

Integrated Analysis of mRNA and Long Noncoding RNA Profiles in Peripheral Blood Mononuclear Cells of Patients with Bronchial Asthma

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Abstract

Background: Bronchial asthma is a heterogeneous disease with distinct disease phenotypes and underlying pathophysiological mechanisms. Long non-coding RNAs (lncRNAs) are involved in numerous functionally different biological and physiological processes. The aim of this study was to identify differentially expressed lncRNAs and mRNAs in patients with asthma and further explore the functions and interactions between lncRNAs and mRNAs.

Methods: Ten patients with asthma and 9 healthy controls were enrolled in this study. RNA was isolated from peripheral blood mononuclear cells. We performed microarray analysis to evaluate lncRNA and mRNA expression. The functions of the differentially expressed mRNAs were analyzed by Gene Ontology and Kyoto Encyclopedia of Genes and Genomes pathway analyses. A global signal transduction network was constructed to identify the core mRNAs. An lncRNA–mRNA network was constructed. Five mRNAs showing the greatest differences in expression levels or high degrees in the gene-gene functional interaction network, with their correlated lncRNAs, were validated by real-time quantitative polymerase chain reaction.

Results: We identified 2,229 mRNAs and 1,397 lncRNAs between the asthma and control groups. Kyoto Encyclopedia of Genes and Genomes pathway analysis identified many pathways associated with inflammation and cell survival. The gene-gene functional interaction network suggested that some core mRNAs are involved in the pathogenesis of bronchial asthma. The lncRNA–mRNA co-expression network revealed correlated lncRNAs. *CXCL8*, *FOXO3*, *JUN*, *PIK3CA*, and *GOS2* and their related lncRNAs NONHSAT115963, AC019050.1, MTCYBP3, KB-67B5.12, and HNRNPA1P12 were identified according to their differential expression levels and high degrees in the gene-gene network.

Conclusions: We identified the core mRNAs and their related lncRNAs and predicted the biological processes and signaling pathways involved in asthma.

1 Background

Bronchial asthma is a heterogeneous disease characterized by chronic airway inflammation, reversible airway obstruction, and bronchial hypersensitivity [1]. At present, the number of people suffering from asthma worldwide has reached more than 300 million, making asthma one of the most common chronic lung diseases worldwide [2]. Asthma has long been considered as a T helper 2-cell-mediated disease; furthermore, basophils, type 2 innate lymphoid cells, and mast cells can produce T helper 2-cell-associated cytokines [3]. It has been suggested that asthma is a highly heterogeneous disease with distinct disease phenotypes and underlying pathophysiological mechanisms [4]. Omics technologies may help to reveal the complex mechanisms underlying the pathophysiology of asthma.

Long noncoding RNA (lncRNA) is a type of noncoding RNA composed of more than 200 nucleotides [5]. Initially, lncRNAs were regarded as the ‘noise’ in the process of genomic transcription, and they were assumed to not have biological functions. However, increasing numbers of studies have indicated that

lncRNAs are closely related to many physiological activities and pathological processes, such as epigenetic modification, cell differentiation, and development and transcriptional regulation [5]. The largest public dataset from the GENCODE project provides information on 15,877 human lncRNA genes (<http://www.genencodegenes.org/stats/archive.html#a21>). Based on these data, several studies have shown that lncRNAs are involved in a variety of biological and physiological processes and are directly related to various cancers, coronary artery disease, and many other diseases affecting humans [6–8]. Technological advances have allowed for a better understanding of the complex pathogenesis of asthma. To provide clues regarding the crucial pathways and pivotal pathogenic genes involved in the pathogenesis of asthma, we identified the lncRNAs and mRNAs in peripheral blood mononuclear cells (PBMCs) from patients with bronchial asthma. Furthermore, we comprehensively analyzed the function and interactions of lncRNA and mRNA.

2 Materials And Methods

2.1 Study Population

Ten patients with bronchial asthma and 9 healthy controls participated in the study conducted at China-Japan Friendship Hospital (Beijing, China) between July 2017 and December 2018. Bronchial asthma was diagnosed according to the guidelines of Global Initiative for Asthma. The inclusion criteria for the patients were: 1) age ≥ 45 and ≤ 75 years; 2) medical reports of treating physicians, symptoms, and use of medications for asthma were available; 3) reversible airflow limitation and a forced expiratory volume in the first second (FEV₁) reversibility $> 12\%$ and 200 mL after post-bronchodilator spirometry; and 4) no active asthma or exacerbation. Ten age- and sex- matched controls from the general population who underwent physical examinations at the China-Japan Friendship Hospital were recruited for this study; these subjects had 1) no previous or present diagnosis of chronic obstructive pulmonary disease, asthma, or other respiratory diseases; 2) no history of allergic diseases, including skin symptoms and nasal symptoms; and 3) no history of chronic cough, wheezing, shortness of breath, or expectoration. Patients and controls were excluded based on the following criteria: 1) pregnancy or breast feeding; 2) liver and kidney insufficiency; 3) past or present malignant tumors; and 4) diagnosis of acute infectious disease. All participants signed a written informed consent form. Furthermore, the study was approved by the Ethics Committee of the China-Japan Friendship Hospital (2017–19). The study was carried out in accordance with the Declaration of Helsinki.

2.2 PBMC Isolation and RNA Extraction

PBMCs were isolated from the peripheral blood as previously reported [9]. We collected venous blood (6 mL) from each participant using an EDTA-anticoagulated tube. PBMCs were isolated via density gradient centrifugation using Ficoll-Paque PLUS (GE Healthcare, Little Chalfont, UK) and stored at -80°C . Total RNA was isolated using TRIzol reagent (Life Technologies, Carlsbad, CA, USA) and purified with an RNeasy Mini Kit (Qiagen, Hilden, Germany). RNA was quantitatively detected using a spectrophotometer (NanoDrop 1000, Thermo Fisher Scientific, Waltham, MA, USA; 260 nm absorbance).

2.3 Microarray Profiling and Data Analysis

The Cnkingbio Biotechnology Corporation (Beijing, China) provided the protocols for assessing the expression profiles using microarray analysis. Biotinylated cDNA was prepared according to the standard Affymetrix protocol using 250 ng total RNA with an Ambion® WT Expression Kit (Austin, TX, USA). The labeled cDNA was hybridized at 45°C for 16 h using the genechip (Clariom D Assay, human, Thermo Fisher Scientific). An Affymetrix Jet Station 450 (Santa Clara, CA, USA) was used to stain and clean genes. All arrays were scanned using the Affymetrix GeneChip Command Console software, which was installed on the GeneChip Scanner 3000 7G.

The 'limma' R package (version 3.36.5) was used to filter the differentially expressed genes (DEGs) identified by microarray analysis. Concurrently, for statistical analysis, Student's *t*-test was used to screen genes with differential expression. The Benjamini–Hochberg method was used to correct for multiple tests, and the false discovery rate was used to adjust the P-values for multiple comparisons [10]. The cutoff criteria of differentially expressed mRNAs were a fold-change (IFC) > 1.5 and P-value < 0.05. Hierarchical clustering was performed based on differentially expressed mRNAs using the R package 'pheatmap' (version: 1.0.12).

2.4 GO Analysis and KEGG Pathway Analysis

Gene Ontology (GO) analysis was performed to determine the primary functions of the differentially expressed mRNAs. Kyoto Encyclopedia of Genes and Genomes (KEGG) is a knowledge databank for systematic analysis of gene functions and significantly altered pathways, which links genomic information with higher-order functional information. Two-sided Fisher's exact test was used as the statistical analysis method, and the Benjamini–Hochberg method was used for multiple test correction. The analysis product of Cnkingbio Biotechnology Corporation was used, and the internal name is GO_Enrichment_Analysis/Pathway_Enrichment_Analysis. The threshold set for the significantly changed pathways was P-value < 0.05.

2.5 Global Signal Transduction Network

A global signal transduction network (signal-net) was constructed to identify interactions between DEGs in patients with asthma. We used Cytoscape software (version 3.6.0) for network visualization. In the network graph, edges and nodes represented the internal connections and genes, respectively. The number of edges from one node to the other denoted the degree. Genes with higher degrees have a powerful ability to modulate other genes and were the core key genes in the signal-net.

2.6 Co-expression Network Analysis (lncRNA–mRNA)

lncRNA–mRNA co-expression network analysis was conducted based on the normalized signal intensity of differentially expressed mRNAs and lncRNAs. The R function 'cor.test' (Hmisc and corrplot) was used to calculate the Pearson's correlations for each pair of mRNA–lncRNA. The mRNA–lncRNA pairs with Pearson's correlations of > 0.9 were included to construct the visualization network using Cytoscape software (version 3.6.0). The degree was calculated to measure the centrality of a gene or lncRNA within a network.

2.7 Real-Time Quantitative Polymerase Chain Reaction (RT-qPCR)

Some of the core differentially expressed lncRNAs and mRNAs identified in microarray analysis were validated by RT-qPCR. The gene primers are listed in Supplementary Table 1. The housekeeping gene GAPDH was used as an internal control. We performed RT-qPCR using SYBR green reagent (Bio-Rad, Hercules, CA, USA) as per the manufacturer's instructions and calculated the sample mean to ensure data stability. The relative expression levels of lncRNAs and mRNAs were calculated using the comparative CT method ($2^{-\Delta\Delta CT}$).

2.8 Statistical Analysis

The software programs used for statistical analysis were GraphPad Prism 7.0 (GraphPad, Inc., La Jolla, CA, USA) and SPSS version 22.0 (SPSS, Inc., Chicago, IL, USA). Expression levels of lncRNAs and mRNAs between patients with asthma and healthy controls were evaluated by Student's *t*-tests. $|FC| > 1.5$ and *P*-values < 0.05 were considered as statistically significant. For GO and KEGG analysis, two-tailed Fisher's exact tests were performed, and *P*-values < 0.05 were considered statistically significant. The experimental data for RT-qPCR analysis were represented as the mean \pm standard deviation. Validation analysis was carried out using *t*-test, and a *P*-value < 0.05 was considered statistically significant.

3 Results

3.1 Baseline Characteristics

Ten patients with asthma and 9 healthy controls participated in this study. No differences in age, sex, body mass index, pulse oximetry saturation, smoking history, FEV₁, FEV₁ as the percentage of the predicted value, or complications were observed between the two groups. FEV₁ to forced vital capacity and fractional exhaled nitric oxide were significantly higher in patients with asthma than in normal controls ($p < 0.05$). The asthma control test and asthma control questionnaire-7 scores of patients with asthma were 20.5 ± 2.7 and 0.76 ± 0.4 , respectively. Table 1 shows the baseline clinical and demographic characteristics of the participants in this study.

Table 1
Baseline Characteristics of the Study Participants

Characteristics	Asthma (n = 10)	Control (n = 9)
Age (years)	32.6 ± 5.1	35.1 ± 6.6
Gender (male, %)	50	55.5
BMI (kg/m ²)	24.3 ± 1.9	23.6 ± 4.4
SpO ₂ (%)	94.9 ± 5.7	98.6 ± 0.7
Current/ex-smoker	1	0
FEV1	3.1 ± 0.7	3.1 ± 0.6
FEV1% pred	82.3 ± 29.7	97.8 ± 8.6
FEV1/FVC	77.5 ± 5.1	83.1 ± 5.2*
FENO	82.0 ± 60.7	18.8 ± 9.6*
ACT score	20.5 ± 2.7	NA
ACQ-7 score	0.76 ± 0.4	NA
Medication status		
SABA	3	0
LABA	0	0
LAMA	0	0
LABA + ICS	1	0
LAMA + ICS	7	0
Theophylline	1	0

Note: Data are presented as the mean ± standard deviation unless otherwise stated. *P-value < 0.01. Abbreviations: BMI, body mass index; SpO₂, pulse oximetry saturation; FEV1, forced expiratory volume in 1 second; FEV1% predicted, forced expiratory volume in 1 second as the percentage of the predicted value; FVC, forced vital capacity; FENO, fractional exhaled nitric oxide; ACT, asthma control test; ACQ-7, asthma control questionnaire-7; SABA, short-acting beta-2 agonist; LABA, long-acting beta-2 agonist; ICS, inhaled corticosteroid; LAMA, long-acting muscarinic antagonist; NA, not available.

3.2 Identification and Differential Expression of mRNAs and lncRNAs in Patients with Asthma vs. Healthy Controls

Microarray analysis of the whole transcriptome was performed to explore the differential expression of mRNAs and lncRNAs between patients with bronchial asthma and healthy controls. The cutoff criteria of

statistical significance were as mentioned above ($|FC| > 1.5$ and $P\text{-value} < 0.05$). Microarray analysis detected 75,550 lncRNAs in the PBMC samples, among which 1,397 lncRNAs (884 upregulated and 513 downregulated lncRNAs) showed significant differences (Fig. 1A). A total of 20,666 mRNAs was detected, including 337 upregulated and 1,942 downregulated mRNAs between patients with asthma and healthy controls (Fig. 1B). Hierarchical clustering of lncRNAs and mRNAs was performed to distinguish patients with asthma from healthy controls using Pearson's correlation (Fig. 1C and D). The top-ten most significant lncRNAs and mRNAs are shown in Supplementary Tables 2 and 3.

3.3 Functional Annotation and Pathway Analysis

To further analyze the differentially expressed mRNA, we performed KEGG pathway and GO analyses. upregulated GO functions included chromatin remodeling, cell-cell adhesion, positive regulation of transcription, response to gamma radiation, viral process, RNA export from the nucleus, and gene silencing via RNA (Fig. 2A). Downregulated GO functions included neutrophil degranulation, cellular response to DNA damage stimuli, mRNA splicing, protein deubiquitination, positive regulation of chemokine production, and double-strand break repair (Fig. 2B). The upregulated pathways in KEGG pathway analysis included RNA transport, apoptosis, the retinoic acid-inducible gene I (RIG-I)-like receptor signaling pathway, and T helper 17 cell differentiation (Fig. 2C). Downregulated pathways included the Toll-like receptor (TLR) signaling pathway, nucleotide-binding oligomerization domain (NOD)-like receptor signaling pathway, Forkhead Box class O (FOXO) signaling pathway, and interleukin signaling pathway (Fig. 2D).

3.4 Global Signal Transduction Network (signal-net)

A global signal transduction network was constructed to identify the core key genes and mRNAs (Fig. 3). *NFKB1* showed the highest degree value and centrality range, reflecting its centrality in the network as it connects the most genes. In addition, *PRKACB*, *PIK3CA*, *PLCB1*, *PLCG1*, *PLCG1*, *GNAI3*, *JUN*, *P53*, *KRAS*, *NRAS*, *JAK2*, *FOXO3*, and a few other genes were determined to have important functions in the signal-net with high degree values (Table 2).

Table 2
Core mRNAs selected using the Gene-Gene Functional Interaction Network

Gene Symbol	Style	Betweenness Centrality	Degree
<i>NFKB1</i>	up	0.052151	35
<i>PRKACB</i>	down	0	32
<i>PIK3CA</i>	down	0.033763	29
<i>PLCB1</i>	down	0.04064	24
<i>PLCG1</i>	up	0.02381	23
<i>GNAI3</i>	down	0.0408	21
<i>JUN</i>	down	0.030527	20
<i>TP53</i>	up	0.040001	19
<i>KRAS</i>	down	0.026864	18
<i>NRAS</i>	down	0.026311	18
<i>JAK2</i>	down	0.011193	18
<i>FOXO3</i>	down	0.024722	17
<i>FOS</i>	down	0.019986	17
<i>ENPP3</i>	down	0.014337	16
<i>STAT1</i>	down	0.014575	12
<i>IFNG</i>	down	0.013518	12
<i>PLA2G4A</i>	down	0.006587	12
<i>PLD1</i>	down	0.005553	12
<i>CREB1</i>	down	0.006042	11
<i>IL6</i>	down	0.00833	11

Betweenness centrality ≥ 0.005 . Betweenness centrality is an indicator of a gene's centrality in a network. It is equal to the number of shortest paths from all vertices to all others that pass through that gene. The degree of a gene was defined as the number of directly linked genes within a network.

3.5 lncRNA–mRNA Co-expression Network

The 519 mRNAs and 380 lncRNAs showed a co-expression relationship. The lncRNA–mRNA co-expression network was mainly used to identify relationships between these mRNAs and lncRNAs, comprising 899 nodes and 1,146 edges (Supplementary Fig. 1). We focused on the core mRNAs with high

degrees in signal-net and highly expressed mRNAs with high FCs in microarray analysis. We found that lncRNA KB-67B5.12 was negatively correlated with *PIK3CA*, whereas lncRNA MTCYBP3 and lncRNA AC019050.1 were positively correlated with *JUN* and *FOXO3*, respectively. Interleukin-8/C-X-C motif chemokine ligand 8 (*CXCL8*) and *G0/G1* switch gene 2 (*GOS2*) show the largest difference in FC. lncRNA NONHSAT115963 was positively correlated with *CXCL8* and NONHSAT122646 was negatively correlated with *GOS2* (Table 3).

Table 3
Correlation Between lncRNAs and Core mRNAs

mRNA	lncRNA	Pearson's correlation	Regulation
<i>CXCL8</i>	NONHSAT115963	0.917**	Positive
<i>FOXO3</i>	AC019050.1	-0.925**	Negative
<i>JUN</i>	MTCYBP3	0.925**	Positive
<i>PIK3CA</i>	KB-67B5.12	0.935**	Positive
<i>GOS2</i>	HNRNPA1P12	-0.901**	Negative

3.6 RT-qPCR Validation

We conducted RT-qPCR to verify the stability and reliability of the microarray analysis. Five core mRNAs and their related five lncRNAs were selected for RT-qPCR (Fig. 4). The results of RT-qPCR analysis of nine genes were consistent with the microarray results. AC019050.1 was upregulated, whereas *JUN*, *GOS2*, *CXCL8*, *FOXO3*, *PIK3CA*, *MTCYBP3*, *KB-67b5.12*, and *NONHSAT115963* were downregulated. Only the lncRNA of *HNRNPA1P12* showed an opposite trend between RT-qPCR and microarray analysis.

4 Discussion

Asthma is a common health issue which poses an economic and social burden on patients. However, the pathogenesis of this disease remains poorly understood. Recent studies have highlighted the potential roles of lncRNA in the pathogenesis of asthma. Lin reported that the lncRNA TUG1 promoted airway smooth muscle cell proliferation and migration, contributing to asthma [11]. The lncRNA ANRIL/miR-125a axis may be related to inflammation, exacerbation, and the severity of bronchial asthma [12]. In this study, 884 upregulated and 513 downregulated lncRNAs were identified between 10 patients with asthma and 10 healthy controls; these factors may serve as biomarkers for bronchial asthma.

GO and KEGG pathway analyses were performed to identify enriched biological functions. According to the results of the GO analysis, DEGs were involved in processes such as cell-cell adhesion, neutrophil degranulation, cellular response to DNA damage stimuli, positive regulation of chemokine production. Previous studies demonstrated that these are important biological processes in asthma. Dysregulation of cell-cell adhesion leads to epithelial barrier destruction, which may facilitate penetration of environmental

allergens, thereby activating the innate immune responses and increasing asthma severity and susceptibility [13]. Airway neutrophilia is associated with asthma severity and is refractory to treatment with corticosteroids. Neutrophils can kill pathogens but may also damage the airways by affecting proteases and reactive oxygen species [14]. Airway inflammation, oxidative damage, DNA damage, and repair protein levels in asthma have been found to be increased [15]. Asthma is known as a chronic inflammatory disease, and processes related to chemokine production, MAPK activation, and NF- κ B activation were detected in this study.

KEGG pathway analysis revealed that the differentially expressed mRNAs are mainly related to apoptosis and *TLR*, RIG-I-like receptor, NOD-like receptor, *FOXO*, and T helper 17 signaling pathway. Some of these pathways have been demonstrated crucial to the onset of asthma. For example, CD9 + B cells induce effector T cell cycle arrest in sub G0/G1 and apoptosis in asthmatic mice [16]. RIG-I has long been known to serve as a pattern recognition receptor for viral detection. However, this receptor was also found to serve several functions as an RNA-responsiveness protein for various cellular activities, including cell development and proliferation [17]. However, the role of RIG-I in asthma requires further study. TLRs recognize microbial, endogenous molecules, and environmental allergens and play an immune-modulatory role in asthma development [18]. Bacterial infection-mediated activation of NOD-like receptors triggers allergic asthma by activating eosinophils, which interact with bronchial epithelial cells in the airway [19]. *FOXO* has multiple biological functions, such as modulation of embryonic endothelial stem cell survival [20], regulation of ischemic brain injury [21], and vascular disease [22], which is a newly investigated aspect of asthma. In this study, we identified some classical biological processes and new processes in asthma, providing a foundation for further study.

We constructed a global signal transduction network to identify core genes with crucial roles in the pathogenesis of asthma. The mRNAs *NFKB1*, *PRKACB*, *PIK3CA*, *PLCB1*, and *PLCG1* had the top five degrees in the gene signal network. *NFKB1* is the NF- κ B p105 subunit, a typical inflammation pathway gene, and is associated with asthma. The protein kinase cAMP-dependent catalytic subunit β (*PRKACB*) is a member of the serine/threonine protein kinase family. *PRKACB* plays a key role in apoptosis and cell differentiation and proliferation [23]. The phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*) gene encodes the PI3K-p110 α protein, which activates the PI3K pathway and leads to dysregulated cell proliferation [24]. *PLCB1* encodes the protein phospholipase C β 1 and plays an important role in intracellular transduction of many extracellular signals [25]. The main function of *PLCG1* is to encode phospholipase C γ 1, which mainly catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate to generate second messenger molecules [26]. Most of these genes have not been sufficiently studied in asthma and require further investigation. However, some mRNAs showing high degrees were not correlated with lncRNAs.

lncRNAs exert their functions by modulating mRNA processing and post-transcriptional regulation. Based on the lncRNA–mRNA co-expression network, gene signal network, and FC in the based expression list, we identified the following lncRNAs: AC019050.1, MTCYBP3, KB-67B5.12, NONHSAT122646, HNRNPA1P12, and their related mRNAs (*FOXO3*, *JUN*, *PIK3CA*, *CXCL8*, and *GOS2*). *FOXO* is a subfamily of

forkhead transcription factors and plays key roles in regulating many pathways that regulate processes such as apoptosis, insulin signaling, DNA repair, oxidative stress resistance, and longevity [27, 28]. As the core component of the AP-1 transcription factor complex, JUN is an important factor in cell survival, cell proliferation, and movement [29]. According to previous studies, c-Jun can be a therapeutic target for cancer, vascular remodeling, acute inflammation, and rheumatoid arthritis [30]. There is no clear conclusion regarding the functions of G0S2 in asthma. G0S2 is highly expressed in the liver, heart, and skeletal muscle [31, 32] and is expressed at low levels in some tumors [33]. The roles of these lncRNAs and related mRNAs in asthma require further analysis.

Our study had some limitations. First, the patients were assessed during a stable period of asthma, and some patients took medications, which may have altered their gene expression. Second, the functions of the differentially expressed mRNAs and lncRNAs were based on bioinformatics analysis, and therefore must be verified in animal experiments. Finally, there may be differences in the composition of PBMCs between the healthy control and asthma groups which may lead to the detection of different genes. If single-cell sequencing technology can be used to detect gene expression in different components of cells, the results may be more accurate; this will be the focus of our future study.

In conclusion, using microarray analysis, we identified the core mRNAs and their related lncRNAs, as well as predicted the possible altered biological processes and signaling pathways. This study provides insight into the investigated targets of asthma, which may be useful as lncRNA-mediated therapy for asthma.

List Of Abbreviations

C-X-C motif chemokine ligand 8, CXCL8

Forkhead box class O, FOXO

Forced expiratory volume in first second, FEV1

G0/G1 switch gene 2, G0S2

Gene ontology, GO

Kyoto Encyclopedia of Genes and Genomes, KEGG

Long noncoding RNA, lncRNA

Nucleotide-binding oligomerization domain, NOD

Peripheral blood mononuclear cells, PBMCs

Real-time polymerase quantitative chain reaction, RT-qPCR

Retinoic acid-inducible gene I, RIG-I

Toll-like receptor, TLR

Declarations

Ethics approval and consent to participate

All methods of this study were carried out in accordance with relevant guidelines and regulation. This study was approved by the Ethics Committee of the China-Japan Friendship Hospital (2017–19), and written informed consent of the study including genetic analysis was obtained from each participant.

Consent for publication

Consent for publication was obtained from all participants.

Availability of data and materials

The datasets used or analyzed during the current study were deposited at NCBI, under accession number GSE165934 (available at: <https://www.ncbi.nlm.nih.gov/bioproject/?term=GSE165934>).

Competing Interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Authors' contributions

HC and RD participated in study design, data analysis, and interpretation of the data. TY, CC, and HN collected data of the patients with asthma and healthy controls. HC wrote the manuscript. KHuang and KHao revised the manuscript prior to submission. TY and CW obtained funding.

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Tables

Table 1. Baseline Characteristics of the Study Participants

Characteristics	Asthma (n = 10)	Control (n = 9)
Age (years)	32.6 ± 5.1	35.1 ± 6.6
Gender (male, %)	50	55.5
BMI (kg/m ²)	24.3 ± 1.9	23.6 ± 4.4
SpO ₂ (%)	94.9 ± 5.7	98.6 ± 0.7
Current/ex-smoker	1	0
FEV1	3.1 ± 0.7	3.1 ± 0.6
FEV1% pred	82.3 ± 29.7	97.8 ± 8.6
FEV1/FVC	77.5 ± 5.1	83.1 ± 5.2*
FENO	82.0 ± 60.7	18.8 ± 9.6*
ACT score	20.5 ± 2.7	NA
ACQ-7 score	0.76±0.4	NA
Medication status		
SABA	3	0
LABA	0	0
LAMA	0	0
LABA + ICS	1	0

LAMA + ICS	7	0
Theophylline	1	0

Note: Data are presented as the mean \pm standard deviation unless otherwise stated. *P-value < 0.01. Abbreviations: BMI, body mass index; SpO₂, pulse oximetry saturation; FEV₁, forced expiratory volume in 1 second; FEV₁% predicted, forced expiratory volume in 1 second as the percentage of the predicted value; FVC, forced vital capacity; FENO, fractional exhaled nitric oxide; ACT, asthma control test; ACQ-7, asthma control questionnaire-7; SABA, short-acting beta-2 agonist; LABA, long-acting beta-2 agonist; ICS, inhaled corticosteroid; LAMA, long-acting muscarinic antagonist; NA, not available.

Table 2. Core mRNAs selected using the Gene-Gene Functional Interaction Network

Gene Symbol	Style	Betweenness Centrality	Degree
<i>NFKB1</i>	up	0.052151	35
<i>PRKACB</i>	down	0	32
<i>PIK3CA</i>	down	0.033763	29
<i>PLCB1</i>	down	0.04064	24
<i>PLCG1</i>	up	0.02381	23
<i>GNAI3</i>	down	0.0408	21
<i>JUN</i>	down	0.030527	20
<i>TP53</i>	up	0.040001	19
<i>KRAS</i>	down	0.026864	18
<i>NRAS</i>	down	0.026311	18
<i>JAK2</i>	down	0.011193	18
<i>FOXO3</i>	down	0.024722	17
<i>FOS</i>	down	0.019986	17
<i>ENPP3</i>	down	0.014337	16
<i>STAT1</i>	down	0.014575	12
<i>IFNG</i>	down	0.013518	12
<i>PLA2G4A</i>	down	0.006587	12

<i>PLD1</i>	down	0.005553	12
<i>CREB1</i>	down	0.006042	11
<i>IL6</i>	down	0.00833	11

Betweenness centrality ≥ 0.005 . Betweenness centrality is an indicator of a gene's centrality in a network. It is equal to the number of shortest paths from all vertices to all others that pass through that gene. The degree of a gene was defined as the number of directly linked genes within a network.

Table 3. Correlation Between lncRNAs and Core mRNAs

mRNA	lncRNA	Pearson's correlation	Regulation
<i>CXCL8</i>	NONHSAT115963	0.917**	Positive
<i>FOXO3</i>	AC019050.1	-0.925**	Negative
<i>JUN</i>	MTCYBP3	0.925**	Positive
<i>PIK3CA</i>	KB-67B5.12	0.935**	Positive
<i>G0S2</i>	HNRNPA1P12	-0.901**	Negative

Figures

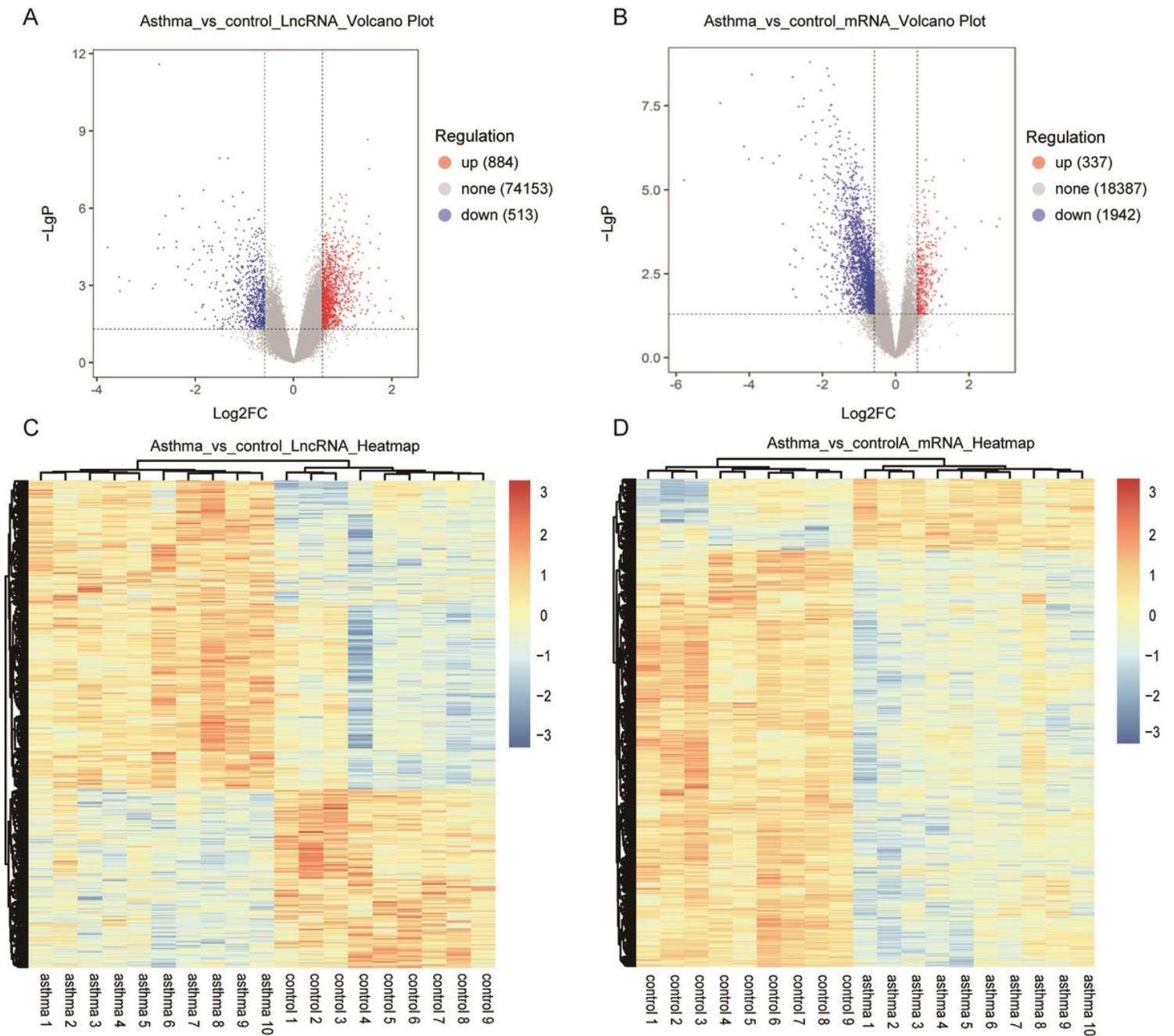


Figure 1

Differential expression of lncRNAs and mRNAs in patients with asthma. Volcano plots are used to distinguish between differentially expressed lncRNAs (A) and mRNAs (B). Red and blue indicate up- and down-regulation, respectively. Hierarchical clustering analysis of dysregulated lncRNAs (C) and mRNAs (D). Relative lncRNA or mRNA expression is depicted according to the color scale. Red indicates elevated expression and blue indicates reduced expression.

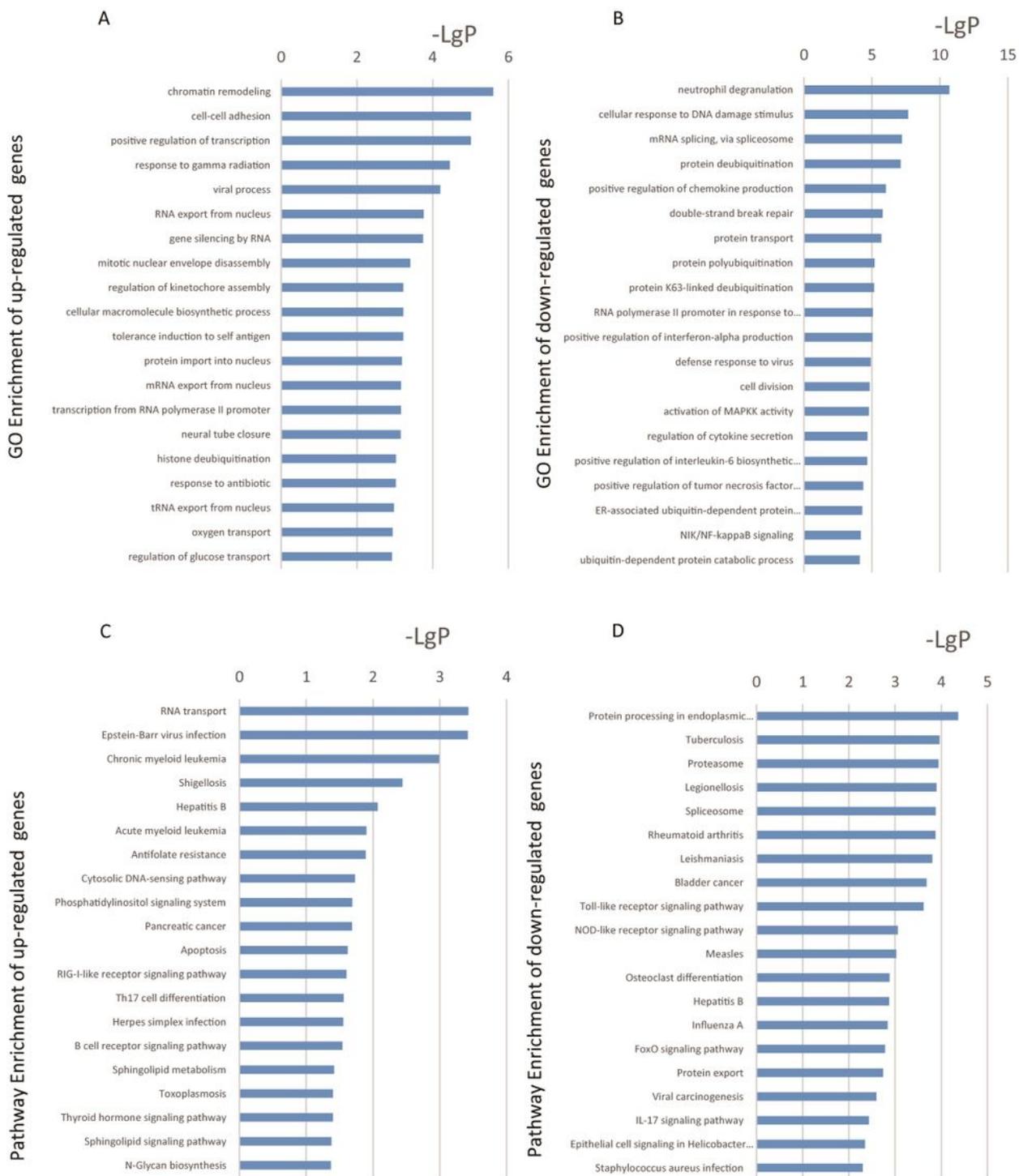


Figure 2

GO and KEGG pathway analyses. GO analysis of significantly upregulated (A) and downregulated (B) mRNAs clustered in the BP. KEGG pathways analysis of differentially upregulated (C) and downregulated mRNAs (D). The abscissa shows $-\text{LogP}$, and the ordinate shows GO terms or KEGG pathways. GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; BP, biological process.

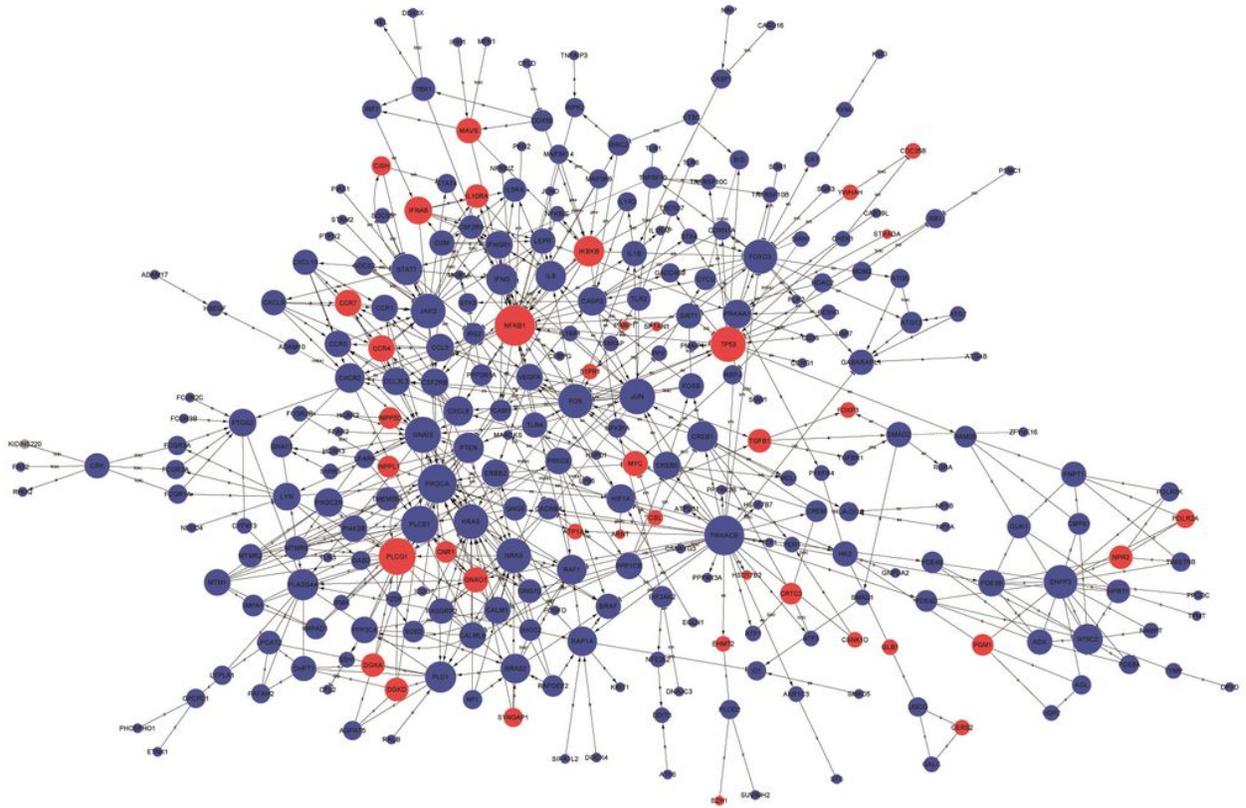


Figure 3

Global signal transduction network analysis of asthma mRNAs. In the constructed network, nodes represent mRNAs, the size of the node's area represents the value of the degree, and red indicates up-regulation and blue indicates down-regulation. The nodes are connected by an edge. The acronyms a, b, c, p, inh, and ind (e) indicate activation, binding, compound, phosphorylation, inhibition, and indirect effect, respectively.

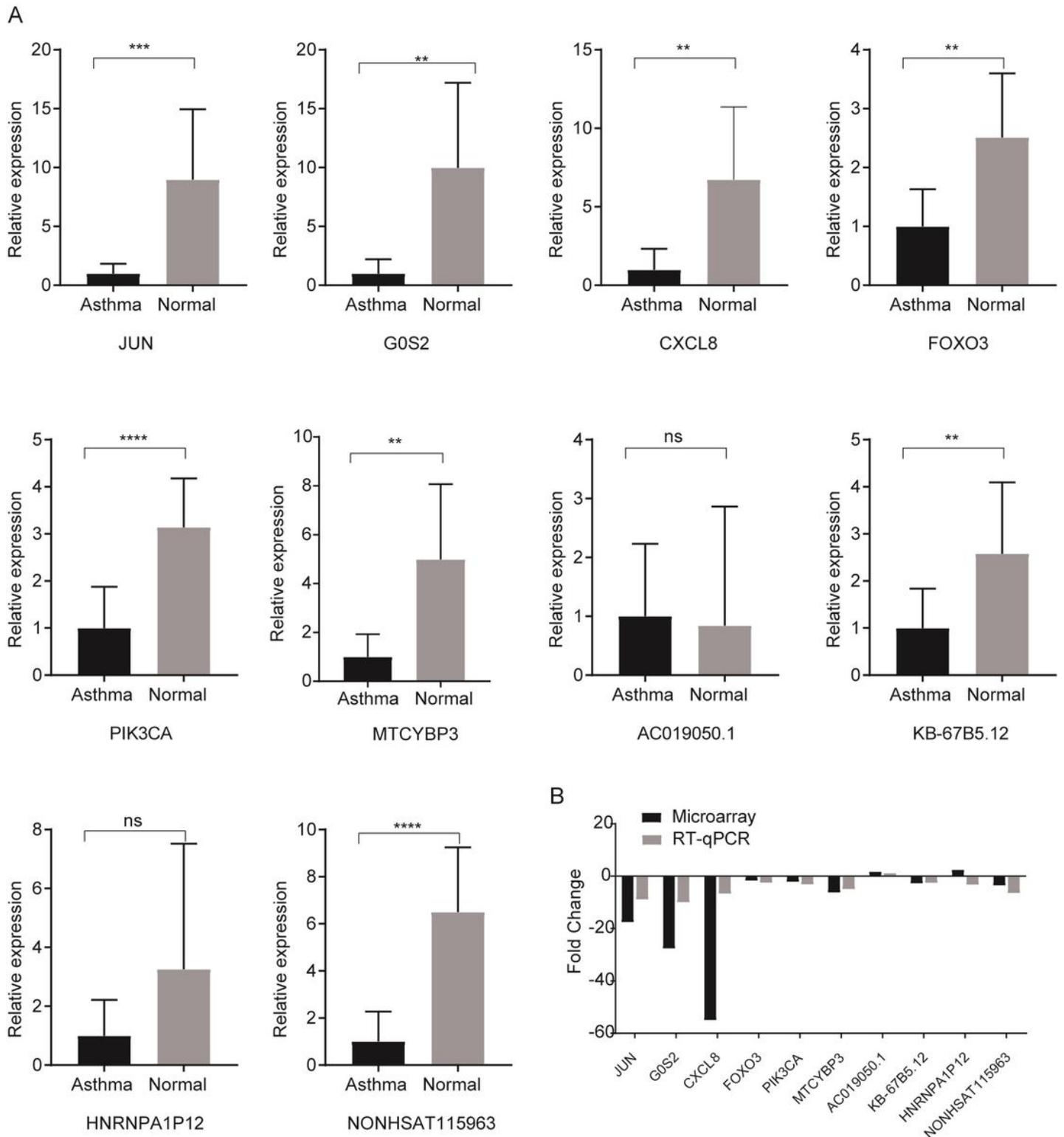


Figure 4

RT-qPCR validation of selected mRNAs and lncRNAs. (A) Relative expression levels of the mRNAs and lncRNAs between the patients with asthma and healthy control assessed by RT-qPCR. The y-axis represents the relative expression ($2^{-\Delta\Delta CT}$). Data are presented as the mean \pm standard error of the mean. All data were normalized to GAPDH expression. (B) Comparison of mean fold-changes between

microarray data and RT-qPCR results. ****P-value < 0.0001; **P-value < 0.001; ns, no significance. RT-qPCR, real-time quantitative polymerase chain reaction.

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