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Children with severe asthma have unique oxidative stress-associated metabolomic profiles

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Summary

Children with severe asthma have unique metabolic derangements associated with oxidative stress-related pathways. These metabolic differences may contribute to corticosteroid insensitivity in severe asthma and may be novel targets for further study and therapeutic intervention.

Keywords

Asthma; severe asthma; children; metabolomics; glutathione; cysteine; thiol; oxidative stress; lipid peroxidation

To the Editor

Children with severe asthma are a challenging group of patients who are extremely difficult to treat. Although inhaled corticosteroids (**ICS**) are the cornerstone of asthma treatment, children with severe asthma have decreased responsiveness to high-dose corticosteroid therapy¹ associated with airway inflammation and remodeling.² Given the limited efficacy of corticosteroids in severe asthma, our efforts have focused on the role of oxidative stress, namely thiol redox disturbances, in the modulation of the disorder.³ Indeed, we have previously demonstrated greater oxidation and decreased concentrations of the thiols cysteine and glutathione in the airways⁴ and systemic circulation⁵ of children with severe asthma that were further associated with increased airway inflammation and impaired cellular function.⁶

Recognizing that the treatment of severe asthma might ultimately require novel approaches to restore corticosteroid sensitivity,¹ we performed metabolomic analyses of plasma samples from a highly characterized sample of children with severe asthma treated with high-dose ICS and long-acting beta agonists (**LABA**) and children with mild-to-moderate asthma

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treated with ICS or ICS/LABA combination therapy. We report that children with severe asthma are differentiated by two metabolic pathways associated with oxidative stress: 1) the glycine, serine and threonine metabolism pathway and 2) the N-acylethanolamine and N-acyltransferase pathway. These findings support the biological plausibility of oxidative stress as a contributory factor to corticosteroid insensitivity in severe asthma and potentially highlight novel therapeutic targets for further confirmation and study.

Enrolled children had confirmed asthma with a history of $\geq 12\%$ reversibility in the forced expiratory volume in one second (**FEV₁**) after bronchodilator (albuterol) administration or airway hyperresponsiveness to methacholine evidenced by a methacholine PC₂₀ ≤ 16 mg/mL. All children underwent comprehensive phenotypic characterization consisting of spirometry with bronchodilator reversibility, exhaled nitric oxide testing and venipuncture for total serum IgE and whole blood eosinophils as described in the Online Repository. Children who met criteria for severe asthma also underwent formal corticosteroid responsiveness testing with systemic triamcinolone acetonide (1 mg/kg intramuscular injection). Children with severe asthma were considered to have corticosteroid “responsiveness” if symptoms, lung function, bronchodilator reversibility and exhaled nitric oxide values normalized two weeks after the intervention.⁷ Cut-points for “normal” were defined as symptoms less than twice weekly, FEV₁ $> 80\%$ predicted with FEV₁/FVC > 0.85 , bronchodilator reversibility $< 12\%$ after albuterol administration, and exhaled nitric oxide values ≤ 25 ppb. Plasma samples were obtained for metabolomic studies prior to triamcinolone administration to avoid carryover effects from the corticosteroid intervention.

Metabolic profiling was accomplished with high performance liquid chromatography whereby plasma was separated on a C₁₈-reverse phase column and analyzed by Fourier Transform ion cyclotron resonance (**FTICR**) mass spectrometry (see Online Repository). Mass-to-charge (*m/z*) features were analyzed for each plasma sample on two separate occasions, in triplicate. Data from the two replicate analyses were averaged to obtain single *m/z* values for each subject. Metabolomic data were processed using the R package apLCMS. False discovery rate (**FDR**) analyses and orthogonal partial least squares discriminant analyses (**OPLS-DA**) were applied to the *m/z* features to determine which discriminated significantly between the two groups. Pathway analysis was performed using an in-house program that considers all common derivatives when comparing *m/z* values to the theoretical molecular weights of metabolites. Comparison was guided by a calibration curve on the FTICR mass spectrometer, which agrees with the inverse correlation between the mass spectrometer resolution and *m/z*. The Kyoto Encyclopedia of Genes and Genomes (**KEGG**) human metabolomic database was used for analyses of pathways and compounds and pathways were examined in more detail with GeneGo software (Thomson Reuters, New York, NY) using Enrichment analysis in Pathway Maps.

Features of enrolled children are shown in Table I. By definition, children with severe asthma were treated with higher doses of ICS (median 1000 vs. 250 μ g fluticasone equivalent/day, $p < 0.001$), yet they had greater healthcare utilization for asthma as well as increased airflow limitation and more impaired asthma control and quality of life (Table I). Additionally, no child with severe asthma displayed complete corticosteroid responsiveness per our composite definition (**Tables EI**). Rather, 31 children (89%) had only partial responsiveness while 4 children (11%) had no discernible clinical response to systemic triamcinolone administration (**Table EII**).

The extracted metabolomic data consisted of 8,953 high-resolution *m/z* features. Of these, 5,629 had $< 5\%$ missing values. Partial least squares-discriminant analysis with orthogonal signal correction with autoscaling showed complete separation of the groups using predictive component 1 (y-axis) (Figure 1A). The first orthogonal component accounted for

18% of the variation; after removal of the first orthogonal component, the first predictive component accounted for 93% of the remaining variation. To address the discriminatory features in greater detail, a false discovery rate (**FDR**) of 0.01 was used to identify features that differed significantly between groups. The Manhattan plot for significant m/z features identified by C_{18} column separation (1,843 features) is shown in Figure 1B. With a FDR of 0.01, the C_{18} features matched 164 metabolites in the KEGG human metabolomics database and were distributed across the metabolic pathways (Figure E1). The metabolites identified were significantly associated with two metabolic pathways: 1) the glycine, serine and threonine metabolism pathway (m/z : 9/123; p -value = $3.485e^{-5}$) and 2) the N-acyl ethanolamine and N-acyltransferase pathway (m/z : 4/32; p -value = $2.980e^{-4}$). Significant metabolites mapped into these pathways are shown in **Tables EII and EIII**.

These preliminary observations from an unsupervised metabolomic analysis confirm that severe, corticosteroid refractory asthma in children is associated with a vast array of metabolic derangements which are most notably associated with oxidative stress. Glycine, serine and threonine are closely related amino acids that share common biochemical pathways (Figure E2). Serine participates in the biosynthesis of purines and pyrimidines and is a precursor for the synthesis of phosphoglycerides, sphingolipids and glycolipids. Additionally, serine is a precursor to several amino acids including glycine and cysteine, which are essential components of the vital antioxidant, glutathione.³ The fact that this pathway most strongly differentiated children with severe versus mild-to-moderate asthma is in keeping with our previous observations of altered cysteine and glutathione redox balance in this population.⁴⁻⁶ Similarly, N-acyl ethanolamine phospholipids, which are formed by membrane-associated N-acyltransferases, are metabolized to N-acyl ethanolamines in response to cellular injury⁸ and play important roles in signal transduction⁹ and cytoprotection against oxidative stress.¹⁰ However, the high number of carbon double bonds that we observed in the identified metabolites suggests that significant lipid peroxidation may be occurring in severe asthma. In keeping with this hypothesis, we have previously observed higher concentrations of lipid peroxidation biomarkers in children with severe asthma that were further associated with thiol redox disturbances.^{2, 4}

Collectively, these observations from an unsupervised analytical approach support the biological plausibility of oxidative stress as a contributory factor to corticosteroid refractory severe asthma in children. To our knowledge, this is the first study to undertake metabolomic analyses in this population. Important strengths of the study are the careful phenotyping of enrolled children and confirmation of corticosteroid insensitivity in children with severe asthma, suggesting that the observed differences cannot be solely attributed to corticosteroid adherence. However, the precise mechanisms underlying the metabolic differences in children with severe asthma are not yet known. It is also unclear whether similar differences would have been detected in airway versus plasma samples. Furthermore, the effect of the environment (i.e., the “exposome”) on our findings is not understood. Questions about confounding by indication, namely asthma medication exposure and co-morbid conditions such as obesity, sinus disease and obstructive sleep apnea which may accompany severe asthma, also remain and require further study. While independent replication and longitudinal validation of our results is essential and further studies with diseased and healthy controls are needed, these preliminary findings are intriguing and suggest that oxidative stress-related derangements in these metabolic pathways may be potential therapeutic targets for future study. However, we recognize that our statistical approach utilized very stringent thresholds for false discovery and we therefore submit that there may be other metabolic pathways with lower abundance metabolites that also warrant further study. Integration of various “omic” strategies through a systems biology approach is also necessary to ultimately unravel mechanisms of asthma and asthma severity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

(A) Analysis of identified mass-to-charge (m/z) features using partial least squares-discriminant analysis with orthogonal signal correction demonstrates complete separation of the groups. Replicate values (averaged from triplicate analysis) are shown for each subject as a circle (mild-to-moderate asthma) or square (severe asthma).

(B) Manhattan plot demonstrating significantly different m/z features (individual points) between children with mild-to-moderate asthma and severe asthma.

Table I
Features of the subjects

Data represent the median (IQR) or the frequency (%).

	Mild-to-moderate asthma (N = 22)	Severe refractory asthma (N = 35)
Age (years)	12 (9, 14)	13 (10, 17)
Male	12 (55)	23 (66)
Non-white	15 (68)	33 (94) [†]
Asthma duration (years)	10 (4, 12)	11 (9, 15) [‡]
BMI percentile		
<85 th	8 (36)	15 (43)
85 th to 95 th	7 (32)	9 (26)
>95 th	7 (32)	11 (31)
Atopic history		
Allergic rhinitis	20 (91)	35 (100)
Atopic dermatitis	11 (50)	21 (60)
Asthma medical history		
Emergency room visit (previous year)	6 (27)	26 (74) [‡]
Hospitalization (previous year)	3 (14)	12 (34)
Intubation (ever)	5 (23)	21 (60) [‡]
Self-reported asthma symptoms at least weekly	7 (32)	32 (91) [‡]
Asthma Control Questionnaire score	0.29 (0.04, 0.64)	1.29 (0.86, 2.54) [‡]
Asthma Quality of Life Questionnaire score	6.02 (5.69, 6.45)	5.06 (4.00, 6.00) [‡]
Serum IgE (kU/L)	78 (9, 136)	193 (78, 335) [†]
Exhaled nitric oxide (ppb)	24 (9, 64)	31 (21, 58)
Blood eosinophils (%)	3.8 (2.6, 6.1)	5.2 (3.5, 8.2)
Baseline spirometry		
FVC (% predicted)	112 (105, 119)	100 (92, 116) [†]
FEV ₁ (% predicted)	106 (91, 108)	91 (78, 100) [‡]
FEV ₁ /FVC	0.83 (0.77, 0.84)	0.74 (0.70, 0.82) [†]
FEF ₂₅₋₇₅ (% predicted)	86 (77, 95)	66 (53, 77) [†]
FEV ₁ bronchodilator reversibility (%)	9 (5, 12)	15 (8, 25) [†]

[†] p < 0.05,

[‡] p < 0.01 versus non-severe asthma.