Analysis of Bronchoalveolar Lavage Fluid Metatranscriptomes among Patients with COVID-19 Disease

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Abstract

To better understand the potential relationship between COVID-19 disease and hologenome microbial community dynamics and functional profiles, we conducted a multivariate taxonomic and functional microbiome comparison of publicly available human bronchoalveolar lavage fluid (BALF) metatranscriptome samples amongst COVID-19 ($n=32$), community acquired pneumonia (CAP) ($n=25$), and uninfected samples ($n=29$). We then performed a stratified analysis based on mortality amongst the COVID-19 cohort with known outcomes of deceased ($n=10$) versus survived ($n=15$). Our overarching hypothesis was that there are detectable and functionally significant relationships between BALF microbiomes and the severity of COVID-19 disease. We observed 34 functionally discriminant gene ontology (GO) terms in COVID-19 disease compared to the CAP and uninfected cohorts, and 21 GO terms functionally discriminant to COVID-19 mortality ($q<0.05$). A Dirichlet multinomial mixtures clustering analysis resulted in a best model fit using three distinct clusters that were significantly associated with COVID-19 disease and mortality. We additionally observed discriminant taxonomic differences associated with COVID-19, CAP, and uninfected BALF. Some positive correlations between COVID-19 mortality include an increase in the in *Sphingomonas, Variovorax,* and taxa belonging to the order Bacteroidales. Collectively, while this data does not speak to causality nor directionality of the association, it does demonstrate a significant relationship between the human microbiome, COVID-19 and CAP. The results from this study have rendered testable hypotheses that warrant further investigation to better understand the causality and directionality of host–microbiome–pathogen interactions.

Introduction

- The lung microbiome contains a dynamic community of microorganisms encompassing potential pathogens and commensal microbes. Unraveling the relationships between commensals, pathogens, and the whole microbial community may lead to the discovery of new therapeutics and improve our understanding of different disease progression.
- The role of the human microbiome in SARS-CoV-2 infection is poorly understood, but it remains important to study, since it could be a significant contributor to the observed variations in COVID-19 disease severity and resiliency between patients. A previous 16S rRNA gene study found that COVID-19 patient endotracheal aspirates had lower microbial diversity compared to uninfected individuals.
- We computationally evaluated microbial insights drawn from BALF metatranscriptomes. Our analysis specifically evaluated the microbial taxonomic and functional profiles of publicly available BALF metatranscriptomes.
- BALF specimens from individual subjects were grouped into one of three categorical classes:
  1. Uninfected controls
  2. Community acquired pneumonia (CAP) patients
  3. COVID-19 patients with moderate to severe disease, including death

Methods

Results

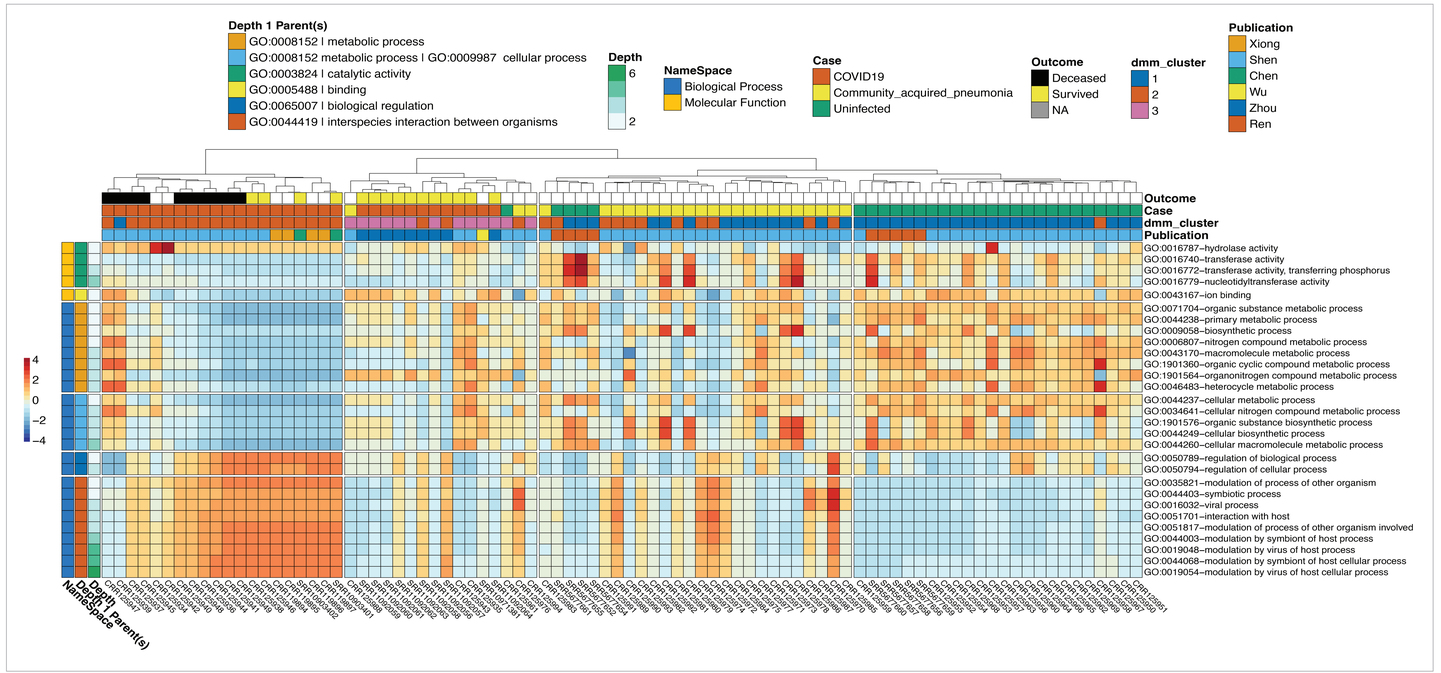

Figure 1: Heatmap with notable microbially-derived gene ontology functional annotations associated with COVID-19 ($n=32$), as compared to community acquired pneumonia ($n=29$) and uninfected ($n=25$) cohorts. Cells are colored via z-scale calculations of the total read counts for each GO term by sample. Rows are sorted by parental GO terms (depth = 1), and columns are clustered by Euclidean distance using ward D2 clustering. Comparisons were conducted using MaAsLin2, controlling for publication and patient ID with Benjamini Hochberg multiple test comparison ($q<0.05$).

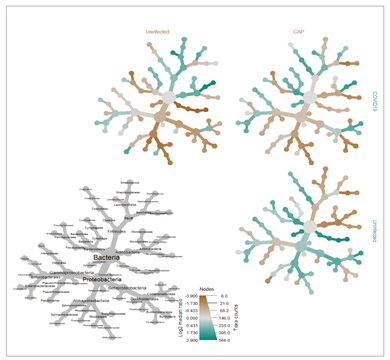

Figure 2: Heat tree matrix visualizing distinct COVID-19 vs. uninfected and community acquired viral pneumonia taxonomic profiles. This taxonomic heat tree data matrix visualization depicts the log2 median ratio differences across the three different cohorts. The taxa colored brown are more abundant among the cohort labelled in the columns, whereas taxa colored green are more abundant in the cohort labelled in the rows.

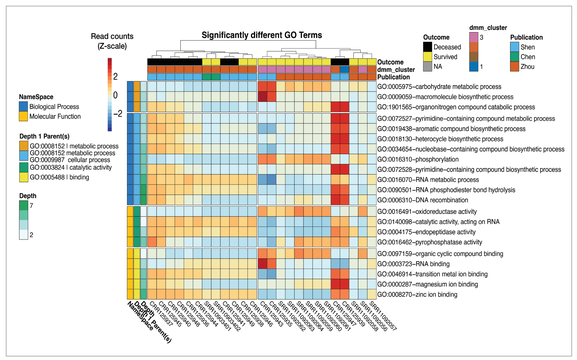

Figure 3: Heatmap of significantly different gene ontology terms associated with COVID-19 mortality comparing deceased ($n=10$) versus survived ($n=15$). Cells are colored via z-scale calculations of the total read counts by sample (x axis) and by GO term (y axis). Rows are sorted by parental GO terms (depth = 1) and columns are clustered by Euclidean distance using ward D2 clustering. Comparisons were conducted using MaAsLin2, controlling for patient ID with Benjamini Hochberg multiple test comparison ($q<0.05$).

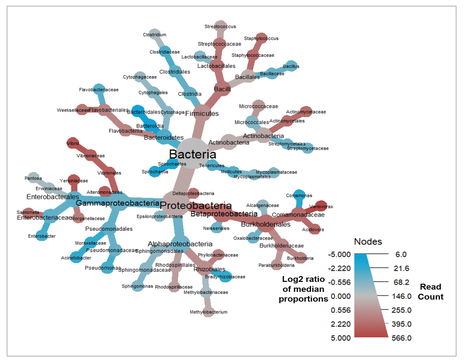

Figure 4: Heat tree demonstrating the BALF metatransciptome profiles associated with COVID-19 mortality. Taxa colored in red were more prevalent amongst COVID-19 patients who died, and nodes in blue represent taxa that were more prevalent amongst patients who survived COVID-19. Notable increases were observed in the log2 median ratios in the family Comamonadaceae, genus and significant decreases in the log2 median ratios of order Bacteroidia and class Bacteroidales.

Conclusions

- We observed significantly unique discriminant taxonomic and functional features in bronchoalveolar lavage fluid (BALF) metatranscriptomes in association with COVID-19 disease and its mortality
- Discriminant taxonomic differences associated with COVID-19 disease and mortality included the following:
  - *Sphingomonas* significantly increased with COVID-19 disease compared to the uninfected cohort and to a lesser extent with COVID-19 disease compared to the CAP cohort.
  - *Variovorax* significantly increased with COVID-19 mortality.
  - Bacteroidia significantly decreased with COVID-19 mortality.
- Different gene transcripts and metabolic pathways were enriched in different cohorts. Some notable examples include:
  - Histidine biosynthesis pathway increased among the COVID-19 deceased cohort, suggesting histidine could be an important contributor to the survival and pathogenicity of opportunistic bacteria of COVID-19 patients.
  - The enrichment of the oxidoreductase activity GO term [GO:0016491] among the COVID-19 survived cohort included underlying genes such as quinone oxidoreductase, pyruvate dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, and glyceraldehyde-3-phosphate dehydrogenase. Lung disease may become more severe in COVID-19 with increased oxidative stress, and it is possible that bacterial response in the COVID-19 survived cohort helped to reduce the oxidative stress.

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