Metabolomics identifies disturbances in arginine, phenylalanine, and glycine metabolism as differentiating features of exacerbating atopic asthma in children

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Abstract

Background: Asthma exacerbations are highly prevalent in children, but only a few studies have examined the biologic mechanisms underlying exacerbations in this population.

Objective: High-resolution metabolomics analyses were performed to understand the differences in metabolites in children with exacerbating asthma who were hospitalized in a pediatric intensive care unit for status asthmaticus. We hypothesized that compared with a similar population of stable outpatients with asthma, children with exacerbating asthma would have differing metabolite abundance patterns with distinct clustering profiles.

Methods: A total of 98 children aged 6 through 17 years with exacerbating asthma (n = 69) and stable asthma (n = 29) underwent clinical characterization procedures and submitted plasma samples for metabolomic analyses. High-confidence metabolites were retained and utilized for pathway enrichment analyses to identify the most relevant metabolic pathways that discriminated between groups.

Results: In all, 118 and 131 high-confidence metabolites were identified in positive and negative ionization mode, respectively. A total of 103 unique metabolites differed significantly between children with exacerbating asthma and children with stable asthma. In all, 8 significantly enriched pathways that were largely associated with alterations in arginine, phenylalanine, and glycine metabolism were identified. However, other metabolites and pathways of interest were also identified.

Conclusion: Metabolomic analyses identified multiple perturbed metabolites and pathways that discriminated children with exacerbating asthma who were hospitalized for status asthmaticus. These results highlight the complex biology of inflammation in children with exacerbating asthma

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and argue for additional studies of the metabolic determinants of asthma exacerbations in children because many of the identified metabolites of interest may be amenable to targeted interventions.

Asthma exacerbations are highly prevalent, affecting nearly half of all children with current asthma in the United States who are younger than 18 years,[1] and they are a substantial driver of pediatric asthma–related health care costs.[2] Although asthma exacerbations are primarily managed at home or in outpatient settings, 8.8 of every 100,000 children in the US population younger than 18 years (and approximately 1 of every 20 children with asthma) progress to status asthmaticus and require hospitalization.[1] Hospitalization rates are even higher in socially vulnerable populations of children.[3] Yet despite the substantial personal and societal impact of hospitalization for asthma, effective management of these children in the hospital setting remains a substantial knowledge gap.[4,5] This is particularly concerning because hospital admission for asthma is a primary predictor of future hospital readmission[6] and significantly increases the risk of asthma-related death.[7]

Although advances have been made in understanding the biologic bases of asthma in stable outpatient populations, there is a paucity of studies that have examined biologic mechanisms underlying asthma exacerbations in children. Indeed, much of what is known about the inflammatory underpinnings of asthma exacerbations stems from work in mice[8-10] and work in stable populations of children, in which predetermined biomarkers are associated with exacerbation risk in either a prospective or retrospective manner.[5,11] Only a few studies have examined inflammatory mechanisms in patients during acute exacerbations.[12-14] Even fewer studies have examined inflammatory mechanisms in hospitalized children with status asthmaticus at the time of admission. As a result, hospital treatment of asthma exacerbations remains largely symptomatic[7] and is not currently directed toward specific inflammatory pathways.

To address this important knowledge gap, we performed metabolomics analyses using HPLC with high-resolution, accurate mass spectrometry to understand the differences in metabolite patterns in children with exacerbating asthma who were hospitalized in a pediatric intensive care unit (PICU) for status asthmaticus. We hypothesized that compared with a similar population of stable outpatients with asthma, children with exacerbating asthma would have differing metabolite patterns with distinct clustering profiles.

METHODS

Participants

Children aged 6 through 17 years with physician-diagnosed asthma evaluated at Children's Healthcare of Atlanta were eligible for inclusion. Two groups of children were enrolled. Children with exacerbating asthma were enrolled within 48 hours of admission from a 36-bed PICU after receipt of any of the following interventions in the emergency department: (1) a third continuous nebulized albuterol treatment; (2) noninvasive respiratory

support delivered by high-flow nasal cannula, bilevel positive airway pressure, or invasive mechanical ventilation; (3) an 80%:20% helium-oxygen mixture; or 4) at least a 50% fraction of inspired oxygen by Venturi mask or positive pressure ventilation strategies to maintain oxygen saturations of 92% or higher. The children with stable asthma were outpatient children participating in research-only clinic visits. Stable children were enrolled from an asthma specialty clinic if they had historical evidence of at least 12% reversibility in their FEV_1 value relative to baseline after bronchodilator administration and if they had at least 1 asthma exacerbation necessitating treatment with systemic corticosteroids in the preceding year.[15] In the event that an outpatient participant had a recent exacerbation treated with systemic corticosteroids, the research visit was postponed until at least 2 weeks after the last dose of systemic corticosteroids. Children were excluded from either group if they had a history of hematopoietic stem cell or solid organ transplant, an oncologic diagnosis, sickle cell anemia, a rheumatologic diagnosis, pulmonary aspiration, gastroesophageal reflux requiring acid suppression medication, bronchiectasis, congenital airway anomalies, a history of premature birth before 35-weeks' gestation, or a personal history of smoking or vaping. Permission to proceed with this study was granted by the Emory University and Children's Healthcare of Atlanta institutional review boards. Informed written consent was obtained from legal guardians. Verbal assent was obtained from children aged 6 to 10 years, and written assent was obtained from children and adolescents aged 11 to 17 years. All study procedures were performed in accordance with the relevant guidelines and regulations in the Declaration of Helsinki.

Participant characterization procedures

Participants and their caregivers completed demographic and medical history questionnaires, the 8-item Pediatric Asthma Impact Scale (PAIS),[16] and the Asthma Control Test (ACT)[17] or Childhood ACT (C-ACT).[18] The PAIS is measured with a T-score, with higher scores reflecting worse impact.[16] ACT scores lower than 20 reflect uncontrolled asthma.[19] Severe asthma was defined by a European Respiratory Society–American Thoracic Society guideline as asthma requiring treatment with high-dose inhaled corticosteroids plus a second controller to prevent it from becoming uncontrolled or asthma that remains uncontrolled despite this therapy.[20] Total serum IgE level, aeroallergen sensitization as determined by specific IgE testing, and lung function data (while the patient's asthma was stable) were abstracted from the electronic medical record of hospitalized patients within 12 months of PICU admission. Aeroallergen sensitization was assessed in outpatients by specific IgE testing with 8 extracts: tree mix, grass mix, weed mix, mold mix (*Alternaria alternata, Aspergillus fumagatis*, and *Cladosporium herbarum*), dog dander, cat dander, *Blatella germanica*, and dust mite mix (*Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*) (Greer Laboratories, Lenoir, NC). Spirometry (KoKo PDS, Ferraris, Louisville, Colo) was performed in outpatients according to technical standards[21] from the best of 3 forced vital capacity (FVC) maneuvers. All spirometry data from the hospitalized patients and outpatients, including FVC, FEV_1 value, and forced expiratory flow at 25% to 75% of FVC, were interpreted according to Global Lung Function Initiative prediction equations[22] and expressed as percentages of predicted values. All participating children also submitted blood samples for plasma cytokines and plasma metabolomics. If hospitalized children were missing serum IgE values, these values were obtained with a commercially

available ELISA kit (Invitrogen, Waltham, Mass). Plasma IFN-γ, IL-4, IL-5, and IL-13 levels were quantified with a commercial assay according to the manufacturers' instructions (Millipore Sigma Milliplex MAP Human High Sensitivity T-Cell Panel, Burlington, Mass). A subset of participants also submitted blood samples for quantification of blood eosinophils (Children's Healthcare of Atlanta, Atlanta, Ga).

Metabolomics

Detailed methods are presented in the Online Repository (available at www.jaci-global.org). Briefly, mass spectrometry was performed on plasma samples with a Vanquish Horizon Binary ultrahigh-performance liquid chromatography system coupled with a Q Exactive High-Field Hybrid Orbitrap mass spectrometer (ThermoFisher). Data were analyzed by using Compound Discoverer 3.3 (ThermoFisher). Metabolite retention times were aligned across runs, and sample extracts combined as a global pooled sample were used as quality controls to correct instrumental response draft and evaluate measurement reproducibility (relative SD < 30%). Metabolites were considered high-confidence annotations and retained for analysis if they included accurate mass (5 ppm) match and either (1) retention time (±5%) matching to previously analyzed reference standards, and/or (2) matching to mass spectrometry[2] spectra in mzCloud (mzcloud.org) with at least 1 product ion match.

Metabolite validation analyses

Validation of selected metabolites was performed in an independent sample of 215 outpatient children with asthma from metropolitan Atlanta, Georgia, who were considered at high risk for exacerbation. Details of the sample, participant characterization, and the metabolomics methodology were published previously.[23] Metabolites were compared in children with acute uncontrolled asthma versus in children with stable asthma. Acute uncontrolled asthma was defined by either an Asthma Control Questionnaire 6-item score higher than 1.5[24] or Global Initiative for Asthma criteria for uncontrolled asthma,[7] which include at least 3 of the following: (1) daytime asthma symptoms more than twice per week, (2) any night awakening due to asthma, (3) short-acting bronchodilator use for symptoms more than twice per week, and (4) any activity limitation due to asthma.[7] Metabolites were also compared in children with severe versus nonsevere asthma.

Exploratory analyses

Exploratory analyses focused on associations between significant metabolites and PAIS scores, lung function measures (FEV_1 value and FEV_1/FVC ratio), and asthma severity.

Statistical analyses

Features between groups were compared by performing chi-squared analyses and t tests using IBMSPSS Statistics Software (version 28, IBM, Armonk, NY). Data that were not normally distributed were logarithmically transformed before statistical analyses. Metabolomics data were analyzed with RStudio software.[25] Metabolites were log_{10} transformed, median normalized, and range scaled to minimize outliers and produce more normal distributions. Univariate analyses were performed on each ionization mode separately.[26] Metabolite comparisons between exacerbating and stable asthma groups

were made by ANOVA, adjusting for race as a binary variable (Black versus non-Black race). Results were adjusted for multiple comparisons by using the Benjamini-Hochberg procedure, with a false discovery rate of 10%.[27] Metabolites were considered significantly different only if they displayed an unadjusted P value less than .05 and adjusted q value less than 0.1. Partial least squares discriminant analysis (PLS-DA) was then performed by using MetaboAnalyst 5.0[28] on all significantly different metabolites between groups. The predictive ability of the PLS-DA model was tested by using 10-fold cross-validation.[29] Pathway enrichment analysis, which considers the connectedness of metabolites within a pathway, was conducted by using MetaboAnalyst 5.0 with utilization of the Kyoto Encyclopedia of Genes and Genomes (KEGG) database, a hypergeometric test, and relative betweenness centrality.[30] For validation analyses, mass-to-charge features for preselected metabolites were z score–normalized and compared with the Welch t test values without assumption of equal SDs. For exploratory analyses, associations were assessed with Pearson correlation analyses and Welch t tests. Validation and exploratory analyses utilized a significance level of .05 without adjustment for multiple comparisons.

RESULTS

A total of 98 children were enrolled (69 with exacerbating asthma and 29 with stable asthma) (Table I). The groups did not differ by age, sex, weight percentile, family history of asthma, daily asthma controller medications, exposures, or asthma-related health care utilization. However, there were slightly more self-reported Black children in the exacerbating group. Although asthma control in the prior 4 weeks (as reflected by ACT or C-ACT scores) did not differ between the groups, asthma impact in the previous week (as reflected by PAIS scores) was significantly worse in the exacerbating children. Although not specifically a requirement for inclusion, more than 93% of children in both groups had aeroallergen sensitization confirmed by specific IgE testing. Both groups also had similar elevations in serum IgE level. Blood eosinophil counts during hospitalization were elevated (ie, >150 cells/μL) in the exacerbating children but were significantly lower than those of the stable children. Lung function values for FVC; FEV_1; forced expiratory flow at 25% to 75% of FVC (obtained while the patient was stable before or after hospitalization in the case of exacerbating patients); and plasma IFNγ, IL-4, IL-5, and IL-13 concentrations were also not different between groups (Table I). Although respiratory viral panels were not performed in all exacerbating children, in the subset in which they performed (n = 33), a respiratory virus was identified in 61% of children (n = 20), with enterovirus and/or rhinovirus present in 95% of the positive samples (n = 19).

Metabolomic analyses

In all, 118 and 131 high-confidence metabolites were identified in positive and negative ionization mode, respectively. A total of 103 unique metabolites differed significantly between the exacerbating and stable asthma groups. These metabolites, along with the log_2 fold change and q value, are listed in Tables E1 and E2 (in the Online Repository at www.jaci-global.org). PLS-DA, which is a "supervised" version of principal components analysis, was performed to visualize the variability and assess the discriminatory and predictive ability of metabolite profiles for the predetermined patient groups (Fig 1, A

and B). Component 1 and component 2 explained 22.1% to 24.5% and 6.3% to 7% of the variance in positive and negative modes, respectively. The exacerbating children had higher within-group variability than did the stable children, as indicated by the size of the ellipse over the point spread (Fig 1, A and B). Classification performance of the PLS-DA model was evaluated for accuracy, goodness of fit (R^2), and predictive ability (Q^2) for the top 5 components for positive and negative modes. With use of 10-fold cross-validation, the model with 3 components best classified the positive mode model, with an accuracy of 0.97, an R^2 value of 0.89, and a Q^2 value of 0.79 (see Fig E1, A in the Online Repository at www.jaci-global.org). The model with 2 components best classified the negative mode model, with an accuracy of 0.99, an R^2 value of 0.85, and a Q^2 value of 0.78 (see Fig E1, B), confirming that the models were predictive and not overfitted.

Variable importance of projection (VIP) scores were then generated to estimate the importance of each metabolite in the projection used in the PLS-DA model. Metabolites with a VIP score close to or higher than 1 were considered important in the model. The metabolites that had the highest predictive power in classifying exacerbating and stable asthma were then visualized by the plot of the first component of normalized metabolites by group for positive and negative modes. This approach yielded 15 predictive metabolites in positive mode and 15 metabolites in negative mode (Fig 2, A and B). The plots of the normalized concentrations of the top 12 metabolites shown in the VIP score plot, regardless of ionization mode, are shown by group in Fig 3, A-L. These 12 metabolites included prolylleucine, trans-4-hydroxy-L-proline, hydroquinone, acetylcholine, 2-hydroxybutyrate, 3-methyl-L-histidine, L-ergothioneine, citrulline, O-acetylcarnitine, 3-(2-hydroxyphenyl) propanoate, 3-methylxanthine, and 3-methyl-2-oxovalerate.

Metabolite pathway analysis

Pathway enrichment analysis was then performed by using the KEGG database to identify the most relevant metabolic pathways involved in discriminating children with exacerbating versus stable asthma. Of the 103 unique metabolites that differed between groups (see Tables E1 and E2), 83 had KEGG identification numbers and 8 significant metabolic pathways were identified (Fig 4). These significantly enriched pathways, in order of significance from highest to lowest, included (1) arginine and proline metabolism; (2) aminoacyl-tRNA synthesis (substrates that determine how the genetic code is interpreted as amino acids); (3) phenylalanine metabolism; (4) glycine, serine, and threonine metabolism; (5) valine, leucine, and isoleucine (branched-chain amino acids) biosynthesis; (6) histidine metabolism; (7) phenylalanine, tyrosine, and tryptophan biosynthesis; and (8) butanoate metabolism. The interconnectedness of the identified pathways is shown in Fig 5.

Metabolite validation analyses

To determine whether the identified metabolites might be generalizable to other samples of similar children, we performed validation analyses in an independent sample of 215 outpatient children with asthma from metropolitan Atlanta who were who were at high risk for exacerbation. The top 12 metabolites shown in the VIP score plot differentiating exacerbating from stable asthma in the present study were compared between children with acute uncontrolled asthma and children with stable asthma. Features of the validation sample

are shown in Table E3 (in the Online Repository at www.jaci-global.org). The z score–normalized mass-to-charge ratios for 9 of these 12 metabolites, including prolylleucine, trans-4-hydroxy-L-proline, acetylcholine (as acetyl-β-methylcholine), 2-hydroxybutyrate, 3-methyl-L-histidine, citrulline, O-acetylcarnitine, 3-(2-hydroxyphenyl) propanoate, and 3-methyl-2-oxovalerate, were available for analysis. This validation analysis confirmed the following 5 metabolites in children with acute uncontrolled versus stable asthma: (1) higher 3-(2-hydroxyphenyl) propanoate concentration, (2) lower trans-4-hydroxy-L-proline concentration, (3) lower acetyl-β-methylcholine concentration, (4) lower 3-methyl-L-histidine concentration, and (5) lower citrulline concentration (Fig 6, A-E). Further analysis by asthma severity, irrespective of asthma control, also demonstrated higher 3-(2-hydroxyphenyl) propanoate, lower acetyl-β-methylcholine, lower 3-methyl-L-histidine, and lower citrulline concentrations in children with severe (n = 76) versus nonsevere (n = 139) asthma Fig E2 (in the Online Repository at www.jaci-global.org).

Exploratory analyses of potential clinical relevance

We then explored whether the 103 high-confidence metabolites that differed between exacerbating and stable asthma were associated with PAIS scores, lung function, and/or severe asthma. Of the 103 metabolites that differentiated exacerbating asthma, 31 were also associated with PAIS scores (see Table E4 in the Online Repository at www.jaci-global.org). In all, 7 metabolites were associated with FEV_1 value and 8 metabolites were associated with FEV_1/FVC ratio (see Table E5 in the Online Repository at www.jaci-global.org), but only 2 metabolites, acetoacetate and lactate, were common between FEV_1 value and FEV_1/FVC ratio (see Fig E3 in the Online Repository at www.jaci-global.org). Of the 103 high-confidence metabolites, 15 were also associated with severe asthma (see Table E6 in the Online Repository at www.jaci-global.org).

DISCUSSION

In this study, we performed metabolomic analyses to understand differences in plasma metabolite patterns in children with exacerbating asthma who were hospitalized for status asthmaticus. Consistent with our hypothesis, we observed distinct metabolomic profiles in children with exacerbating versus stable asthma that were largely associated with alterations in arginine, phenylalanine, and glycine metabolism. Other pathways of interest and other high-confidence metabolites that could not be mapped to specific pathways but were also identified warrant further investigation. Interestingly, these identified metabolic alterations were not associated with systemic perturbations in classical type 1 (ie, IFN-γ) and type 2 (ie, IL-4, IL-5, and IL-13) cytokines. These results highlight the complex biology of inflammation in children with exacerbating asthma and argue for additional studies of the metabolic determinants of asthma exacerbations in children because many of the identified metabolites of interest may be amenable to targeted interventions.

Metabolomic studies in isolated plasma have previously been performed in children with asthma,[31-34] but to our knowledge, this is the first study to perform metabolomic analyses on children during an acute exacerbation. This is also one of very few studies to perform molecular characterization of hospitalized children with status asthmaticus. Whereas

other pediatric metabolomic studies have primarily compared metabolites in children with asthma as a whole versus in healthy controls or other nonasthma populations,[31-34] this study utilized an asthma comparison group, which permitted study of the unique metabolites that are altered during an acute exacerbation necessitating hospitalization. Therefore, unlike other studies with healthy controls, our study did not identify eicosanoids (derived from oxidation of arachidonic acid or other polyunsaturated fatty acids),[34] nicotinamide pathway metabolites (which regulate glycolysis, β-oxidation, and oxidative phosphorylation),[32] or fatty acids (which drive inflammation through production of arachidonic acid, linoleic acid, eicosapentaenoic acid, and docosahexaenoic acid),[31,33,34] even though our chromatographic technique was optimized for hydrophilic molecules. Nonetheless, our findings do have biologic plausibility. In our previous metabolomic analysis of children with severe asthma refractory to treatment with high-dose inhaled and systemic corticosteroids, children with severe asthma, compared with children with mild-to-moderate asthma, were differentiated by alterations in glycine, serine, and threonine metabolism, which are closely linked to arginine and proline metabolism and biosynthesis of valine, leucine, and isoleucine (branched-chain amino acids).[35] We have also previously highlighted metabolomic perturbations in children with exacerbation-prone asthma (defined as those children having 3 or more exacerbations in the previous year) that were largely associated with arginine, lysine and methionine metabolism.[23] In that study, comparison of the identified metabolite concentrations with reported values for healthy controls revealed that most of the metabolites were decreased in asthma, but significantly more so in children with exacerbation-prone asthma.[23] Other studies have similarly identified disruptions in phenylalanine metabolism[36] and histidine metabolism[37] in children with severe asthma who failed to respond to systemic corticosteroids or omalizumab, respectively.

Arginine is a versatile amino acid that serves as a precursor for synthesis of nitric oxide, urea, creatine, polyamines, proline, glutamate, agmatine, and homoarginine. Metabolism of arginine is complex because multiple enzymes utilize arginine as a substrate. Furthermore, cells may contain multiple arginine pools, not all of which are accessible to arginine metabolic enzymes.[38] Therefore, whether arginine metabolite concentrations are "increased" or "decreased" in children with asthma is difficult to assess and may be influenced by the chronicity of disease and current asthma control. For example, previous work in children with chronic, well-controlled asthma observed lower concentrations of arginine but significantly increased concentrations of ornithine and citrulline, which may represent a compensatory response.[39] A separate study of children with exacerbating asthma presenting to an emergency department similarly noted decreased arginine concentrations and also lower citrulline concentrations compared with the concentrations in healthy controls.[40] We also observed decreased plasma citrulline concentrations in otherwise stable children with exacerbation-prone asthma[23] and children with obese asthma whose asthma was uncontrolled.[41] However, other studies in young stable children with mild persistent asthma have shown no associations between arginine or its metabolites and features of asthma (such as allergic sensitization) or exacerbations over 48 weeks.[42] Studies of older school-age children with asthma have also found no differences[43] or increases[44] in arginine metabolite concentrations compared with those in healthy controls.

The aromatic amino acid phenylalanine is considered inflammatory, with higher concentrations of phenylalanine reported in adults with coronary artery disease[45,46] and infants at risk for cardiovascular disease with more frequent infections during the first year of life.[47] Previous studies have shown that phenylalanine concentrations are higher in children with acute exacerbations of asthma than in healthy controls,[48] in obese children with uncontrolled versus controlled asthma,[41] and in children with severe asthma who failed to respond to systemic corticosteroid therapy.[36] Likewise, imbalances in the glycine, serine, and threonine metabolism pathway are also considered inflammatory and may promote sustained oxidative stress. Serine is a precursor of cysteine, which is itself a precursor of glutathione. Disturbances in serine biosynthesis may account for alterations in the cysteine and glutathione redox balance that we have previously reported in children with severe refractory asthma.[49,50] The present study did not detect cysteine or glutathione disulfide, prohibiting evaluation of their respective redox balances. However, we did identify methionine and 2 of its oxidation products (methionine sulfoxide and dehydromethionine), with methionine being a precursor of cysteine and glutathione. Methionine sulfoxide concentration was significantly higher in children with exacerbating asthma and may be associated with neutrophil-mediated lung damage, similar to what is seen in children with cystic fibrosis.[51]

Serine is also a critical reagent for the synthesis of sphingolipids, including sphingomyelins, which have a phosphocholine head group. We found that both serine and choline concentrations were decreased in children with exacerbating asthma, whereas the concentrations of 2 sphingomyelins were increased. Sphingolipids play key roles in regulation and function of immune cells[52] and may drive lung inflammation.[53] Although the precise roles of sphingolipids in asthma is unclear, decreased sphingolipid synthesis due to increased expression of the *ORMDL3* (orosomucoid-like 3) gene, which inhibits serine palmitoyl transferase,[54] has been implicated in increased susceptibility to wheezing and early-onset asthma.[55-59] However, increased sphingolipid levels may drive asthma progression because higher levels of ceramides (in particular long-chain ceramides) and sphingosine-1-phosphate have been noted in adult patients with asthma,[60-63] especially in patients whose asthma is uncontrolled.[62]

Strengths of the study include characterization of the included patients, who were essentially all sensitized with mild airflow limitation and poor asthma control in the 4 weeks before enrollment, which permits generalization to a very large group of children with asthma encountered in both primary care and specialty settings. Another strength is the high-confidence annotations in our metabolites. Nonetheless, this study does have limitations. First, given the acute nature of children with exacerbating asthma, the findings in this group might result from hypoxia, infection, acidosis, and other factors rather than from asthma itself. Although our groups were clinically similar, some confounding by indication is also expected because the medications and interventions used for management of acute asthma differ. Therefore, the observations observed here might be an epiphenomenon and therefore require more validation. Second, pathway enrichment analyses were not performed on all metabolites because many of these could not be mapped to the KEGG database. These metabolites may be of clinical importance. For example, the dipeptide prolylleucine was the most significant single metabolite identified, yet it is not included in the KEGG metabolic

pathways. Indeed, the dipeptide isoleucine/leucineproline (isomeric with prolylleucine, and indistinguishable by our current method) has been negatively associated with FEV_1 value and positively associated with lung damage and bronchiectasis in children with cystic fibrosis, potentially through the proteases such as neutrophil elastase.[64,65] Furthermore, viral panels were not clinically indicated and were not performed in all exacerbating children. Therefore, we are unable to make inferences related to exacerbation triggers. However, in the subset of hospitalized children in whom viral panels were performed, a respiratory virus was identified in 61% of cases, with enterovirus and/or rhinovirus present in 95% of the positive samples, which is consistent with the results of other pediatric studies.[66] We were also potentially limited by the cross-sectional analyses and cannot comment on the temporal stability of the metabolome in either group. Future studies of the same patient before and after hospital discharge would also be informative.

In summary, targeted metabolomic analyses permitted discrimination of children with exacerbating versus stable asthma, largely owing to alterations in arginine, phenylalanine, and glycine metabolism. Although independent validation studies are warranted, this work highlights the biologic complexity of asthma exacerbations in children but also offers some insight into individual metabolites and pathways that might be amenable to future intervention.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

DISCLOSURE STATEMENT

Abbreviations used

ACT	Asthma Control Test
FVC	Forced vital capacity
KEGG	Kyoto Encyclopedia of Genes and Genomes
PAIS	Pediatric Asthma Impact Scale
PICU	Pediatric intensive care unit
PLS-DA	Partial least squares discriminant analysis
VIP	Variable importance of projection

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Key messages

- Children with exacerbating asthma have significant perturbations in their plasma metabolites that are largely associated with alterations in arginine, phenylalanine, and glycine metabolism.

- The identified metabolic alterations are not associated with systemic perturbations in classical type 2 inflammation and highlight the complex biology of inflammation in children with exacerbating asthma.

- Additional studies of the metabolic determinants of asthma exacerbations in children are warranted because many of the identified metabolites of interest may be amenable to targeted interventions.
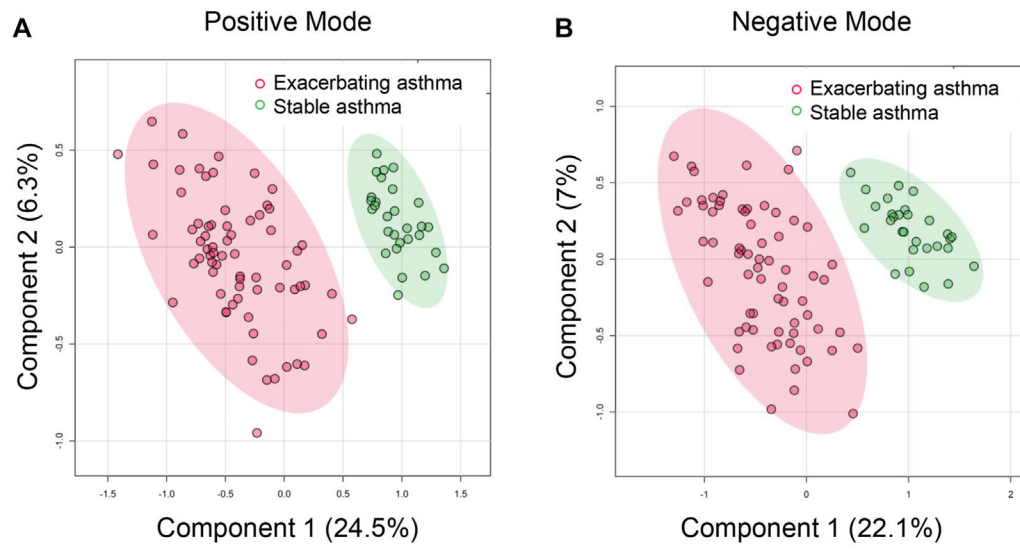
FIG 1.
A and **B,** PLS-DA of metabolites from positive mode (**A**) and negative mode (**B**) that discriminated between exacerbating asthma (*red circles*) and stable asthma (*green circles*) in children. The number in parentheses reflects the variance explained by each component.
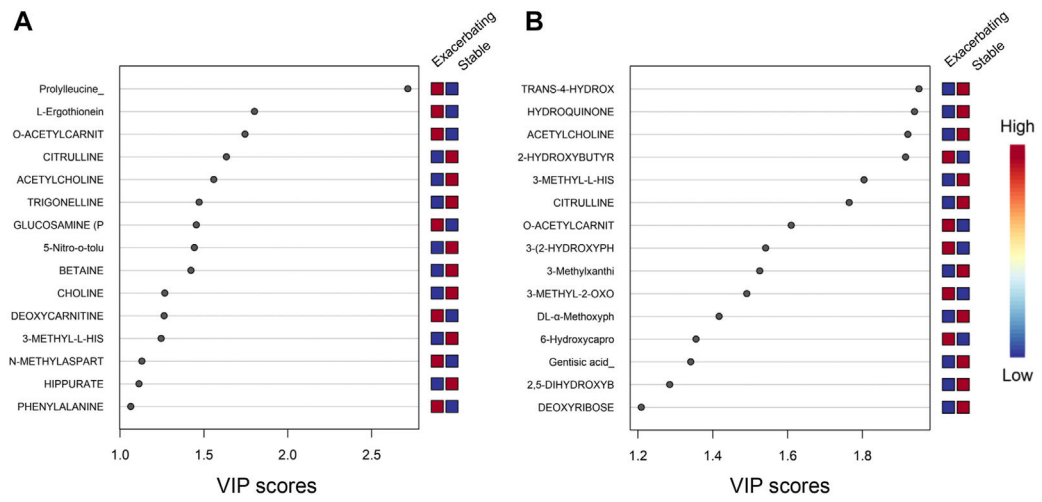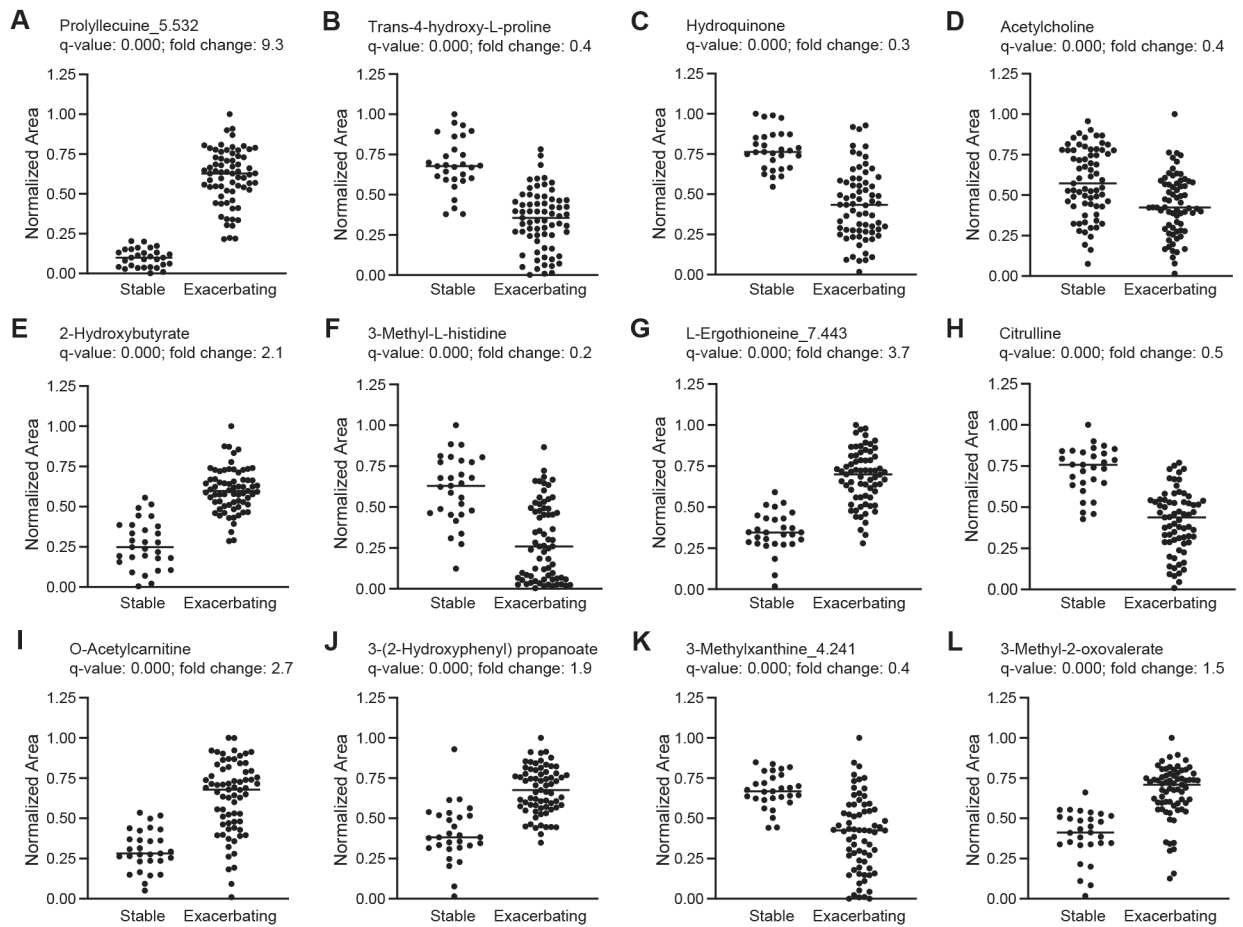
FIG 2.
A and **B,** VIP score plot of normalized metabolites by group in positive mode (**A**) and negative mode (**B**). Higher concentrations are in red and lower concentrations are in blue.

FIG 3.

Scatterplots of normalized concentrations of the top 12 metabolites explaining the variable importance of projection (VIP) of the first component in the PLS-DA. Data reflect either positive or negative mode for prolylleucine (**A**), trans-4-hydroxy-L-proline (**B**), hydroquinone (**C**), acetylcholine (**D**), 2-hydroxybutyrate (**E**), 3-methyl-L-histidine (**F**), L-ergothioneine (**G**), citrulline (**H**), O-acetylcarnitine (**I**), 3-(2-hydroxyphenyl) propanoate (**J**), 3-methylxanthine (**K**), and 3-methyl-2-oxovalerate (**L**).
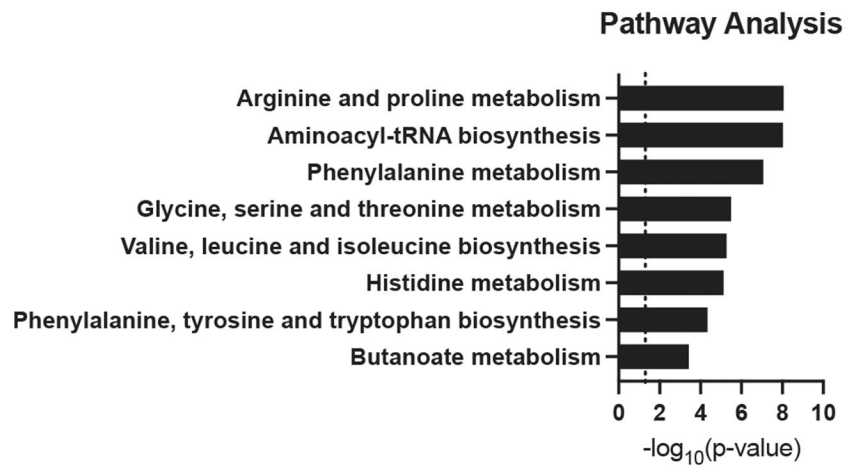
Pathway Analysis

FIG 4.

Pathway enrichment analysis showing the differences between children with exacerbating versus stable asthma. Significant metabolic pathways with an impact of .05 or higher are shown. *tRNA*, Transfer RNA.
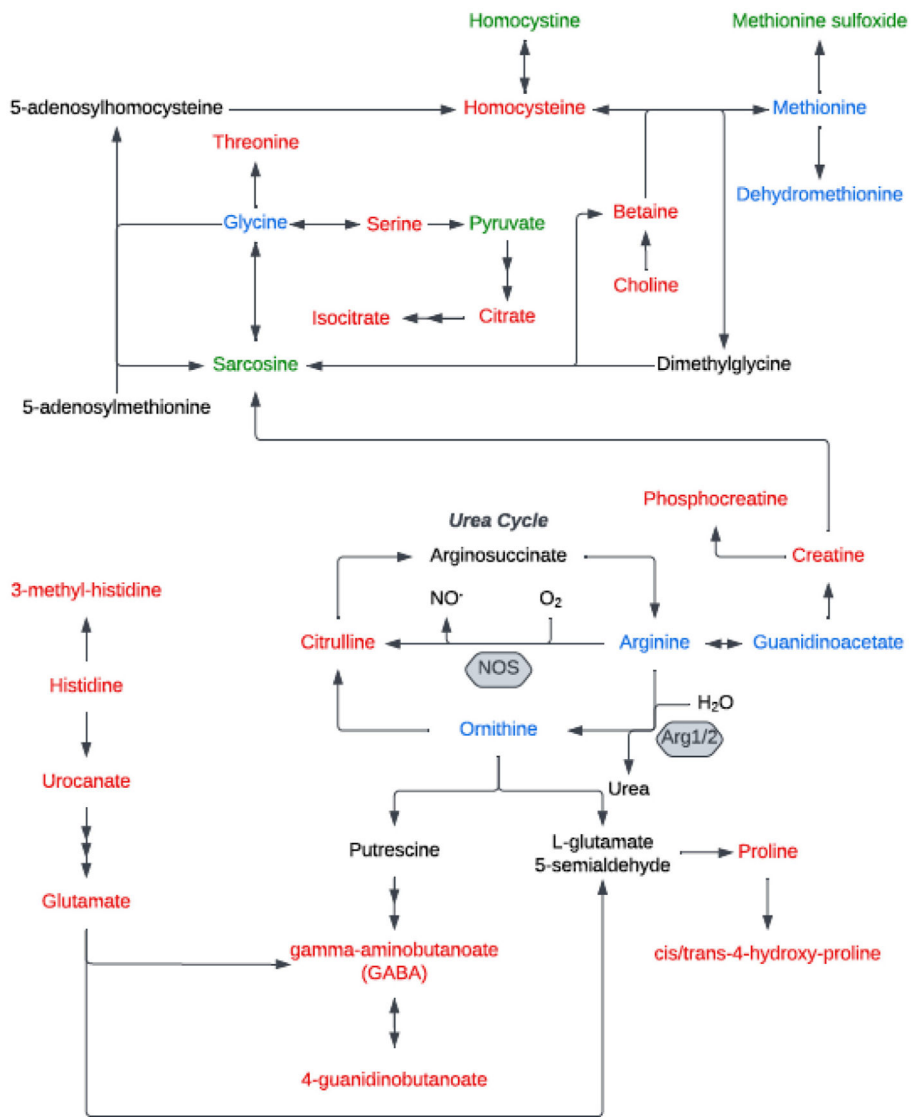
FIG 5.
Interconnectedness of significant pathways from pathway enrichment analysis. Metabolites with concentrations that were decreased in children with exacerbating asthma are shown in red, metabolites with concentrations that were increased are shown in green. Metabolites that were identified but did not differ in terms of concentration are shown in blue, and metabolites that were not identified by our methods are shown in black.
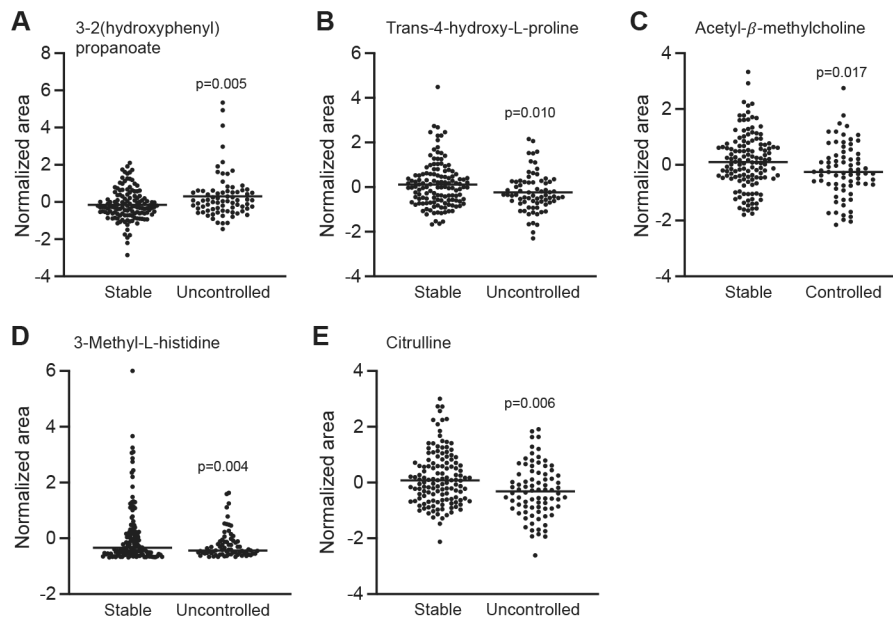
FIG 6.
Scatterplots of normalized concentrations of 5 significant (of 9 in total) metabolites assessed in a validation sample of outpatient children with uncontrolled and stable asthma, including 3-(2-hydroxyphenyl) propanoate (**A**), trans-4-hydroxy-L-proline (**B**), acetyl-β-methylcholine (**C**), 3-methyl-L-histidine (**D**), and citrulline (**E**).

TABLE I.

Features of the participants

Feature	With exacerbating asthma (n = 69)	With stable asthma (n = 29)	P value
Age (y), median (25th, 75th percentile)	11 (9, 14)	10 (8, 14)	.328
Males, no. (%)	42 (60.9)	14 (48.3)	.250
Self-reported race, no. (%)			.020
White	4 (5.8)	8 (27.6)	
Black	58 (84.1)	19 (65.5)	
Asian or American Indian	2 (2.9)	2 (6.9)	
1 Race	3 (4.3)	0	
Declined to answer	2 (2.9)	0	
Weight percentile, median (25th, 75th percentile)	76 (54, 99)	77 (60, 99)	.500
Family history, no. (%)			
Mother with asthma	23 (33.3)	10 (35.7)	.823
Father with asthma	20 (29.0)	8 (28.6)	.967
Daily asthma controller medications, no. (%)			
Montelukast	28 (40.6)	15 (51.7)	.310
Inhaled corticosteroid	39 (56.5)	22 (75.9)	.071
Long-acting β-agonist	23 (33.3)	10 (34.5)	.912
Exposures, no. (%)			
Smokers in the home	23 (33.3)	6 (20.7)	.211
Dog or cat in the home	22 (31.9)	9 (31.0)	.934
Previous PICU admission for asthma (ever in lifetime), no. (%)	26 (37.7)	16 (55.2)	.110
Intubation for asthma (ever), no. (%)	5 (7.2)	5 (17.2)	.136
Unscheduled visits for asthma symptoms (in past 12 mo), median (25th, 75th percentile)*	2 (1, 3)	2 (1, 4)	.466
Severe asthma, median (25th, 75th percentile)*	38 (55.1)	11 (37.9)	.121
ACT or C-ACT score, median (25th, 75th percentile)	14 (11, 16)	18 (14, 21)	.181
ACT or C-ACT score of <20, no. (%)	49 (71.0)	18 (62.1)	.116
PAIS score, median (25th, 75th percentile)	58 (54, 66)	53 (46, 57)	.010
Aeroallergen sensitization, no. (%)†	53 (98.1)	27 (93.1)	.240

Feature	With exacerbating asthma (n = 69)	With stable asthma (n = 29)	P value
Serum IgE (kU/L), median (25th, 75th percentile)	443 (202, 697)	346 (167, 765)	.359
Blood eosinophils (per µL), median (25th, 75th percentile)[‡]	217 (59, 551)	396 (217, 551)	.001
Lung function (% predicted), median (25th, 75th percentile)[§]			
FVC	95 (83, 114)	99 (88, 108)	.284
FEV_1	87 (72, 104)	89 (79, 99)	.180
FEV_1/FVC	91 (77, 94)	91 (84, 99)	.401
FEF_{25-75}	70 (42, 80)	73 (59, 86)	.165
Plasma cytokines (pg/mL), median (25th, 75th percentile)			
IFN-γ	10.1 (5.3, 16.4)	9.0 (5.8, 12.3)	.286
IL-4	20.1 (8.3, 39.8)	17.6 (10.1, 65.9)	.935
IL-5	3.1 (2.3, 4.0)	2.0 (1.0, 4.5)	.230
IL-13	1.7 (1.1, 0.8)	1.6 (0.8, 9.1)	.974

C-ACT, Childhood ACT; *FEF25-75,* forced expiratory flow at 25% to 75% of forced vital capacity.

[*] Defined as the requirement for high-dose inhaled corticosteroids plus an additional controller medication for asthma control.

[†] Exacerbating asthma (n = 54).

[‡] Exacerbating asthma (n = 35).

[§] Exacerbating asthma (n = 43).