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Systematic Mendelian randomization reveals the causal roles of the microbiome on the risk of Parkinson's disease

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Abstract. Parkinson's Disease (PD) is a neurodegenerative disorder whose etiology is not fully understood. The human gut microbiome, a complex community of microorganisms residing in the digestive tract, has recently emerged as a potential factor influencing various health conditions, including neurodegenerative diseases. The causal involvement of the human gut microbiome in Parkinson's disease (PD) remains elusive, primarily due to the challenge of distinguishing causation from mere correlation, and the presence of contradictory findings in existing research. The relationship between the gut microbiome and PD was assessed using a two-sample Mendelian randomization (MR) approach. Assessing the impact of microbial traits using data from independent genome-wide association studies (GWAS) (comprising 2,259 samples) on PD within a cohort of 15,056 cases and 449,056 controls. We performed multiple sensitivity analyses to validate our findings. Preliminary MR analysis indicated that modifications in the bacterial composition of the *Firmicutes* and *Proteobacteria* phyla are associated with changes in PD risk. Additionally, bacteria in other genera also play a role in this causal association. We provide a comprehensive discussion of the results obtained from our MR analysis and highlight the distinctions between our study and prior research efforts.

Keywords: Microbiome, Parkinson's disease, Mendelian randomization (MR)

1. Introduction

Parkinson's disease (PD) is a common neurodegenerative disorder characterized by tremor, stiffness of movement, and a unique gait [1, 2]. Research shows that the prevalence of PD in people over 60 years old is 1.37% (95% confidence interval 1.02%-1.73%) [3]. It can be estimated that the number of PD patients in China may be up to 3.62 million. So it is necessary to identify new modifiable risk factors to prevent and treat the disease.

Much evidence shows that the human intestinal microbiome is very important to human health [4]. New research shows a link between the complexity and diversity of our gut microbiome and Parkinson's disease. The microbiome and the brain communicate with each other. The gut microbiome has been identified as the gut-brain function's key regulator. Many biological mechanisms exist that could potentially explain how the gut microbiome affects the underlying biology and physiology of age-related, psychiatric, neurodevelopmental, and neurodegenerative diseases.

Research shows that people diagnosed with Parkinson's disease often exhibit an imbalance in the composition of their gut microbiota. More than 30% of tested genes, species, and pathways show altered abundance in PD individuals, and these gut microbiota are associated with various pathways associated

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with PD development [5] . Notably, the findings in this case primarily established correlations, which makes establishing causality challenging. In animal models, humanized mice transplanted with Parkinson's disease-associated microbiota had worsened motor symptoms compared with healthy controls. The results suggest that alpha-synuclein (αSyn) overexpression and dysbiosis interact to influence disease outcome in mice [6] . However, whether specific microbial species play a role in the disease process remains unknown.

Mendelian randomization (MR) is a method of causal inference. It operates on the fundamental principle of inferring the impact of biological factors on disease by leveraging the influence of naturally assigned genotypes on phenotypes [7]. In MR, researchers analyze data from Genome-Wide Association Studies (GWAS) to pinpoint specific genetic variations, commonly referred to as Single Nucleotide Polymorphisms (SNPs). These SNPs serve as instrumental variables that are linked to biological traits of interest. Utilizing these instrumental variables, researchers can then estimate the causal impact of these biological traits on disease outcomes, thereby providing a more robust understanding of disease etiology. Since SNPs are randomly assigned and unaffected by confounding factors, employing genetic variation as a means to investigate causality helps to mitigate potential confounders and enhance the reliability of causal inference [8]. By applying the knowledge gained from GWAS in human genetics, we can obtain more precise evaluations of the makeup and variability of our microbiome. Additionally, this knowledge allows us to gain valuable insights into its functional capabilities, particularly in relation to the host and the gut-brain axis [4]. It is important to highlight that the microbiome is remarkably diverse, constantly changing, and highly responsive to external factors. These characteristics make it a promising target for potential therapeutic interventions. However, in order to investigate innovative microbiome-based therapies, establishing causal relationships is crucial. Therefore, an increasing number of researchers are using MR to examine the impact of gut microbiome variation on various health outcomes.

This article examines the properties of two-sample MR analysis of genetic effects based on host-microbiome relationships to investigate potential causal relationships between human gut microbiome variation and Parkinson's disease risk.

2. Methods

2.1. Gut microbiome GWAS data and instrument selection.

The dataset comprises 16S sequencing data from 2,259 samples in the Flemish Gut Flora Project, encompassing 152 microbial trait genome-wide association analyses (EGA European Genome-Phenome Archive) [9]. Significant SNPs were identified from the pooled GWAS data using screening criteria ($p < 5\times10^{-7}$, linkage disequilibrium $r^2 = 0.1$) to ensure SNP independence and mitigate the impact of genetic pleiotropy on the results.

2.2. Parkinson's disease GWAS data.

Parkinson's disease GWAS data consisted of 15,056 European ancestry cases, 18,618 European ancestry proxy cases, and 449,056 European ancestry controls obtained from the European Bioinformatics Institute [10].

2.3. Mendelian randomization methods.

This article uses the inverse variance weighting method. The IVW method analyzes effect estimates for all SNPs [11]. Using multiple SNPs with relaxed p-value thresholds in MR analyzes increases the likelihood of weak instrument bias and introduces horizontal pleiotropic pathways between SNPs and outcomes [12].

The MR-Egger regression method was applied to test for horizontal pleiotropy and the effect estimates were compared with those obtained by the IVW method. The MR-Egger regression slope between multiple SNP outcomes and SNP exposure associations can be viewed as an unbiased causal effect between exposure (microbial signature) and outcome (PD), assuming any level of pleiotropic

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effects versus SNP exposure effects [13, 14].This article uses maximum likelihood (ML) to estimate probability distribution parameters [15].The TwoSampleMR R package (version 0.5.7) is used for analyse and data visualization.

2.4. Sensitivity analysis.

Horizontal pleiotropy. Horizontal pleiotropy, a potential source of bias in causal effect estimation using the gIVW method, can be effectively identified using the intercept gEgger regression. This statistical approach provides an estimate of the directional pleiotropic effect [16].

Heterogeneity. When multiple independent risk variants coexist within a locus, the amalgamation of these signals may relocate the primary association from causal variants to a nearby non-causal variant [17]. Such shifts can also arise due to variations in the quality of variant genotype imputation, leading to fluctuations in association signal statistics among adjacent variants in linkage disequilibrium [18].

Directionality test. To mitigate MR signals influenced by reverse causality, we employed the MR Steiger test [19], a method designed to assess the directionality of the causal effects estimated through MR. Subsequently, we eliminated all MR signals displaying reverse directionality.

3. Results

3.1. Forward MR reveals gut bacteria that causally affect PD risk.

Using Mendelian Randomization (MR) analyses, we found 6 bacterial species that could potentially affect PD risk through a causal path. For example, higher bacterial abundance in the Coriobacteriaceae family was associated with a slightly increased risk of Parkinson's disease, approximately 1% for each standard deviation (SD) increase in bacterial abundance (odds ratio [OR]: 1.02). Likewise, each SD increase in Firmicutes bacteria was associated with a 3% increased risk of PD (OR: 1.03). Each SD increase in the unclassified group and other bacteria within the phylum Proteobacteria was associated with a 5% and 2% lower risk of PD, respectively (OR: 0.95 and 0.98).

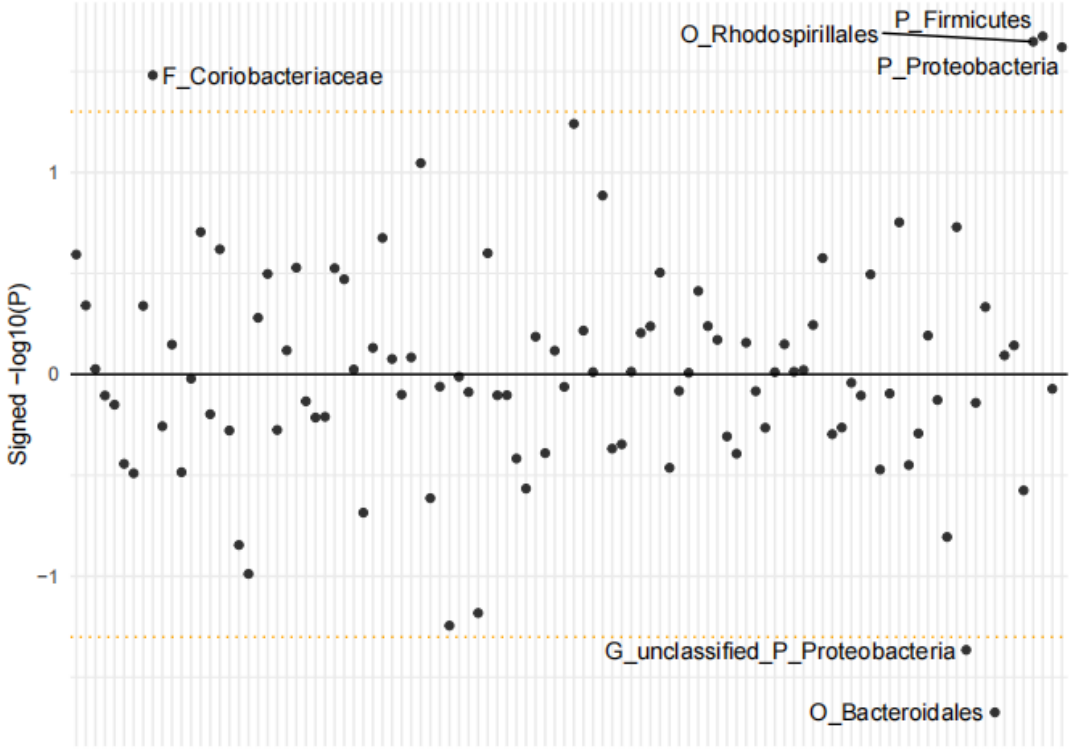

Figure 1. The letters in the name of a microbial trait represent the classification level of the microbial trait, where "F", "G", "O" and "P" represent "family", "genus", "order" and "phylum".

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3.2. Effects of PD on gut microbial traits.

Alterations in the abundance of bacteria were observed in various groups, including Bacteroides, Coprococcus, Fusicatenibacter, Sutterella, as well as families such as Peptostreptococcaceae and Sutterellaceae, with marginal changes of per standard diviation in a range of up and down 10% (OR range: 0.90–1.10) in PD patients. Conversely, bacteria in other groups (Aestuariispira, Faecalitalea, Lactobacillus, and Prevotella) and the Gammaproteobacteria class exhibited more pronounced responses to PD. Notably, bacteria in the Collinsella group, an unclassified group under the Rhodospirillaceae family, and the Alphaproteobacteria class were significantly affected by PD.

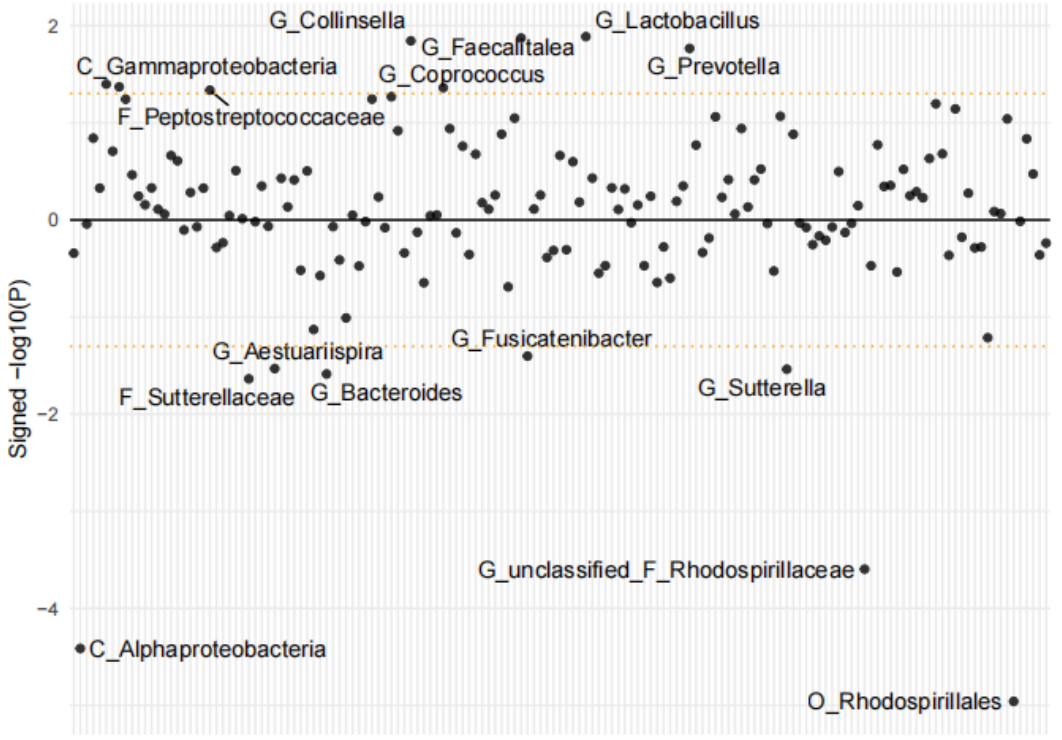

Figure 2. The letters in the name of a microbial trait represent the classification level of the microbial trait, among which “C”, “F”, “G”, “O” and “P” represent “class”, “family”, “genus”, “order” “ and “phylum.”

4. Discussion

The MR results allow us to identify numerous microbiota that either influence or are influenced by Parkinson’s disease. Our findings partially overlap with those of previous studies, showing some fluctuation in certain microbiota groups, which introduces a degree of contradiction in relation to prior research.

A study utilizing 16S rRNA gene sequencing demonstrated a clear decrease in both the abundance and diversity of microbiota in MPTP-treated mice. Specifically, the abundance of P. *Coriobacteriaceae* was markedly reduced [20]. However, another study employing next-generation sequencing examined 64 Italian patients with Parkinson’s disease, revealing a significant increase in the presence of *Coriobacteriaceae* in their gut microbiota composition [21]. A study conducted in China utilized fecal samples from 20 patients and selected the V4 region of 16S ribosomal ribonucleic acid for high-throughput sequencing analysis. This study also proposed the concept of an increase in *Coriobacteriaceae* [22]. Our study’s results align with those of the last two studies, showing 2% per standard diviaion increase of its abundancy (OR: 1.02).

The *Firmicutes* phylum is to elevate the risk of Parkinson’s disease by increasing its abundance. This result is corroborated by prior studies. In a study involving a model organism, specifically rotenone-treated mice, the *Firmicutes* ratio increased after three weeks of treatment [23]. One study demonstrated

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the enrichment of 25 MEs in the PD group, and among these, six were associated with inflammatory indicators and an increased abundance of two biomarkers from the *Firmicutes* phylum [24]. This suggests that alterations to MEs in the gut microbiota may contribute to changes in the inflammatory response. The *Coprococcus* genus within this phylum contributes to these alterations. In our study, we observed an increase in the abundance of *Coprococcus* due to Parkinson's disease. However, multiple reports have demonstrated alterations in *Coprococcus* in both directions, reflecting the bidirectional influence of PD on the gut microbiome and the gut microbiome on PD [25]. Studies containing results of *Coprococcus* being more abundant in control subjects compared to those with PD also exist [26]. Research demonstrates that bacteria capable of producing short-chain fatty acids, such as *Coprococcus*, exhibit a decrease in the microbiome of individuals with PD compared to controls [27, 28]. *Lactobacillus* genus in this phylum increased due to PD, which in a study this group were positively associated with enriched microbiota-associated epitope in PD [24]. Furthermore, they conducted HUMAnN2 analysis to discern significant functional pathways associated with microbiota in the PD group. *Lactobacillus* exhibited a negative correlation with isopropanol biosynthesis and a positive correlation with monocyte count. This finding further suggests that *Lactobacillus* may influence immunity through microbiota-associated epitopes.

The results of the analysis of the *Proteobacteria* phylum using MR indicate that an increase in *Proteobacteria* is associated with a decreased risk of PD, with an odds ratio value of 0.98. However, the majority of studies conclude that Proteobacteria are more abundant in individuals with PD than in controls [26, 29,30]. By examining the class, order, family, and genus within this phylum, we can elucidate an explanation for this phenomenon. Our findings indicate an increase in the *Gammaproteobacteria* class and a decrease in the *Alphaproteobacteria* class in PD patients, which aligns with the results of the majority of previous studies [31, 32]. A decrease in the *Sutterellaceae* family and the *Sutterella* genus is also supported by previous research [21].

Increases in the order Bacteroidetes were associated with reduced risk of Parkinson's disease, and Bacteroidetes genus in this order was found to be reduced in patients with Parkinson's disease. One study showed that PBM treatment over 12 weeks resulted in a trend toward an increase in the number of genera within the order Bacteroidetes [33]. The genus Bacteroidetes is generally associated with a healthy microbiome, being more abundant in high-fiber diets and less abundant in high-fat diets. Additionally, people with Parkinson's disease have been consistently found to have a reduced microbiome [34, 35].

The microbiome's impact on Parkinson's disease does not exhibit a robust causal relationship. Conversely, Parkinson's disease exerts a more pronounced influence on microbiome abundance. The *Rhodospirillales* order (HB), impacted by PD, displays a 30 % decrease per standard deviation (OR: 0.70), while the *Collinsella* genus exhibits a 37% increase per SD (OR: 1.37). Various other genera also demonstrate a fluctuation within the range of 12% decrease to 25% increase per SD (OR: 0.82-1.25).

Certain bacterial groups, such as the *Peptostreptococcaceae* family [36] and *Fusicatenibacter* genus [37] have limited influence on PD and lack comprehensive studies regarding their functional alterations. Microbiota exhibiting a robust causal relationship with PD, such as Collinsella, have previously been associated with low dietary fiber intake and weight loss. Therefore, further investigation of the link between PD and diet is warranted [38].

5. Conclusion

We utilized two-sample MR to reveal the causal relationship between the abundance of gut microbiomes and the risk of Parkinson's disease. We uncover that a small number of gut bacteria can contribute to the risk of PD, albeit with limited impact. Parkinson's disease can influence numerous gut microbiomes, and certain ones exhibit more pronounced effects. Among these microbiomes, some have already been verified to have associations with human weight and dietary patterns. Going forward, we can investigate the causal relationship among these three factors using multivariable Mendelian randomization. This analysis will help determine if specific diets can mitigate PD risk by influencing the gut microbiome, potentially offering avenues for PD prevention.

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