

SMC3 may play an important role in atopic asthma development

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Abstract

Introduction: As a common disease with a complex risk, including genetic and environmental factors, atopic asthma is prevalent but treatable. The aim of the study was to predict the underlying mechanism of asthma and identify target genes for the disease.

Methods: The affymetrix chip data, GSE18965, were available from Gene Expression Omnibus and the differentially expressed genes (DEGs) between nine atopic asthmatic specimens and seven healthy nonatopic samples were identified by R. Then Gene Ontology and pathway enrichment analyses were performed to these DEGs. Further, search tool for the retrieval of interacting genes/proteins (STRING) was used to select protein–protein interaction (PPI) for DEGs, and then the network was visualized by Cytoscape. Finally, transcription factor binding site analysis was conducted to the hot gene.

Results: Total 565 DEGs were identified, including 535 upregulated and 30 downregulated genes. The upregulated genes, such as structural maintenance of chromosome (SMC)3, significantly affected cellular component of extracellular matrix ($P = 1.56E-04$). Otherwise, DEGs were remarkably enriched in three pathways, including transforming growth factor-beta signaling pathway ($P = 0.005252649$). Further, SMC3 was detected as hot gene in PPI network, and NET (Elk3) was predicted as the significant transcription factor for this gene.

Conclusion: SMC3 may play an important role in atopic asthma development; therefore, it has the potential to be the target for the disease. Moreover, our findings provide more knowledge about the mechanism of atopic asthma and help the researchers to explore it in future.

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Abbreviations:

AA	atopic asthmatic
AEC	airway epithelial cell
BP	biological process
CC	cellular component
CEP290	centrosomal protein 290 kDa
DAVID	Database for Annotation, Visualization and Integrated Discovery
DEG	differentially expressed gene
EMT	epithelial–mesenchymal transition
GEO	Gene Expression Omnibus
GO	Gene Ontology
HN	healthy nonatopic
KEGG	Kyoto Encyclopedia of Genes and Genomes
MF	molecular function

Key words

asthma – differentially expressed genes – functional analysis – protein–protein interaction network – transcription factor binding site

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Ethics

This study was conducted based on bioinformatics analysis and the affymetrix chip data were downloaded from the Gene Expression Omnibus.

Conflict of interest

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

*The first author.

PPI	protein–protein interaction
SMC	structural maintenance of chromosome
TAC	tachykinin
TCF	ternary complex factor
TFBS	transcription factor binding site
TGF-beta	transforming growth factor-beta

Introduction

Bronchial asthma is one of the most common chronic respiratory diseases in the world, and it has become a serious public health problem with the increasing incidence. Nakagome *et al.* have reported that asthma is a chronic disorder characterized by airway inflammation, reversible airway obstruction, irreversible airway remodeling and airway hyperresponsiveness (1). Airway remodeling refers to the structural changes in the airway wall caused by chronic inflammation, resulting in the persistent or progressive damage in lung function, which play an important role in severe and refractory asthma pathogenesis. Currently, the mechanism of airway remodeling is still far from clear. Traditionally, airway remodeling mainly contains the proliferation and hypertrophy of airway smooth muscle cell, deposition of extracellular matrix, thickening of basement membrane, inflammation cell infiltration and gland hypertrophy. However, in recent years, many studies have showed that airway epithelial cell (AEC) plays an important role in airway remodeling, which may be the source and induction factor for interstitial cells in airway remodeling, such as fibroblasts (2, 3). The stimulation of allergens and environment factors, as well as the release of inflammatory mediators and cytokines, may induce the damage of airway epithelial barrier, the reduced adhesion among epithelial cell, the downregulated epithelial gene and upregulated interstitial cells gene (4). Epithelial cells may participate in airway remodeling through epithelial–mesenchymal transition (EMT) (5).

Up to now, it has been reported that asthma incorporates numbers of syndromes with varying clinical manifestations (6). Most patients with asthma can be symptom free if they receive appropriate medical treatment, modify their environment to reduce or eliminate exposure to allergens and irritants, and use inhaled corticosteroid when prescribed (7). Generally, asthma is treated with glucocorticoid, which may alleviate the disease; however, it has little effect on refractory asthma, such as hormone-resistant and hormone-dependent asthma. Moreover, the long-term high-dose use of glucocorticoid may cause several side effects, such as growth inhibition, obesity and femoral head necrosis. Although many studies have been conducted to treat this disease and obtained certain of results, the underlying mechanism of asthma is still not very clear.

Gagliardo *et al.* have reported the role of transforming growth factor-beta (TGF-beta) 1 in airway inflammation of childhood asthma (8). In a word, many genetic and other factors have discovered associated

with asthma development and progression. However, the pathogenesis of asthma has not been fully explored. Therefore, there is an urgent need to explore the potential mechanism of asthma and discover new treatment for asthma, especially for asthma remodeling. In the present research, the differentially expressed genes (DEGs) between asthma and healthy samples were screened out, and their functions in asthma development were predicted. In addition, the protein–protein interactions (PPIs) were selected by STRING, and the hot genes were chosen. Then the PPI network for hot genes was constructed and the significant transcription factor binding site (TFBS) for hot gene was predicted. Our study explored the potential mechanism of atopic asthma and provided the target for the disease. Thus, our findings may shed new insight for atopic asthma research and treatment.

Materials and methods

Affymetrix chip data

The Gene Expression Omnibus (GEO, <http://www.ncbi.nlm.nih.gov/geo/>) database in National Center for Biotechnology Information is currently the largest fully public gene expression resource, which includes 214 268 samples and 4500 platforms (9). In the present study, the chip data GSE18965 consisted of 16 AECs samples collected by bronchial brushing from children (2.4–19.6 years old) before elective surgery with no use of inhaled or oral glucocorticosteroids for at least 1 month before surgery were downloaded from GEO. There were nine atopic asthmatic (AA) samples from AA children with a documented history of wheeze in the 12 months prior to recruitment and seven healthy nonatopic (HN) samples from healthy nonatopic children with no history of wheeze (10). The platform was GPL96 [HG-U133A] Affymetrix Human Genome U133A Array.

Data preprocessing and DEGs identifying

The affy package is an R package of functions and classes for the analysis of oligonucleotide arrays (11). Data preprocessing was performed using robust multi-array average in Affy package in R, including background-adjusted, normalized and log-transformed values (12). Then the original CEL file data were transformed to probe-level data. Further, the probe-level data were converted to gene symbols by perl procedure. Given that when several probes correspond to one gene symbol, the gene expression value is the mean of probes. Then the DEGs between seven AA

and nine HN samples were identified by samr package for R with $|\log \text{fold change}| (|\log \text{FC}|) > 1$ (13, 14).

Functional analysis

In the present study, DAVID (Database for Annotation, Visualization and Integrated Discovery) was applied to conduct Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses to DEGs. DAVID bioinformatics resource consists of an integrated biological knowledgebase and analytic tools aimed at systematically extracting biological meaning from large gene or protein lists (15). GO terms are significantly overrepresented in a set of genes from three aspects, including cellular component (CC), molecular function (MF) and biological process (BP) (16). KEGG is a knowledge base for systematic analysis of gene functions (17). In the present research, the significant GO terms and KEGG pathways were screened out with $P < 0.05$.

PPI analysis

STRING was utilized to select the interactional genes for DEGs, and the genes with PPI more than 10 were set as hot genes. The STRING database (<http://string-db.org/>) provides a global protein interactions and associations (18). Then the Cytoscape was used to visualize the PPIs, which formed PPI network (19).

TFBS analysis

TFBS implements objects representing specificity profile matrices, binding sites and sets thereof, pattern generators and pattern database interfaces (20). In this work, whole genome rVista (<http://genome.lbl.gov/cgi-bin/WGRVistaInput5.pl>) was used to select TFBS for genes (21). The TFBS was performed from 5 kb to the transcription start site, and the significant TFBS was selected with $P < 0.01$.

Results

Identified DEGs

The original data were well preprocessed, and a total of 565 DEGs between nine AA and seven HN samples, which included 535 upregulated and 30 downregulated genes, were identified.

Enrichment analysis

Furthermore, the functional analyses, including GO and KEGG pathway enrichment, were performed for

the upregulated and downregulated genes. In our study, the top five significant GO terms for upregulated and downregulated genes between AA and HN samples were selected for analysis, respectively (Table 1). For the upregulated genes, the most significant GO term was regulation of RNA metabolic process ($P = 8.82\text{E-}05$) with 77 genes enriched in it, including structural maintenance of chromosome (SMC)3, zinc finger protein 208 (ZNF208), BTB and CNC homology 2 (BACH2), N- α -acetyltransferase 15 (NAA15) and zinc finger protein 674 (ZNF674). On the other hand, the most remarkable GO term for downregulated genes was glucose catabolic process ($P = 1.42\text{E-}04$), with GPI, G6PD, PGD and OGDH enriched in it.

On the other hand, the KEGG pathway analysis discovered that the DEGs were significantly enriched in three pathways including TGF- β signaling pathway, retinol metabolism and pentose phosphate pathway. It also revealed that several DEGs, such as GPI, G6PD, PGD, BMP2 and ADH1B, may disturb these pathways (Table 2).

PPI network for hot genes

By using STRING, the genes with PPIs number more than 10 were set as hot genes, and their PPI network was constructed and shown in Fig. 1. Only two downregulated genes, NOL6 and SF3A3, were discovered in the network. Total four key genes, including SMC3, BRX1 (biogenesis of ribosomes, homolog), tachykinin (TAC)1 and centrosomal protein 290 kDa (CEP290), were found in the network, and all the key genes were upregulated in the AA samples (Fig. 1).

TFBS analysis

The TFBS analysis was conducted by Whole Genome RVista and the potential sites for SMC3 were predicted, visualized and displayed in Fig. 2. From Fig. 2, we found that NET was the significant transcription factor for SMC3.

Discussion

Given that asthma is the most common chronic disease of childhood and inner city residents (22), there is an urgent need to explore the underlying mechanism of the disease. In our present research, bioinformatics approach was utilized to predict the potential pathogenesis of asthma. First, the DEGs between AA and HN samples were screened out, and then their functions were analyzed by GO and KEGG pathway enrichment.

Table 1. The top five GO terms for up- and down-regulated genes between atopic asthmatic and healthy normal samples ($P < 0.05$), respectively

DEGs	Term ID	Description	Count	P value	Gene
Upregulated	BP	Regulation of RNA metabolic process	77	8.82E-05	ZNF208, BACH2, NAA15, ZNF674, etc
	GO:0051252				
	BP	Regulation of transcription, DNA-dependent	75	1.33E-04	ZNF208, BACH2, NAA15, ZNF674, etc
	GO:0006355				
	CC	Extracellular matrix	22	1.56E-04	WNT5A, HAPLN1, SMC3, CRISP3, etc
	GO:0031012				
	CC	Proteinaceous extracellular matrix	21	1.58E-04	WNT5A, HAPLN1, SMC3, CRISP3, etc
Downregulated	GO:0005578				
	CC	Extracellular region part	43	2.49E-04	WNT5A, OLFM4, SMC3, KERA, etc
	GO:0044421				
	BP	Glucose catabolic process	4	1.42E-04	GPI, G6PD, PGD, OGDH
	GO:0006007				
	BP	Hexose catabolic process	4	2.38E-04	GPI, G6PD, PGD, OGDH
	GO:0019320				
	BP	Monosaccharide catabolic process	4	2.59E-04	GPI, G6PD, PGD, OGDH
	GO:0046365				
	BP	Alcohol catabolic process	4	3.82E-04	GPI, G6PD, PGD, OGDH
	GO:0046164				
	BP	Cellular carbohydrate catabolic process	4	4.40E-04	GPI, G6PD, PGD, OGDH
	GO:0044275				

DEGs is differentially expressed genes. Count represents the DEGs enriched in the corresponding term. BP and CC mean biological process and cellular component.

GO, Gene Ontology.

Further, the PPI network for hot genes, including SMC3, was constructed and the TFBS were predicted.

First, the DEGs between AA and HN samples were identified and analyzed. The expressions of these genes were changed significantly during atopic asthma development. This result indicates that these genes may have a close relationship with atopic asthma development. Moreover, the functions of these genes were analyzed by GO and KEGG pathway enrichment. From the GO analysis results, we discovered that the downregulated genes, including GPI, G6PD, PGD and OGDH, mainly enriched in five BP terms, which contained catabolic processes of glucose, hexose, monosaccharide, alcohol and cellular carbohydrate. This finding suggests that the four downregulated genes may induce atopic

asthma by disturbing these five BP, which means that the five BP may be suppressed in AA samples.

On the other hand, numbers of upregulated genes were found enriched in two BP terms and three CC terms. Interestingly, SMC3 was enriched in all the three CC terms, including extracellular matrix ($P = 1.56E-04$), proteinaceous extracellular matrix ($P = 1.58E-04$) and extracellular region part ($P = 2.49E-04$). Conserved in all eukaryotes examined and most prokaryotes, SMC participates in chromosome folding and dynamics (23). Extracellular matrix is a biochemical target for TGF-beta and a dynamic niche in cancer progression (24). Extracellular matrix is a complex network of macromolecules with distinctive biomechanical, physical and biochemical properties.

Table 2. The significant KEGG pathways with $P < 0.05$

Term	Description	P value	Genes
hsa04350	TGF-beta signaling pathway	0.005252649	BMP2, RBL2, INHBE, TGFBR1, SMAD5, SMURF2, BMP5, BMP8B (up)
hsa00830	Retinol metabolism	0.042819904	ADH1B, UGT2B4, RPE65, CYP1A2, UGT2A3 (up)
hsa00030	Pentose phosphate pathway	0.001486249	GPI, G6PD, PGD (down)

Down and up indicate the downregulated and upregulated genes enriched in the pathway.

KEGG, Kyoto Encyclopedia of Genes and Genomes; TGF-beta, transforming growth factor-beta.

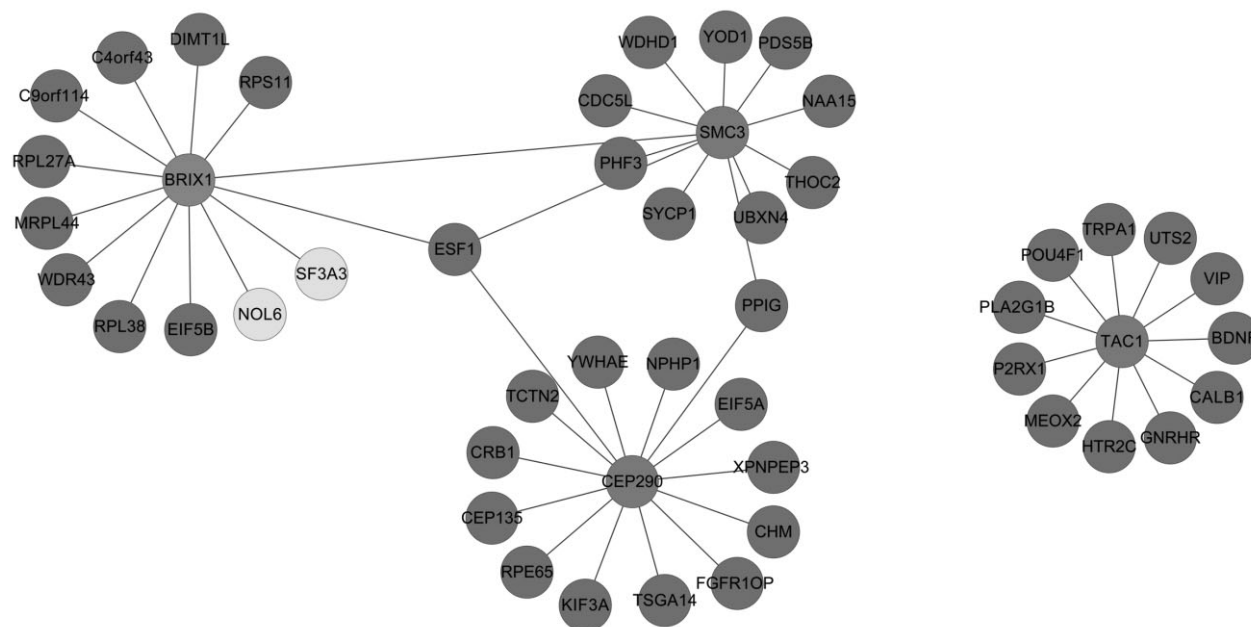


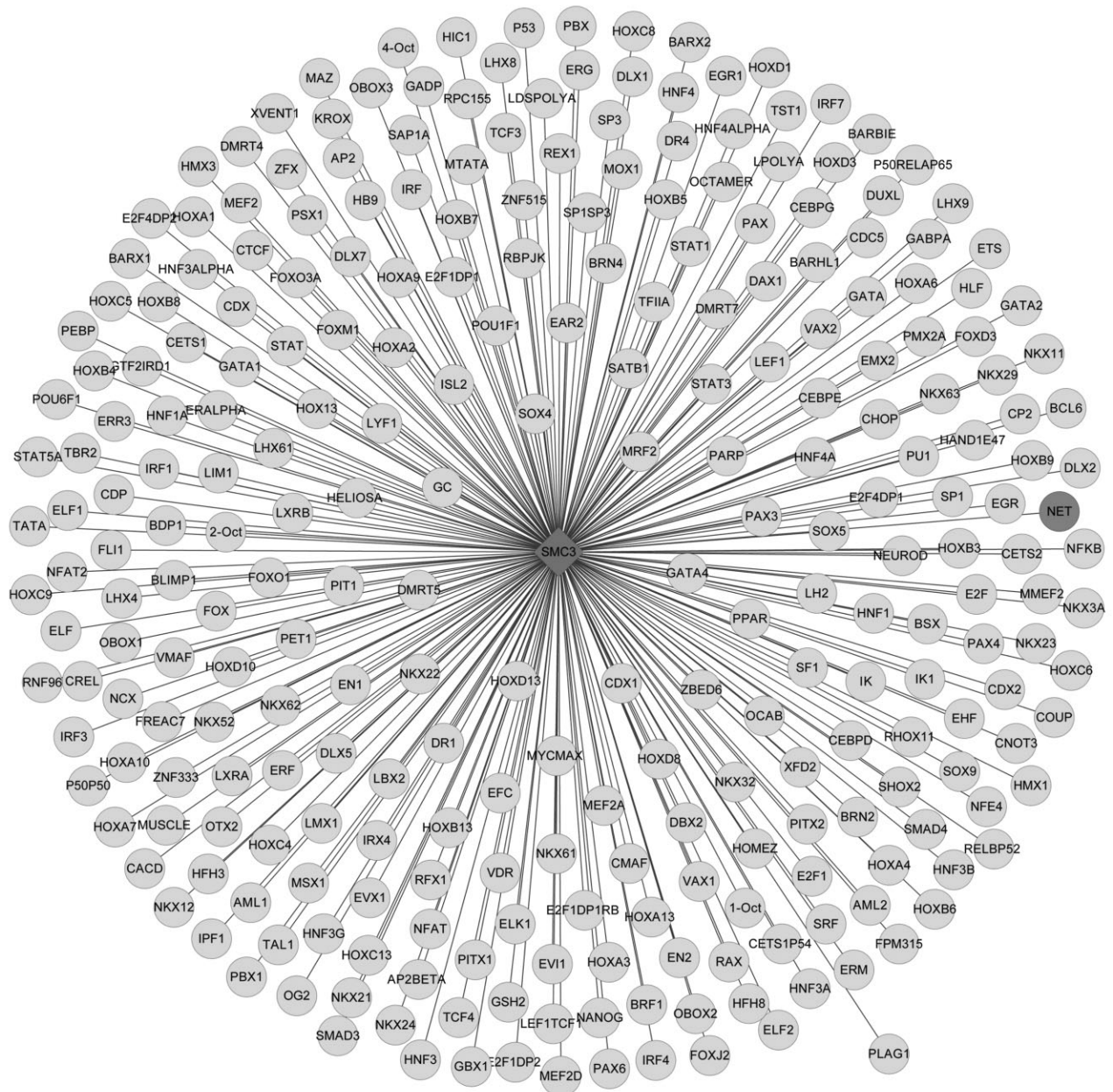
Figure 1. The protein–protein interaction (PPI) network for hot genes with PPIs more than 10. The red, green and pink colors represent overregulated, downregulated and hot genes.

Extracellular region part describes any constituent part of the extracellular region, the space external to the outermost structure of a cell. For cells without external protective or external encapsulating structures, this refers to space outside of the plasma membrane. This term covers constituent parts of the host cell environment outside an intracellular parasite. The overexpressed genes were detected enriched in these three CC terms, indicating that the upregulated genes may result in asthma by affecting these terms. The upregulated SMC3 may induce asthma by affect these three CC terms, which may be the mechanism of atopic asthma.

In addition, DEGs were discovered mainly enriched in three pathways, including TGF-beta signaling pathway, retinol metabolism and pentose phosphate pathway. These pathways have been reported to play important roles in diseases, such as renal disease, hepatic fibrosis and chronic liver disease (25). Moreover, TGF-beta can promote cancer progression by the induction of EMT, leading to increased motility and invasion (26). Studies in a wide range have demonstrated that TGF-beta is a central mediator of fibrogenesis, and it is upregulated and activated in fibrotic diseases (27). Interestingly, two pathways were disturbed by upregulated genes, and only one pathway was enriched with downregulated genes. This result indicates that the development of atopic asthma may be significantly related with the overexpressed genes.

Furthermore, the PPIs of the DEGs were selected by STRING, and the DEGs with PPIs more than 10 were selected as hot genes. Finally, total four hot genes were selected, which included SMC3, BRIX1, TAC1 and CEP290. Overexpression of SMC3 has been reported to have the ability to cause cell transformation (28), suggesting SMC3 might be involved in asthma. As a family of structurally related neuropeptides, TACs are distributed in the central nervous system and have roles as neurotransmitters and/or neuromodulators (29). Some studies have reported that neuropeptides participate in asthmatic airway inflammation, and substance P has a role in asthmatic rat airway smooth muscle cell proliferation, migration and cytoplasmic calcium concentration *in vitro* (30, 31). The previous studies have verified that the mutation of CEP290 cause a number of debilitating and phenotypically distinct human diseases (32). In our present research, these four genes were identified had more PPIs than other DEGs, which suggested that these genes may play important roles in atopic asthma development.

Finally, TFBS analysis was performed to SMC3, and the significant transcription factor, NET, was screened out. NET, a member of the Elk/Ternary Complex Factor (TCF) subfamily of ETS E26 transformation-specific (ETS) factors, has similar DNA-binding characteristics with Elk-1, and the C-terminal sequences of NET could strongly inhibit the complex formation with serum response factor (33, 34). The TCF



NET/Elk3 has been discovered downregulated in hypoxia and participates in the induction by hypoxia of several genes (35). Elk3 (NET) is a transcriptional repressor of nitric-oxide synthase-2. In our study, our results indicate that NET has the most possibility to regulate SMC3 expression, thus to induce development of atopic asthma.

several genes were selected as the new targets for the disease. By comparing the AA and HN samples, many genes were upregulated, which may disturb several functions and pathways. SMC3, an overexpressed gene, may affect three CC and may be regulated by NET. Thus, we predicted that the upregulated SMC3 may play an important role in atopic asthma development, especially in asthma EMT, by disturbing several GO terms, and it may be the new target for asthma. Our

study may contribute to the study of atopic asthma pathogenesis and treatment.

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