* Thank you for allowing me to present our ongoing research on behalf of the COVIRT microbial subgroup. I have no conflicts of interest of financial disclosures. **[NEXT SLIDE]**
* In order to better understand the potential relationship between COVID-19 disease severity and microbial community dynamics / functional profiles from a hologenome standpoint, \*\*\* we conducted an analysis using human bronchoalveolar lavage fluid (BALF) metatranscriptomes sample sequences sourced from 8 different publications that were made available from public repositories. **[NEXT SLIDE]**
* \*\*\* These samples comprise 3 main cohorts case types consisting of uninfected control cohort, community acquired pneumonia or CAP patients, or COVID19 patients, \*\*\* with a secondary analysis of disease severity amongst a subset of the COVID19 cohort broken down by survival outcome. \*\*\* The objectives of the study are to compare the **COVID19 cohort** amongst **uninfected** and **CAP** patients **BALF metatranscriptomes** and identify \*\*\* Changes in **microbial** derived **community dynamics** / **gene ontologies** associated with **COVID19 \*\*\* and** Predict **outcomes** amongst **COVID19** based on metatranscriptomes profiling \*\*\*With the overarching hypothesis that there is a potential informative relationship between the BALF microbiome and the severity of COVID-19 disease onset and progression. **[NEXT SLIDE]**
* Raw sequencing reads were download from either SRA or CRA followed by
* Read preprocessing consisted of adapter trimming,QA/QC, and filtering of human and low complexity reads using Trimmomatic, fastQC, Kraken2, FastP, followed by
* Taxonomic classification via Kraken2 and functional annotation via Seqscreen
* Taxonomic classifications were decontaminated against negative controls when applicable using the library decontam in R, followed by statistical analysis and visualization using the bioinformatic software package phyloseq , vegan, and metacoder in R.
* Functional annotations gene ontologies counts derived from seqscreen outputs were parent propagated using covirt-micro conda package generated by Mike Lee at NASA.
* Parent propagated Gene ontology terms counts were then subjected to a Dirichlet mixture modeling (or Dmm clustering) and comparative analysis using multivariable associations with linear models Maaslin2 in R.
* All Scripts, intermediate files, and results can be found in the OSF, and covirt-micro githubs repos and conda packages. **[NEXT SLIDE]**
* After read filtering and batch effect sample removal, sample cohorts consisted of 29 Uninfected sampled, 25 CAP samples, and 32 COVID19 samples, bringing the total n to 86. \*\*\*
* Amongst the COVID19 cohort with known survival outcomes, 10 were deceased and 15 were survived. **[NEXT SLIDE]**
* 30 out of a total of 13,534 Gene ontologies were associated with COVID19 when compared to community acquired pneumonia and uninfected patients using a pvalue cutoff of 0.05 with BH mutltiple test correction (Controlling for random effects of publication and patient).
* Sig. go terms were comprised of 6 Depth 1 Parents involving catalytic activity, binding, metabolic and cellular processes, biological regulation, and interspecies interaction between organisms. **[NEXT SLIDE]**
* Significant Terms of interest associated with COVID19 include hydrolase/transferase activity transferring phosphorus, and nucleotidyltransferase activity, and ion binding.
* Additionally, Results from the Dirichlet Multinomial Mixtures clustering analysis using all 13,534 Gene ontologies counts resulted in a best model fit using 3 distinct dmm clusters that were significantly associated with each case type p<0.0001\*\*\*. **[NEXT SLIDE]**
* Taxonomic analysis revealed a statistically significant decrease in log2 median ration of several species \*\*\* belonging to the genus *Sphingomonas* when compared to both the uninfected (p<0.0001, q <0.001) and CAP cohorts (p<0.005,q <0.05) cohorts (table X). This finding supports previous reports regarding an association with *Sphingomonas*, which is commonly known as an opportunistic pathogen found in healthcare-associated pneumonia. Sig. GO Terms derived from *Sphingomonas* proteins in the COVID19 samples were hydrogen peroxide catabolic process, response to oxidative stress, catalase activity, heme binding and metal ion binding.
* The catalase protein decomposes hydrogen peroxide into water and oxygen; serves to protect cells from the toxic effects of hydrogen peroxide.
* So as a discussion point perhaps Sphingomonas is responding to COVID-19 conditions in the patient by expressing genes that help it to survive well under the COVID-19 disease conditions. **[NEXT SLIDE]**
* We then conducted a subsequent Maaslin2 analysis amongst COVID19 samples with known survival outcomes analysis revealing notable functional profiles associated Phosphate / phosphorylation, Metal ion binding (mg,zn,etc) Nucleotide terms (DNA/RNA) Lytic activity (hydrolase, endopeptidase,etc). **[NEXT SLIDE]**
* These findings were similar in nature to the GO Terms associated with COVID19 versus the uninfected and community acquired pneumonia patient cohorts **[NEXT SLIDE]**
* Collectively, while this data does cannot speak to causality or directionality of the association, it does demonstrate a significant relationship between the human microbiome and severity of COVID-19, rendering further testable hypotheses that warrant further investigation. **[NEXT SLIDE]**
* In conclusion, we observed unique and taxonomic and functional discriminant features in the brochoalveolar lavage metatranscriptomes associated with COVID19 disease and death.  Taxa of interested included genera from the Sphingomonadacae Class, and function annotated Gene ontologies of interest included associated with:Phosphate / phosphorylation, metal ion binding (mg,zn,etc), nucleotide terms (DNA/RNA), Lytic activity (hydrolase, endopeptidase,etc) **[NEXT SLIDE]**
* With that Iwould like to thank the COVIRT microbial subgroup team members and give special acknowledgment to John Fonner and the Texas Advanced Computing Center (TACC) at The University of Texas at Austin for providing HPC resources that have contributed to the research results reported. I would also like to thank my PI Dr. Kjersti Aagaard, Mike Lee at NASA, viktoria zakas at the University of Chicago, Kristen Curry and Todd, and the entirety of the Treangen lab at Rice, and our fearless leader Krista Ternus at Signature science.