

# Dynamic Urinary Proteome Changes in Ovalbumin-Induced Asthma Mouse Model Using Data-Independent Acquisition Proteomics

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**Background:** In this work, we aim to investigate dynamic urinary proteome changes during asthma development and to identify potential urinary protein biomarkers for the diagnosis of asthma.

**Methods:** An ovalbumin (OVA)-induced mouse model was used to mimic asthma. The urinary proteome from asthma and control mice was determined using data-independent acquisition combined with high-resolution tandem mass spectrometry.

**Results:** Overall, 331 proteins were identified, among which 53 were differentially expressed (26, 24, 14 and 20 on days 2, 8, 15 and 18, respectively; 1.5-fold change, adjust  $P < 0.05$ ). Gene Ontology annotation of the differential proteins showed that the acute-phase response, innate immune response, B cell receptor signaling pathway, and complement activation were significantly enriched. Protein-protein interaction network revealed that these differential proteins were partially biologically connected in OVA-induced asthma, as a group. On days 2 and 8, after two episodes of OVA sensitization, six differential proteins (CRAMP, ECP, HP, F2, AGP1, and CFB) were also reported to be closely associated with asthma. These proteins may hold the potential for the early screening of asthma. On days 15 and 18, after challenged with 1% OVA by inhalation, seven differential proteins (VDBP, HP, CTSE, PIGR, AAT, TRFE, and HPX) were also reported to be closely associated with asthma. Thus, these proteins hold the potential to be biomarkers for the diagnosis of asthma attack.

**Conclusion:** Our results indicate that the urinary proteome could reflect dynamic pathophysiological changes in asthma progression.

**Keywords:** OVA-induced asthma, mice, urine, proteome, data-independent acquisition

## Introduction

Asthma is a heterogeneous disease that is usually characterized by chronic airway inflammation. It is characterized by variable symptoms, such as wheeze, dyspnea, and chest tightness and/or cough that vary over time and intensity, together with variable expiratory airflow limitation.<sup>1</sup> Asthma is a major public health concern worldwide, with global prevalence ranging from 1% to 21% in adults<sup>2</sup> and up to 32% in children aged 6–7 years.<sup>3</sup> The predominant mechanism involved in the pathogenesis of asthma is a Type 2 helper T cell (Th2) cytokine-mediated eosinophilic airway inflammation associated with hyper-responsiveness of the lungs.<sup>4</sup> Asthma is a heterogeneous disease; many clinical phenotypes of asthma have been identified, including allergic and nonallergic asthma, late-onset asthma,

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Received: 18 July 2021  
Accepted: 30 October 2021  
Published: 9 November 2021



asthma with persistent airflow limitation, and asthma with obesity.<sup>5,6</sup> Many of these and other phenotypes of asthma are diagnosed based on the clinical characteristics.

Spirometry is by far the primary diagnostic approach for asthma, which reflects disease severity rather than disease activity.<sup>7</sup> Moreover, its measurements strictly depend on patient's compliance, physician's expertise, and data interpretation.<sup>8</sup> Especially for children aged under 6 years, the diagnosis is more difficult because they are too young to undergo spirometry. Besides, clinical symptoms on which pulmonologists rely for a correct diagnostic approach are subjective and nonspecific. The differential diagnosis in a patient with suspected asthma is based on the age of onset, reversibility of airflow limitation, symptom variability, and atopy. A significant proportion of patients with chronic respiratory symptoms (9–55%) has diagnoses and/or features of both asthma and chronic obstructive pulmonary disease (COPD).<sup>9</sup> Distinguishing asthma from chronic airflow limitation due to COPD becomes problematic, particularly among smokers.<sup>10,11</sup> Thus, there is an urgent need for noninvasive biomarkers for early diagnosis, evaluation of lung functional impairment, and prognosis in asthma.

Mass spectrometry (MS)-based proteomics has dramatically improved and emerged as a prominent tool in the field of biomarker study. Many protein biomarkers of asthma and/or COPD have been described and categorized as primarily blood and bronchoalveolar lavage fluid (BALF) biomarkers.<sup>12,13</sup> For example, MS-based proteomic analysis revealed serum ApoE to be significantly downregulated in atopic asthmatics compared to healthy volunteers.<sup>14</sup> Comparative proteomics analysis of patients with quick development and slow development COPD, provided 15 potential serum biomarkers for efficiently predicting the development phenotypes of COPD.<sup>15</sup>

Urine, as an attractive resource for biomarker research, can be collected noninvasively and continuously, which has arrested more and more concerns. Mass spectrometry-based proteomics based on the association between different protein profiles and pathogenic processes is gaining an important role in pulmonary medicine.<sup>16–18</sup> Urinary proteomic studies have identified several candidate biomarkers for pulmonary disease, such as lung cancer,<sup>19</sup> pulmonary fibrosis, and tuberculosis.<sup>20,21</sup> However, several candidate biomarkers of asthma have been described primarily in the sputum, blood, and bronchoalveolar lavage fluid. Besides, the urinary proteome could reflect changes in disease progression at the early stage. For

example, the urinary proteome profiles changed significantly even in the absence of clinical manifestations or histopathological damage to the lung tissue in bleomycin-induced pulmonary fibrosis<sup>21</sup> and Walker-256 tail-vein injection-induced lung metastasis<sup>22</sup> rat models. Therefore, urine can sensitively reflect pathophysiological changes in the lung tissue at an early stage and is, thus, a promising resource for studying the biomarkers of lung diseases.

This study aimed to observe dynamic urinary proteome changes during asthma development and to identify potential urinary protein biomarkers for the diagnosis of asthma. The data-independent acquisition (DIA) approach was used to profile the urinary proteome of OVA-induced asthma mouse model.

## Materials and Methods

### Mice and Asthma Induction

The C57BL/6J male mice (6-week-old) were purchased from Charles River (Beijing, China) and housed in light (12 h light: 12 h dark cycle) and temperature-controlled ( $22 \pm 3$  °C) rooms. Throughout the experiments, the mice had free access to standard laboratory chow and were provided with tap water ad libitum. The mice were treated humanely according to international guidelines and the experiment was adhered to the Guide for the Care and Use of Laboratory Animals and approved by the Animal Ethics Committee of Qingdao Municipal Hospital, Qingdao, China.

Twelve specific pathogen-free C57BL/6 mice were used and divided randomly into control (n=6) and asthma (n=6) groups. During the induction of asthma, the mice were sensitized by intraperitoneal injection of 50 µg ovalbumin grade V (OVA) (Sigma, Germany) adsorbed on Al(OH)<sub>3</sub> (AlumInject©, Perbio, Belgium) on days 1 and 7. Animals were fed 50 µg of OVA via nasal drops on days 14–17, as previously described.<sup>23</sup> Each control mouse was administered 0.9% NaCl as placebo.

### Histological Analysis

On day 17, the mice were anesthetized by intraperitoneal injection of 10% pentobarbital. After anesthesia induction, a catheter was intubated through the trachea. The left lung was removed after ligation of the left main bronchus, and the lung tissues were then quickly fixed in 10% neutral-buffered formalin. The formalin-fixed tissues were embedded in paraffin sectioned (4 mm) and stained with hematoxylin and eosin (H&E) to identify histopathological lesions.

## ELISA and Flow Cytometry

The blood samples were collected by cardiac puncture, and centrifuged at 3000 rpm for 5 min at 4 °C. IgE level was measured by an ELISA kit (BD Bioscience) according to the manufacturer's instructions.

After the left lung was removed, 1 mL of iced PBS was slowly injected into the trachea with a syringe. The bronchoalveolar lavage fluid (BALF) was washed several times, counted on a blood cell counting plate, and  $5 \times 10^5$  cells were added to the 7AAD-percP (BD Biosciences), CD45-eflour 450 (Biolegend), CD11b-ITC (Biolegend), and SiglecF-PE (Biolegend), according to the specification. Antibody surface staining of BALF cells. After 30 min of light staining on ice, 200  $\mu$ L PBS was added, the solution was centrifuged at 5000 r/min for 5 min, and excess antibody was washed off. The cells were resuspended in 300  $\mu$ L of PBS or a 1:1 mixture of PBS and 4% paraformaldehyde and then transferred to a flow tube for on-machine detection using flow cytometry (ACEA Novo Cyte™).

## Urine Collection and Sample Preparation

Urine samples were collected from six asthmatic mice at days 2, 8, 15, and 18 and six control mice. The mice were individually housed in the metabolic cages for 4 hours. During urine collection, no food was provided to the mice to avoid urine contamination. After collection, the urine samples were centrifuged at 2000 g for 30 min at 4°C immediately and then stored at -80°C.

For tryptic digestion, the proteins were digested with trypsin (Promega, USA) using filter-aided sample preparation methods.<sup>24</sup> Briefly, 100  $\mu$ g of the protein sample was loaded on the 10-kD filter unit (Pall, USA). The protein solution was reduced with 4.5 mM DTT for 1 h at 37°C and then alkylated with 10 mM of indoleacetic acid for 30 min at room temperature in the dark. The proteins were digested with trypsin (enzyme-to-protein ratio of 1:50) for 14 h at 37°C. The peptides were desalted on Oasis HLB cartridges (Waters, USA) and lyophilized for trap column fractionation and LC-DIA-MS/MS analysis.

## Spin Column Separation

The pooled peptides sample were fractionated using a high-pH reversed-phase peptide fractionation kit (Thermo Pierce, USA) according to the manufacturer's instructions. Briefly, 60  $\mu$ g of the pooled peptide sample was loaded onto the spin column. Ten different fractions were collected by centrifugation, including flow-through

fraction, wash fraction, and eight step gradient sample fractions (5, 7.5, 10, 12.5, 15, 17.5, 20, and 50% acetonitrile). The fractionated samples were lyophilized and used for the LC-DDA-MS/MS analysis.

## Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) Analysis

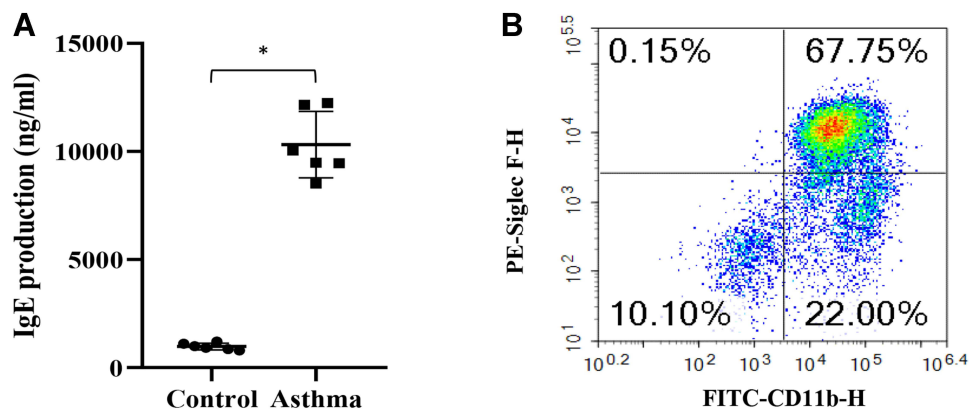
The Orbitrap Fusion Lumos Tribid Mass Spectrometer coupled with an EASY-nLC 1200 HPLC system (Thermo Scientific, Germany) was used for the DDA-MS and DIA-MS analysis. The digested peptides were dissolved in 0.1% formic acid and loaded on a trap column (75  $\mu$ m  $\times$  2 cm, 3  $\mu$ m, C18, 100 Å). The eluent was transferred to a reversed-phase analytical column (50  $\mu$ m  $\times$  250 mm, 2  $\mu$ m, C18, 100 Å). The eluted gradient was 5–30% buffer B for 90 min. The calibration kit (iRT kit from Biognosys, Switzerland) was spiked at a concentration of 1:20 v/v in all samples. DDA-MS: full scan, 350–1550 m/z at 60,000; cycle time: 3 secs (top speed mode); AGC 2e5; MS/MS scans in the Orbitrap at a resolution of 15,000; isolation window 2 Da, HCD 32%, AGC 5e5, 50 ms. DIA-MS: 32 windows.

## DIA Quantification Analysis

The raw data files were processed using Proteome Discoverer (version 2.1; Thermo Scientific, Germany) with SEQUEST HT against the SwissProt *ratus* database (released in May 2019, containing 8086 sequences). The search parameters consisted of the parent ion mass tolerance, 10 ppm; fragment ion mass tolerance, 0.02 Da; fixed modifications, carbamidomethylated cysteine (+58.00 Da); and variable modifications, oxidized methionine (+15.995 Da) and deamidated glutamine and asparagine (+0.984 Da). Other settings included the default parameters.<sup>25</sup> The DIA-MS raw files were imported to Spectronaut Pulsar with the default settings. In brief, all results were filtered by an FDR of 1%. Peptide intensity was calculated by summing the peak areas of their respective fragment ions for MS2.

## Bioinformatics Analysis

Bioinformatics analysis was conducted in order to better study the biological function of the dysregulated proteins. Gene Ontology (GO) analysis was performed on the urinary differentially altered proteins identified at the discovery phase (<http://www.geneontology.org/>).<sup>26,27</sup> Protein-protein interaction (PPI) networks were constructed using the STRING database (<http://www.string-db.org/>), which is a database of



**Figure 1** The ELISA and flow cytometric analysis results. **(A)** Blood samples were collected and level of serum IgE was measured (n=6). **(B)** The ratio of eosinophils in bronchoalveolar lavage fluid (BALF) in asthmatic mice. \* $p < 0.001$  compared to control group.

known and predicted protein interactions, including direct (physical) and indirect (functional) associations.

The “Wu Kong” platform (<https://www.omicsolution.org/wkomics/main/>) was used for statistical analysis. The differential proteins were selected using one-way ANOVA, and p-values were adjusted using the Benjamini–Hochberg method. Significance was set at a p-value of  $< 0.05$  and a fold change of 1.5.

## Results

### Characterization of OVA-Induced Asthmatic Mice

To investigate the dynamic urinary proteome changes in asthma progression, we established an OVA-induced asthma model. After challenging with OVA, the serum IgE levels were measured (Figure 1A). The results showed that the IgE levels in the OVA-treated group were increased. Next, flow cytometry was performed on eosinophils in BALF. After removing the dead cells (7AAD<sup>+</sup>), the proportion of eosinophils in the leukocytes (CD45<sup>+</sup>) was analyzed (Figure 1B). The ratio of eosinophils (CD11b<sup>+</sup>siglecF<sup>+</sup>) in BLAF was 67.75% after 17 days of OVA induction.

The hematoxylin-eosin (HE) stained microscopic features of the lung from control mice showed a normal structure: the alveolar wall structure is intact, there is no thickening, and there is no exudate in the alveolar cavity (Figure 2A), and alveolar epithelial cells are arranged in order (Figure 2B). Compared to the control group, HE-stained of the lung in asthma group showed alveolar destruction and alveolar wall thickening (Figure 2C), as well as pulmonary fibrosis and neutrophil infiltration (Figure 2D). These pathological changes reveal the success of OVA-induced asthma modeling.

### Dynamic Urinary Proteome Changes in OVA-Induced Asthmatic Mice

For a preliminary investigation of changes in the urinary proteome with asthma progression, three urine samples from the control group and 12 samples taken at four time points (days 2, 8, 15, and 18) from the asthma group were analyzed by LC-DIA-MS/MS workflow.

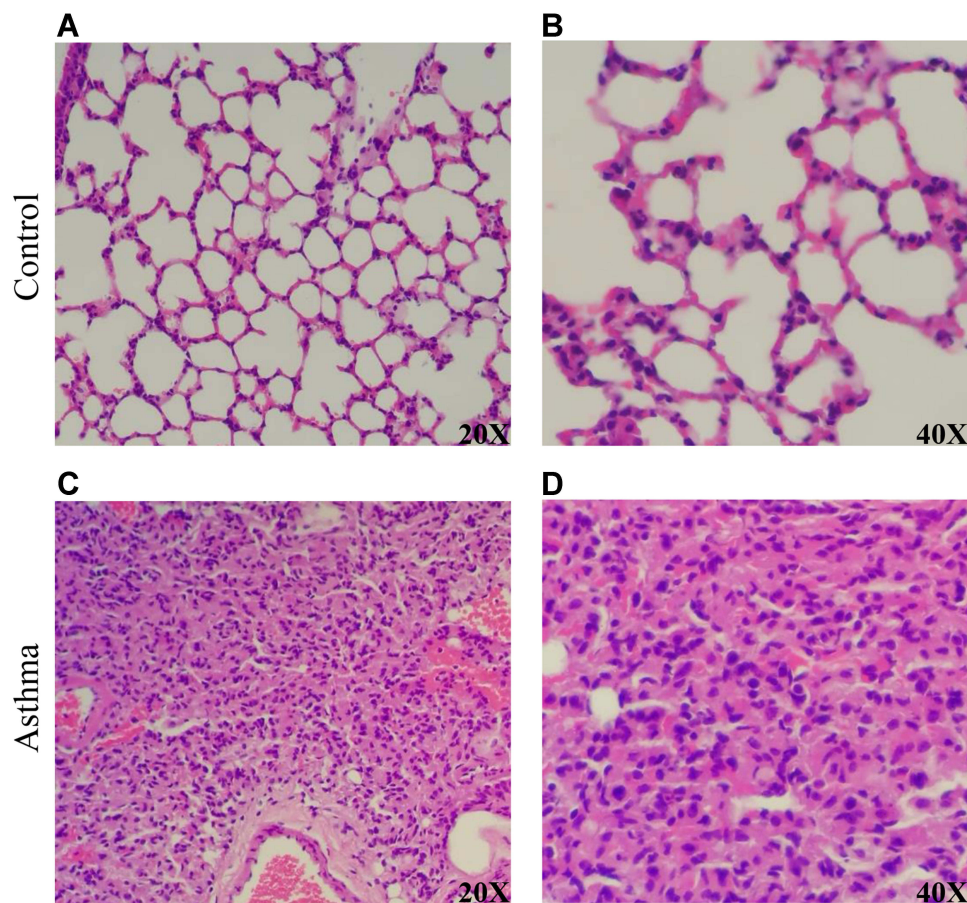
Overall, a total of 331 urinary protein groups were identified; all details are listed in [Supporting Table S1](#). Among these, 53 proteins were significantly changed in asthmatic mice compared to control mice (1.5-fold change, adjust  $P < 0.05$ ; Table 1).

At day 2, 26 differential proteins, 21 of which increased and five of which decreased, were identified. At day 8, 24 differential proteins, 15 of which increased and nine of which decreased, were identified. At day 15, 14 differential proteins, six of which increased and nine of which decreased, were identified. At day 18, 20 differential proteins, seven of which increased and 13 of which decreased, were identified (Figure 3).

### Gene Ontology Analysis of Differential Proteins

The GO functional annotation was performed on the differentially expressed proteins in asthmatic mice. A total of 53 dysregulated proteins, at four time points, were annotated and classified to be involved with certain biological processes (Figure 4).

The GO enrichment analysis showed that inflammation or immune responses, such as acute-phase response, innate immune response, B cell receptor signaling pathway, complement activation, positive regulation of B cell activation, and phagocytosis, were the mainly involved biological



**Figure 2** Pathological results of hematoxylin and eosin (HE) staining in the lungs of asthma and control mice. (A) The lung of normal control mice (20×). (B) The lung of normal control mice (40×). (C) The lung of asthmatic mice (20×). (D) The lung of asthmatic mice (40×).

processes at the four time points. Coagulation factor II, haptoglobin, and alpha-1-acid glycoprotein 1 were enriched in acute-phase response. Beta-2 microglobulin, cathelicidin antimicrobial peptide, complement factor B, immunoglobulin heavy chain, immunoglobulin heavy constant gamma 2A, immunoglobulin kappa constant, and lipocalin 2 were enriched in innate immune response. These results indicate that urine can reflect biological responses in the body during the progression of asthma.

In the cellular component category, most of these differential proteins were extracellular region, extracellular space, blood microparticle, extracellular exosome, and immunoglobulin complex proteins (Figure 4B). In the molecular function category, serine-type endopeptidase activity, immunoglobulin receptor binding, hormone activity, and serine-type peptidase activity were overrepresented on days 2 and 8. Immunoglobulin receptor binding, insulin-activated receptor activity, small molecule binding, and transporter activity were overrepresented on days 15 and 18 (Figure 4C).

## Protein-Protein Interaction Network of the Differential Proteins

To better understand the pathogenic mechanisms of OVA-induced asthma, the PPI network for 53 differential proteins was constructed using STRING (Figure 5). The STRING PPI network analysis revealed that the average node degree is 3.84, average local clustering coefficient is 0.443, and PPI enrichment p-value is  $<1.0e-16$ . This indicates that these proteins have more interactions among themselves than what would be expected for a random set of proteins of similar size drawn from the genome.

## Differential Urinary Proteins Related to Asthma

Among these 53 differential proteins, 18 that had human orthologs were reported to be closely associated with asthma (Table 2). The first 13 differential proteins shown in the table were changed on days 2 and 8, which was the early stage at which a low dose of OVA was intraperitoneally injected for

**Table 1** Details of the Differential Urinary Proteins in OVA-Induced Asthmatic Mice

UniProt ID	Protein Name	Fold Change			
		D2	D8	D15	D18
PI9221	Prothrombin	2.15	3.02	2.02	2.19
PI5501	Prostatic spermine-binding protein	15.94		7.56	0.15
Q61646	Haptoglobin	7.12	3.24		1.71
Q9D3H2	Odorant-binding protein 1a	4.23	10.98		1.86
Q8C6C9	Protein LEG1 homolog	2.78		1.51	1.89
P01878	Ig alpha chain C region		2.68	2.01	2.13
P01864	Ig gamma-2A chain C region secreted form	0.29	0.27	0.33	
Q02596	Glycosylation-dependent cell adhesion molecule 1		0.36	0.60	0.61
Q9JI02	Secretoglobin family 2B member 20	81.62	21.94		
P51437	Cathelicidin antimicrobial peptide	7.93	17.65		
Q60590	Alpha-1-acid glycoprotein 1	4.49	1.61		
P04186	Complement factor B	2.56	2.92		
P97426	Eosinophil cationic protein 1	1.88	1.59		
Q9EP95	Resistin-like alpha	0.19	0.10		
PI1588	Major urinary protein 1	4.12			0.39
P21614	Vitamin D-binding protein	0.55		0.38	
P01633	Ig kappa chain V19-17	0.42			
Q9ET22	Dipeptidyl peptidase 2		0.56	0.49	
Q9JHY4	WAP four-disulfide core domain protein 15B		0.61		0.40
P01837	Ig kappa chain C region		0.59		0.40
P00688	Pancreatic alpha-amylase			0.62	0.59
Q92111	Serotransferrin			0.59	0.36
Q91X72	Hemopexin			0.56	0.44
P42567	Epidermal growth factor receptor substrate 15	22.40			
P02816	Prolactin-inducible protein homolog	4.29			
Q8K1H9	Odorant-binding protein 2a	3.58			
Q99P86	Resistin-like beta	2.38			
P01887	Beta-2-microglobulin	2.28			
Q07456	Protein AMBP	1.83			
P07759	Serine protease inhibitor A3K	1.69			
P20918	Plasminogen	1.61			
Q61398	Procollagen C-endopeptidase enhancer 1	1.60			
G5E861	Sodium channel and clathrin linker 1	1.58			
Q80T19	Hepcidin-2	0.19			
Q60997	Deleted in malignant brain tumors 1 protein		5.20		
P62806	Histone H4		25.93		
PI1672	Neutrophil gelatinase-associated lipocalin		5.87		
P60710	Actin, cytoplasmic 1		3.21		
Q91VW3	SH3 domain-binding glutamic acid-rich-like protein 3		2.25		
Q8K426	Resistin-like gamma		1.74		
Q9Z0M9	Interleukin-18-binding protein		0.55		
P23780	Beta-galactosidase		0.54		
P0CW02	Lymphocyte antigen 6C1		0.51		
P70269	Cathepsin E			1.97	
O08997	Copper transport protein ATOX1			1.72	
O70570	Polymeric immunoglobulin receptor			0.60	
Q6SJQ5	CMRF35-like molecule 3				4.26
Q9JLB4	Cubilin				1.62
A2BIM8	Major urinary protein 18				0.67

(Continued)

**Table 1** (Continued).

UniProt ID	Protein Name	Fold Change			
		D2	D8	D15	D18
P07724	Serum albumin				0.66
P04939	Major urinary protein 3				0.63
Q00897	Alpha-1-antitrypsin 1-4				0.50
Q60932	Voltage-dependent anion-selective channel protein 1				0.47

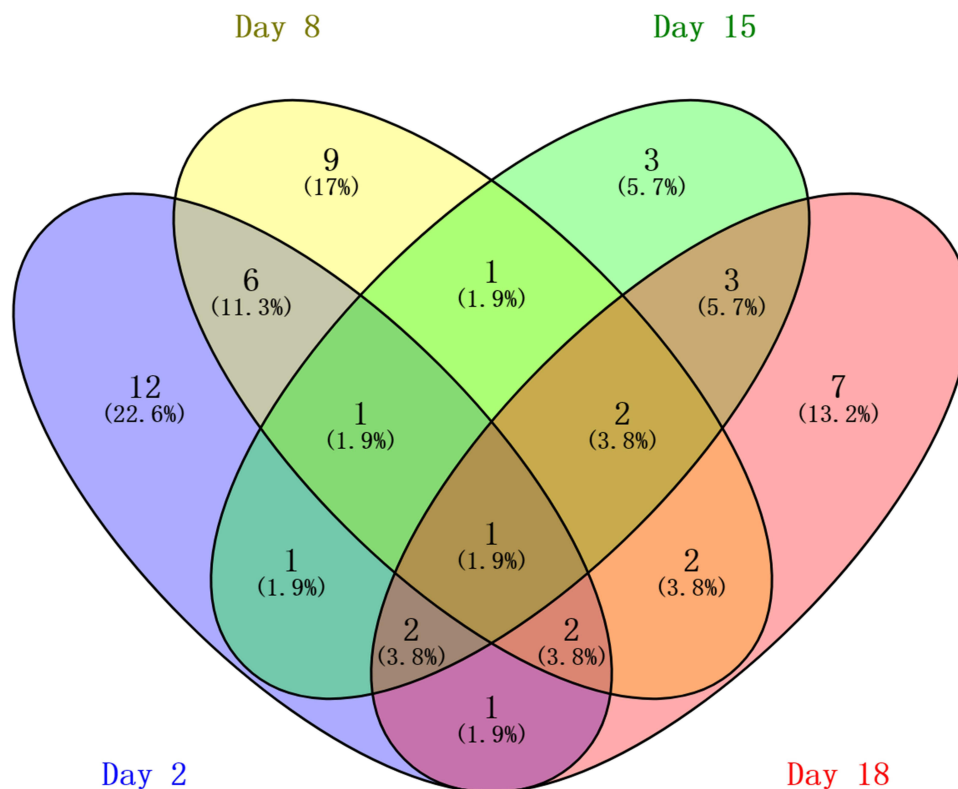
sensitization of mice, and the last seven differential proteins were changed on days 15 and 18, during which a high dose of OVA was administered via nasal inhalation to provoke asthma.

## Discussion

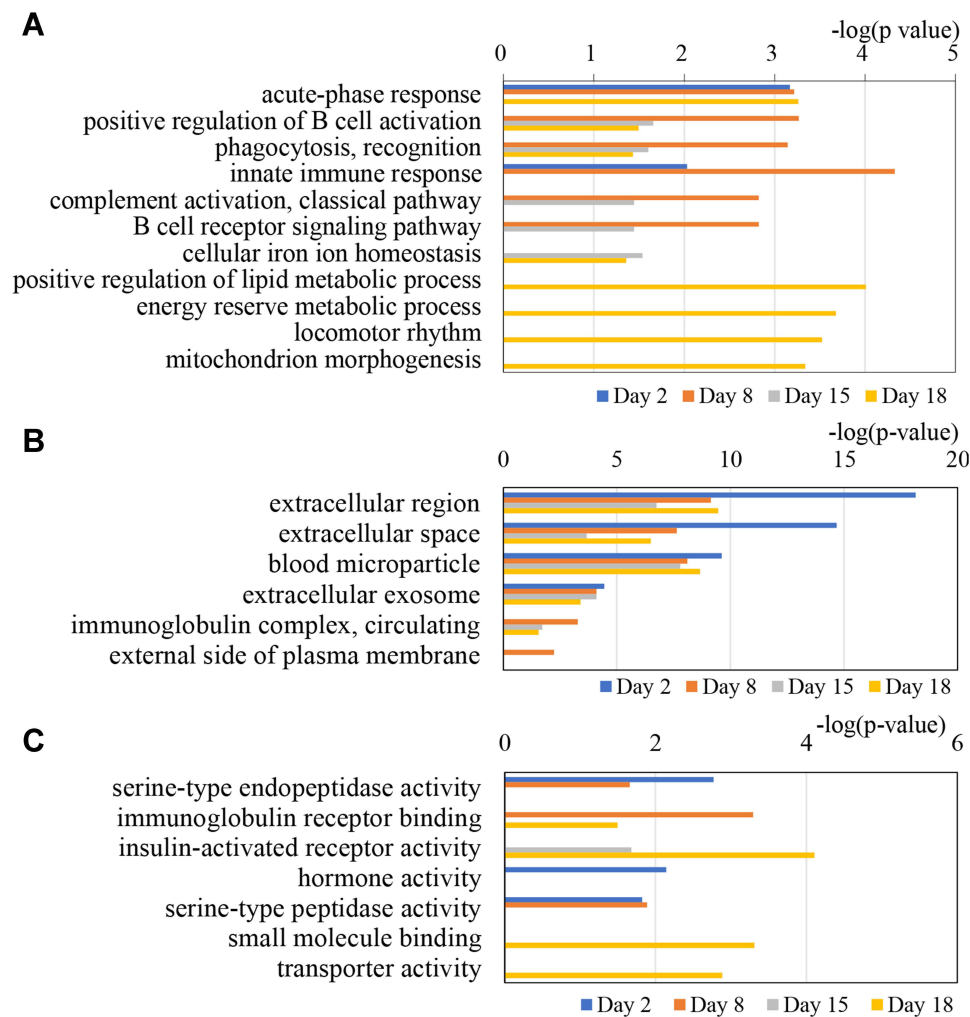
In this study, urine samples were collected from six control mice and six asthmatic mice on days 2, 8, 15, and 18 for proteome analysis to identify potential biomarkers for asthma. Compared to control mice, 53 proteins were found to have undergone significant changes in the urine samples of asthmatic mice.

On days 2 and 8, 13 differential proteins that had human orthologs were reported to be closely associated

with asthma. Among these, six urinary proteins were changed on days 2 and 7, and the trend was consistent at each time point; these proteins hold the potential to be biomarkers for the early screening of asthma attack. For example, cathelicidin antimicrobial peptide (CRAMP) significantly enhanced the infiltration of inflammatory cells and accumulation of pro-inflammatory Th2 cytokine IL-13 and IL-33 in BALF and exacerbated lung tissue inflammation and airway goblet cell hyperplasia in a previous study.<sup>28</sup> CRAMP plays an important role in the accumulation of inflammatory monocyte-derived dendritic cells in the peribronchiolar tissue in allergic airway inflammation.<sup>29</sup> Serum eosinophil cationic protein 1 (ECP) was positively correlated with the severity of an



**Figure 3** Venn diagram of the differential urinary proteins in asthmatic mice compared with control mice.

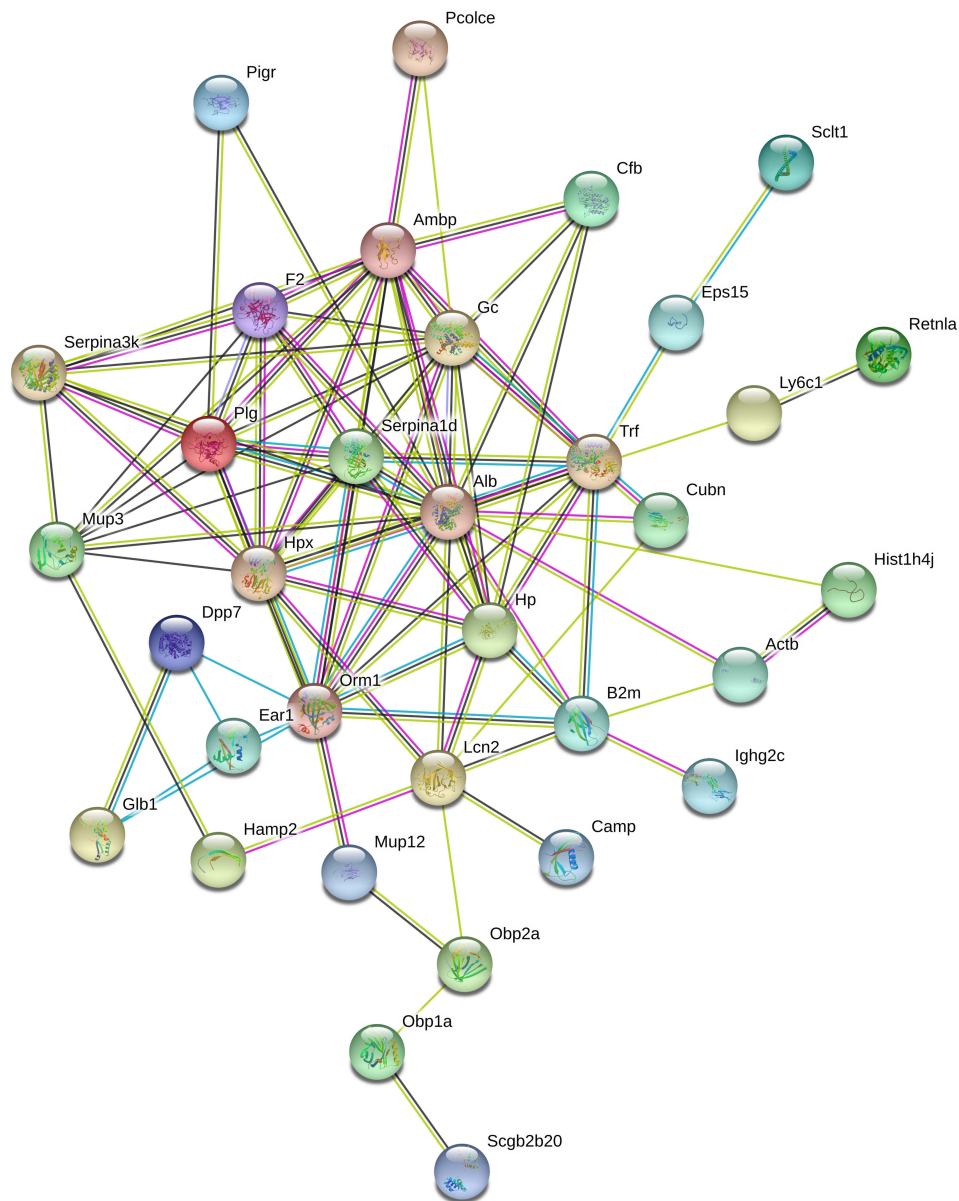


**Figure 4** GO analysis of the differential proteins at days 2, 8, 15 and 18 in OVA-induced mice. **(A)** Biological process; **(B)** Cellular component; **(C)** Molecular function.

asthma attack.<sup>30,31</sup> Elevated serum ECP level had the highest likelihood of fixed airflow obstruction among patients with asthma.<sup>32</sup> Serum ECP level is helpful in assessing the efficacy of inhaled corticosteroids therapy in bronchial asthma.<sup>33</sup> Increased serum haptoglobin (HP) level is seen in patients with asthma, asthma exacerbation and 24 hours after allergen challenge in late responders.<sup>34</sup> As part of its tissue repair function, HP can induce differentiation of fibroblast progenitor cells into lung fibroblasts in airway remodeling in patients with asthma.<sup>35</sup> Patients with asthma had 61% higher maximal prothrombin (PT) conversion rate than controls.<sup>36</sup> Plasma PT level was significantly higher in patients with nonallergic asthma than in controls.<sup>37</sup> Alpha-1-acid glycoprotein 1 was altered in patients with atopic asthma, and the presence of asthma symptoms correlated with increased glycan branching of AGP in both plasma and BALF.<sup>38</sup> Rhinovirus infection is

the most common trigger of the acute exacerbations of asthma; the infection stimulates the expression of not only complement factor B but also the novel pro-inflammatory cytokine IL-32.<sup>39</sup>

On days 15 and 18, the acute attack stage on which a high-dose of OVA was nasal inhalation to provoke asthma, seven differential proteins that had human orthologs were reported to be closely associated with asthma. Besides HP and vitamin D-binding protein (VDBP), which were altered on day 2, another five differential proteins also showed changes; these proteins hold the potential to be biomarkers for the diagnosis of asthma attack. For example, serum VDBP was significantly upregulated in the steroid-resistant asthma group compared with the steroid-sensitive asthma group in a previous study.<sup>40</sup> Another study reported upregulated VDBP expression both in serum and BALF of isocyanate-occupational asthma



**Figure 5** STRING PPI network analysis of the 53 differential proteins in OVA-induced asthma mouse model. The average node degree is 3.84, average local clustering coefficient is 0.443, and PPI enrichment p-value is  $<1.0e-16$ .

(OA) patients compared with controls.<sup>41,42</sup> These findings were consistent with those of studies in which VDBP was identified in the BALF of patients with asthma using proteomic analysis.<sup>43,44</sup> In vivo, Cathepsin E deficiency resulted in reduced lymphocyte influx after allergen sensitization and challenge in both investigated airway inflammation models.<sup>45</sup> Polymeric immunoglobulin receptor protein level was decreased in the bronchial epithelium from patients with asthma,<sup>46</sup> whereas PIGR demonstrated significantly enhanced upregulation in the BALF of OVA-sensitized mice.<sup>47</sup> The prevalence of alpha-1-antitrypsin 1–4 deficiency was low in patients with severe persistent

asthma.<sup>48</sup> Serotransferrin was upregulated in patients with methylene diphenyl diisocyanate (MDI)-induced occupational asthma and was used as serologic markers of MDI-OA.<sup>49</sup> Hemopexin was upregulated in the local lymph nodes and serum of toluene-2,4-diisocyanate-induced asthmatic mice.<sup>50</sup> Individuals with asthma–COPD overlap exhibited significantly elevated serum levels of HPX compared with healthy control subjects.<sup>51</sup>

Urine can be collected noninvasively and continuously, which is a good resource of biomarker for asthma. Despite the advantage of urine as a promising biomarker source, urine proteins are affected by many factors. Therefore, to

**Table 2** Differential Urinary Proteins Related to Asthma

UniProt ID	Protein Name	Human Homolog	Trend	Asthma
P51437	Cathelicidin antimicrobial peptide	P49913	↑	OVA-induced airway inflammation
P97426	Eosinophil cationic protein 1	P12724	↑	Bronchial asthma
P19221	Prothrombin	P00734	↑	Asthma
Q60590	Alpha-1-acid glycoprotein 1	P02763	↑	Asthma
P04186	Complement factor B	P00751	↑	Rhinovirus
Q99P86	Resistin-like protein beta	Q9BQ08	↑	Asthma, allergic asthma
P01887	Beta-2-microglobulin	P61769	↑	Asthma
P62806	Histone H4	P62805	↑	OVA-induced airway inflammation
P11672	Neutrophil gelatinase-associated lipocalin	P80188	↑	Asthma
Q9Z0M9	Interleukin-18-binding protein	O95998	↓	Asthma
P23780	Beta-galactosidase	P16278	↓	Neonatal and pediatric asthma
Q61646	Haptoglobin	P00738	↑	Asthma
P21614	Vitamin D-binding protein	P02774	↓	Asthma, isocyanate-occupational asthma
P70269	Cathepsin E	P14091	↑	Allergic asthma
O70570	Polymeric immunoglobulin receptor	P01833	↓	Asthma, OVA-induced airway inflammation
Q00897	Alpha-1-antitrypsin 1-4	P01009	↓	Severe asthma
Q92111	Serotransferrin	P02787	↓	Occupational asthma
Q91X72	Hemopexin	P02790	↓	TDI-induced asthma, asthma and COPD

sort out factors associated with any particular pathophysiological condition, especially in human samples, is challenging. To circumvent this problem, simpler systems, such as animal models, should be used to establish the direct relation between disease progression and urine changes. To avoid contamination, the mice were individually housed in the metabolic cages, and no food was provided during urine collection. To avoid protein degradation, the urine samples were centrifuged immediately after 4 hours collection, and the stored at  $-80^{\circ}\text{C}$ .

Overall, this study was a preliminary study with a small number of OVA-induced asthmatic mice. In future studies, the urinary protein biomarkers should be further evaluated in urine samples of patients with asthma to test their sensitivity and specificity. The findings of these studies will have potential applications in monitoring in treatment and prevention studies.

## Conclusions

Our results suggest that the urinary proteome could reflect dynamic pathophysiological changes in asthma; therefore, these differential proteins could be potential biomarkers for the early diagnosis of asthma.

## Acknowledgments

The National Key Research and Development Program of China (2018YFC0910202, 2016YFC1306300); National Natural Science Foundation of China (82000881);

Beijing Natural Science Foundation (7172076); Beijing Cooperative Construction Project (110651103); Beijing Normal University (11100704).

## Disclosure

The authors declare that they have no competing interests.

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