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Respiratory Virus–Specific Nasopharyngeal Lipidome Signatures and Severity in Infants With Bronchiolitis: A Prospective Multicenter Study

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Background. In infant bronchiolitis, recent evidence indicates that respiratory viruses (eg, respiratory syncytial virus [RSV], rhinovirus [RV]) contribute to the heterogeneity of disease severity. Of the potential pathobiological molecules, lipids serve as signaling molecules in airway inflammation. However, little is known about the role of the airway lipidome in between-virus heterogeneity and disease severity.

Methods. In this multicenter prospective study of 800 infants hospitalized for RSV or RV bronchiolitis, we analyzed nasopharyngeal lipidome data. We examined discriminatory lipids between RSV and RV infection and the association of the discriminatory lipids with bronchiolitis severity, defined by positive pressure ventilation (PPV) use.

Results. We identified 30 discriminatory nasopharyngeal lipid species and 8 fatty acids between RSV and RV infection. In the multivariable models adjusting for patient-level confounders, 8 lipid species—for example, phosphatidylcholine (18:2/18:2) (adjusted odds ratio [aOR], 0.23 [95% confidence interval {CI}, .11–.44]; false discovery rate [FDR] = 0.0004) and dihydroceramide (16:0) (aOR, 2.17 [95% CI, 1.12–3.96]; FDR = 0.04)—were significantly associated with the risk of PPV use. Additionally, 6 fatty acids—for example, eicosapentaenoic acid (aOR, 0.27 [95% CI, .11–.57]; FDR = 0.01)—were also significantly associated with the risk of PPV use.

Conclusions. In infants hospitalized for bronchiolitis, the nasopharyngeal lipidome plays an important role in the pathophysiology of between-virus heterogeneity and disease severity.

Keywords. ceramide; glycerophospholipid metabolism; lipids; phosphatidylcholine; sphingolipid metabolism.

Infant bronchiolitis is an important public health problem in the United States (US). While 40% of infants develop clinical bronchiolitis in the first 2 years of life, its severity ranges from a minor nuisance to fatal [1]. Bronchiolitis is the leading cause of hospitalization in US infants, accounting for approximately 110 000 hospitalizations each year [2]. Most cases of bronchiolitis requiring hospitalization are caused by 2 distinct viruses—respiratory syncytial virus (RSV) and rhinovirus (RV). Emerging evidence suggests that the difference in infecting virus contributes to the heterogeneity in the disease course (eg, ventilatory support use [3], prolonged hospital length of stay [4–6]) and host immune responses [7, 8]. For example, a previous study has reported that infants with RSV infection had a significantly longer hospital length of stay than those with RV infection [6]. Yet, the exact mechanisms underlying virus-disease heterogeneity link remain unclear.

Of the various pathobiological pathways, lipids play an important role in respiratory diseases as cell membrane substrates and ligand messengers [9, 10]. Airway lipids are also involved in a variety of pathobiology, including airway inflammation, endothelial permeability, surfactant synthesis, and cell apoptosis [11, 12]. Recent studies using the metabolome data of infants with bronchiolitis have suggested that distinct nasopharyngeal and serum lipids (eg, sphingolipids) are associated with between-virus heterogeneity and disease severity [13–15]. Despite the clinical and research importance, no study has yet applied lipidomics—the comprehensive characterization of complex lipids—to delineate the virus-severity relationship in infant bronchiolitis.

To address this knowledge gap, we investigated the virus-specific lipidome signature and its relationship with disease severity in infants hospitalized for bronchiolitis. A better understanding of the role of the virus-specific lipidome in bronchiolitis would reveal the pathobiology of virus-disease heterogeneity and disease severity and lead to the development of a potential therapy for bronchiolitis.

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METHODS

Study Design, Setting, and Participants

We analyzed data from a multicenter prospective cohort study of infants hospitalized for bronchiolitis—the 35th Multicenter Airway Research Collaboration (MARC-35) study [16, 17]. MARC-35 is coordinated by the Emergency Medicine Network (www.emnet-usa.org), an international research collaboration with 247 participating hospitals. Details of the study design, setting, participants, data collection, and testing are shown in the Supplementary Methods. In brief, MARC-35 investigators at 17 sites across 14 US states enrolled 1016 infants (age <1 year) who were hospitalized with an attending physician diagnosis of bronchiolitis during 3 bronchiolitis seasons (from 1 November through 30 April) from 2011 to 2014 (Supplementary Table 1). The diagnosis of bronchiolitis was made according to the American Academy of Pediatrics bronchiolitis guidelines, defined as an acute respiratory illness with some combination of rhinitis, cough, tachypnea, wheezing, crackles, or retraction [18]. We excluded infants with preexisting heart and lung disease, immunodeficiency, immunosuppression, or gestational age <32 weeks. All patients were treated at the discretion of the treating physicians. The study was approved by the Institutional Review Board of Partners Human Research Committee (Protocol code 2017P001637). The institutional review board at each participating hospital approved the study with written informed consent obtained from the parent or guardian.

Of infants who were enrolled in MARC-35, the current analysis investigated 800 infants with solo-RSV or RV infection who underwent nasopharyngeal lipidome profiling. Additionally, the current study also analyzed the nasopharyngeal transcriptome data from randomly selected 312 infants in MARC-35 [19].

Data Collection

Clinical data (patients' demographic characteristics, medical history, environmental, and family, and details of the acute illness) were collected via structured interview and chart reviews using a standardized protocol [16, 17]. All data were reviewed at the Emergency Medicine Network Coordinating Center at Massachusetts General Hospital (Boston, Massachusetts) [20]. In addition to the clinical data, investigators also collected nasopharyngeal airway specimens within 24 hours of hospitalization using a standardized protocol [17]. These specimens underwent (1) viral testing of respiratory viruses (eg, RSV and RV) using real-time polymerase chain reaction assays at Baylor College of Medicine (Houston, Texas) [21, 22]; (2) complex lipidomic profiling at Metabolon (Durham, North Carolina) [23]; and (3) transcriptome profiling using RNA sequencing (RNA-seq) at the University of Maryland, Baltimore [19].

Nasopharyngeal Complex Lipidomic Profiling

The detail of complex lipidomic profiling is described in the Supplementary Methods. In brief, lipids were extracted from the nasopharyngeal specimens by a modified Bligh–Dyer extraction using methanol/water/dichloromethane in the presence of deuterated internal standards [24]. The extracts were dried under nitrogen and reconstituted in ammonium acetate dichloromethane: methanol. The extracts were then transferred to vials for infusion–mass spectrometry analysis, performed on a Shimadzu liquid chromatography with nano PEEK tubing (Shimadzu Scientific Instruments, Kyoto, Japan) and the SCIEX SelexIon-5500 QTRAP (SCIEX, Toronto, Canada). The specimens were analyzed by both positive and negative mode electrospray. Individual lipid species were then quantified by taking the ratio of the signal intensity of each target compound to that of its internal standard, then multiplying by the concentration of internal standard added to the specimen.

Nasopharyngeal Transcriptomic Profiling

The details of RNA extraction, RNA-seq, quality control, and transcriptome profiling are described in the Supplementary Methods. In brief, after total RNA extraction, DNase treatment, and ribosomal RNA reduction, we performed RNA-seq with NovaSeq6000 (Illumina, San Diego, California) using an S4 100PE Flowcell (Illumina). All RNA-seq samples had high sequence coverage after quality control. The transcript abundances were estimated with Salmon using the human genome hg38 and the mapping-based mode [25].

Outcome Measures

The clinical outcomes of interest were acute severity of bronchiolitis. More specifically, the primary outcome was the use of positive pressure ventilation (PPV), defined as the use of continuous positive airway pressure ventilation and/or mechanical ventilation during the index hospitalization [16]. The secondary outcome was respiratory support use, defined as the use of PPV or high-flow oxygen therapy [26].

Statistical Analyses

The analytic workflow is summarized in Figure 1. First, we preprocessed the lipidome data (eg, Pareto normalization [27] and removal of lipid species with near-zero variance [28]). Second, to examine discriminatory lipid species between RSV and RV infection, we performed sparse partial least squares discriminant analysis (sPLS-DA) with Lasso penalization and 5-fold cross-validation (repeated 50 times) to minimize potential overfitting by using R *mixOmics* package [29]. The sPLS-DA method has an advantage in that it handles correlated variables (eg, lipids). To examine discriminatory fatty acids, we also fit unadjusted logistic regression models. For the downstream analyses, we selected the 30 most between-virus (RSV vs RV)

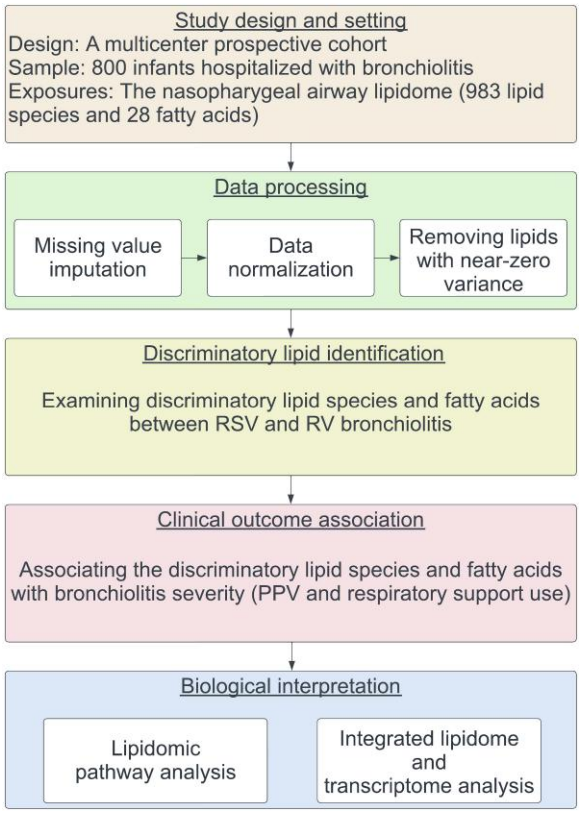

Figure 1. Analytic workflow. The analytic cohort consisted of 800 infants hospitalized for respiratory syncytial virus (RSV) or rhinovirus (RV) bronchiolitis in a multicenter prospective cohort study, MARC-35. First, by using the nasopharyngeal lipidome data, we performed missing value imputation and data normalization and removed lipid species with near-zero variance. Second, we determined discriminatory lipid species and fatty acids between RSV and RV bronchiolitis. Third, we examined the associations of the discriminatory lipid species and fatty acids with bronchiolitis severity (eg, positive pressure ventilation [PPV] and respiratory support use). Last, we investigated the biological interpretation by conducting pathway analysis and integrated lipidome and transcriptome analysis.

discriminatory lipid species that had a high variable importance and discriminatory fatty acids that had a false discovery rate (FDR) of <0.10. We then computed Spearman correlations between the respiratory virus genomic load (measured as the inverse cycle threshold) and discriminatory lipids to examine the discriminatory ability of these lipids. Third, to investigate the associations of the between-virus discriminatory lipids with the outcomes of interest, we fit multivariable logistic regression models. In the multivariable models, we adjusted for potential confounders (age, sex, race/ethnicity, prematurity, corticosteroid use during lifetime, breastfeeding, and RSV infection) that were selected based on clinical plausibility and a priori knowledge [4, 15, 30–32]. In the sensitivity analysis, we repeated these analyses excluding infants with RSV/RV coinfection (ie, comparisons between solo-RSV infection and RV without RSV infection). Last, to examine the biological function of these lipids, we performed lipidomic pathway and integrated lipidomic-transcriptomic pathway analyses by comparing the infants who underwent PPV to those who did not, based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) database as the reference [33] by using MetaboAnalyst 5.0 [34]. In the integrated lipidomic-transcriptomic pathway analysis, we used both between-virus discriminatory lipids and mRNA ($n = 312$) that are identified through R DESeq2 package [35]. We conducted the statistical analysis by using R version 4.1.0 (R Foundation, Vienna, Austria). All P values were 2-tailed, with $P < .05$ considered statistically significant. We accounted for multiple testing using the Benjamini–Hochberg FDR method that allows for the interpretation of statistical significance in the context of multiple hypothesis testing [36].

RESULTS

Patient Characteristics

Of 800 infants hospitalized for bronchiolitis who underwent nasopharyngeal lipidome profiling, 588 infants had solo-RSV infection and 212 infants had RV infection. Among 212 infants with RV infection, 59 infants had solo-RV infection. Overall, the median age was 3 months (interquartile range, 2–5 months) and 40% were female; 5% underwent PPV and 18% received respiratory support during the hospitalization. Between the 2 virus groups, infants with solo-RSV infection were younger and less likely to be male and have received corticosteroid use ($P < .05$) compared to those with RV infection (Table 1).

Virus-Specific Discriminatory Lipids Are Associated With Bronchiolitis Severity

The complex lipidomic profiling measured 982 lipid species and 28 fatty acids in the nasopharyngeal airway of infants with bronchiolitis. Of these, the analysis identified 30 most virus-discriminatory lipid species (eg, phosphatidylcholine [PC] [18:2/18:2], dihydroceramide [DCER] [16:0]) and 8 discriminatory fatty acids (eg, eicosapentaenoic acid [an omega-3 fatty acid]). These differences were consistent with the correlations of quantitative virus status (genomic load) with the discriminatory lipid species (Figure 2*A*) and fatty acids (Figure 2*B*). RSV genomic load was significantly negatively correlated with 23 lipid species and positively correlated with 4 lipid species (all FDR <0.05); Specifically, RSV genomic load was negatively correlated with phosphatidylcholines and dihydroceramides. RSV genomic load was also significantly negatively correlated with 6 fatty acids (all FDR <0.05).

In the multivariable models, the lower concentrations of 5 lipid species—for example, PC (18:2/18:2) (adjusted odds ratio [aOR], 0.23 [95% confidence interval {CI}, .11–.44], FDR = 0.0004 for PPV use; aOR, 0.57 [95% CI, .42–.76], FDR = 0.01 for respiratory support use)—were significantly associated with the risk of both PPV use and respiratory support use (FDR <0.05; Figures 3 and Supplementary Figure 1). In contrast, the concentration of DCER (16:0) was significantly

Table 1. Characteristics and Clinical Course of Infants Hospitalized for Bronchiolitis by Respiratory Virus

Patient Characteristics	Solo-RSV (n = 588)	RV (n = 212)	*P* Value
Demographics			
Age, mo, median (IQR)	3 (1–5)	4 (2–6)	<.001
Male sex	335 (57)	143 (67)	.01
Race/ethnicity			.18
Non-Hispanic White	269 (45)	80 (38)	
Non-Hispanic Black	130 (22)	57 (27)	
Hispanic	165 (28)	68 (32)	
Other or unknown	24 (4)	7 (3)	
C-section delivery	217 (37)	65 (31)	.14
Prematurity (32–36.9 wk)	105 (18)	48 (23)	.16
History of eczema	80 (14)	31 (15)	.80
Corticosteroid use during lifetime	63 (11)	40 (19)	.004
Mostly breastfed during 0–2.9 mo	253 (49)	81 (46)	.44
Cigarette smoke exposure at home	95 (16)	23 (11)	.08
Maternal smoking during pregnancy	86 (15)	29 (14)	.85
Clinical presentation			
Weight, kg, median (IQR)	5.7 (4.6–7.3)	6.4 (4.9–8.0)	<.001
Respiratory rate (breaths/min), median (IQR)	48 (40–60)	48 (40–60)	.31
Oxygen saturation at presentation			.65
<90%	53 (9)	17 (8)	
90%–93%	93 (16)	29 (14)	
≥94%	427 (75)	161 (78)	
Respiratory virus			
RSV	588 (100)	121 (57)	<.001
RV	0 (0)	212 (100)	<.001
Other pathogens[a]	0 (0)	52 (25)	<.001
Solo-RV	0 (0)	59 (28)	<.001
Laboratory data			
Any IgE sensitization[b]	120 (20)	40 (19)	.70
Clinical outcomes			
PPV use[c]	33 (6)	8 (4)	.39
Respiratory support use[d]	111 (19)	35 (17)	.51
Length of hospital stay, d, median (IQR)	2 (1–3)	2 (1–3)	.06

Data are presented as No. (%) of infants unless otherwise indicated. Percentages may not equal 100 because of rounding and missingness.

Abbreviations: IgE, immunoglobulin E; IQR, interquartile range; PPV, positive pressure ventilation; RSV, respiratory syncytial virus; RV, rhinovirus.

[a]Adenovirus, bocavirus, *Bordetella pertussis*, enterovirus, human coronavirus (NL63, OC43, 229E, or HKU1), human metapneumovirus, influenza A or B virus, *Mycoplasma pneumoniae*, and parainfluenza virus 1–3.

[b]Defined by having 1 or more positive values for allergen-specific IgE at index hospitalization.

[c]Defined as the use of continuous positive airway pressure ventilation and/or mechanical ventilation during the hospitalization.

[d]Defined as the use of high-flow oxygen therapy, continuous positive airway pressure ventilation, and/or mechanical ventilation during the hospitalization.

associated with a higher risk of PPV use (aOR, 2.17 [95% CI, 1.12–3.96]; FDR = 0.04). Of the 8 discriminatory fatty acids, the concentrations of 6 fatty acids—for example, eicosapentaenoic acid (aOR, 0.27 [95% CI, .11–.57], FDR = 0.01)—were significantly associated with the risk of PPV use (FDR <0.05; Supplementary Figure 2). Similarly, the concentration of eicosapentaenoic acid was significantly associated with the risk of respiratory support use (aOR, 0.55 [95% CI, .37–.79]; FDR = 0.01; Supplementary Figure 3).

Pathway Analysis Shows the Biological Role of the Nasopharyngeal Lipidome in Bronchiolitis

The lipidomic pathway analysis using the between-virus discriminatory lipid species data demonstrated that 4 pathways (eg, glycerophospholipid and sphingolipid metabolism pathways) were differentially expressed between infants who underwent PPV and those who did not (all FDR <0.05; Figure 4*A*). Similarly, the integrated lipidomic-transcriptomic analysis using the between-virus discriminatory lipid species and mRNA also found that the glycerophospholipid metabolism and sphingolipid metabolism pathways were differentially expressed (both FDR <0.05; Figure 4*B*).

Sensitivity Analysis Supports the Robustness of the Findings

In the sensitivity analysis excluding infants with RSV/RV coinfection (n = 679), we identified 20 discriminatory lipid species and 5 discriminatory fatty acids between RSV and RV infection. These differences were consistent with the correlations

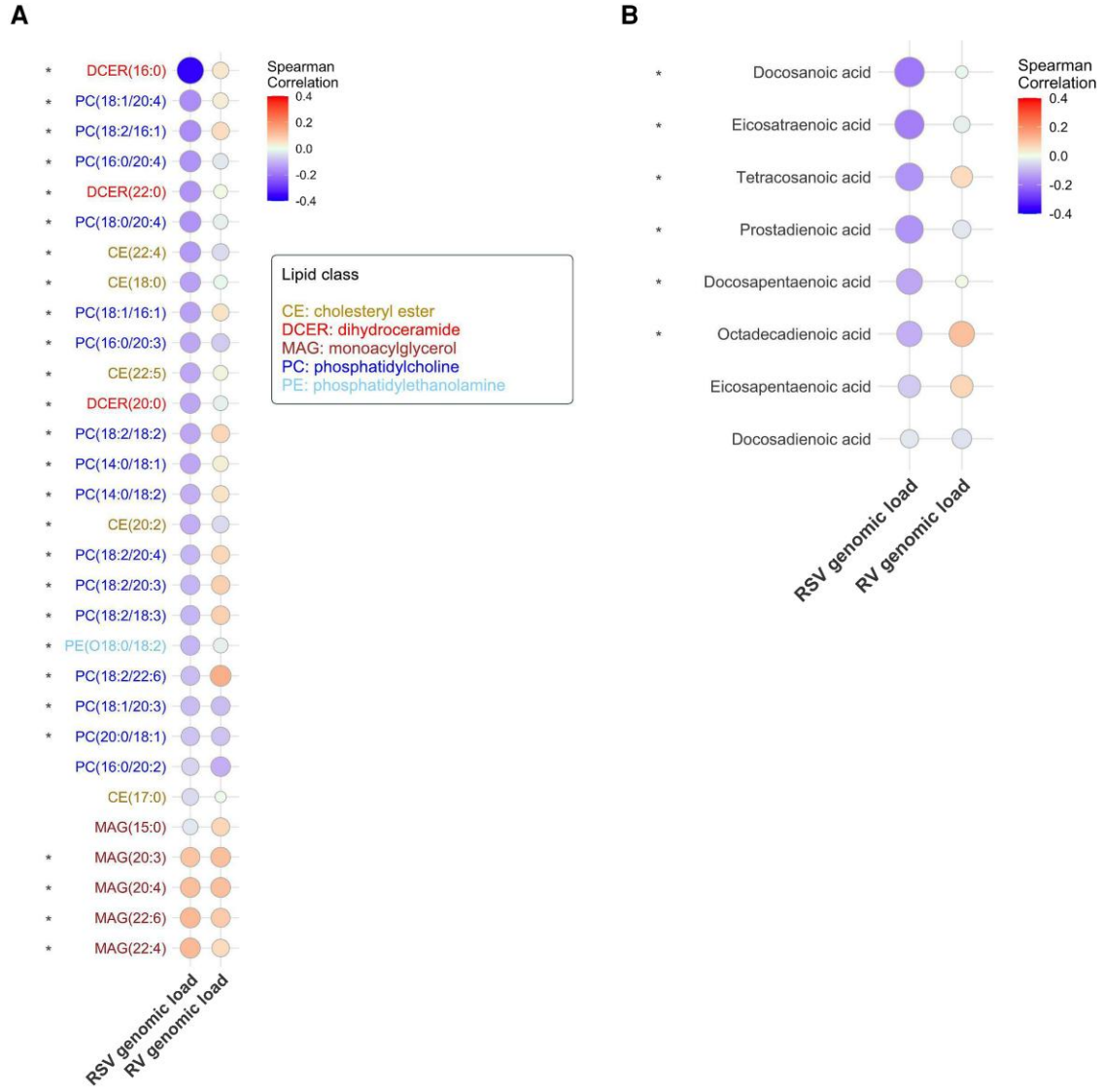

Figure 2. Correlations between respiratory virus genomic load and discriminatory lipids in infants hospitalized for bronchiolitis. *A*, Heatmap showing Spearman correlations between the genomic load of respiratory viruses and 30 discriminatory lipid species. The sizes of circles and colors represent the correlation coefficients. *False discovery rate (FDR) <0.05, estimated by Spearman correlation coefficient between respiratory syncytial virus (RSV) genomic load and lipid species. *B*, Heatmap showing Spearman correlations between the genomic load of respiratory viruses and 8 discriminatory fatty acids. The sizes of circles and colors represent the correlation coefficients. *FDR <0.05, is estimated by Spearman correlation coefficient between the corresponding RSV genomic load and fatty acid. Abbreviations: CE, cholesteryl ester; DCER, dihydroceramide; FDR, false discovery rate; MAG, monoacylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; RSV, respiratory syncytial virus; RV, rhinovirus.

between quantitative virus status and lipids intensity (Figure 5). RSV genomic load was significantly negatively correlated with 14 lipid species and 3 fatty acids (all FDR <0.05). Similar to the primary analysis, 9 lipid species—for example, PC (18:2/18:2) (aOR, 0.28 [95% CI, .13–.53]; FDR = 0.001) and DCER (16:0) (aOR, 2.20 [95% CI, 1.12–4.06]; FDR = 0.02)—were significantly associated with the risk of PPV use (FDR <0.05; Figure 6). Additionally, 3 fatty acids—for example, eicosapentaenoic acid (aOR, 0.22 [95% CI, .08–.50]; FDR = 0.004)—were significantly associated with the risk of PPV use (FDR <0.05; Supplementary Figure 4).

DISCUSSION

Based on the analysis of the nasopharyngeal lipidome data from a multicenter prospective study of 800 infants hospitalized for bronchiolitis, we identified the discriminatory lipids between RSV and RV infection. We also found that, of these discriminatory lipids, the lower concentrations of PC species (eg, PC [18:2/18:2]) and eicosapentaenoic acid were significantly associated with disease severity. In contrast, the higher concentration of DCER (16:0) was significantly associated with the risk of PPV use. To the best of our knowledge, this is the first investigation that has examined the relationship of virus-specific

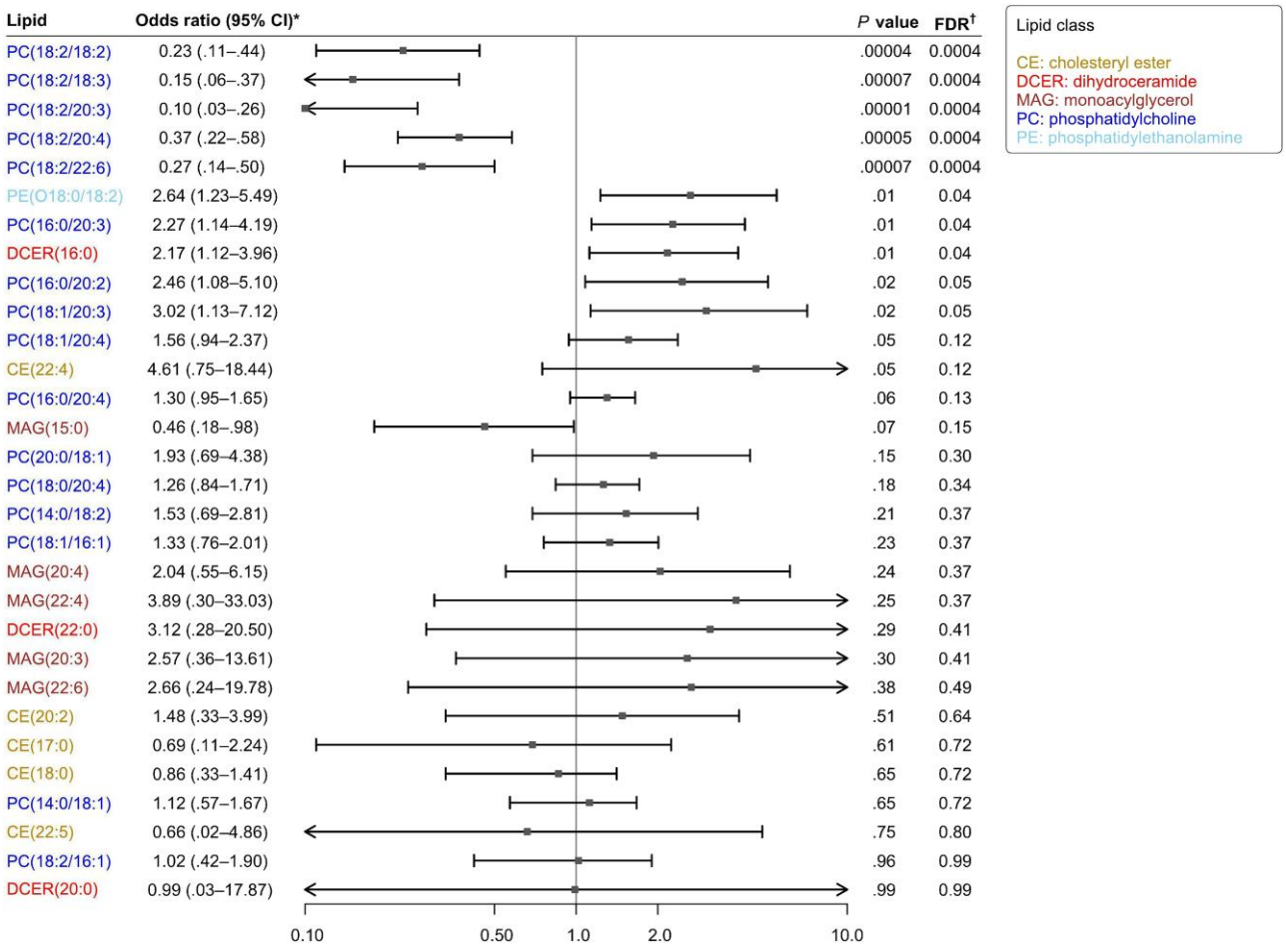

Figure 3. Adjusted associations of between-virus discriminatory lipid species with severity in infants hospitalized for bronchiolitis. The forest plot shows multivariable-adjusted associations of each of the 30 between-virus discriminatory lipid species with the risk of positive pressure ventilation use. The odds ratio was estimated for a square root of the standard deviation change in the concentration of the corresponding lipid species. Arrows indicate that the 95% confidence interval of the odds ratio exceeds the lower or higher limit of the x-axis. *Estimated by fitting logistic regression model adjusting for 7 potential confounders (age, sex, race/ethnicity, prematurity, corticosteroid use during lifetime, breastfeeding, and respiratory syncytial virus infection). †The Benjamini-Hochberg false discovery rate method was used to account for multiple testing. Abbreviations: CE, cholesteryl ester; CI, confidence interval; DCER, dihydroceramide; FDR, false discovery rate; MAG, monoacylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine.

nasopharyngeal lipidome signature with disease severity among infants with bronchiolitis.

Bronchiolitis has been conventionally considered a single disease entity [1]. Yet, recent research has reported between-virus heterogeneity in clinical outcomes [4, 5] and host immune responses [3, 7]. Indeed, metabolomics (ie, not lipidomics) studies have suggested that the upper airway and serum lipids may contribute to between-virus heterogeneity in bronchiolitis [13, 14]. In agreement with our findings, a previous smaller-scale (N = 144) analysis of bronchiolitis has reported that the upper airway sphingolipid metabolism was significantly enriched in infants with PPV use [16]. Similarly, a previous study (N = 140) of serum data has found that 1-palmitoyl-2-palmitoleoyl-glycerophosphocholine was associated with the risk of PPV use [17]. In contrast, a study (N = 80) using urine metabolome data has reported no significant difference in the metabolite profiles between infants with RSV infection and those with RV infection [37]. These differences may be attributable to the difference in the study sample, specimens, measurement methods, or any combination of these factors. Nevertheless, the current multicenter study—with a sample size many times larger than any other previous study—builds on these prior metabolomics reports and extends them by demonstrating the relationship of the nasopharyngeal lipidome with infecting virus and disease severity among infants hospitalized for bronchiolitis.

The mechanisms underlying the associations of nasopharyngeal lipids (eg, PC [18:2/18:2], DCER [16:0], eicosapentaenoic acid) with infecting virus and disease severity remain to be elucidated. First, PC is one of the main components of the

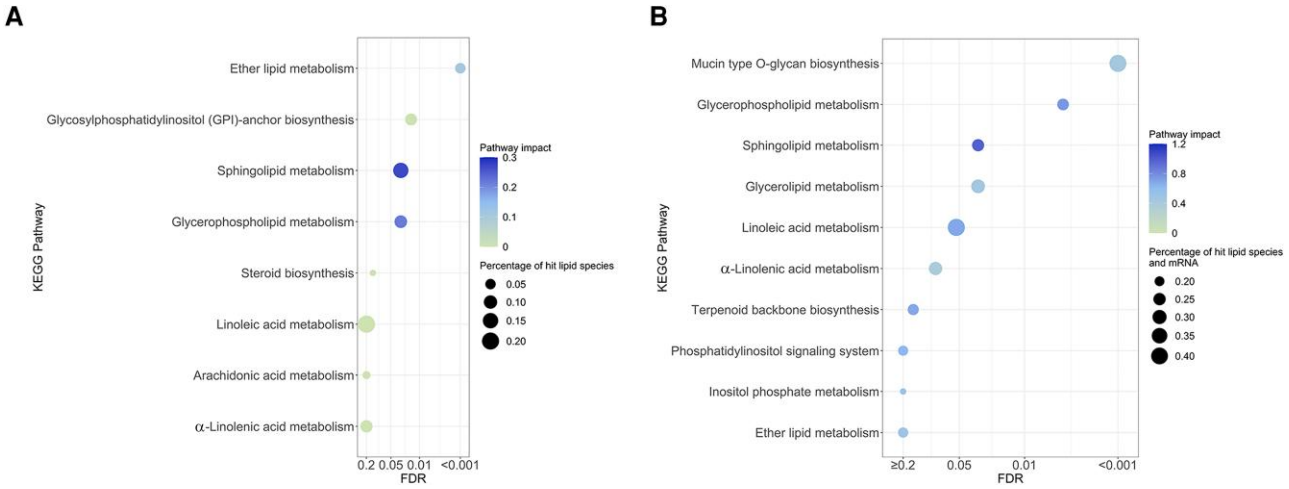

Figure 4. Lipidomic and integrated lipidomic-transcriptomic pathway analysis among infants hospitalized for bronchiolitis. *A*, Lipidomic pathway analysis: The concentrations of the between-virus discriminatory lipid species were used for the pathway analysis based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. The color of each dot represents the pathway impact. The pathway impact is calculated as the sum of the importance measures of the matched lipids normalized by the sum of the importance measures of all metabolites in each pathway [49]. The size of each dot represents the proportion of hit lipid species in the corresponding pathway. *B*, Integrated lipidomic-transcriptomic pathway analysis: The concentrations of the between-virus discriminatory lipid species and nasopharyngeal messenger RNA (mRNA) data were used for the integrated pathway analysis based on the KEGG database. The color of each dot represents the pathway impact. The size of each dot represents the proportion of hit lipid species and mRNA in the corresponding pathway. Glycerophospholipid and sphingolipid metabolism pathways were significantly differentially expressed in infants with positive pressure ventilation use in both lipidomic and integrated lipidomic-transcriptomic pathway analyses. Abbreviations: FDR, false discovery rate; KEGG, Kyoto Encyclopedia of Genes and Genomes; mRNA, messenger RNA.

mitochondrial membrane and synthesizes mature cardiolipins, which are essential for maintaining mitochondrial function [9]. Indeed, an animal model study has reported that respiratory virus infection in mice decreased cardiolipin content and induced mitochondrial shrinkage in alveolar type 2 cells in vivo [38]. Additionally, an animal model study has shown that treatment with cytidine 5′-diphospho-choline—a precursor of phosphatidylcholine—on influenza virus–infected mice attenuated hypoxemia, pulmonary edema, and lung mechanics in vivo [39]. These reports support the current finding that depleted PC was associated with bronchiolitis severity. Additionally, phosphatidylcholines are targets of the potential therapies. Second, DCER and ceramides—precursors of sphingolipids—play important roles in immune response and inflammation [9, 11]. Indeed, an animal model study has reported that ceramide accumulation in the airways causes pulmonary inflammation and death of airway epithelial cells in a cystic fibrosis model in vivo [40]. Of the dihydroceramide, an animal model study has shown that the concentration of DCER (16:0) in the cardiac ventricle significantly increased under hypoxic conditions in vivo [41]. Furthermore, endothelial barrier function is regulated by sphingolipids through 2 distinct pathways (sphingosine-1-phosphate [S1P] and acid sphingomyelinase [ASMase]). Animal studies has reported that S1P receptor antagonist induced loss of capillary integrity in lungs [42], and intervention of inositol-triphosphate suppressed ASMase activity and reduced epithelial apoptosis in neonatal acute lung injury model in vivo [43]. With regard to respiratory viruses, an experimental study using lung epithelial cells has reported that RSV activated both extracellular signal-related kinase and protein kinase B signaling involved in the S1P activation and cell survival in vitro [44]. Given the inverse correlation between ceramide and S1P, this study supports our findings that showed RSV genomic load was negatively correlated with dihydroceramides. Another experimental study using HeLa epithelial cells has shown that inhibition of ASMase prevented the generation of ceramide-enriched membrane platforms and blocked cellular uptake of rhinovirus in vitro [45]. Thus, while RSV and RV are implicated in sphingolipid metabolism in the infection process, the role of sphingolipid metabolism in RSV and RV infection may differ. These data also support our findings that dihydroceramide and sphingolipid metabolism are involved in the pathobiology of virus-disease heterogeneity and disease severity. Third, eicosapentaenoic acid—an omega-3 fatty acid—modulates inflammation related to oxidative stress and improves immune function [46]. A meta-analysis of 10 randomized controlled trials reported that the use of omega-3 fatty acids and antioxidants in patients with acute respiratory distress syndrome reduced the length of intensive care unit stay and ventilator days, albeit with relatively low quality of evidence [47]. The earlier evidence also supports our finding that the depletion of eicosapentaenoic acid is associated with bronchiolitis severity. Notwithstanding the complexity of these potential mechanisms, our lipidomics data—in conjunction

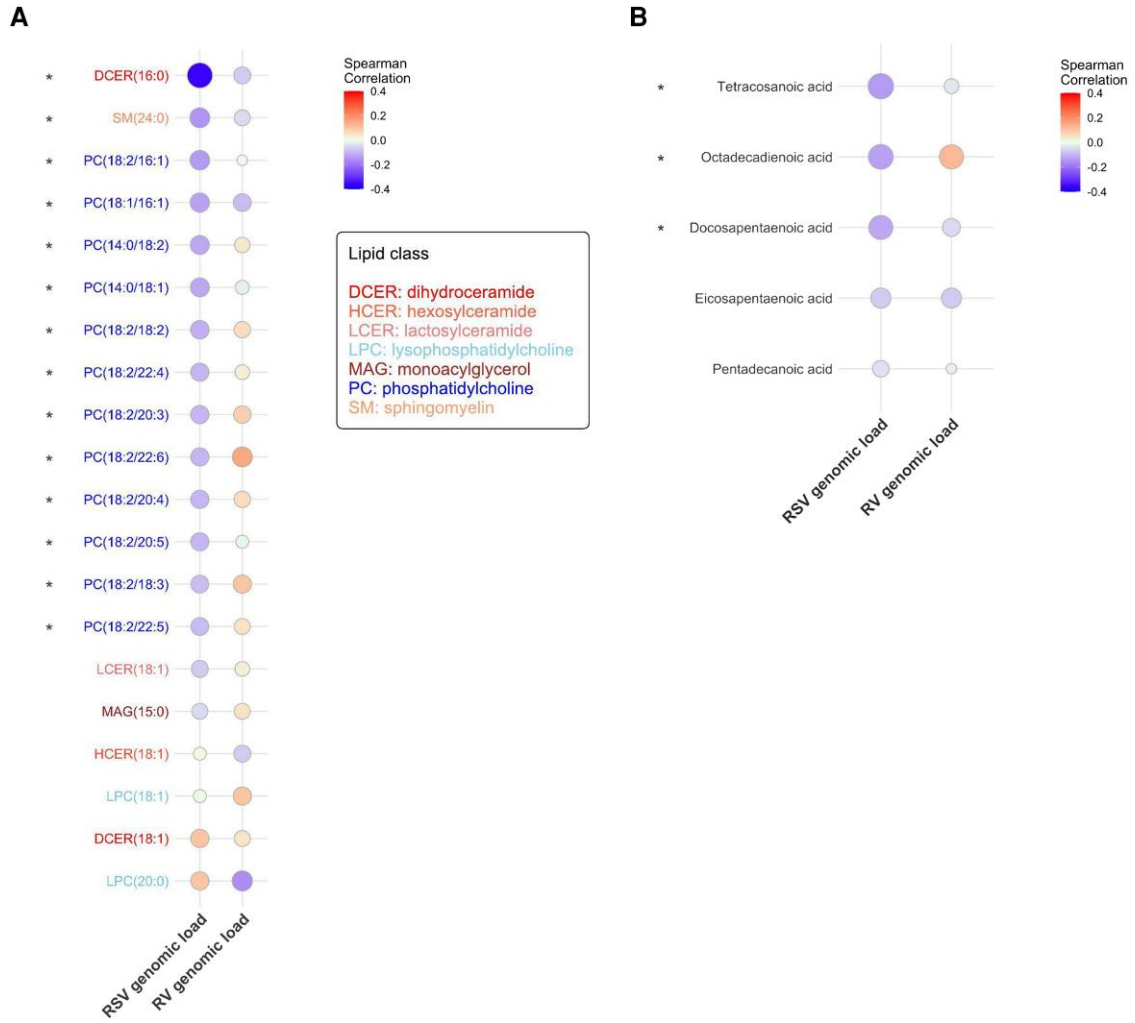

Figure 5. Sensitivity analysis: Correlations between respiratory virus genomic load and discriminatory lipids in infants hospitalized for bronchiolitis. *A*, Heatmap showing Spearman correlations between the genomic load of respiratory viruses and 20 discriminatory lipid species. The sizes of circles and colors represent the correlation coefficients. *False discovery rate (FDR) <0.05, estimated by Spearman correlation coefficient between respiratory syncytial virus (RSV) genomic load and lipid species. *B*, Heatmap showing Spearman correlations between the genomic load of respiratory viruses and 5 discriminatory fatty acids. The sizes of circles and colors represent the correlation coefficients. *FDR <0.05, estimated by Spearman correlation coefficient between the corresponding RSV genomic load and fatty acid. Abbreviations: DCER, dihydroceramide; HCER, hexosylceramide; LCER, lactosylceramide; LPC, lysophosphatidylcholine; MAG, monoacylglycerol; PC, phosphatidylcholine; RSV, respiratory syncytial virus; RV, rhinovirus; SM, sphingomyelin.

with the existent literature—should advance research into the underlying mechanisms of the pathobiology and heterogeneity of infant bronchiolitis.

This study has several potential limitations. First, the current study did not have nondisease controls. However, the aim of this study was not to assess the role of the nasopharyngeal lipidome in the development of bronchiolitis but its relationship with infecting virus and disease severity among infants with bronchiolitis. Second, bronchiolitis involves inflammation of the lower airway in addition to the upper airway, while we used nasopharyngeal airway specimens to examine the lipidome and transcriptome. Yet, previous research has demonstrated that the data from the upper airway specimens offer a reliable representation of lung inflammation profiles [48]. Third, the RV infection group included coinfection because a previous study reported that the risk of prolonged hospital length of stay was lower in infants with RSV/RV coinfection compared to those with RSV infection [6]. However, the sensitivity analysis excluding infants with coinfection showed results that are consistent with the main analysis. Fourth, the lipidome was profiled at a single time point, and the current study did not have data on the exact timing of specimen sampling and the use of PPV or high-flow oxygen therapy. Therefore, caution may be warranted when interpreting the associations of airway lipids with severity. Nonetheless, the findings of this study in the early course of bronchiolitis are clinically and biologically

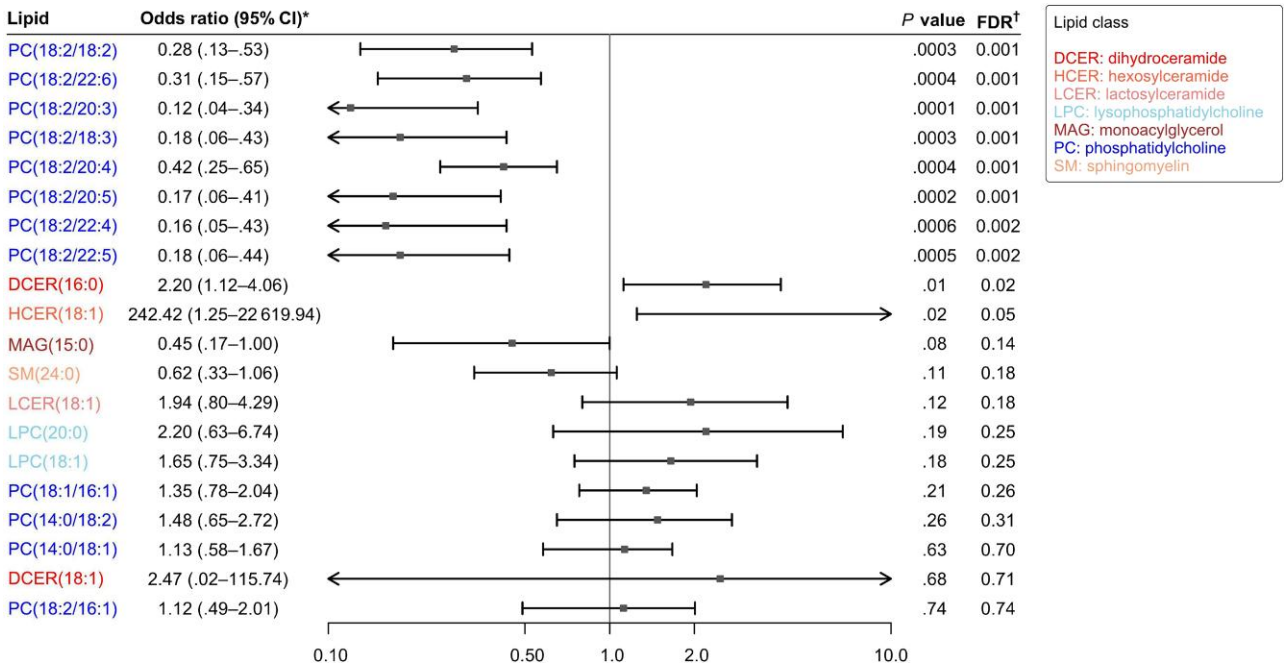

Figure 6. Sensitivity analysis. Adjusted associations of between-virus discriminatory lipid species with severity in infants hospitalized for bronchiolitis. In this sensitivity analysis excluding infants with respiratory syncytial virus (RSV)/rhinovirus coinfection (n = 679), the forest plot shows multivariable-adjusted associations of each of the 20 between-virus discriminatory lipid species with the risk of positive pressure ventilation use. The odds ratio was estimated for a square root of the standard deviation change in the concentration of the corresponding lipid species. Arrows indicate that the 95% confidence interval of the odds ratio exceeds the lower or higher limit of the x-axis. *Estimated by fitting logistic regression model adjusting for 7 potential confounders (age, sex, race/ethnicity, prematurity, corticosteroid use during lifetime, breastfeeding, and RSV infection). †The Benjamini-Hochberg FDR method was used to account for multiple testing. Abbreviations: CI, confidence interval; DCER, dihydroceramide; FDR, false discovery rate; HCER, hexosylceramide; LCER, lactosylceramide; LPC, lysophosphatidylcholine; MAG, monoacylglycerol; PC, phosphatidylcholine; SM, sphingomyelin.

significant. Fifth, as with any observational study, our causal inference may have been biased due to unmeasured confounding factors (eg, host genetics, nutrition). Sixth, while we applied approaches to minimize overfitting (eg, Lasso penalization and cross-validations), external validation in an independent cohort is warranted. Additionally, our findings warrant further validation in a mechanistic study. Finally, although the current cohort consisted of racially/ethnically and geographically diverse US infants, the inference may not be generalized beyond infants hospitalized for bronchiolitis (ie, mild-to-moderate illness). Regardless, our data are directly relevant to 110 000 hospitalized US infants each year [2].

CONCLUSIONS

Based on the nasopharyngeal lipidome data from a multicenter prospective study of infants hospitalized for bronchiolitis, we identified lipids that discriminate between RSV and RV bronchiolitis. Of these, specific lipids at the species (eg, PC [18:2/18:2], DCER [16:0]) and fatty acid (eg, eicosapentaenoic acid) levels were associated with bronchiolitis severity. The lipidome signatures were also related to distinct biological pathways (eg, glycerophospholipid metabolism, sphingolipid metabolism). Our observations suggest that the nasopharyngeal lipidome plays an important role in the pathophysiology of between-virus heterogeneity and the severity of bronchiolitis. Our findings provide clinicians with the opportunity to identify infants with bronchiolitis at risk for higher severity. Furthermore, our data should advance research into the pathobiological mechanisms of bronchiolitis and the development of potential therapy for this population with a large morbidity burden.

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

Author contributions. M. K. carried out the statistical analysis, drafted the initial manuscript, and approved the final manuscript as submitted. Z. Z., R. S., and M. F. carried out the statistical analysis, reviewed and revised the initial manuscript, and approved the final manuscript as submitted. J. M. M. collected the study data, reviewed and revised the initial manuscript, and approved the final manuscript as

submitted. C. A. C. and K. H. conceptualized the study, obtained funding, supervised the statistical analysis, reviewed and revised the initial manuscript, and approved the final manuscript as submitted.

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Potential conflicts of interest. The authors: No reported conflicts.

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