

# Metabolomic Clusters and Their Association with Small Airway Disease in Asthma

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**Background:** Asthma is a heterogeneous airway disease in which small airway dysfunction (SAD) plays a central role, but its biochemical basis are not yet fully understood. This study aimed to identify distinct serum metabolomic clusters in asthma and to evaluate their associations with physiologic and structural indicators of small airway involvement.

**Methods:** Fifty-four adults with well-controlled asthma were enrolled. Serum metabolites were quantified using proton nuclear magnetic resonance spectroscopy and liquid chromatography–tandem mass spectrometry. Hierarchical clustering of 40 metabolites identified metabolic subgroups. SAD was assessed using impulse oscillometry and structural airway remodeling was quantified using chest computed tomography. Multivariable linear regression was used to assess associations between metabolite concentrations and small airway indices after adjustment for demographic and clinical factors.

**Results:** Three metabolomic clusters were identified with distinct biochemical and clinical features. Cluster 1, enriched in lipid and fatty acid metabolites, showed higher airway resistance, thicker airway walls, and increased serum C-reactive protein levels, suggesting more pronounced small airway involvement. Cluster 2 was characterized by elevated levels of sugars and alkylamines while maintaining relatively preserved airway function. Cluster 3 had reduced concentrations of amino acids and tricarboxylic acid cycle metabolites, along with increased proportion of current smokers. Phenylalanine was inversely associated with airway resistance and a positive correlation with reactance, while methanol and dimethylamine showed opposite patterns. These relationships remained significant after multivariable adjustment, indicating independent metabolic correlates of small airway disease.

**Conclusion:** Systemic metabolic profiles are associated with small airway inflammation and structural remodeling through bidirectional interactions between circulating metabolites and airway pathophysiology.

**Keywords:** airway resistance, asthma, cluster analysis, metabolomics, small airway disease

## Introduction

Asthma is a heterogeneous chronic airway disease characterized by diverse phenotypes and endotypes with distinct biological mechanisms.<sup>1</sup> Standardized guideline-based therapies including inhaled corticosteroids (ICSs) often show heterogeneous treatment responses depending on the underlying endotype and phenotype.<sup>2,3</sup> Although asthma involves various biologic mechanisms contributing to its heterogeneity, small airway dysfunction (SAD) has been consistently recognized as a fundamental and universal feature of asthma pathogenesis. Indeed, the prevalence of SAD has been reported consistently high across asthma populations.<sup>4</sup> SAD is frequently observed even in patients with preserved spirometry and has been linked to poor symptom control, increased risk of exacerbations, and structural airway changes.<sup>4,5</sup> Complementary approaches such as impulse oscillometry (IOS) and advanced radiologic tools are needed

to detect SAD, because conventional spirometry alone is insufficient to capture these abnormalities.<sup>5–7</sup> Notably, a previous study demonstrated that IOS performed well in diagnosing small airway and obstructive diseases among hospitalized subjects, highlighting its utility as a sensitive and effort-independent modality in clinical settings where conventional spirometry often provides low diagnostic yield.<sup>8</sup> SAD is increasingly recognized as a treatable trait that may help identify patients at high risk and guide precision-based asthma management.<sup>9</sup> However, the mechanisms driving the development of SAD remain incompletely understood, which necessitates approaches such as metabolomics to figure out systemic biochemical pathways contributing to small airway pathology.

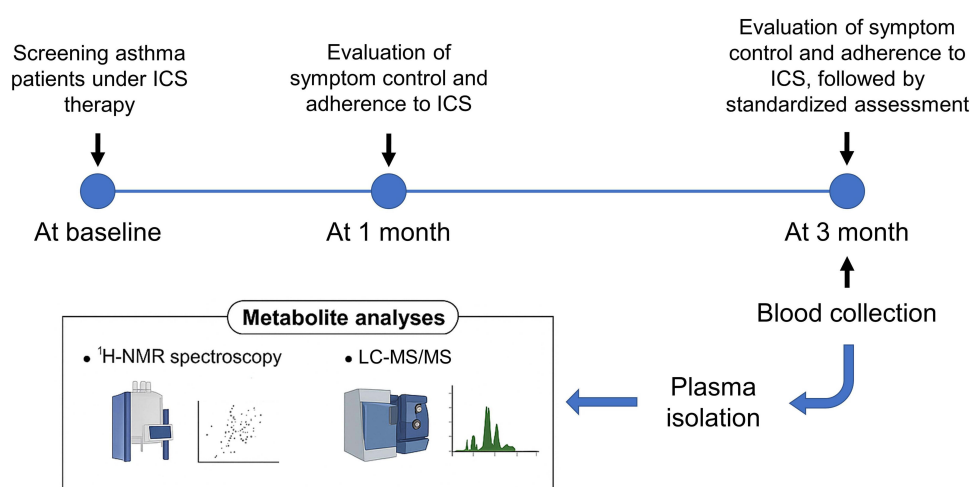
Metabolomics provides a comprehensive characterization of low-molecular weight metabolites that reflect the integrated outcome of genetic, transcriptomic, proteomic, and environmental influences on disease biology.<sup>10</sup> By capturing systemic biochemical alterations, metabolomic profiling has been considered as a potential tool to elucidate the molecular pathways that link systemic metabolism with airway inflammation, oxidative stress, and tissue remodeling.<sup>11,12</sup> Metabolomics studies have revealed that alterations in energy, amino acid, and lipid pathways, including impaired fatty acid metabolism and stearyl-coenzyme A desaturase activity, are linked to asthma pathogenesis, lung function, disease severity, and corticosteroid resistance.<sup>13–17</sup> However, most investigations have relied predominantly on spirometry-based endpoints or disease severity, providing limited insight into SAD. Integrating metabolomic signatures with physiologic measures has the potential to uncover novel biomarkers and provide insight into the mechanisms driving SAD.

Our study aimed to characterize serum metabolomic profiles in well-controlled asthma through clustering analysis and to evaluate their associations with SAD parameters.

## Materials and Methods

### Study Design and Experimental Setting

This cross-sectional observational study prospectively enrolled patients with specialist-diagnosed asthma who visited a teaching hospital between July 2023 and August 2024. Asthma was diagnosed on the basis of variable respiratory symptoms and objective evidence of variable expiratory airflow limitation, consistent with the Global Initiative for Asthma (GINA) guidelines. At the time of screening, patients receiving ICS therapy were evaluated to confirm the accuracy of the asthma diagnosis and to determine eligibility for longitudinal follow-up (Figure 1). Treatment adherence and symptom control were monitored for one month to ensure consistent ICS use and adequate asthma control, defined as an Asthma Control Test (ACT) score of 20 or higher. At three months from the screening visit, patients who continued to demonstrate stable asthma control and sustained adherence to ICS therapy underwent standardized clinical assessments and peripheral blood sampling after providing written informed consent.



**Figure 1** Study design and analysis workflow.

## Participants and Eligibility Criteria

Participants were eligible for inclusion if they met the following criteria: (1) a specialist-confirmed diagnosis of asthma, (2) continuous use of the same inhaled therapy, including both component and dose of ICS, for at least three months, (3) well-controlled asthma during the preceding three months, defined as an ACT score  $\geq 20$ , (4) no use of systemic corticosteroids within the preceding three months, (5) no history of acute exacerbation within the preceding three months, and (6) adequate treatment adherence, defined as a medication possession ratio (MPR)  $> 80\%$ .

Patients were excluded if they had concomitant pulmonary diseases other than asthma, such as chronic obstructive pulmonary disease (COPD), bronchiectasis, interstitial lung disease including pulmonary fibrosis, cystic fibrosis, lung cancer, or chronic respiratory infections. Individuals with rare genetic or structural airway disorders, including alpha-1 antitrypsin deficiency or primary ciliary dyskinesia, were also excluded. Uncontrolled systemic comorbidities with the potential to influence inflammatory or metabolic profiles were considered exclusionary, including active infections, autoimmune or hematologic disorders, hepatic, renal, cardiovascular, or endocrine diseases, as well as malignancies. In addition, patients receiving medications known to affect systemic inflammation or metabolism, such as systemic corticosteroids, immunosuppressants, antidiabetic agents, or lipid-lowering therapies, were not eligible for inclusion.

## Clinical Data Collection

Demographic and clinical information, including age, sex, body mass index (BMI), and age at asthma onset, was obtained at enrollment. Smoking history was categorized as never smoker, ex-smoker, or current smoker, and cumulative exposure was quantified in pack-years. Asthma severity was classified as mild, moderate, or severe according to clinical evaluation, with mild asthma corresponding to GINA steps 1–2, moderate asthma to steps 3–4, and severe asthma to step 5. Symptom control was assessed using the ACT score. A history of acute exacerbations during the preceding year was also recorded.

Pulmonary function testing included pre- and post-bronchodilator spirometry, with forced expiratory volume in one second (FEV<sub>1</sub>) and forced vital capacity (FVC) reported as percentages of predicted values. Fractional exhaled nitric oxide (FeNO) was measured concurrently to assess airway inflammation. IOS was used to evaluate small airway physiology, including resistance at 5 Hz minus 20 Hz (R5-R20), area of reactance (AX), reactance at 5 Hz (X5), and resonant frequency (Fres).

Peripheral blood analyses included routine chemistry tests (aspartate aminotransferase [AST], alanine aminotransferase [ALT], total bilirubin, and prothrombin time–international normalized ratio [PT-INR]), total immunoglobulin (Ig) E levels, multiple allergen simultaneous test (MAST) results, differential leukocyte counts, and serum C-reactive protein (CRP).

High-resolution computed tomography (CT) of the chest was performed to evaluate structural airway abnormalities and emphysematous changes. Airway wall thickness was quantified using the square root of wall area at an internal perimeter of 10 mm (Pi10), and airway wall area percentage was calculated. The extent of emphysema was expressed as the percentage of low attenuation areas (LAA) below  $-950$  Hounsfield units.

Asthma treatment regimens, including both inhaled therapies (ICS or ICS-containing combinations) and oral therapies, were documented at enrollment.

## Blood Collection and Metabolomic Profiling

Peripheral venous blood (10 mL) was collected into EDTA-treated vacutainer tubes. Samples were placed on ice immediately and transported under controlled temperature conditions for same-day processing. Plasma was separated by centrifugation at  $12,000 \times g$  for 15 minutes at  $4^{\circ}\text{C}$  and stored at  $-80^{\circ}\text{C}$  until analysis.

Metabolomic profiling was performed using  $^1\text{H}$  nuclear magnetic resonance (NMR) spectroscopy on a Bruker Avance III HD 600 MHz spectrometer (Bruker Biospin, Germany). Plasma samples were prepared in deuterium oxide ( $\text{D}_2\text{O}$ , pH 7.0) with 3-(trimethylsilyl)propionic-2,2,3,3- $\text{d}_4$  acid (TSP- $\text{d}_4$ ) as the internal standard. Spectra were acquired with a Carr–Purcell–Meiboom–Gill (CPMG) pulse sequence, processed using TopSpin software (Bruker Biospin,

Germany), and referenced to TSP. Metabolites were identified and quantified with Chenomx NMR Suite (Chenomx, Canada), supported by two-dimensional NMR experiments and cross-referenced with the Human Metabolome Database.

Targeted quantification of short-chain fatty acids (SCFAs) was additionally performed using liquid chromatography–tandem mass spectrometry (LC–MS/MS) with a Shimadzu Nexera X2 system coupled to an LCMS-8050 mass spectrometer. Chromatographic separation was achieved on a CORTECS C18 column (Waters, USA) with a binary gradient of water containing 0.1% formic acid (solvent A) and acetonitrile (solvent B). Optimized electrospray ionization conditions were applied for detection.

## Statistical Analysis

Clustering based on metabolites was performed to identify patient subgroups according to serum metabolomic profiles. K-means clustering of 40 quantified metabolites was applied, resulting in the classification of patients into three clusters. This classification was based on the optimal separation of metabolic patterns, as determined by the Gap statistic and Silhouette analysis. Principal component analysis (PCA) was subsequently conducted to visualize the distribution of patients in a reduced dimensional space, confirming distinct separation of the three metabolomic clusters. These clusters served as the analytic framework for all subsequent comparisons.

Baseline characteristics were summarized using descriptive statistics. Continuous variables were expressed as means with standard deviations, and compared across clusters using one-way analysis of variance (ANOVA). Post hoc pairwise comparisons were performed with Bonferroni correction. Categorical variables were presented as counts and percentages and analyzed using chi-squared tests or Fisher's exact tests.

Differences in individual metabolite concentrations among clusters were examined using ANOVA or nonparametric equivalents, with post hoc pairwise comparisons performed where indicated. Heatmaps were generated to visualize Z-score–transformed metabolite levels and highlight cluster-specific metabolic signatures.

Associations between serum metabolites and small airway disease (SAD) parameters measured by IOS (R5–R20, X5, Fres, and AX) were explored using univariable linear regression models. Metabolites showing nominal associations ( $P < 0.05$ ) were further assessed in multivariable regression models adjusted for age, sex, body mass index (BMI), and smoking status.

All analyses were performed using R software (version 4.5.0; R Foundation for Statistical Computing, Vienna, Austria). Two-sided  $P$ -values  $< 0.05$  were considered statistically significant.

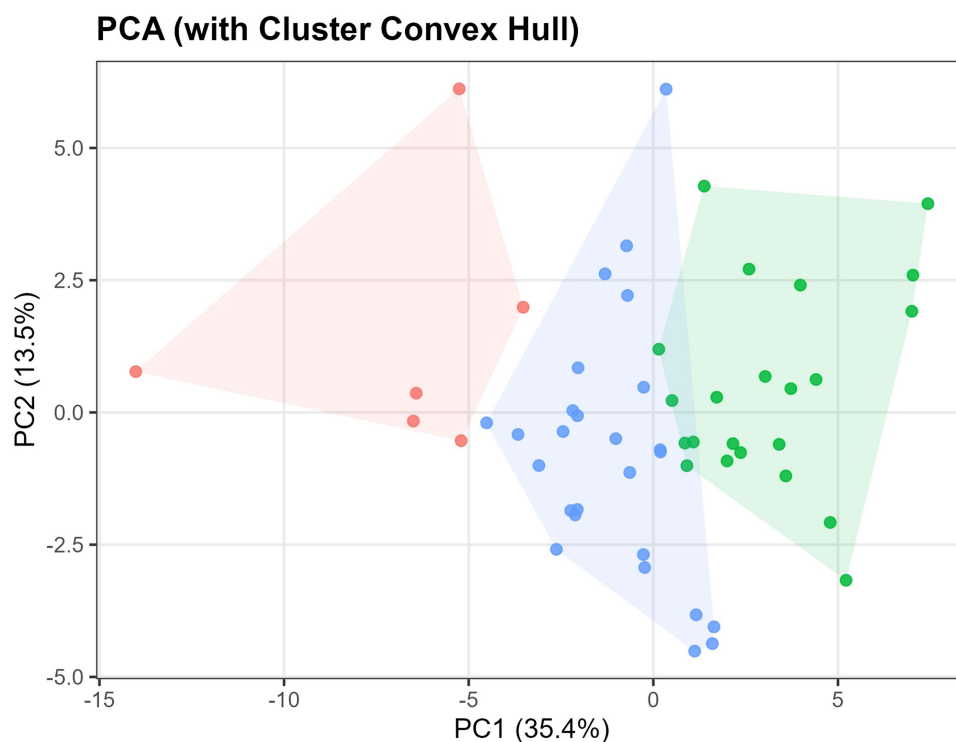
## Ethics

The study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki and complied with all applicable regulatory requirements. Written informed consent was obtained from all participants at the time of enrollment. The study protocol received approval from the Institutional Review Boards of the participating institutions, including the Seoul Metropolitan Government–Seoul National University Boramae Medical Center (IRB No. 30–2023–14) and the Seoul National University Institutional Review Board (IRB No. E2308/003-009).

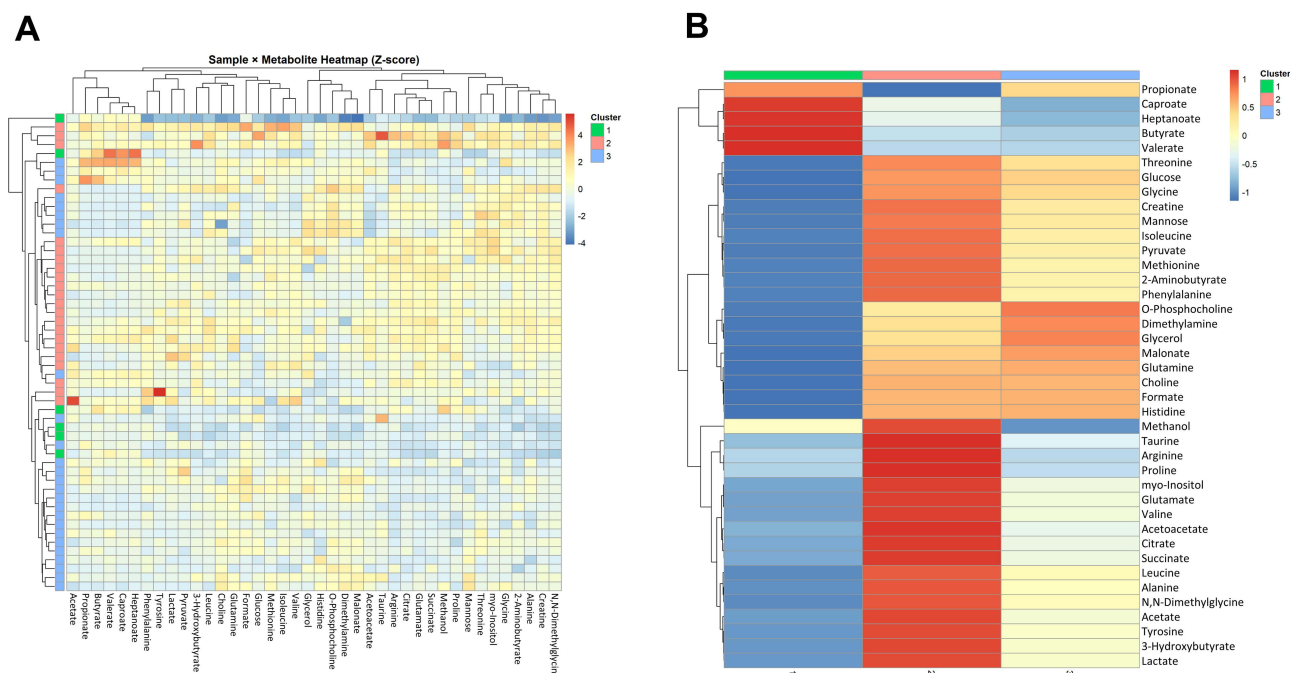
## Results

### Metabolite Profiles and Cluster-Specific Signatures

A total of 54 patients with physician-diagnosed asthma were enrolled, and hierarchical clustering of serum metabolomic profiles classified them into three distinct clusters (Cluster 1,  $n=6$ ; Cluster 2,  $n=22$ ; Cluster 3,  $n=26$ ). In the PCA plot, the three clusters were distributed in distinguishable patterns (Figure 2). The top ten metabolite loadings for PC1 and PC2 are presented in [Supplementary Figure 1](#). In addition, heatmap analysis revealed distinct cluster-specific metabolite profiles (Figure 3). Detailed plasma metabolite profiles according to asthma clusters are provided in [Supplementary Table 1](#). Cluster 1 showed an enrichment of metabolites related to lipid and fatty acid metabolism. Cluster 2 was characterized by elevated levels of sugars and alkylamines, while Cluster 3 exhibited reduced levels of amino acids and metabolites related to the tricarboxylic acid (TCA) cycle.



**Figure 2** Principal component analysis (PCA) of metabolomic clusters. Principal component analysis (PCA) plot of 54 asthma patients based on ~40 serum metabolites. Each dot represents one individual, colored according to the assigned cluster (red = Cluster 1, green = Cluster 2, blue = Cluster 3). Ellipses indicate the 95% confidence intervals for each cluster. The first two principal components (PC1 and PC2) explain 35.4% and 13.5% of the variance, respectively, demonstrating partial separation of three metabolic phenotypes.



**Figure 3** Cluster-specific metabolomic profiles. (A) Heatmap of Z-score-standardized metabolite levels (rows = metabolites, columns = individual patients). Patients are ordered by cluster assignment (Cluster 1, 2, 3). (B) Average Z-score profiles of each cluster across all metabolites. Cluster 1 is characterized by relatively higher levels of fatty acid metabolites; Cluster 2 by sugars and alkylamines; and Cluster 3 by lower levels of amino acids and TCA cycle-related metabolites.

## Patient Characteristics

Baseline demographic and clinical characteristics of each cluster are summarized in Table 1. Patients in Cluster 1 were older than those in Clusters 2 and 3 although the difference was not statistically significant. The distribution of smoking status demonstrated a significant difference, with a higher proportion of current smokers in Cluster 3 compared with Clusters 1 and 2 ( $P=0.024$ ). Sex, BMI, asthma severity, and ACT scores were comparable among clusters.

**Table 1** Baseline Characteristics According to Metabolomic Clusters

|                              | Cluster 1<br>(N=6) | Cluster 2<br>(N=22) | Cluster 3<br>(N=26) | p-value |
|------------------------------|--------------------|---------------------|---------------------|---------|
| Age                          | 75.7±14.3          | 57.5±15.1           | 55.7±21.7           | 0.065   |
| Male sex                     | 4 (66.7%)          | 10 (45.5%)          | 14 (53.8%)          | 0.628   |
| BMI                          | 23.1±4.8           | 24.8±3.8            | 23.9±4.1            | 0.594   |
| Smoking_status               |                    |                     |                     | 0.024   |
| Non-smoker                   | 1 (16.7%)          | 13 (59.1%)          | 10 (38.5%)          |         |
| Ex-smoker                    | 5 (83.3%)          | 6 (27.3%)           | 7 (26.9%)           |         |
| Current smoker               | 0 (0.0%)           | 3 (13.6%)           | 9 (34.6%)           |         |
| Smoking pack-year (PY)       | 33.8±28.6          | 10.2±22.3           | 18.9±24.5           | 0.097   |
| Asthma onset                 |                    |                     |                     | 0.499   |
| Adult onset                  | 6 (100%)           | 21 (95.5%)          | 23 (88.5%)          |         |
| Childhood onset              | 0 (0%)             | 1 (4.5%)            | 3 (11.5%)           |         |
| Asthma severity              |                    |                     |                     | 0.218   |
| mild                         | 3 (50.0%)          | 11 (50.0%)          | 8 (30.8%)           |         |
| Moderate                     | 3 (50.0%)          | 6 (27.3%)           | 15 (57.7%)          |         |
| Severe                       | 0 (0.0%)           | 5 (22.7%)           | 3 (11.5%)           |         |
| mMRC                         |                    |                     |                     | 0.334   |
| 0                            | 1 (16.7%)          | 6 (27.3%)           | 6 (23.1%)           |         |
| 1                            | 4 (66.7%)          | 13 (59.1%)          | 20 (76.9%)          |         |
| 2                            | 1 (16.7%)          | 3 (13.6%)           | 0 (0.0%)            |         |
| Spirometry                   |                    |                     |                     |         |
| PreBD FEV1 (%pred)           | 84.5±23.5          | 86.9±24.5           | 96.9±16.4           | 0.181   |
| PostBD FEV1 (%pred)          | 88.3±21.5          | 88.8±25.9           | 99.4±14.2           | 0.168   |
| PreBD FVC (%pred)            | 91.5±10.9          | 93.4±21.0           | 98.8±16.6           | 0.487   |
| PostBD FVC (%pred)           | 92.3±11.0          | 94.1±19.9           | 100.0±16.7          | 0.416   |
| FeNO (ppb)                   | 64.0±93.5          | 47.6±51.2           | 51.6±54.9           | 0.832   |
| IOS parameters               |                    |                     |                     |         |
| R5-R20                       | 0.3±0.3            | 0.1±0.1             | 0.1±0.1             | 0.032   |
| AX                           | 2.4±2.5            | 1.5±1.1             | 1.0±0.9             | 0.056   |
| X5                           | -0.2±0.1           | -0.2±0.1            | -0.2±0.1            | 0.419   |
| Fres                         | 21.5±7.2           | 18.8±6.2            | 17.2±5.2            | 0.233   |
| Blood neutrophil (%)         | 59.7±5.9           | 57.8±12.5           | 55.1±9.0            | 0.502   |
| Blood eosinophil (%)         | 3.3±3.0            | 4.6±5.5             | 4.9±3.2             | 0.706   |
| Blood eosinophil count (/μL) | 257.9±260.7        | 340.5±460.4         | 331.3±240.4         | 0.874   |
| Serum CRP (mg/dL)            | 1.3±1.4            | 0.4±0.6             | 0.4±0.6             | 0.032   |
| AST (U/L)                    | 22.8±7.4           | 22.0±6.6            | 23.2±7.6            | 0.827   |
| ALT (U/L)                    | 20.2±8.6           | 18.4±7.7            | 20.3±10.3           | 0.758   |
| Total bilirubin (mL/dL)      | 0.6±0.3            | 0.6±0.3             | 0.5±0.2             | 0.537   |
| PT-INR                       | 1.0±0.0            | 1.0±0.1             | 1.1±0.1             | 0.611   |
| Creatinine (mg/dL)           | 1.0±0.4            | 0.9±0.2             | 0.8±0.2             | 0.369   |
| IgE (IU/mL)                  | 341.0±409.4        | 449.3±640.5         | 325.3±390.4         | 0.699   |
| MAST positive                | 2 (33.3%)          | 10 (45.5%)          | 9 (34.6%)           | 0.713   |

(Continued)

**Table 1** (Continued).

|                                         | Cluster 1<br>(N=6) | Cluster 2<br>(N=22) | Cluster 3<br>(N=26) | p-value |
|-----------------------------------------|--------------------|---------------------|---------------------|---------|
| CT parameters                           |                    |                     |                     |         |
| Pi10 (mm)                               | 4.9±0.4            | 4.5±0.7             | 4.0±0.6             | 0.002   |
| Wall area (%)                           | 70.4±4.5           | 67.3±7.1            | 61.6±6.8            | 0.005   |
| LAA <-950 HU (%)                        | 3.0±3.9            | 2.5±3.6             | 2.6±2.4             | 0.941   |
| Treatment (Inhaler)                     |                    |                     |                     | 0.629   |
| ICS                                     | 0 (0.0%)           | 1 (4.5%)            | 0 (0.0%)            |         |
| ICS + LABA                              | 5 (83.3%)          | 18 (81.8%)          | 20 (76.9%)          |         |
| ICS + LAMA                              | 0 (0.0%)           | 1 (4.5%)            | 0 (0.0%)            |         |
| ICS + LABA + LAMA                       | 1 (16.7%)          | 2 (9.1%)            | 6 (23.1%)           |         |
| Treatment - LTRA                        | 2 (33.3%)          | 12 (54.5%)          | 9 (34.6%)           | 0.337   |
| Past exacerbation history within 1 year | 0 (0.0%)           | 0 (0.0%)            | 1 (3.8%)            | 0.578   |

**Abbreviations:** BMI, body mass index; PY, pack-year; mMRC, modified Medical Research Council; BD, bronchodilator; FEV<sub>1</sub>, forced expiratory volume in 1 second; FVC, forced vital capacity; FeNO, fractional exhaled nitric oxide; IOS, impulse oscillometry; R5-R20, difference between resistance at 5 Hz and 20 Hz; AX, Area of Reactance; X5, reactance at 5 Hz; Fres, resonant frequency; CRP, C-reactive protein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; PT-INR, prothrombin time-international normalized ratio; IgE, immunoglobulin E; MAST, multiple allergen simultaneous test; CT, computed tomography; Pi10, square root of wall area of a hypothetical airway with an internal perimeter of 10 mm; LAA, low attenuation area; HU, Hounsfield unit; ICS, inhaled corticosteroid; LABA, long-acting  $\beta_2$ -agonist; LAMA, long-acting muscarinic antagonist; LTRA, leukotriene receptor antagonist.

Pulmonary function tests revealed no statistically significant differences in pre- or post-bronchodilator FEV<sub>1</sub> and FVC across clusters, although Cluster 3 showed a tendency for higher values relative to the other clusters. FeNO levels were similar among groups. In contrast, IOS results showed significant difference in small airway parameters among the clusters. In the overall analysis, R5-R20 and serum CRP levels showed significant heterogeneity across clusters ( $P=0.032$  for both), exhibiting a trend of being highest in Cluster 1, intermediate in Cluster 3, and lowest in Cluster 2. However, post-hoc pairwise comparisons with Bonferroni correction indicated that these differences did not reach statistical significance between specific subgroups ([Supplementary Table 2](#)). The associations between metabolites and IOS parameters were identified in univariable analyses ([Supplementary Table 3](#)). In contrast, structural airway parameters showed robust differences between clusters. Cluster 1 exhibited the greatest airway wall thickness (Pi10, 4.9 mm;  $P=0.002$ ) and wall area percentage (70.4%;  $P=0.005$ ), and pairwise comparisons confirmed significantly higher values in Cluster 1 and Cluster 2 compared to Cluster 3.

## Serum Metabolites and SAD Parameters

Linear regression analyses identified five metabolites including dimethylamine, glycerol, malonate, methanol, and phenylalanine that were associated with at least one of the SAD indices derived from impulse oscillometry (R5-R20, X5, Fres and AX) ([Supplementary Table 4](#)). Cluster specific differences were observed for dimethylamine, malonate, methanol, and phenylalanine ([Table 2](#)). In multivariable regression models adjusted for age, sex, BMI and smoking status, three metabolites were significantly associated with SAD parameters derived from IOS ([Table 3](#)). Phenylalanine

**Table 2** Differences in Metabolites Associated with SAD Across Metabolomic Clusters

|               | Cluster 1<br>(N=6) | Cluster 2<br>(N=22) | Cluster 3<br>(N=26) | p-value |
|---------------|--------------------|---------------------|---------------------|---------|
| Dimethylamine | 10.5±4.1           | 16.1±3.1            | 17.6±2.6            | <0.001  |
| Methanol      | 4.2±4.2            | 6.2±3.1             | 2.5±1.1             | <0.001  |
| Phenylalanine | 15.8±7.0           | 24.1±5.3            | 20.9±3.3            | 0.001   |
| Malonate      | 166.2±58.4         | 252.6±39.4          | 265.0±30.6          | <0.001  |

**Abbreviation:** SAD, small airway disease.

**Table 3** Multivariate Analysis of the Associations Between SAD Parameters and SAD-Associated Metabolites

| SAD Parameters | Metabolites   | $\beta$ [95% CI]        | p-value |
|----------------|---------------|-------------------------|---------|
| R5-R20         | Phenylalanine | −0.034 [−0.066, −0.002] | 0.036   |
|                | Dimethylamine | −0.031 [−0.064, 0.003]  | 0.074   |
|                | Methanol      | 0.039 [0.009, 0.068]    | 0.012   |
|                | Malonate      | −0.027 [−0.063, 0.009]  | 0.136   |
| AX             | Phenylalanine | −0.398 [−0.727, −0.069] | 0.019   |
|                | Dimethylamine | −0.403 [−0.747, −0.058] | 0.023   |
|                | Methanol      | 0.353 [0.038, 0.668]    | 0.029   |
|                | Malonate      | −0.257 [−0.631, 0.117]  | 0.174   |
| Fres           | Phenylalanine | −1.050 [−2.654, 0.554]  | 0.194   |
|                | Methanol      | 1.039 [−0.483, 2.561]   | 0.176   |
|                | Dimethylamine | −1.061 [−2.735, 0.613]  | 0.208   |
|                | Malonate      | −0.253 [−2.037, 1.530]  | 0.776   |
| X5             | Phenylalanine | 0.034 [0.011, 0.057]    | 0.004   |
|                | Dimethylamine | 0.016 [−0.009, 0.042]   | 0.204   |
|                | Methanol      | −0.012 [−0.036, 0.011]  | 0.300   |
|                | Malonate      | 0.014 [−0.013, 0.041]   | 0.302   |

**Notes:** Adjusted variables include age, sex, BMI, smoking status R5-R20, difference between resistance at 5 Hz and 20 Hz; AX, Area of Reactance; X5, reactance at 5 Hz; Fres, resonant frequency.

was significantly associated with lower R5-R20 ( $\beta$ =−0.034 [95% CI −0.066, −0.002];  $P$ =0.036) and AX ( $\beta$  =−0.398 [−0.727, −0.069];  $P$ =0.019), and higher X5 ( $\beta$ =0.034 [0.011, 0.057];  $P$ =0.004). Dimethylamine was inversely associated with AX ( $\beta$ =−0.403 [−0.747, −0.058];  $P$ =0.023). Methanol showed positive associations with R5-R20 ( $\beta$  =0.039 [0.009, 0.068];  $P$ =0.012) and AX ( $\beta$  =0.353 [0.038, 0.668];  $P$ =0.029).

## Discussion

In this study, hierarchical clustering of serum metabolomic profiles from patients with well-controlled asthma identified three distinct clusters with differentiated metabolic signatures. Cluster 1, enriched with lipid and fatty acid metabolites, was associated with adverse small airway characteristics, including higher R5-R20, increased airway wall thickness, greater wall area percentage, and elevated systemic inflammation as reflected by CRP levels. Cluster 2, characterized by higher concentrations of sugars and alkylamines, demonstrated relatively preserved small airway indices, whereas Cluster 3, defined by lower levels of amino acids and tricarboxylic acid cycle intermediates, showed intermediate impairment in airway physiology and a higher prevalence of current smokers. When examining specific metabolites, phenylalanine, dimethylamine, and methanol were significantly correlated with impulse oscillometry parameters such as R5-R20, X5, indicating their potential contribution to the pathogenesis of SAD. These findings delineate discrete systemic metabolic phenotypes that parallel variations in small airway physiology, emphasizing interconnected metabolic–inflammatory pathways underlying asthma pathobiology.

Previous studies have emphasized the heterogeneity of asthma pathogenesis and the need to identify subgroups underlying different biological mechanisms.<sup>18</sup> Metabolomics has emerged as a promising approach to characterize systemic biochemical changes that may play a role in airway inflammation and remodeling.<sup>19,20</sup> Several studies have reported correlations between amino acid metabolism, particularly phenylalanine and branched-chain amino acids, and the clinical manifestations of asthma.<sup>21–23</sup> Dimethylamine lies on the asymmetric dimethylarginine (ADMA)–dimethylarginine dimethylaminohydrolase (DDAH) axis, which regulates nitric oxide bioavailability and reactive oxygen species generation. This association connects the metabolite to oxidative stress and inflammatory responses in cardiorespiratory and systemic contexts.<sup>24–27</sup> Our findings enhance these observations by relating specific metabolites, including

phenylalanine, dimethylamine, and methanol, with SAD parameters derived from IOS, which is recognized as a treatable trait before the onset of irreversible airway remodeling.<sup>9</sup>

The metabolomic differences observed across clusters may reflect various biochemical pathways contributing to SAD pathogenesis in asthma.<sup>28</sup> Lipid and fatty acid metabolites enriched in Cluster 1 are closely linked to airway inflammation and remodeling processes, as altered lipid signaling can modulate epithelial barrier integrity, mucus hypersecretion, and immune cell recruitment.<sup>29,30</sup> Elevated systemic CRP levels in this cluster may indicate shared inflammatory activity between systemic and airway compartments, linking metabolic and local inflammatory processes.<sup>31</sup> Amino acid metabolism, particularly phenylalanine, has been implicated in the regulation of oxidative stress and nitric oxide pathways, which may explain its associations with IOS parameters such as R5-R20, AX, and X5.<sup>32</sup> Reduced phenylalanine levels correlated with improved small airway indices in our study, suggesting that perturbations in aromatic amino acid metabolism could influence airway compliance and resistance.<sup>33</sup> Among transcriptomic markers, *ALDH2* has been associated with asthma endotypes, and given its role in aldehyde detoxification and one-carbon metabolism, its link to metabolites such as methanol and dimethylamine suggests that metabolomic profiling may provide endotype-specific insights into the mechanisms of SAD.<sup>34</sup> Methanol, which correlated positively with adverse SAD parameters, may reflect increased oxidative stress or microbial metabolic activity in the small airway.<sup>35</sup> Interestingly, in contrast to previous reports linking dimethylamine to oxidative stress and inflammatory responses,<sup>24–27</sup> our study found protective associations with SAD indices, suggesting that its role may differ depending on disease context and that one-carbon metabolism and methylation balance could modulate airway inflammatory responses.<sup>36</sup> It is reasonable to propose that specific metabolic alterations may regulate oxidative stress, epithelial remodeling, and immune activation, thereby linking systemic biochemical shifts with localized small airway pathology.

Radiologic findings in chest CT such as bronchial wall thickening and emphysema represent clinically important phenotypes, as they are associated with the disease course and prognosis of asthma.<sup>37,38</sup> Growing evidence indicates that metabolomic alterations can be closely associated with radiologic manifestations of airway and parenchymal disease. In COPD, distinct metabolic signatures have been linked to emphysema severity and air-trapping patterns on computed tomography, supporting the concept that systemic metabolic states may reflect localized structural abnormalities.<sup>39</sup> Metabolomic analyses, when combined with radiomic features, have enhanced the prediction of tumor heterogeneity and treatment response in cancer treatment.<sup>40</sup> Our study demonstrates that serum metabolomic profiles in asthma can be integrated with imaging-based indices such as Pi10 and airway wall area, connecting systemic biochemical alterations with structural remodeling of the small airways. Such integrative approaches provide a multidimensional perspective on asthma biology and hold possibility for refining patient stratification.

This study has several limitations. First, it was conducted at a single center with a relatively small sample size, which may limit statistical power and the generalizability of the findings. Second, the cross-sectional design precludes conclusions regarding temporal or causal relationships between metabolomic alterations and SAD. Third, despite careful selection of participants with stable asthma and consistent ICS use, residual confounding factors such as dietary patterns, environmental exposures, or microbiome composition were not fully accounted for and may have influenced systemic metabolomic profiles. Fourth, the analysis was restricted to serum metabolites without integration of other omics layers such as transcriptomics or proteomics, which could provide a more comprehensive understanding of the biological pathways involved. Finally, external validation in independent cohorts is necessary to confirm the robustness and reproducibility of these associations.

In conclusion, specific metabolites, including phenylalanine, dimethylamine, and methanol, were associated with IOS-derived parameters and radiologic measures. Metabolomic clustering of patients with well-controlled asthma revealed distinct biochemical profiles associated with SAD, indicating interrelated metabolic states and airway mechanisms that underlie asthma pathophysiology.

## AI Use Statement

Artificial intelligence tools were not used in the conception, design, analysis, or original drafting of this manuscript. ChatGPT was utilized solely to assist with English language editing. All scientific content was developed, analyzed, and written exclusively by the author.

## Data Sharing Statement

The data supporting this study's findings are available upon reasonable request from the corresponding author. Restrictions may apply due to ethical considerations.

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