Am J Transl Res 2022;14(10):7350-7361
www.ajtr.org /ISSN:1943-8141/AJTR0146037

*Original Article*

# Identification of potential key autophagy-related genes in asthma with bioinformatics approaches

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Received August 20, 2022; Accepted September 28, 2022; Epub October 15, 2022; Published October 30, 2022

**Abstract:** Objectives: Asthma is a chronic respiratory disease characterized by airway remodeling and inflammation. Recent studies have demonstrated that multiple autophagy-related genes are involved in the pathogenesis of asthma. However, the roles of many of these autophagy-related genes in asthma remain unclear, particularly with regard to the diagnosis of asthma. Methods: In this study, autophagy-related differentially expressed genes (DEGs) in asthma were identified by bioinformatics analysis of the GSE76262 datasets. Hub genes were screened by protein-protein interaction (PPI) network and module analyses. Gene Ontology and Kyoto Encyclopedia of Genes and Genomes pathway enrichment analyses were used to explore potential signaling pathways. Receiver operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic value of autophagy-related biomarkers in asthma. Results: A total of 17 autophagy-related DEGs were identified, most of which were involved in autophagy and protein processing in the endoplasmic reticulum signaling pathway. ROC curve analysis demonstrated that the hub genes (*HIF1A*, *ERN1*, and *DNAJB1*) identified from the PPI network exhibited good performance in the diagnosis of asthma. The GSE137268 and GSE43696 databases were used to verify the expression of 17 autophagy-related DEGs in asthma. Interestingly, *ERN1* was an overlapping gene defined by the intersection of hub autophagy-related DEGs and key modules (including *HIF1A*, *ERN1*, and *DNAJB1*). We also analyzed the interaction between miRNAs and mRNAs for 14 autophagy-related DEGs with an area under the curve > 0.7. The identified genes were involved in the glypican, interferon-gamma, and plasma membrane estrogen receptor signaling pathways. Conclusions: The results of this study indicate that specific signaling pathways and autophagy-related DEGs are potential diagnostic biomarkers related to the inception and progression of asthma.

**Keywords:** Autophagy-related genes, asthma, ERN1, bioinformatics

## Introduction

Asthma, a chronic heterogeneous lung disease characterized by chronic inflammation, airway hyper-reactivity, mucus overproduction, airway remodeling, and airway narrowing, affects more than 300 million individuals worldwide [1]. Clinically, patients with asthma suffer from typical respiratory symptoms, including dyspnea, wheezing, chest tightness, and cough [2]. The disease can be of varying severity, ranging from very mild to severe. Although only a small percentage of patients have a level of disease that is considered severe, asthma is associated with excess morbidity, mortality, and economic costs linked to reduction in productivity [3].

Many genes, including some autophagy-related genes, are involved in and regulate the pathogenesis of asthma [4]. Earlier studies reported that autophagy-related genes are associated with airway remodeling and mechanical impairment of the respiratory system in asthma [5-7].

However, the mechanism by which autophagy ultimately modulates the onset of asthma remains to be elucidated.

Autophagy is an evolutionarily highly conserved catabolic process in eukaryotic cells. Through this process, damaged and misfolded proteins, along with aged or damaged organelles, are delivered to lysosomes and degraded [8]. Accumulating evidence suggests that autophagy plays a critical role in the pathogenesis of a variety of respiratory diseases, including asthma [9]. Autophagy may be a cellular mechanism that promotes transforming growth factor-β1 airway remodeling and loss of lung function in asthma. Notably, genetic variants of the autophagy-related gene 5 (*ATG5*) are significantly associated with lung function and airway remodeling [10]. Another study revealed that interleukin (IL)-4 induces autophagy in B cells, thus exacerbating asthma symptoms [11]. Autophagy is closely correlated with the severity of asthma via eosinophilic inflammation [12]. Additionally, genetic inhibition of autophagy-related genes (*ATG5* and *ATG14*) in airway epithelial cells treated with IL-13 was shown to lead to goblet cell hypertrophy and reduced mucin secretion [13], as well as reduced C-C motif chemokine ligand 26 (CCL26/EOTAXIN-3) secretion, respectively. These findings imply that autophagy promotes an epithelial T helper 2 (Th2) response in asthma [14]. Although studies have suggested a genetic relationship between autophagy and asthma, many autophagy-related genes associated with asthma remain unidentified, especially with regard to severe allergic asthma.

In this study, we explored autophagy-related differentially expressed genes (DEGs) in asthma by analyzing the GSE76262 dataset from the Gene Expression Omnibus (GEO) database. Receiver operating characteristic (ROC) curve monofactor analysis was performed to evaluate the diagnostic value of autophagy-related biomarkers in asthma. The results of this study may enhance our understanding of the role of autophagy in asthma, and provide a new approach for the treatment of asthmatic airway inflammation through targeted autophagy.

## Materials and methods

### *Acquisition of microarray datasets*

Expression profiles of the GSE76262 array (118 asthma cases and 21 healthy controls) [15] were retrieved from the GEO database (http://www.ncbi.nlm.nih.gov/geo/). DEGs were analyzed using the following cut-off criteria: adjusted *p* value < 0.05 and fold-change of 1.5 as the threshold. A total of 222 genes were obtained from The Human Autophagy Database (http://www.autophagy.lu/index.html).

### *Analysis of autophagy-related DEGs*

The raw data were preprocessed using R project (R version 4.0.5), and the preprocessing included background correction, normalization, and calculation of gene expression level. We subsequently identified autophagy-related DEGs using the "limma" package [16, 17] of the R software (adjusted *p* value < 0.05, fold-change > 1.5). Volcano and box plots, as well as a heatmap, were constructed using "ggplot2" and "heatmap" packages of the R software, respectively.

### *Protein-protein interaction (PPI) network analysis and module selection*

PPI network analysis of autophagy-related DEGs was conducted using the STRING online database (http://string-db.org) [18]. The string interaction file was downloaded, and the constructed PPI networks were visualized using the Cytoscape software (version 3.8.2). The "Corrplot" package of the R software was used to determine the correlations between autophagy-related DEGs.

### *Gene ontology and Kyoto encyclopedia of genes and genomes pathway enrichment analyses of autophagy-related DEGs*

Gene ontology (GO) is a tool for gene annotation that uses a defined, structured, and controlled vocabulary [19]. GO analyses encompass biological process (BP), cellular component (CC), and molecular function (MF) pathways. GO and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were conducted in R software using the package "GO plot".

### *Validation of autophagy-related DEGs*

The expression profiles of autophagy-related DEGs were validated using the GSE137268 [20] and GSE43696 [21] datasets. The survival ROC package of the R software was used to plot ROC curves and determine the area under

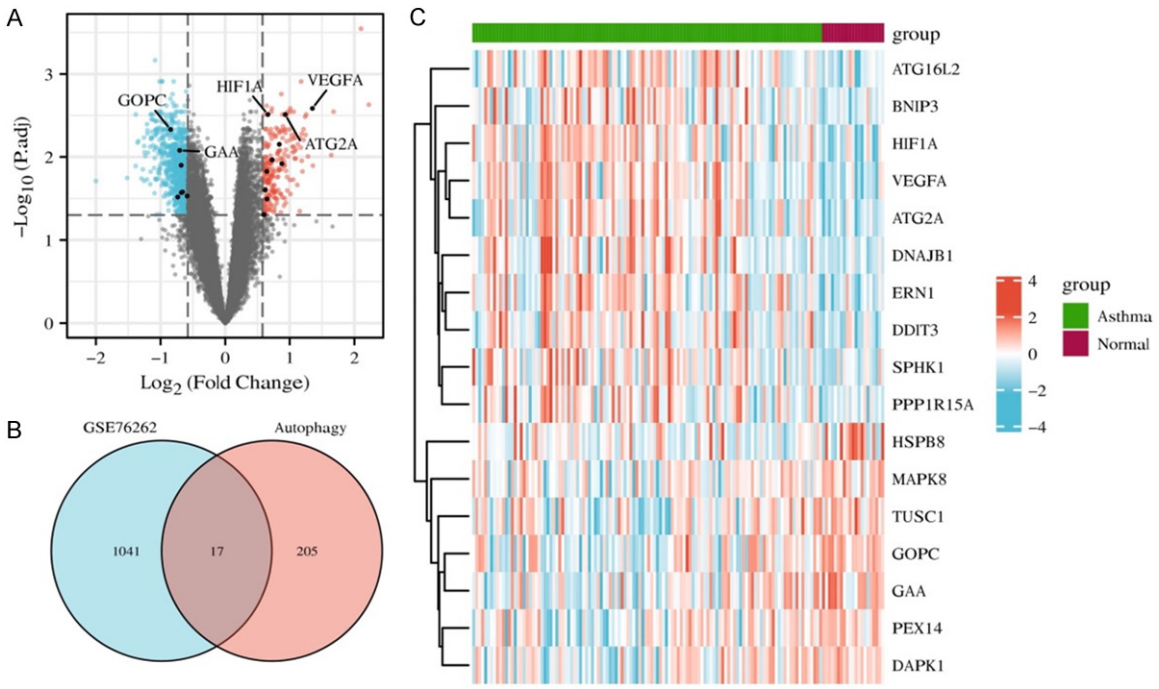



**Figure 1.** Autophagy-related DEGs in asthma patients and healthy subjects. A. Volcano plot of DEGs based on the criteria of fold-change > 1.5 and adjusted *p* value < 0.05. Blue indicates downregulated genes, and red indicates upregulated genes. B. Intersection of DEGs and genes from The Human Autophagy Database. C. The 17 autophagy-related DEGs shown in a heatmap, with red representing increased gene expression and blue representing lower gene expression.

the curve (AUC). ROC curve analyses were conducted for the autophagy-related DEGs, and AUC values were used to evaluate the diagnostic accuracy of the autophagy-related DEGs for asthma.

*Gene-miRNA interaction networks*

miRWalk 2.0 was used to predict key target miRNAs and construct a gene-miRNA interaction network related to autophagy [22]. The prediction results from the MiRTarBase and mirWalk databases were cross-examined to ensure the accuracy of the results.

*Statistical analysis*

Statistical analyses were performed using the R software (version 4.0.5). Correlations were analyzed using Spearman's rank-order correlation. A *p* value < 0.05 denoted statistically significant differences.

## Results

*Identification of autophagy-related DEGs in asthma*

Of the 1,058 DEGs identified, 230 and 828 were upregulated and downregulated, respectively. **Figure 1A** shows volcano plots of the identified DEGs. DEGs that overlapped with the human autophagy database were filtered using the Venn package of the R software. A total of 17 overlapping genes were identified: *VEGFA*, *ATG2A*, *DNAJB1*, *ERN1*, *DDIT3*, *HIF1A*, *BNIP3*, *ATG16L2*, *SPHK1*, *PPP1R15A*, *DAPK1*, *MAPK8*, *TUSC1*, *HSPB8*, *GAA*, *PEX14*, and *GOPC* (**Table 1**). A Venn diagram and heatmap of the autophagy-related DEGs are shown in **Figure 1B** and **1C**. In addition, boxplots illustrate the difference in expression patterns of the 17 autophagy-related DEGs between patients with asthma and healthy subjects (**Figure 2A** and **2B**). A total of 7 downregulated and 10 upregulated autophagy-related DEGs were identified (**Table 1**).

*PPI network and correlation analysis of autophagy-related DEGs*

A PPI network analysis was conducted to determine the interactions between autophagy-related DEGs. The interacting autophagy-related DEGs are shown in **Figure 3A**, and the number of interacting partners of each DEG is shown in **Figure 3B**. The Cytoscape application MCODE was used for cluster analysis of the autophagy-related DEG networks to draw key

**Table 1.** The 17 autophagy-related DEGs in asthma samples compared to healthy samples

| Gene Symbol | logFC | Changes | *P*.Value | *P*.Value |
|---|---|---|---|---|
| VEGFA | 1.350345498 | Up | $3.15\times10^{-7}$ | $2.60\times10^{-3}$ |
| ATG2A | 0.930055691 | Up | $9.00\times10^{-6}$ | $3.08\times10^{-3}$ |
| DNAJB1 | 0.878172868 | Up | $4.65\times10^{-4}$ | $1.21\times10^{-2}$ |
| ERN1 | 0.837842783 | Up | $1.04\times10^{-4}$ | $7.04\times10^{-3}$ |
| DDIT3 | 0.726061873 | Up | $3.34\times10^{-4}$ | $1.08\times10^{-2}$ |
| HIF1A | 0.661928325 | Up | $7.34\times10^{-6}$ | $3.08\times10^{-3}$ |
| BNIP3 | 0.649008724 | Up | $3.83\times10^{-3}$ | $3.21\times10^{-2}$ |
| ATG16L2 | 0.645989339 | Up | $7.80\times10^{-4}$ | $1.50\times10^{-2}$ |
| SPHK1 | 0.621758378 | Up | $2.29\times10^{-3}$ | $2.50\times10^{-2}$ |
| PPP1R15A | 0.600647623 | Up | $8.49\times10^{-3}$ | $4.95\times10^{-2}$ |
| DAPK1 | -0.589957265 | Down | $3.25\times10^{-3}$ | $2.95\times10^{-2}$ |
| MAPK8 | -0.66039329 | Down | $2.56\times10^{-3}$ | $2.64\times10^{-2}$ |
| TUSC1 | -0.678870759 | Down | $2.66\times10^{-3}$ | $2.69\times10^{-2}$ |
| HSPB8 | -0.681707336 | Down | $5.17\times10^{-4}$ | $1.26\times10^{-2}$ |
| GAA | -0.705543704 | Down | $1.67\times10^{-4}$ | $8.34\times10^{-3}$ |
| PEX14 | -0.734914804 | Down | $3.44\times10^{-3}$ | $3.04\times10^{-2}$ |
| GOPC | -0.844868252 | Down | $2.95\times10^{-5}$ | $4.67\times10^{-3}$ |

modules. Three genes (i.e., *HIF1A*, *ERN1*, and *DNAJB1*) were identified in the key module drawing (**Figure 3C**). Additionally, we investigated the correlations in the expression of the autophagy-related DEGs. **Figure 4A** shows the relationships between the 17 autophagy-related DEGs in the GSE76262 dataset. Correlation analyses were also performed for the key genes (**Figure 4B**).

*Pathway enrichment analysis of autophagy-related DEGs*

The potential biological functions of the identified autophagy-related DEGs were examined using GO and KEGG enrichment analyses with the R software. The results of the GO enrichment analysis showed that the BP component of the autophagy-related DEGs was mainly enriched in terms of processes involving autophagic mechanisms, autophagy, regulation of autophagy, and macroautophagy. The CC of the autophagy-related DEGs was mainly enriched in terms pertaining to neuron to neuron synapse, postsynaptic specialization, asymmetric synapse, and postsynaptic density. The MF component of the autophagy-related DEGs was mainly enriched in terms related to heat shock protein (Hsp) binding, Hsp70 protein binding, Hsp90 protein binding, and platelet-derived growth factor receptor binding (**Figure 5A** and **5B**; Supplementary Table 1). In addition, according to the KEGG pathway analysis, the autophagy-related DEGs were strongly associated with autophagy and protein processing in the endoplasmic reticulum (ER) signaling pathway (**Figure 5C** and **5D**; Supplementary Table 2).

*Diagnostic value of autophagy-related DEGs in asthma*

Fourteen of the 17 autophagy-related DEGs identified in the GSE76262 dataset exhibited an AUC value > 0.7 (**Figure 6A** and **6B**). The collective AUC of these 14 autophagy-related DEGs was 0.873 ($P < 0.05$) (**Figure 6C**). The AUC of the ROC curve for the diagnostic accuracy of the key genes (i.e., *HIF1A*, *ERN1*, and *DNAJB1*) for asthma was 0.805 (**Figure 6D**).

*Validation of the expression profiles of 17 autophagy-related DEGs using the GSE137268 and GSE43696 datasets*

We verified the expression profiles of the 17 autophagy-related DEGs in asthma using the GSE137268 and GSE4369 external datasets. As shown in **Figure 7**, the expression of *ERN1*, *PEX14*, and *SPHK1* was also upregulated in patients with asthma versus healthy controls. By examination of the intersection of hub autophagy-related DEGs and key modules (including *HIF1A*, *ERN1*, and *DNAJB1*), *ERN1* was identified as an overlapping gene.

*Further miRNA interactions and data mining*

The mRNAs of 14 autophagy-related DEGs with an AUC > 0.7 were evaluated using the miRWalk 2.0 software. A total of 38 miRNA expression genes were uploaded to Funrich. The results of the enrichment analysis indicated that the BP was significantly enriched in protein serine/threonine kinase activity, transcription factor activity, and transcription regulator activity. The biological pathways that were enriched included the glypican, interferon-gamma, and plasma membrane estrogen receptor signaling pathways (**Figure 8**).

## Discussion

Asthma is a chronic airway disease with increasing global prevalence that exhibits multiple

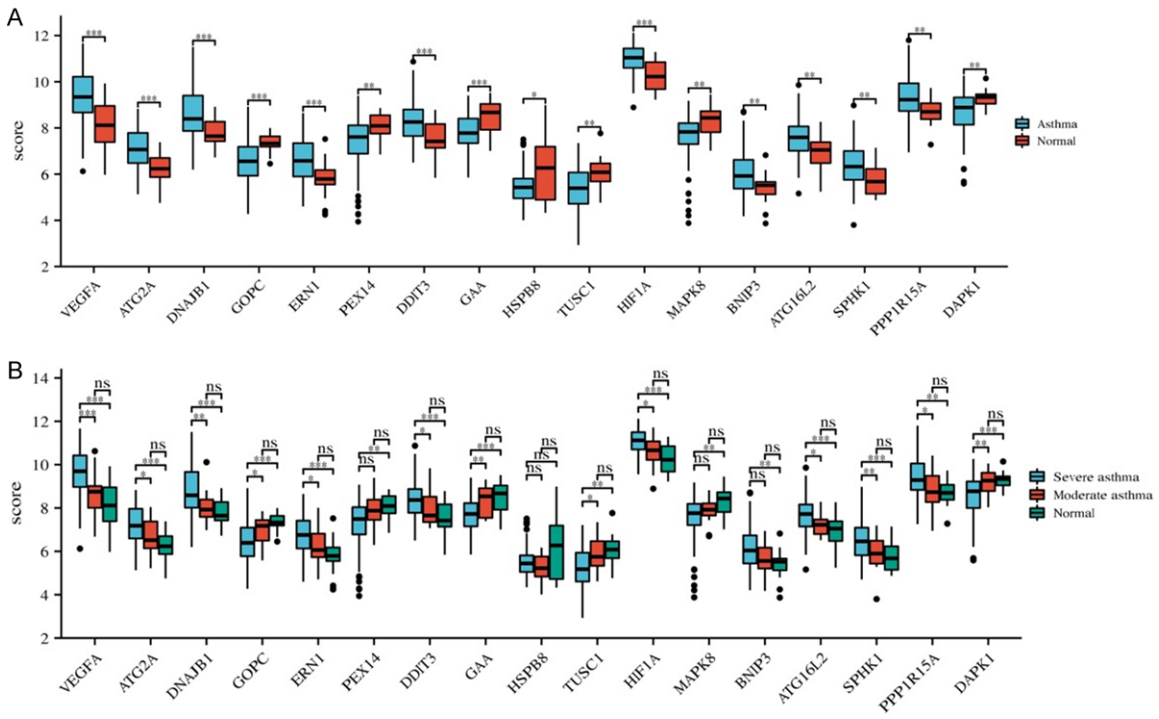



**Figure 2.** Boxplot of 17 autophagy-related DEGs in asthma patients and healthy subjects. A. Boxplot of 17 autophagy-related DEGs in asthma patients and healthy subjects. B. Boxplot 17 autophagy-related DEGs in patients with severe and moderate asthma and healthy subjects.

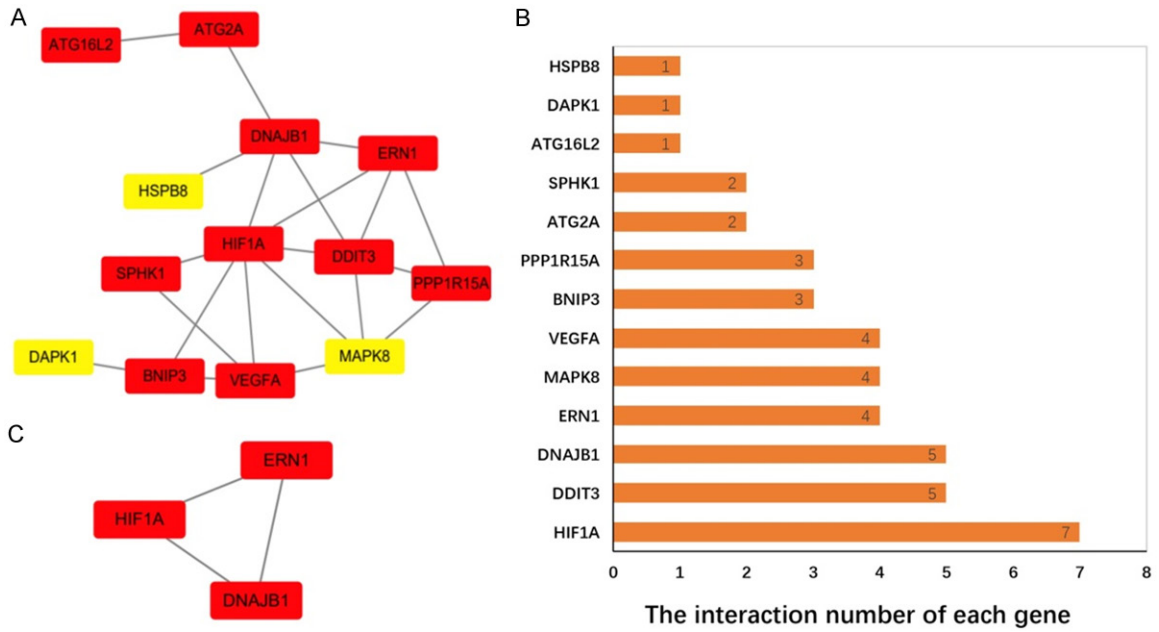



**Figure 3.** Protein-protein interaction (PPI) network and modular analysis of autophagy-related DEGs. A. PPI network among autophagy-related DEGs. B. One module was obtained from the PPI network. C. Number of interactions for each autophagy-related DEG.

phenotypes based on interactions between genetic and environmental factors [23]. Recent studies have indicated that autophagy and genetic predisposition play roles in the develop-

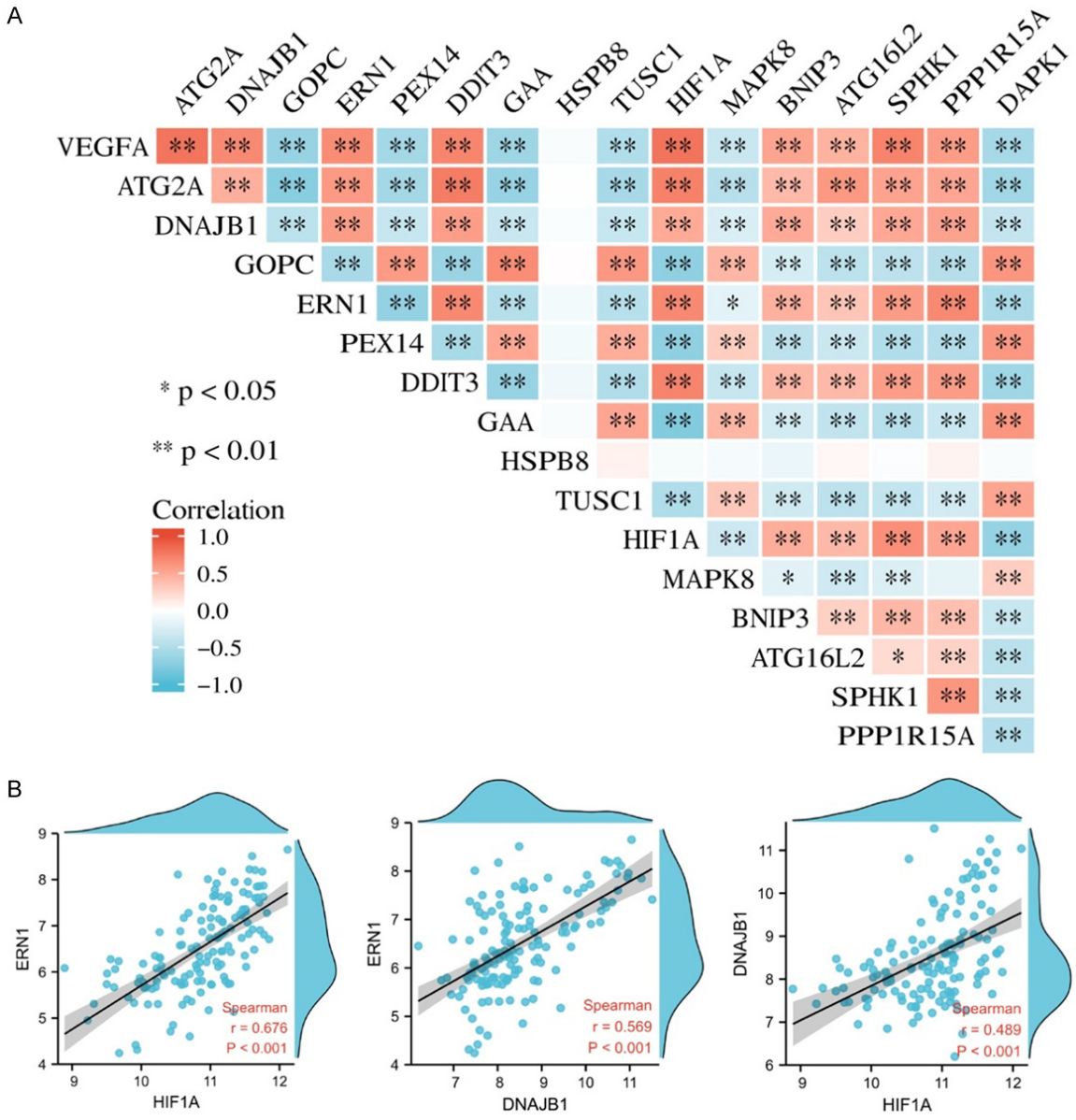
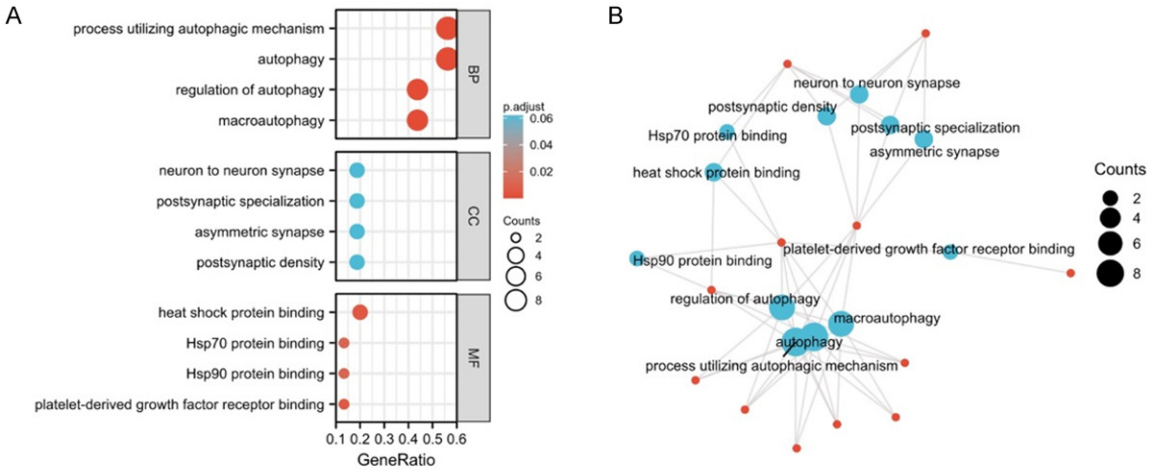



**Figure 4.** Spearman correlation analysis. A. Correlation analysis of the 17 autophagy-related DEGs. B. Correlation analysis of the key module.

ment of asthma. Autophagy is an evolutionarily conserved, lysosome-mediated degradation process. Multiple autophagy-related genes, such as microtubule-associated protein 1 light chain 3 (*LC3*), sequestosome 1 and *ATG5* [24], have been implicated in asthma. In addition, it has been shown that polymorphisms in these genes are associated with asthma [25, 26]. Evidence has indicated that autophagy-related genes are involved in the progression and prognosis of asthma and regulate the immune microenvironment [27]. Nevertheless, few studies have investigated the diagnostic value of autophagy-related biomarkers in asthma. Thus far, the role of autophagy in asthma has not been fully addressed, particularly with regard to severe allergic asthma. Therefore, further investigations are warranted to identify autophagy-related genes involved in asthma.

In this study, we identified 17 autophagy-related DEGs (10 upregulated genes and 7 downregulated genes) among DEGs in the GSE76262 (118 patients with asthma and 21 healthy con-




| ID | Description |
|---|---|
| hsa04140 | Autophagy - animal |
| hsa04141 | Protein processing in endoplasmic reticulum |
| hsa04137 | Mitophagy - animal |
| hsa04210 | Apoptosis |
| hsa05017 | Spinocerebellar ataxia |
| hsa04932 | Non-alcoholic fatty liver disease |
| hsa05219 | Bladder cancer |
| hsa05010 | Alzheimer disease |
| hsa05167 | Kaposi sarcoma-associated herpesvirus infection |
| hsa04370 | VEGF signaling pathway |

**Figure 5.** Gene Ontology (GO) (A, B) and Kyoto Encyclopedia of Genes and Genomes (KEGG) (C, D) analyses of 17 autophagy-related DEGs. Abbreviations: BP, Biological Process; CC, Cellular Component; MF, Molecular Function.

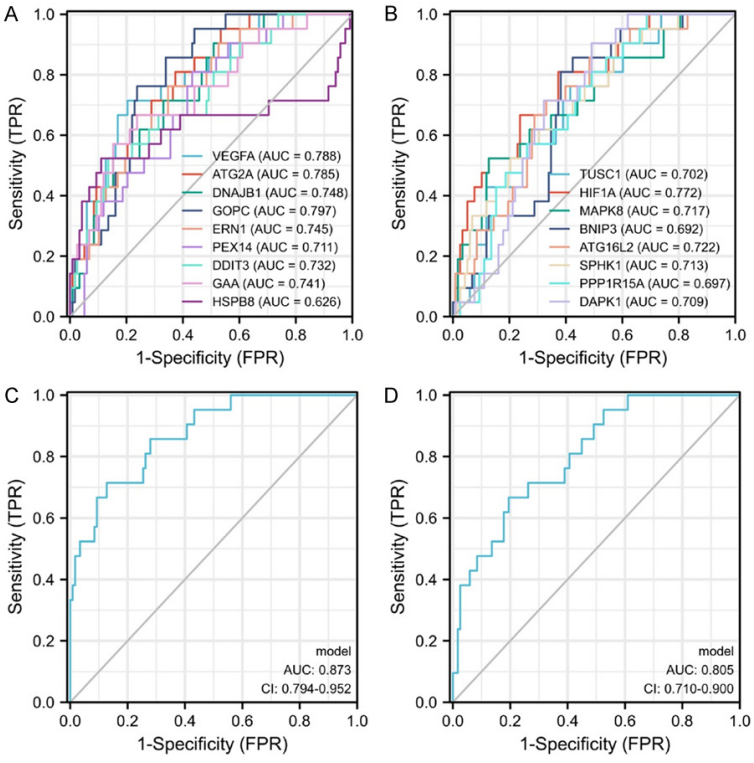



**Figure 6.** ROC curve analysis of 17 autophagy-related DEGs in the GSE76262 dataset. A, B. Autophagy-related DEGs associated with asthma that exhibited an area under the curve (AUC) > 0.7 in the ROC curve analysis. C. AUC values of the 14 autophagy-related DEGs in asthma. D. AUC value of the key genes (*HIF1A*, *ERN1*, and *DNAJB1*) in asthma.

trols) and human autophagy database. We conducted a comprehensive analysis of autophagy-related DEGs to clarify the relationship between autophagy and asthma, and explore the diagnostic value of autophagy-related DEGs in this setting. GO and KEGG pathway analyses of the autophagy-related DEGs revealed several enriched terms strongly related to autophagy and protein

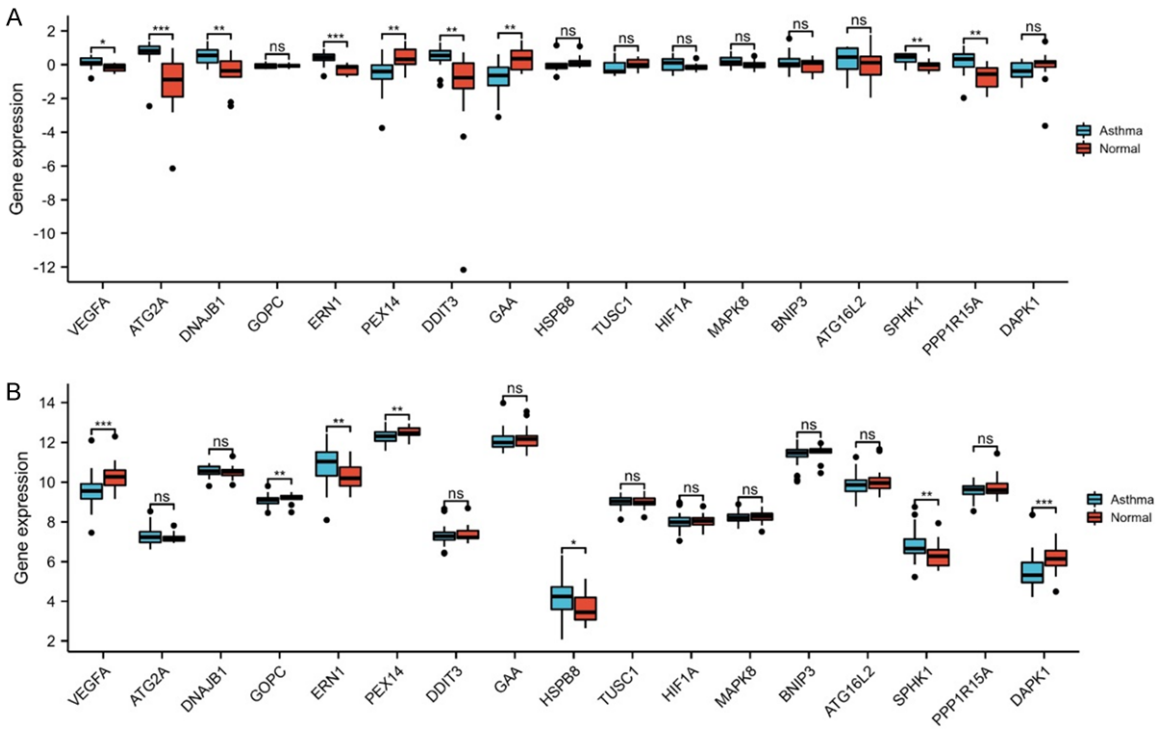



**Figure 7.** Expression profiles of 17 autophagy-related DEGs validated using the GSE137268 and GSE43696 datasets. A. Verification of expression profiles of 17 autophagy-related DEGs in the GSE137268 dataset (15 healthy controls vs. 12 severe-asthma patients). B. Verification of expression profiles of 17 autophagy-related DEGs in the GSE43696 dataset (20 healthy controls vs. 37 severe-asthma patients).

processing in the ER signaling pathway. Several studies have also revealed that ER stress and activation of the unfolded protein response (UPR) induce autophagy and, therefore, play a critical role in the pathogenesis of severe or steroid-resistant bronchial asthma [28, 29]. For example, a trial investigating the pathophysiology of pediatric asthma revealed that mitophagy and ER stress associated with high nuclear factor-κβ and cytokines activity play an important role [30]. Despite its clear importance, the detailed pathogenesis of asthma has not been fully elucidated.

Three hub genes (i.e., *HIF1A*, *ERN1*, and *DNAJB1*) were identified according to the PPI network constructed using Cytoscape. ROC curve analysis was performed to evaluate the sensitivity and specificity of the 17 autophagy-related DEGs with regard to asthma. We found that the AUC of 14 autophagy-related DEGs was 0.873 ($P < 0.05$) (**Figure 6C**), and the diagnostic accuracy of the ROC curves of key genes (i.e., *HIF1A*, *ERN1*, and *DNAJB1*) for asthma was 0.805 (**Figure 6D**). The results demonstrated good diagnostic capacity. Subsequently, we verified the 17 autophagy-related DEGs in asthma using the GSE137268 and GSE4369 external datasets. As shown in **Figure 7**, *ERN1*, *PEX14*, and *SPHK1* were also upregulated in patients with asthma versus healthy controls. Interestingly, *ERN1* was overlapped with the key module (including *HIF1A*, *ERN1*, and *DNAJB1*). *ERN1*/inositol requiring enzyme 1 (*IRE1*) is a primary inducer of the UPR. Accumulating evidence suggests that the UPR and ER-associated degradation pathways [31] promote autophagy and apoptosis upon exposure to different types of ER stress [32]. ER stress stimulates ERN1 oligomerization in the ER membrane, and stimulation of *ERN1* induces dissociation of the BECN1-BCL2 complex and autophagy via augmentation of mitogen-activated protein kinase 8 (MAPK8/JNK) activity. These effects result in cellular apoptosis with persistent activation [32, 33]. Research has shown that the ERN1-MAPK8/9/10 axis played a critical role in autophagy induction and autophagy-mediated cell death following exposure of MARC-145 monkey kidney cells to

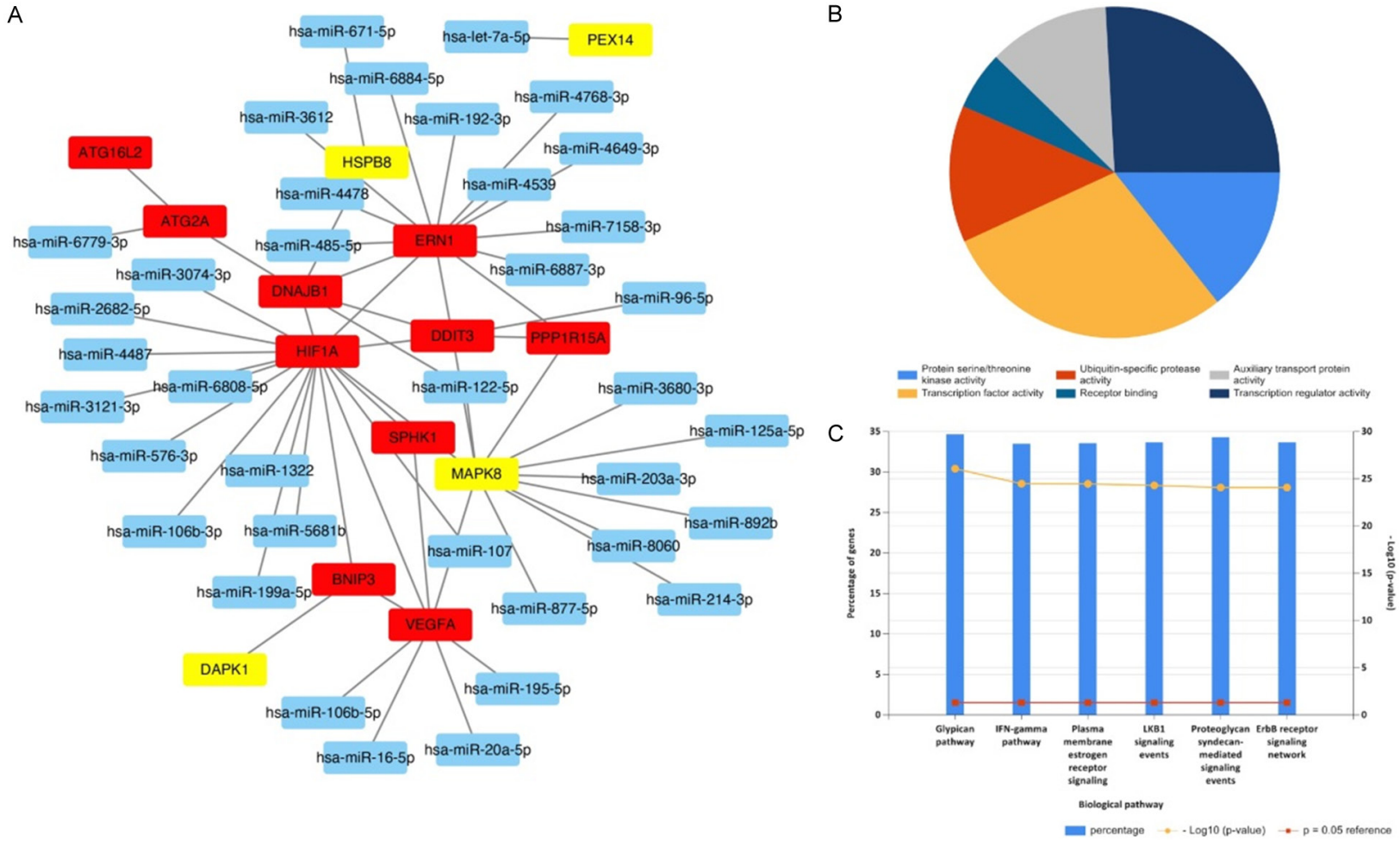



**Figure 8.** Interaction network between 14 autophagy-related DEGs and targeted miRNAs. (A) Gene-miRNA interaction network. Upregulated genes are colored in red, downregulated genes are colored in yellow, and miRNAs are colored in blue. Interaction network of 14 autophagy-related DEGs with an AUC > 0.7 and enriched items in GO (B) and KEGG (C) analyses.

fumonisin B1 [34]. However, the role of ERN1 as a molecular target in asthma has not been examined and warrants further investigation.

Studies have proposed pro-apoptotic IRE1α-miRNAs (e.g., miR-17, miR-34a, and miR-125b) as potential targets in the treatment of certain neurodegenerative diseases [35]. Krammes *et al*. suggested that miR-34a-5p directly targets the IRE1α branch of the UPR in neuronal cells to identify efficient ways to modulate cellular UPR stress response to reestablish cell homoeostasis and combat neurodegeneration [36]. Based on this, we performed miRNA and mRNA interaction analyses for the 14 autophagy-related DEGs with an AUC > 0.7. A total of 38 miRNAs were involved in the glypican, interferon-gamma, and plasma membrane estrogen receptor signaling pathways. Our results indicated that the ERN1-miRNA chain may play a role in patients with asthma. However, further studies should be carried out to determine the specific molecular mechanism involved in this process.

This study had some limitations. Firstly, results were obtained through bioinformatics analysis of data from a single dataset (GSE76262). In addition, there were an insufficient number of clinical tissue samples for validation due to limitations in the experimental conditions, particularly with regard to induced sputum cell counts. Furthermore, we only verified the expression levels of autophagy-related DEGs using data from other databases; hence, we did not examine potential hub genes in cellular and mouse models of asthma. Finally, further verification through functional experiments is needed to elucidate the exact molecular mechanism underlying the role of *ERN1* in asthma.

## Conclusion

Through the present bioinformatics analysis, we identified 17 potential autophagy-related DEGs associated with asthma. The findings indicated that the key gene *ERN1* may affect the development of asthma by regulating autophagy, suggesting that this gene may have noninvasive diagnostic value. These results expand our understanding of asthma and suggest that targeted inhibition of *ERN1* might be useful in the treatment of asthma.

## Acknowledgements

We thank all the families for participating in this research project.

This study was supported by the National Natural Science Foundation of China (Grant No. 81901632, 82001576, 81971509, and 81971513); Suzhou Science and Technology Support Program (SS2019066, SYS2019098); the Primary Research & Development Plan of Jiangsu Province (BE2022736); Fujian Natural Science Foundation Project (2020J011262) and Putian Science and Technology Foundation Project (2020S3F002).

## Disclosure of conflict of interest

None.

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