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Serum Metabolome Is Associated With the Nasopharyngeal Microbiota and Disease Severity Among Infants With Bronchiolitis

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Background. Emerging evidence suggests relationships between the nasopharyngeal metabolome and both the microbiota and severity of bronchiolitis. However, the influence of host systemic metabolism on disease pathobiology remains unclear. We aimed to examine metabolome profiles and their association with more-severe disease, defined by use of positive pressure ventilation (PPV), in infants hospitalized for bronchiolitis.

Methods. In 140 infants with bronchiolitis, metabolomic profiling was performed on serum; samples from 70 were in a training data set, and samples from 70 were in an independent test data set. We also profiled the nasopharyngeal airway microbiota and examined its association with the serum metabolites.

Results. Serum metabolome profiles differed by bronchiolitis severity ($P < .001$). In total, 20 metabolites in the training data set were significantly associated with the risk of PPV, of which 18 remained significant following adjustment for confounders (false-discovery rate [FDR], < 0.10). Phosphatidylcholine metabolites were associated with higher risks of PPV use, while metabolites from the plasmalogen subpathway were associated with lower risks. The test data set validated these findings (FDR < 0.05). *Streptococcus* abundance was positively associated with metabolites that are associated with higher risks of PPV.

Conclusions. Serum metabolomic signatures were associated with both the nasopharyngeal microbiota and the severity of bronchiolitis. Our findings advance research into the complex interrelations between the airway microbiome, host systemic response, and pathobiology of bronchiolitis.

Keywords. Bronchiolitis; severity; serum; metabolomics; microbiota.

Bronchiolitis is an important public health problem in the United States, accounting for 290 000 emergency department visits and 130 000 hospitalizations each year [1–3]. While approximately 40% of children develop clinical bronchiolitis in the first 2 years of life, its severity ranges from a minor nuisance to fatal [3]. However, these differences in acute severity are not explained by traditionally identified clinical risk factors (eg, age, lower body weight, and prematurity). Indeed, the pathobiology of bronchiolitis remains to be elucidated [3].

Although bronchiolitis is typically caused by a viral infection, emerging evidence about its pathobiology suggests a complex interrelationship of respiratory viruses, the airway microbiome, and local and systemic host immune responses [4–7]. In the airways, a *Streptococcus*-dominant microbiome and increased sphingolipid metabolism has been linked to more-severe disease [4]. Moving beyond the local site of disease, a recent single-center study of children with RSV infection reported microbiome-specific systemic immune responses—eg, a *Streptococcus*-dominant microbiome was associated with overexpression of genes linked to Toll-like receptor and neutrophil activation in whole-blood specimens [8]. However, it remains unclear how these microbes and the host systemic response influence clinical outcomes by perpetuating downstream functional molecules. Metabolomics addresses this knowledge gap via comprehensively characterizing small-molecule metabolites that are a function of an infant's genetic makeup and of environmental factors, such as the microbiome [9].

In the current study, we aimed to investigate the interrelationships between serum metabolome, nasopharyngeal airway microbiome, and bronchiolitis severity. We tested the hypotheses that serum metabolites are associated with the risk of positive pressure ventilation (PPV) use and that nasopharyngeal airway bacteria are associated with these identified metabolites among infants with bronchiolitis.

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METHODS

Study Design, Setting, and Participants

The current study is part of the 35th Multicenter Airway Research Collaboration (MARC-35) [7], a multicenter, prospective cohort study of 1016 infants (age, <1 year) hospitalized for bronchiolitis. This cohort was coordinated by the Emergency Medicine Network, a collaboration of 245 hospitals [10]. The study design, setting, participants, and methods of data collection have been reported previously [7, 11]. Using a standardized protocol, investigators at 17 sites across 14 US states (Supplementary Table E1) enrolled infants hospitalized with a diagnosis of bronchiolitis from an attending physician during any of 3 consecutive bronchiolitis seasons from 1 November to 30 April in 2011–2014. Bronchiolitis was defined by the American Academy of Pediatrics guidelines as follows: acute respiratory illness with some combination of rhinitis, cough, tachypnea, wheezing, crackles, and retractions [12]. We excluded infants who were transferred to a participating hospital >24 hours after the original hospitalization, those who provided consent >24 hours after hospitalization, and those with known heart-lung disease, immunodeficiency, or gestational age <32 weeks. The institutional review board at each of the participating hospitals approved the study. We obtained written informed consent from the parent or guardian.

In addition to the clinical data (Supplementary Methods), trained site investigators collected serum and nasopharyngeal airway samples within 24 hours of hospitalization and stored them at −80°C, using a standardized protocol [11]. The present analysis investigated a subset of 140 infants among whom, by design, rhinovirus infection was overrepresented (Supplementary Table E2), given its relevance to respiratory outcomes in childhood [3]. We generated separate training and test data sets, with samples from 70 infants in each, and profiled their serum metabolome and nasopharyngeal microbiota.

Ethical approval for this study was obtained from the Human Research Committee at Massachusetts General Hospital (protocol 2017P001861).

Metabolomic Profiling of Serum Samples

Metabolomic profiling used 100 μL of each serum sample and was performed by Metabolon (Durham, NC). All samples in each data set were blinded to Metabolon and processed in a random order. Metabolome profiling used ultrahigh-performance liquid chromatography–tandem mass spectrometry. The training and testing data sets were tested separately (in March and September 2017, respectively). Details of the metabolome profiling are in the Supplementary Methods. Instrument variability was 4%, as determined by calculating the median relative standard deviation for the internal standards that were added to each sample before the sample was injected into the mass spectrometer.

Microbiota and Metabolomic Testing of Nasopharyngeal Airway Samples

A complete description of the sequencing and metabolomic protocols for nasopharyngeal samples is provided in the Supplementary Methods.

Statistical Analyses

The primary outcome was the use of PPV—defined as the use of continuous positive airway pressure and/or intubation—at any time during the index hospitalization [13, 14]. Use of PPV is a clinically relevant and important outcome.

Analysis was first conducted on the training data set. To examine the differences in the serum metabolome profiles between infants with and those without PPV use, we performed sparse partial least-squares discriminant analysis (sPLS-DA) with 2000-permutations testing [15]. To determine individual discriminatory metabolites that were significantly associated with the risk of PPV use, we performed orthogonal partial least-squares discriminant analysis (OPLS-DA) and a 2-tailed Welch *t* test in MetaboAnalyst 3.0 [16], as well as multivariable linear regression in R, version 3.3 [17]. In the multivariable models, we adjusted for potential confounders (ie, age, sex, weight at presentation, breastfeeding status, and virus). *P* values were adjusted for multiple comparisons, using the Benjamini-Hochberg false-discovery rate (FDR) method [18]. Resulting FDRs of <0.10 were considered statistically significant, for the training data set, and FDRs of <0.05 were considered statistically significant, for the test data set. For the test data set, only the significant metabolites derived in the training data set were analyzed, and the same analysis work flow was used.

To determine the associations between the relative abundance of dominant bacterial genera in nasopharyngeal airway samples and the intensity of derived serum metabolites, we performed sPLS regression analysis in canonical mode, using MixOmics [19]. Last, to examine the relations between the airway and circulating metabolome in 70 infants with the paired samples, we computed the Pearson correlations between the nasopharyngeal airway and serum individual metabolites, using an existing metabolomic data set of nasopharyngeal airway samples from the MARC-35 cohort [4]. Pearson correlations were plotted in R with the *corrplot* package, and FDR-adjusted *P* values were calculated in R with the *psych* package.

RESULTS

Study Population

As part of a 17-center prospective cohort study of 1016 infants with severe bronchiolitis, the current investigation analyzed 140 serum samples (70 in the training data set and 70 in the test data set) and nasopharyngeal airway samples obtained at the start of the index hospitalization. The analytic and nonanalytic cohorts had no significant differences in most patient characteristics ($P > .05$; Supplementary Table E2), except the analytic cohort had a lower proportion of infants with respiratory syncytial

virus (RSV) infection and a higher proportion with rhinovirus infection. Of the 70 infants in the training data set, the median age was 3.7 months (interquartile range, 1.4–5.4 months), and 14 (20%) underwent PPV during hospitalization (Table 1). While most characteristics did not differ between infants who underwent PPV and those who did not (all $P > .20$), the PPV group was significantly younger and had a significantly lower body weight ($P < .05$ for both comparisons).

Serum Metabolomic Profiles Were Associated With a Risk of PPV Use Among Infants With Bronchiolitis

Serum metabolomic testing of infants with bronchiolitis identified 772 metabolites in the training data set and 828 metabolites in the test data set, of which 86% were identified in both data sets. These metabolites were from 91 subpathways, contained within 8 superpathways. sPLS-DA showed that metabolomic profiles of infants with PPV use clustered distinctly from profiles among infants without PPV use ($P < .001$ by the permutation test; Figure 1A).

In the training data set, 20 metabolites were significantly associated with a risk of PPV use, of which 5 were associated with a higher risk of PPV use and 15 were associated with a lower risk of PPV use (FDR < 0.10; Table 2). 1-palmitoyl-2-palmitoleoyl-GPC (16:0/16:1; phosphatidylcholine subpathway) and glucuronate (amino sugar metabolism subpathway) were the most significant metabolites associated with higher risks of PPV use.

All significant metabolites were from unique subpathways, with the exception of 6 metabolites from the plasmalogen subpathway—1-(1-enyl-palmitoyl)-2-oleoyl-GPE (P-16:0/18:1), 1-(1-enyl-stearoyl)-2-linoleoyl-GPE (P-18:0/18:2), 1-(1-enyl-palmitoyl)-2-linoleoyl-GPE (P-16:0/18:2), 1-(1-enyl-palmitoyl)-2-arachidonoyl-GPE (P-16:0/20:4), 1-(1-enyl-stearoyl)-2-oleoyl-GPE (P-18:0/18:1), and 1-(1-enyl-stearoyl)-2-arachidonoyl-GPE (P-18:0/20:4)—and 2 metabolites from the fatty acid metabolism subpathway—lignoceroylcarnitine (C24) and ximenoylcarnitine (C26:1) (Supplementary Figure E1). These plasmalogen metabolites were associated with significantly lower risks of PPV use. Of the 20 metabolites that were significantly associated with the risk of PPV use, 18 remained significant following adjustment for confounders, and the direction of significant associations was the same in both analyses (Table 2). Likewise, in the OPLS-DA analysis, these significant metabolites were also among the most discriminatory between the group with and the group without PPV use (Figure 1B). The heatmap of the 20 significant metabolites also showed clustering of infants by PPV use (Figure 1C).

To address the possibility of reverse causation (ie, PPV use alters serum metabolomic profiles), we repeated the analysis with the use of another severity outcome—hospital length

Table 1. Patient Characteristics of Infants Hospitalized for Bronchiolitis, by Use of Positive Pressure Ventilation (PPV)

	Training Data Set (n = 70)			Test Data Set (n = 70)		
Variable	PPV Use (n = 14)	No PPV Use (n = 56)	*P*	PPV Use (n = 24)	No PPV Use (n = 46)	*P*
Demographic/clinical characteristic						
Age, mo	1.5 (1.0–2.8)	3.1 (1.8–5.5)	.04	1.9 (1.2–5.0)	4.9 (2.5–8.0)	.051
Male sex	9 (64)	40 (71)	.85	10 (42)	28 (61)	.20
Race/ethnicity			.47			.49
Non-Hispanic white	9 (64)	23 (41)		9 (38)	13 (28)	
Non-Hispanic black	2 (14)	13 (23)		4 (17)	10 (22)	
Hispanic	3 (21)	19 (34)		9 (38)	22 (48)	
Other	0 (0)	1 (2)		2 (8)	1 (2)	
Maternal smoking during pregnancy	2 (14)	9 (16)	.99	2 (8)	4 (9)	.99
Prematurity (32–37 wk)	3 (21)	16 (29)	.84	6 (25)	9 (20)	.83
Vaginal delivery	10 (71)	39 (70)	.99	14 (58)	25 (54)	.95
Mostly breastfed for the first 3 mo of age	7 (50)	29 (52)	.75	12 (50)	18 (39)	.53
Smoke exposure at home	0 (0)	5 (9)	.56	2 (8)	8 (17)	.50
Clinical presentation						
Weight at presentation, kg	5.2 (4.1–6.2)	6.1 (4.6–8.0)	.049	4.7 (4.0–6.6)	6.9 (5.3–8.2)	.007
Respiratory rate at presentation, breaths/min	40 (36–48)	49 (41–60)	.051	57 (44–72)	49 (40–60)	.09
Received corticosteroids during prehospitalization visit	0 (0)	7 (13)	.37	2 (8)	7 (15)	.66
Virological characteristic			.41			.07
Sole RSV infection	10 (71)	26 (46)		11 (46)	16 (35)	
Sole rhinovirus infection	1 (7)	10 (18)		3 (13)	14 (30)	
RSV + rhinovirus coinfection	1 (7)	8 (14)		1 (4)	6 (13)	
Other infecting pathogen(s)	2 (14)	12 (21)		9 (38)	10 (22)	

Data are no. of infants (%) or median value (interquartile range).

Abbreviation: RSV; respiratory syncytial virus

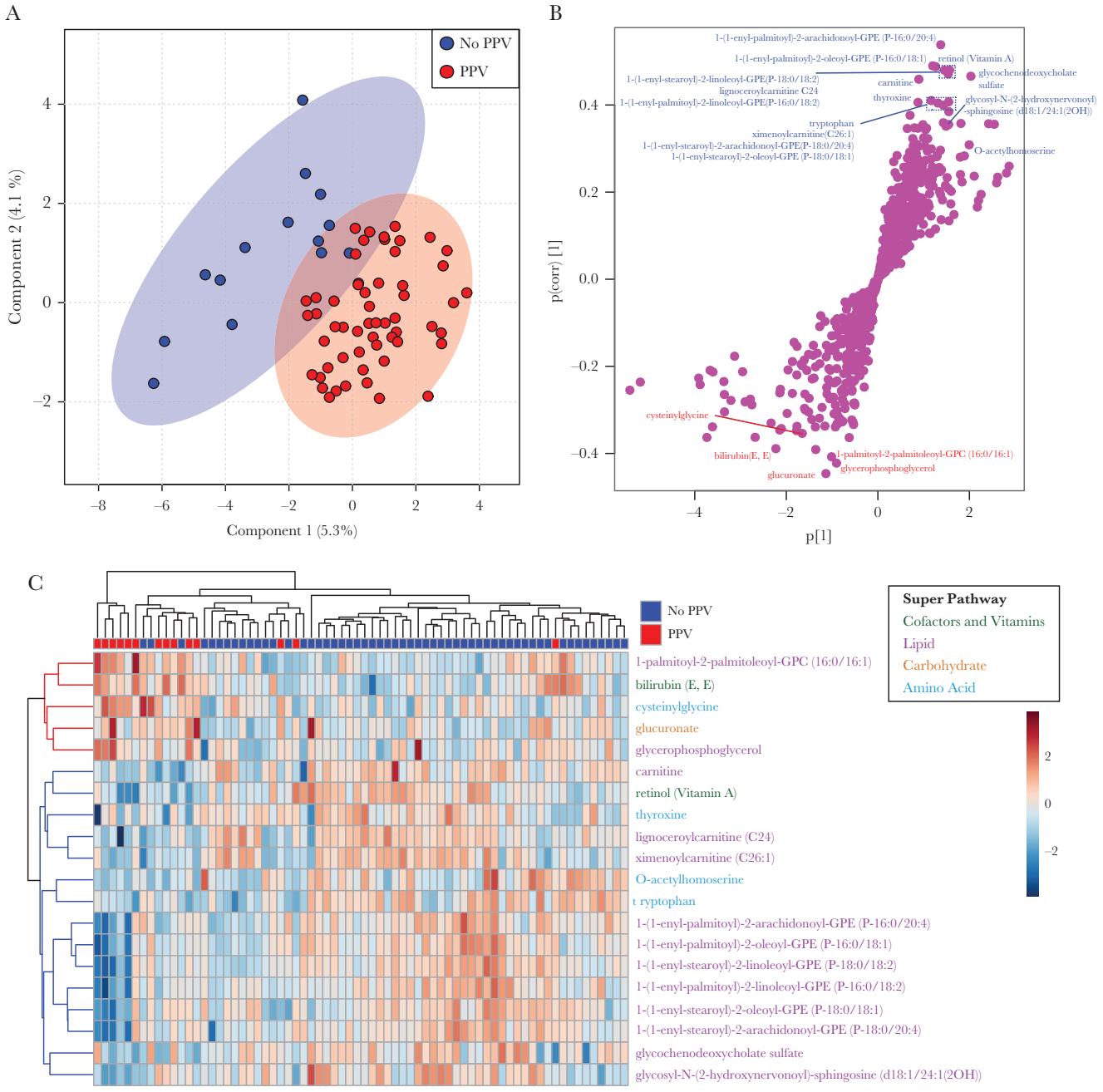

Figure 1. Association between serum metabolome and positive pressure ventilation (PPV) use in the training data set. *A*, Sparse partial least-squares discriminant analysis score scatterplot per the use of PPV ($P < .001$ with 2000 random permutations). Each dot represents the serum metabolomic profile of a single patient in a 2-dimensional space. The ellipses represent 95% confidence intervals. *B*, Orthogonal partial least-squares discriminant analysis loadings plot. The 20 metabolites that are significantly different between PPV use and non–PPV use are shown. Metabolites are colored according their association with a higher (red) or lower (blue) risk of PPV use. *C*, Heatmap of the 20 metabolites with significantly different intensities between PPV use and non–PPV use. Clustering is based on Euclidean distance and Ward linkage. The patients are shown in columns, with PPV use in red and the non–PPV use in blue. The log-transformed intensity of metabolites is shown as low (with dark blue denoting the lowest intensity) and high (with dark red denoting the highest intensity). The row dendrogram shows whether the metabolite intensity is higher in PPV use (red) or non–PPV use (blue).

of stay (LOS) of ≥3 days [11]. In this sensitivity analysis, metabolomic profiles of infants also differed significantly by hospital LOS ($P < .001$ by the permutation test; Supplementary Figure E2). Likewise, 12 of 20 metabolites with a significant association with the risk of PPV use were also significantly associated with hospital LOS in the unadjusted model, with 8 remaining significant following adjustment for confounders (FDR < 0.1; Supplementary Table E3). In accordance with the analysis for PPV use, 1-palmitoyl-2-palmitoleoyl-GPC (16:0/16:1) was the metabolite most significantly associated

Table 2. Serum Metabolites Significantly Associated With a Risk of Positive Pressure Ventilation Use in the Training and Test Data Sets

Metabolite	Subpathway	Superpathway	Training Data Set[a]		Test Data Set[b]	
			Unadjusted Model FDR	Adjusted Model FDR[c]	Unadjusted Model FDR[a]	Adjusted Model FDR[c]
Higher risk of PPV use						
1-palmitoyl-2-palmitoleoyl-GPC (16:0/16:1)[d]	Phosphatidylch oline (PC)	Lipid	0.055	0.047	<0.001	0.005
Bilirubin (E,E)	Hemoglobin and porphyrin metabolism	Cofactors and vitamins	0.090	0.180	0.740	0.360
Cysteinylglycine	Glutathione metabolism	Amino acid	0.090	0.096	0.200	0.606
Glucuronate	Amino sugar metabolism	Carbohydrate	0.029	0.011	0.110	0.890
Glycerophosphoglycerol	Glycerolipid metabolism	Lipid	0.072	0.120	0.360	0.760
Lower risk of PPV use						
1-(1-enyl-palmitoyl)-2- arachidonoyl-GPE (P- 16:0/20:4)	Plasmalogen	Lipid	0.003	<0.001	0.001	0.055
1-(1-enyl-palmitoyl)-2-linoleoyl- GPE (P-16:0/18:2)[d]	Plasmalogen	Lipid	0.009	0.005	<0.001	0.014
1-(1-enyl-palmitoyl)-2-oleoyl- GPE (P-16:0/18:1)[d]	Plasmalogen	Lipid	0.003	<0.001	0.002	0.007
1-(1-enyl-stearoyl)-2- arachidonoyl-GPE (P- 18:0/20:4)	Plasmalogen	Lipid	0.072	0.012	0.013	0.220
1-(1-enyl-stearoyl)-2-linoleoyl- GPE (P-18:0/18:2)[d]	Plasmalogen	Lipid	0.009	0.002	0.001	0.014
1-(1-enyl-stearoyl)-2-oleoyl-GPE (P-18:0/18:1)	Plasmalogen	Lipid	0.070	0.015	0.005	0.068
Carnitine	Carnitine metabolism	Lipid	0.012	0.008	0.059	0.501
Glycochenodeoxycholate sulfate	Primary bile acid metabolism	Lipid	0.009	0.003	0.009	0.150
Glycosyl-N-(2- hydroxynervonoyl)-sphingosine (d18:1/24:1(2OH))	Ceramides	Lipid	0.072	0.030	0.095	0.097
Lignoceroylcarnitine (C24)	Fatty acid metabolism	Lipid	0.025	0.010	0.023	0.15
O-acetylhomoserine	Glycine, serine, and threonine metabolism	Amino acid	0.090	0.003	0.260	0.980
Retinol (vitamin A)[d]	Vitamin A metabolism	Cofactors and vitamins	0.009	0.011	0.001	0.010
Thyroxine[d]	Tyrosine metabolism	Amino acid	0.090	0.012	0.009	0.037
Tryptophan[d]	Tryptophan metabolism	Amino acid	0.072	0.009	<0.001	0.001
Ximenoylcarnitine (C26:1)	Fatty acid metabolism	Lipid	0.090	0.075	0.062	0.720

Abbreviations: GPC, glycerophosphorylcholine; GPE, glycerophosphoethanolamine; FDR, false-discovery rate.

[a]Values of <0.10 are considered statistically significant.

[b]Values of <0.05 are considered significant.

[c]Adjusted for age, sex, weight at presentation, breastfeeding status, and virus.

[d]Significant in all analyses.

with a longer LOS, while plasmalogen metabolites were associated with a shorter LOS.

The 20 Discriminatory 20 Metabolites From the Training Data Set Also Separated Infants With From Those Without PPV Use in the Test Data Set

Using the 20 metabolites with a significant association with the risk of PPV use that were derived from the training data set, the metabolomic profiles remained significantly different (P = .002 by the permutation test) between infants who did and those who did not use PPV in the test data set (Figure 2A). 1-palmitoyl-2-palmitoleoyl-GPC (16:0/16:1) was the only metabolite that remained significantly associated with a higher risk of PPV use (unadjusted FDR < 0.001 and adjusted FDR = 0.005; Table 2). Eleven of 15 metabolites associated with a lower risk of PPV use retained their significant association in the test data set in the unadjusted model (FDR < 0.05; Table 2); 6 metabolites remained significant following adjustment (FDR < 0.05; Table 2). In the heatmap, consistent with the training data set analysis, the infants generally clustered by PPV use. All metabolites associated with higher risks of PPV use clustered distinctly from the metabolites associated with lower risks of PPV use (Figure 2B).

Analysis of Nasopharyngeal Airway Microbiota and Serum Metabolites Showed That the *Streptococcus* Relative Abundance Correlates With the Metabolites Associated With a Risk of PPV Use

Streptococcus, *Moraxella*, and *Haemophilus* genera dominated the nasopharyngeal microbiota, accounting for 80% of the overall relative abundance, in this cohort of infants with bronchiolitis. Next, we examined how these dominant bacterial bacteria are related to the intensity of the 20 derived metabolites. The relative abundance of *Streptococcus* was positively correlated with the metabolites associated with a higher risk of

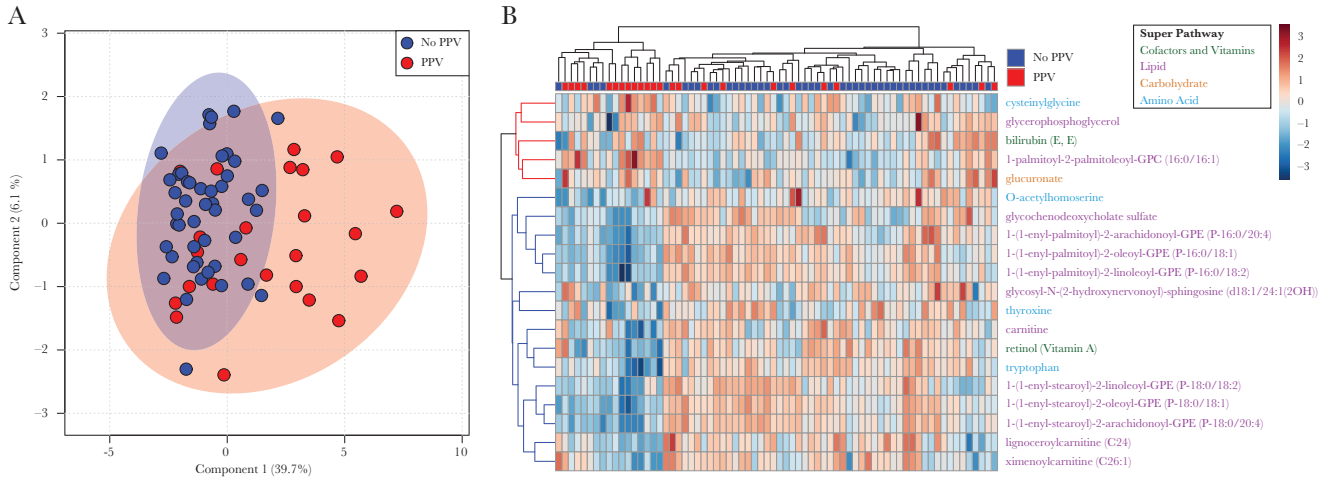

Figure 2. Association of serum metabolome with positive pressure ventilation (PPV) use in the test data set. Data based on serum metabolites among 24 PPV use (n = 24) and 46 non–PPV use. *A*, Sparse partial least-squares discriminant analysis score scatterplot of the test data set, grouped according to PPV use (P = .002 with 2000 random permutations). Each dot represents the serum metabolomic profile of a single patient in a 2-dimensional space. The ellipses represent 95% confidence intervals. *B*, Heatmap analysis of the test data set, based on the 20 metabolites significantly different between PPV use and non–PPV use that are derived in the training data set. Clustering based on Euclidean distance and Ward linkage. The patients are shown in columns, with PPV use in red and non–PPV use in blue. The log-transformed intensity of metabolites is shown as low (with dark blue denoting the lowest intensity) and high (with dark red denoting the highest intensity). The row dendrogram shows whether the metabolite intensity is higher in PPV use (red) or non–PPV use (blue).

PPV use (eg, glucuronate and 1-palmitoyl-2-palmitoleoyl-GPC [16:0/16:1]) and negatively correlated with metabolites associated with a lower risk of PPV use, including all metabolites from the plasmalogen subpathway (ie, GPEs), in both the training and test data sets (Figure 3). In contrast, the abundance of *Moraxella* organisms had the opposite correlation patterns.

Nasopharyngeal Airway and Serum Metabolite Intensities Are Not Correlated

In the analysis of 70 infants with paired nasopharyngeal and serum metabolome data, a total of 225 metabolites from were identified in both data sets (36%). Metabolomic profiles clustered distinctly between nasopharyngeal and serum samples (P < .001 by the permutation test; Supplementary Figure E3*A*) Of these metabolites detected in both biofluids, 222 of the 225 metabolites had no statistically significant correlation in intensity between nasopharyngeal airway and serum samples (all FDRs > 0.05; Supplementary Figure E3*B*).

DISCUSSION

In the current analysis of a multicenter prospective cohort of infants with bronchiolitis, we used a metabolomics approach to investigate serum metabolites and their association with the risk of PPV and hospital LOS. We found that serum metabolomic profiles differed by disease severity. A total of 20 metabolites were associated with a risk of PPV use in the training data set, with 18 metabolites remaining statistically significant after adjustment for potential confounders. Most metabolites significantly associated with a risk of PPV were from unique pathways, with the exception of the plasmalogen pathway, from which 6 metabolites (eg, GPEs) were associated with a lower risk of PPV use. The test data set provided validation of the training data set. Integrating the nasopharyngeal airway microbiota data from these infants, we also found that the relative abundance of *Streptococcus*—one of the pathological bacteria in respiratory health [4, 11, 20, 21]—was negatively associated with plasmalogen pathway metabolites. To the best of our knowledge, this is the first study that has investigated the interrelations between airway microbiota, circulating metabolome, and disease severity in infants with bronchiolitis.

While bronchiolitis is caused by a viral infection, recent studies have suggested that a complex interplay between the airway, gut, and host systemic response contributes to the severity of bronchiolitis (Supplementary Figure E4). In the airways, we recently demonstrated in the MARC-35 cohort that infants with a *Streptococcus*-dominant microbiota had more-severe illness; this finding was externally validated in a separate cohort of 307 children hospitalized for bronchiolitis [11] and was also observed in 234 Australian infants from the Childhood Asthma Study cohort [6]. Furthermore, studies have also reported interactions between the nasopharyngeal airway microbiota and airway immune response—which is represented by CCL5 [22] and proinflammatory lipid mediators (eg, sphingolipid metabolites) [4] in the airway—with regard to acute severity of bronchiolitis. In the gut, a *Bacteroides*-dominant fecal microbiota and enhanced sphingolipid metabolic pathway were associated with a higher likelihood of infant bronchiolitis [23, 24]. In relation to the host systemic response, a recent single-center study of 132 children with RSV infection examining nasopharyngeal microbiota and whole-blood gene expression

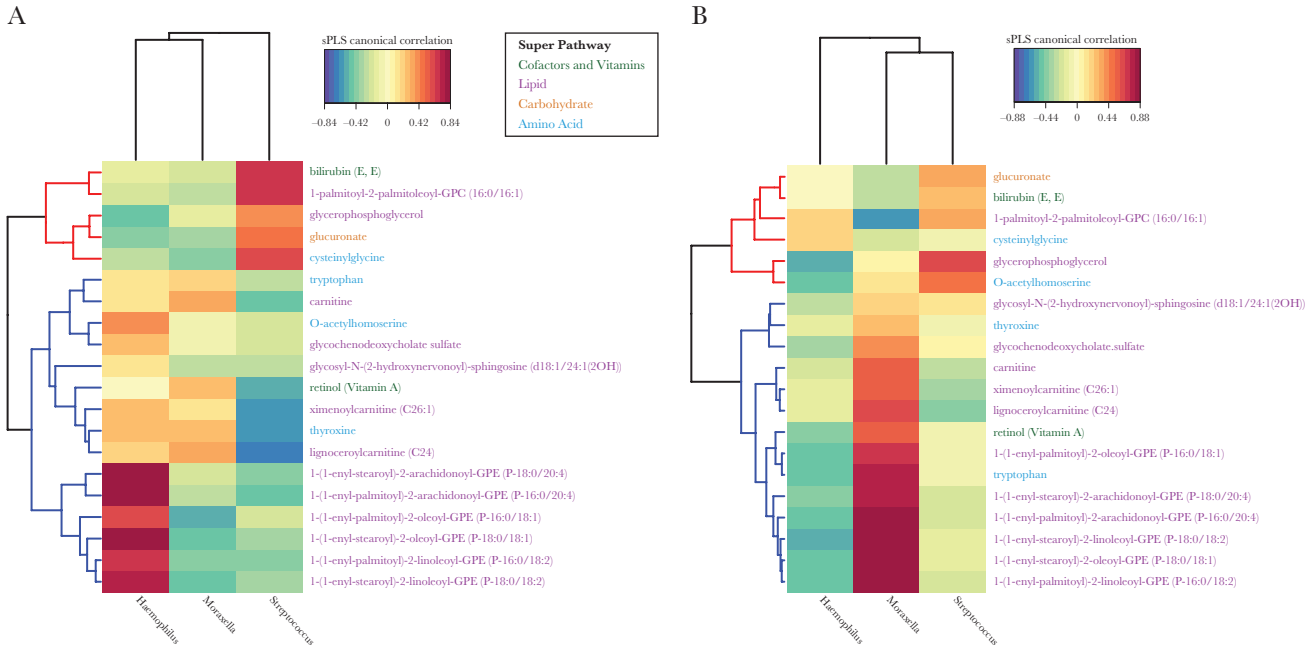

Figure 3. Canonical correlation between nasopharyngeal microbiota associated with positive pressure ventilation (PPV) use. *A*, Training data set. *B*, Test data set. The top 3 dominant bacterial genera (*Haemophilus, Moraxella,* and *Streptococcus*), which together represent 80% of the overall abundance, are shown. Relative abundance for the 3 dominant genera is based on 16S ribosomal RNA gene sequencing. The 20 discriminatory metabolites are shown in rows, with the dendrogram colored according to whether the metabolite is associated with a higher (red) or lower (blue) risk of PPV use. sPLS, sparse partial least squares.

reported microbiota-specific systemic immune responses [8]. Specifically, children with a *Streptococcus*-dominant nasopharyngeal microbiota had overexpression of genes linked to Toll-like receptor signaling and chemotactic factors (eg, interleukin 8) in whole-blood specimens [8]. The current multi-omic analysis corroborates findings from these earlier studies and extends them by demonstrating the interrelations between the serum metabolome (eg, glycerophospholipids), airway microbiota (eg, *Streptococcus* abundance), and risk of PPV use in infants with bronchiolitis.

The mechanisms underlying these interrelations remain to be elucidated. The literature has suggested that different body sites (and, thus, sample types) have distinct metabolome profiles [25, 26] and endogenous bacterial communities [27]. Consistently, we also observed no statistically significant correlations for most individual metabolites between nasopharyngeal airway and serum samples. It is possible that the unique airway microbiome profile influenced the systemic inflammatory response [8], thereby altering the serum metabolome profile. Alternatively, the microbiome of distant organs and circulating metabolites might have pertubated the microbiome and local inflammatory response in the airway. These mechanisms are not mutually exclusive. For example, the "gut-lung axis" is bidirectional, with changes in either site having subsequent effects on the other [28]. Previous work has reported that exposing the lungs of mice to bacterial lipopolysaccharide resulted in changes to the cecum microbiome [29]. Studies have also demonstrated the effects of the gut microbiome on circulating bacteria-derived metabolites (eg, short-chain fatty acids)—which play a role in the regulation of T-helper type 2 cells and regulatory T cells—and inflammation in the airway [30, 31]. These observations from the earlier studies and the present study should advance investigations into the mechanisms underlying the complex interplay between the microbiome, metabolome, and local and systemic immune responses in infants with bronchiolitis.

Among the 772 serum metabolites identified in the current study, 6 (eg, GPEs) from the plasmalogen subpathway were associated with a lower risk of PPV use. Plasmalogens are a specific glycerophospholipid class containing a vinyl ether moiety at the sn-1 position and polyunsaturated fatty acids at the sn-2 position of the glycerol backbone [32]. Plasmalogens are primarily considered structural cellular membrane components and reservoirs for second lipid messengers. Additionally, lung plasmalogens are particularly enriched in arachidonic acid and have important roles in inflammation and immunity [32, 33]. Plasmalogens also act as endogenous antioxidants and can protect endothelial cells from hypoxic stress and reactive oxygen species [34]. Reduced levels of plasmalogens in a range of diseases have been reported in the literature. In the airways, reduced plasmalogens have been associated with bronchopulmonary dysplasia in preterm infants [35], with improved outcomes in infants who received higher amounts of plasmalogen in surfactant preparations [36]. In a cohort of adults with chronic obstructive pulmonary disease, smoking

was associated with decreased plasmalogen synthesis in the lung, and subsequent plasmalogen deficiency in the serum [37]. These studies and our data collectively suggest a potentially protective effect of plasmalogens on respiratory health.

We also found that higher cysteinylglycine levels are associated with a higher risk of PPV use in the training data set. This metabolite is related not only to liver diseases (as the product of the reaction catalyzed by gamma-glutamyl transferase) but also to respiratory diseases (eg, asthma and acute respiratory distress syndrome). Cysteinylglycine is a substrate of glutathione (gamma-glutamyl-cysteinylglycine), which is central to the redox defense during oxidative stress due to oxidant and antioxidant imbalance [38, 39].

The current study has several potential limitations. First, the metabolome and microbiota were measured at a single time point early in the hospital course of critical illness. While the consistent findings both in the PPV use and hospital LOS outcomes address, at least in part, potential reverse causation, longitudinal sampling would facilitate further investigation into temporal changes preceding disease onset. Second, we did not have information from a nonbronchiolitis control group. Regardless, the study aim was not to evaluate the relationships between the metabolome and microbiota and the development of bronchiolitis, but to investigate their associations with acute disease severity among infants with bronchiolitis. Third, the association of serum metabolites with bronchiolitis severity represents a novel area of investigation, and the applicability of serum as a biomarker for airway disease requires additional validation and mechanistic interpretation. However, targeted analysis of serum has revealed that lower levels of serum LL-37 are associated with increased severity of disease [40], providing additional evidence of serum components as potential biomarkers of bronchiolitis severity. Finally, although our cohort comprised racially/ethnically diverse samples, all subjects were hospitalized for bronchiolitis; the inferences may not be generalizable to infants with mild-to-moderate bronchiolitis. Nevertheless, our findings are of direct relevance to approximately 130 000 US children hospitalized annually with bronchiolitis [2].

In conclusion, in this multi-omic analysis of the multicenter data of 140 infants hospitalized for bronchiolitis, we found that serum metabolome profiles differed by disease severity. In particular, plasmalogen metabolites, such as GPEs, were associated with a lower risk of PPV use in both training and test data sets. When we integrated the nasopharyngeal airway microbiota data, we also found that the relative abundance of *Streptococcus* organisms was negatively correlated with these plasmalogen pathway metabolites. While the causal relationship remains to be elucidated, prior studies and the current study collectively suggest that the interrelations between the airway microbiota and host systemic inflammatory processes—reflected by the circulating metabolome profiles—may contribute to bronchiolitis severity (Supplementary Figure E4). Our data advance understanding of the mechanism(s) underlying the complex interplay between the airway microbiota and host immune response, which will facilitate the development of novel preventive and therapeutic strategies in infants with bronchiolitis.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Disclaimer. The content of this article is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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Potential conflicts of interest. J. M. M. has provided bronchiolitis-related consultation for Regeneron. N. J. A. and J. F. P. own shares at Diversigen, a microbiome research company. All other authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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