Unique SARS-CoV-2 Variants and Inflammatory Milieu in Urine of Severe COVID-19 Participants

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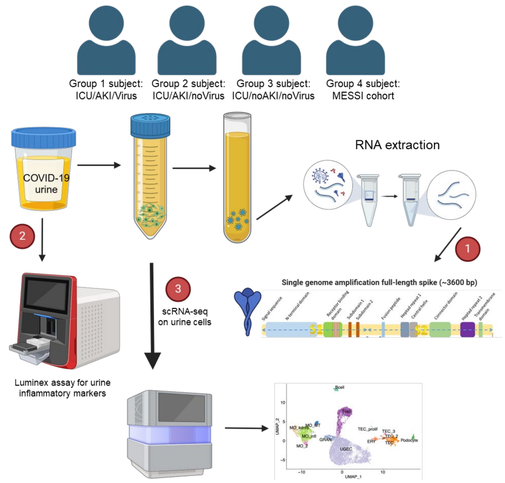
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BACKGROUND

- Acute kidney injury (AKI) is a common complication in people hospitalized with COVID-19.
- To understand the pathophysiology of AKI in people with COVID-19, we investigated the presence of SARS-CoV-2 RNA in urine samples from participants with mild or severe disease and explored the associated inflammatory profiles.

METHODS

- Urine specimens were obtained from 66 participants with COVID-19 hospitalized in the intensive care unit (ICU) and 26 with mild symptoms (MESSI Cohort).



- We performed single genome amplification and sequence analysis of the full-length SARS-CoV-2 spike gene in urine and nasal swab samples.
- scRNAseq of urine-derived cells and multiplex analyses of inflammatory markers in urine supernatants were performed to compare renal inflammatory signatures in mild and severe COVID-19 participants with or without viral shedding in urine.

Our data demonstrate the ability to use urine samples to noninvasively study renal infection, characterize cellular diversity and identify altered pathways in the setting of COVID-19 AKI.

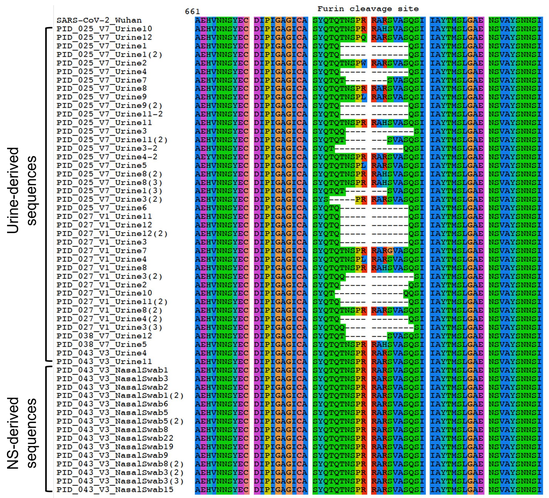


Figure 1. SARS-CoV-2 spike sequences from urine and nasal swab samples of 4 COVID-19 participants with acute kidney injury. Shown is an alignment of 40 urine-derived and 15 nasal swab-derived SARS-CoV-2 sequences, revealing the presence of a dominant deletion in the furin-cleavage site of SARS-CoV-2 found only in the urine-derived sequences.

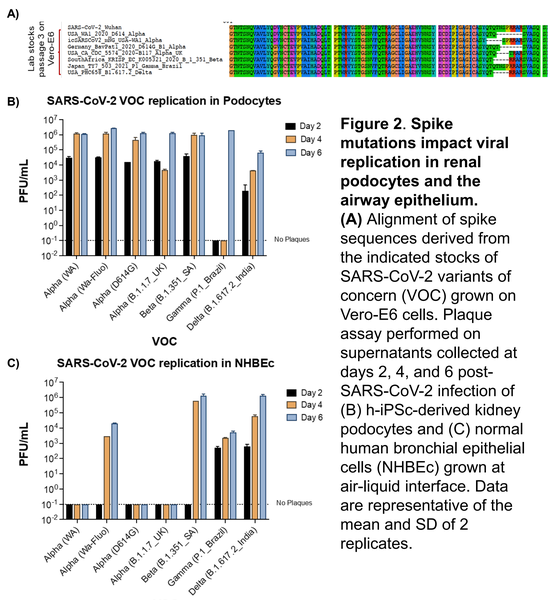


Figure 2. Spike mutations impact viral replication in renal podocytes and the airway epithelium. **(A)** Alignment of spike sequences derived from the indicated stocks of SARS-CoV-2 variants of concern (VOC) grown on Vero-E6 cells. Plaque assay performed on supernatants collected at days 2, 4, and 6 post-SARS-CoV-2 infection of (B) h-iPSc-derived kidney podocytes and (C) normal human bronchial epithelial cells (NHBEc) grown at air-liquid interface. Data are representative of the mean and SD of 2 replicates.

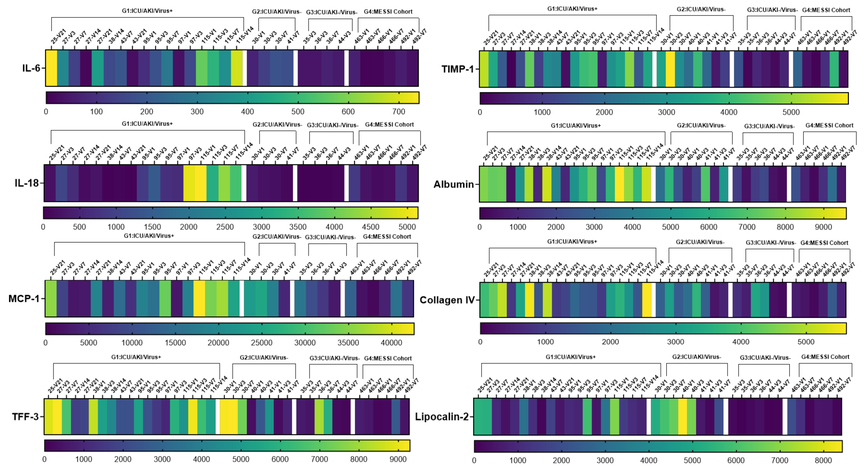


Figure 3: **Multiplexed analysis of inflammatory markers in urine from participants with COVID-19**. Heat maps show the mean fluorescent intensity over the background for the indicated cytokines and kidney injury biomarkers in urine samples of each group of participants.

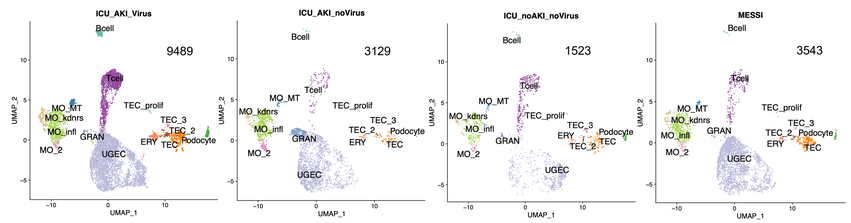


Figure 4. **Single-cell RNA sequencing of urine-derived cells.** Uniform manifold approximation and projections (UMAPs) of 17,684 total single urine-derived cells from the indicated groups of COVID-19 participants, showing the differences in cell populations based on AKI status and viral shedding in urine. MO: Monocytes/Macrophages, TECs: Tubular epithelial cells, UGECs: Urogenital epithelial cells, GRAN: Granulocyte, ERY: Erythrocyte.

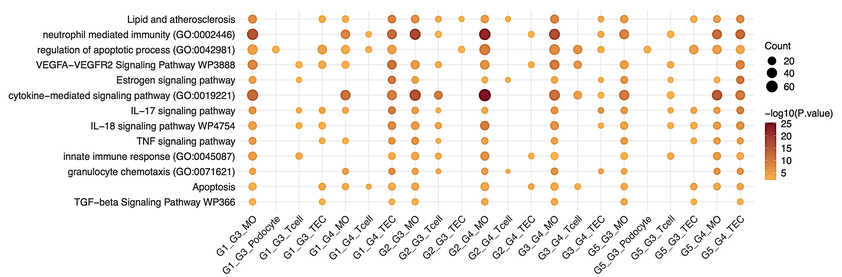


Figure 5. **Comparison of pathway enrichment analyses among the indicated groups of COVID-19 participants for the four major cell types found in urine.** Dot plot shows the differences in cellular processes enriched in MO, TECs, T cells and podocyte clusters for the indicated comparisons. The size of the dot indicates the number of DEGs enriched in the pathway, and the darkness of the color indicates -log10(P value) of the enrichment tested by one-sided hypergeometric test. Group 5 (G5) comprises all participants with AKI (G1+G2).

RESULTS

- Analysis of urine-derived SARS-CoV-2 spike sequences from seven COVID-19 ICU participants revealed that deletions and mutations of the SARS-CoV-2 furin-cleavage site at position 686 and neighboring amino acids were the predominant mutations observed in urine.
- SARS-CoV-2 VOC with a deletion in the furin-cleavage site demonstrated a replicative advantage in renal podocytes.
- We found that COVID-19 ICU participants with AKI and viral shedding in urine had higher levels of IL-6, IL-8, IL-18, and MCP-1 compared to the other groups.
- All the COVID-19 ICU participants with AKI showed increased levels of TIMP-1, Collagen IV, TFF-3, Lipocalin-2, Albumin and cystatin C compared to the other groups.
- scRNAseq analysis of urine-derived cells revealed higher shedding of renal tubular cells, podocytes, macrophages, and T cells in G1:ICU/AKI/Virus compared to other groups.
- Preliminary pathway analysis of tubular cells showed enrichment of cytokine, VEGFA, and neutrophil-mediated signaling in the G1 vs. G4 and G5 (G1+G2) vs. G4 comparisons.
- Neutrophil-mediated immunity and cytokine-mediated signaling pathways were enriched in the MO cluster of all the groups irrespective of AKI or severity of disease.

CONCLUSIONS

- Our study demonstrates SARS-CoV-2 viral shedding in the urine of participants with severe COVID-19 and identified unique inflammatory signatures that discriminate people with severe COVID-19, AKI, and viral shedding in urine from participants with mild symptoms.

ACKNOWLEDGEMENTS

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