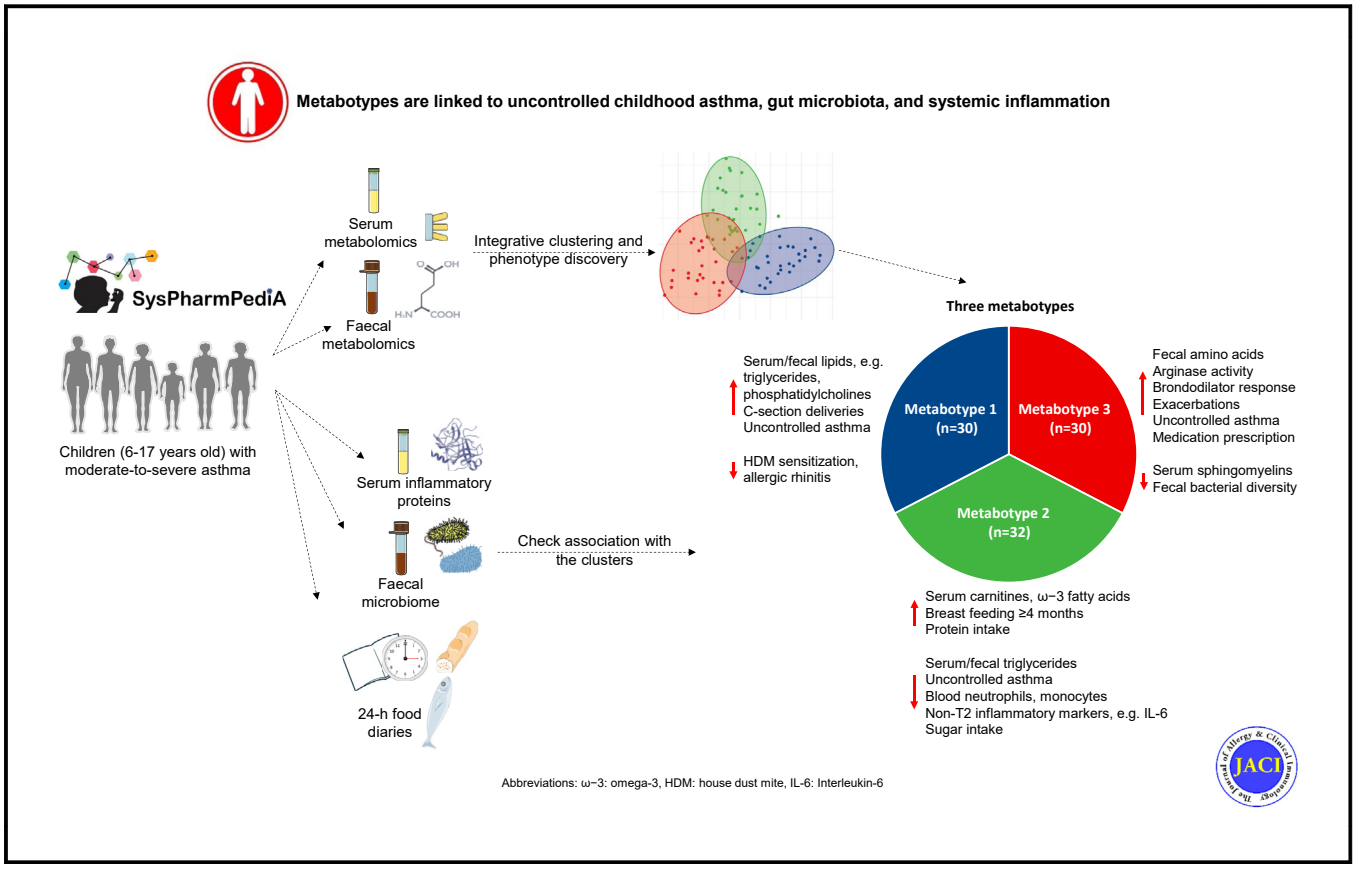
# Metabotypes are linked to uncontrolled childhood asthma, gut microbiota, and systemic inflammation

**Mahmoud I. Abdel-Aziz, PhD, Simone Hashimoto, MD, PhD, Anne H. Neerincx, PhD, Eric G. Haarman, MD, PhD, Alexander Cecil, PhD, Jutta Lintelmann, PhD, et al**

## GRAPHICAL ABSTRACT




**Capsule summary:** Serum and fecal-based metabotyping identified 3 childhood asthma subtypes linked with asthma burden, and gut microbiota, and non-T2 inflammation supporting the role of certain, for example, lipid pathways in the development of severe childhood asthma.

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# Metabotypes are linked to uncontrolled childhood asthma, gut microbiota, and systemic inflammation

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**Background: Childhood asthma has been linked to distinct metabolomic profiles.**

**Objective: We sought to identify phenotypes (metabotypes) in children with moderate to severe asthma through integrative fecal and serum metabolome analysis.**

*Abbreviations used*
FDR: False-discovery rate
FENO: Fractional exhaled nitric oxide

**Methods: Children from the Systems Pharmacology Approach to Uncontrolled Pediatric Asthma cohort with Global Initiative for Asthma treatment step 3 or higher were recruited. Asthma control was defined by the Asthma Control Test and annual exacerbation history. Targeted metabolomic profiling of feces and serum was performed using liquid chromatography and flow injection electrospray ionization-triple quadrupole mass spectrometry. Similarity network fusion integrated fecal and serum metabolome profiles, followed by spectral clustering. Clusters were analyzed for differences in asthma characteristics, food diaries, fecal microbiota composition, and levels of serum inflammatory markers and blood cells.**

**Results: Integrative fecal and serum metabolome analysis of 92 children with moderate to severe asthma (median age, 11.5 years, 34% female) revealed 3 metabotypes. Metabotype 1 had the lowest percentage of allergic rhinitis, with elevated serum ceramides and triglycerides. Metabotype 2 had higher odds of asthma control, the highest percentage of children with 4 or more months of breast-feeding, reduced sugar intake, lowest levels of blood neutrophils and serum inflammatory markers, and elevated serum acylcarnitines and ω-3 fatty acids. Metabotype 3 included the highest percentage of uncontrolled asthma patients, with decreased serum cholesteryl esters, phosphatidylcholines, and sphingomyelins, elevated fecal amino acids, and reduced fecal microbiota diversity.**

**Conclusions: Metabotypes in children with moderate to severe asthma are linked to asthma control, distinct fecal microbiota, and systemic inflammatory patterns. The findings suggest that metabotyping can be valuable in precision medicine approaches for asthma. (J Allergy Clin Immunol 2025;156:339-51.)**

***Key words:*** *Moderate to severe childhood asthma, metabotyping, gut microbiota, inflammatory markers, precision medicine*

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Asthma is the most common chronic disease in children, characterized by airway inflammation with heterogeneity in clinical manifestations and treatment responses.[1] The burden of childhood asthma increases with its increasing severity and lack of control due to the increased health-related complications and the lack of suitable treatment options.[2]

Currently, asthma diagnosis and management are based on the evaluation of clinical symptoms, lung function tests, fractional exhaled nitric oxide (FENO), inflammatory biomarkers, and allergy testing.[3] However, these methods have limitations in accurately reflecting the heterogeneity of asthma, particularly in childhood.[4] Pinpointing asthma heterogeneity into clinically identifiable phenotypes (phenotyping) and understanding their underlying mechanisms (endotyping) will help to improve the care and treatment of asthma.[4] Omics-guided classification of subjects with asthma has been emerging as a systems medicine strategy for phenotyping and endotyping asthma.[4] It relies on using omics to help subtyping subjects with asthma while uncovering molecular pathways underlying the different subtypes. This may help to target specific molecular pathways for diagnosing or treating the different asthma subtypes.

Metabolites are small molecules involved in various biological processes, such as energy metabolism, immunity, inflammation, and oxidative stress.[5] In addition, metabolites are influenced by the genetic makeup and environmental factors,[6,7] making them potential biomarkers reflecting the cellar state of asthma and its heterogeneity. Metabotyping is the process of subtyping individuals on the basis of their metabolome profiles and is thought to help in subtyping subjects with different diseases and in guiding personalized therapeutic or nutritional decisions for each individual subject.[8] In adults with asthma, metabotyping has been successfully performed using the metabolome profiles in different sampling compartments, such as exhaled breath and serum, showing strong associations to asthma severity and inflammatory biomarkers.[9,10] Yet, limited research has been performed on childhood asthma. We hypothesize that metabolome profiles in serum and feces can reveal distinct clusters (metabotypes) in children with moderate to severe asthma.

The aims of this study were to (1) perform unsupervised integrative clustering of serum and fecal metabolome profiles of children with moderate to severe asthma and assess whether they are linked to distinct disease characteristics and (2) elucidate the possible underlying nutritional and biological connections of the revealed phenotypic clusters by investigating 24-hour food diaries, the fecal microbiome, and inflammatory markers.

## METHODS

### Study design

Systems Pharmacology Approach to Uncontrolled Pediatric Asthma (SysPharmPediA) is a European multicenter observational study involving children aged 6 to 17 years with physician-diagnosed asthma from the Netherlands, Germany, Spain, and Slovenia.[11] Ethics approval was obtained, informed consent was given by parents/caregivers, and assent was given by children where appropriate. The study is registered at ClinicalTrials.gov (NCT04865575).

### Participants

Children (n = 145) with moderate to severe asthma (Global Initiative for Asthma treatment step ≥3) were included and 92 subjects provided paired samples for the analysis (see Fig E1 in this article's Online Repository at www.jacionline.org). Uncontrolled asthma was defined by (childhood) Asthma Control Test score 19 or less and/or severe exacerbations requiring hospitalization or emergency room visits or oral corticosteroid use in the past year.[12,13] Subjects were evaluated for allergy, atopy, spirometry, FENO, blood inflammatory biomarkers, and medical history.

### Sample collection and omics analysis

Sample collection and detailed omics analyses are described in this article's Online Repository at www.jacionline.org. Subjects with available paired fecal and serum samples were compared with subjects not included in the study across different characteristics (see Table E1 in this article's Online Repository at www.jacionline.org).

### Fecal and serum metabolomics analysis

Metabolomic profiling was performed using the Biocrates MxP Quant 500 kit (BIOCRATES Life Sciences AG, Innsbruck, Austria), which resulted in hundreds of metabolites belonging to various classes (see Table E2 in this article's Online Repository at www.jacionline.org) and their relevant ratios. Metabolites with more than 40% missing values were excluded. Remaining values below the limit of detection were imputed,[14] and those with more than 25% coefficient of variation were excluded. Spearman correlation analysis was conducted between shared metabolites in serum and feces, and $P$ values were adjusted by Benjamini-Hochberg false-discovery rate (FDR).

### Fecal 16S V3-V4 rRNA microbiome sequencing

Amplicon sequencing was performed.[15] Quality control was performed by DADA2 pipeline[16] with SILVA database version 138.[17] The microbiome data are deposited on BioProject (PRJNA867125).

### Serum cytokines and chemokines

Differential blood count was performed using fluorescence flow cytometry, and a panel of serum cytokines and chemokines was measured using a Luminex multiplex assay as described.[18] Protein data were imputed similarly to the metabolites.

### Food diaries

Participants completed a 24-hour dietary recall diary 1 day before the study visits to estimate food intake.[19] Nutrient calculations were performed using the "eetmeter" tool.[20]

### Data and statistical analysis

The general data analysis workflow is shown in Fig E2 (in the Online Repository available at www.jacionline.org) and described in detail in the Online Repository. Briefly, clustering was based on serum and fecal metabolomics using similarity network fusion.[21] Both omics layers were normalized, scaled, and converted into Euclidean distances, then into patient affinity matrices. The matrices were fused into a single similarity matrix (parameters, $K = 10$, $\alpha = 0.5$, and $T = 20$) as recommended.[21] The optimum number of clusters was determined using eigengap,

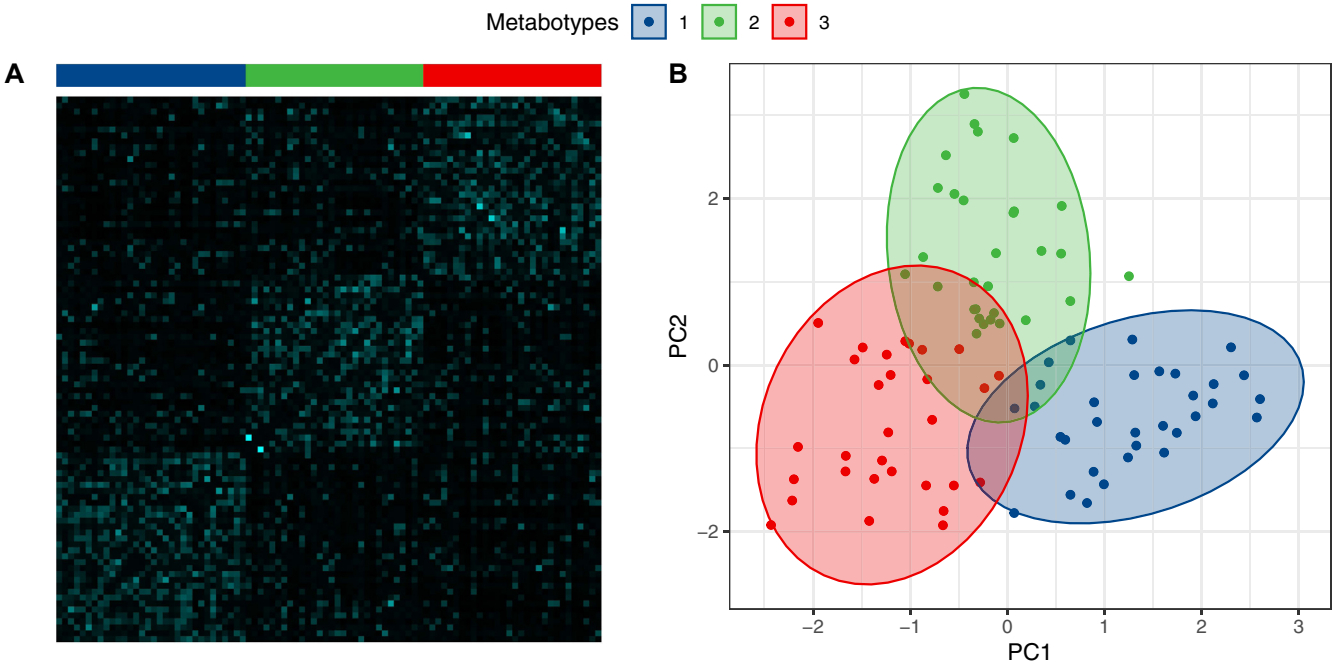


**FIG 1. A,** Heatmap of similarity network fusion of serum and fecal metabolomics in the SysPharmPediA moderate to severe childhood asthma cohort, showing 3 identifiable clusters (metabotypes). **B,** Kernel principal-component analysis plot showing the separation of the metabotypes based on their similarity network of the 2 metabolomics blocks based on the 2-dimensional depiction of the first 2 principal components with the 95% confidence ellipses. *PC,* Principal component.

rotation cost, silhouette's width, within cluster sum of squares, and gap statistics.[21] A heatmap and kernel principal-component analysis were used to visualize the similarity network. The metabotypes were checked for differences in asthma characteristics using chi-square tests with Monte-Carlo simulation (10,000 permutations) or Kruskal-Wallis H tests as appropriate, followed by *post hoc* analyses for nominally significant associations.[22]

Covariates identified using directed acyclic graph (see Fig E3 in this article's Online Repository at www.jacionline.org) included age, sex, ethnicity, country of inclusion, body mass index *z* score, recent antibiotic intake, season of inclusion, corticosteroid (nasal/inhaled/oral) intake, and Medication Adherence Report Scale-5 scores were adjusted for in a multivariate logistic regression model (asthma control was the dependent outcome and metabotypes and covariates were included as independent parameters). An additional model with a reduced number of covariates (age, sex, and country of inclusion) was then fitted to minimize the risk of overfitting. As a sensitivity analysis, atopic sensitization was included as an additional covariate to assess whether the association between metabotypes and asthma control was explained by atopy.

To benchmark whether the integrative clustering based on dual metabolomics blocks offers more clinical relevance than each individual metabolomics block, independent clustering was performed on separate omics layers and compared in relation to asthma burden using chi-square tests with Monte-Carlo simulation.

### Differences in omics layers between the clusters

Fecal and serum metabolites, serum cytokines, and nutrient intake values were compared between metabotypes using the Kruskal-Wallis H test, followed by FDR correction. *Post hoc* analysis identified specific enriched/depleted metabolites per metabotype, and then grouped by metabolic classes. Differences in microbial richness and Shannon α diversity were assessed between the metabotypes, and weighted UniFrac β diversity was analyzed using PERMANOVA after adjusting for confounders. Microbiome Multivariable Associations with Linear Models 2 (MaAsLin 2[23]) checked differences in bacterial genera with FDR correction, while adjusting for most significant confounders. In addition, we assessed the direct correlation between fecal microbiome diversity (α and β) and metabolic classes by calculating mean *z* scores for metabolic classes and applying Spearman correlation with FDR correction, and PERMANOVA while adjusting for multiple confounders.

## RESULTS

### Combining fecal and serum metabolome revealed 3 different metabotypes

Ninety-two subjects provided dual serum and fecal samples that successfully underwent metabolomics profiling. The correlation between paired fecal and serum metabolites was generally low, with a mean ± SD of 0.06 ± 0.14. Only 5 metabolites showed statistically significant correlations between paired sample types (correlation coefficients $r_s > 0.3$ and $q$ value $< 0.05$), specifically 1,2-diacylglycerol (C16:0/C18:1), lyso-phosphatidylcholine (C16:0), triacylglycerol (C16:0/C34:1), triacylglycerol (C18:0/C36:2), and triacylglycerol (dC18:1/C36:0). Similarity network fusion based on serum and fecal metabolome profiles yielded 3 distinct clusters (ie, metabotypes) by spectral clustering (Fig 1), as evaluated by the majority

vote of multiple indices (see Table E3 and Fig E4, *A-E*, in this article's Online Repository at www.jacionline.org). The combination of serum and fecal metabolomics yielded more coherent clusters than each single omics block at all clustering K solutions (K 2:10, Fig E4, *F*). In addition, each omics block separately divided the subjects into an inconsistent number of clusters (Table E3), in contrast to the combined omics integration, which divided the subjects consistently into 3 clusters using different statistical indices.

## The metabotypes show distinct clinical characteristics and are associated with asthma control, allergy, and blood neutrophils

Subjects' baseline characteristics are presented in Table I. The median age of the included participants was 11 years, 46% were female, and 80% were White. The metabotypes showed statistically significant differences in mode of delivery, breast-feeding, country of inclusion, history of atopic sensitization (house dust mite, dog dander aeroallergens, and nuts food allergens), physician-diagnosed allergic diseases (allergic rhinitis and allergic conjunctivitis), blood inflammatory cells (neutrophils and monocytes), bronchodilator response, and leukotriene antagonist add-on asthma medication. Particularly, metabotype 1 had the highest percentage of subjects with C-section deliveries (n = 11 of 30 [37%]) and the lowest percentage of subjects with house dust mite atopic sensitization (n = 13 of 30 [43%]), allergic rhinitis (n = 17 of 29 [59%]), and allergic conjunctivitis (n = 15 of 28 [54%]). Furthermore, it had notable sensitization to pollens, mold, cat, and dog, but these findings were not statistically significant. Metabotype 2 had the highest percentage of subjects with 4 or more months of breast-feeding (n = 25 of 32 [78%]), lowest percentage of subjects with nut allergens (1 of 29 [3%]), the lowest blood monocytes (median, 0.46; interquartile range, 0.36-0.54) and neutrophil counts (median, 2.60; interquartile range, 1.92-3.54), and the lowest percentage of subjects prescribed leukotriene antagonists (n = 0 of 32 [0%]). Metabotype 3 had the highest percentage of subjects with dog dander aeroallergen sensitization (n = 13 of 28 [46%]). Moreover, the metabotypes showed differences in characteristics associated with asthma burden, including asthma control, severe exacerbations in the past year, more than 10 missed school days, and non–infection-related dry cough during the past year. In particular, metabotype 3 showed the largest percentage of subjects with uncontrolled asthma (unadjusted odds ratio, 4.41, 95% CI, 1.42-15.7, and 4.37, 95% CI, 1.39-15.8, relative to metabotype 2 and metabotype 1, respectively, all $P$ values < .05). However, after adjusting for multiple confounders, both metabotype 3 and 1 showed statistically significantly higher odds of uncontrolled asthma in comparison to metabotype 2 (see Table E4 in this article's Online Repository at www.jacionline.org; adjusted odds ratio, 11.7, 95% CI, 2.57-88, for metabotype 3 and 7.34, 95% CI, 1.22-68.9, for metabotype 1, both relative to metabotype 2, all $P$ values < .05). There were no statistically significant associations between atopic sensitization or sensitization to house dust mite and asthma control, either alone (data not shown) or after adjusting for covariates, including metabotype classification (see Tables E5 and E6 in this article's Online Repository at www.jacionline.org). Box 1 shows detailed summary of the phenotypic differences between the metabotypes.

## Integrative clustering exhibits stronger clinical relevance than single omics blocks

Six different clustering solutions for the individual serum and fecal layers were obtained (based on $K = 2{:}4$ highlighted in Table E3), none of which was significantly associated with asthma burden (including asthma control status, severe asthma exacerbations in the past year, and missed school >10 days in the past year, all $P$ values > .05, data not shown), with exception of the dry cough episodes in the past year, which merely showed associations to serum-based metabolome clustering solutions ($P$ values range, 0.012-0.087, with a lower effect size compared with clustering based on dual-omics integration) but not fecal-based metabolome clustering (data not shown). The significant association to different parameters of asthma burden is mainly seen when the clustering is performed on the integrative information of both omics blocks.

## The metabotypes reveal characteristic differences in metabolic classes

**Serum metabolome.** Hundreds (n = 377) of serum metabolites and associated ratios were differentially abundant between the metabotypes after FDR correction (see Excel file E1 in this article's Online Repository at www.jacionline.org). Differences in serum metabolic classes are described in Table II. Metabotype 1 demonstrated enriched serum levels of ceramides, cholesteryl esters, phosphatidylcholines, triglycerides, and diglycerides compared with the other metabotypes, but no characteristic depleted serum profiles. Metabotype 2 showed enriched serum levels of acylcarnitines (particularly tetradecadienylcarnitine), fatty acids (particularly docosahexenoic acid, an omega-3 fatty acid) and α-aminobutyric acid, and depleted serum levels of triglycerides, diglycerides, and amino acids (particularly glutamate) compared with the other metabotypes. Metabotype 3 showed elevated serum levels of triglycerides compared with metabotype 2, but less compared with metabotype 1 as well as an elevated ratio of ornithine-to-arginine (ie, ornithine synthesis) compared with the other metabotypes. Moreover, it showed characteristic depleted serum levels of sphingomyelins, phosphatidylcholines, cholesteryl esters, and ceramides as well as ratio of citrulline-to-ornithine (ie, citrulline synthesis) compared with the other metabotypes. Notably, no differences in steroid hormones (cortisol and cortisone) among the 3 metabotypes were observed.

**Fecal metabolome.** Similarly, several fecal metabolites (n = 136) and associated ratios were differentially abundant between the 3 metabotypes after FDR correction (see Excel file E2 in this article's Online Repository at www.jacionline.org). Differences in fecal metabolic classes are presented in Table III. Metabotype 1 displayed enriched fecal levels of phosphatidylcholines, sphingomyelins, and triglycerides, and depleted fecal levels of fecal amino acids compared with the other metabotypes. Metabotype 2 did not show characteristic enriched levels of fecal metabolic classes, but showed depleted fecal levels of ceramides, phosphatidyl cholines, sphingomyelins, triglycerides, and diglycerides compared with the other metabotypes. Metabotype 3 expressed elevated fecal levels of amino acids and ceramides compared with the other metabotypes, but did not show distinct depleted levels of fecal metabolic classes.

**TABLE I.** Demographic and clinical characteristics of the metabotypes

| Characteristics | All subjects (N = 92) | Metabotype 1 (n = 30) | Metabotype 2 (n = 32) | Metabotype 3 (n = 30) | *P* value |
|---|---|---|---|---|---|
| Age (y), median (IQR) | 11.54 (9.65 to 13.44) | 11.74 (9.65 to 14.11) | 11.58 (9.59 to 12.84) | 11.16 (9.65 to 13.20) | .880 |
| Sex: female, n (%) | 31 of 92 (34%) | 10 of 30 (33%) | 14 of 32 (44%) | 7 of 30 (23%) | .249 |
| White Caucasian, n (%) | 73 of 92 (79%) | 26 of 30 (87%) | 27 of 32 (84%) | 20 of 30 (67%) | .120 |
| BMI *z* score, median (IQR) | 0.38 (−0.37 to 1.34) (n = 91) | 0.25 (−0.60 to 1.41) (n = 29) | 0.33 (−0.32 to 1.11) (n = 32) | 0.44 (−0.33 to 1.26) (n = 30) | .895 |
| **Mode of delivery (C-section), n (%)** | **20 of 91 (22%)** | **11 of 30 (37%)** | **6 of 32 (19%)** | **3 of 29 (10%)** | **.048** |
| **Breast-feeding (≥4 mo), n (%)** | **51 of 91 (56%)** | **12 of 30 (40%)** | **25 of 32 (78%)** | **14 of 29 (48%)** | **.006** |
| Residential location, n (%) | | | | | .193 |
| ● City | 30 of 90 (33%) | 5 of 30 (17%) | 13 of 32 (41%) | 12 of 28 (43%) | |
| ● City center | 8 of 90 (9%) | 2 of 30 (7%) | 2 of 32 (6%) | 4 of 28 (14%) | |
| ● Rural area | 15 of 90 (17%) | 6 of 30 (20%) | 6 of 32 (19%) | 3 of 28 (11%) | |
| ● Village | 37 of 90 (41%) | 17 of 30 (57%) | 11 of 32 (34%) | 9 of 28 (32%) | |
| Smoking exposure, n (%) | 25 of 90 (28%) | 8 of 30 (27%) | 10 of 32 (31%) | 7 of 28 (25%) | .874 |
| **Uncontrolled asthma,* n (%)** | **58 of 92 (63%)** | **16 of 30 (53%)** | **17 of 32 (53%)** | **25 of 30 (83%)** | **.019** |
| **Severe asthma exacerbations in the past 12 mo, n (%)** | **50 of 92 (54%)** | **12 of 30 (40%)** | **15 of 32 (47%)** | **23 of 30 (77%)** | **.010** |
| **Dry cough apart from infection in the past 12 mo, n (%)** | **70 of 90 (78%)** | **26 of 29 (90%)** | **18 of 32 (56%)** | **26 of 29 (90%)** | **.001** |
| **Dry cough at night apart from infection in the past 12 mo, n (%)** | **63 of 90 (70%)** | **25 of 29 (86%)** | **17 of 32 (53%)** | **21 of 29 (72%)** | **.013** |
| **>10 d of missed school due to asthma in the past 12 mo, n (%)** | **14 of 90 (16%)** | **6 of 29 (21%)** | **1 of 32 (3%)** | **7 of 29 (24%)** | **.049** |
| **Country of inclusion, n (%)** | | | | | **$1 \times 10^{-04}$** |
| ● **Spain** | **49 of 92 (53%)** | **4 of 30 (13%)** | **30 of 32 (94%)** | **15 of 30 (50%)** | |
| ● **Germany** | **22 of 92 (24%)** | **19 of 30 (63%)** | **0 of 32 (0%)** | **3 of 30 (10%)** | |
| ● **The Netherlands** | **12 of 92 (13%)** | **5 of 30 (17%)** | **0 of 32 (0%)** | **7 of 30 (23%)** | |
| ● **Slovenia** | **9 of 92 (10%)** | **2 of 30 (7%)** | **2 of 32 (6%)** | **5 of 30 (17%)** | |
| Atopy,† n (%) | 79 of 90 (88%) | 23 of 30 (77%) | 29 of 32 (91%) | 27 of 28 (96%) | .061 |
| ● Aeroallergen combined | 78 of 91 (86%) | 22 of 30 (73%) | 29 of 32 (91%) | 27 of 29 (93%) | .067 |
| ○ **HDM** | **68 of 91 (75%)** | **13 of 30 (43%)** | **29 of 32 (91%)** | **26 of 29 (90%)** | **$1 \times 10^{-04}$** |
| ○ Grass pollens | 43 of 88 (49%) | 18 of 30 (60%) | 11 of 30 (37%) | 14 of 28 (50%) | .213 |
| ○ Mold | 7 of 70 (10%) | 3 of 23 (13%) | 0 of 22 (0%) | 4 of 25 (16%) | .199 |
| ○ Cat | 28 of 82 (34%) | 11 of 30 (37%) | 4 of 23 (17%) | 13 of 29 (45%) | .109 |
| ○ **Dog** | **24 of 80 (30%)** | **8 of 28 (29%)** | **3 of 24 (12%)** | **13 of 28 (46%)** | **.029** |
| ● Food allergen combined | 19 of 66 (29%) | 6 of 14 (43%) | 4 of 29 (14%) | 9 of 23 (39%) | .057 |
| ○ **Nuts** | **15 of 64 (23%)** | **6 of 12 (50%)** | **1 of 29 (3%)** | **8 of 23 (35%)** | **.001** |
| ○ Egg | 6 of 57 (11%) | 1 of 9 (11%) | 1 of 29 (3%) | 4 of 19 (21%) | .124 |
| ○ Milk | 8 of 60 (13%) | 3 of 11 (27%) | 1 of 29 (3%) | 4 of 20 (20%) | .078 |
| ○ Fish | 1 of 55 (2%) | 0 of 9 (0%) | 1 of 29 (3%) | 0 of 17 (0%) | 1 |
| Comorbid allergy diagnosed (ever), n (%) | | | | | |
| ● **Allergic rhinitis** | **68 of 87 (78%)** | **17 of 29 (59%)** | **27 of 30 (90%)** | **24 of 28 (86%)** | **.006** |
| ● **Allergic conjunctivitis** | **63 of 85 (74%)** | **15 of 28 (54%)** | **27 of 30 (90%)** | **21 of 27 (78%)** | **.005** |
| ● Atopic dermatitis | 39 of 85 (46%) | 8 of 23 (35%) | 19 of 32 (59%) | 12 of 30 (40%) | .145 |
| (childhood) Asthma Control Test *z* score, median (IQR) | 0.95 (0.33 to 1.55) (n = 91) | 1.03 (0.35 to 1.55) (n = 30) | 1.19 (0.72 to 1.69) (n = 31) | 0.72 (−0.17 to 1.28) (n = 30) | .132 |
| Spirometry | | | | | |
| ● $FEV_1$ presalbutamol *z* score, median (IQR) | −0.45 (−1.45 to 0.24) (n = 91) | −0.62 (−1.55 to 0.02) (n = 29) | −0.33 (−1.18 to 0.13) (n = 32) | −0.42 (−1.48 to 0.26) (n = 30) | .781 |
| ● $FEV_1$ postsalbutamol *z* score, median (IQR) | −0.12 (−0.93 to 0.65) (n = 90) | −0.63 (−1.28 to 0.66) (n = 28) | −0.09 (−0.74 to 0.48) (n = 32) | −0.10 (−0.70 to 0.65) (n = 30) | .595 |
| ● **Positive bronchodilator response ($FEV_1$ ≥200 mL and ≥12%), n (%)** | **19 of 91 (21%)** | **4 of 29 (14%)** | **4 of 32 (12%)** | **11 of 30 (37%)** | **.016** |
| $F_{ENO}$ (ppb), median (IQR) | 15.00 (8.60, 36.30) (n = 85) | 15.00 (7.35, 31.00) (n =27) | 12.70 (8.70, 32.85) (n = 31) | 17.00 (10.35, 47.15) (n = 27) | .631 |
| White blood cell count ($\times 10^9$/L), median (IQR) | | | | | |
| ● Eosinophils | 0.40 (0.27 to 0.65) | 0.34 (0.21 to 0.47) | 0.50 (0.29 to 0.84) | 0.38 (0.28 to 0.62) | .151 |
| ● **Neutrophils** | **3.06 (2.30 to 3.93)** | **3.40 (2.75 to 4.12)** | **2.60 (1.92 to 3.54)** | **3.14 (2.45 to 4.30)** | **.047** |
| ● Lymphocytes | 2.58 (2.23 to 3.09) | 2.88 (2.23 to 3.10) | 2.50 (2.31 to 3.10) | 2.58 (2.05 to 3.04) | .864 |
| ● Basophils | 0.05 (0.03 to 0.07) | 0.05 (0.03 to 0.06) | 0.05 (0.03 to 0.08) | 0.04 (0.03 to 0.06) | .175 |

*(Continued)*

**TABLE I**. *(Continued)*

| Characteristics | All subjects (N = 92) | Metabotype 1 (n = 30) | Metabotype 2 (n = 32) | Metabotype 3 (n = 30) | *P* value |
|---|---|---|---|---|---|
| **● Monocytes** | **0.52 (0.40 to 0.60) (n = 83)** | **0.56 (0.46 to 0.66) (n = 21)** | **0.46 (0.36 to 0.54) (n = 32)** | **0.54 (0.42 to 0.62) (n = 30)** | **.030** |
| Inclusion season, n (%) | | | | | .180 |
| ● Winter | 20 of 92 (22%) | 4 of 30 (13%) | 8 of 32 (25%) | 8 of 30 (27%) | |
| ● Spring | 29 of 92 (32%) | 10 of 30 (33%) | 10 of 32 (31%) | 9 of 30 (30%) | |
| ● Summer | 29 of 92 (32%) | 14 of 30 (47%) | 6 of 32 (19%) | 9 of 30 (30%) | |
| ● Autumn | 14 of 92 (15%) | 2 of 30 (7%) | 8 of 32 (25%) | 4 of 30 (13%) | |
| Asthma medications, n (%) | | | | | |
| ● ICS dose | | | | | .388 |
| ○ Low | 49 of 92 (53%) | 18 of 30 (60%) | 18 of 32 (56%) | 13 of 30 (43%) | |
| ○ Medium | 27 of 92 (29%) | 7 of 30 (23%) | 11 of 32 (34%) | 9 of 30 (30%) | |
| ○ High | 16 of 92 (17%) | 5 of 30 (17%) | 3 of 32 (9%) | 8 of 30 (27%) | |
| ● SABA | 82 of 92 (89%) | 26 of 30 (87%) | 28 of 32 (88%) | 28 of 30 (93%) | .768 |
| ● LABA | 89 of 92 (97%) | 30 of 30 (100%) | 32 of 32 (100%) | 27 of 30 (90%) | .070 |
| ● OCS | 1 of 92 (1%) | 0 of 30 (0%) | 0 of 32 (0%) | 1 of 30 (3%) | .654 |
| **● LTRA** | **13 of 92 (14%)** | **5 of 30 (17%)** | **0 of 32 (0%)** | **8 of 30 (27%)** | **.008** |
| ● Anticholinergics | 9 of 92 (10%) | 5 of 30 (17%) | 1 of 32 (3%) | 3 of 30 (10%) | .176 |
| ● Biologicals (omalizumab or mepolizumab) | 7 of 92 (8%) | 3 of 30 (10%) | 1 of 32 (3%) | 3 of 30 (10%) | .554 |
| ● GINA step | | | | | .528 |
| ○ Step 3 | 46 of 92 (50%) | 17 of 30 (57%) | 17 of 32 (53%) | 12 of 30 (40%) | |
| ○ Step 4 | 39 of 92 (42%) | 10 of 30 (33%) | 14 of 32 (44%) | 15 of 30 (50%) | |
| ○ Step 5 | 7 of 92 (8%) | 3 of 30 (10%) | 1 of 32 (3%) | 3 of 30 (10%) | |
| MARS-5 (≥21), n (%) | 81 of 86 (94%) | 26 of 28 (93%) | 30 of 31 (97%) | 25 of 27 (93%) | .727 |

For continuous measures (variables), the number of samples available for a specific measure is provided only when data were missing. *P* values were calculated using Pearson $\chi^2$ test with Monte-Carlo simulation (10,000 permutations) or the Kruskal-Wallis H test as appropriate.
Entries with statistically significant *P* values (<.05) are shown in boldface.
*BMI*, Body mass index; *FENO*, fractional exhaled nitric oxide; *GINA*, Global Initiative for Asthma; *HDM*, house dust mite; *ICS*, inhaled corticosteroid; *IQR*, interquartile range; *LABA*, long-acting β-agonist; *LTRA*, leukotriene antagonist; *MARS-5*, Medication Adherence Report Scale-5; *OCS*, oral corticosteroid; *SABA*, short-acting β-agonist.
*Uncontrolled asthma is defined on the basis of (childhood) Asthma Control Test score ≤19 and/or severe exacerbations requiring hospitalization or emergency room visits or OCS use in the past year.
†Atopic sensitization refers to physician history (ever) of sensitization to aeroallergens or food allergens by a positive skin prick test result (wheal diameter ≥3 mm) and/or positive allergen-specific IgE (≥0.35 kU/L).

### The metabotypes were modestly associated with short-term dietary habits, but strongly associated with fecal microbiome

Seventy-three (79.3%) of the 92 subjects completed 24-hour food diaries before the baseline visit. The metabotypes showed nominally significant associations with total sugar and protein intake (see Fig E5 in this article's Online Repository at www.jacionline.org); however, the results were not significant after FDR corrections.

Metabotype 3 showed significantly decreased richness and Shannon α diversity in comparison to metabotype 2 and metabotype 1 in fecal microbiome (see Fig E6 in this article's Online Repository at www.jacionline.org). Moreover, PERMANOVA analysis revealed that the metabotypes were significantly different in the weighted UniFrac β-diversity measure (adjusted $R^2 = 0.076$; *P* value = .001). Fig E7 (in the Online Repository available at www.jacionline.org) depicts the relative separation of the metabotypes based on the β diversity. Fig 2 shows differentially abundant bacterial genera between the metabotypes. In particular, *Christensenellaceae R−7 group*, *Oscillospiraceae UCG−005*, *Oscillospiraceae NK4A214 group*, *Coprococcus*, *Anaerovoracaceae Family XIII AD3011 group*, and *Victivallis* were more abundant, whereas *Lachnoclostridium* was less abundant in metabotype 2 in comparison to metabotype 3. No differentially abundant bacterial genera were found between metabotype 1 and metabotype 3 after FDR correction (Fig 2), and between metabotype 1 and metabotype 2 (data not shown for the latter). At the metabolic class level, the fecal microbiome showed more statistically significant associations with the fecal metabolome than with the serum metabolome at both α- and β-diversity levels (see Fig E8 and Table E7 in this article's Online Repository at www.jacionline.org).

### The metabotypes exhibit dysregulation of non-T2 asthma biomarkers

In metabotype 2, decreased levels of serum IL-6 and IL-7 inflammatory markers were observed, whereas metabotype 1 showed elevated levels of TIMP-4 compared with the other metabotypes (all *q* values < 0.05, Fig 3).

## DISCUSSION

Using integrative analyses of fecal and serum metabolome profiles, we found that children with moderate to severe asthma could be stratified into 3 metabotypes that differed significantly by (1) asthma burden (asthma control, severe exacerbations, dry cough episodes, and missed school days), early-life exposures (breast-feeding and mode of delivery), and inflammatory biomarkers (blood neutrophils and monocytes), (2) gut microbiota

**Box 1.** Summary overview for the differences in the study characteristics between the metabotypes

**Metabotype 1**

Metabotype 1 included 30 (33%) subjects, with a large percentage of children recruited from Germany (n = 19 of 30 [63%]; overall $P = 1 \times 10^{-04}$, *post hoc q* = <.001). Metabotype 1 had the lowest percentage of subjects with history of ≥4 mo of breast-feeding (n = 12 of 30 [40%]; overall $P = .006$, *post hoc q* = 0.031), highest percentage of subjects with a cesarian-section mode of delivery—however, the latter was not statistically significant at *post hoc* level (n = 11 of 30 [37%]; overall $P = .048$, *post hoc q* = 0.106)—, lowest percentage of subjects with history of house dust mite atopic sensitization (n = 13 of 30 [43%]; overall $P = 1 \times 10^{-04}$, *post hoc q* = <0.001), and lowest percentage of subjects with physician-diagnosed allergic rhinitis (n = 17 of 29 [59%]; overall $P = .006$, *post hoc q* = 0.011) and allergic conjunctivitis (n = 15 of 28 [54%]; overall $P = .005$, *post hoc q* = 0.015). However, it had the highest percentage of subjects with nut allergens sensitization (n = 6 of 12 [50%]; overall $P = .001$, *post hoc q* = 0.048). This metabotype did not show association with asthma control at *post hoc* level (n = 16 of 30 [53%]; overall $P = .019$, *post hoc q* = 0.359); however, it showed higher odds for uncontrolled asthma when compared with metabotype 2 after adjusting for multiple covariates. In addition, it showed the highest percentage of subjects with dry cough episodes at night, with a trend of statistically significant difference at *post hoc* level (n = 25 of 29 [86%]; overall $P = .013$, *post hoc q* = 0.062).

**Metabotype 2**

Metabotype 2 included 32 (35%) subjects, and was dominated by children recruited from Spain (n = 30 of 32 [94%]; overall $P = 1 \times 10^{-04}$, *post hoc q* = <0.001). Metabotype 2 had the highest percentage of subjects with history of ≥4 mo of breast-feeding (n = 25 of 32 [78%]; overall $P = .006$, *post hoc q* = 0.002), a high percentage of subjects with history of house dust mite atopic sensitization (n = 29 of 32 [91%]; overall $P = 1 \times 10^{-04}$, *post hoc q* = 0.030), a trend for the lowest percentage of subject with dog dander aeroallergen sensitization at *post hoc* level (n = 3 of 24 [12%]; overall $P = .029$, *post hoc q* = 0.076), lowest percentage of subjects with nut allergens (n = 1 of 29 [3%]; overall $P = .001$, *post hoc q* = 0.004), and the highest percentage of subjects with physician-diagnosed allergic conjunctivitis (n = 27 of 30 [90%]; overall $P = .005$, *post hoc q* = 0.041). This metabotype showed the most favorable clinical profile in terms of asthma burden and inflammatory cell profile among the 3 metabotypes. In particular, both metabotype 3 and metabotype 1 showed higher odds for uncontrolled asthma after adjusting for covariates (adjusted odds ratio, 11.7, 95% CI, 2.57-88, for metabotype 3 and 7.34, 95% CI, 1.22-68.9, for metabotype 1, relative to metabotype 2, all *P* values < .05). In addition, it showed the lowest percentage of subjects with dry cough episodes (n = 18 of 32 [56%]; overall $P = .001$, *post hoc q* = 0.002), a trend for the lowest percentage of subjects with >10 d of missed school days at *post hoc* level (n = 1 of 32 [3%]; overall $P = .049$, *post hoc q* = 0.094), the lowest blood monocyte counts (median, 0.46, IQR, 0.36-0.54, overall $P = .030$, *post hoc q* = 0.039 in comparison to metabotype 1 [median, 0.56, IQR, 0.46-0.66], and *post hoc q* = 0.084 in comparison to metabotype 3 [median, 0.54, IQR, 0.42-0.62]), and a trend for lowest blood neutrophils counts (median, 2.60, IQR, 1.92-3.54, overall $P = .047$, *post hoc q* = 0.059 in comparison to metabotype 1 [median, 3.40, IQR, 2.75-4.12], and *post hoc q* = 0.100 in comparison to metabotype 3 [median, 3.14, IQR, 2.45-4.30]). Moreover, this metabotype had the lowest percentage of subjects who were prescribed leukotriene antagonists add-on asthma medication (n = 0 of 32 [0%]; overall $P = .008$, *post hoc q* = 0.027).

**Metabotype 3**

Metabotype 3 included 30 (33%) subjects. Metabotype 3 had a high percentage of subjects with history of house dust mite atopic sensitization (n = 26 of 29 [90%]; overall $P = 1 \times 10^{-04}$, *post hoc q* = 0.050), and a trend for the highest percentage of subjects with dog dander aeroallergen sensitization at *post hoc* level (n = 13 of 28 [46%]; overall $P = .029$, *post hoc q* = 0.076). This metabotype was the most associated with increased asthma burden. In particular, this metabotype showed the highest percentage of subjects with uncontrolled asthma (n = 25 of 30 [83%]; overall $P = .019$, *post hoc q* = 0.030) and had the highest odds for uncontrolled asthma in comparison to metabotype 2 after adjusting for covariates (adjusted odds ratio, 11.7, 95% CI, 2.57-88, all *P* value < .05). In addition, it showed the highest percentage of subjects with severe asthma exacerbation in the past year (n = 23 of 30 [77%]; overall $P = .010$, *post hoc q* = 0.017), a trend for the highest percentage of subjects with a positive bronchodilator response (n = 11 of 30 [37%]; overall $P = .016$, *post hoc q* = 0.056), and the highest percentage of subjects who were prescribed leukotriene antagonists add-on asthma medication (n = 8 of 30 [27%]; overall $P = .008$, *post hoc q* = 0.049).

*IQR*, Interquartile range.

**TABLE II.** Number of statistically significant enriched or depleted serum metabolites grouped by metabolic classes across the 3 metabotypes

| | Enriched serum metabolome | | | | Depleted serum metabolome | | | |
|---|---|---|---|---|---|---|---|---|
| **Metabolite class** | **Metabotype 1** | **Metabotype 2** | **Metabotype 3** | ***P* value*** | **Metabotype 1** | **Metabotype 2** | **Metabotype 3** | ***P* value*** |
| Amine oxides | 0 | 1 | 0 | <.0001 | 0 | 0 | 0 | <.0001 |
| Amino acids | 0 | 3 | 2 | | 0 | 4 | 2 | |
| Carnitines | 0 | 3 | 0 | | 1 | 0 | 1 | |
| Ceramides | 12 | 0 | 0 | | 0 | 0 | 4 | |
| Cholesteryl esters | 10 | 0 | 0 | | 0 | 0 | 8 | |
| Cresols | 0 | 0 | 0 | | 0 | 0 | 0 | |
| Fatty acids | 0 | 4 | 0 | | 0 | 0 | 0 | |
| Phosphatidylcholines | 46 | 5 | 0 | | 1 | 1 | 18 | |
| Sphingomyelins | 1 | 0 | 0 | | 0 | 0 | 7 | |
| Triglycerides | 73 | 0 | 26 | | 0 | 144 | 0 | |
| Diglycerides | 5 | 1 | 2 | | 0 | 5 | 0 | |
| Vitamins and cofactors | 1 | 0 | 0 | | 0 | 0 | 0 | |

**P* values were calculated for overall metabolic classes.

**TABLE III.** Number of statistically significant enriched or depleted fecal metabolites grouped by metabolic classes across the 3 metabotypes

| | Enriched stool metabolome | | | | Depleted stool metabolome | | | |
|---|---|---|---|---|---|---|---|---|
| **Metabolite class** | **Metabotype 1** | **Metabotype 2** | **Metabotype 3** | ***P* value*** | **Metabotype 1** | **Metabotype 2** | **Metabotype 3** | ***P* value*** |
| Amino acids | 0 | 2 | 12 | <.0001 | 6 | 0 | 1 | .016 |
| Bile acids | 0 | 0 | 0 | | 0 | 0 | 0 | |
| Biogenic amines | 0 | 0 | 1 | | 0 | 0 | 0 | |
| Carboxylic acids | 0 | 0 | 1 | | 0 | 0 | 0 | |
| Carnitines | 0 | 1 | 0 | | 0 | 1 | 0 | |
| Ceramides | 0 | 0 | 3 | | 0 | 15 | 0 | |
| Fatty acids | 0 | 0 | 0 | | 0 | 0 | 0 | |
| Phosphatidylcholines | 16 | 0 | 0 | | 1 | 9 | 0 | |
| Sphingomyelins | 2 | 0 | 0 | | 0 | 7 | 0 | |
| Triglycerides | 2 | 0 | 0 | | 0 | 19 | 0 | |
| Diglycerides | 0 | 0 | 0 | | 0 | 6 | 0 | |
| Hormones and related | 0 | 0 | 1 | | 0 | 0 | 0 | |
| Monosaccharides | 0 | 0 | 0 | | 0 | 0 | 0 | |

*$P$ values were calculated for overall metabolic classes.

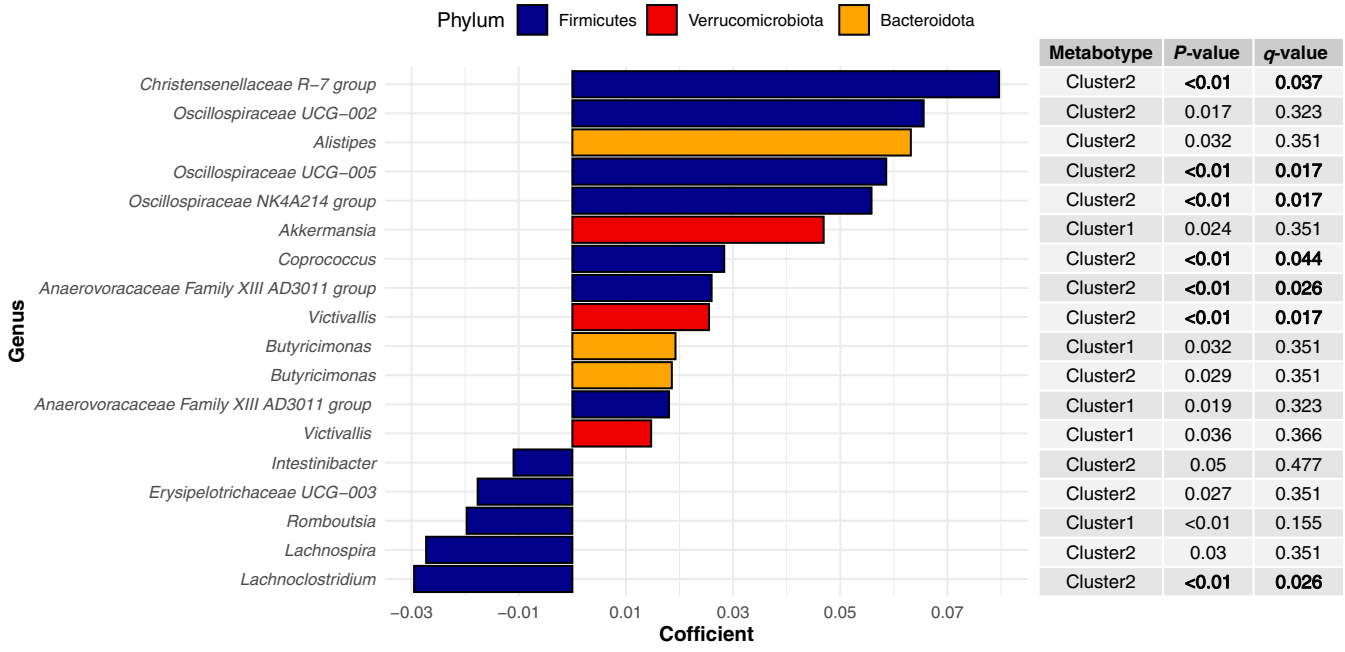


**FIG 2.** Microbiome Multivariable Associations with Linear Models 2 (MaAsLin 2) plot showing differentially abundant bacterial genera between the metabotypes ($P$ values < .05), whereas those with statistically significant associations after multiple testing correction ($q$ values < 0.05) are highlighted in boldface. The bars of bacterial genera were colored according to their respective phyla. Metabotype 3 with the least diverse microbial profile was chosen as a reference group for comparison.

composition, and (3) serum inflammatory proteins. This study is regarded the first, to-date, to delineate multiomics and clinical characteristics of metabotypes in moderate to severe childhood asthma.

Metabotype 1 showed the lowest percentage of subjects with allergic rhinitis and allergic conjunctivitis, as well as the lowest percentage of subjects with atopic sensitization to house dust mite. However, it showed the highest percentage of atopic sensitization to food allergens, particularly nuts, and showed notable sensitization to other aeroallergens such as pollens, mold, cat, and dog. These contrasting patterns of allergen sensitization warrant further investigation to determine the link between specific sensitizations and the metabolome. This metabotype showed higher odds for uncontrolled asthma compared with only metabotype 2 after adjusting for confounders, and showed the highest levels of blood neutrophils and monocytes, particularly in comparison to metabotype 2. Metabotype 1 exhibited the highest levels of serum (ceramides, cholesteryl esters, phosphatidylcholines, triglycerides, and diglycerides) and fecal (sphingomyelins, phosphatidylcholines, and triglycerides) lipids compared with the other metabotypes. Furthermore, it demonstrated the lowest levels of fecal amino acids. Our results are partly in line with other findings described in the literature. It was reported that serum triglycerides were elevated in adults with asthma compared

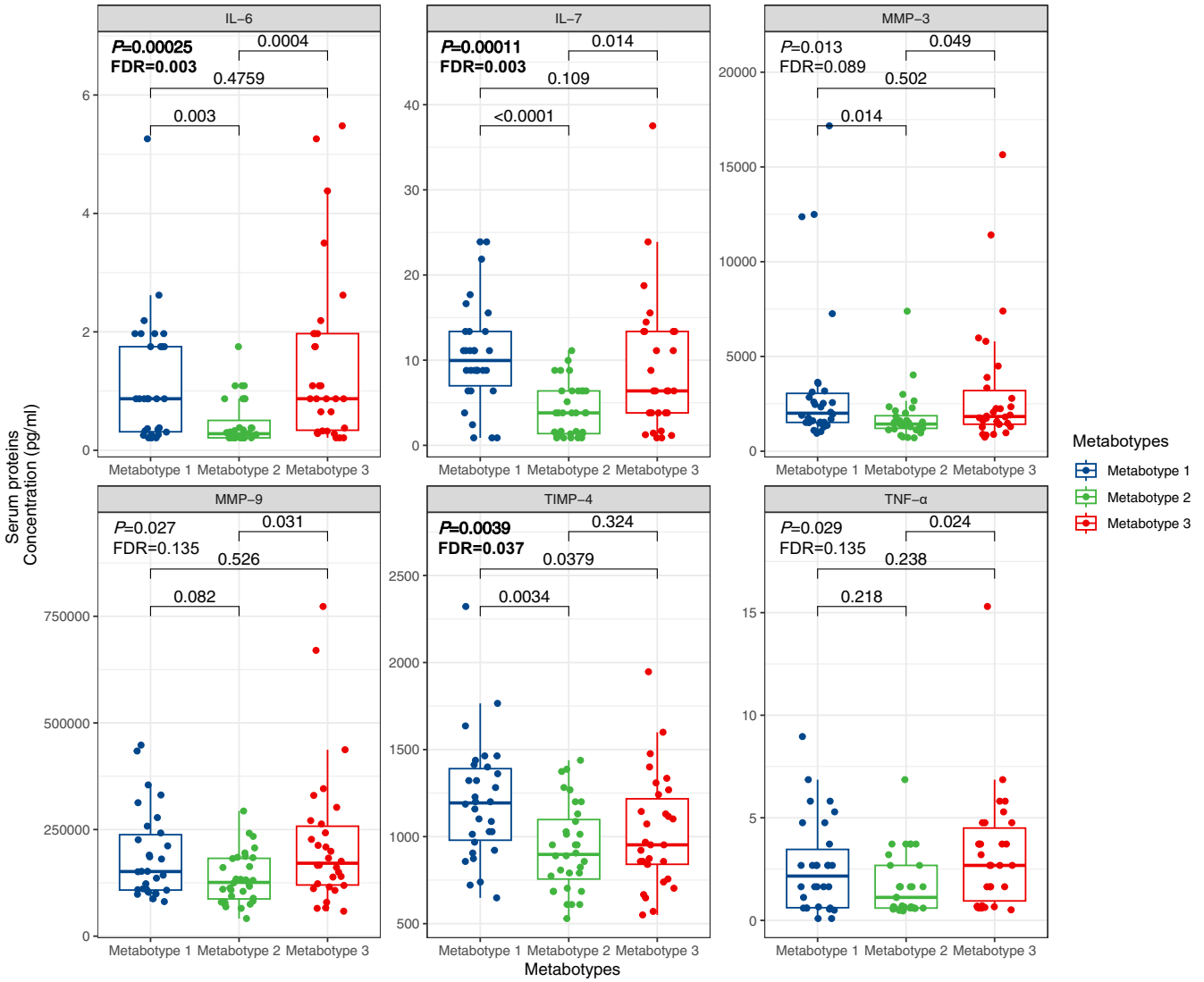


**FIG 3.** Box-and-Whisker plots of the statistically significant serum cytokines and chemokines in the 3 metabotypes, whereas those with FDR-corrected *P* values less than .05 are shown in boldface.

with healthy controls, even after adjusting for body mass index, inflammatory markers, and medication intake.[24] In another study, serum ceramides, particularly C16:0 and C24:0, were elevated in uncontrolled asthma compared with controlled asthma, whereas high levels of CD66+ neutrophils were associated with high levels of sphingosine and C16:0 ceramide.[25] Our findings suggest that elevation of specific lipids may be associated with uncontrolled asthma development. The high levels of both fecal and serum lipids may be partly associated with the high intake of a sugary diet in metabotype 1, particularly in comparison to metabotype 2. However, results were not statistically significant after multiple testing correction for the nutrients, which could be attributed to the small sample size of subjects/parents who filled in the food diaries. Interestingly, we did not observe differences between the metabotypes in fat intake or body mass index, suggesting that we cannot fully capture the dietary habits of these children using the 24-hour food diaries. Therefore, further research is needed to investigate the consequence of long-term dietary intake over the metabolomics profiles. Moreover, metabotype 1 is distinguished by elevated levels of tissue inhibitor of metalloproteinase-4 (TIMP-4) compared with the other metabotypes. TIMP-4 plays a crucial role in regulating the activity of matrix metalloproteinases, which are involved in tissue remodeling and inflammation.[26,27] Limited research has been performed to investigate the role of TIMP-4 in severe asthma pathophysiology; however, a previous study reported its link to chronic obstructive pulmonary disease,[28] suggesting its potential role in airway inflammation, which requires further investigation.

Metabotype 2 showed the most favorable clinical profile (highest odds for asthma control and lowest percentage of dry cough episodes in the past year) compared with the other metabotypes. Metabotype 2 had the lowest levels of blood neutrophils and monocytes compared with the other metabotypes. Furthermore, it showed the most favorable metabolic profile characterized by depletion of triglycerides in feces and serum and elevation of omega 3 fatty acids, acylcarnitines, and α-aminobutyric acid in serum, which partly corresponds to reported evidence in the literature. For instance, a study demonstrated that elevated levels of omega-3 fatty acids in erythrocyte membranes positively correlate with improved asthma control

in children.[29] Another study revealed that maternal intake of fish oil–derived omega-3 fatty acids during pregnancy was linked to a reduction in asthma risk later in life.[30] Moreover, children with moderate persistent asthma exhibited reduced levels of serum carnitines compared with healthy controls; meanwhile, supplementation of L-carnitine to these asthmatic children showed improvement in asthma control relative to those receiving a placebo.[31] Red meats, followed by poultry, fish, and dairy products, are the main dietary sources of carnitines. Therefore, the elevated levels of carnitines in metabotype 2 may be partly explained by the high reported amounts of protein intake assessed by the 24-hour food diaries within this metabotype. Metabotype 2 also showed the most favorable microbial profile, characterized by enrichment of some beneficial and/or butyrate-producing bacteria,[32-34] such as *Christensenellaceae R−7* group, *Coprococcus,* and members of *Oscillospiraceae* family particularly in comparison to metabotype 3. Moreover, metabotype 2 showed decreased levels of non-T2 proinflammatory proteins in comparison to the other metabotypes, which is in line with their favorable clinical, metabolomics, and microbial profiles. The decreased levels of non-T2 proinflammatory markers, such as IL-6, matrix metalloproteinase 3, and TNF-α, in this metabotype may be partly explained by the elevated serum levels of α-aminobutyric acid within this metabotype. α-Aminobutyric acid is reported to inhibit polarization and activity of M1 macrophages that can secrete these cytokines.[35] Moreover, these markers play a significant role in asthma pathophysiology and exacerbations,[27,36] suggesting that metabotype 2 has a lower inflammatory status compared with the other metabotypes.

Metabotype 3 exhibited the highest burden of asthma in comparison to the other metabotypes, as characterized by the highest percentage of patients with uncontrolled asthma and the highest percentage of at least 1 severe asthma exacerbation requiring a burst of oral corticosteroid intake for 3 or more days in the past 12 months. Subjects within this group were more likely to be prescribed add-on asthma medications, particularly leukotriene antagonists, suggesting that these medications were prescribed to control their severe symptoms. This metabotype exhibited elevated ornithine-to-arginine ratio in serum compared with the other metabotypes. Ornithine-to-arginine ratio is a marker of arginase activity, where arginase is an enzyme that catalyzes the conversion of arginine to ornithine in the final step of the urea cycle.[37] This finding is in line with a previous study also showing an increased arginase activity in serum of children with asthma or wheezing (n = 21) admitted to emergency room with acute exacerbations compared with controls (n = 15).[38] Increased arginase activity and decreased citrulline synthesis can reflect inducible nitric oxide synthase (iNOS) inhibition (ie, decreased endogenous nitric oxide production) in this metabotype,[39] while inhibition of iNOS is thought to promote bronchoconstriction and airway remodeling and worsen disease control.[40,41] In addition, this metabotype showed dysregulated lipid profiles in serum characterized by the depletion of sphingomyelins, as well as other lipid classes, including phosphatidylcholines, cholesteryl esters, and ceramides. Sphingomyelins and phosphatidylcholines are classes of phospholipids; therefore, their depletion may suggest an imbalance in phospholipid metabolism. Phospholipids and ceramides are components of the lipid membranes as well as the airway surfactants,[42] and decreased levels could reflect abnormalities in normal structure and function of the airways. Thus, the direct relationship between serum levels of these metabolites and airway function requires further investigation. Notably, this metabotype showed elevation of some triglycerides in comparison to metabotype 2; however, the overall number of elevated triglycerides was significantly less than metabotype 1. Again, this highlights that elevated triglycerides are associated with childhood asthma severity. Moreover, this metabotype showed characteristic elevated fecal levels of amino acids and ceramides compared with the other metabotypes. Increased levels of fecal amino acids have been observed in other inflammatory diseases, such as inflammatory bowel disease,[43] suggesting that this may underline an ongoing inflammatory state in metabotype 3.

Distribution of subjects within the metabotypes was significantly different according to the country of inclusion. This is expected, considering that subjects recruited from different countries have different dietary habits and are exposed to different environments. Yet, we could still detect significant differences in asthma characteristics after adjusting for countries and other potential confounders, including medication intake. This suggests that the metabolic processes are strongly linked to childhood asthma control and may play a role in disease development. It has been reported that factors such as age, sex, ethnicity, and corticosteroid intake can influence the metabolome profiles of individuals.[44,45] In contrast, we did not observe significant differences in these factors, and neither the dose of inhaled corticosteroids nor oral corticosteroids intake among the 3 metabotypes. Interestingly, a larger percent of subjects within metabotype 2 were breast-fed for equal to or more than 4 months compared with the other metabotypes, while the mode of delivery for a larger percent of subjects within metabotype 1 was C-section. These findings suggest that early-life exposures are associated with the metabolic makeup of children with asthma. Further research is needed to evaluate whether these early-life exposures have causal effects on the metabolic profiles later in life.

The association of the metabolic profiles with disease burden in children with moderate to severe asthma can have clinical implications. Asthma endotyping is crucial for the appropriate choice of the medications and the personalized disease management. In contrast to adulthood asthma, endotyping in childhood asthma is challenging due to the difficulty of obtaining sputum and/or invasive samples. Therefore, metabolomics profiling from minimally invasive/noninvasive specimens could be a solution. Moreover, our findings highlight potential metabolic pathways that can be targeted to improve disease control in children with moderate to severe asthma. In metabotype 2, favorable metabolic (eg, increased acylcarnitines and ω-3 fatty acids and decreased triglycerides in serum) and gut microbiome (eg, increased diversity and butyrate-producing bacteria) profiles were associated with improved asthma control in those children. Therefore, further investigations are required to assess whether targeting microbiome (eg, probiotics) or metabolome (eg, ω-3 or carnitines) or diet-based supplement/healthy diet can play a role in the therapeutic management of severe childhood asthma.

This study has several strengths. First, we comprehensively assessed the metabolome profiles using a large panel of targeted metabolic profiling that encompass several metabolic classes across 2 sampling compartments (serum and feces). This allowed us to adequately characterize children with moderate to severe asthma with respect to their metabolic phenotyping, while linking it to other omics features. Second, the analysis recruited children from 4 different European countries, making the findings more generalizable than single-center studies. Third, the analysis approach used is unsupervised and its clinical relevance is driven

mainly by the metabolome profiles. Finally, this study is regarded as one of the first attempts to uncover integrative metabolome-driven endotypes and elucidate targets for precision medicine within children with moderate to severe asthma.

However, this study has some limitations. First, although we recruited children from 4 European countries, the overall cohort sample size with both fecal and serum metabolome was limited, mainly due to difficulties in recruiting children with moderate to severe disease and their reluctance to provide some sample types, such as feces.[11] This raises the question of whether there are more metabotypes in children with asthma that our sample size could not adequately cover. However, this highlights that multicenter collaboration is essential in the multiomics investigation of moderate to severe childhood asthma. Second, we investigated the metabolome at one time point. Longitudinal shifts in the metabolomics profiles have been previously reported[46] and further research should examine the stability of the metabotypes and their relationship to the asthma pathophysiology over time. Third, we have collected the 24-hour food diaries only in a subset of the cohort, which limits statistical power. In addition, the 24-hour history may not necessarily reflect the long-term dietary habits, which can have a stronger influence on the metabolome profiles. Fourth, we were not able to validate these findings in an external cohort. This is attributed to the scarceness of moderate to severe childhood asthma cohorts where comprehensive metabolic profiling has been performed. Finally, given the study's observational nature, bias due to selection and unmeasured confounders cannot be ruled out.

## Conclusions

This study has delineated 3 distinct metabotypes among children with moderate to severe asthma, revealing significant differences in asthma burden, and underlying molecular and inflammatory profiles. These findings underscore the importance of metabolic profiling in understanding childhood asthma and highlight the potential for metabolomics to aid in asthma endotyping and personalized management. Further research is needed to investigate whether targeting specific metabolic pathways could offer safe and effective therapeutic options in children with uncontrolled disease.

## DISCLOSURE STATEMENT

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Some of the drawn objects in the Graphical Abstract were adopted from Servier, Somersault18:24, and National Institutes of Health BioArt corporations, which are used under creative commons license.

**Clinical implications: Metabotyping reveals distinct childhood asthma subtypes with unique inflammatory profiles and gut microbiota composition, offering novel insights for targeted therapies and personalized management of moderate to severe childhood asthma.**

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