Cord blood sphingolipids are associated with atopic dermatitis and wheeze in the first year of life

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Abstract

Background: Allergen-sensitized pregnant mice have increased plasma levels of the lipids β-glucosylceramides (βGlcCers) that are transplacentally transferred to the fetus, increased subsets of proinflammatory dendritic cells in the fetal liver and pup lung, and increased allergen-induced offspring lung inflammation.

Objective: Our aim was to determine whether these preclinical observations extend to a human association of βGlcCers with wheeze and allergic disease in the prospective Wisconsin Infant Study Cohort.

Methods: We measured 74 lipids in cord blood plasma by using mass spectrometry detection of sphingolipids, eicosanoids, and docosinoids, as well as an ELISA for 13-hydroxyoctadecadienoic acid. Lipid profiles were determined by unbiased Uniform Manifold Approximation and Projection dimensional reduction machine learning. Lipid profiles and a proinflammatory lipid index were analyzed for association with maternal allergy and childhood outcomes of wheeze, atopic dermatitis, cord blood leukocytes, and total IgE level at age 1 year.

Results: Uniform Manifold Approximation and Projection analysis of lipids defined 8 cluster-specific plasma lipid profiles. Cluster 6 had significantly lower levels of plasma βGlcCers and a higher frequency of cord blood plasmacytoid dendritic cells that mediate anti-inflammatory responses, which is consistent with an anti-inflammatory profile. For clusters and for each infant,

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a proinflammatory lipid index was calculated to reflect the sum of the proinflammatory lipids minus the anti-inflammatory lipids that were significantly different than in cluster 6. The cluster proinflammatory lipid index was associated with cord blood basophil frequency and with wheeze and atopic dermatitis in the first year of life. The infant inflammatory lipid index was associated with increased risk of wheeze in the first year of life.

Conclusion: The cord blood proinflammatory lipid index is associated with early-life atopic dermatitis and wheezing.

Keywords

Allergy; β-glucosylceramide; dendritic cells; basophils; lipidomics; maternal; birth cohort

The rates of asthma and other allergic diseases have increased dramatically in the past 40 years.[1] The development of asthma is associated with several risk factors, including maternal allergic disease and early-life atopic dermatitis and wheeze.[2,3] Reports have identified associations between childhood onset of asthma and maternal asthma.[4] In humans and animals, offspring of mothers with allergy have increased responsiveness to allergens.[5–7] In mechanistic murine studies addressing why maternal atopy is a strong risk factor,[7] we demonstrated that lipid changes in mothers with allergy and the transfer of these lipids to offspring enhance development of allergic disease and increase the numbers of subsets of proinflammatory dendritic cells (DCs) in the fetal liver and offspring.[4,8] Also, DCs from pups of mothers with allergy have enhanced allergen presentation function.[8] Moreover, in mice, transfer of DCs (but not macrophages) from neonates of mothers with allergy to recipient neonates from mothers without allergy enhances neonates' allergen responsiveness.[4,8] Thus, offspring of mothers with allergy have functionally distinct subsets of DCs that enhance allergen responsiveness.

During allergic inflammation in humans and mice, the levels of lipids such as resolvins, lipoxins, sphingosine-1-phosphate, and ceramides are altered.[9–14] Also, maternal sphingomyelins are positively associated with wheeze by age 3 years[15] and lipids are actively transported on high-density lipoproteins and low-density lipoproteins across the placenta,[16,17] whereas allergens and maternal IgE, IL-4, and IL-13 do not pass to the fetus from the mother.[4,18–20] We reported that elevated plasma sphingosine-1-phosphate of mothers with allergy is catabolized in the placenta and is not elevated in the fetus, whereas maternal β-glucosylceramides (βGlcCers) are transported to the fetal liver and enhance the numbers of offspring DCs.[7] It is not known whether these preclinical observations for sphingolipid regulation of offspring development of allergy extend to humans or whether early-life changes in the anti-inflammatory lipids resolvins and lipoxins influence the development of wheeze early in life.

Lipids have different classes, have several chain lengths, and are present at different concentrations *in vivo* for regulation of biologic functions. In lipidomic studies of lipid dysregulation, the sums of lipids and generation of proinflammatory lipid indexes have been used.[21–23] Dei Cas et al[22] utilized indexes for lipids based on the average fold change (FC) of significant lipids in volcano plots in lipidomics studies. The sum of $\log_2$ FC ($\log_2$FC) of

the omics data[23–25] and the sum of the average fold increases for lipid classes are used to determine association with experimental parameters.[22]

It is unknown whether sphingolipids, eicosanoid lipids, and docosanoid lipids are altered in the fetus of mothers with allergy or whether the profiles of these cord blood plasma lipids are associated with the development of allergic diseases. To address this knowledge gap, we analyzed cord blood plasma for 74 lipids in these lipid classes and developed a proinflammatory lipid index in a well-characterized birth cohort to test for associations with maternal allergic disease, early-life childhood clinical outcomes, and cord blood immune cell profiles.

METHODS

Study cohort and clinical outcomes

Cord blood plasma samples were obtained from the Wisconsin Infant Study Cohort (WISC).[26,27] The WISC birth cohort recruited pregnant women who worked or lived on animal farms and pregnant woman from the same rural communities who had no exposure to animal farms. This was not a high-risk cohort, and it was not selected by parental atopy. The parent WISC study was designed to determine the impact of early-life farming exposures on immune development, respiratory health, and allergic diseases. For the ancillary study reported in this article, cord blood samples from all 174 WISC participants collected between 2013 and 2017 were analyzed (Table I). Questionnaires were completed prenatally and during in-person or phone appointments during the offspring's first year of life (at 2, 6, 9, and 12 months of age); the questionnaires assessed environmental exposures, parental history of allergic disease, and child clinical outcomes (including atopic dermatitis and wheezing).[26] Atopic dermatitis was defined as reported,[27] and parent-reported wheezing episodes were determined from International Study of Asthma and Allergies in Childhood (ISAAC)-validated questions.[26,28] Child plasma total IgE level was measured at age 1 year in a clinical laboratory. All studies were approved by the human subjects institutional review boards at the Marshfield Clinic Health System, the University of Wisconsin, and the University of Indiana School of Medicine.

Sample collection and processing

Cord blood samples were collected at delivery from enrolled pregnant women who had provided consent; the samples were collected by using standardized sample collection kits. Cord blood samples were collected in sodium heparin tubes maintained at ambient temperature and processed within 24 hours of collection at a single study site, after which the plasma was stored at −80°C to preserve lipids, as previously described.[29]

Flow cytometry

Cord blood mononuclear cells were analyzed by immunolabeling and flow cytometry, as detailed in the Supplementary Methods (available in the Online Repository at www.jaci-global.org). Cord blood mononuclear cells were immunolabeled by using optimized flow cytometry panels[30] and fluorescence analysis performed with Fortessa or LSR II cytometers (BD Biosciences, East Rutherford, NJ). Data were analyzed by using FlowJo, version10.1

(FlowJo LLC, Ashland, Ore) (for the gating strategy, see Fig E1 in the Online Repository at www.jaci-global.org).

Lipid measurement by mass spectrometry

Lipidomics for 74 lipids in the panel of sphingolipids and panel of eicosanoids and docosanoids was performed at the Lipidomics Core at Virginia Commonwealth University as described in Supplementary Methods. Briefly, internal lipid standards were added to the samples and the lipids were extracted and analyzed by mass spectrometry as previously described.[7] Because βGlcCer and β-galactosylceramide (βGalCer) are coeluted by the UHPLC-MS/MS method, biologic samples containing both were separated with an LC-Si column and then analyzed by mass spectrometry, as described in detail in the Supplementary Methods.

13-HODE ELISA

Cord blood plasma (100 μL) was used for measurement of 13-hydroxyoctadecadienoic acid (13-HODE) by ELISA (Enzo Life Sciences, Farmingdale, NY; catalog no ADI-900–108).

Statistics and visualization

For unsupervised clustering of cord blood plasma lipids with dimensional reduction of Pareto scaled lipidomics data (74 distinct lipids over 174 study participants with multiple clinical and demographic features), we used Uniform Manifold Approximation and Projection (UMAP) for dimension reduction, which generated 8 distinguishable clusters of study participants with a similar lipid profile and then associated clinical and demographic features specific with the clusters. This was done by using the R package uwot for UMAP generation, the kmeans R base package for cluster identification, and the factoextra and Nbclust[31] packages for determination of optimal numbers of clusters.[32–36] Optimum number of clusters is determined by using the friz_nbclust function in the factoextra R package, which calculates a goodness of clustering measure, the "gap" statistic. The highest gap value was selected because it is considered to be the predicted optimum number of clusters. As in the UMAP website instructions, we also performed visual inspection of clusters defined by k-means (n = 5–10) overlaid on UMAP plots and compared k-mean clusters with hierarchic clusters in the heatmap to ensure predicted clusters optimally partitioned cluster members with proper partitioning of the visually distinguishable cluster members. UAMP clustering of lipids has recently been described for other human epidemiology studies, including studies of brain development or patients with trauma.[24,25]

For a heatmap of the lipidomics data, raw data were first normalized in a range from −1 to 1 by applying the formula $(x - mean)/max(abs[x - mean])$ with use of the clusterSim package,[37] and normalized data combined with selected clinical meta features were used to generate a heatmap by the ComplexHeatmap package.[38]

For volcano plots, false discovery rate (FDR) by Benjamini-Hochberg correction–adjusted P values generated by a pairwise Wilcoxon test (Wilcoxon rank and signed test) of the cluster mean of the Pareto transformed value[39] was used as the y-axis, and $\log_2$ fold differences in

the mean raw lipid concentration between clusters were used as the x-axis. FDR and $\log_2$FC values were generated by using the rstatix package[40] and plotted with the ggplot2 package.[41]

We calculated a proinflammatory lipid index for analysis of associations of lipid profiles with clinical features, similar to lipid profile analyses in other diseases.[21–24,42] The *P* value cutoffs for significant lipids were adjusted for FDR as in the volcano plot analysis in comparison with cluster 6. The proinflammatory lipid index for each cluster or for each infant was based *a priori* on preclinical data and studies in humans for proinflammatory functions of sphingolipids[7,15] and the anti-inflammatory functions reported for lipoxin A4 and resolvins[11,43]; the index was generated by using the lipids that had significant increases in the volcano plots for the clusters compared with cluster 6. The means for the lipids in cluster 6 were used as reference values (see Table E3 in the Online Repository at www.jaci-global.org) to calculate the $\log_2$FC for lipids in clusters or for each infant. Next, the proinflammatory lipid index was generated by adding the mean $\log_2$FC of the proinflammatory lipids (mean $\log_2$FC βGlcCer, mean $\log_2$FC βGalCer, and mean $\log_2$FC in sphingomyelin level) minus the $\log_2$FC of the anti-inflammatory lipids (mean $\log_2$FC in lipoxin A4 level and mean $\log_2$FC in resolvin D level). In the analysis of lipids, the mean $\log_2$FC is used instead of absolute lipid concentrations because the lipids occur at different magnitudes and because absolute plasma or tissue concentrations for sphingolipid chain lengths do not reflect proinflammatory and anti-inflammatory potency.[7,44]

The statistical analyses for outcomes were based on the biologic function of lipids and preclinical data in mice and associations in humans.[7,15,45,46] Boxplots for the immune cell phenotypes of each of the clusters were generated by ggplot2, and the statistical significance of proportions of each of the phenotypes among clusters was calculated by using wilcox.test in the rstatix package.

Three-dimensional plots were created by using the scatter3d function in the car[47] package. The lm function in the base R package[48] and Spearman correlation method were used to determine the *P* values, R^2 for linear regression, and *R* values for correlation plots. For regression model fitting of the proinflammatory lipid index, the gam function in the mgcv package was used.[49] The mgcv package was chosen for nonlinear polynomial regression model fitting by its generalized additive model (GAM) function. The advantage of GAM over glm is that GAM does not assume *a priori* any specific form of this relationship and can be used to reveal and estimate nonlinear effects of the covariate on the dependent variable. The mgcv package offers the ability to build the models and smoothers, automatically estimates the penalty values, and optimizes the smoothers, requiring less user manipulation to prevent model overfitting. Descriptive statistics, including mean, median, SD, and interquantile range, were summarized for continuous covariates by clusters; frequencies were summarized for categoric covariates by clusters. Plurality and birth order are categoric variables with 3 and 2 levels, respectively. To test the intercluster variability of other covariates that may need to be adjusted in the model, Kruskal-Wallis tests were performed to compare continuous covariates between clusters, and Fisher exact tests were performed to compare categoric covariates between clusters. All of the statistical computations were done with table by function in the arsenal package,[50] and the resulting analysis table was created by the knitr package.[51] Analyses included analyses for maternal asthma, maternal allergy,

allergy symptoms during pregnancy, maternal eczema, cesarean section, infant wheeze, and infant atopic dermatitis. The Kruskal-Wallis test, followed by the Dunn test between pairs with FDR adjustment, was performed for analysis of leukocytes between clusters. Relative risk was calculated in comparison with the first quantile for the proinflammatory lipid index. P values and FDR values less than .05 were considered statistically significant.

RESULTS

Subject characteristics

The study subjects included 174 mothers and their infants participating in the prospective WISC birth cohort study. Among the 174 maternal-infant dyads, 18% of mothers had some allergic disease symptoms during pregnancy, and 8% and 9% of mothers had active asthma or atopic dermatitis during pregnancy, respectively. In the offspring, there was a 25% incidence of wheezing and 17% incidence of atopic dermatitis in the first year of life (Table I).

Unsupervised clustering of cord blood plasma lipids

Cord blood plasma was quantified by mass spectrometry for 74 sphingolipid and eicosanoid and docosanoid lipid analytes. Unbiased UMAP and k-means clustering analysis distinguished 8 clusters of subjects with similar concentrations of 74 lipids within each cluster (Fig 1, A). The covariates infant sex, infant race, number of children in the household, maternal prenatal body mass index, and household smoke exposure did not significantly differ across the 8 clusters (see Table E1 in the Online Repository at www.jaci-global.org). The clusters (see Table E1) and individual lipids (data not shown) were not significantly associated with maternal allergy or infant atopic dermatitis or wheezing. This analysis was limited, as it did not include different levels and opposing functions of proinflammatory and anti-inflammatory lipids. This led to further approaches to incorporate the biologic function of proinflammatory and anti-inflammatory lipids into the analyses.

Heatmap analysis identified specific lipid profiles of the subjects in the UMAP-derived lipid clusters (Fig 1, B). Because the levels of βGlcCers as a lipid class regulated responses to allergen in our neonate mouse studies,[7] we addressed the dimensionality of the 10 βGlcCers as a class. The total mean scaled βGlcCer of the 10 chain lengths was significantly lower in cluster 6 than in all the other clusters (Fig 1, C). We next tested for differences in the lipid profiles of the clusters as compared with that of the cluster with low βGlcCer (cluster 6), because in preclinical mouse studies allergen responsiveness and altered subsets of proinflammatory DCs occurred in neonatal mice with elevated βGlcCer levels.[7] In volcano plots (Fig 2), the colored dots indicate significantly different lipids in the clusters (FDR-adjusted P value < .05, indicated as $-\log_{10}$FDR < 1.3 and significant [>1.5-fold] change, indicated as $\log_2$FC > 0.6 & <−0.6) as compared with in cluster 6. Most of the lipids with significantly increased levels in Fig 2 were βGlcCers and βGalCers (see Table E2 in the Online Repository at www.jaci-global.org). The clusters also differed in concentrations of the anti-inflammatory docosanoids lipoxin A4, resolvin D1, and resolvin D2. Cluster 6 had higher lipoxin A4 levels than clusters 1, 3, 4 and 5 did and higher levels of resolvin D1 than clusters 3 and 4 did (Fig 2 and see Table E2).

A cord blood plasma proinflammatory lipid index was correlated with wheeze and atopic dermatitis in the first year of life

To assess the relationships between clinical outcomes and cord blood plasma lipid profiles that have both proinflammatory and anti-inflammatory lipids, we generated a proinflammatory lipid index for each UMAP cluster with the lipids that were different in the volcano plots in Fig 2, using the lipid profile of cluster 6 as a reference point (see Table E3). The proinflammatory lipid index for each cluster in Fig 3, A was determined by adding the mean $\log_2$FC of proinflammatory lipids (βGlcCer, βGalCer, and sphingomyelins) minus the $\log_2$FC of anti-inflammatory lipids (lipoxin A4 and resolvin D1 and D2 [Fig 3, A and see Table E2]) with levels that were significantly elevated in the volcano plots in Fig 2. The cord blood plasma proinflammatory lipid index values for the clusters were significantly associated with wheeze during the first year of life (adjusted $R^2 = 0.69$; $P = .0068$ [Fig 3, B]) and also with atopic dermatitis during the first year of life (adjusted $R^2 = 0.54$; $P = .02$ [Fig 3, C]). In contrast, the cluster proinflammatory lipid index was not associated with cluster plasma total IgE level in the first year of life ($P = .27$ [data not shown]), maternal allergy symptoms during pregnancy ($P = .58$ [data not shown]), or cohort farm group status ($P = .91$ [data not shown]).

Associations of cluster lipid profiles with cord blood mononuclear cells

The UMAP clusters were compared for relative frequency of mononuclear cell subsets in the cord blood. Cluster 6 had a significantly higher frequency of plasmacytoid DCs (pDCs), which mediate anti-inflammatory responses in atopic diseases, as compared with in clusters 2, 5, and 7 (Fig 4, B). There were no significant differences among the clusters for myeloid DCs, B cells, and monocytes, and there was a trend toward lower basophil frequency in cluster 6 (Fig 4, A and C–E). There was a significant linear association between the cluster proinflammatory lipid index and the cluster median frequency for cord blood basophils ($R = 0.607$; $P = .0138$ [Fig 4, F]). There was no association between cluster proinflammatory lipid index and cord blood pDC frequency (Fig 4, G), even though there were significantly more pDCs in cluster 6, suggesting that the proinflammatory lipid index does not encompass all components of clusters influencing cord blood pDC frequency.

Three-dimensional plots of mean scaled lipids were generated to graphically depict relationships among proinflammatory βGlcCer and anti-inflammatory lipoxin A4, resolvins, and pDCs for the clusters (Fig 5) because there were significant associations with these parameters in Figs 1, C and 2–4. In the 3-dimensional plots, cluster 6 is separated from the other clusters, is closest to the origin on the βGlcCer axis, and has higher numbers of pDCs (Fig 5).

Individual infant cord blood plasma proinflammatory lipid index association with cord blood basophils and relative risk of wheeze in the first year of life

In addition to assessing association with lipid clusters, we assessed individual infant risk of development of wheeze by cord blood lipid profiles. A proinflammatory lipid index was generated for each infant sample, using the cluster 6 lipid concentrations as a reference point (see Table E3). The individual proinflammatory lipid index was determined by adding the mean $\log_2$FC of proinflammatory lipids (βGlcCer, βGalCer, and sphingomyelins) minus

the log$_2$FC of the anti-inflammatory lipids (lipoxin A4 and resolvin D). Although there was no association of infant proinflammatory lipid index with atopic dermatitis (data not shown), the quantiles for cord blood plasma proinflammatory lipid index were associated with frequency of infants with wheeze (adjusted R^2 = 0.59; P = .026 [Fig 6, A]). There was an increased relative risk of wheeze in the first year of life with increasing cord blood proinflammatory lipid index for infants (adjusted R^2 = 0.85; P = .0085 [Fig 6, B]).

DISCUSSION

This study investigated the relationship between cord blood plasma lipids within the context of sphingolipids, eicosanoids, and docosanoids with maternal allergic diseases and also with early-life childhood clinical outcomes of wheezing and atopic dermatitis. There were no significant associations between individual lipids or lipid clusters with either maternal allergy or infant health outcomes, but this simple analysis did not incorporate opposing influences by proinflammatory and anti-inflammatory lipids in the clusters. To gain insight into the overall role of the cord blood lipids, children were clustered on the basis of their lipid profiles; we then calculated an index that considered both the proinflammatory and anti-inflammatory functions of lipids. This proinflammatory lipid index was significantly correlated with the cluster frequencies of infant wheeze and atopic dermatitis. Moreover, cord blood proinflammatory lipid index values for individual infants were associated with the development of infant wheeze and the relative risk of wheeze in the first year of life.

Preclinical studies have demonstrated that levels of βGlcCers are significantly elevated (up to 2.5-fold) in pregnant mice during allergic responses and that these βGlcCers are transported to the fetus.[7] Although fetal livers undergo lipogenesis, the maternal lipids that were transported to the fetal liver were sufficient and necessary for elevated offspring responsiveness to allergen in mice.[7] In the preclinical studies, reducing the levels of βGlcCers in mothers with allergy to the levels in mothers without allergy blocked development of allergen-stimulated inflammation in their offspring.[7] Also, administration of βGlcCers to mothers without allergy was sufficient to induce offspring responsiveness to allergen.[7] βGlcCers may regulate immune cell function or cell signaling by being metabolized to gangliosides or globosides that may alter lipid rafts or cell signaling.[52,53] Also, a recent report indicated that maternal third-trimester sphingomyelins were positively associated with an increased odds ratio for wheeze in offspring by age 3 years,[15] whereas in mice with allergy sphingomyelin levels were not elevated, suggesting that there are some species or environmental differences. Nevertheless, βGlcCer levels were elevated in mice with allergy in the preclinical studies[7] and also within the lipid clusters of human cord blood that had higher frequencies of wheeze. In the WISC study, for clusters or individual infants, a proinflammatory lipid index that reflected higher levels of proinflammatory sphingolipids (including βGlcCers and sphingomyelins) and incorporated anti-inflammatory docosanoids was positively associated with infant wheeze and atopic dermatitis. These clinical outcomes are risk factors for subsequent development of allergic diseases, including asthma.[54–56] The cluster cord blood proinflammatory lipid index was not significantly associated with cluster frequency of mothers who reported allergic symptoms during their pregnancies.

One limitation of this analysis is that the severity of maternal allergic disease was not measured for this cohort. Another limitation is that we developed the proinflammatory lipid index for this study and this approach needs to be validated in an independent cohort. Future studies using cohorts with an increased number of subjects with allergy could better define the relationship between maternal allergic disease, cord blood plasma lipid profiles, and early-life clinical outcomes.

In contrast to sphingolipids, which perform proinflammatory functions, lipoxin A4 and resolvins have well-characterized anti-inflammatory effects. These anti-inflammatory lipids modulate IgE production, T-cell responses, and proresolving phagocytes.[9,57,58] In children, resolvin D1 is positively correlated with lung function in children in studies of asthma.[10] With regard to lipoxin A4, blood levels of lipoxin A4 are low in infants (aged 6–36 months) with wheeze as compared with the levels in healthy controls.[11] Although children (aged 2–75 months) with asthma have lower serum lipoxin A4 than controls, serum lipoxin A4 alone did not predict asthma.[43] Notably, previous analyses did not include lipidomics to enable the consideration of both proinflammatory and anti-inflammatory lipids. Of the 8 clusters of infants defined by their cord blood lipids, cluster 6 had an anti-inflammatory profile with significantly lower levels of βGlcCers, higher levels of the anti-inflammatory lipid lipoxin A4, higher proportions of cord blood anti-inflammatory pDCs, and a lower frequency of cord blood basophils. Cluster 7 qualitatively had a frequency of wheeze most similar to that of cluster 6. Although cord blood levels of βGlcCers, pDCs, and basophils were greater in cluster 7 than in cluster 6, the levels of lipoxin A4 and resolvin D2 were also higher in cluster 7 than in cluster 6, suggesting that these anti-inflammatory lipid factors may limit the development of atopic dermatitis and wheeze. These findings in infants and the data from preclinical studies suggest that the balance between sphingolipids and the anti-inflammatory lipids resolvin and lipoxin could affect the onset of atopic dermatitis and wheezing diseases.

Lipids with levels elevated during allergic or asthmatic responses can be transported from the mother to the fetus and alter the lipid compositions in the fetal blood. In adults, levels of plasma lipids, including ceramides and eicosanoids, can be elevated with allergy or allergic asthma. Reports of nonpregnant humans and animal models have demonstrated that an increased plasma ceramide level in adults correlates with adult severe asthma, but lipidomics were not reported.[13,44] Also, in adults with asthma the levels of eicosanoids and docosanoids 12-hydroxyeicosatetraenoic acid, 15-hydroxyeicosatetraenoic acid, leukotriene B_4, prostaglandin E_2, 13-HODE, and docosahexaenoic acid are higher than in healthy controls.[14] In the WISC cohort, levels of eicosanoids, docosanoids, and ceramides defined UMAP clusters. Some clusters had small increases for a few ceramides or eicosanoids as compared with in cluster 6. The role of eicosanoids in development of early-life atopy is being examined in ongoing studies.

Wheezing in infancy is often secondary to respiratory viruses, and the frequency of blood pDCs and basophil progenitors has been associated with risk of wheezing with respiratory viral infection. Briefly, in asthmatic children, the frequency of pDCs is lower than in adults.[59] Also, a lower number of peripheral blood pDCs in infants of families with history of atopy is a risk factor for more frequent and more severe respiratory tract infections, wheezing, and a diagnosis of asthma.[60,61] It has been reported that wheeze with acute

respiratory infections is associated with cord blood eosinophil and basophil progenitor numbers, but in those studies, lipid associations with wheeze and leukocytes were not analyzed.[62] In our lipidomics analyses of the WISC cohort cord blood, cluster 6, which had the lowest proportion of participants with wheeze or atopic dermatitis, had a higher frequency of cord blood pDCs and a lower frequency of basophils. There was also an association of cord blood proinflammatory lipid index with the frequency of cord blood basophils.

Lastly, the protective effect of early-life farm exposure on allergic disease and respiratory illnesses has been reported.[63,64] Also, differences in the environments of different farms can influence rates for asthma on farms.[65,66] In the WISC cohort, we previously reported an inverse association between infant atopic dermatitis and farm exposures, whereas farm status was not significantly related to either maternal atopic dermatitis or asthma.[27,67] In this analysis, we did not find a significant difference in cord blood lipids for early-life farm exposure versus rural nonfarm exposure. The protective effects of farm exposures may operate independently of lipid alterations at birth, and studies are ongoing within our WISC cohort to further define the impact of farming exposures on lipid and metabolic plasma profiles.

In conclusion, unbiased dimensional reduction clustering of targeted lipidomics of cord blood plasma revealed clusters of children with distinct lipid profiles that informed calculation of a proinflammatory lipid index. This index, which reflects proinflammatory lipid function, was associated with infant wheeze, atopic dermatitis, and cord blood basophils. Thus, a proinflammatory index that incorporates proinflammatory lipids and anti-inflammatory lipids, which may be influenced by gene-environment interactions, may explain at least some of the variation in development of wheeze early in life. Moreover, these plasma lipid signatures required less than 300 μL of cord blood plasma and could inform studies involving additional children to assess predictive value for allergic disease. A predictive index based on lipidomics could be a useful biomarker in babies to evaluate the risk of development of allergic diseases or asthma.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Abbreviations used

βGalCer	β-Galactosylceramide
βGlcCer	β-Glucosylceramide
DC	Dendritic cell
FC	Fold change
FDR	False discovery rate
GAM	Generalized additive model
13-HODE	13-Hydroxyoctadecadienoic acid
log₂FC	Log$_2$ fold change
pDC	Plasmacytoid dendritic cell
UMAP	Uniform Manifold Approximation and Projection
WISC	Wisconsin Infant Study Cohort

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Clinical implications:

The association of lipid profiles in cord blood with early-life wheeze and atopic dermatitis may lead to novel early-life interventions to reduce risk of wheeze and atopic dermatitis.
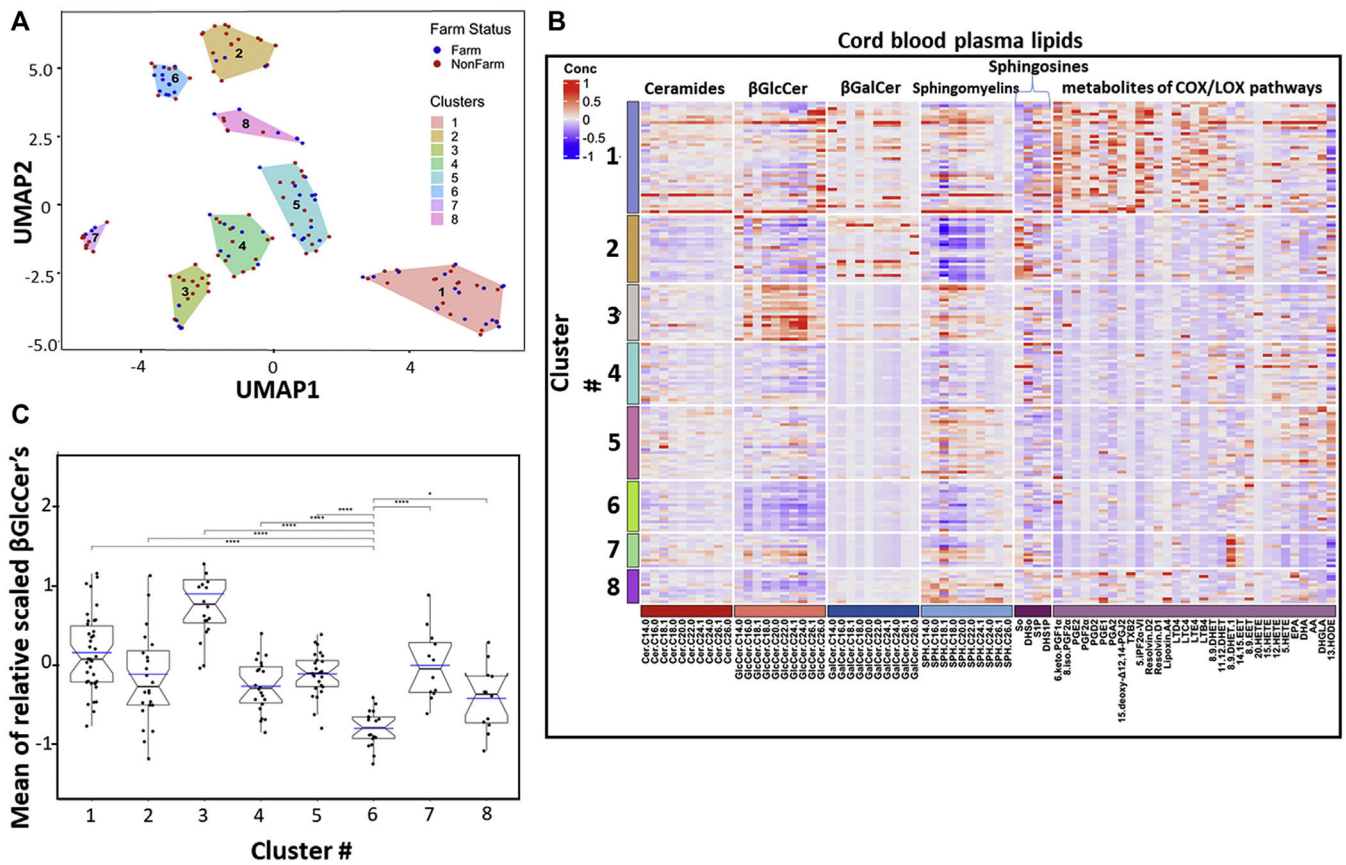
FIG 1.

Lipidomics for lipids in UMAP clusters with lower βGlcCer levels in cluster 6. **A,** UMAP
of cord blood lipids with cluster-specific annotation defines 8 clusters. Cord blood plasma
sphingolipids and eicosanoids were measured by mass spectrometry. Cord blood plasma
13-HODE was measured by ELISA. **B,** Heatmap of lipids for individuals in clusters from
UMAP. **C,** Mean of the relative scaled βGlcCers by cluster in the heatmap. *P < .05 or
****P < .0001 as compared with cluster 6 with adjustment for multiple comparison by a
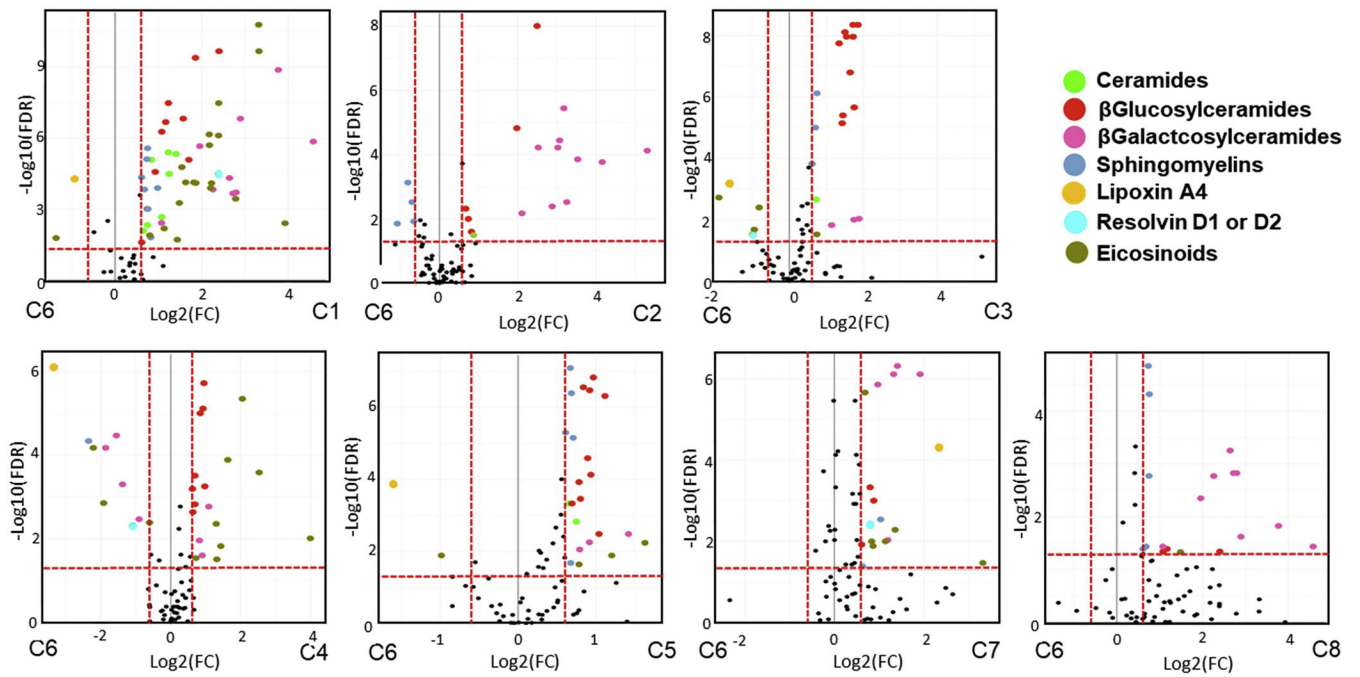Benjamini-Hochberg procedure.

FIG 2.

Relative levels of lipids for clusters 1, 2, 3, 4, 5, 7, and 8 compared to cluster 6. Clusters C1 to C8 from Fig 1. Colored dots represent lipids with significant FC versus in cluster 6. Black dots represent lipids that are not different. Red dotted lines represent cutoffs for $\log_2 FC > 0.6$ and < -0.6 and $-\log_{10}(FDR) < 1.3$. Significantly different lipids are listed in Table E2.

A Pro-inflammatory lipid index for clusters

cluster #	1	2	3	4	5	6	7	8
βGlcCers (mean log2FC)	1.33	1.34	1.60	0.79	0.90	0	0.76	1.55
βGalCers (mean log2FC)	2.74	3.30	1.59	0.93	1.04	0	1.36	2.74
sphingomyelins (mean log2FC)	0.75		0.69		0.67	0	0.83	0.71
Lipoxin A4 (log2FC)							-2.34	
Resolvin D2 (log2FC)	-2.39						-0.81	
pro-inflammatory lipid index	2.44	4.64	3.88	1.72	2.61	0	-0.19	5.00
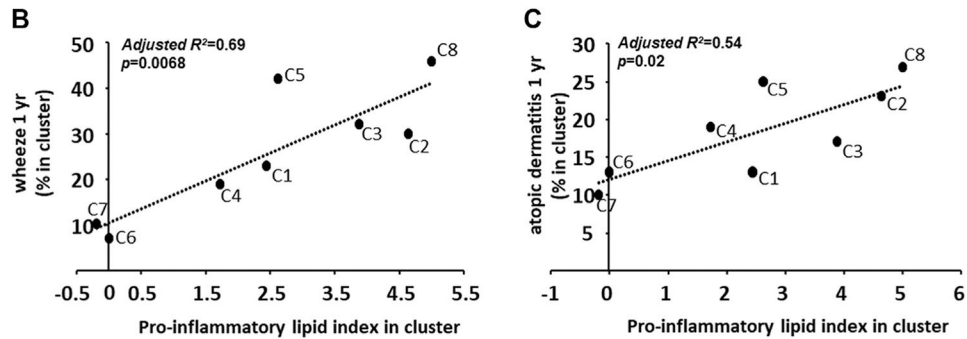
FIG 3.

Cluster cord blood lipids are associated with frequency of infant wheeze or atopic dermatitis in clusters. **A,** The proinflammatory lipid index for clusters was based on the biology of significant lipids in volcano plots in Fig 2. For each of these significant lipids, the $\log_2$FC value is calculated as compared with the mean values for the lipids in cluster 6 (see Table E3). The means of the $\log_2$FC for isoforms of βGlcCer, βGalCer, and sphingomyelins in each cluster are listed in (**A**). The cluster proinflammatory lipid index equals the sum of the $\log_2$FC values for the proinflammatory lipids βGlcCer, βGalCer, and sphingomyelin minus the sum of the $\log_2$FC values for the anti-inflammatory lipids resolvin D2 and lipoxin A4. **B**, Percentage of infants in clusters with wheeze in year 1 of life versus the cluster proinflammatory lipid index. **C,** Percentage of infants in clusters with atopic dermatitis in year 1 of life versus the cluster proinflammatory lipid index. *C*, Cluster.
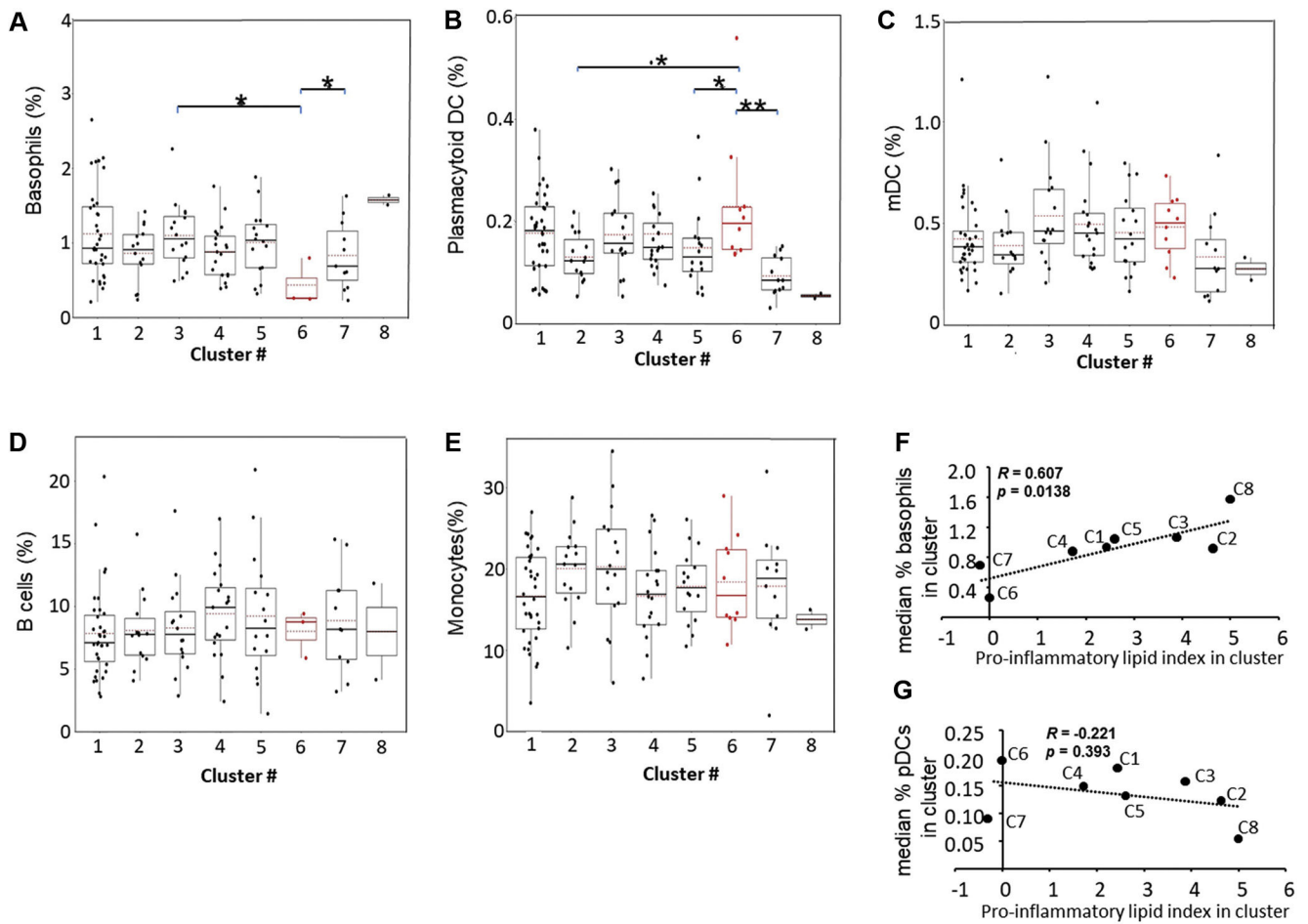
FIG 4.

Cord blood leukocytes in clusters. Cord blood cells were immunolabeled and analyzed by flow cytometry as in Fig E1. Presented are the percentages of cord blood leukocytes for each cluster (**A-E**) or versus the proinflammatory lipid index (**F** and **G**). *$P < .05$; **$P < .01$; ***$P < .005$. Solid horizontal line indicates the median. Dotted horizontal line indicates the mean. C1 to C8 are the cluster numbers. *mDC*, Myeloid DC.
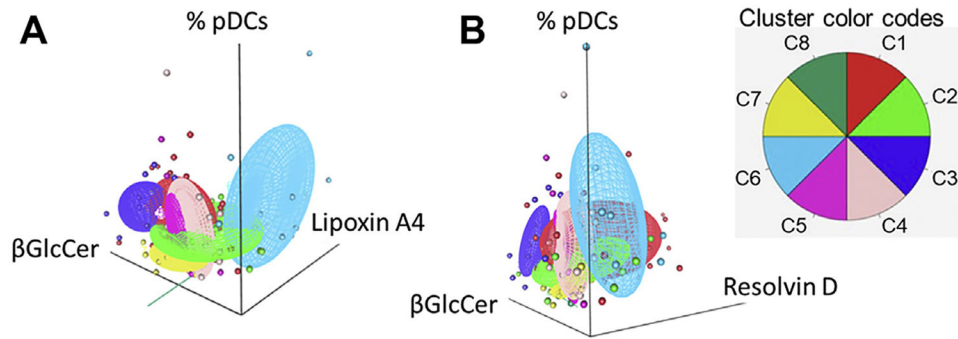
FIG 5.
Three-dimensional plot analyses of clusters for pDCs, βGlcCer, and lipoxin A4 or resolvin D1 and resolvin D2. Shown are the mean scaled values for βGlcCer, the resolvins D1 and D2, and lipoxin A4. Axis origins are in front of the plot for visibility of separation of cluster 6 from the other clusters and cluster 6 location near the βGlcCer axis origin. The axis origin is rotated to the front.
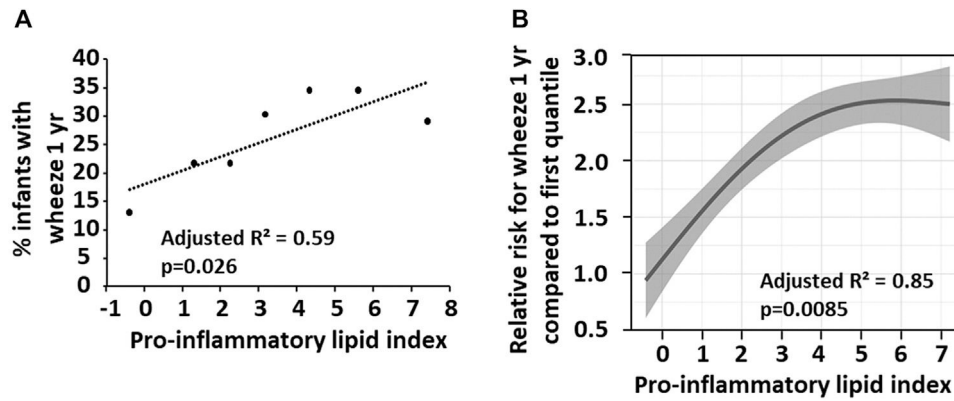
FIG 6.
Cord blood proinflammatory lipid index for each infant and relative risk of wheeze. A proinflammatory lipid index was calculated for each infant as follows: reference values were defined as the average for each lipid in cluster 6 (see Table E3); then, the $\log_2$FC value for each lipid for each infant was determined as compared with the average lipid concentration in cluster 6 in Table E3; next, for each infant, the proinflammatory lipid index, which equals the sum of the $\log_2$FC values for the proinflammatory lipids βGlcCer, βGalCer, and sphingomyelin minus the sum $\log_2$FC for the anti-inflammatory lipids resolvin D2 and lipoxin A4, was calculated. Wheeze occurred in 25% of infants in the first year of life, whereas 17% of infants had atopic dermatitis in the first year of life (Table I) and only 4% had both wheeze and atopic dermatitis. **A,** Percentage of infants with wheeze in first year of life versus quantiles (n = 23 per quantile) of the proinflammatory lipid index (n = 162). **B,** Relative risk of wheeze with increasing levels of the proinflammatory lipid index as compared with the first quantile of the proinflammatory lipid index.

TABLE I.

Study participant characteristics

Characteristic	Value
Maternal (n = 174)	
Prenatal BMI (kg/m^2), median (IQR)	25.76 (23.03–30.58)
Delivery by cesarean section, no. (%)	44 (25%)
Any allergic disease ever, no. (%)[*]	77 (45%)
Any allergic disease symptoms during pregnancy, no. (%)	31 (18%)
Asthma	
Ever, no. (%)	28 (16%)
Active during pregnancy, no. (%)	14 (8%)
Eczema	
Ever, no. (%)	35 (20%)
Active during pregnancy, no. (%)	16 (9%)
Allergic rhinitis	
Ever, no. (%)	56 (32%)
Active during pregnancy, no. (%)	14 (8%)
Infant (n = 174)	
Sex (female), no. (%)	90 (52%)
Wheeze during first year of life, no. (%)[†]	41 (25%)
Atopic dermatitis during first year of life, no. (%)[‡]	27 (17%)
Environment	
Smoke exposure, no. (%)[*]	19 (11%)
Nonfarm status, no. (%)	73 (42%)

BMI, Body mass index; *IQR,* interquartile range.

[*] Missing data: n = 2.

[†] Missing data: n = 12.

[‡] Missing data: n = 14.