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LETTER

The nasopharyngeal and salivary microbiomes in COVID-19 patients with and without asthma

To the Editor,

So far, our understanding of the associations between respiratory infections and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in the context of asthma is limited. Although our previous study and others did not find a correlation between preexisting asthma and increased risks of severe coronavirus disease 2019 (COVID-19) outcomes,[1–3] people with asthma usually have an increased vulnerability to conventional respiratory viral infections. Thus, continuous investigation on SARS-CoV-2 infection in people with asthma is important.

People with asthma harbor altered airway microbiota, which has been suggested to mediate an increased susceptibility to severe illnesses upon viral respiratory infections.[4] However, the microbiomes of patients with asthma during SARS-CoV-2 infection have not yet been characterized. To this end, we performed a microbiome study using nasopharyngeal samples and saliva samples from COVID-19 patients with and without preexisting asthma.

This study was approved by the Institutional Review Board of Washington University in St. Louis (IRB number 202003085), and all patients who were enrolled in the study provided informed consent. A total of 105 samples were collected from patients with COVID-19 within 14 days from the onset of any relevant symptoms between March and September of 2020. For the nasopharyngeal samples, seven were from patients with asthma and 41 were from patients with no asthma diagnosis. For the salivary samples, 16 were from patients with asthma and 41 were from patients with no asthma diagnosis. Demographics and clinical characteristics of the COVID-19 patients are shown in Table S1. Study participants were enrolled in both outpatient and inpatient settings. Nine patients ($n = 3$ asthma, and $n = 6$ non-asthma) provided both saliva and nasopharyngeal samples. The detailed methods and sequencing analysis procedures are presented in the Appendix S1. The read number of each sample and rarefaction curves are plotted in Figure S1A, B. The microbial communities of the nasopharyngeal and saliva samples were significantly different in alpha diversity represented by the Shannon Index (p-value <0.001, Figure 1A) and beta diversity based on weighted UniFrac distances (p-value = 0.001, Figure 1B). For the 48 nasopharyngeal samples, seven were from COVID-19 patients with asthma and 41 were from those who did not have an asthma diagnosis. There were no marked differences in relative abundance for any of the top five abundant phyla in nasopharyngeal samples between the asthma and non-asthma groups (Figure 1C). For the 57 saliva samples, the relative abundance of phylum Actinobacteria was significantly decreased in COVID-19 patients with asthma compared with those without preexisting asthma (adjusted p-value = 0.02, Figure 1D). The top ten abundant genera in the nasopharyngeal samples and saliva samples are displayed in Figure 1E, F, respectively.

Differences at the genus-level, but not at the community level (Figure S2), were observed in the nasopharyngeal and salivary microbiomes between patients with and without preexisting asthma. In differential abundance tests using DESeq2 for nasal samples, seven genera were significantly different between the two groups, with all being less abundant (including *Porphyromonas*, *Haemophilus*, *Alloprevotella*, *Moraxella*, *Facklamia*, *Campylobacter*, and *Janibacter*) in those with preexisting asthma compared with those without asthma (Figure 2A). In the saliva samples, seven genera were significantly different between the two groups (Figure 2B), with three being less abundant (including *Centipeda*, *Staphylococcus*, *and Actinomyces)* and four being more abundant (including *Porphyromonas*, *Capnocytophaga*, *Bergeyella*, and *Neisseria)* in those with preexisting asthma compared with those without asthma. The normalized counts of each identified genus were displayed by asthma status, as shown in Figure 2C, D. We then compared the relative abundance of these identified genera based on the treatment of inhaled corticosteroids (ICS) only or a combination of ICS and Long-Acting Beta-Agonists (LABA). As shown in Figure S3, there were no significant differences in relative abundance based on the use of ICS/LABA. However, this may be due to the small sample size.

To further investigate opportunistic pathogenic bacteria, we compared the prevalence of selected bacterial genera between the asthma and non-asthma groups. The five selected genera, including *Haemophilus*, *Moraxella*, *Neisseria*, *Streptococcus*, and *Staphylococcus*, have previously been associated with increased vulnerabilities to respiratory viral infections in patients with asthma. However, as shown in Table S2, their prevalence was similar in both groups. Some of the bacteria even had a decreased prevalence in the asthma group, although the trends were not significant.

The nasopharynx and oropharynx are both important entry ports and reservoirs of SARS-CoV-2.[5] Interactions between invading SARS-CoV-2 viruses and commensal microbes residing in the pharynx are essential in mediating viral loads and host immune responses.[5,6] As patients with asthma harbor imbalanced

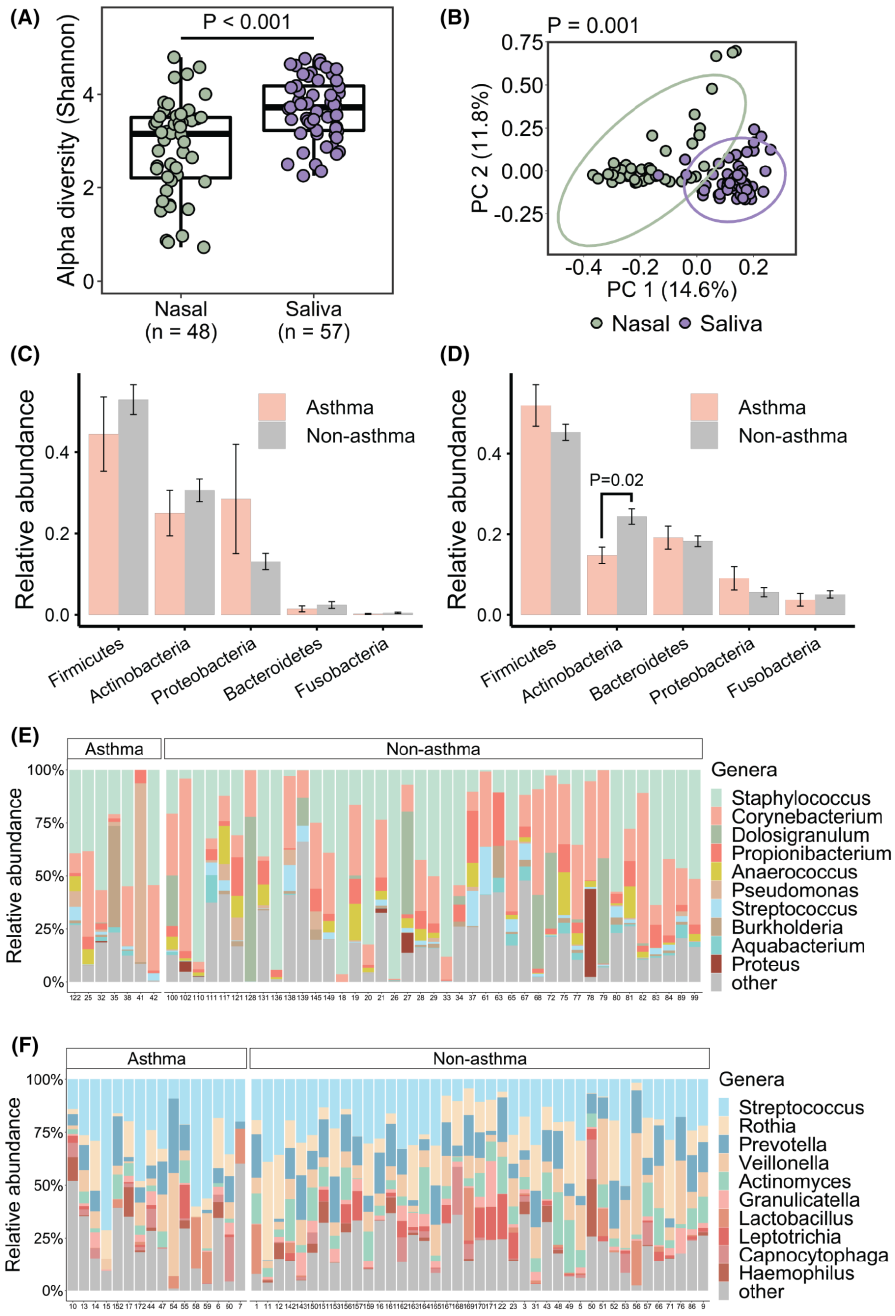

FIGURE 1 Nasopharyngeal and salivary microbial profiles of COVID-19 patients with and without preexisting asthma. (A) Shannon Index comparison between the nasopharyngeal and salivary microbiomes. Statistical significance was assessed by the Wilcoxon rank-sum test. (B) Principal coordinates (PC) analysis based on weighted UniFrac distances between the nasopharyngeal and salivary microbiomes. Statistical significance was assessed by the ADONIS test. Relative abundance of the top five most abundant bacterial phyla in nasopharyngeal samples (C) and saliva samples (D). Statistical significance was assessed by the Wilcoxon rank-sum test with multiple comparison adjustments using the Benjamini–Hochberg method. Relative abundance of the top 10 bacterial genera in nasopharyngeal samples (E) and saliva samples (F)

microbial communities, which is one of several factors that underlie asthma-associated susceptibility to conventional respiratory viral infections, it is critical to characterize these microbial factors in COVID-19 patients with and without preexisting asthma.[4,7,8] To our knowledge, there have been no such investigations on SARS-CoV-2 infection in patients with asthma. Our study starts to fill this important knowledge gap and provides novel insights into asthma-associated nasopharyngeal and salivary microbial features during SARS-CoV-2 infection.

We found that COVID-19 patients with asthma had a decreased abundance of Actinobacteria, including its genus, *Actinomyces*, in the salivary samples compared with those without asthma. Interestingly, a study reported that a reduced abundance of Actinobacteria and *Actinomyces* in the oropharyngeal microbiome was associated with more severe COVID-19 outcomes.[9] Therefore, further studies are needed to validate our findings and investigate whether Actinobacteria and *Actinomyces* could be mechanistically linked to COVID-19 disease severity in asthma patients.

Previous studies have shown that asthma patients harbor airway microbial dysbiosis featuring an increase in pathogenic bacteria.[10] We found oral *Neisseria* was more abundant in the asthma group compared with non-asthma group during SARS-CoV-2 infection. However, interestingly, several other opportunistic pathogens, including *Moraxella*, *Haemophilus*, and *Staphylococcus*, in the nasopharyngeal or saliva samples were absent or less abundant in the asthma group compared with non-asthma group.

In conclusion, our results suggest that patients with preexisting asthma exhibit taxa-level, but not community-level differences, in

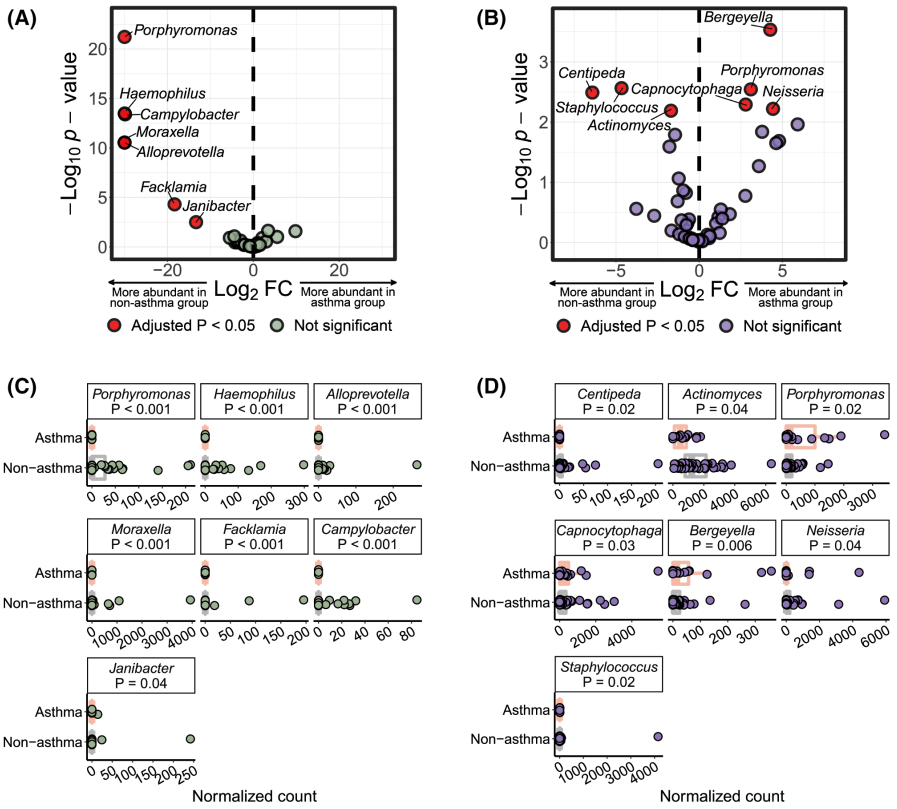

FIGURE 2 Differentially abundant bacterial genera identified in the nasopharyngeal and salivary microbiomes between COVID-19 patients with and without preexisting asthma. DESeq2 models were used at the genus level including age, sex, race, and the use of antibiotics as covariates. Volcano plot of log2 fold change (FC) vs statistical significance in the nasopharyngeal microbiome (A) and saliva microbiome (B). Genera with a prevalence greater than 5% and base mean greater than 5 are displayed in the volcano plots. The red circles and labels indicate genera that were significantly different between the asthma and non-asthma groups. FC, fold change. Normalized counts by asthma status in the nasopharyngeal samples (C) and the saliva samples (D). The *p*-values were from Wald test adjusted for multiple comparisons using the Benjamini–Hochberg method

the airway and oral microbiomes during SARS-CoV-2 infection compared with those without preexisting asthma. We did not observe a significantly increased prevalence of pathogenic bacterial genera in patients with preexisting asthma compared with patients without asthma. Together, our results characterize, for the first time, the nasopharyngeal and salivary microbiomes in COVID-19 patients with asthma. This information contributes to our understanding of the relationship between asthma and COVID-19 and opens the door for future applications of the microbiome as biomarkers and treatment strategies. A major limitation of this study is the number of asthma patients, which may be too small to detect certain differences between asthma and non-asthma groups. Future studies with larger sample sizes are needed to validate our results and investigate the clinical relevance of our microbiome findings by probing associations between microbiota that were found to be differentially abundant between COVID-19 patients with and without asthma and factors such as COVID-19 disease severity, angiotensin converting enzyme 2, and antiviral immunity.

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CONFLICT OF INTEREST

No conflict of interest in relation to this work was reported by the authors.

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REFERENCES

1. Cao L, Lee S, Krings JG, et al. Asthma in patients with suspected and diagnosed coronavirus disease 2019. *Ann Allergy Asthma Immunol*. 2021;126(5):535-541.
2. Chhiba KD, Patel GB, Vu THT, et al. Prevalence and characterization of asthma in hospitalized and nonhospitalized patients with COVID-19. *J Allergy Clin Immunol*. 2020;146(2):307-314.
3. Eggert LE, He Z, Collins W, et al. Asthma phenotypes, associated comorbidities, and long-term symptoms in COVID-19. *Allergy*. 2022;77(1):173-185.
4. Huang YJ, Nariya S, Harris JM, et al. The airway microbiome in patients with severe asthma: Associations with disease features and severity. *J Allergy Clin Immunol*. 2015;136(4):874-884.
5. Xiang Z, Koo H, Chen Q, Zhou X, Liu Y, Simon-Soro A. Potential implications of SARS-CoV-2 oral infection in the host microbiota. *J Oral Microbiol*. 2020;13(1):1853451.
6. Rosas-Salazar C, Kimura KS, Shilts MH, et al. SARS-CoV-2 infection and viral load are associated with the upper respiratory tract microbiome. *J Allergy Clin Immunol*. 2021;147:1226-1233.
7. Durack J, Lynch SV, Nariya S, et al. Features of the bronchial bacterial microbiome associated with atopy, asthma, and responsiveness to inhaled corticosteroid treatment. *J Allergy Clin Immunol*. 2017;140(1):63-75.
8. Lukacs NW, Huang YJ. Microbiota-immune interactions in asthma pathogenesis and phenotype. *Curr Opin Immunol*. 2020;66:22-26.
9. Merenstein C, Liang G, Whiteside SA, et al. Signatures of COVID-19 Severity and Immune Response in the Respiratory Tract Microbiome. *mBio*. 2021;12(4):e0177721.
10. Kozik AJ, Huang YJ. The microbiome in asthma: Role in pathogenesis, phenotype, and response to treatment. *Ann Allergy Asthma Immunol*. 2019;122(3):270-275.

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