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Causal association between gut microbiome and polycystic ovary syndrome: A bidirectional Mendelian randomization study

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Abstract

Through implementing a bidirectional Mendelian randomization (MR) study, the causal effects between gut microbiome and polycystic ovary syndrome (PCOS) were analyzed. Summary statistics for PCOS were acquired from the FinnGen consortium R8 release data, which included 27,943 cases and 162,936 controls. The inverse-variance weighting (IVW) method was adopted for analysis. Additionally, the causality involving exposure plus the outcome was evaluated by means of MR-Egger, weighted median, simple mode methods, as well as weighted mode. The IVW estimate showed that the *genera Streptococcus* plus *Enterorhabdus* served as protectors of PCOS. By contrast, the *phylum Tenericutes*, *genera Anaerofilum*, *Coprococcus 2*, *Lachnospiraceae ND3007 group* and *Ruminiclostridium 5* were identified as risk elements of PCOS. Based on reverse MR analyses from PCOS on the intestinal microbiome based on the IVW method, the *phyla Tenericutes*, *Actinobacteria*, *class Actinobacteria*, *genera Ruminococcaceae UCG004* and *Christensenellaceae R 7group* exhibited a down-regulation effect after PCOS onset. The *genera Bacteroides*, *Barnesiella*, *Erysipelotrichaceae UCG003*, *Ruminococcus gnavus group*, and *Veillonella* were up-regulated. No horizontal pleiotropy or significant IV heterogeneity was observed. We conclude that there is a causality relationship between gut microbiome and PCOS, where some bacterial taxa can be used as biomarkers to promote targeted diagnosis and therapy for PCOS. *(Afr J Reprod Health 2024; 28 [12]: 127-138).*

Keywords: Causal effect; gut microbiome; Mendelian randomization; polycystic ovary syndrome

Résumé

Grâce à la mise en œuvre d'une étude de randomisation mendélienne (MR) bidirectionnelle, les effets causals entre le microbiome intestinal et le syndrome des ovaires polykystiques (SOPK) ont été analysés. Les statistiques récapitulatives sur le SOPK ont été acquises à partir des données de diffusion R8 du consortium FinnGen, qui comprenaient 27 943 cas et 162 936 contrôles. La méthode de pondération de variance inverse (IVW) a été adoptée pour l'analyse. De plus, la causalité impliquant l'exposition et le résultat a été évaluée au moyen des méthodes MR-Egger, de la médiane pondérée, du mode simple, ainsi que du mode pondéré. L'estimation IVW a montré que les genres Streptococcus et Enterorhabdus servaient de protecteurs du SOPK. En revanche, le phylum Tenericutes, les genres Anaerofilum, Coprococcus 2, le groupe Lachnospiraceae ND3007 et Ruminiclostridium 5 ont été identifiés comme éléments de risque du SOPK. Sur la base d'analyses IRM inverse du SOPK sur le microbiome intestinal basées sur la méthode IVW, les phyla Tenericutes, Actinobacteria, classe Actinobacteria, genres Ruminococcaceae UCG004 et Christensenellaceae R 7group ont présenté un effet de régulation négative après l'apparition du SOPK. Les genres Bacteroides, Barnesiella, Erysipelotrichaceae UCG003, le groupe Ruminococcus gnavus et Veillonella étaient régulés positivement. Aucune pléiotropie horizontale ni hétérogénéité IV significative n'a été observée. Nous concluons qu'il existe une relation de causalité entre le microbiome intestinal et le SOPK, certains taxons bactériens pouvant être utilisés comme biomarqueurs pour promouvoir un diagnostic et un traitement ciblés du SOPK. *(Afr J Reprod Health 2024; 28 [12]: 127-138).*

Mots-clés: Effet causal; microbiome intestinal ; Randomisation mendélienne ; syndrome des ovaires polykystiques

Introduction

As a frequently-occurring endocrine metabolic disturbance, polycystic ovary syndrome (PCOS) influences about 5-10% of reproductive-aged females worldwide[1]. PCOS is highly heterogeneous and complex, mainly characterized as sparse ovulation or anovulation, hyperandrogenism, insulin resistance, and polycystic ovary[2]. It has been shown that PCOS is correlated with elevated risks of infertility, pregnancy complications, and miscarriage[3,4]. In addition, it induces further

complications like metabolic syndrome[5], depression, type 2 diabetes mellitus, anxiety, and cardiovascular diseases[6]. Until now, the treatment for PCOS remained unclear and it was considered a chronic condition requiring long-term management[7]. Thus, the preventing or delaying the occurrence of PCOS is particularly important. Despite suggestions of genetic[8], gestational environment[9], and lifestyle factors[10] contributing to the development of PCOS, the complete understanding of its pathogenesis remains elusive.

Recently, gut microbiota has been proposed to have a role in PCOS pathogenesis. The human microbiome consists of over 100 trillion microorganisms, forming a mutually beneficial symbiotic relationship with the human body and contributing to the maintenance of human health[11]. In 2012, Tremellen studied PCOS and pointed out the Dysbiosis of Gut Microbiota (DOGMA) theory[12]. The gastrointestinal tract of a healthy person is home to a wide variety of microbes called intestinal flora. Once the internal and external environment of the body changes, the sensitive intestinal bacteria are inhibited, and the uninhibited bacteria take the opportunity to multiply, resulting in microbial imbalance[13]. Since then, the correlation between intestinal microbiome and PCOS has been extensively explored. Several studies identified variations in the intestinal microbiome composition between healthy controls and PCOS females. For example, Jobira et al. reported lower *Bacteroidetes*, and the increased relative abundance percent (%RA) of the *phyla Actinobacteria*[14]. However, *Firmicutes* and *Proteobacteria* remained similar in PCOS females with obesity compared to controls. A study by Li *et al.* revealed the increased abundance of *Bacteroides*, but lower abundance of *Firmicutes* in PCOS females[15]. These findings suggest that intestinal microbiome dysbiosis has potential effects on PCOS development. However, the results have been inconsistent, and whether the intestinal microbiome has a causality with PCOS remains unclear.

Considering the confounding factors in addition to probable reverse causality, it is challenging to establish a causal effect through clinical observational research. It is of importance to ascertain whether the changes in the intestinal microbiome or PCOS onset occurred first, given that numerous prior investigations examining the relationship between PCOS and the intestinal microbiome rely on case–control study designs.

In the last decade, genome-wide association studies (GWASs) have been carried out to identify gut microbiome- or PCOS-associated genetic loci, which are increasing in number[16,17]. Such findings put forward new ideas on the causality between intestinal microbiome and PCOS from a genetic perspective.

The implementation of Mendelian randomization (MR) by virtue of GWAS-derived summary statistics) offers a valuable means of probing the causality of intestinal microbiome with PCOS. In MR, the exposure is established via instrumental variables (IVs) sourced from genetic variants, allowing for the estimation of the causality between an exposure and an outcome[18]. Because of the random transmission of genotypes from parents to offspring, common confounders have no impact on the relation between genetic variants and outcomes, providing a plausible causal sequence[19]. To date, there has been no MR research to investigate the plausible causality between gut microbiome and PCOS, hence the need for this study.

Methods

Study design and MR assumptions

To establish the causality of PCOS with the gut microbiome, we acquired summary-level data from publicly available GWAS studies. The potential association of PCOS with the intestinal microbiome was examined utilizing a bidirectional two-sample MR analysis[20]. Initially, the intestinal microbiome and PCOS were designated as the exposure variables and outcome variables, respectively, to study the preventive or promoting roles of intestinal microbiome in PCOS. Furthermore, in reverse, we investigated the causality of PCOS on intestinal microbiome. According to Bownden and his colleague[21], two-sample MR analysis followed the following three assumptions: (1) instrumental variables (IVs) chosen from the data sets were associated with the exposed variables; (2) IVs demonstrated no association with any undisclosed confounders of the exposured variables; (3) IVs correlated with results only by exposing variables, not by other means (Figure 1).

Number of SNPs

The IEU OpenGWAS (https://gwas.mrcieu.ac.uk/), originating from a study conducted by the international MiBioGen consortium, was retrieved for GWAS-derived summary data on the gut microbiome [16]. The data included a diverse cohort of 18,340 individuals from 24 cohorts across 11 countries. Notably, there was a predominance of European ancestry, totaling 14,306 individuals.

To investigate the microbial composition as well as taxonomic classification, the study focused on specific 16S rRNA gene regions, namely, V4, V3-V4, as well as V1-V2. This characterization utilized direct taxonomic binning[16]. Additionally, host genetic variants in relation to the bacterial taxon abundance in intestinal microbiota were distinguished using microbiota quantitative trait loci (mbQTL) mapping analysis. The intention of this analysis was to map genetic loci that exhibited associations with bacterial taxon abundance obtained from intestinal microbiota. The results concerned 9, 16, 20, 35 and 131 phyla, classes, orders, families, and genera, respectively, with a mean abundance >1%. However, 12 unknown genera and three unknown families existed. Consequently, the present study analyzed 196 taxa, excluding 15 unknown taxa. The GWAS summary statistics for PCOS were acquired from the FinnGen consortium R8 release data[22]. The phenocode "E4_PCOS_CONSORTIUM" was utilized in the present study. Such a particular GWAS comprised 27,943 cases of Finnish females and 162,936 controls, encompassing a set of SNPs[22].

IVs selection

To ensure a comprehensive analysis, we applied the same threshold for SNP selection as instrumental variables (IVs), whether considering the gut microbiome or PCOS to be the exposures. The IVs were chosen as per the criteria listed below: (1) Potential IVs were selected from single nucleotide polymorphisms (SNPs) linked to exposure (intestinal microbiome or PCOS) to reach a full-site significance threshold ($P < 1.0\times10–5$)[23]; (2) The linkage disequilibrium (LD) among SNPS was calculated through the sample data of the European 1000 Genomes Project as a reference panel. The SNPs showing an $r^2 < 0.001$ (utilizing a clumping window size of 10,000 kb) were included[24]; (3) Palindromic SNPs were discarded; and (4) No proxy-SNPs were used in the case of missing IVs in the data sets of the outcome.

Statistical methods

This study utilized five commonly employed methods to examine the causality concerning the exposure and outcome variables. These methods consist of weighted median, inverse-variance weighting (IVW), simple mode, weighted mode, as well as MR-Egger. By employing these diverse approaches, a robust assessment of the causality was achieved. Meta-analysis was adopted for the IVW method, which involved combining Wald estimates for every individual SNP. This allowed for the calculation of an overall estimate of the exposure affecting the outcome[25]. The weighted median method provided estimates of bias, even with up to 50% of the information available[26]. The weighted mode method maintained consistency in the presence of invalid instrumental variables by relying on the most abundant estimates of causal effect through individual instruments, which are derived from valid instruments[27]. MR-Egger provided the causality by means of the slope coefficient generated by the Egger regression and detected small study bias[21]. Lastly, the empirical density function for causal estimation in an unweighted mode was realized by the simple mode[28].

In the present study, a causality between exposure and result was considered significant if the IVW $P < 0.05$. To assess heterogeneity, Cochran's Q statistics were employed with IVW methods. If instrumental variables were heterogeneous ($p < 0.05$), the effect of heterogeneity needed to be taken into consideration[29].

Horizontal pleiotropy occurs when outcomes have correlations with instrumental variables through mechanisms instead of causality, where false-positive results may be produced ($p < 0.05$)[30]. In this study, whether the incorporated SNPs demonstrated latent horizontal pleiotropy was assessed using MR-Egger regression, whereas any findings indicating horizontal pleiotropy ($p < 0.05$) were removed from the analysis[21].

Sensitivity analysis involved the utilization of Mendelian randomization pleiotropy residual sum and outlier (MR-PRESSO), thereby identifying the outliers that could introduce polymorphic deviations, potentially affecting MR-Egger regression from the perspective of statistical efficacy and precision. Following the exclusion of outliers,

the MR analysis was repeated to reevaluate the causal effects[31]. To assess if a single SNP exerted a strong influence on the causality of the outcome and exposure, a leave-one-out analysis was implemented. This analysis involved sequentially omitting each SNP from the instrumental variables while utilizing coordinated outcome and exposure data. The TwoSampleMR package was utilized to identify potential outliers during this analysis[32].

The F-statistic calculated based on formula $F = R2 \times (N-1-K) / (1-R2) \times K$ was applied to evaluate IV strength. In this formula, R2 means the percentage of variance in the exposure variable explained by the genetic variants. K indicates the instrument quantity. N means the sample size[33]. The resulting F-statistic greater than 10 indicated no significant weak instrumental bias[33].

The MR Steiger test was employed for examining the validity of the outcome-induced exposure hypothesis[34]. This test determined the variance interpreted in the exposure variable, besides the outcome variable, via the instrumental SNPs, thus, allowing the assessment of whether the outcome variance was lower than the exposure variance. A "TRUE" MR Steiger result indicated anticipated causality, while a "FALSE" result suggested causality in the reverse direction.

Utilizing R software (version 4.2.2), statistical analyses were implemented. The primary R packages utilized in our manuscript included TwoSampleMR, MR-PRESSO, and Mendelian randomization. The GitHub repository containing the resources plus code for this research can be retrieved from https://github.com/1527311/20221004.

Ethics approval and consent to participate

This research has been conducted using published studies and consortia providing publicly available summary statistics. All original studies have been approved by the corresponding ethical review board, and the participants have provided informed consent. In addition, no individual-level data was used in this study.
Therefore, no new ethical review board approval was required.

Results

Causal influence of the intestinal microbiome on PCOS

2,037 IVs (Table S1) were analyzed for the causal influence of the intestinal microbiome on PCOS. The F-statistics of the IVs was between 20.349 and 27.853, and thus, the bias of weak IVs was eliminated (Table S2). Notably, the identical SNPs were selected from *class Mollicutes* and *phylum Tenericutes*, and therefore, we retained *phylum Tenericutes* in the analysis. Based on IVW methods, six bacterial genera, specifically, *Anaerofilum,Coprococcus 2, Enterorhabdus, Lachnospiraceae ND3007 group, Ruminiclostridium5* and *Streptococcus*, as well as *phylum Tenericutes* were significant (Table S3).

The IVW estimate suggested that the *genera Enterorhabdus* (OR = 0.9, 95% CI: 0.8-1.0, P = 0.0) plus *Streptococcus* (OR = 0.9, 95% CI: 0.8-1.0, P = 0.0) were protectors of PCOS. The *phylum Tenericutes* (OR = 1.1, 95% CI: 1.0-1.2, P = 0.0), *genera Anaerofilum* (OR = 1.1, 95% CI: 1.0-1.2, P = 0.0), *Coprococcus 2* (OR = 1.1, 95% CI: 1.0-1.2, P = 0.0), *Lachnospiraceae ND3007 group* (OR = 1.3, 95% CI: 1.0-1.6, P = 0.0), as well as *Ruminiclostridium 5* (OR = 1.2, 95% CI: 1.0-1.3, P = 0.0) were proved as risk factors for PCOS (Figure 2). Among the seven causal associations, the IVs were not significantly heterogeneous based on the Cochran's IVW Q test results (P > 0.05, Table S4). Horizontal pleiotropy was not observed at the intercepts of MR-Egger regression (P > 0.05, Table S5). Additionally, we used the MR-PRESSO method to validate the MR-Egger regression results, indicating no evidence of outliers (global test P > 0.05, Table S6). Intestinal microbiome had no SNP-derived correlation with PCOS, as shown by leave-one-out analysis (Figure 3). The MR Steiger test demonstrated that the direction of causal effect from gut microbiome to PCOS was true, and indicated no reverse causality (Table S7).

Causal impact of PCOS on the gut microbiome

A total of 5,828 SNPs satisfied the criteria for determining the causal influence of PCOS on intestinal microbiome (Table S8).

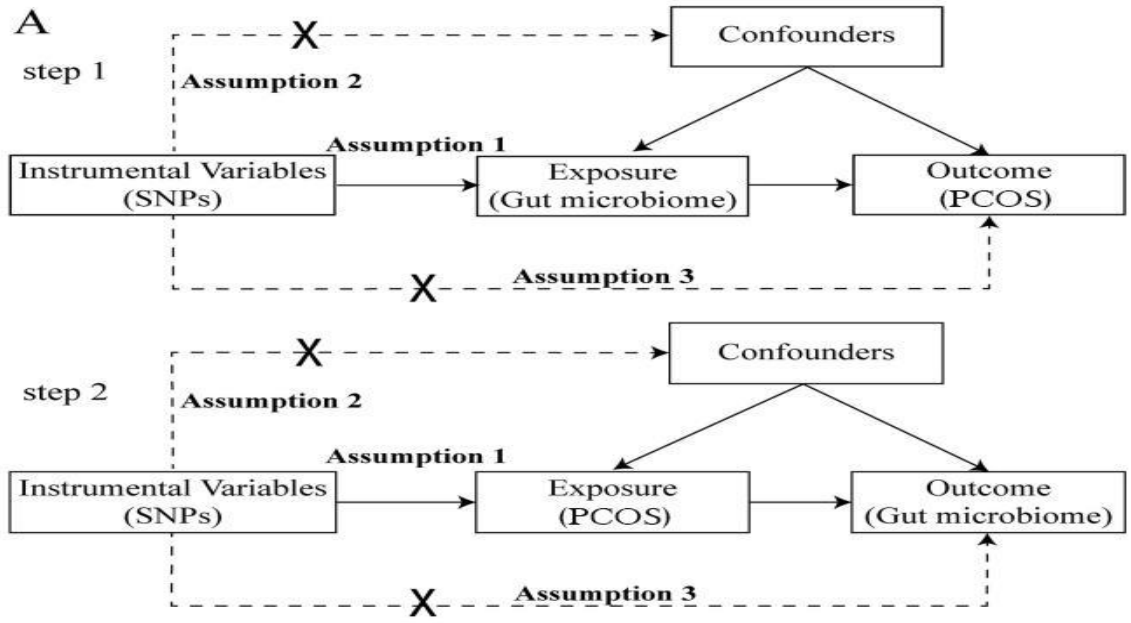

Figure 1: The bidirectional Mendelian randomization process was divided into two distinct steps, with each step encompassing a two-sample MR analysis and incorporating three assumptions (assumptions 1, 2, and 3). These steps were executed to identify and appraise the causality of the outcome and exposure variables.

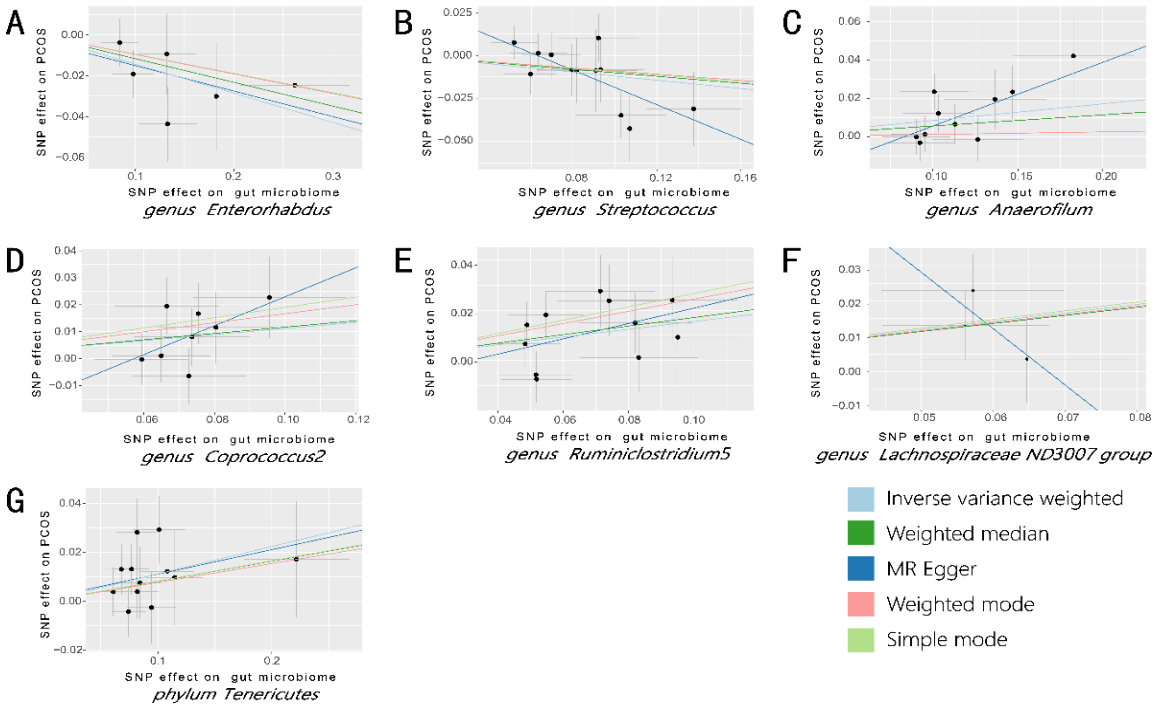

Figure 2: Scatter plots for the causal effects of the gut microbiome on PCOS.

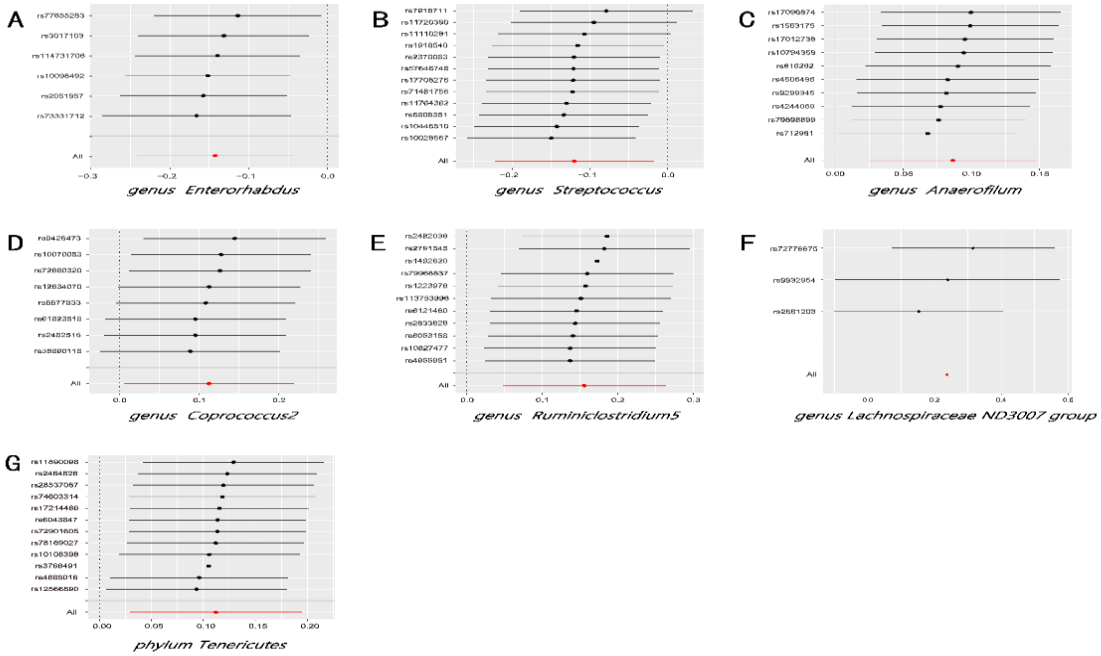

Figure 3: Causal effects of the gut microbiome on PCOS, plotted by leave-one-out analysis.

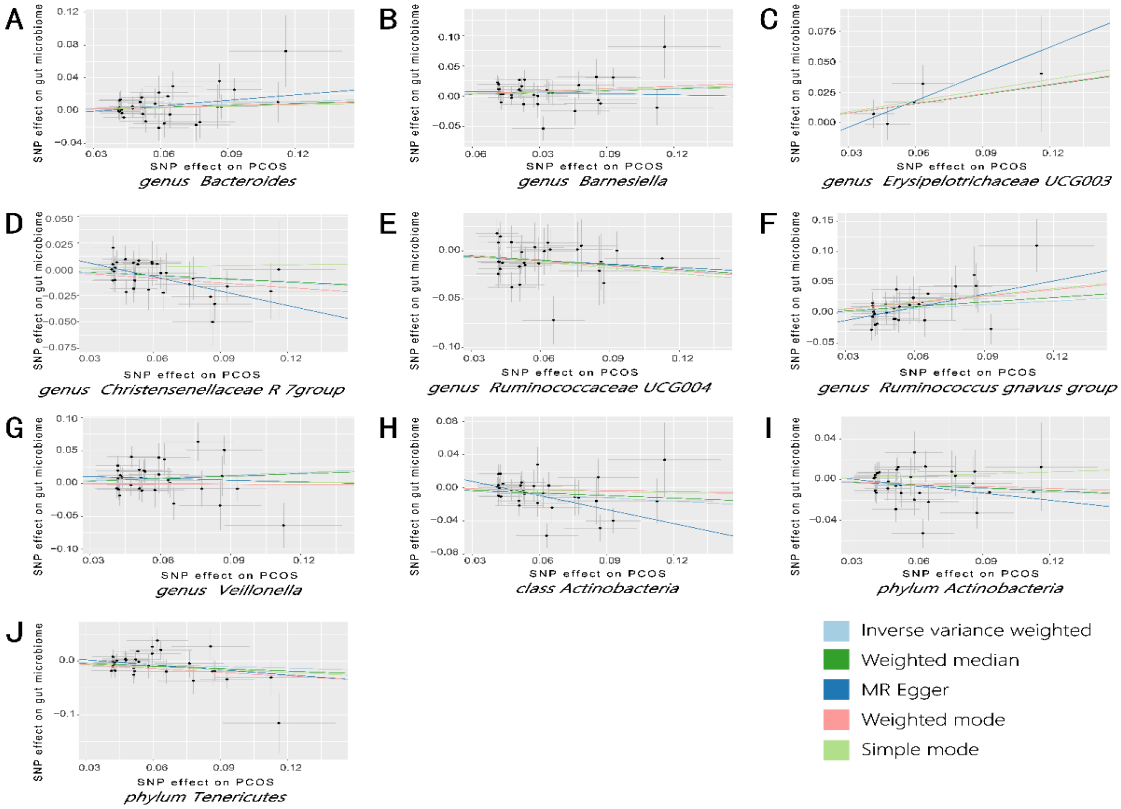

Figure 4: Scatter plots for the causal effects of PCOS on the gut microbiome

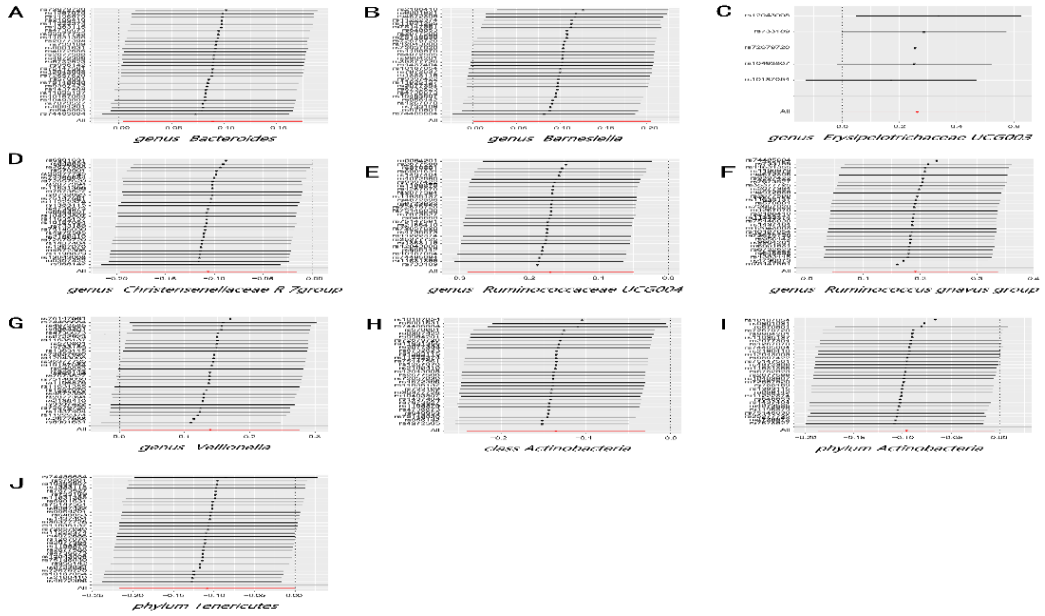

Figure 5: Causal effects of PCOS on the gut microbiome plotted by the leave-one-out analysis

The F-statistics for the independent variables (IVs) ranged between 20.463 and 25.130, effectively mitigating the bias caused by weak IVs (Table S9). PCOS had a causal effect on two phyla, two classes, one family and seven genera, as revealed by MR analysis (Table S10). Notably, the selected SNPs of *class Mollicutes* were the same as those selected of *phylum Tenericutes*, in addition, the same SNPs were extracted from *family Bacteroidaceae* and *genus Bacteroides*, thus *phylum Tenericutes* and *genus Bacteroides* were remained to analysis.

According to the IVW method, the *phyla Tenericutes* (OR = 0.9, 95% CI: 0.8-1.0, P = 0.0) and *Actinobacteria* (OR = 0.9, 95% CI: 0.8-1.0, P = 0.0), *class Actinobacteria* (OR = 0.9, 95% CI: 0.8-1.0, P = 0.0), as well as *genera Ruminococcaceae UCG004* (OR = 0.8, 95% CI: 0.7-1.0, P = 0.0) and *Christensenellaceae R 7group* (OR = 0.9, 95% CI: 0.8-1.0, P = 0.0) were down-regulated after the onset of PCOS. The *genera Bacteroides* (OR = 1.1, 95% CI: 1.0-1.2, P = 0.0), *Barnesiella* (OR = 1.1, 95% CI: 1.0-1.2, P = 0.0), *Erysipelotrichaceae UCG003* (OR = 1.3, 95% CI: 1.0-1.7, P = 0.0), *Ruminococcus gnavus group* (OR = 1.2, 95% CI: 1.0-1.4, P = 0.0), and *Veillonella* (OR = 1.2, 95% CI: 1.0-1.3, P = 0.0) were up-regulated (Figure 4). MR-PRESSO suggested that the outlier rs10187054 existed in the *genus Bifidobacterium*, *family Bifidobacteriaceae*, and *order Bifidobacteriales*. After removing it, the P values were no longer significant based on the IVW method (*order Bifidobacteriales*, P = 0.1, *family Bifidobacteriaceae*, P = 0.1, *genus Bifidobacterium*, P = 0.1). No evidence of outliers was obtained from the results of the rest of the taxa (Table S13). None of the IVs exhibited heterogeneity statistics (Table S11). The IVs and the rest of the intestinal microbiome did not exhibit horizontal pleiotropy between them (Table S12). The SNPs presented no significant relationship on the results based on leave-one-out analysis (Figure 5).

The MR Steiger test suggested that there was a true causal direction for PCOS plus the gut microbiome (Table S14).

Discussion

Our findings revealed that the gut microbiome had a reciprocal interaction with PCOS, indicating a bidirectional causal effect. Recent studies have provided evidence of microbiota dysbiosis in animal models of PCOS and PCOS patients[35-38]. Furthermore, previous studies also highlighted that the gut microbiome affects PCOS via different mechanisms, including involvement in the brain-gut axis, promoting chronic inflammatory state, regulating the short-chain fatty acid (SCFA) pathway, increasing intestinal permeability, and altering bile acid metabolism[39].

Upon analyzing the gut microbiome in relation to PCOS, we made some noteworthy observations. Specifically, we found that the presence of the

genera Streptococcus and *Enterorhabdus* had beneficial effects on PCOS. In a cross-sectional study implemented by Zhou and his colleague, involving 30 PCOS females with obese (OG) and 30 without obese (NG), respectively, as well as healthy, but obese females (OC, n=11) besides healthy females (NC, n=30) as controls, it was observed that there was a negative correlation between *Streptococcus* and insulin levels in both the NG and OG groups[40]. Additionally, Deng *et al*. revealed a positive correlation between *Enterorhabdus* and tryptophan, as well as a negative correlation with kynurenine in a murine model of chronic restraint stress[41]. In reference to such information, the *genus Enterorhabdus* was assumed to influence PCOS through the kynurenine metabolic pathway of the microbial-intestine-brain axis. In contrast, we identified several genera, namely *Anaerofilum, Coprococcus 2, Lachnospiraceae ND3007 group, and Ruminiclostridium 5*, as risk factors for PCOS. Zhou *et al.* conducted a study that compared healthy but obese women to obese PCOS patients and found a remarkably elevated abundance of the *genus Coprococcus 2*. The linear discriminant analysis (LDA) further highlighted *Coprococcus 2* as a characteristic gut bacterium in obese PCOS females, suggesting its potential as a targeted clinical diagnostic marker[40].

However, Liang *et al*. investigated PCOS in the Chinese Han population and found that fasting insulin, HOMA-IR, and testosterone levels exhibited negative relationships to the *Lachnospiraceae ND3007 group and Ruminiclostridium 5*[42]. However, those data contradict our findings. That inconsistency could be attributed to differences in sample size and ethnicity, suggesting the need for further research to elucidate the correlation between PCOS and those genera.

Liu *et al*. observed a decline in *Ruminococcaceae* in females with PCOS[43]. Dong *et al.* also discovered that the PCOS group displayed less abundant *Christensenellaceae spp* than healthy group[44]. Fu *et al*. identified a negative correlation between *Christensenellaceae* and BMI[45]. *Christensenellaceae* has been recognized as a characteristic of a healthy gut[46]. Jillian L. highlighted the benefits of *Christensenellaceae*, which may be attributed to linkage with protein- and fiber-rich diets[47]. These research findings offer novel insights for PCOS pathogenesis and treatment. Additionally, the cultured representatives of *Christensenellaceae* as a therapeutic probiotic should be contemplated for interventions in PCOS patients[48].

Numerous studies have consistently observed an increase in *Bacteroides* in PCOS patients. Those findings agree with our own findings of an upregulation in *Bacteroidaceae* and *Bacteroides*. According to Zeng *et al.*, PCOS women with insulin resistance exhibited higher *Bacteroides* levels than their healthy counterparts. Increased inflammation, insulin resistance, and sex hormone levels exhibited positive relations to *Bacteroidaceae* levels[49]. Zhang et al. also pointed out an enrichment of *Bacteroides* in the PCOS group[50]. Moreover, Liu *et al*. revealed that *Bacteroides* and ghrelin were negatively correlated, which acts as a brain-gut axis mediator[43]. Grasset *et al*. showcased the detrimental roles of a distinct cluster of pro-inflammatory bacteria in the ileum, such as *Bacteroides*, on the functionality of the GLP-1R (glucagon-like peptide-1 receptor) from the enteric nervous system[51]. This impairment disrupts the production of nitric oxide induced by GLP-1, leading to the inhibition of the gut-brain-periphery axis, which is responsible for regulating gastric emptying and insulin secretion. Such a mechanism operates on the basis of a NOD2/TLR4/CD14-dependent pathway. Considering these findings, it is promising to design drug for PCOS patients by targeting *Bacteroides*. Future research should focus on leveraging this knowledge to develop effective interventions for PCOS management.

The correlation of the increased prevalence of *Erysipelotrichaceae* with metabolic disorders has reported in multiple studies[52]. In line with such findings, Gulnar Mammadova observed significant high *Erysipelotrichaceae* abundance from PCOS group[53]. As revealed by Dong et al., PCOS patients presented with a higher abundance of *Ruminococcus gnavus*, which was positively correlated with markers containing HOMA-IR, BMI, weight, TG, FINS, and TP[44]. Furthermore, Hall *et al*. observed temporary surges in the abundance of *Ruminococcus gnavus*, which correlated with heightened inflammatory bowel disease (IBD) activity[54]. Changes in the abundance of *Ruminococcus gnavus* was shown to increase intestinal permeability while influencing gut barrier integrity in IBD patients. In a study on PCOS etiology, increased intestinal permeability potentially facilitated LPS translocation to the systemic circulation from Gram-

negative colonic bacteria. Those data suggest that the *genus Ruminococcus gnavus* may contribute to increased intestinal permeability, which in turn affects the progression of PCOS. Additionally, Zhou *et al*. analyzed fecal metabolites obtained from an obese PCOS group, illustrating a positive relationship between fecal arachidonic acid and *Veillonella* and *Lachnospira* abundance[55]. Arachidonic acid plays a significant role in cholesterol esterification, inflammatory responses, muscle growth, and platelet activation[56]. Moreover, Khajeh *et a*l. revealed a crucial effect of arachidonic acid on embryo development and oocyte maturation by influencing oocyte ovulation and meiosis via the cAMP/PKC pathway[57]. Based on this understanding, it is plausible to speculate that *Veillonella* may regulate the arachidonic acid-associated metabolic pathway to impact obese PCOS females in terms of the concentration of cholesterol, blood glucose, and lipids. We can further explore the mechanisms underlying the inflammatory processes and metabolic disruptions in PCOS.

Our research has provided promising insights into the diverse effects of various intestinal flora on the pathogenesis of PCOS. Moreover, we observed that PCOS itself induces variations in the composition of the intestinal flora. As we deepen our understanding of the microbiome, it has become increasingly evident that human health can be sustained by specific taxa and their metabolic pathways, and the interactions between taxa. Consequently, investigating such aspects has emerged as a new priority in microbiome research. By unraveling the intricate relationships between specific microbial taxa and their metabolic activities, we can gain a comprehensive understanding of their contributions to PCOS onset and advancement. This knowledge not only sheds light on the underlying mechanisms driving the condition but also opens up new possibilities for early diagnosis and targeted interventions. With this enhanced understanding of the microbiome, we can explore innovative approaches for the early detection of PCOS. By identifying specific microbial signatures associated with the condition, we can develop diagnostic tools that enable timely interventions and management. Furthermore, insights into the metabolic pathways influenced by the intestinal flora offer potential avenues for targeted interventions. The deepened comprehension of the functions of the microbiome in PCOS will undoubtedly pave the way for improved strategies for diagnosis, prevention, and treatment of this complex disorder.

This study had several notable strengths that contribute to the robustness of the findings. First, a bidirectional MR analysis, accompanied by the Steiger test, was employed to prove the causality of PCOS with intestinal microbiota. This approach effectively mitigated the influence of confounding elements, solves the issue of reverse causality, and enhances the effectiveness of the causal reasoning. Additionally, the genetic variants linked to the gut microbiome came from a robust GWAS meta-analysis, ensuring reliable instrumental variables were applied in the MR analysis. The potential horizontal pleiotropy was explained by means of MR-Egger regression intercept terms, MR-PRESSO as well as other tests. Furthermore, for the purpose of reducing bias due to racial differences, the study incorporated data exclusively from individuals of European descent that were sourced from the MiBioGen and FinnGen consortia. The use of nonoverlapping exposure and outcome summary-level data further strengthened the credibility of the results[58].

It is crucial to recognize the constraints and shortcomings of this study while interpreting the findings. As the analysis was conducted using summary statistics instead of raw data, subgroup analyses based on factors covering obesity status were not feasible. Another limitation arises from the fact that the exposure dataset only provided information at the genus level, which precluded a deeper exploration of the causality of PCOS and the intestinal microbiome. The inclusion of larger numbers of genetic variations as instrumental variables would be more beneficial to sensitivity analysis together with horizontal pleiotropy detection. Consequently, the SNPs utilized for analysis failed to obtain the significance threshold for traditional GWAS, potentially leading to an increased risk of false positives. Furthermore, while efforts were made to account for potential bias due to sex by excluding genetic variants on sex chromosomes and adjusting for sex in the analysis, the influence of sex-related factors cannot be entirely ruled out. MR studies investigating the causality between intestinal microbiota and PCOS in the future should consider exploring the impact of various mediating factors, including BMI, lifestyle,

diet, blood glucose, and serum lipid levels to offer a more comprehensive comprehension of the relationship.

Conclusion

The present bidirectional MR study found that the gut microbiome was linked to PCOS in a causal manner. Specific bacterial taxa with potential as novel biomarkers were identified and are expected to help develop targeted diagnoses and therapies for PCOS. The role of probiotics in preventing PCOS and the underlying specific mechanisms need to be elucidated through in-depth randomized controlled trials.

Availability of data and materials

The datasets analyzed during the current study are available from the IEU OpenGWAS(https://gwas.mrcieu.ac.uk/), and the FinnGen repository(https://console.cloud.google.com/storage/browser/finngen-public-data-r8/summary_stats;tab=objects?prefix=&forceOnObjectsSortingFiltering=true).

Competing interests

The authors declare that they have no competing interests.

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