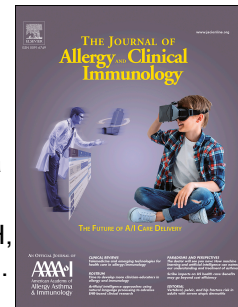


Journal Pre-proof

Metabolome subtyping of severe bronchiolitis in infancy and risk of childhood asthma

Zhaozhong Zhu, ScD, Carlos A. Camargo, Jr., MD, DrPH, Yoshihiko Raita, MD, MPH, MMSc, Michimasa Fujiogi, MD, Liming Liang, PhD, Eugene P. Rhee, MD, Prescott G. Woodruff, MD, MPH, Kohei Hasegawa, MD, MPH



PII: S0091-6749(21)00899-X

DOI: <https://doi.org/10.1016/j.jaci.2021.05.036>

Reference: YMAI 15150

To appear in: *Journal of Allergy and Clinical Immunology*

Received Date: 19 February 2021

Revised Date: 29 April 2021

Accepted Date: 28 May 2021

Please cite this article as: Zhu Z, Camargo Jr. CA, Raita Y, Fujiogi M, Liang L, Rhee EP, Woodruff PG, Hasegawa K, Metabolome subtyping of severe bronchiolitis in infancy and risk of childhood asthma, *Journal of Allergy and Clinical Immunology* (2021), doi: <https://doi.org/10.1016/j.jaci.2021.05.036>.

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

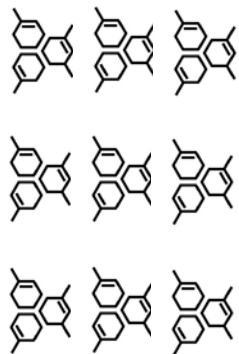
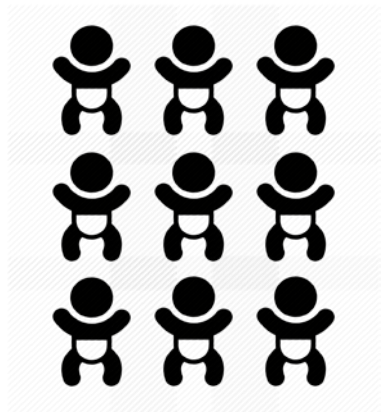
© 2021 Published by Elsevier Inc. on behalf of the American Academy of Allergy, Asthma & Immunology.



Metabolome subtyping of severe bronchiolitis in infancy, and risk of childhood asthma

Metabolome subtyping

Nasopharyngeal metabolome profiling of 918 infants with severe bronchiolitis



Consensus clustering analysis



Derived metabolotypes

A: glycerophosphocholine-high (n=79)

B: amino acid-high, polyunsaturated fatty acid-low (n=72)

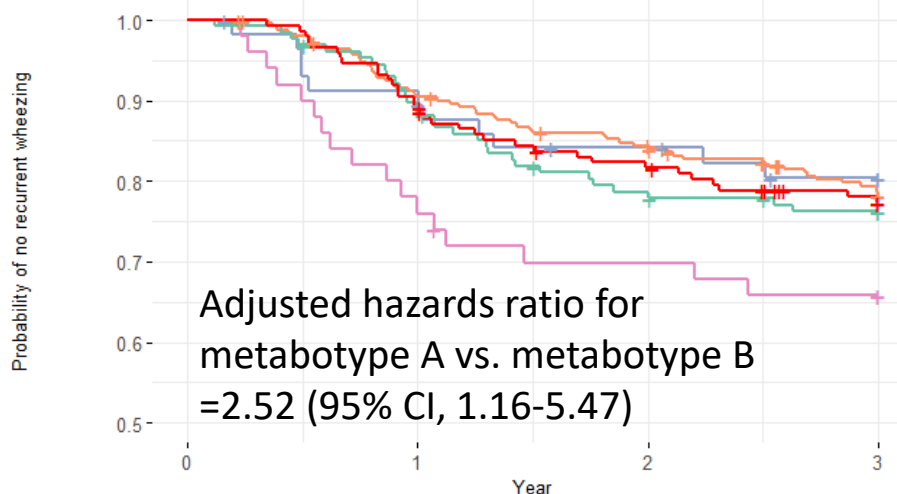
C: amino acid-high, glycerophospholipid-low (n=363)

D: glycerophospholipid-high (n=177)

E: mixed (n=227)

Association of metabolotypes with clinical outcomes

Recurrent wheeze by age 3 years with asthma at age 5 years



Asthma at age 5 years

Adjusted odds ratio for metabolotype A vs. metabolotype B = 2.18 (95% CI, 1.03-4.71)

Metabolome subtyping of severe bronchiolitis in infancy and risk of childhood asthma

Zhaozhong Zhu, ScD¹; Carlos A. Camargo, Jr., MD, DrPH¹; Yoshihiko Raita, MD, MPH, MMSc¹; Michimasa Fujiogi, MD¹; Liming Liang, PhD^{2,3}; Eugene P. Rhee, MD⁴; Prescott G. Woodruff, MD, MPH⁵; and Kohei Hasegawa, MD, MPH¹

¹ Department of Emergency Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA.

² Program in Genetic Epidemiology and Statistical Genetics, Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA, USA.

³ Department of Biostatistics, Harvard T.H. Chan School of Public Health, Boston, MA, USA.

⁴ The Nephrology Division and Endocrine Unit, Department of Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA.

⁵ Division of Pulmonary and Critical Care Medicine, Department of Medicine and CVRI, University of California San Francisco, San Francisco, CA, USA.

Corresponding author:

Dr. Zhaozhong Zhu, Department of Emergency Medicine, Massachusetts General Hospital, 125 Nashua Street, Suite 920, Boston, MA 02114. (E-mail: zzhu5@mgh.harvard.edu; Tel: 617-726-3925. Fax: 617-724-4050)

Conflict of interests statement: Dr. Zhu reports grants from National Institutes of Health during the conduct of the study. Dr. Camargo reports grants from National Institutes of Health during the conduct of the study. Dr. Woodruff reports personal fees from Sanofi, personal fees from

Regeneron, personal fees from Astra Zeneca, personal fees from Glenmark Pharma, personal fees from Theravance, personal fees from NGM Pharma, personal fees from GSK, outside the submitted work. Dr. Hasegawa reports grants from National Institutes of Health during the conduct of the study; grants from Novartis, outside the submitted work. All other authors have indicated that they have no financial relationships relevant to this article to disclose.

Funding: This study was supported by grants from the National Institutes of Health (Bethesda, MD): K01 AI-153558, U01 AI-087881, R01 AI-114552, R01 AI-127507, R01 AI-134940, R01 AI-137091, R01 AI-148338, and UG3/UH3 OD-023253. The content of this manuscript is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. The funding organizations were not involved in the collection, management, or analysis of the data; preparation or approval of the manuscript; or decision to submit the manuscript for publication.

Main manuscript word count: 3281

ABSTRACT (250250 words)

Background: Infants with bronchiolitis are at increased risk for developing asthma. Growing evidence suggests bronchiolitis is a heterogeneous condition.

Objective: To identify biologically-distinct subgroups based on the metabolome signatures (metabotypes) in infants with severe bronchiolitis and to examine their longitudinal relationships with asthma development.

Methods: In a multi-center prospective cohort study of infants (aged <12 months) hospitalized for bronchiolitis, the nasopharyngeal airway metabolome was profiled at hospitalization. Using a clustering approach, we identified mutually-exclusive metabotypes. We also examined their longitudinal association with the risk of developing asthma by age five years.

Results: Of 918 infants hospitalized for bronchiolitis (median age, 3 months), we identified 5 distinct metabotypes—characterized by their nasopharyngeal metabolome profile: A) glycerophosphocholine-high; B) amino acid-high, polyunsaturated fatty acid (PUFA)-low; C) amino acid-high, glycerophospholipid-low; D) glycerophospholipid-high; and E) mixed. Compared with metabotype A infants (who clinically resembled “classic” bronchiolitis), metabotype B infants had a significantly higher risk for developing asthma (23% vs. 41%; $OR_{adjusted}$, 2.22; 95%CI, 1.07-4.69). The pathway analysis showed that metabotype B had enriched amino acid (e.g., methionine, histidine, glutathione) and α -linolenic/linoleic acid metabolism pathways (all $FDR < 5 \times 10^{-14}$). Finally, the transcriptome analysis revealed that metabotype B infants had up-regulated interferon- α and interleukin-6/Janus kinase/signal transducer activator of transcription (IL-6/JAK/STAT3) pathways and down-regulated fatty acid metabolism pathways (both $FDR < 0.05$).

Conclusion: In this multicenter prospective cohort study of infant severe bronchiolitis, the clustering analysis of metabolome data identified biologically-distinct metabolotypes, including one characterized by high inflammatory amino acids and low PUFAs that is at significantly increased risk for developing asthma.

Capsule Summary: Among infant with severe bronchiolitis, clustering analysis of the nasopharyngeal airway metabolome identified biologically distinct metabolotypes, including one characterized by high inflammatory amino acids and low PUFAs that is at increased risk for developing asthma.

Keywords: severe bronchiolitis, heterogeneity, subtype, asthma, metabolome, inflammatory amino acids, polyunsaturated fatty acids, transcriptome.

Key Messages:

- Growing evidence suggests that bronchiolitis is a heterogeneous syndrome.
- Using data of the nasopharyngeal airway metabolome from a multicenter cohort of infants with severe bronchiolitis, unsupervised clustering analysis identified five biologically distinct “metabotypes”.
- These metabotypes were also clinically meaningful. For example, the metabotype characterized by high abundance of inflammatory amino acids and low abundance of PUFAs had the highest risk for developing asthma.

Abbreviations:

92	
93	DNA: Deoxyribonucleic acid
94	FDR: False discovery rate
95	GP: Glycerophospholipid
96	GPC: Glycerophosphocholine
97	GR- α : Glucocorticoid receptor-alpha
98	HODE: Hydroxyoctadecadienoic acid
99	IFN- α : Interferon-alpha
100	IL-4: Interleukin-4
101	IL-6: Interleukin-6
102	IMPaLA: Integrated Molecular Pathway Level Analysis
103	IQR: Interquartile range
104	JAK: Janus kinase
105	MARC-35: The 35th Multicenter Airway Research Collaboration
106	NF- κ B: Nuclear factor kappa-light-chain-enhancer of activated B cells
107	OR: Odds ratio
108	PUFA: Polyunsaturated fatty acid
109	RSV: Respiratory syncytial virus
110	SAM: S-adenosylmethionine
111	STAT3: Signal transducers and activators of transcription-3
112	

INTRODUCTION

Bronchiolitis is the most common lower respiratory tract infection among infants, accounting for ~110,000 hospitalizations (“severe bronchiolitis”) in the U.S. annually¹. In addition to the acute morbidity, its chronic morbidity burden is also substantial—e.g., of these infants with severe bronchiolitis, approximately 30% develop recurrent wheeze and asthma in childhood²⁻⁷.

Although bronchiolitis was traditionally considered a single disease entity with similar pathobiology⁷, there is growing evidence suggesting that bronchiolitis is a heterogeneous condition⁸⁻¹⁰. For example, recent epidemiological studies have identified⁸ and validated⁹ the presence of multiple phenotypes that have differential risks for developing recurrent wheeze. However, these phenotypes were identified only based on major clinical characteristics. The pathobiological mechanisms underlying the heterogeneity remains unclear. This insufficient understanding of pathobiology of bronchiolitis during infancy—a critical period of airway development—has hindered efforts to develop targeted bronchiolitis treatment and asthma prevention strategies. Metabolomics is well-suited to address this knowledge gap by broadly profiling small molecules (metabolites), which are a function of an individual’s genetic makeup and environmental factors¹¹. Regardless, no study has yet investigated the biologically-distinct subgroups of bronchiolitis based on metabolome signatures (“metabotypes”¹²).

In this context, we aimed to identify distinct nasopharyngeal airway metabotypes by applying an unsupervised clustering approach to the metabolome data from a multicenter prospective study of infants with severe bronchiolitis. We also sought to investigate the longitudinal relationships of the metabotypes with recurrent wheeze and childhood asthma. This study provides further evidence that “bronchiolitis” represents several diseases with distinct biological mechanisms and differential risks of chronic airway morbidities in childhood.

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^{13, 14}. MARC-35 is coordinated by the Emergency Medicine Network (EMNet, www.emnet-usa.org), an international research collaboration with 247 participating hospitals. Details of the study design, setting, participants, data collection, testing, and statistical analysis may be found in the **Supplementary Methods**. Briefly, MARC-35 investigators at 17 sites, across 14 U.S. states, enrolled infants (aged <1 year) who were hospitalized with an attending physician diagnosis of bronchiolitis during three bronchiolitis seasons (November 1 to April 30) from 2011 to 2014 (**Table E1**). The diagnosis of bronchiolitis was made according to the American Academy of Pediatrics bronchiolitis guidelines, defined as acute respiratory illness with a combination of rhinitis, cough, tachypnoea, wheezing, crackles, or retraction¹⁵. We excluded infants with a preexisting heart and lung disease, immunodeficiency, immunosuppression, or gestational age of <32 weeks. All patients were treated at the discretion of the treating physicians. The institutional review board at each participating hospital approved the study with a written informed consent obtained from parent or guardian.

Data Collection

Clinical data (patients' demographic characteristics, and family, environmental, and medical history, and details of the acute illness) were collected via structured interview and chart reviews using a standardized protocol^{13, 14}. After the index hospitalization for bronchiolitis, trained

interviewers began interviewing parents/legal guardians by telephone at 6-month intervals in addition to medical record review by physicians. All data were reviewed at the Emergency Medicine Network Coordinating Center at Massachusetts General Hospital (Boston, MA, USA)¹⁶. Nasopharyngeal specimens were collected within 24 hours of hospitalization using a standardized protocol¹⁴. The details of the data collection and measurement methods are described in the **Supplementary Methods**.

Nasopharyngeal Metabolome Profiling

The details of metabolome profiling are described in **Supplementary Methods**. Briefly, the metabolome profiling was conducted by Metabolon (Durham, NC, USA) using liquid chromatography–tandem mass spectrometry. All specimens were blinded to the laboratory and processed in a random order. Instrument variability was 4%, as determined by calculating the median relative standard deviation for the internal standards. We used the metabolome data of 278 nasopharyngeal metabolites from 76 sub-pathways within seven super-pathways.

Nasopharyngeal Transcriptome Profiling

The details of RNA extraction, RNAseq, quality control, and transcriptome profiling are described in **Supplementary Methods**. Briefly, after total RNA extraction, DNase treatment, and rRNA reduction, we performed RNAseq with Illumina NovaSeq6000 (Illumina, San Diego, CA, USA). All RNAseq samples had high sequence coverage after quality control. The transcript abundances were estimated with *Salmon*¹⁷ using the human genome (hg38) and the mapping-based mode.

Outcome Measures

The primary outcome was asthma at age five years. The secondary outcome was the development of recurrent wheeze by age three years stratified by asthma status, to account for the heterogeneity of recurrent wheeze according to prior research¹⁶. The definition of asthma was based on a commonly-used epidemiologic definition of asthma^{16, 18}—physician-diagnosis of asthma by age five years, plus either asthma medication use (e.g., albuterol inhaler, inhaled corticosteroids, montelukast) or asthma-related symptoms in the preceding year. The definition of recurrent wheeze was based on the U.S. asthma guidelines—defined as having at least two corticosteroid-requiring exacerbations in six months or at least four wheezing episodes in one year that last at least one day and affect sleep¹⁹.

Statistical Analysis

In the current study, our objectives were to identify biologically-distinct severe bronchiolitis subgroups based on the metabolome signatures (clustering/description) and to relate them to the outcomes (association). The analytic workflow is summarized in **Figure 1**.

Data Normalization and Clustering to Identify Metabotypes

We first adjusted the metabolome data for potential batch effect by using empirical Bayes models (ComBat method)²⁰. Then, we normalized the data using total sum scaling method to account for potentially differential dilutions. To identify individuals with a similar metabolome

signature (metabotype), we applied consensus clustering with partitioning around medoids algorithm. We determined the optimal number of clusters by a combination of the average silhouette score, consensus matrix heatmap (**Figure E1**), and biological plausibility^{11, 21, 22}.

Association of Metabotypes with Clinical Outcomes

To investigate the longitudinal association of metabotypes with asthma status at age five years (binary outcome), we fitted unadjusted and adjusted logistic regression models. To investigate the relation of metabotypes with the development of recurrent wheeze (time-to-event) outcomes, we constructed Cox proportional hazards models. Patients were censored at time of the recurrent wheeze development, loss to follow-up, or age 36-months, whichever occurred first. For both models, we adjusted for potential confounders (age, sex, parent history of asthma, number of previous breathing problems, respiratory syncytial virus infection and rhinovirus infection) based on *a priori* knowledge^{13, 14, 16, 23, 24}.

To examine the robustness of inferences, we conducted a series of sensitivity analyses. First, we repeated the outcome regression model by restricting the study sample to those without previous breathing problems. Second, we also examined the robustness of the metabotype-outcome associations by repeating the analysis with a different number of metabotypes. We also computed E-values to examine the robustness of the causal inference by using the R *Evalue* package²⁵. The E-value indicates the minimum strength of association that an unmeasured confounder(s) would need to have with both the exposure and outcome to fully explain away a specific association, conditional on the measured covariates²⁵. For example, an E-value of 2.5 means that unmeasured confounders that were associated with both the metabotypes and

outcome by an odds ratio of 2.5 could explain away the estimate, but weaker confounding could not.

Pathway Analyses

To identify biologically-meaningful pathways, we conducted a metabolite-set enrichment analysis by comparing the reference metabotype with each of the other metabotypes using MetaboAnalyst 4.0²⁶ and the Small Molecule Pathway Database²⁷ library as the reference. To investigate whether gene expression for specific biological pathways are enriched, we conducted a functional class scoring analysis using R *fgsea* package²⁸. To detect differentially-expressed pathways by integrating the metabolome and transcriptome data, we performed a Wilcoxon pathway enrichment analysis using the Integrated Molecular Pathway Level Analysis (IMPaLA) method²⁹.

All statistical analyses were performed using R version 4.0.2. All P-values are two-tailed, with $P < 0.05$ considered statistically significant. We further calculated the false discovery rate (FDR) that allows for the interpretation of statistical significance in the context of multiple hypothesis testing. The FDR value was controlled using Benjamini-Hochberg method³⁰.

RESULTS

Of 921 infants with severe bronchiolitis enrolled into this longitudinal cohort, the current study included 918 infants (>99%) who had information for the nasopharyngeal airway metabolome. Among the analytic cohort, the median age was 3 (IQR, 2-6) months, 40% were female, and 44% were non-Hispanic white. Subsequently, 32% developed recurrent wheeze by age 3 years and 27% developed asthma by age 5 years.

Identification of Metabotypes and Their Characteristics

By applying the consensus clustering approach on the nasopharyngeal airway metabolome data, we identified five distinct metabotypes (**Figure E1**): A) glycerophosphocholine (GPC)-high (8.6%); B) amino acid-high, polyunsaturated fatty acid (PUFA)-low (7.8%); C) amino acid-high, glycerophospholipid (GP)-low (39.5%); D) GP-high (19.3%); and E) mixed (24.7%) (**Figure 2**).

Across these metabotypes, several clinical characteristics (e.g., age, number of previous breathing problems, and infecting virus) were significantly different ($P < 0.05$; **Table 1**).

Descriptively, infants with a metabotype A were characterized by “classic” clinical presentation of bronchiolitis (e.g., young age and a low proportion of previous breathing problems, lifetime corticosteroid use, and parental asthma and eczema) and high abundance of GPC (**Table 1**, **Figure 2**). Metabotype B was characterized by a high proportion of lifetime corticosteroid use and parental asthma, a high abundance of amino acids and hydroxyoctadecadienoic acid (HODE) metabolites, and low abundance of PUFAs (**Figures 2-3**, **Figure E2**). Metabotype C was characterized by a high proportion of rhinovirus-C infection, high abundance of amino acids, and low abundance of GP (**Figure 2**, **Figure E3**). Metabotype D was characterized by a high

proportion of parent history of eczema and IgE sensitization, and high abundance of GP (**Figure 2, Figure E4**). Lastly, the metabotype E was characterized by a high proportion of intensive treatment use, and a mixed metabolite profile (**Figure 2, Figure E5**).

Association of Metabotypes with Chronic Respiratory Outcomes

To investigate longitudinal association of the metabotypes with clinical outcomes, we compared the outcome risks between infants with a metabotype A (clinically “classic” bronchiolitis) and those with each of the other metabotypes (**Table 2**). Compared with metabotype A infants, metabotype B infants had a significantly higher risk of developing asthma (23.4% vs. 40.6% [binary outcome]; OR, 2.24; 95%CI, 1.10-4.63; $P=0.03$). This association remained significant after adjusting for the confounders (OR_{adj}, 2.18; 95%CI, 1.03–4.71; $P=0.04$; E-value=2.32). By contrast, those with a metabotype C, D, or E did not have significantly differential risks.

Likewise, compared with metabotype A infants, metabotype B infants had a significantly higher rate of recurrent wheeze that resulted in asthma (19.0% vs. 34.0%; HR_{adj}, 2.52; 95%CI, 1.16–5.47; $P=0.02$; E-value=3.18; **Table E2**). By contrast, for the rate of recurrent wheeze that did not result in asthma, there were no significant differences between the metabotypes A and B (20.3% vs. 19.5%; HR_{adj}, 0.88; 95%CI, 0.36–2.17; $P=0.78$).

Distinct Biological Characteristics of Metabotypes

Based on principal component analysis, the global metabolome profiles were distinct between the reference (metabotype A) and highest asthma risk (metabotype B) groups (**Figure 3A**). To

better understand their functional difference, we conducted pathway analyses using the nasopharyngeal metabolome and transcriptome data. In the metabolite-set enrichment analysis, the differentially-enriched (FDR<0.05) pathways for metabotype B infants included amino acid (e.g., methionine, betaine, histidine, glutathione) and fatty acid (α -linolenic acid and linoleic acid) metabolism pathways (all FDR< 5×10^{-14} ; **Figure 3B**). Additionally, in the gene-set enrichment analysis of the transcriptome data, infants with a metabotype B had differentially-enriched pathways, such as up-regulated interferon- α (IFN- α) and interleukin-6/Janus kinase/signal transducers and activators of transcription-3 (IL-6/JAK/STAT3) pathways as well as down-regulated fatty acid metabolism pathway. The integrated analysis of both metabolome and transcriptome data also identified differentially-enriched pathways in metabotype B, such as metabolism of lipids (FDR= 5×10^{-27} ; **Figure E2**). For the comparisons of metabotype A vs. the other metabolotypes (C, D, and E), the detailed functional differences are summarized in **Figures E3-E5**.

Sensitivity Analysis

In the subgroup analysis restricting the study sample to the infants without previous breathing problems, compared with metabotype A, metabotype B had a non-significantly higher risk of developing asthma (OR_{adj}, 1.76; 95%CI, 0.75-4.16; $P=0.19$; **Table E3**). Second, we also examined different numbers of metabolotypes. The alluvial plot (**Figure E6**) showed a consistency of the original 5 metabolotypes (A-E) across the different numbers of metabolotypes. With the use of 6 metabolotypes, for example, the metabotype 1 had 98.6% concordance with the original metabotype A and the metabotype 2 had 96.5% concordance with the original metabotype B (**Table E4**). Similar to the primary analysis, the metabolite-set enrichment analysis demonstrated

that, compared to metabotype 1 (which is concordant with metabotype A), metabotype 2 (which is concordant with metabotype B) infants also had differentially-enriched metabolism of amino acids and PUFAs (**Figure E7**). In addition, compared to metabotype 1, metabotype 2 infants had a non-significantly but consistently higher risk of asthma (23.9% vs. 41.8%; OR_{adj}, 2.19; 95%CI, 0.97-5.01; $P=0.06$; **Table E5**) and significantly higher rate of recurrent wheeze that resulted in asthma (20.4% vs. 37.8%; HR_{adj}, 2.55; 95%CI, 1.13-5.76; $P=0.02$; E-value=3.21; **Table E6**).

DISCUSSION

By applying a clustering approach to the nasopharyngeal airway metabolome data from a multicenter prospective cohort study of 918 infants with severe bronchiolitis, we identified five biologically-distinct metabotypes. In particular, compared to infants with metabotype A (who resemble “classic” bronchiolitis), those with a metabotype B (characterized by a high proportion of parental asthma, high abundance of amino acids, and low abundance of PUFAs) had a significantly higher risk for developing recurrent wheeze and asthma. To the best of our knowledge, this is the first study that has identified biologically-distinct airway metabotypes in infants with severe bronchiolitis and demonstrated their longitudinal relations with the risk of chronic respiratory outcomes.

Bronchiolitis has been traditionally viewed as a single disease condition with similar mechanisms. Indeed, the current U.S. guidelines for diagnosis and management of bronchiolitis rely on this assumption^{15, 31}. Yet, concordant with the results of the present study, a growing body of evidence supports the complexity of bronchiolitis, as reflected by heterogeneous clinical characteristics^{8, 9}, transcriptome³²⁻³⁴, microRNA³³, cytokine^{35, 36}, and upper airway

microbiome³⁷⁻³⁹. Downstream of these transcriptional and translational processes, the metabolome—the global collection of small molecules (e.g., sugars, amino acids, lipids)—provides “proximal reports” of a system’s functional state¹¹. Recent studies have suggested the relationship of the metabolome with disease severity among infants with severe bronchiolitis^{13, 14, 24, 40-42}. For example, among 140 infants with severe bronchiolitis in the U.S., *serum* metabolome profiles (e.g., glutathione) was associated with a higher severity of illness²⁴. Furthermore, in a single-center study of 52 infants with bronchiolitis in Italy, urine amino acids (e.g., methionine) were associated with a higher risk of recurrent wheeze during the 2-year follow-up period⁴³. These earlier reports have supported the heterogeneity of bronchiolitis and discovered potential mechanisms that underlie the bronchiolitis-chronic respiratory outcomes link. The current study—with a sample size many times larger than any other prior study—corroborates earlier findings and extends them by identifying biologically-distinct severe bronchiolitis metabotypes that have differential risks of developing recurrent wheeze and asthma.

There are several potential mechanisms linking severe bronchiolitis—in particular metabotype B (amino acid-high, PUFA-low)—with subsequent respiratory outcomes. First, studies have shown inflammatory amino acids (such as betaine, methionine, and histidine) involved in DNA methylation in the asthma pathobiology. Specifically, betaine provides the methyl groups for synthesizing methionine, which further generates S-adenosylmethionine (SAM). SAM is a key methyl donor, which contributes to DNA methylation⁴⁴. Indeed, DNA methylation is vital in the activation of the immune response in patients with asthma. For example, a previous study showed an increased DNA methylation level at the IL-4 promoter in cells from patients with asthma after house dust mite stimulation⁴⁵. Second, the role of PUFAs (e.g., ω -3 PUFAs) as

immune modulators has been demonstrated in relation to asthma⁴⁶. For example, α -linolenic acid (an ω -3 PUFA) is converted to eicosapentaenoic acid, which inhibits arachidonic acid thereby suppressing production of eicosanoid inflammatory mediators. Eicosapentaenoic acid also functions as a precursor for pro-resolving mediators (such as resolvins, protectins, and maresins)⁴⁷. These mediators have important anti-inflammatory properties with protective effects on asthma⁴⁶. Observational studies and randomized controlled trials have also shown a protective effect of PUFAs on development of asthma. For example, the supplement of ω -3 PUFAs during pregnancy are associated with a reduced risk of wheezing illness⁴⁸ and asthma^{48, 49} in the offspring. Similarly, lower ω -3 PUFA intake is associated with a higher incidence of asthma in children⁵⁰. Third, in the current study, we also found a high level of 9- and 13-HODE in the metabotype B. Emerging evidence suggests that HODE plays a role in the pathobiology of asthma⁵¹. For example, Panda *et al.* reported, in allergic asthma mouse and human bronchial epithelial cell models, that 13-HODE increases pNF- κ B levels and reduces GR- α transcripts, thereby inducing corticosteroid resistance⁵¹. Lastly, our transcriptome analysis also found that metabotype B had up-regulated IFN- α and IL-6/JAK/STAT3 pathways, which are involved in asthma pathobiology⁵². Notwithstanding the complexity of these mechanisms, we believe that the identification of severe bronchiolitis subtypes based on metabolome data (or metabotypes) and their longitudinal relations with chronic respiratory outcomes are important. Our findings, in conjunction with the existent literature, should advance research into the development of subtype-specific strategies for bronchiolitis treatment and asthma prevention.

Our study has several potential limitations. First, bronchiolitis involves inflammation of the lower airways, in addition to the upper airways. While our data relied on nasopharyngeal

specimens, research has shown that upper airway sampling provides reliable representation of inflammatory profiles in the lower airways⁵³. Furthermore, the use of upper airway specimens is preferable as bronchoscopy or other methods of lower airway sampling would be too invasive in young infants with bronchiolitis. Second, the nasopharyngeal specimens were obtained at a single timepoint. While longitudinal molecular data are also instrumental, the study objective was to identify metabotypes of severe bronchiolitis. Even with single-time point data, we successfully identified biologically-distinct metabotypes that are longitudinally associated with subsequent respiratory outcomes. Third, the current analysis was designed not to examine asthma phenotypes but to use the epidemiological definition of asthma at age five years^{16, 18}. The cohort is being followed up not only to minimize misclassification due to asthma development at a later age, but also to perform comprehensive phenotyping and endotyping of children who develop asthma. Fourth, our causal inference might have been confounded by unmeasured factors (e.g., host genetics, nutrition, inborn errors of metabolism). However, the relatively large E-values implied that confounders would have been strong to explain away our inference. Fifth, the current study used untargeted metabolomics procedure and it is important to conduct independent replication using targeted metabolomics technique to improve the profiling resolution and replicate our findings. Lastly, despite the study cohort consisting of racially/ethnically- and geographically-diverse infants, our inferences may not be generalizable to those with mild-to-moderate bronchiolitis, and hence warrant external validation. Nonetheless, our data remain directly relevant for the 110,000 infants hospitalized yearly in the U.S.¹, a population with substantial morbidity burden.

In conclusion, by applying an unsupervised clustering approach to the nasopharyngeal airway metabolome data from a multicenter prospective cohort study of 918 infants with severe bronchiolitis, we identified five biologically-distinct and clinically-meaningful metabotypes. Specifically, the metabotype characterized by a high abundance of inflammatory amino acids and low abundance of PUFAs had the highest risk for developing asthma. Our data lend significant support to the concept that “bronchiolitis” is a heterogenous syndrome with different biological mechanisms. These observations should facilitate further investigations into the development of metabotype-targeted strategies for bronchiolitis treatment and asthma prevention. Furthermore, future examinations of the relationship between bronchiolitis metabotypes and asthma endotypes (e.g., based on the genome, transcriptome, and metabolome) will also provide a new avenue for the development of endotypes-specific prevention strategies for asthma.

Acknowledgements: We thank the MARC-35 study hospitals and research personnel for their ongoing dedication to bronchiolitis and asthma research (**Table E1**), and Ashley F. Sullivan, MS, MPH and Janice A. Espinola, MPH (Massachusetts General Hospital, Boston, MA) for their many contributions to the MARC-35 study. We also thank Alkis Togias, MD, at the National Institutes of Health (Bethesda, MD) for helpful comments about the study results.

Author contributions: Z.Z. carried out the main statistical analysis, drafted the initial manuscript, and approved the final manuscript as submitted. C.A.C. conceptualized and designed the study, obtained funding, collected the data, supervised the conduct of study and the analysis, critically reviewed and revised the initial manuscript, and approved the final manuscript as submitted. Y.R. and M.F. assisted statistical analysis, reviewed the manuscript, and approved the final manuscript. L.L. and P.W. conceptualized the study and reviewed the manuscript, and approved the final manuscript. E.P.R obtained funding, reviewed the manuscript, and approved the final manuscript. K.H. conceptualized the study, obtained funding, supervised the statistical analysis, reviewed and revised the initial manuscript, and approved the final manuscript as submitted.

REFERENCES

1. Fujiogi M, Goto T, Yasunaga H, Fujishiro J, Mansbach JM, Camargo CA, Jr., et al. Trends in bronchiolitis hospitalizations in the united states: 2000-2016. *Pediatrics* 2019; 144.
2. Kotaniemi-Syrjanen A, Vainionpaa R, Reijonen TM, Waris M, Korhonen K, Korppi M. Rhinovirus-induced wheezing in infancy--the first sign of childhood asthma? *J Allergy Clin Immunol* 2003; 111:66-71.
3. Carroll KN, Wu P, Gebretsadik T, Griffin MR, Dupont WD, Mitchel EF, et al. The severity-dependent relationship of infant bronchiolitis on the risk and morbidity of early childhood asthma. *J Allergy Clin Immunol* 2009; 123:1055-61, 61 e1.
4. Sigurs N, Aljassim F, Kjellman B, Robinson PD, Sigurbergsson F, Bjarnason R, et al. Asthma and allergy patterns over 18 years after severe rsv bronchiolitis in the first year of life. *Thorax* 2010; 65:1045-52.
5. Bacharier LB, Cohen R, Schweiger T, Yin-Declue H, Christie C, Zheng J, et al. Determinants of asthma after severe respiratory syncytial virus bronchiolitis. *J Allergy Clin Immunol* 2012; 130:91-100 e3.
6. Regnier SA, Huels J. Association between respiratory syncytial virus hospitalizations in infants and respiratory sequelae: Systematic review and meta-analysis. *Pediatr Infect Dis J* 2013; 32:820-6.
7. Hasegawa K, Dumas O, Hartert TV, Camargo CA, Jr. Advancing our understanding of infant bronchiolitis through phenotyping and endotyping: Clinical and molecular approaches. *Expert Rev Respir Med* 2016; 10:891-9.
8. Dumas O, Mansbach JM, Jartti T, Hasegawa K, Sullivan AF, Piedra PA, et al. A clustering approach to identify severe bronchiolitis profiles in children. *Thorax* 2016; 71:712-8.
9. Dumas O, Hasegawa K, Mansbach JM, Sullivan AF, Piedra PA, Camargo CA, Jr. Severe bronchiolitis profiles and risk of recurrent wheeze by age 3 years. *J Allergy Clin Immunol* 2019; 143:1371-9 e7.

10. Raita Y, Camargo CA, Jr., Bochkov YA, Celedon JC, Gern JE, Mansbach JM, et al. Integrated-omics endotyping of infants with rhinovirus bronchiolitis and risk of childhood asthma. *J Allergy Clin Immunol* 2020.
11. Zhu Z, Camargo CA, Jr., Hasegawa K. Metabolomics in the prevention and management of asthma. *Expert Rev Respir Med* 2019; 13:1135-8.
12. Reinke SN, Gallart-Ayala H, Gomez C, Checa A, Fauland A, Naz S, et al. Metabolomics analysis identifies different metabotypes of asthma severity. *Eur Respir J* 2017; 49.
13. Stewart CJ, Mansbach JM, Ajami NJ, Petrosino JF, Zhu Z, Liang L, et al. Serum metabolome is associated with the nasopharyngeal microbiota and disease severity among infants with bronchiolitis. *J Infect Dis* 2019; 219:2005-14.
14. Stewart CJ, Mansbach JM, Wong MC, Ajami NJ, Petrosino JF, Camargo CA, Jr., et al. Associations of nasopharyngeal metabolome and microbiome with severity among infants with bronchiolitis. A multiomic analysis. *Am J Respir Crit Care Med* 2017; 196:882-91.
15. Ralston SL, Lieberthal AS, Meissner HC, Alverson BK, Baley JE, Gadomski AM, et al. Clinical practice guideline: The diagnosis, management, and prevention of bronchiolitis. *Pediatrics* 2014; 134:e1474-502.
16. Hasegawa K, Mansbach JM, Bochkov YA, Gern JE, Piedra PA, Bauer CS, et al. Association of rhinovirus c bronchiolitis and immunoglobulin e sensitization during infancy with development of recurrent wheeze. *JAMA Pediatr* 2019; 173:544-52.
17. Patro R, Duggal G, Love MI, Irizarry RA, Kingsford C. Salmon provides fast and bias-aware quantification of transcript expression. *Nat Methods* 2017; 14:417-9.
18. Camargo CA, Jr., Ingham T, Wickens K, Thadhani R, Silvers KM, Epton MJ, et al. Cord-blood 25-hydroxyvitamin d levels and risk of respiratory infection, wheezing, and asthma. *Pediatrics* 2011; 127:e180-7.

19. National Asthma Education and Prevention Program. Expert panel report 3 (epr-3): Guidelines for the diagnosis and management of asthma-summary report 2007. *J Allergy Clin Immunol* 2007; 120:S94-138.
20. Leek JT, Johnson WE, Parker HS, Jaffe AE, Storey JD. The sva package for removing batch effects and other unwanted variation in high-throughput experiments. *Bioinformatics* 2012; 28:882-3.
21. Comhair SA, McDunn J, Bennett C, Fettig J, Erzurum SC, Kalhan SC. Metabolomic endotype of asthma. *J Immunol* 2015; 195:643-50.
22. Kelly RS, Dahlin A, McGeachie MJ, Qiu W, Sordillo J, Wan ES, et al. Asthma metabolomics and the potential for integrative omics in research and the clinic. *Chest* 2017; 151:262-77.
23. Abreo A, Gebretsadik T, Stone CA, Hartert TV. The impact of modifiable risk factor reduction on childhood asthma development. *Clin Transl Med* 2018; 7:15.
24. Hasegawa K, Stewart CJ, Celedon JC, Mansbach JM, Tierney C, Camargo CA, Jr. Serum 25-hydroxyvitamin d, metabolome, and bronchiolitis severity among infants-a multicenter cohort study. *Pediatr Allergy Immunol* 2018; 29:441-5.
25. VanderWeele TJ, Ding P. Sensitivity analysis in observational research: Introducing the e-value. *Ann Intern Med* 2017; 167:268-74.
26. Chong J, Soufan O, Li C, Caraus I, Li S, Bourque G, et al. Metaboanalyst 4.0: Towards more transparent and integrative metabolomics analysis. *Nucleic Acids Research* 2018; 46:W486-W94.
27. Frolkis A, Knox C, Lim E, Jewison T, Law V, Hau DD, et al. Smpdb: The small molecule pathway database. *Nucleic Acids Res* 2010; 38:D480-7.
28. Korotkevich G, Sukhov V, Sergushichev A. Fast gene set enrichment analysis. *bioRxiv* 2019:060012.
29. Kamburov A, Cavill R, Ebbels TMD, Herwig R, Keun HC. Integrated pathway-level analysis of transcriptomics and metabolomics data with impala. *Bioinformatics* 2011; 27:2917-8.

30. Benjamini Y, Hochberg Y. Controlling the false discovery rate: A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society. Series B (Methodological)* 1995; 57:289-300.
31. National Institute for Health and Care Excellence. Bronchiolitis in children: Diagnosis and management. 2015.
32. de Steenhuijsen Piter WA, Heinonen S, Hasrat R, Bunsow E, Smith B, Suarez-Arrabal MC, et al. Nasopharyngeal microbiota, host transcriptome, and disease severity in children with respiratory syncytial virus infection. *Am J Respir Crit Care Med* 2016; 194:1104-15.
33. Hasegawa K, Perez-Losada M, Hoptay CE, Epstein S, Mansbach JM, Teach SJ, et al. Rsv vs. Rhinovirus bronchiolitis: Difference in nasal airway microRNA profiles and nfkb signaling. *Pediatr Res* 2018; 83:606-14.
34. Fujiogi M, Camargo CA, Jr., Bernot JP, Freishtat RJ, Harmon B, Mansbach JM, et al. In infants with severe bronchiolitis: Dual-transcriptomic profiling of nasopharyngeal microbiome and host response. *Pediatr Res* 2020; 88:144-6.
35. Turi KN, Shankar J, Anderson LJ, Rajan D, Gaston K, Gebretsadik T, et al. Infant viral respiratory infection nasal immune-response patterns and their association with subsequent childhood recurrent wheeze. *Am J Respir Crit Care Med* 2018; 198:1064-73.
36. Hasegawa K, Hoptay CE, Harmon B, Celedon JC, Mansbach JM, Piedra PA, et al. Association of type 2 cytokines in severe rhinovirus bronchiolitis during infancy with risk of developing asthma: A multicenter prospective study. *Allergy* 2019; 74:1374-7.
37. Hasegawa K, Mansbach JM, Ajami NJ, Espinola JA, Henke DM, Petrosino JF, et al. Association of nasopharyngeal microbiota profiles with bronchiolitis severity in infants hospitalised for bronchiolitis. *Eur Respir J* 2016; 48:1329-39.

38. Mansbach JM, Hasegawa K, Henke DM, Ajami NJ, Petrosino JF, Shaw CA, et al. Respiratory syncytial virus and rhinovirus severe bronchiolitis are associated with distinct nasopharyngeal microbiota. *J Allergy Clin Immunol* 2016; 137:1909-13 e4.
39. Rosas-Salazar C, Shilts MH, Tovchigrechko A, Schobel S, Chappell JD, Larkin EK, et al. Differences in the nasopharyngeal microbiome during acute respiratory tract infection with human rhinovirus and respiratory syncytial virus in infancy. *J Infect Dis* 2016; 214:1924-8.
40. Hasegawa K, Stewart CJ, Celedon JC, Mansbach JM, Tierney C, Camargo CA, Jr. Circulating 25-hydroxyvitamin d, nasopharyngeal airway metabolome, and bronchiolitis severity. *Allergy* 2018; 73:1135-40.
41. Fujiogi M, Camargo CA, Jr., Raita Y, Bochkov YA, Gern JE, Mansbach JM, et al. Association of rhinovirus species with nasopharyngeal metabolome in bronchiolitis infants: A multicenter study. *Allergy* 2020; 75:2379-83.
42. Fujiogi M, Camargo CA, Jr., Raita Y, Zhu Z, Celedon JC, Mansbach JM, et al. Integrated associations of nasopharyngeal and serum metabolome with bronchiolitis severity and asthma: A multicenter prospective cohort study. *Pediatr Allergy Immunol* 2021.
43. Barlotta A, Pirillo P, Stocchero M, Donato F, Giordano G, Bont L, et al. Metabolomic profiling of infants with recurrent wheezing after bronchiolitis. *J Infect Dis* 2019; 219:1216-23.
44. Sharma S, Litonjua A. Asthma, allergy, and responses to methyl donor supplements and nutrients. *J Allergy Clin Immunol* 2014; 133:1246-54.
45. Wang CM, Chang CB, Chan MW, Wen ZH, Wu SF. Dust mite allergen-specific immunotherapy increases il4 DNA methylation and induces der p-specific t cell tolerance in children with allergic asthma. *Cell Mol Immunol* 2018; 15:963-72.
46. Wendell SG, Baffi C, Holguin F. Fatty acids, inflammation, and asthma. *J Allergy Clin Immunol* 2014; 133:1255-64.

47. Duvall MG, Levy BD. Dha- and epa-derived resolvins, protectins, and maresins in airway inflammation. *Eur J Pharmacol* 2016; 785:144-55.
48. Bisgaard H, Stokholm J, Chawes BL, Vissing NH, Bjarnadottir E, Schoos AM, et al. Fish oil-derived fatty acids in pregnancy and wheeze and asthma in offspring. *N Engl J Med* 2016; 375:2530-9.
49. Lumia M, Luukkainen P, Tapanainen H, Kaila M, Erkkola M, Uusitalo L, et al. Dietary fatty acid composition during pregnancy and the risk of asthma in the offspring. *Pediatr Allergy Immunol* 2011; 22:827-35.
50. Lee-Sarwar K, Kelly RS, Lasky-Su J, Kachroo P, Zeiger RS, O'Connor GT, et al. Dietary and plasma polyunsaturated fatty acids are inversely associated with asthma and atopy in early childhood. *J Allergy Clin Immunol Pract* 2019; 7:529-38 e8.
51. Panda L, Gheware A, Rehman R, Yadav MK, Jayaraj BS, Madhunapantula SV, et al. Linoleic acid metabolite leads to steroid resistant asthma features partially through nf-kappab. *Sci Rep* 2017; 7:9565.
52. Rincon M, Irvin CG. Role of il-6 in asthma and other inflammatory pulmonary diseases. *Int J Biol Sci* 2012; 8:1281-90.
53. Poole A, Urbanek C, Eng C, Schageman J, Jacobson S, O'Connor BP, et al. Dissecting childhood asthma with nasal transcriptomics distinguishes subphenotypes of disease. *J Allergy Clin Immunol* 2014; 133:670-8 e12.

FIGURE LEGENDS**Figure 1. Analytic workflow**

The analytical cohort consists of 918 infants hospitalized for bronchiolitis (severe bronchiolitis) in a multicenter prospective cohort study—the 35th Multicenter Airway Research Collaboration (MARC-35). The nasopharyngeal metabolome data were adjusted for the potential batch effect and normalized by total sum scaling before clustering analysis. To identify metabotypes, the partitioning around medoids algorithm was used in consensus clustering. To choose the optimal number of metabotypes, a combination of the average silhouette score, consensus matrix heatmap, and biological plausibility was used. The between-metabotype differences in the metabolome profile were visualized using a heatmap. The association between the metabotypes and the risk of developing childhood asthma (binary outcome) was estimated by fitting logistic regression models; the rate of developing recurrent wheeze (time-to-event outcome) was estimated by fitting Cox proportional hazards models. To examine the distinct function of each metabotype, 1) metabolite set enrichment analysis examining the metabolome data, 2) gene set enrichment analysis examining the nasopharyngeal transcriptome data, and 3) Wilcoxon pathway enrichment analysis integrating the metabolome and transcriptome data were conducted. In the sensitivity analysis, the concordance between the different numbers of metabotypes was also examined

Abbreviation: LC-MS/MS, liquid chromatography-tandem mass spectrometry

Figure 2. Abundance of selected nasopharyngeal metabolites between five metabotypes of severe bronchiolitis

The normalized metabolomics data (50 metabolites with highest variance and top 10 metabolites in the metabolite-set enrichment analysis) are used to visualize the between-metabotype differences. The top row represents five different metabotypes (A-E). The first column on the left represents a major metabolite class (e.g., amino acids, lipids) for the corresponding metabolite. The tree on the left represents hierarchical clustering of metabolites. Each cell represents the median value of normalized metabolite data within each metabotype—the yellow color represents a high value, and dark purple color represents a low value. Asterisk on some metabolites indicates a compound that has not been confirmed based on a standard, but we are confident in its identity (by Metabolon, Inc).

Figure 3. Principal component analysis and functional pathway analyses in the metabotypes A vs. B comparison

a. Principal component analysis

Principal component analysis of metabotypes A and B based on the first two principal components. The global metabolome profiles were distinct between the metabotypes A and B.

b. Metabolite-set enrichment analysis

Shown metabolite pathways (based on the Small Molecule Pathway Database [SMPDB]) are 20 pathways with a smallest false discovery rate (FDR) that have the number of hits per pathway of ≥ 5 nasopharyngeal metabolites. SMPDB is a 99-metabolite set based on normal human metabolism pathways. The colour of each dot represents the proportion of hit metabolites; the size of each dot represents the number of hit metabolites.

c. Functional class scoring analysis

Shown biological pathways (based on the Molecular Signatures Database [MSigDB] hallmark gene sets) are 20 pathways with a highest absolute value of normalized enriched score. MSigDB hallmark collection contains 50 gene sets, which summarize and represent specific well-defined biological states or processes and display coherent expression. The analysis is based on nasopharyngeal transcriptome (through RNAseq) data. The colour of bars represents $FDR < 0.05$ (green) or ≥ 0.05 (orange).

Figure 4. Kaplan-Meier curves for development of recurrent wheeze by age 3 years, according to severe bronchiolitis metabotypes

a. Recurrent wheeze by age 3 years *with* asthma at age 5 years

Compared with metabotype A infants, the rate of developing recurrent wheeze was not significantly different in metabotype B, C, D, or E infants. However, the rate was significantly higher in metabotype B (amino acid-high/PUFA-low) infants (adjusted HR 2.52; 95% CI 1.16-5.47; $P=0.02$).

b. Recurrent wheeze by age 3 years *without* asthma at age 5 years

Compared with metabotype A infants, the rate of developing recurrent wheeze was not significantly different in metabotype B, C, D, or E. Corresponding hazards ratio estimates are presented in **Table E2**.

Table 1. Baseline characteristics and clinical course of infants, according to severe bronchiolitis metatypes

Characteristics	Metatype A (n=79, 8.6%)	Metatype B (n=72, 7.8%)	Metatype C (n=363, 39.5%)	Metatype D (n=177, 19.3%)	Metatype E (n=227, 24.7%)	P-value
Demographics						
Age (month), median (IQR)	2 (1-3)	3 (2-5)	3 (2-6)	3 (2-6)	4 (2-7)	<0.001
Female sex	36 (45.6)	27 (37.5)	147 (40.5)	74 (41.8)	83 (36.6)	0.63
Race/ethnicity						0.13
Non-Hispanic white	28 (35.4)	35 (48.6)	167 (46.0)	68 (38.4)	101 (44.5)	
Non-Hispanic black	15 (19.0)	17 (23.6)	89 (24.5)	45 (25.4)	44 (19.4)	
Hispanic	34 (43.0)	19 (26.4)	90 (24.8)	59 (33.3)	73 (32.2)	
Other or unknown	2 (2.5)	1 (1.4)	17 (4.7)	5 (2.8)	9 (4.0)	
Prematurity (32-37 weeks)	14 (17.7)	12 (16.7)	67 (18.5)	39 (22.0)	39 (17.2)	0.76
Birth weight (kg), median (IQR)	3.35 (2.95-3.60)	3.33 (2.90-3.60)	3.26 (2.90-3.58)	3.20 (2.86-3.54)	3.32 (2.98-3.63)	0.51
Mode of birth (caesarean delivery)	24 (31.2)	25 (35.2)	126 (35.1)	65 (36.9)	71 (31.8)	0.81
Previous breathing problems (count)						0.001
0	69 (87.3)	58 (80.6)	293 (80.7)	152 (85.9)	160 (70.5)	
1	6 (7.6)	9 (12.5)	61 (16.8)	18 (10.2)	51 (22.5)	
2	4 (5.1)	5 (6.9)	9 (2.5)	7 (4.0)	16 (7.0)	
Previous ICU admission	1 (1.3)	2 (2.8)	4 (1.1)	2 (1.1)	6 (2.6)	0.56
Child history of eczema	5 (6.3)	8 (11.1)	57 (15.7)	24 (13.6)	43 (18.9)	0.07
Lifetime antibiotic use *	24 (30.4)	22 (30.6)	111 (30.6)	49 (27.7)	87 (38.3)	0.19
Lifetime corticosteroid use *	8 (10.1)	13 (18.1)	52 (14.3)	27 (15.3)	38 (16.7)	0.61
Ever attended daycare	10 (12.7)	18 (25.0)	86 (23.7)	31 (17.5)	65 (28.6)	0.02
Cigarette smoke exposure at home	7 (8.9)	12 (16.7)	55 (15.2)	28 (15.8)	35 (15.4)	0.62
Maternal smoking during pregnancy	10 (13.0)	11 (15.7)	50 (13.9)	26 (14.8)	29 (12.9)	0.97
Parental history of asthma	24 (30.4)	25 (34.7)	119 (32.8)	54 (30.5)	82 (36.1)	0.77
Parental history of eczema	11 (13.9)	14 (19.4)	70 (19.3)	39 (22.0)	41 (18.1)	0.64
Clinical presentation						
Weight (kg), median (IQR)	4.99 (4.20-6.17)	5.80 (4.70-7.15)	6.10 (4.70-7.60)	5.90 (4.80-7.60)	6.90 (5.30-8.32)	<0.001
Respiratory rate (per minute), median (IQR)	48 (42-60)	48 (39-60)	48 (40-60)	52 (42-62)	50 (40-60)	0.26
Oxygen saturation						0.50
<88%	6 (7.7)	7 (10.1)	19 (5.3)	10 (5.7)	10 (4.5)	
88%-89.9%	1 (1.3)	5 (7.2)	14 (3.9)	7 (4.0)	6 (2.7)	

90%-93.9%	9 (11.5)	7 (10.1)	51 (14.3)	30 (17.2)	38 (17.2)	
≥94%	62 (79.5)	50 (72.5)	272 (76.4)	127 (73.0)	167 (75.6)	
Blood eosinophilia (≥4%)	9 (12.7)	10 (17.9)	38 (12.0)	10 (6.2)	15 (7.8)	0.06
Any IgE sensitization	16 (20.3)	15 (20.8)	64 (17.6)	47 (26.6)	39 (17.2)	0.13
Aeroallergen sensitization	0 (0.0)	1 (1.4)	6 (1.7)	3 (1.7)	4 (1.8)	0.85
Food allergen sensitization	16 (20.3)	14 (19.4)	60 (16.5)	44 (24.9)	36 (15.9)	0.14
Clinical course						
Positive pressure ventilation use [†]	4 (5.1)	2 (2.8)	14 (3.9)	14 (7.9)	15 (6.6)	0.23
Intensive treatment use [‡]	11 (13.9)	8 (11.1)	49 (13.5)	29 (16.4)	44 (19.4)	0.28
Length-of-stay (day), median (IQR)	2 (1-3)	2 (1-3)	2 (1-3)	2 (1-4)	2 (1-3)	0.24
Respiratory virus						
Any RSV infection	66 (83.5)	61 (84.7)	294 (81.0)	155 (87.6)	173 (76.2)	0.05
Any rhinovirus infection	13 (16.5)	9 (12.5)	79 (21.8)	27 (15.3)	57 (25.1)	0.04
Rhinovirus-A	6 (7.6)	5 (6.9)	31 (8.5)	15 (8.5)	31 (13.7)	0.20
Rhinovirus-B	3 (3.8)	0 (0.0)	5 (1.4)	2 (1.1)	5 (2.2)	0.36
Rhinovirus-C	6 (7.6)	3 (4.2)	41 (11.3)	8 (4.5)	23 (10.1)	0.05

Abbreviations: ICU, intensive care unit; IgE, immunoglobulin E; IQR, interquartile range; RSV, respiratory syncytial virus

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

* Any systemic antibiotic or corticosteroid use from birth up to the index hospitalization for bronchiolitis.

[†] Infants with severe bronchiolitis who underwent continuous positive airway ventilation and/or mechanical ventilation.

[‡] Infants with severe bronchiolitis who were admitted to ICU and/or who underwent positive pressure ventilation.

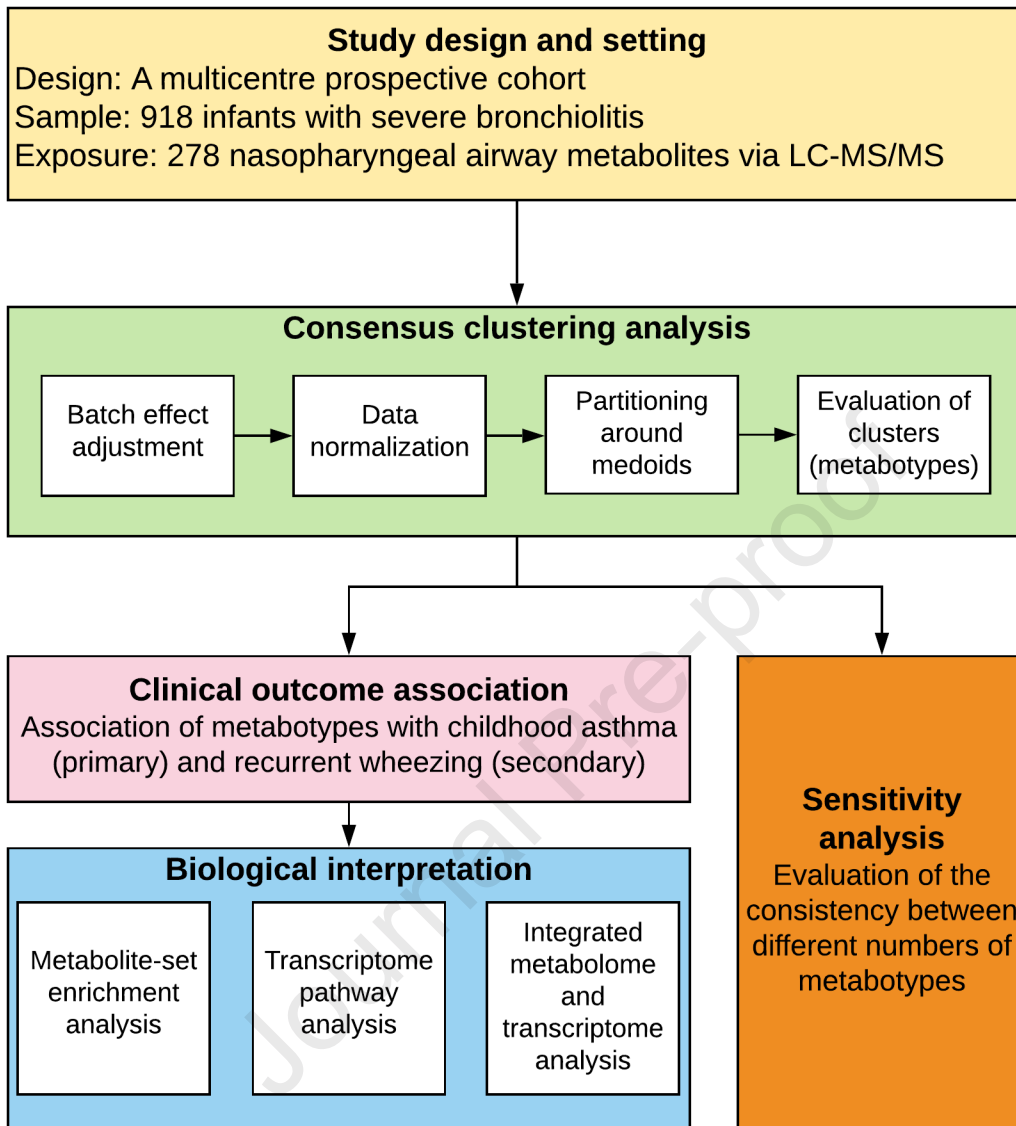
Table 2. Unadjusted and adjusted associations of severe bronchiolitis metabotypes in infants with development of childhood asthma

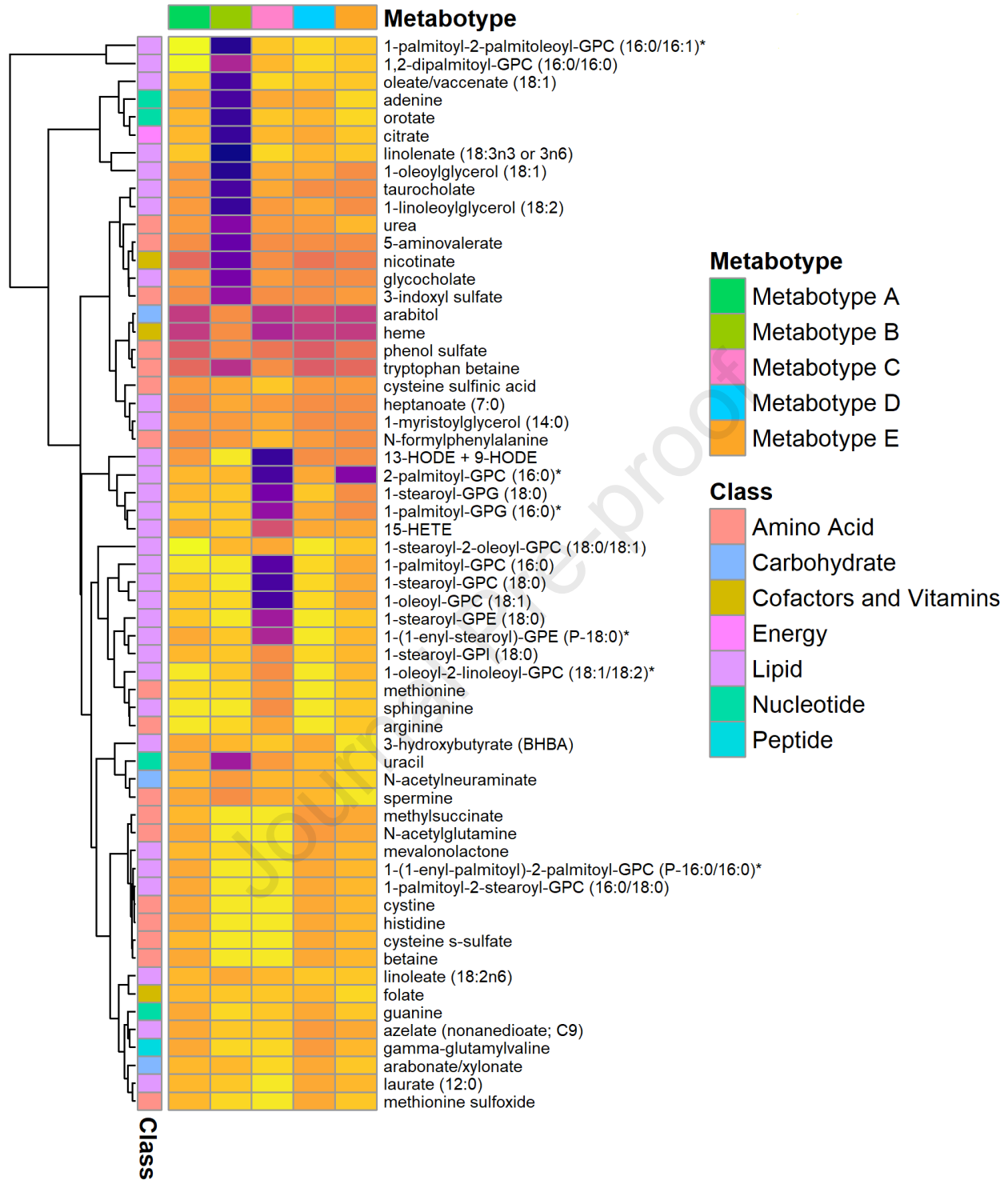
Metabotypes	Childhood asthma*			
	Unadjusted model		Adjusted model†	
	Odds ratio (95% CI)	P-value	Odds ratio (95% CI)	P-value
Metabotype A (GPC-high)	1 [Reference]	–	1 [Reference]	–
Metabotype B (amino acid-high, PUFA-low)	2.24 (1.10-4.63)	0.03	2.18 (1.03-4.71)	0.04
Metabotype C (amino acid-high, GP-low)	1.14 (0.65-2.08)	0.66	1.07 (0.58-2.02)	0.84
Metabotype D (GP-high)	1.23 (0.66-2.34)	0.52	1.25 (0.65-2.49)	0.51
Metabotype E (mixed)	1.12 (0.62-2.11)	0.72	0.87 (0.46-1.72)	0.69

Abbreviations: CI, confidential interval; GP, glycerophospholipid; GPC, glycerophosphocholine; PUFA, polyunsaturated fatty acid.

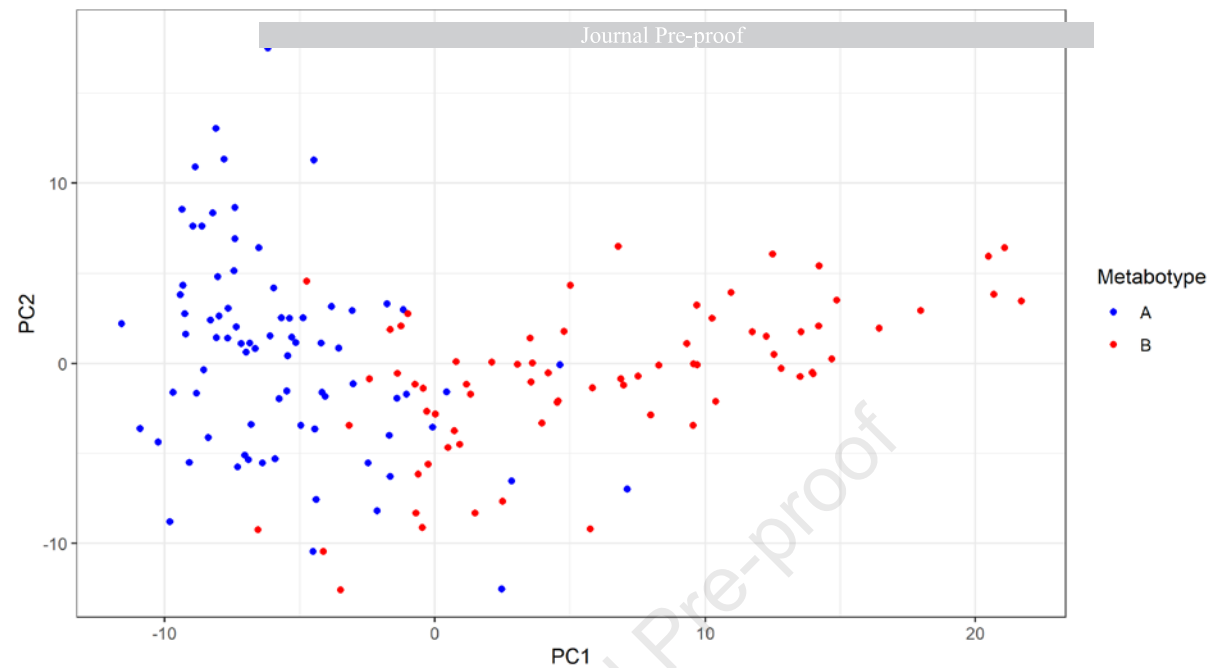
* Asthma was defined as physician-diagnosis of asthma by age 5 years, plus either asthma medication use (e.g., albuterol inhaler, inhaled corticosteroids, montelukast) or asthma-related symptoms in the preceding year. To examine the association between severe bronchiolitis metabotypes (metabotype A as the reference) and the risk of developing childhood asthma, logistic regression model was fit.

† The multivariable logistic regression models adjusted for potential confounders, including patient's age, sex, parent history of asthma, number of previous breathing problems, respiratory syncytial virus infection, and rhinovirus infection.

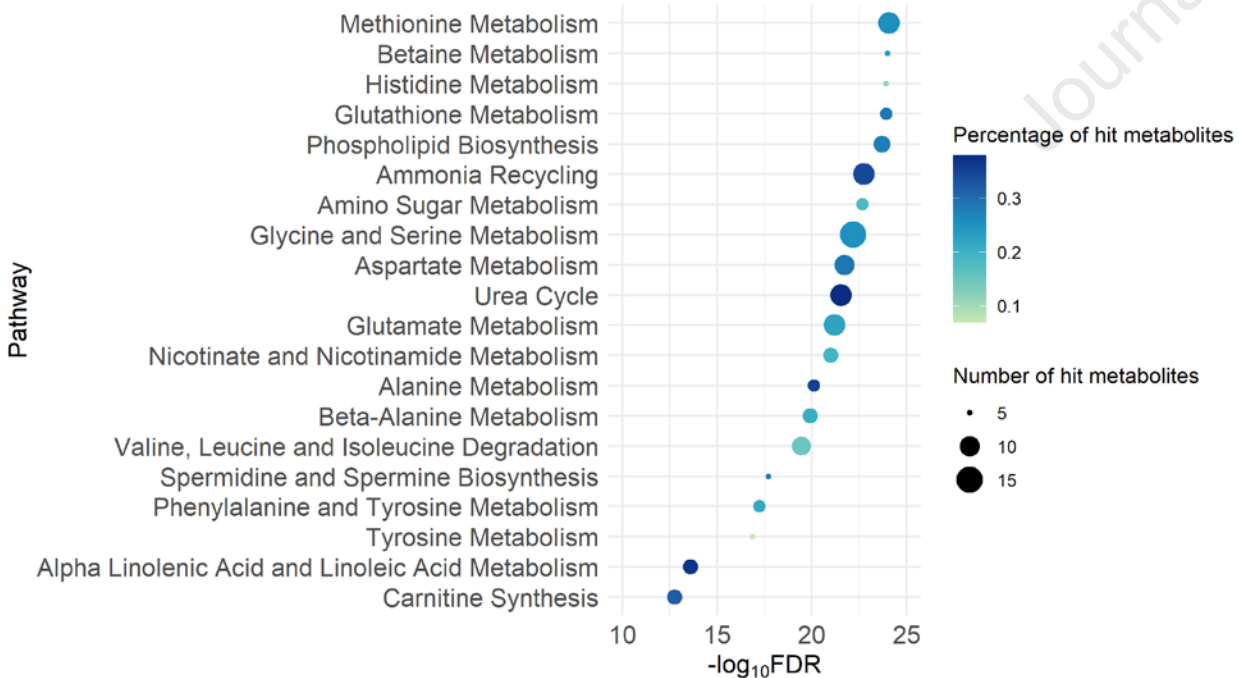




A



B



C

