among structural cells within the airways, bronchial epithelial cells play a pivotal role in several pathological processes, such as airway hyperresponsiveness, inflammation, and remodeling.[13] Therefore, investigating involvement of bronchial epithelial cells in asthma development and identifying effective targets are paramount for

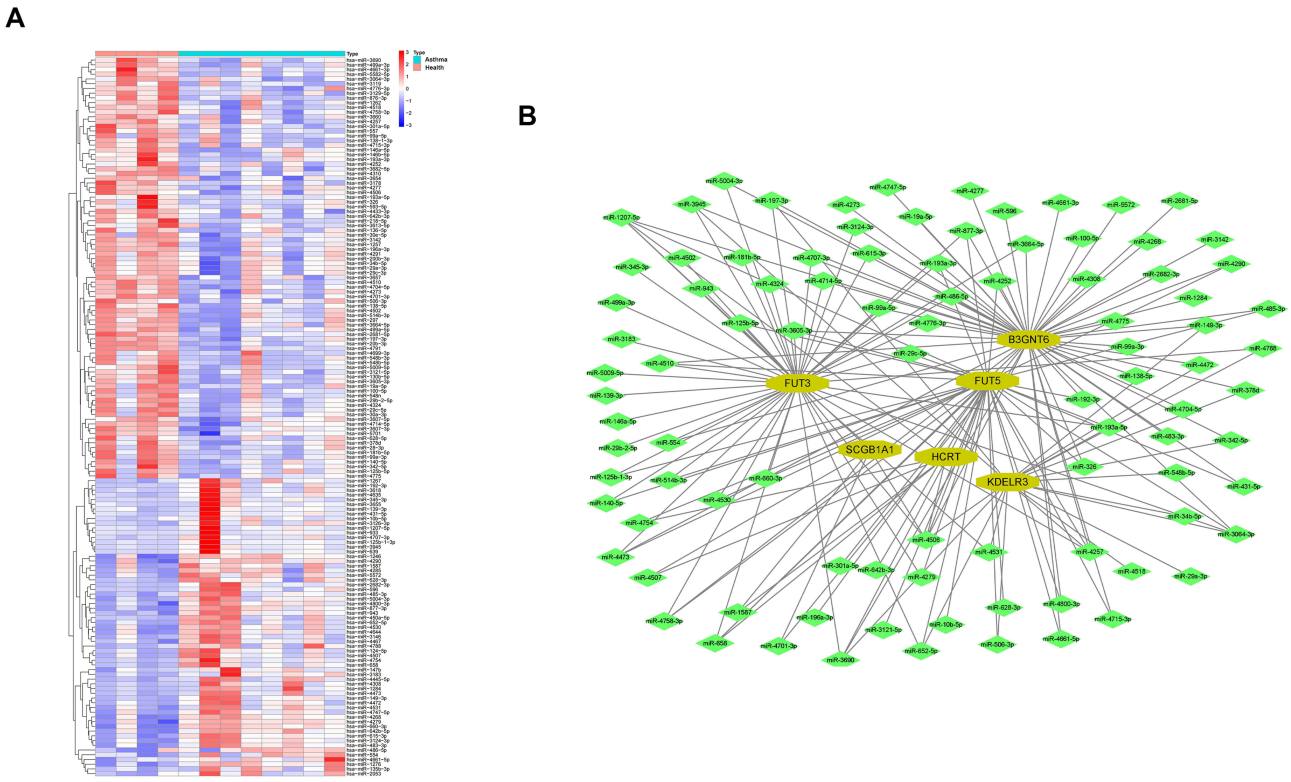

Figure 5 Construction of the miRNA–key gene regulatory network. (**A**) Heatmap of differentially expressed miRNAs in GSE142237. (**B**) MiRNA–key gene regulatory networks.

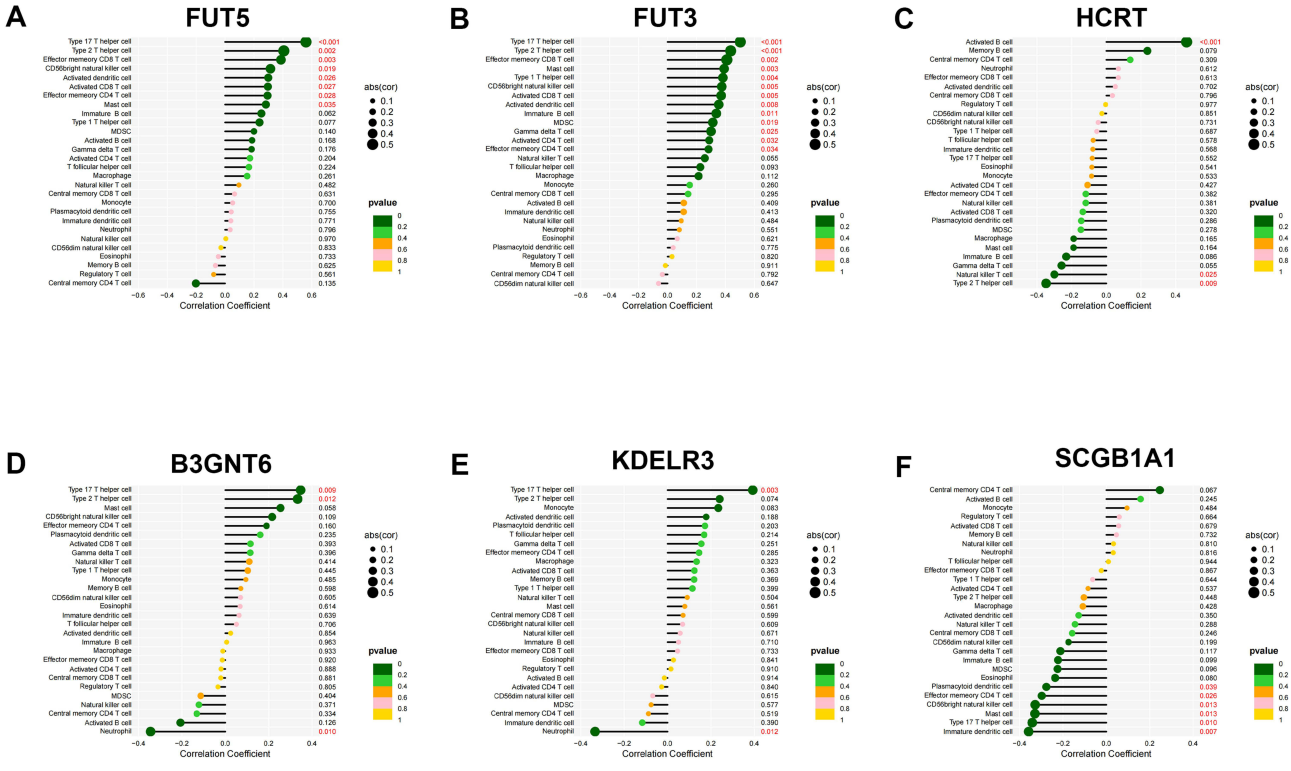

Figure 6 Correlations between key genes and infiltrating immune cells in asthma and normal samples. (**A**) The correlation analysis of FUT5 and immune cells. (**B**) The correlation analysis of FUT3 and immune cells. (**C**) The correlation analysis of HCRT and immune cells. (**D**) The correlation analysis of B3GNT6 and immune cells. (**E**) The correlation analysis of KDELR3 and immune cells. (**F**) The correlation analysis of SCGB1A1 and immune cells.

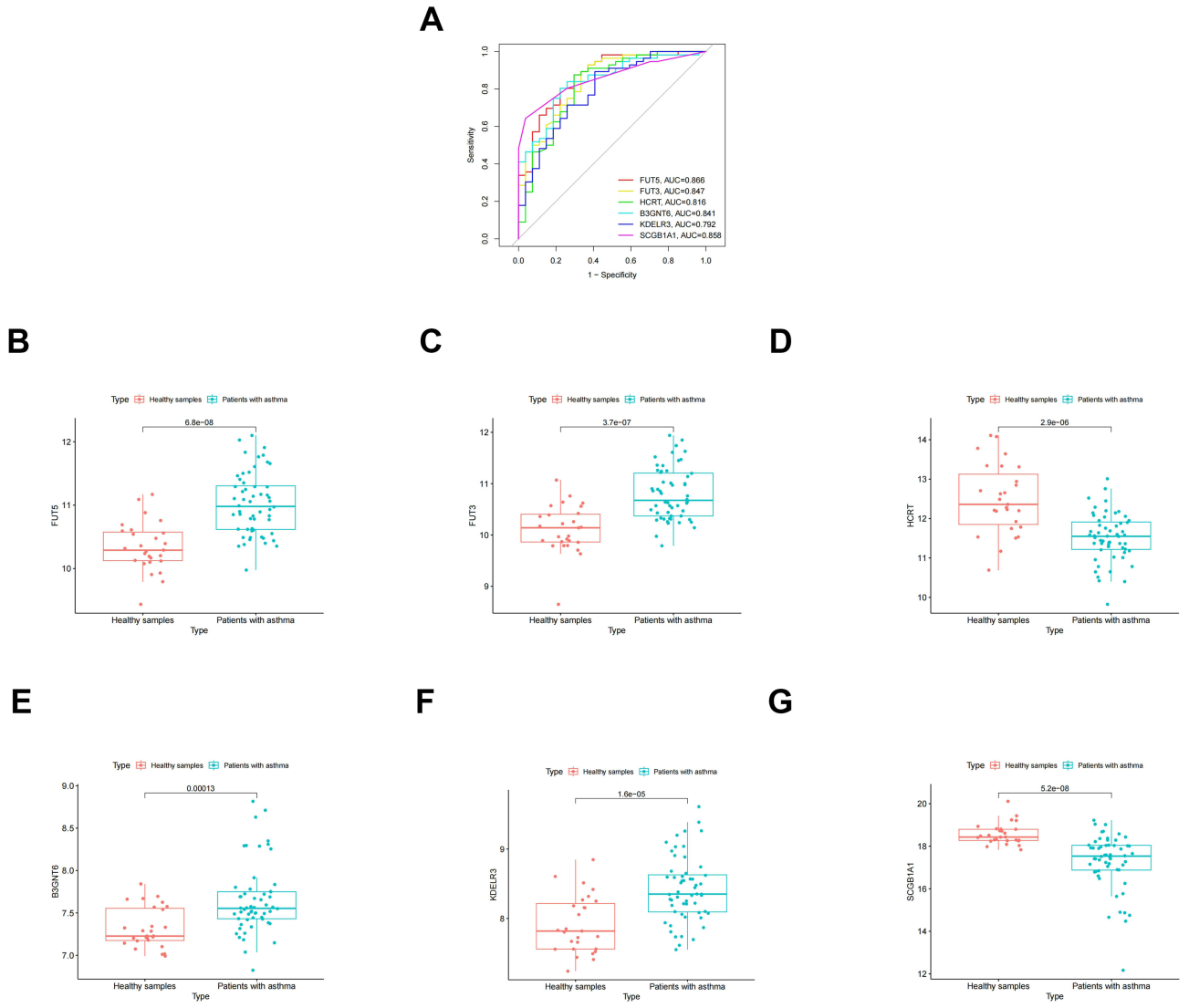

Figure 7 Validation of key glycosylation-related genes. (**A**) ROC curves were plotted to examine the diagnostic potential of key genes in GSE63142 (healthy samples versus patients with asthma). (**B**–**G**) Validation of key glycosylation-related genes in the dataset GSE63142.
Abbreviation: ROC, receiver operating characteristic.

asthma research. Carbohydrates are added to proteins or lipids via glycosylation under the control of glycosylated transferases.[5] This process begins in the endoplasmic reticulum and terminates in the Golgi apparatus.[14] Although glycosylation has been extensively studied in malignant tumors, it has received limited attention in nontumor diseases, particularly asthma. The current study found that the regulation of airway inflammation in asthma is related to glycosylation, but studies on asthma and glycosylation are rare.[15] Therefore, this study explored the pathogenesis of glycosylation in asthma using bioinformatics analysis, providing important evidence for the treatment and prevention of asthma.

In our analyses of the GSE63142 dataset, we identified 132 DEGs, of which 72 were upregulated and 60 downregulated. Through GO and KEGG pathway analysis, it was finally concluded that DEGs were mainly enriched in cellular response to xenobiotic stimulus and the metabolism of xenobiotics by cytochrome P450 pathways, which further confirmed that DEGs may be closely associated with the pathogenesis of asthma. No studies have yet investigated how glycosylation plays a role in asthma through these pathways, and further studies are needed.

In this study, six key asthma-related glycosylation genes (FUT5, FUT3, HCRT, B3GNT6, KDELR3, and SCGB1A1) were screened using WGCNA. FUT5 and FUT3 belong to the fucosyltransferase family, though it is unclear how FUT5

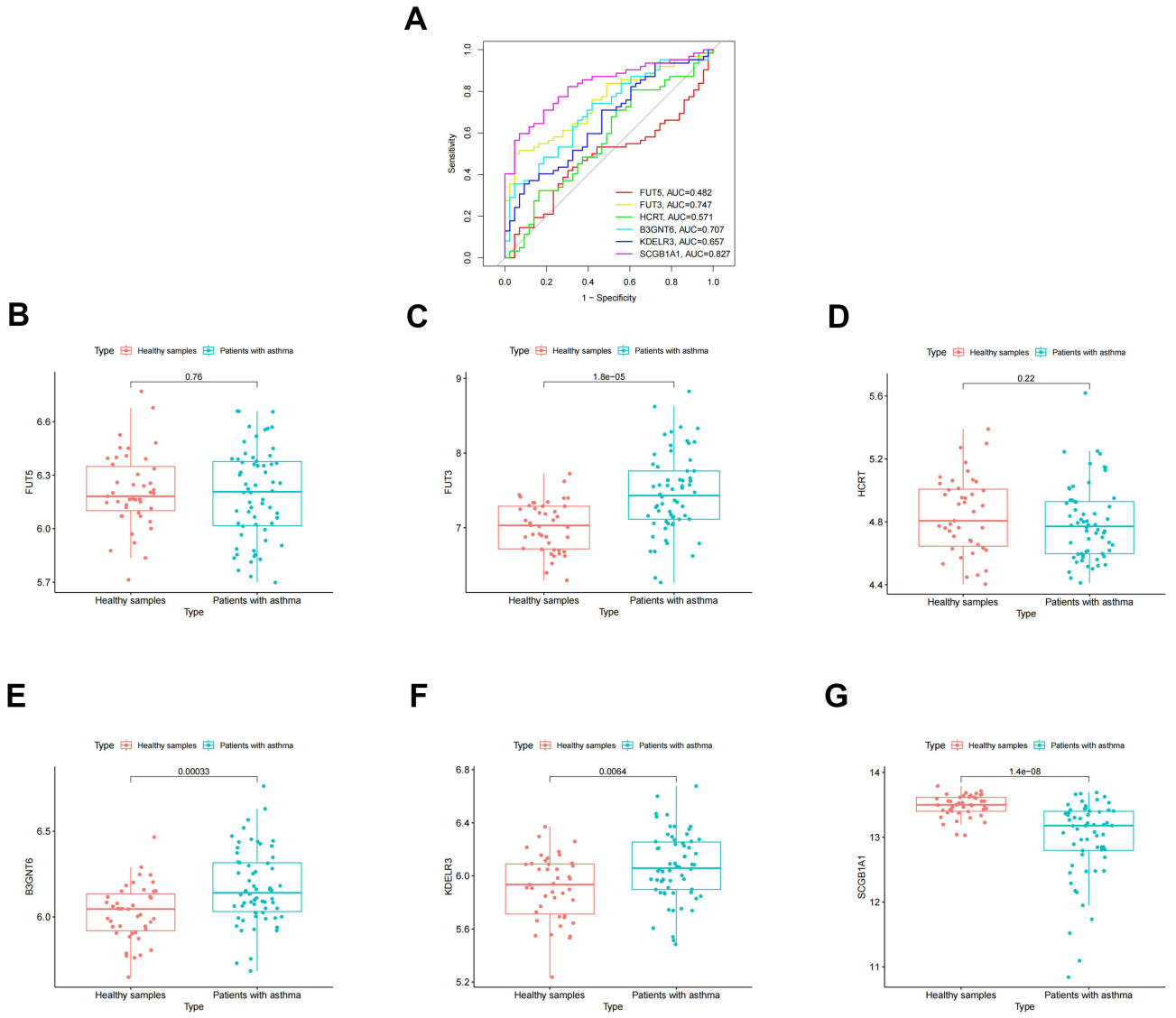

Figure 8 Validation of key glycosylation-related genes. (**A**) ROC curves were plotted to examine the diagnostic potential of key genes in GSE67472 (healthy samples versus patients with asthma). (**B**–**G**) Validation of key glycosylation-related genes in the dataset in GSE67472.
Abbreviation: ROC, receiver operating characteristic.

and FUT3 influence asthma. The fucosyltransferases encoded by FUT3 catalyze the formation of α-(1,4)-fucosylated glycoconjugates and are present only in two hominids (humans and chimpanzees). These genes are closely related and belong to the Lewis FUT5–FUT3 gene cluster, and the corresponding enzymes share 85% sequence similarity due to duplications of ancestral Lewis gene events.[16] Fucosylation is a posttranslational modification in which fucose residues are attached to polysaccharides, which partly determines mucin size and charge heterogeneity.[17] Core 3 synthase, a product of B3GNT6, displays O-GlcNAc glycosyltransferase activity; core 1 synthase synthesizes O-type carbohydrate chains on mucin surfaces.[18] This protein shows a substantial level of glycosylation. Abnormal patterns of O-glycosylation are not only directly related to tumor proliferation and metastasis but also cause other diseases such as asthma, cardiovascular diseases, and Alzheimer's disease.[19] KDELR3 regulates protein synthesis and their secretion into the endoplasmic reticulum cavity.[20] Endoplasmic reticulum stress is caused by various factors that impair the ability of cells to correctly fold and post-translationally modify secreted and transmembrane proteins in the endoplasmic reticulum, resulting in accumulation of misfolded proteins.[21] KDELR3 seems to influence asthma development through endoplasmic reticulum stress pathways.[22] SCGB1A1 (CC16) is a protein mainly produced by nonciliated bronchial

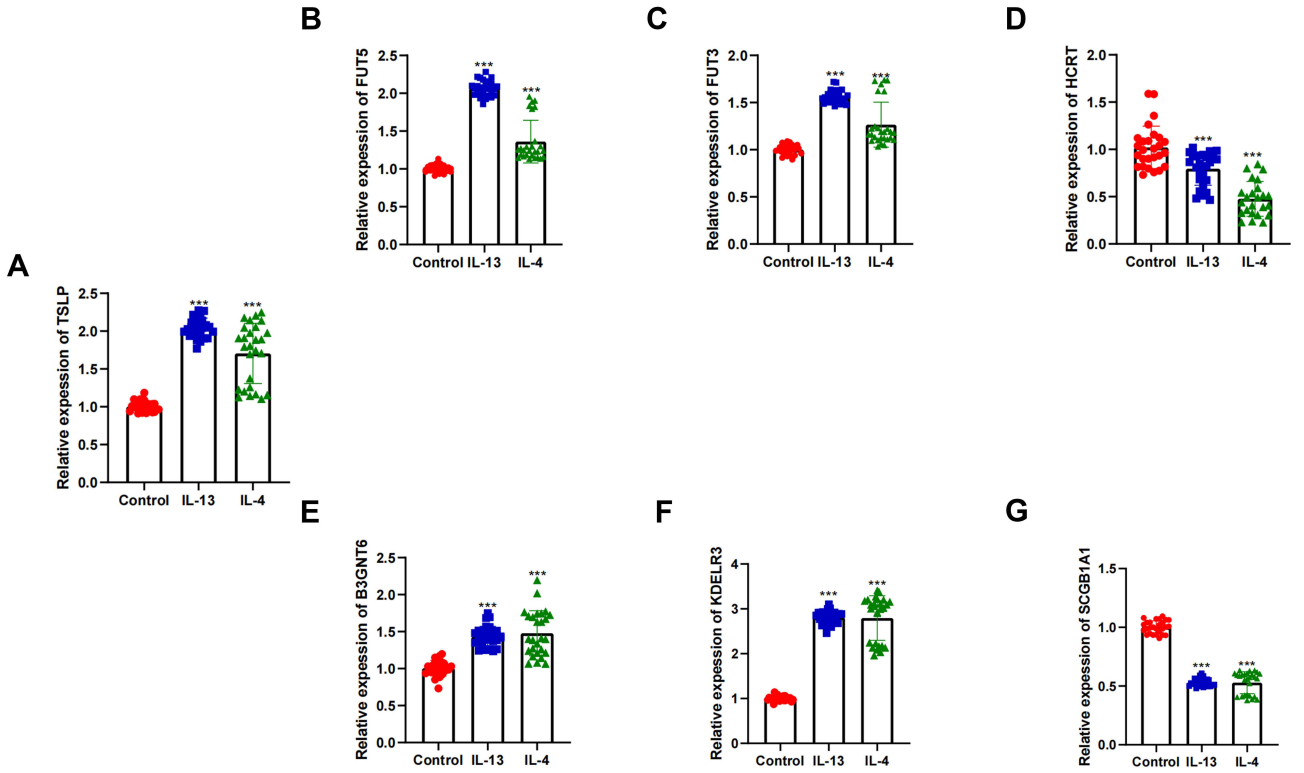

Figure 9 Expression of key genes in the cell model. (**A**) The expression of TSLP verified by RT-qPCR in the cell model. (**B**–**G**) The expression of key genes verified by RT-qPCR in the cell model. ***p < 0.001.
Abbreviation: RT-qPCR, Real-time quantitative PCR.

epithelial cells (BECs) that participates in host defense. Researchers found that low SCGB1A1 mRNA expression levels in BECs are associated with asthma susceptibility, severity, and exacerbations, partially through immunomodulation of T helper 2 (Th2) inflammation.[23] HCRT, while significant, has not yet been extensively researched in the context of glycosylation, suggesting the need for further research. Thus, it can be concluded that glycosylation has a potential but integral role in asthma. Our study enriches the mechanism of glycosylation in asthma through bioinformatics.

Table 1 Correlation Analysis of Key Gene Expression and Clinical Characteristics

Characteristic	FUT5	FUT3	HCRT	B3GNT6	KDELR3	SCGB1A1
Age (years)	−0.199	−0.145	0.014	0.021	0.080	0.147
Gender	−0.076	−0.273	0.106	−0.122	−0.132	−0.284
BMI (kg/m²)	−0.096	−0.112	0.187	−0.128	−0.201	−0.032
FEV1 (L)	−0.222	−0.454**	0.227	−0.335*	−0.330*	0.064
FEV1% predicted (%)	−0.087	−0.254	0.173	−0.179	−0.315	0.403*
FVC (L)	−0.235	−0.427**	0.136	−0.251	−0.249	−0.227
FVC percent predicted (%)	−0.259	−0.312	0.138	−0.188	−0.212	0.108
FEV1/FVC (%)	−0.017	−0.117	0.154	−0.149	−0.191	0.586***
FEV1/FVC percent predicted (%)	0.003	−0.084	0.132	−0.120	−0.187	0.599***
Eosinophils (10^9/L)	0.036	0.324*	−0.538***	0.161	0.144	−0.169
Eosinophils (%)	−0.117	0.111	−0.352*	0.007	0.029	−0.147
Neutrophils (10^9/L)	−0.093	−0.199	0.260	−0.213	−0.212	0.141
Neutrophils (%)	−0.003	−0.156	0.296	−0.025	−0.109	0.081
FeNO (ppb)	−0.022	0.178	−0.314	0.189	0.140	−0.125
IgE (IU/mL)	−0.091	−0.098	−0.097	−0.109	−0.046	−0.045

Notes: *p < 0.05, **p < 0.01, ***p < 0.001.
Abbreviations: BMI, body mass index; FEV1, forced expiratory volume in one second; FVC, forced vital capacity; FeNO, fractional exhaled nitric oxide; IgE, immunoglobulin E.

To understand the mechanisms of action of key asthma-related genes, we constructed three regulatory networks: TFs, RBPs, and miRNAs. TFs refer to a group of protein molecules that specifically bind to specific gene sequences, thereby ensuring expression of target genes with specific strengths and spatiotemporal specificity.[24] We predict that the key genes may be regulated by multiple TFs. YY1 belongs to the zinc finger family and is widely involved in various cell biological processes by activating or inhibiting gene transcription.[25] CD4-specific knockdown of YY1 in mice markedly reduced Th2 cytokine expression, repressed chromatin remodeling, decreased intrachromosomal interactions, and decreased resistance in this animal model of asthma.[26] Our results revealed that these key genes formed a connected regulatory network with TFs, thus suggested that the key genes may play important roles in asthma. RBPs are proteins that bind to RNA through one or more RNA-binding domains and alter its function.[27] We predict that the key genes may be regulated by multiple RBPs. Researchers screened 6 RBPs (FTO, IGF2BP2, RBM15, RBMX, WTAP, and YTHDC1) that were significantly dysregulated in asthma or proinflammatory conditions.[28] A study by the Donnelly Center showed that SP1 plays a role as an RBP in gene regulation by affecting the stability of RNA.[28] *Mahuang decoction* (MHD) is a classic famous traditional Chinese medicine that has various pharmacological effects, including anti-inflammatory and anti-asthma effects. MHD may retard airway inflammation and remodeling by suppressing the SP1/FGFR3/PI3K/AKT axis, which contributes to an extensive understanding of asthma and may provide novel therapeutic options for this disease.[29] Our results revealed that these key genes formed a connected regulatory network with RBPs, thus suggested that the key genes may play important roles in asthma. The discovery of miRNAs has had a profound effect on the understanding of gene expression and is now considered to be part of the epigenetic machinery.[30] It has led to the addition of a new level of gene regulation, adding a layer of complexity to the central dogma. We predict that the key genes may be regulated by multiple miRNAs. Researchers have shown that miR-125b-5p is highly expressed in M2 macrophages and bronchoalveolar lavage fluid cells in *Dermatophagoides farinae* protein 1 (Der f1)-induced asthma. In response to Der f1 challenge, miR-125b-5p knockdown attenuates allergic airway inflammation in mice by preventing M2 macrophage polarization. Mechanistic studies have indicated that the lncRNA AK089514 functions as a competing endogenous RNA for miR-125b-5p, thereby leading to depression of its endogenous target TNF receptor-associated factor 6.[31] These studies indicate that miRNAs are important regulators of asthma pathogenesis.

Immune homeostasis regulated by immune cells and various inflammatory factors have an important role in maintaining normal lung function, and these immune cells include T and B cells, neutrophils, mast cells, monocytes, eosinophils and dendritic cells.[32] FUT5, FUT3, B3GNT6, and KDELR3 were found to correlate with type 17 T helper cells, HCRT correlated with activated B cells, and SCGB1A1 correlated with immature dendritic cells. Recently, it was discovered that glycosylation is essential for immune homeostasis related to chronic inflammatory diseases.[33] Our findings suggest that these key genes participate in asthma progression.

Type 2 asthma and non-type 2 asthma are the main divisions of asthma phenotypes.[34] Type 2 inflammatory biomarkers, including blood and sputum eosinophil counts, fractional exhaled nitric oxide, and serum immunoglobulin E, are helpful for the differentiation of the phenotypes for patients with asthma.[35] These biomarkers can be utilized to determine the level of type 2 inflammation, as well as guide the therapy of type 2 asthma. The 2023 Global Initiative for Asthma guideline suggests considering the addition of biologic therapy based on the inflammatory phenotype for patients with severe and difficult-to-control asthma.[9] Early studies have demonstrated that airway inflammation in type 2 asthma is primarily mediated by cytokines such as IL-4, IL-5, and IL-13, which are mainly derived from Th2 cells.[36] Studies of recent years have discovered that other innate immune cells, besides Th2 cells, can produce cytokines such as IL-4, IL-5, and IL-13 as well. IL-4, IL-5, and IL-13 are both consequently referred to as type 2 cytokines. IL-4 and IL-13 are involved in the pathogenesis of asthma by binding to IL-4R, as well as activating IL-4R signaling pathway. IL-4Rα, a receptor subunit shared by IL-4 and IL-13, holds a crucial role in the signal transduction of both cytokines.[37,38] TSLP is an important regulator of asthma inflammation and plays a key role in the occurrence and persistence of airway inflammation. TSLP can drive the release of downstream type 2 cytokines, which leads to an inflammatory response and increases airway obstruction. Cytokines such as IL-4 and IL-13 can induce TSLP in epithelial cells and immune cells, ultimately promoting airway inflammation. We built asthma cell models by virtue of IL-4 and IL-13.[39] The expression of inflammatory marker (TSLP) was obviously upregulated in the experimental group compared with that in the control group. The upregulated expression of TSLP confirmed that we successfully constructed the cell model of

asthma. The expression of key glycosylation-related genes was obviously up- or downregulated in the experimental group compared with that in the control group. We eventually experimentally validated that asthma-associated glycosylation genes were consistent with the results predicted by bioinformatics.

Correlation analysis of key gene expression and clinical characteristics of asthma patients was performed using Spearman correlation analysis. Expression of the key glycosylation-related genes correlated with lung function and eosinophils, suggesting that these key glycosylation-related genes may be used as novel biomarkers for asthma. The results suggest that key genes may be biomarkers for the diagnosis of asthma and the assessment of asthma severity. However, our sample size is still small. In future studies, we will expand the sample size for further in-depth research.

Conclusions

Ultimately, this study screened 6 key genes in asthma by bioinformatics to provide clues for the pathogenesis of glycosylation in asthma. These key genes have good diagnostic properties in asthma and can be used as potential biomarkers specific to asthma. These results not only identify potential biomarkers and therapeutic targets specific to asthma, but also deepen the understanding of the pathogenesis of asthma.

Data Sharing Statement

All data involved in this study are available from the corresponding author on request. The original GEO datasets used for analysis in this study (including GSE63142, GSE67472, and GSE142237) are available in GEO database (http://www.ncbi.nlm.nih.gov/geo).

Ethics statement

This study was approved by the Academic Advisory Board of the First Affiliated Hospital of Zhengzhou University (approval no. 2019-KY-009).

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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