



CORRESPONDENCE

In infants with severe bronchiolitis: dual-transcriptomic profiling of nasopharyngeal microbiome and host response

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Bronchiolitis is the leading cause of hospitalizations among infants in the U.S.¹ Two major respiratory viruses—respiratory syncytial virus (RSV) and rhinovirus (RV)—account for 85% of severe bronchiolitis (bronchiolitis resulting in hospitalization).² While bronchiolitis has been considered a single disease entity and currently available management does not vary by causative virus,³ emerging evidence suggests heterogeneity in the clinical manifestations and pathobiology of bronchiolitis by virus. For example, epidemiological research has shown that causative viruses are associated with distinct risks of both acute (e.g., illness severity) and chronic (e.g., incident asthma) outcomes in childhood.¹ Recent data also suggest an interplay between respiratory viruses, airway microbiome, and host response.¹ For example, studies have shown virus-specific (e.g., RSV and RV specific) microbiome composition (through 16S rRNA gene sequencing)⁴ and host response (through transcriptomics^{5–7} and metabolomics⁸) in children with acute respiratory infection, including bronchiolitis. Despite the clinical and research importance of bronchiolitis, no study to date has reported an integrated investigation of the between-virus differences in microbiome composition (beyond 16S rRNA gene sequencing) and function, along with host response, in the airway of infants with bronchiolitis. In this context, we performed a pilot study using a dual-transcriptomic approach^{9,10}—simultaneous profiling of microbe's transcriptome (metatranscriptome) and host transcriptome—to test the hypothesis that, in infants with severe bronchiolitis, RSV and RV infection have distinct nasopharyngeal airway microbiome composition and function as well as host response profiles.

Details of the study design, setting, samples, measurement, and analysis may be found in Supplemental Methods. Briefly, in the current pilot study, we performed dual-transcriptomic profiling of the nasopharyngeal samples from five infants with RSV infection and five infants with sole RV infection (i.e., no co-infecting RSV) as part of an ongoing cohort study. This multicenter prospective cohort study—the 35th Multicenter Airway Research Collaboration (MARC-35)—enrolled infants (aged <12 months) hospitalized with bronchiolitis at 17 sites across 14 U.S. states during the 2011–2014 winter seasons. Bronchiolitis was defined by the American Academy of Pediatrics guidelines.³ In addition to phenotypic data measurements, investigators collected nasopharyngeal aspirates within 24 h of hospitalization using a standardized protocol. These samples underwent (1) real-time reverse transcription PCR to test for respiratory viruses—e.g., RSV and RV—and (2) dual transcriptomics through RNAseq to profile the microbiome composition and function, as well as host function, in the nasopharyngeal airway.

The bioinformatic processing and analysis are detailed in Supplemental Methods. First, we used microbial RNA to infer microbial

taxonomic composition through PathoScope.^{11–13} Then, to compare compositional differences (α -diversity indices and individual species abundances) between the RSV and sole RV infection groups, we used linear regression models and Wald test with Cook's distance correction, respectively. We also used the Benjamini–Hochberg method to correct for multiple testing. Second, we used metatranscriptomic contigs to infer microbial function and Gene Ontologies (GOs) and compared their between-group differences. Third, we also computed host gene expression profiles for each sample and examined differential expression. Lastly, we also performed pathway enrichment analysis to examine the virus-specific host response.

The ten infants with severe bronchiolitis had a median age of 7 (IQR, 3–9) months. Between the virus groups, there was no significant difference in the baseline characteristics (all $P > 0.10$; Supplementary Table S1). All RNAseq samples had sufficient sequence depth (mean, 74 million pair-end reads/sample) to obtain a high degree of sequence coverage. The metatranscriptomic analysis obtained 22,115,512 merged sequences and identified 2780 microbial lineages after singleton removal.

COMPOSITION AND FUNCTION OF THE NASOPHARYNGEAL MICROBIOME

Nasopharyngeal microbiome was dominated by *Moraxella catarrhalis* (32%), followed by other bacteria including *Streptococcus mitis* (7%), *Streptococcus pneumoniae* (6%), *Prevotella* spp. (5%), *Prevotella melaninogenica* (5%), and *Haemophilus influenzae* (4%). Comparing the α -diversity between the two groups, Shannon and Simpson indices were generally higher in the RSV group but there were no significant differences ($P > 0.05$; Supplementary Fig. S1). By contrast, at the individual taxon level, the abundance of 52 microbial species were significantly different between the virus groups ($q < 0.05$; Supplementary Fig. S2), including several representatives of some genera, *Moraxella* and *Staphylococcus*.

The functional profiling identified 18 differentially expressed microbial genes between the virus groups ($q < 0.05$; Fig. 1a). In the RSV group, two genes were upregulated—*coaA* and *mazF*, which are involved in pantothenate kinase and nucleic acid metabolism activities, respectively. By contrast, in the sole RV group, 16 genes were upregulated (e.g., *ddc* involved in carboxylase activity). Similarly, the GO-level analysis demonstrated different top ontology terms between the virus groups (Fig. 1b and Supplementary Table S2). While some GO terms were shared between the virus groups (e.g., nucleobase biosynthetic process), most of the enriched terms were exclusive to each group, indicating functional differences in the microbiome by infecting virus. Indeed, the RSV group was enriched with nucleic acid-related metabolism (e.g., adenine metabolic process [GO: 0046083], purine-nucleobase compound salvage [GO: 0043096]; $q < 0.05$). By contrast, the sole RV group was enriched with acylation and acetylation activities (e.g., C-acyltransferase activity [GO: 0016408], C-acetyltransferase activity [GO: 0016453]; $q < 0.05$).

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differential regulation of genes related to Toll-like receptor in blood.⁵ The current study utilizing a novel dual-transcriptomic approach corroborates these earlier studies and extends them by simultaneously determining the virus-specific microbiome function and host response signatures in the airway of infants with bronchiolitis.

The present study has several potential limitations. First, bronchiolitis involves inflammation of the lower airways in addition to the upper airways. While our analyses relied on nasopharyngeal samples, studies have shown that the upper airway sampling provides reliable representation of the lung microbiome¹⁵ and transcriptome profiles.¹⁶ Second, the limited sample size of this current proof-of-concept study precluded us from evaluating the role of RSV/RV co-infection compared to sole infection and from making robust inferences. Third, we did not have a “healthy control” group. Yet, the study objective was not to evaluate the role of metatranscriptome and transcriptome on the development of bronchiolitis (yes/no) but to examine virus-specific (RSV vs. RV) pathobiology within this high-risk population. Lastly, our inferences may not be generalizable beyond infants hospitalized for bronchiolitis. Yet, our findings are directly relevant for infants with severe bronchiolitis, which accounts for 130,000 hospitalizations in the U.S. each year.¹

In summary, based on the dual-transcriptomics analysis in infants with bronchiolitis, we found that RSV and sole RV infection had distinct microbiome composition and function, as well as host response, profiles. In conjunction with earlier studies, our findings suggest that the bronchiolitis pathobiology differs between RSV and RV infection. The current pilot study lends support to the use of dual-transcriptomic approach, which has a potential to delineate the integrated contributions of the airway microbiome and host to bronchiolitis pathobiology. In conjunction with existent evidence, the data should not only inform future adaptive clinical trials (e.g., the use of microbiome and host data as biomarkers) but also facilitate investigations into the development of treatment strategies (e.g., modification of microbiome, immunomodulators) in infants with bronchiolitis.

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AUTHOR CONTRIBUTIONS

M.F. carried out the statistical analysis, drafted the initial manuscript, and approved the final manuscript as submitted. K.H. conceptualized and designed the study, obtained the funding, reviewed and revised the initial manuscript, and approved the final manuscript as submitted. J.P.B., R.J.F., B.H., E.C.-N., and M.P.-L. generated the RNAseq data, carried out the statistical analysis, reviewed and revised the manuscript, and approved the final manuscript as submitted. J.M.M. and C.A.C. conceptualized and designed the study, supervised the conduct of the study, reviewed and revised the manuscript, and approved the final manuscript as submitted.

ADDITIONAL INFORMATION

The online version of this article (<https://doi.org/10.1038/s41390-019-0742-8>) contains supplementary material, which is available to authorized users.

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