Casual effects of gut microbiota on the severity of COVID-19: a two-sample Mendelian randomization and a meta-analysis

Zheng Wang[1], Aimin Jiang[1], Demeng Xia[1], Peng Luo[2], Xinxin Gan[1], Renqi Yao[3], and Linhui Wang[1]

[1]Changhai Hospital
[2]Zhujiang Hospital
[3]Chinese PLA General Hospital

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Aimin Jiang[1+], Zheng Wang[1+], Demeng Xia[2+], Peng Luo[3], Xinxin Gan[1], Renqi Yao[4,5*], Linhui Wang[1*]

[1] Department of Urology, Changhai Hospital, Naval Medical University, Shanghai 200433, China

[2] Department of Orthopedics, Changhai Hospital, Naval Medical University, Shanghai, China

[3] Department of Oncology, Zhujiang Hospital, Southern Medical University, Guangzhou 510260, China.

[4] Translational Medicine Research Center, Fourth Medical Center and Medical Innovation Research Division of the Chinese PLA General Hospital, Beijing 100048, China

[5] Department of Burn Surgery, Changhai Hospital, Naval Medical University, Shanghai 200433, China

[+] Aimin Jiang, Zheng Wang, and Demeng Xia contributed equally to this work.

Correspondence:

Linhui Wang, MD, PhD, Department of Urology, Changhai Hospital, No.168 Road, Yangpu District, Shanghai 200433, People's Republic of China. Tel: (+86) 13901635510; Email:*wanglinhui@smmu.edu.cn*

Renqi Yao, MD, Translational Medicine Research Center, Medical Innovation Research Division and Fourth Medical Center of the Chinese PLA General Hospital, 28 Fuxing Road, Haidian District, Beijing 100853, People's Republic of China. Tel: (+86) 18501689629; Email:*yaorenqixx1995@163.com.*

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Dear Editor:

Since gut represents the largest immunological organ and its resident microbiota are well-characterized to significantly orchestrate host immune responses, there were a growing interest in interrogating the potential impact of the gut microbiota on host susceptibility to SARS-CoV-2. Various studies have confirmed that alterations in gut microbiota composition are significantly associated with COVID-19 progression and severity of illness.[1,2] However, these studies to date have not completely eliminated confounding factors, thereby establishing a definitive causal relationship between COVID-19 and the gut microbiota. Mendelian randomization (MR), as a statistical methodology utilizing genetic variations as instrumental variables, has

increasingly adopted as a promising tool for revisiting causal relationships between exposures and outcomes, with the controlling for confounding factors. MR studies can be conducted by using large-scale genetic data from biobanks, which can provide high statistical power and reduce the potential for bias. Mendelian randomization has been leveraged to investigate the causal effects of multiple influential factors on outcomes and severity of COVID-19.[3-6] These findings hold tremendous potential to inform public health policies and interventions aimed at mitigating the burden of COVID-19.

This study aims to explore the directional relationship between gut microbiota and the severity of COVID-19 using MR method. The genetic variants correlated with exposure, the gut microbiota composition detected in fecal sample, were sourced from the largest genome-wide meta-analysis to date, conducted by the MiBioGen consortium.[7] The GWAS data of COVID-19 patients were obtained from the latest r7 version based on COVID-19 Host Genetics Initiative data (HGI) (*https://www.covid19hg.org/results/r7/*). According to the HGI standard, the outcomes were stratified into three distinct categories (mild, moderate and severe). In MR analysis, various methods were applied, including inverse-variant weighted, MR-Egger, weighted mode, weighted median, and simple median. Only SNPs with a significant threshold ($p < 5\times10\text{-}8$) were considered for instrumental variable selection. Pleiotropy and heterogeneity were assessed *via* MR-Egger, Cochran's Q test and "Leave-one-out" sensitivity analysis (Figure 1). All data used in the current study were publicly accessible.

The results of MR analysis revealed a causal relationship between certain genera of gut microbiota and the severity of COVID-19, as shown in **Table 1** . Notably, increased composition of *Dorea* at genetic level was positively correlated with all outcomes, while*Bifidobacterium* was related to mild and moderate outcomes, and the *Rikenellaceae RC9 gut group* was linked to moderate and severe outcomes (Supplementary Figure 1). After performing sensitivity analysis, we have demonstrated that the causal pathway was not influenced by any potentially influential SNPs, thereby validating the robustness of our findings. Additionally, MR-Egger was performed as well, in which no potentially significant horizontal pleiotropy or outliers were detected (Supplementary Figure 2-5). To further summarize the evidence and verify the results of MR, we conducted a meta-analysis incorporating the original data included in our study. The overall effect sizes were quantified and no significant evidence of heterogeneity was found (Supplementary Figure 6).

Since previous studies concerning the relationship between gut microbiota and COVID-19 susceptibility were limited by a lack of causal relationships, small sample sizes, insufficient evidence due to the timing of fecal sample collection and inability to rule out confounding factors, our study represents the first time that MR has been utilized to confirm the causal impact of specific genera of gut microbiota on the severity of COVID-19. Further investigation into the specific bacteria associated with severity of illness among COVID-19 patients is also warranted. For instance, *Dorea* and *Bifidobacterium* are crucial producers of short-chain fatty acids (SCFAs), which have been found to have potent immunomodulatory properties, along with the ability in mediating $CD8^+$ T cell immune response.[8] Elevated levels of *Rikenellaceae RC9 gut group* have been shown to modulate TLR signaling pathways, thereby increasing the susceptibility of the gut to inflammation.[9] *Bacteroides* play a role in regulating host immunity and suppressing colonic ACE-2 expression. Furthermore, an inverse correlation has been observed between*Bacteroides* levels and the severity of COVID-19 infection.[1] An increased abundance of intestinal*Prevotella* is associated with adverse outcomes in several inflammatory disorders, such as rheumatoid arthritis, ankylosing spondylitis, as well as systemic immune activation in HIV patients.[10] Hence, exploring the microbiota that is intricately linked with COVID-19 has the potential to unravel the exact pathophysiological mechanisms that underpin SARS-CoV-2 infection.

In conclusion, our results implied that targeted screening of gut microbiota compositions in fecal samples for susceptible populations could become an effective yet feasible strategy for preventing moderate to severe cases of COVID-19, paving the way for more personalized treatments.

Abbreviations

ACE-2: angiotensin-converting enzyme 2

COVID-19: Coronavirus Disease 2019

GWAS: Genome-Wide Association Study

MR: Mendelian randomization

RR: risk ratio

SARS-CoV-2: severe acute respiratory syndrome coronavirus 2

SCFA: short-chain fatty acids

SNP: single nucleotide polymorphism

TLR: Toll-like receptors

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Availability of data and materials

All original data were publicly available. The COVID-19 data were obtained from*https://www.covid19hg.org/results/r7/*. The full GWAS statistics of gut microbiota were downloaded from*https://mibiogen.gcc.rug.nl/*.

Competing interest

We declare no competing interests.

Authors' contribution:

All authors contributed to the study concept. LHW designed and supported the study. AMJ analysed the data. ZW and DMX visualized the data and drafted this letter. PL, XXG and RQY revised the manuscript. All authors reviewed and approved the final manuscript.

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Table 1 Results of MR analysis

Figure 1. Flow chart of the study

The flow chart illustrates the causal relationship between gut microbiota and severity of COVID-19 by utilizing genetic variants and Mendelian randomization.

Supplementary Figure 1. Venn diagram

The Venn diagram shows the interactions between different gut microbiota and the severity of COVID-19. The circles represent mild COVID-19 (blue circle), moderate COVID-19 (yellow circle) and severe COVID-19 (red circle). The numbers in the circles represent the types of gut microbiota. The overlapping areas between the circles represent the number of studies in which the same type of gut microbiota was simultaneously involved in different degrees of COVID-19 infection.

Supplementary Figure 2. Scatter plot for correlation between gut microbiota and severity of COVID-19

The plot shows the relationship between gut microbiota (y-axis) and severity of COVID-19 (x-axis) represented by each point. The black point represents the type of gut microbiota, and the colors of the line represent the different methods of Mendelian randomization.

Supplementary Figure 3. Forest plot for effect size of each SNP

The plot shows the effect size of each SNP (y-axis) on the severity of COVID-19 (x-axis) represented by each study. The risk ratio (RR) is depicted by the vertical lines.

Supplementary Figure 4. Funnel plot for the risk ratio of COVID-19 severity associated with gut microbiota

The plot shows the relationship between gut microbiota (y-axis) and severity of COVID-19 (x-axis) represented by each point. The risk ratio (RR) is depicted by the vertical lines. (SE: standard error)

Supplementary Figure 5. Leave-one-out sensitivity analysis

The leave-one-out analysis showed no significant heterogeneity among the SNPs.

Supplementary Figure 6. Forest plot for meta-analysis.

Certain types of gut microbiota were associated with different degrees of COVID-19 infection. (A *Dorea* , B *Bifidobacterium* , C*Rikenellaceae RC9 gut group*)

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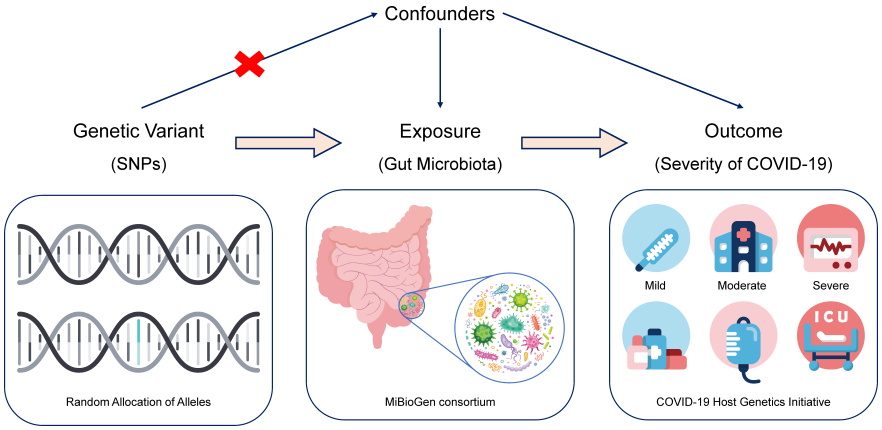

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`Table 1.docx` available at https://authorea.com/users/616221/articles/642297-casual-effects-of-gut-microbiota-on-the-severity-of-covid-19-a-two-sample-mendelian-randomization-and-a-meta-analysis