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Exploring the Link Between Gut Microbiota and Insomnia Diagnosis Based on the Concept of "If the Stomach is Unharmonious, the Sleep will be Restless"

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Abstract

To investigate the association between gut microbiota and the diagnosis and treatment of insomnia. Methods: First, a two-sample Mendelian randomization analysis was conducted between gut microbiota data and insomnia, using the inverse variance weighted method as the primary outcome measure. Subsequently, genetic variants associated with insomnia were annotated to identify corresponding genes. Results: Our analysis identified six pathogenic bacterial taxa associated with insomnia, and found that SUCLG2 and TM9SF2 may serve as key regulatory genes. Conclusion: Our study provides insights for the development of personalized treatment strategies for insomnia and offers preliminary candidate targets for future drug development.

Keywords

Gut Microbiota; Insomnia; Traditional Chinese Medicine.

1. Introduction

Insomnia is a common sleep disorder characterized by difficulties in falling asleep, maintaining sleep, or early morning awakenings. Insomnia not only affects patients' daily life and work efficiency but may also lead to psychological issues such as anxiety and depression. Modern research has found that gut microbiota is closely linked to the occurrence of insomnia. Reduced sleep duration can affect the function of gut microbiota, while metabolic abnormalities in gut microbiota can interfere with the normal sleep cycle. Traditional studies are often limited by small sample sizes and confounding factors, resulting in insufficient evidence for a causal relationship between gut microbiota and insomnia. In this study, we employed a Mendelian randomization approach, utilizing genome-wide association study data with larger sample sizes, to explore the association between gut microbiota and insomnia.

2. Methods

2.1. Date sources

First, gut microbiota data were obtained from the MiBioGen database (https://mibiogen.gcc.rug.nl/). Subsequently, the GWAS database (https://gwas.mrcieu.ac.uk/) was searched using the keyword "insomnia" to select data with more recent publication dates and larger sample sizes. Finally, genetic data for insomnia with GWAS ID "ukb-a-13" were obtained, comprising 33,696 European samples and 10,894,596 single nucleotide polymorphisms (SNPs). Additionally, transcriptomic and single-cell data for insomnia were retrieved from the GEO database (https://www.ncbi.nlm.nih.gov/gds). The transcriptomic data, with accession number GSE208668, included samples from 17 patients and 25 healthy controls, derived from peripheral blood. The single-cell data, with accession number GSE214337, included samples from one patient, derived from the cerebral cortex.

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2.2. Mendelian Randomization Analysi

Gut microbiota was treated as the exposure factor and insomnia as the outcome variable to analyze the causal relationship between gut microbiota and insomnia.

2.2.1. Exposure Data Filtering

First, exposure data were filtered by selecting gut microbiota at the "genus" level, followed by correlation analysis to exclude SNPs with $p > 1e\text{-}05$. Subsequently, SNPs in linkage disequilibrium were removed (parameters set to $r^2 < 0.001$, kb = 10,000). Finally, weak instrumental variables were excluded based on the criterion of an F-statistic > 10.

2.2.2. Statistical Analysis

Statistical analyses were performed using R version 4.3.2 software, utilizing the "TwoSampleMR" package. First, heterogeneity among instrumental variables from different platforms, experiments, or populations may affect the results of Mendelian randomization analysis. Heterogeneity was assessed using IVW and MR-Egger tests, and instrumental variables with $p < 0.05$ were excluded to eliminate heterogeneity. If instrumental variables influence the outcome through pathways other than the exposure factor, this indicates pleiotropy. In this study, pleiotropy was detected using the MR-Egger intercept test, and instrumental variables with $p < 0.05$ were excluded to remove pleiotropic effects. Finally, the inverse variance weighted (IVW) method was employed as the primary MR analysis method to screen instrumental variables significantly associated with the outcome (i.e., insomnia).

2.3. Mapping Gut Microbiota to Genes

Based on the SNPs significantly associated with insomnia identified through the IVW analysis, genetic variants were mapped to their nearest genes using the NCBI-SNP annotation website (https://www.ncbi.nlm.nih.gov/snp/). These genes were further explored to investigate the pathological mechanisms of gut microbiota in insomnia.

3. Results

3.1. Mendelian Randomization

After cleaning and filtering the gut microbiota data, the causal relationships between 119 microbial taxa (genera) and insomnia were analyzed. A forest plot displays the association between each genetic variant and the outcome. A funnel plot indicates the presence of heterogeneity among genetic variants. A leave-one-out analysis shows the impact of removing any single genetic variant on the outcome. A scatter plot illustrates the relationship between each genetic variant and both the exposure (gut microbiota) and outcome (insomnia). Ultimately, after accounting for pleiotropy and sensitivity analyses, six gut microbial genera were found to be significantly associated with insomnia (Figure 1).

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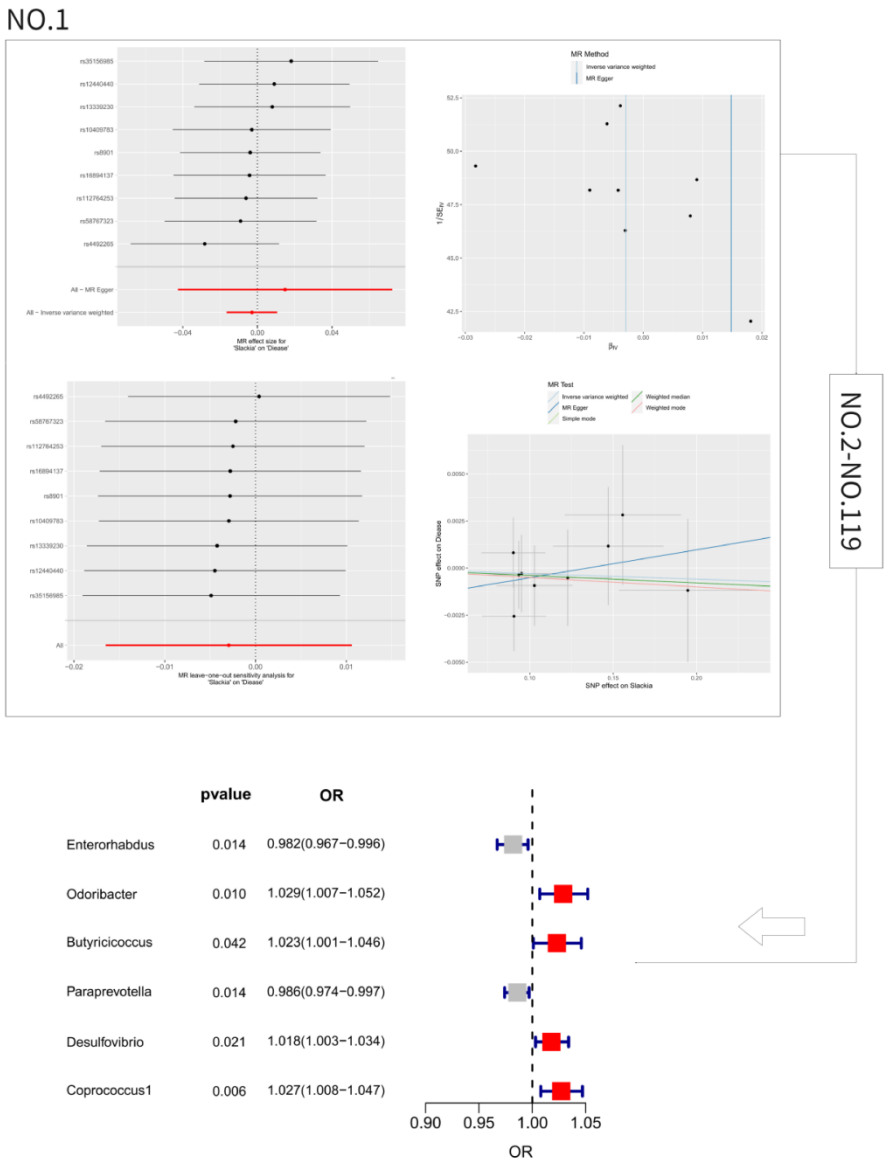

Figure 1: Mendelian Randomization Results

Figure 1 Mendelian Randomization Results;** a SNP forest plot showing the impact of SNPs corresponding to bacterial genera on the outcome; b Funnel plot assessing heterogeneity based on the symmetry of SNP distribution; c Sensitivity analysis (leave-one-out) examining the effect of removing individual SNPs on the results; d Scatter plot where a slope > 0 indicates the bacterial genus is a risk factor for insomnia, and a slope < 0 indicates it is a protective factor; e Forest plot of microbial genera, where an OR > 1 indicates the genus is a risk factor for insomnia, and an OR < 1 indicates it is a protective factor.

Enterorhabdus and Paraprevotella were found to be negatively associated with the onset of insomnia, suggesting these two genera may be protective factors. Conversely, Desulfovibrio, Butyricicoccus, Coprococcus1and Odoribacter showed positive associations with insomnia onset, indicating these four genera may be risk factors for insomnia. Finally, through SNP-gene mapping, 13 genes regulating beneficial genera and 30 genes regulating harmful genera were identified.

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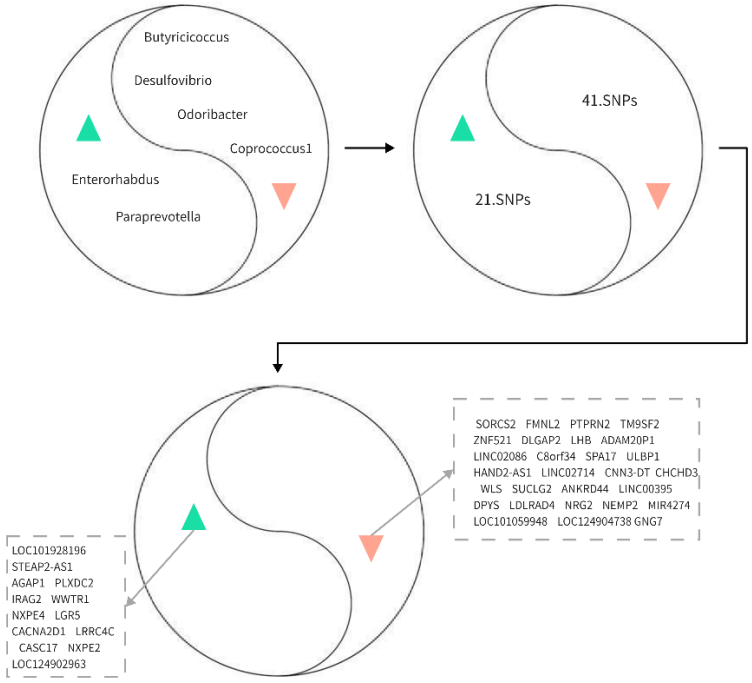

Figure 2 Gut Microbiota - SNP - Gene Network

4. Discussion

In this study, we identified Enterorhabdus and Paraprevotella as microbial genera associated with favorable outcomes in insomnia. Simultaneously, Desulfovibrio, Butyricicoccus, Coprococcus1, and Odoribacter were identified as gut microbiota associated with unfavorable outcomes. Research has found that Enterorhabdus is negatively correlated with the concentration of pro-inflammatory cytokines and plays an important role in maintaining gut homeostasis [1]. Paraprevotella produces short-chain fatty acids, which positively influence intestinal electrolyte balance, energy metabolism, immune regulation, and anti-inflammatory processes [2]. Desulfovibrio, a sulfate-reducing bacterium, not only produces harmful substances like hydrogen sulfide but also inhibits the function of short-chain fatty acids, leading to intestinal tissue apoptosis and inflammation. Published research on the other genera is limited, warranting attention in future studies. Therefore, we hypothesize that microbiota associated with favorable insomnia outcomes may reduce patients' inflammatory levels, while microbiota associated with unfavorable outcomes may exacerbate intestinal inflammatory responses.

In summary, our study is the first to employ a Mendelian randomization design to investigate the relationship between gut microbiota and insomnia. We successfully identified gut microbiota and related genes associated with insomnia, encompassing both beneficial and harmful microbial communities.

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