Original Article

ISSN: 1735-0344 *Tanaffos 2025; 24(3): 223-228*

TANAFFOS

Network Analysis of Severe Asthma in Female Patients to Find Possible Drug Targets

Hamideh Moravvej [1], Zahra Razzaghi [2], Mitra Rezaei [3], Babak Arjmand [4,5], Nastaran Asri [6], Mostafa Rezaei Tavirani [7]

[1] Skin Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran, [2] Laser Application in Medical Sciences Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran, [3] Clinical Tuberculosis and Epidemiology Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran, [4] Cell Therapy and Regenerative Medicine Research Center, Endocrinology and Metabolism Molecular-Cellular Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran, [5] Iranian Cancer Control Center (MACSA), Tehran, Iran, [6] Celiac Disease and Gluten Related Disorders Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran, [7] Proteomics Research Center, System Biology Institute, Faculty of Paramedical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Received: 4 December 2024
Accepted: 23 May 2025

Correspondence to: Rezaei Tavirani M
Address: Proteomics Research Center, System Biology Institute, Faculty of Paramedical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email address: taviranim@gmail.com

Background: Severe asthma (SA) has been a challenging health issue worldwide. In this view, a complementary study of gene expression profiles could be helpful. Protein-protein interaction network analysis could help better understand the molecular changes and, ultimately, the underlying mechanisms of treatment approaches. The identification of the critical genes involved in severe asthma is the main aim of this study.

Materials and Methods: Data were retrieved from the Gene Expression Omnibus (GEO) database and pre-evaluated via GEO2R to find the significantly differentially expressed genes (DEGs). Cytoscape software and its plugin were used to facilitate protein-protein interaction (PPI) network analysis and gene ontology enrichment.

Results: A total of 30 significant DEGs were identified as the genes that differentiate severe asthma from control samples. PPI network analysis led to the identification of four hub-bottlenecks, including TP53, AKT1, ACTB, and EGFR, and two hubs (GAPDH and PTEN). DLG4 was highlighted as a bottleneck node. "Nitric-oxide synthase", "lymphocyte apoptotic process", "regulation of cyclin-dependent protein serine/threonine kinase activity", and "positive regulation of miRNA maturation" biological processes were linked to the central genes.

Conclusion: AKT1 and EGFR, and the related biological processes, can be considered as the possible drug targets for severe asthma.

Keywords: Asthma, Severe Asthmatic Patients, Epithelial airway, Gene Expression, Gene ontology

INTRODUCTION

The quality of life significantly decreases in patients with severe asthma (SA); in addition, many complications, as serious as death, can accompany this health condition (1). In addition, it is the most common respiratory problem that the WHO has announced as an important worldwide problem (2). Clinical and physiological approaches alone do not suffice for achieving accurate diagnosis and treatment objectives (3). Therefore, immediate attention in the health care system is required to address this issue. One approach is to study the molecular mechanisms of this type of disease. This disease has a complex biology and is

known with heterogenous and made it challenging to diagnose and treat, as it shows different phenotypes (4). Moreover, molecular examination in this regard is not adequate, and not many biomarkers are available for asthma after years of research. One of the identified biomarkers in asthma is type 2 inflammatory response, including immunoglobulin E (IgE), which is a useful indicator for patient diagnosis and outcome; however, not specific to any phenotype or endotype of Asthma (2), and also interleukins (IL)-4, IL-5, and IL-13 as well (5). The other introduced biomarkers are eosinophils, nitric oxide, periostin, and allergy (6).

Molecular studies like proteomics are still in their early stages, and thorough analysis is required for further progress (7). Gene expression profiling is one of the ways that could facilitate the process of identifying asthma biomarkers identifications (8). PPI network analysis is a well-known method to study interactions between a set of genes or proteins. This tool is applied to explore the molecular mechanism of many diseases (9, 10). Two well-known topological parameters of a PPI network are degree (K) and betweenness centrality (BC). The genes that are high in values for these parameters are called hubs and bottlenecks, respectively (11). These two features are offered by Cytoscape's applied software, which examines them through topological analysis. These fundamentals are crucial for maintaining the integrity and robustness of a scale-free network. In a protein-protein interaction (PPI) network, which is a scale-free network, the removal or dysregulation of these nodes could be harmful or even lethal to organisms (12, 13). From this point of view, studying these elements could assist in better understanding the related mechanism of Asthma. DEG of central and peripheral epithelial cells of airways between healthy and severe asthma samples were used in our study for protein-protein interaction network analysis.

MATERIALS AND METHODS

Data collection

In the original study, severe asthma gene expression profiling in comparison with healthy cases was handled considering the criteria of collecting epithelial cells from central and peripheral parts of the airways. This analysis (GSE64913 using platform GPL570) was published in 2017 and is available in the GEO database (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE64913). For our study, gene expression profiles of epithelial cells of central and peripheral airways from female cases were compared in severe and healthy conditions. These include 12 severe cases and 17 healthy cases.

Pre-evaluation analysis

Data visualization and comparability of data were carried out with regard to Box plot, expression density plot, and mean-difference plot analysis by using the GEO2R program. The significant DEGs were introduced based on an adjusted p-value<0.05.

PPI network analysis

After defining differentially expressed genes, which are 30 genes, they were introduced to the Cytoscape software to construct a PPI network and identify central genes. The central nodes in the network are defined as hubs and bottlenecks. Hub-bottlenecks are the most significant nodes in the physical interaction network. STRING database (https://string-db.org/) (14), was the source for interaction network construction in the Cytoscape platform. For further analysis, data were evaluated via gene ontology enrichment by using the ClueGO+ CluePedia plugin of Cytoscape software (15). The related biological processes were identified for the queried DEGs.

Statistical analysis

The PPI network was formed using a confidence score cutoff = 0.5 (16). The kappa statistic was a statistical method for gene ontology assessment. The kappa score cut off was set as default= 0.4; however, other statistical criteria, including the number of genes per group and their percentage of contribution, were modified to 2 and 3, respectively. In addition, we used the Bonferroni correction method to control false discovery rates.

RESULTS

Pre-evaluation analysis results

Plots derived by R programming demonstrating the comparability of two groups of samples (severe asthma and healthy), as it is shown in Figures 1, 2, and 3.

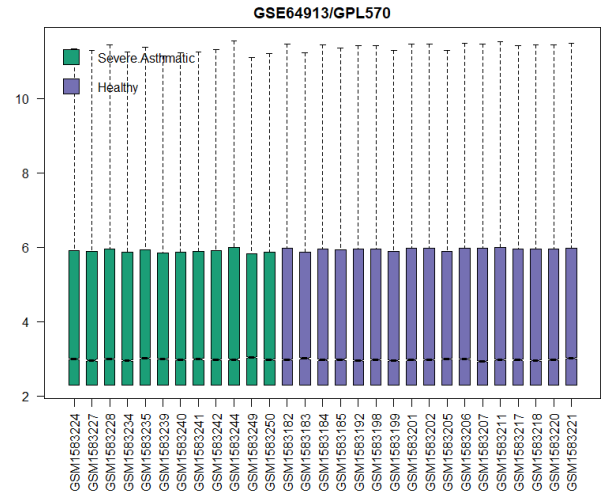

Figure 1. Boxplot view of two groups of healthy and severe asthmatic females. Severe asthmatic patients are indicated by green, while healthy samples are in purple

As depicted in Figure 1, the samples are median-centered; as a result, they are comparable for more assessment based on box plotting. Expression density plot (see Figure 2) refers to a similar pattern of relationship between intensity and density for the two compared groups of samples.

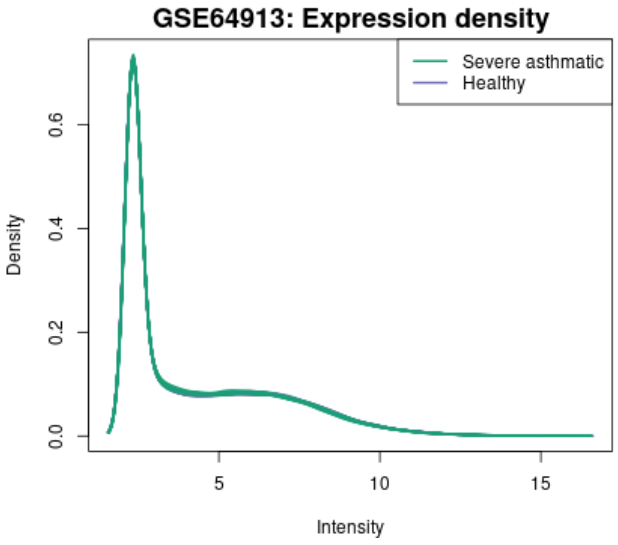

Figure 2. Expression density plot for severe asthmatic and healthy groups

The results of the mean-difference plot are shown in Figure 3. As shown in Figure 3, there are several significantly up and down-regulated DEGs.

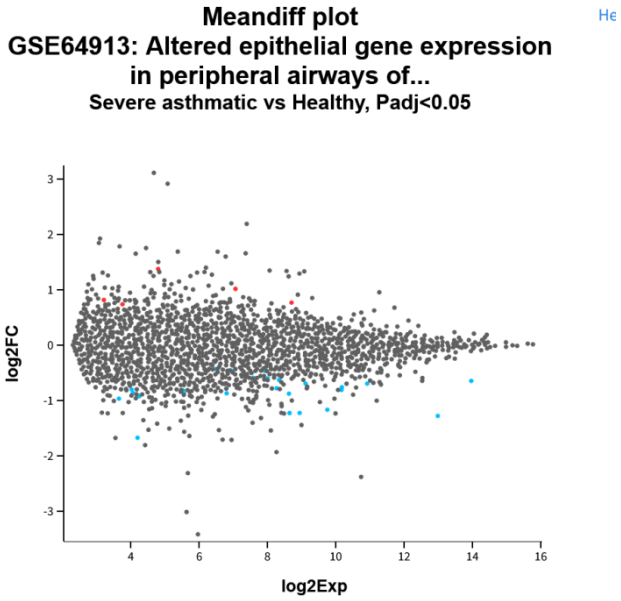

Figure 3. Mean-difference plot of severe asthmatic-healthy groups analysis

PPI network analysis results

After cleaning, 23 significant DEGs remained for PPI analysis. A total of 22 DEGs were recognized by the STRING database. In the protein-protein interaction network analysis, by adding 50 First neighbors, 72 genes and 936 links were obtained (data not shown).

Centrality analysis was handled by analyzing network features in the Cytoscape plug-in that provides essential different gene properties on an interaction scale. The identified hubs, bottleneck nodes, and hub-bottlenecks are presented in Table 1.

Table 1. The list of hubs and bottlenecks considering 10% nodes with the highest degree value. Hub-bottlenecks are assigned with an asterisk

Row	Gene name	Degree	Betweenness centrality
1	TP53*	50	0.04
2	AKT1*	49	0.03
3	ACTB*	48	0.04
4	GAPDH	47	0.02
5	PTEN	47	0.01
6	EGFR*	46	0.03

TP53 is the highest valued hub-bottleneck in Table 1. Four hub-bottlenecks and two hubs, including GAPDH and PTEN, are presented in Table 1. DLG4 (it is not included in Table 1) was the top bottleneck gene with a betweenness centrality of 0.06 and a very low degree value.

In Figure 4, enrichment of important hubs and bottlenecks for biological process identification is visualized. Each group of terms is indicated by different colors, and the associated genes are shown in connection with the terms. Some of the genes are connected with more than one group (see Figure 4). "Nitric-oxide synthase", "lymphocyte apoptotic process", "regulation of cyclin-dependent protein serine/threonine kinase activity", and "positive regulation of miRNA maturation" biological processes were linked to the central genes.

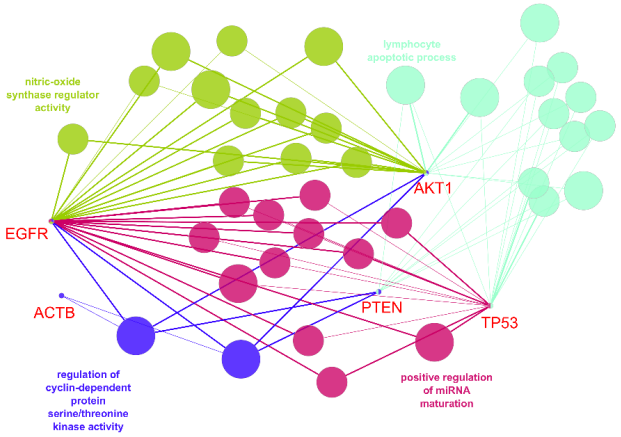

Figure 4. Biological process network by ClueGO+CluePedia using kappa score statistics. Five genes were included in the network based on the defined statistical criteria. EGFR and AKT1 are the most involved genes in biological processes

DISCUSSION

Protein-protein interaction network analysis could assist in understanding critical biomarkers in the disease condition by adding more value to their role. In a way that the biomarkers centrality feature analysis could provide additional information in this regard (17, 18). Genes with high value in centrality in a network are more prominent at the network scale and ultimately in the underlying disease mechanism (18). In severe conditions of asthma, there is no exception, and the biomarkers with these properties could play more vital roles in the severity mechanism. A network of highly significant differential genes in asthma was constructed by Cytoscape software. These genes are from an original study of females with severe asthma in comparison with healthy samples. The DEGs were introduced in the network query based on their statistical significance, and with that respect, 30 genes were identified as qualified. Before that, all data analysis for gene profile comparison was handled by GEO2R, which indicated statistically significant results for more analysis.

There are six genes in the PPI network of Asthma that show a key role in the network strength and foundation stability. These genes are TP53, AKT1, ACTB, GAPDH, PTEN, and EGFR. In addition, DLG4 is an essential bottleneck with the highest value of betweenness centrality in the Asthma network. All these identified genes are designated for further analysis to understand their role and background in asthma. The first-ranked gene in the asthma network is the famous gene in cancer, the tumor suppressor gene (TP53). Previous studies regarding the involvement of this gene in asthma have been reported. DNA methylation of TP53 is one of the molecular processes in Asthma (19). The studies indicate that this gene is not functional in this type of disease. It has a vast involvement in different types of disease because of its role in glycolysis regulation. In Asthma, dysregulation of TP53 could trigger an inflammation cascade (20). This gene is not among the queried DEGs; it belongs to the additional first neighbor genes.

The next gene is serine/threonine-protein kinases (AKT1); that is also not among the differentially expressed genes. Based on previous reports on asthma, this gene could be activated after a cascade of other gene expression (21, 22). Actin B (ACTB) is the next high-ranked housekeeping hub-bottleneck that is also not among the DEGs. However, proteomics and genomics studies identified ACTB down-regulation in children with asthma and other asthmatic patients, respectively (23, 24). The 4th-ranked important gene is a hub, GAPDH, also known as

Glyceraldehyde-3-phosphate dehydrogenase, that has a vast domain of functions (25). This gene is a housekeeping gene and has a key role in glycolysis (26).

In a gene expression study, the expression level of this gene has been decreased (24). Phosphatidylinositol 3,4,5-trisphosphate 3-phosphatase (PTEN) is the next hub gene that is again not among the queried DEGs in the sample of the study, while previous studies indicated the relationship of this gene and asthma. Also, this gene is famous for cancer initiation and development. The expression level of these genes has been reported to be low in asthma (27). EGFR Epidermal growth factor receptor (EGFR) is the last hub-bottleneck of our list that is also not among the DEGs. The literature review of this gene shows that its activation plays a key role in exacerbating the asthmatic condition in mice (28). In a way that this gene can increase the epithelial damage in these patients (29). Disks large homolog 4 (DLG4) is a bottleneck that is the only differentially expressed gene from the main study. This gene is upregulated in asthmatic conditions. The relationship between this gene and asthma is unclear, and this gene plays a critical role in brain function and related diseases (30). DLG4, the pivotal gene in neural signaling, is identified as an essential protein in the inflammatory response of microglial cells (31). As depicted in Figure 4, EGFR and AKT1 are highly involved in common biological processes, indicating their role in similar terms. Therefore, while these hub-bottlenecks and hubs are not reported as DEGs in the original gene expression profile, they are important in Asthma based on previous findings. In addition, DLG4 is an important bottleneck whose presence among DEGs adds to its key role. However, previous studies have not yet identified its role in asthma, despite its involvement in the inflammation process. This aspect requires further investigation to explore any potential connection. In this view, the complementary study of these factors and their contribution to asthma is essential for understanding the molecular basis of asthma.

CONCLUSION

It can be concluded that AKT1 and EGFR are the crucial genes that promote severe asthma. These genes are associated with "Nitric-oxide synthase", "lymphocyte apoptotic process", "regulation of cyclin-dependent protein serine/threonine kinase activity", and "positive regulation of miRNA maturation" directly or indirectly. AKT1, EGFR, and the related biological processes can be considered as the possible drug targets for severe asthma.

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