

Distinct Serum Sphingolipid Profiles among School-aged Children with Exercise-induced Wheeze and Asthma Persistence

To the Editor:

Alterations in sphingolipids have been associated with chronic lung diseases (1). A role for altered sphingolipid metabolism in asthma pathogenesis is suggested by single-nucleotide polymorphisms in the most replicated gene locus associated with childhood asthma, 17q21, which includes the gene for ORM-like protein isoform 3, a regulator of sphingolipid synthesis (2). Also, decreased sphingolipid synthesis is associated with airway hyperreactivity (3).

Childhood asthma includes multiple disease phenotypes, the disentangling of which would lead to better understanding of the underlying etiologies and potential treatments. Exercise-induced wheezing (EIW) in school-aged children has been identified as an asthma phenotype that includes both atopic and nonatopic asthma (4). Because many school-aged children with asthma will not continue to have asthma symptoms, identifying risk factors that predict persistence of wheezing has been challenging.

We hypothesized that serum sphingolipids would be altered with asthma symptoms, EIW, and asthma persistence among a case-control study of children with and without asthma, the New York City Neighborhood Asthma and Allergy Study, described previously (4). Briefly, during home visits, spirometry, fractional exhaled nitric oxide testing, and waist circumference measurements were conducted and serum was collected in 7- to 8-year-old children. Three years after enrollment, parents of all the willing subjects with asthma were queried about ongoing asthma symptoms.

Children were classified as cases on the basis of symptoms or asthma medication use in the past year (4). Children classified as a case at baseline (age 7–8 yr) were considered to have asthma persistence if they again met the case definition at age 10 to 11 years. EIW was defined as an affirmative response to questions about (1) wheezing after running or active play, and/or (2) child's chest sounding wheezy during or after exercise in the past 12 months. Children with specific IgE greater than or equal to 0.35 IU/ml against

any of the inhalant allergens tested were considered seroatopic. Sphingolipids were analyzed by mass spectrometry (5).

Multivariable analyses to examine asthma outcomes (dependent variables) were conducted using logistic regression. Variables conventionally considered as potential confounders were included in the multivariable models, including sex, race, Hispanic ethnicity, maternal asthma, environmental tobacco smoke, and material hardship.

Serum was available for 243 children at age 7 to 8 years, of whom 152 were classified as having asthma. Of those 152 subjects with asthma at age 7 to 8 years, 147 participated in the follow-up at age 10 to 11 years. Of those 147 children meeting the case definition of asthma at age 7 to 8 years, 102 (69%) also met that definition 3 years later and were considered to have asthma persistence between ages 7 to 8 and 10 to 11 years. Clinically, children with asthma had mostly mild disease with relatively normal lung function; however, they had significantly lower FEV₁/FVC, higher total IgE, and were more likely to be seroatopic than the children without asthma. Children included in these analyses did not differ significantly from those lost to follow-up.

Seventeen sphingolipid species were measurable in serum. Dihydroceramides C16 and C18:1 and long-chain bases sphinganine and sphingosine were below the limit of detection in most samples and were excluded from analyses.

In multivariable analyses, there were no statistically significant differences in sphingolipid concentrations among the children with and without asthma (Table 1). Among the children with asthma, concentrations of dihydroceramide C18 (DHC18); ceramides C18, C20, C24, and deoxy C24:1; and sphingomyelin SM24:1 were higher among those with EIW than among those without EIW. Asthma persistence between ages 7 to 8 years and 10 to 11 years was associated with increased serum DHC18 and C20:0 (Figure 1).

To our knowledge, this is the first study to evaluate the association between serum sphingolipid profiles and asthma in children. We identified a distinct serum dihydroceramide and ceramide profile associated with EIW and found that elevated concentrations of DHC18 and C20:0 at age 7 to 8 years predicted asthma persistence to age 10 to 11 years. These associations were not altered by seroatopy, suggesting a mechanism independent of allergic sensitization. This is consistent with the observation that nonallergic childhood asthma was the major phenotype found associated with single-nucleotide polymorphisms that affect expression of ORM-like protein isoform 3 (6). Although it is not known whether serum sphingolipid profiles reflect those tissues of the respiratory tract, these results strongly support that altered systemic sphingolipid metabolism is present with EIW and that increased serum DHC18 and C20 may predict asthma persistence. We acknowledge the possibility of spurious findings, given the number of sphingolipids tested in these analyses. As such, these findings must be interpreted with caution. Still, we believe that these findings are compelling for replication in future studies.

All serum sphingolipids that distinguished EIW and asthma persistence were higher than levels measured among control subjects. Notably, these serum signatures are opposite from what we found in murine models with impaired *de novo* sphingolipid synthesis demonstrating airway hyperreactivity. In this mouse model, genetic or pharmacologic inhibition of serine

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Table 1. Odds Ratio for Asthma Outcomes with Each Interquartile Range Increase in Serum Sphingolipid

	Cases vs. Control Subjects (n = 243)	EIW vs. Non-EIW (Cases and Control Subjects)* (n = 243)	EIW vs. Non-EIW (Cases)† (n = 152)	Asthma Persistence vs. Remission (n = 147)
Dihydroceramides (d18:0)				
DHC18	1.2 (0.82–1.7)	1.7 [‡] (1.1–2.5)	1.9 [‡] (1.1–3.0)	2.3 [§] (1.3–4.4)
DHC24:1	0.88 (0.61–1.3)	1.3 (0.88–2.0)	1.7 (0.97–2.9)	1.3 (0.70–2.4)
DHC24	0.96 (0.68–1.4)	1.3 (0.87–1.8)	1.4 (0.87–2.2)	1.0 (0.60–1.7)
Ceramides (d18:1)				
C16	1.2 (0.78–1.8)	1.6 [‡] (1.05–2.4)	1.7 (0.99–2.8)	1.5 (0.80–2.7)
C18	1.1 (0.76–1.5)	1.5 (1.0–2.2)	1.7 [‡] (1.1–2.9)	1.3 (0.78–2.2)
C20	0.86 (0.59–1.2)	1.5 (0.99–2.2)	1.9 [‡] (1.2–3.1)	1.8 [‡] (1.0–3.1)
C22	0.87 (0.59–1.3)	1.2 (0.77–1.8)	1.3 (0.78–2.3)	1.5 (0.84–2.8)
C24	0.92 (0.66–1.3)	1.4 (0.95–2.0)	1.8 [‡] (1.1–3.0)	1.1 (0.62–1.9)
C24:1	0.81 (0.52–1.3)	1.1 (0.71–1.8)	1.4 (0.78–2.5)	1.6 (0.81–3.1)
Long-chain bases (d18:1)				
S1p	1.4 (0.89–2.4)	1.2 (0.78–2.2)	1.1 (0.61–1.9)	1.5 (0.72–2.9)
Sa1p	1.3 (0.80–2.0)	1.2 (0.78–2.0)	1.1 (0.69–1.9)	1.7 (0.92–3.3)
Sphingomyelins (d18:1)				
SM C16	0.99 (0.68–1.4)	1.2 (0.80–1.9)	1.2 (0.73–1.9)	1.7 (0.96–3.0)
SM C18	1.1 (0.75–1.7)	1.3 (0.84–2.1)	1.3 (0.77–2.2)	1.6 (0.86–2.9)
SM C18:1	1.2 (0.81–1.7)	1.5 (0.94–2.3)	1.4 (0.85–2.4)	1.3 (0.74–2.3)
SM C24:1	1.0 (0.66–1.5)	1.6 (1.0–2.5)	1.7 [‡] (1.0–3.0)	1.5 (0.83–2.8)
Alanine-derived ceramides (m18:1)				
DeoxyC24:1	0.93 (0.68–1.3)	1.3 (0.94–1.9)	1.7 [‡] (1.04–2.7)	1.0 (0.62–1.8)

Definition of abbreviation: EIW = exercise-induced wheezing.

Adjusted for sex, race, Hispanic ethnicity, seroatopy, season of serum collection, assay batch, and number of freeze/thaws of serum before analyses. To standardize across sphingolipids, odds ratios were calculated for an interquartile range increase in sphingolipid.

*Analyses were conducted comparing the children with EIW to those without EIW. The group without EIW included cases with asthma and control subjects without asthma.

†Analyses were conducted comparing the children with EIW to those with asthma without EIW. The group without EIW only included subjects with asthma and did not include control subjects without asthma.

[‡] $P < 0.05$.

[§] $P < 0.01$.

palmitoyltransferase, the rate-limiting step of *de novo* sphingolipid synthesis, results in airway hyperreactivity and is associated with decreased dihydroceramide and ceramide levels in the lungs (3). In the current study, sphingolipid synthesis

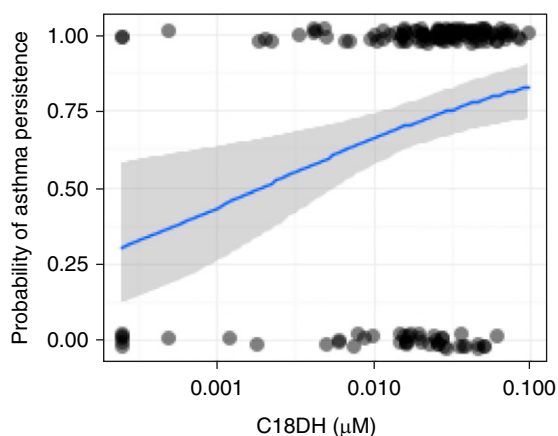


Figure 1. Association between dihydroceramide C18 (C18DH) and asthma persistence. The blue line represents logistic regression estimated odds ratio, with the 95% confidence interval in gray. The dots along the top and bottom of the figure represent individual children. Those in line with 0 on the y-axis did not have persistence, whereas those in line with 1 had asthma that persisted. $P = 0.005$ for unadjusted model (depicted), and $P = 0.008$ for the adjusted model described in Table 1.

cannot be directly assessed, as sphinganine (a specific product of *de novo* sphingolipid synthesis) was not measurable. The maintenance of ceramide and dihydroceramide levels is complex and subject to compensatory regulation. *De novo* sphingolipid synthesis may have only minor contributions to the total serum sphingolipid pool, with other regulatory feedback mechanisms involving ceramide synthases and sphingosine kinase playing a role. It is therefore possible that dihydroceramides and ceramides in serum originate from recycling pathways that are up-regulated when serine palmitoyltransferase is inhibited (7).

Overall, the alterations of distinct sphingolipid species in the serum of children with an asthma phenotype not defined by allergic sensitization suggest that sphingolipids are associated with childhood asthma. This metabolic pathway should be subject to further exploration for improved identification and characterization of asthma phenotypes and as a potential target for therapy. ■

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Monitoring of Pneumothorax Appearance with Electrical Impedance Tomography during Recruitment Maneuvers

To the Editor:

Pneumothorax can be defined as the abnormal accumulation of air in the pleural space. In patients with acute respiratory distress syndrome (ARDS), its incidence averages 8–10% (1), reaching 42% (2) in some reports, as a result of barotrauma. In patients with ARDS, especially those with moderate and severe forms of the syndrome, death after the occurrence of pneumothorax occurs in about 50% of the cases (1). Curiously, the incidence of pneumothorax does not decrease with lower values of external positive end-expiratory pressure (PEEP) (1). Therefore, irrespective of the ventilatory strategy applied, but especially during procedures involving higher risk for barotrauma, such as lung recruitment maneuvers, close monitoring for the occurrence of pneumothoraces is appealing.

Our group has systematically used electrical impedance tomography (EIT) to monitor changes in local air content during recruitment maneuvers at the bedside. This practice was established on the basis of a previous experimental study in pigs monitored with EIT that showed that an automatic pneumothorax detector had high sensitivity to detect small pneumothoraces in controlled settings (3). This detector, however, has not yet been cleared for routine clinical use.

We monitored 15 consecutive patients diagnosed with ARDS who were submitted to a standardized recruitment maneuver as part of an ongoing clinical trial to assess the effect on survival of lung recruitment maneuvers and high PEEP (ClinicalTrials.gov: NCT01374022). The recruitment maneuver consisted of three incremental PEEP steps of 25, 30, and 35 cmH₂O with a fixed driving pressure of 15 cmH₂O, thus achieving a maximum plateau pressure of 50 cmH₂O. In some patients, recruitment maneuvers were performed more than once during their intensive care unit stay. We detected the occurrence of one episode of barotrauma, described in detail here and illustrated in Figure 1. In the remaining 25 recruitment maneuvers (96%) performed in 14 patients, EIT imaging did not detect any instances of barotrauma. The absence of pneumothorax in these cases was supported by prolonged clinical follow-up with daily chest radiographies and close clinical evaluation. Figure 2 shows the usual behavior of EIT variables during a recruitment maneuver in one representative case among the 14 patients who underwent a recruitment maneuver and did not develop pneumothorax.

Case Report

A 49-year-old man was admitted to the intensive care unit because of septic shock caused by hospital-acquired *Pseudomonas aeruginosa* pneumonia. The patient's past medical history was

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