SCIENCE CHINA
Life Sciences

•LETTER TO THE EDITOR•

July 2022 Vol.65 No.7: 1473–1476
https://doi.org/10.1007/s11427-021-2091-0

Metatranscriptomic analysis of host response and vaginal microbiome of patients with severe COVID-19

Meng Xiao[1,2†], Bo Lu[3,4†], Rui Ding[1,2], Xia Liu[5], Xian Wu[1,2], Yaqian Li[6], Xudong Liu[6], Lin Qiu[5], Zhibo Zhang[5], Jing Xie[7], Yu Chen[1,2], Dong Zhang[1,2], Liting Dong[3,4], Meiling Zhang[3], Jinying Peng[3], Hua Yang[5], Timothy Kudihna[8,9], Yingchun Xu[1,2], Taisheng Li[7], Chengqi Yi[3,4*] & Lan Zhu[5*]

[1]*Department of Laboratory Medicine, State Key Laboratory of Complex Severe and Rare Diseases, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing 100730, China;*
[2]*Beijing Key Laboratory for Mechanisms Research and Precision Diagnosis of Invasive Fungal Diseases, Beijing 100730, China;*
[3]*State Key Laboratory of Protein and Plant Gene Research, School of Life Sciences, Peking University, Beijing 100871, China;*
[4]*Peking-Tsinghua Center for Life Sciences, Peking University, Beijing 100871, China;*
[5]*Department of Obstetrics and Gynecology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing 100730, China;*
[6]*Medical Science Research Center, Peking Union Medical College Hospital, Chinese Academy of Medical Science and Peking Union Medical College, Beijing 100730, China;*
[7]*Department of Infectious Diseases, Peking Union Medical College Hospital, Chinese Academy of Medical Science and Peking Union Medical College, Beijing 100730, China;*
[8]*School of Biomedical Sciences, Charles Sturt University, Orange NSW 2800, Australia;*
[9]*NSW Health Pathology, Regional and Rural, Orange Hospital, Orange NSW 2800, Australia*

Received January 23, 2022; accepted March 12, 2022; published online April 14, 2022

Citation: Xiao, M., Lu, B., Ding, R., Liu, X., Wu, X., Li, Y., Liu, X., Qiu, L., Zhang, Z., Xie, J., et al. (2022). Metatranscriptomic analysis of host response and vaginal microbiome of patients with severe COVID-19. Sci China Life Sci 65, 1473–1476. https://doi.org/10.1007/s11427-021-2091-0

Dear Editor,

The pandemic of coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has become a global public health threat. Here we use a TRACE-seq-based metatranscriptomic analysis to compare host responses and vaginal microbiome of postmenopausal female patients with underlying severe COVID-19 disease with those of healthy females, thereby providing insights into the changes in the microenvironment of women's reproductive system.

This study comprised 16 postmenopausal women diagnosed with COVID-19 and admitted to the intensive care unit (ICU) at hospitals in Wuhan, China, between February 4 and March 29, 2020, and 5 women volunteers with no COVID-19 as a healthy control group. Total nucleic acids were extracted from vaginal samples of the 21 individuals studied, and metatranscriptomic analysis was carried out using TRACE-seq (Lu et al., 2020). For host transcriptional profiling, multidimensional scaling (MDS) and differential gene expression analysis were performed, with significantly upregulated genes subjected to Gene Ontology (GO) enrichment analysis. Microbial taxonomic classification was performed on complete bacterial, fungal and human viral genomes from the NCBI RefSeq database, and principal coordinate analysis (PCoA) was also performed. The expression profile of antimicrobial resistance genes was evaluated against the Com-

†Contributed equally to this work
*Corresponding authors (Chengqi Yi, email: chengqi.yi@pku.edu.cn; Lan Zhu, email: zhu_julie@vip.sina.com)

prehensive Antibiotic Resistance Database (CARD). Detailed methodology for the whole metatranscriptomic analysis process is given in Files S1 and S2 in Supporting Information. The raw sequence data were deposited into the China National Microbiology Data Center with accession numbers NMDC40003555 to NMDC40003575.

Characteristics of the 16 COVID-19 patients are summarized in File S3 in Supporting Information. All the patients had severe/critical type COVID-19, and the common coexisting diseases were hypertension (8/16, 50.0%), diabetes (5/16, 31.3%), and coronary heart disease (3/16, 18.8%).

The proportion of reads mapped to human, viruses, bacteria, and fungi for individual samples are summarized in File S4A in Supporting Information. For host transcriptome analysis, an average of 8,919 human genes with FPKM>1 were detected per sample (File S4B in Supporting Information). The initial inspection of human gene expression data using an MDS plot clearly segregated COVID-19 patient samples from those of the healthy individuals, with one COVID-19 patient (PT02) being identified as an outlier (Figure S4C in Supporting Information). In the rest of the 15 COVID-19 patients studied, 200 differentially expressed genes were identified, 140 of which were upregulated (File S4D in Supporting Information). GO enrichment analysis identified the following top most upregulated biological processes in COVID-19 patients: defense response, immune response, response to other organisms, cytokine production, response to biotic stimulus, cellular response to cytokine stimulus, regulation of cytokine production, etc., among others (Figure S4E in Supporting Information). In contrast, the top most downregulated biological processes included biological adhesion, membrane organization, and establishment of protein localization to the membrane (File S4F in Supporting Information).

When specifically focusing on immune response related to upregulated genes in the COVID-19 patients, we observed a series of upregulated genes involved in inflammatory responses (*IL1A*, *CXCL8*, *CXCL2*, *IL36A*, *IL36G*, *IL36RN*, *IL1RN*, *ECM1*, *TGFB1*, *ANXA1*, *FPR1*, *TNFAIP3*, *TICAM1*, *KRT16*, *NFKBIZ*, *PROK2*, *HMOX1*, *S100A12*, *S100A8*). In addition, a series of genes related to the cellular response to cytokine stimulus (*IFITM1*, *IFITM2*, *IFITM3*, *EREG*, *NFKBIA*, *PELI1*, *ANXA1*, *NFIL3*, *ZFP36*, *IL1R2*, *IL1RN*, *CXCL8*, *CXCL2*, *IL1A*, *IL36A*, *IL36G*, *IL36RN*, *FPR1*, *TGFB1*, *HMOX1*) were also upregulated. Furthermore, a series of genes involved in innate immune response (*CRISP3*, *TGFB1*, *NCF2*, *SH2D1B*, *SIRPB1*, *SEC14L1*, *RNASE7*, *PRDM1*, *IFITM1*, *IFITM2*, *IFITM3*, *ANXA1*, *TICAM1*, *IL36A*, *IL36G*, *IL36RN*, *KRT16*, *S100A8*, *S100A12*), were also upregulated. Also noteworthy was the upregulation of a series of genes involved in the host's response to bacteria (*BCL3*, *ADM*, *TGFB1*, *CXCL8*, *TNFAIP3*, *TICAM1*, *CXCL2*, *NFKBIA*, *ZFP36*, *RNASE7*, *PELI1*, *S100A12*, *S100A8*) (Figure 1A and File S4G in Supporting Information).

We also noted upregulation of *IL-1* superfamily cytokines, including *IL-1* subfamily cytokines *IL1A* and *IL1RN* and *IL-36* subfamily cytokines. Whereas the IL-1 pathway has previously been reported to be associated with COVID-19 (Shin et al., 2020), we report here for the first time that the IL-36 pathway is also potentially associated with COVID-19. It was previously suggested that the IL-36 pathway is linked to the pathogenesis of skin diseases and is involved in human defense and immune responses to genital infections by bacterial and viral pathogens in women (Gardner et al., 2020; Ngo et al., 2021). Considering that epithelial cells are predominantly responsible for secreting the *IL-36* cytokines (Gardner et al., 2020), and in light of the findings of this study, we propose that the reproductive tract epithelial cells play an important role in defense and immune responses in women with COVID-19 disease.

The IL-36 pathway could activate dendritic cells and may play a role in polarizing T-helper cell responses (Ngo et al., 2021). By binding to *IL1RL2*/*IL-36R* receptors, *IL36A* and *IL36G* could activate NF-κB signaling pathways in target cells linked to a proinflammatory response. Our findings also indicate activation of NF-κB signaling pathway in COVID-19 patients' reproductive systems, as the level of expression of the genes *NFKBIA*, *TICAM1*, *CXCL8*, *CXCL2*, *BCL2A1*, and *TNFAIP3* was higher in COVID-19 patient samples than in those of the healthy controls. NF-κB serves as a primary transcription factor regulating various cellular responses. Thus activation of the NF-κB pathway could promote the expression of cytokines, chemokines, adhesion molecules, phase proteins, and inducible effector enzymes and may trigger a cytokine storm that is associated with increased severity and mortality of COVID-19 (Shin et al., 2020). Therefore, inhibiting activation of the NF-κB pathway, directly or indirectly, has been suggested as a potential treatment route for COVID-19 (Shin et al., 2020). We also observed that Type I interferon signaling pathway genes were upregulated, with a high-level expression of *IFITM1*, *IFITM2*, and *IFITM3* genes in the vaginal samples of COVID-19 patients. The role of Type I interferons in the development of severe COVID-19 disease has been validated and it has also been confirmed that both NF-κB and Type I interferon signaling pathways are regulated by a papain-like protease (Shin et al., 2020). Therefore, we propose that the IL-1/IL-36 pathway and downstream NF-κB pathway, as well as Type I interferon signaling pathways, are essential in the female reproductive system's response to infection.

To date, the effect of COVID-19 on the vaginal microbiome of women has not been investigated. As per metatranscriptomic data from this study, no SARS-CoV-2 was detected from the vaginal fluid of women with COVID-19. However, notable differences in the vaginal microbiome were observed between the COVID-19 patient group and the

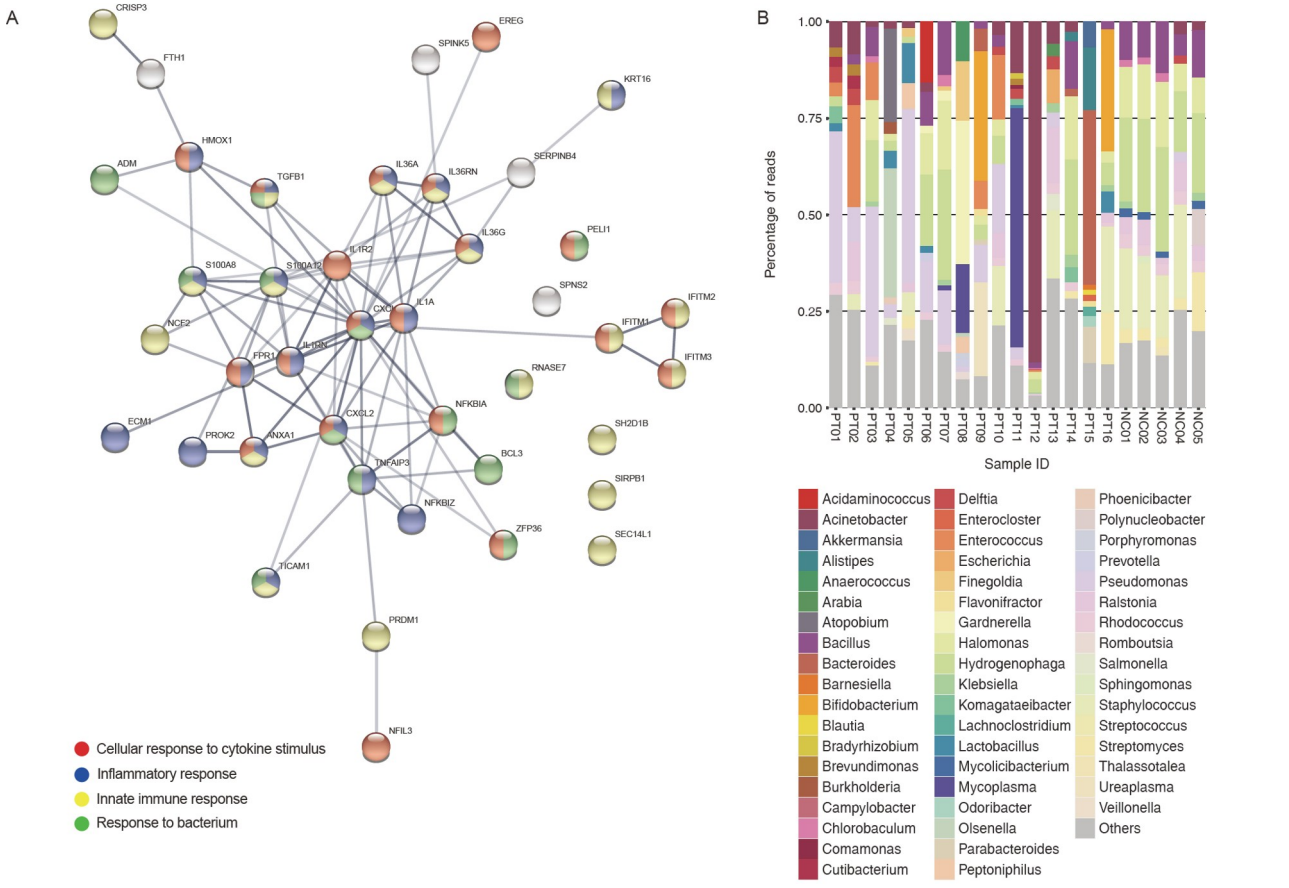

Figure 1 TRACE-seq-based metatranscriptomic analysis of vaginal samples. A, STRING analysis of immune response-related upregulated genes (Red: genes involved in "cellular response to cytokine stimulus" [GO:0071345]; Blue: genes involved in "inflammatory response" [GO:0006954]; Yellow: genes involved in "innate immune response" [GO:0045087]; Green: genes involved in "response to bacterium" [GO:0009617]). B, relative abundance of the 10 most abundant bacterial genera in the vagina.

healthy control group. Generally, bacterial and fungal transcripts accounted for only 1.3% and 1.0% of the total transcriptomic data, respectively, among the healthy controls, with insignificant intra-individual differences (bacteria 1.2%–1.5%, fungi 0.8%–1.1%) (File S4A in Supporting Information). Rarefaction analysis was conducted at the genus level, and the rarefaction curves of all patient samples, except PT14, reached a plateau (File S5A in Supporting Information), suggesting that sequencing depth was adequate for analysis. Compared with the healthy control group, the proportions of bacterial and fungal transcripts in the COVID-19 group were significantly higher (bacteria 19.9% and fungi 2.4%) and varied among individuals (bacteria 1.0%–84.3%, fungi 0.2%–5.6%) (File S4A in Supporting Information). PCoA was performed based on the relative abundance of the bacterial microbiome at the genus level. It was observed that COVID-19 patients harbored a vaginal microbiome that was significantly different from that of the healthy controls, whereas changes in mycobiome was insignificant between the two groups (File S5B in Supporting Information).

Further investigation into the abundant bacterial genera detected in each sample revealed that the intragroup heterogeneity of vaginal microbiota structure among COVID-19 patients was also higher than that among healthy individuals (Figure 1B). In addition, vaginal dysbacteriosis was more frequent in COVID-19 patients, with bacteria causing vaginosis or nosocomial infections being much more abundant. For instance, an exceptionally high frequency of the following microbes, previously reported as bacterial vaginosis (BV) pathogens (Park et al., 2021), was observed in 6/16 (37.5%) COVID-19 patients: *Mycoplasma* spp. (61.8% in patient PT11 and 17.8% in patient PT08, with >99% reads matched to *Mycoplasma hominis*), *Gardnerella* spp. (37.1% in patient PT08, all matched to *Gardnerella vaginalis*), *Ureaplasma* spp. (24.3% in patient PT09, with >99% matched to *Ureaplasma urealyticum*), *Bacteroides* spp. (45.2% in patient PT15) and *Atopobium* spp. (24.2% in patient PT04), whereas none were detected in the healthy control group. *Enterococcus* spp., a bacterium that is associated with aerobic vaginitis (AV) (Donders et al., 2017), was also highly abundant in one COVID-19 patient (16.8% in PT10, >90% matched to *Enterococcus faecium*). In addition, *Acinetobacter* spp. were exceptionally high in 3/16 patients (88.4% in patient PT12, 13.4% in patient PT11, and 6.8% in patient PT01,

with >90% matched to *Acinetobacter baumannii* complex, a major nosocomial pathogen) (Peleg et al., 2008).

As shown in Figure S5C in Supporting Information, more antimicrobial resistance genes were expressed in COVID-19 patient samples than in the healthy controls. Genes conferring resistance to beta-lactam antimicrobials, including cephalosporin, penicillin, penem, and monobactam, were highly abundant in 87.5% (14/16) of COVID-19 patients. In addition, genes conferring resistance to tetracycline, aminoglycoside, macrolide, fluoroquinolone, phenicol, rifamycin, diaminopyrimidine, aminocoumarin, lincosamide, cephamycin, and carbapenem drugs, were also expressed at noticeably high levels in one (6.3%) to seven (43.8%) COVID-19 patient samples whereas none of these were overexpressed in the healthy controls.

The unfavorable microbiome changes in the COVID-19 patients might be attributed to immunosuppression (Fajgenbaum and June, 2020) or the administration of a broad range of antimicrobials during their stay in ICU (Ferrer et al., 2017) or both. Furthermore, it has been previously reported that the vaginal microbiome of menopausal women is distinct from that of the premenopausal population. This difference in vaginal microbiome has been attributed to the decline in estrogen secretion, which leads to vaginal atrophy and decreased glycogen levels, and the depletion of lactobacilli that protect women from adverse gynecological sequelae (Gliniewicz et al., 2019). This may make postmenopausal COVID-19 women patients more vulnerable than premenopausal women.

The study has several limitations. First, we primarily focused on the impact of COVID-19 on women's reproductive system in a critically ill subpopulation, which can be considered a bias. It would have been desirable to also include a subgroup of COVID-19 patients with milder symptoms. Secondly, due to the rapid control of the COVID-19 epidemic in China, only 16 women with COVID-19 were enrolled, which limited the statistical power of the study. Thirdly, all the subjects used in this study were elderly women, which prohibits the application of our results to other women population age groups. Fourthly, our study was not conclusive on whether the observed activation of the host immune responses was due to systematic immune response to SARS-CoV-2 or alteration of the vaginal microbiota, as there are also reports on upregulation of *IL36*G in women with BV (Gardner et al., 2020). We recommend that future studies use a large sample size of patients and controls, including women in other age groups and those with normal and mild symptoms, as well as the asymptomatic ones.

Compliance and ethics *The authors have filed a patent application related to TRACE-seq (patent application number: 202011520074.X).*

Acknowledgements *This work was supported by the National Key Research and Development Program of China (2020YFC0861000), Beijing Nova Program (Z201100006820127), International Innovation Resource Cooperation Project, Beijing Municipal Science and Technology Commission (Z201100008320024), the National Natural Science Foundation of China (31861143026, 91940304 and 21825701) and Epidemic Prevention and Control Special Project, Peking University. The funders played no role in study design, data collection, analysis, interpretation, report writing, or decision to submit the paper for publication. The authors would like to thank Vazyme Biotech in Nanjing, China, for assisting in library procedure optimization and providing library preparation kits. In addition, the authors would like to thank National Center for Protein Sciences at Peking University in Beijing, China, for assistance with experiments. Part of the analysis was performed on the High Performance Computing Platform of the Center for Life Science (Peking University).*

References

Donders, G.G.G., Bellen, G., Grinceviciene, S., Ruban, K., and Vieira-Baptista, P. (2017). Aerobic vaginitis: no longer a stranger. Res Microbiol 168, 845–858.

Fajgenbaum, D.C., and June, C.H. (2020). Cytokine storm. N Engl J Med 383, 2255–2273.

Ferrer, M., Méndez-García, C., Rojo, D., Barbas, C., and Moya, A. (2017). Antibiotic use and microbiome function. Biochem Pharmacol 134, 114–126.

Gardner, J.K., Łaniewski, P., Knight, A., Haddad, L.B., Swaims-Kohlmeier, A., and Herbst-Kralovetz, M.M. (2020). Interleukin-36γ is elevated in cervicovaginal epithelial cells in women with bacterial vaginosis and *in vitro* after infection with microbes associated with bacterial vaginosis. J Infect Dis 221, 983–988.

Gliniewicz, K., Schneider, G.M., Ridenhour, B.J., Williams, C.J., Song, Y., Farage, M.A., Miller, K., and Forney, L.J. (2019). Comparison of the vaginal microbiomes of premenopausal and postmenopausal women. Front Microbiol 10, 193.

Lu, B., Dong, L., Yi, D., Zhang, M., Zhu, C., Li, X., and Yi, C. (2020). Transposase-assisted tagmentation of RNA/DNA hybrid duplexes. eLife 9, e54919.

Ngo, V.L., Kuczma, M., Maxim, E., and Denning, T.L. (2021). IL-36 cytokines and gut immunity. Immunology 163, 145–154.

Park, S.Y., Lee, E.S., Lee, S.R., Kim, S.H., and Chae, H.D. (2021). Vaginal microbiome is associated with vulvodynia, vulvar pain syndrome: a case-control study. Sex Med 9, 100314.

Peleg, A.Y., Seifert, H., and Paterson, D.L. (2008). *Acinetobacter baumannii*: emergence of a successful pathogen. Clin Microbiol Rev 21, 538–582.

Shin, D., Mukherjee, R., Grewe, D., Bojkova, D., Baek, K., Bhattacharya, A., Schulz, L., Widera, M., Mehdipour, A.R., Tascher, G., et al. (2020). Papain-like protease regulates SARS-CoV-2 viral spread and innate immunity. Nature 587, 657–662.

SUPPORTING INFORMATION

The supporting information is available online at https://doi.org/10.1007/s11427-021-2091-0. The supporting materials are published as submitted, without typesetting or editing. The responsibility for scientific accuracy and content remains entirely with the authors.