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Causal relationship between gut microbiota and polycystic ovary syndrome: a literature review and Mendelian randomization study

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Introduction: Numerous studies have suggested an association between gut microbiota and polycystic ovarian syndrome (PCOS). However, the causal relationship between these two factors remains unclear.

Methods: A review of observational studies was conducted to compare changes in gut microbiota between PCOS patients and controls. The analysis focused on four levels of classification, namely, phylum, family, genus, and species/genus subgroups. To further investigate the causal relationship, Mendelian randomization (MR) was employed using genome-wide association study (GWAS) data on gut microbiota from the MiBioGen consortium, as well as GWAS data from a large meta-analysis of PCOS. Additionally, a reverse MR was performed, and the results were verified through sensitivity analyses.

Results: The present review included 18 observational studies that met the inclusion and exclusion criteria. The abundance of 64 gut microbiota taxa significantly differed between PCOS patients and controls. Using the MR method, eight bacteria were identified as causally associated with PCOS. The protective effects of the genus *Sellimonas* on PCOS remained significant after applying Bonferroni correction. No significant heterogeneity or horizontal pleiotropy was found in the instrumental variables (IVs). Reverse MR analyses did not reveal a significant causal effect of PCOS on gut microbiota.

Conclusion: The differences in gut microbiota between PCOS patients and controls vary across observational studies. However, MR analyses identified

specific gut microbiota taxa that are causally related to PCOS. Future studies should investigate the gut microbiota that showed significant results in the MR analyses, as well as the underlying mechanisms of this causal relationship and its potential clinical significance.

KEYWORDS

gut microbiota, polycystic ovarian syndrome, review, Mendelian randomization, causation

1 Introduction

Polycystic ovary syndrome (PCOS) is a highly prevalent endocrine disorder in females, particularly among women of reproductive age. It is estimated that the incidence of PCOS in women of reproductive age ranges from 4% to 20% (1–3). Currently, Rotterdam criteria are recommended for the diagnosis of PCOS, which requires the presence of at least two of the following manifestations: oligo-anovulation, hyperandrogenism, and polycystic ovarian morphology (PCOM). However, the diagnosis of PCOS is challenging due to the diverse and varying features associated with this syndrome. The heterogeneity in the presentation of PCOS is observed in various aspects, including ultrasound assessment of ovarian morphology, overall length of the menstrual cycle, length of follicular phase, and length of luteal phase. Although the etiology of PCOS is not fully understood, some scholars have proposed possible mechanisms, including ones related to genetics, endocrinology, and metabolism (4–6). Tremellen et al. introduced the Dysbiosis of Gut Microbiota (DOGMA) theory in 2012, proposing that intestinal microorganisms may contribute to the typical manifestations of PCOS (7). Several observational studies have provided evidence supporting the association between PCOS and gut microbiota (8–10). For example, a significant reduction in the phylum *Tenericutes* has been reported in women with PCOS (11). PCOS patients have shown an increase in *Bacteroides vulgatus*, possibly influenced by a gut microbiota/bile acid/interleukin-22 axis (12). Furthermore, several studies have suggested that probiotic supplementation may be effective in treating PCOS, highlighting the potential therapeutic value of microbial treatment (13, 14). However, the findings of observational studies on the gut microbiota and PCOS are inconsistent, and it remains unclear whether there is a causal relationship between PCOS and the gut microbiota, as well as a reverse causal relationship between the two.

Investigating the causal relationship between gut microbiota and PCOS holds potential clinical value. It is important to conduct a comprehensive review of observational studies on the relationship between gut microbiota and PCOS, as well as to further explore the causal relationship between the two. Mendelian randomization

(MR) is an analytical approach used to determine the causal association between an exposure or risk factor and a clinically relevant outcome, particularly when a randomized controlled trial is not feasible and observational studies may have biased associations due to confounding or reverse causality (15). MR is based on three assumptions and is considered a cost-effective and time-efficient approach with the use of publicly accessible genome-wide association study (GWAS) data (16, 17). MR analysis allows the determination of the causal relationship between gut microbiota and PCOS.

In the present study, a review of observational studies on changes in gut microbiota in patients with PCOS was conducted. Furthermore, two-sample MR and reverse MR analyses were performed to explore the bidirectional causal relationship between gut microbiota and PCOS.

2 Materials and methods

2.1 Literature search strategy and study selection criteria

Cohort and cross-sectional studies comparing changes in the gut microbiota of PCOS patients and controls were searched from inception to May 10, 2023. The following search terms were used: “gut microbiota,” “gastrointestinal microbiome,” “intestinal flora,” “microbiome,” and “microbiota” combined with “PCOS” and “polycystic ovary syndrome.” The inclusion criteria for the literature were as follows: (i) cohort studies or cross-sectional studies that compared gut microbiota changes between PCOS patients and healthy controls, with clear definitions of the subgroups in these studies; (ii) data included the first author, year of publication, country, study type, diagnostic criteria, sample size, age range, microbiota analysis technique, and statistical results related to the gut microbiota; (iii) fecal samples were used for gut microbiota analysis; and (iv) methods for identifying gut microbiota were clearly described. The exclusion criteria were as follows: (i) individual bacterial taxa not provided; (ii) any systematic analyses, reviews, case reports, or conference reports; and (iii) original data that could not be extracted from the literature.

2.2 Data extraction and quality assessment

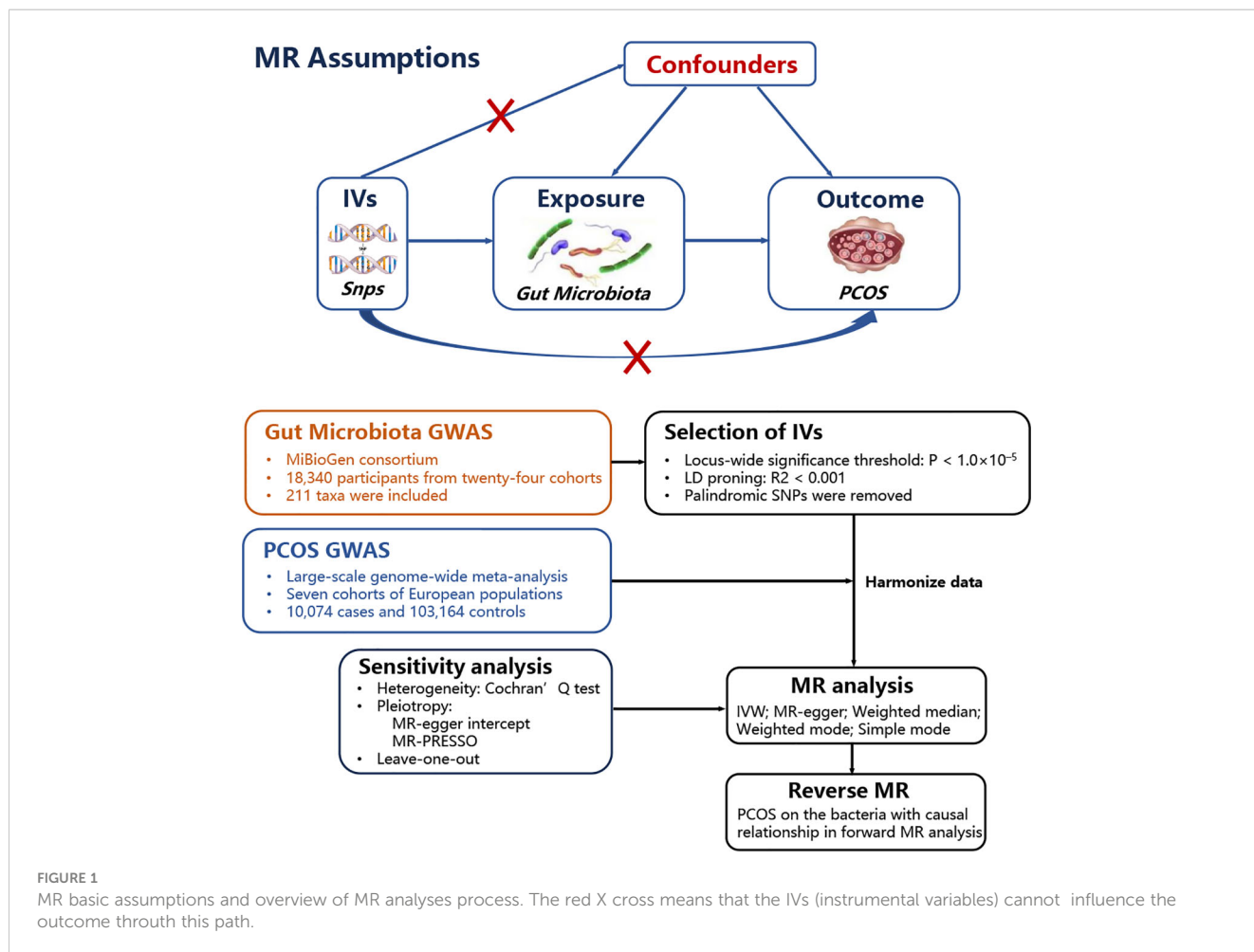
Data were obtained from the texts, tables, and figures of each study. The following data were collected: first author, year of publication, country, study type, diagnostic criteria, sample size, age range, microbiota analysis technique, and statistical results related to the gut microbiota. All outcomes from the included studies were categorized into tables, offering a comprehensive overview of the variations in the gut microbiota between subjects with PCOS and control subjects. The tables provided detailed information from the phylum level down to the species level. Regarding the composition of the gut microbiota, a descriptive literature synthesis was performed, considering the variations in age, body mass index (BMI), assessment methods, small sample sizes, limited data, and the quality of the studies included. The risk of bias assessment tool for nonrandomized studies (RoBANS) was used to evaluate the methodological quality and risk of bias of each included study. The RoBANS tool contains six domains and is a valid tool for evaluating nonrandomized controlled trials (18).

2.3 MR study design and data sources

A two-sample MR study was performed to investigate the causal relationship between gut microbiota and PCOS. Genetic variants

were used as instrumental variables (IVs) for the exposure because they are randomly allocated and not influenced by reverse causation or other confounding factors. The availability of publicly accessible GWAS data on gut microbiota and PCOS allowed efficient screening for suitable genetic IVs, making the two-sample MR approach cost-effective and time-efficient. A positive result from the MR analysis would provide support for a causal relationship between gut microbiota and PCOS. Additionally, a reverse MR was performed to examine whether PCOS also contributes to changes in gut microbiota. Valid estimates from the MR analyses were obtained when the following assumptions were satisfied: (i) a correlation between the single nucleotide polymorphisms (SNPs) and gut microbiota; (ii) SNPs influenced PCOS only through their impact on gut microbiota; and (iii) SNPs were not influenced by any confounding factors that may affect the relationship between gut microbiota and PCOS. Figure 1 shows the MR analysis design that accompanied the basic MR assumptions.

The largest known GWAS dataset for gut microbiota was obtained from the MiBioGen consortium. The GWAS data involved 24 cohorts, consisting of 18,340 participants, most of whom (n = 13,266) were of European descent. The authors used microbiota quantitative trait loci (mbQTL) mapping analysis to identify host genetic variants related to the abundance of gut microbiota. The V1–V2, V3–V4, and V4 variable regions of the 16S rRNA gene were used to determine microbial composition. The



GWAS data included 211 taxa from 9 phyla, 16 classes, 20 orders, 35 families, and 131 genera. Additional details about the microbiota data have been previously reported (19), and the comprehensive GWAS data is available at <https://mibiogen.gcc.rug.nl/>. GWAS data for PCOS were obtained from a large meta-analysis conducted by Day et al. (20), which involved 113,238 subjects, including 10,074 cases and 103,164 controls, all of whom were of European ancestry. Taking into account the characteristics of the GWAS of gut microbiota, MR analysis was performed at the five taxa levels (phylum, class, order, family, and genus) of gut microbiota. The GWAS data used in this study received ethical approval from their respective institutions and were publicly available online.

2.4 IV extraction and statistical analysis

The selection of IVs was based on the following criteria: (i) if $P < 5 \times 10^{-8}$ was taken as the genome-wide significance threshold, the number of selected IV SNPs was too small; therefore, $P < 1.0 \times 10^{-5}$ was taken as the threshold for screening IV in this study (21–24); (ii) based on the Europe-based 1,000 Genome project, a clustering distance of 10,000 kb and a threshold of $r^2 < 0.001$ were used in linkage disequilibrium (LD) analysis; and (iii) palindromic SNPs were removed to avoid the influence of alleles.

In the present study, five high-efficiency MR analysis methods were used, and the inverse variance weighted (IVW) method was the main MR method. The IVW method is based on the three assumptions of MR and the idea of a randomized experiment, and it estimates the causality using genetic variation to explore the causality in observational data. The key advantage of the IVW method is its utilization of naturally occurring genetic variation as an IV to estimate the causal effect between exposure and outcomes. Genetic variation adheres to the principle of random distribution, allowing it to simulate a random experiment (25). However, it is important to note that the IVW method is not without limitations. In addition to the IVW method, four other MR analysis methods are commonly used as complementary methods, including the MR-Egger, weighted median, weighted mode, and simple mode methods. The specific principles, advantages, and disadvantages of each method have been previously reported (16, 26, 27).

Because two-sample MR has several limitations that can potentially impact the reliability of the MR results, effective testing methods were utilized to verify the results in the present study. Horizontal pleiotropy refers to when IVs have a direct impact on PCOS through alternative pathways, bypassing the influence of gut microbiota. MR-Pleiotropy RESidual Sum and Outlier (MR-PRESSO) was used to examine the outliers that may indicate pleiotropic biases. If the SNPs used as IVs had significant heterogeneity, the results of MR may be biased. The heterogeneity of the selected SNPs was assessed using Cochran's Q statistic and leave-one-out sensitivity analysis. Weak IVs refer to the SNPs that are associated with exposure, but the strength of the association is not high. The strength of the IVs was examined using the F-statistic, which was calculated with the following formula $F = \frac{R^2 \times (N-1-K)}{(1-R^2) \times K}$; where R^2 is the fraction of variance in exposure explained by IVs; K is the number of IVs; and N is the sample size. An F-statistic greater

than 10 indicates that the corresponding IV was not considered a weak IV (28). To obtain a more stringent interpretation of causality, Bonferroni correction based on 211 bacterial taxa was used as follows: $0.05/211$ (3.81×10^{-4}).

Reverse MR analysis was conducted on the bacteria identified as causally associated with PCOS in the forward MR analysis to examine the reverse causality between the gut microbiota and PCOS. The methods and settings used in reverse MR analysis were consistent with those used in the forward MR analysis.

R software (version 4.3.0) was used for statistical analysis.

3 Results

3.1 Literature search and basic characteristics

A total of 261 studies were identified using the search strategy. After removing 63 duplicate studies, the remaining 198 studies were reviewed based on the inclusion and exclusion criteria. Ultimately, 18 articles were included in the present study. The process of literature screening is illustrated in Figure 2, and a summary of the included studies is shown in Table 1. Four studies were cross-sectional studies (12, 32, 33, 40), and 18 studies were cohort studies (11, 29–31, 34–39, 41–44). Most studies used the Rotterdam criteria for the diagnosis of PCOS, except for the study by Jobira, which used the National Institutes of Health (NIH) criteria (35). The study by Lüll et al. did not specify which diagnostic criteria were used (40). In addition, 16S rRNA gene sequencing with different region specifications was used as a microbiome assessment method in most studies, and whole-genome shotgun sequencing was used in the studies by Qi and Chu (12, 37). Seven studies divided participants into PCOS and healthy controls (11, 12, 30, 32, 33, 38, 40, 44), whereas the other studies subdivided participants into subgroups based on weight, insulin resistance, and other conditions. The studies were conducted in the following regions: 11 studies were conducted in East Asia (China) (12, 29, 31, 33, 34, 36, 37, 39, 41, 42, 44); four studies were conducted in Europe (11, 30, 32, 40); one study was conducted in North America (America) (35); and one study was conducted in Western Asia (Turkey) (38).

3.2 Summary of bacterial taxa changes in observational studies

All studies included in the review utilized high-throughput sequencing methods, specifically whole-genome shotgun sequencing and 16S rRNA gene sequencing. The abundance of gut microbiota was expressed using various parameters, including observed operating taxonomic unit (OTU) counts, richness, Chaos1, Shannon index, and evenness. To determine changes in the abundance of specific gut microbiota taxa, a species was considered to be increased or decreased if its relative abundance differed significantly ($P < 0.05$) at the phylum, family, genus, or species/genus subgroups levels.

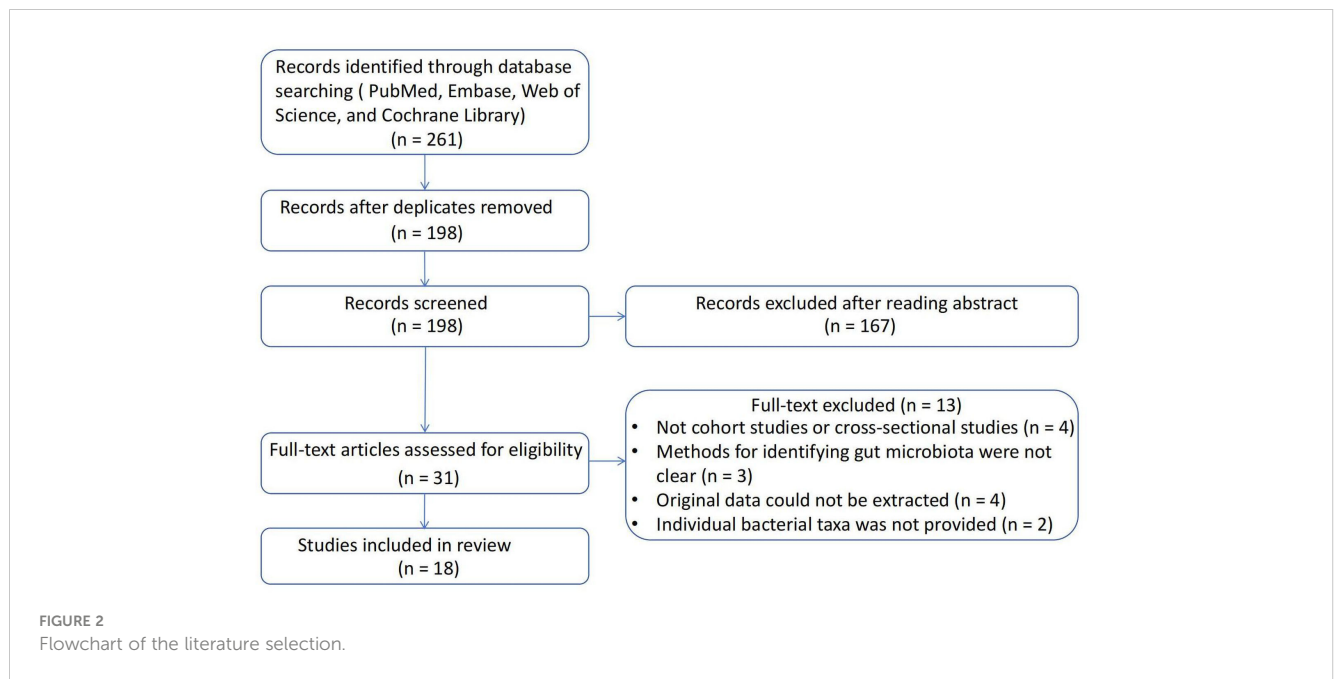


TABLE 1 Characteristics of the observational studies included in the review.

First Author	Year	Country	Study Design	Definition of PCOS	Major Microbiome Identification Method	Group	Patients, n	Age, years
Liu (29)	2017	China	Cross-sectional study	Rotterdam criteria	16S rRNA gene sequencing	Obese PCOS	21	29.3 ± 6.5
						Non-obese PCOS	12	25.5 ± 4.3
						Obese control	6	33 ± 5.4
						Non-obese control	9	32.2 ± 5.9
Lindheim (11)	2017	Austria	Cross-sectional study	Rotterdam criteria	16S rRNA gene sequencing	PCOS	24	27 (5.9)
						Healthy control	20	32 (12.0)
Insenser (30)	2018	Spain	Cross-sectional study	Rotterdam criteria	16S rRNA gene sequencing	PCOS	15	26.7 ± 7.2
						Healthy control	16	27.3 ± 5.2
Zeng (31)	2018	China	Cross-sectional study	Rotterdam criteria	16S rRNA gene sequencing	PCOS with insulin resistance	9	25.1 ± 4.3
						PCOS without insulin resistance	8	26.1 ± 7.1
						Healthy control	8	26.4 ± 3.9
Torres (32)	2018	Poland	Cohort study	Rotterdam criteria	16S rRNA gene sequencing	PCOS	73	27.4 ± 4.9

(Continued)

TABLE 1 Continued

First Author	Year	Country	Study Design	Definition of PCOS	Major Microbiome Identification Method	Group	Patients, n	Age, years
						Healthy control	48	29.4 ± 4.9
						PCOM Only	42	29.8 ± 5.3
Qi (12)	2019	China	Cohort study	Rotterdam criteria	Whole-genome shotgun sequencing	PCOS	50	20-40
						Healthy control	43	20-40
Zhang (33)	2019	China	Cohort study	Rotterdam criteria	16S rRNA gene sequencing	PCOS	38	27.6 ± 3.8
						Healthy control	26	26.7 ± 2.0
Zhou (34)	2020	China	Cross-sectional study	Rotterdam criteria	16S rRNA gene sequencing	Obese PCOS	30	26.9 ± 4.9
						Non-obese PCOS	30	25.1 ± 4.3
						Obese control	11	25.3 ± 1.6
						Non-obese control	30	22.1 ± 1.6
Jobira (35)	2020	America	Cross-sectional study	NIH	16S rRNA gene sequencing	Obese PCOS	37	16.1 ± 0.3
						Obese control	21	14.5 ± 0.4
Liang (36)	2020	China	Cross-sectional study	Rotterdam criteria	16S rRNA gene sequencing	Obese PCOS	8	27.1 ± 3.5
						Non-obese PCOS	10	25.7 ± 3.5
						Healthy control	9	27.9 ± 3.6
Chu (37)	2020	China	Cross-sectional study	Rotterdam criteria	Whole-genome shotgun sequencing	Non-overweight PCOS	7	27.14 ± 4.56
						Non-overweight control group	7	30.29 ± 3.90
						Overweight PCOS	7	29.14 ± 2.87
						Overweight control group	7	28.57 ± 2.72
Eyupoglu (38)	2020	Turkey	Cross-sectional study	Rotterdam criteria	16S rRNA gene sequencing	PCOS	17	20 (19-22)
						Healthy control	15	22 (18-27)
Chen (39)	2021	China	Cross-sectional study	Rotterdam criteria	16S rRNA gene sequencing	PCOS-LB	98	29.48 ± 3.39

(Continued)

TABLE 1 Continued

First Author	Year	Country	Study Design	Definition of PCOS	Major Microbiome Identification Method	Group	Patients, n	Age, years
						PCOS-HB	50	29.64 ± 4.06
						Healthy control	38	29.26 ± 4.18
Lüll (40)	2021	Estonia	Cohort study	–	16S rRNA gene sequencing	PCOS	102	46
						Healthy control	201	46
Liang (41)	2021	China	Cross-sectional study	Rotterdam criteria	16S rRNA gene sequencing	Lean PCOS	10	24.13 ± 2.45
						Overweight PCOS	10	28.94 ± 6.13
						Lean healthy control	10	25.08 ± 3.59
						Overweight healthy control	10	30.12 ± 5.20
Dong (42)	2021	China	Cross-sectional study	Rotterdam criteria	16S rRNA gene sequencing	PCOS	37	26-35
						Healthy control	45	28-35
Mammadova (43)	2021	Turkey	Cross-sectional study	Rotterdam criteria	16S rRNA gene sequencing	Lean PCOS	24	19.0-22.5
						Lean healthy control	22	22.0-24.25
Li (44)	2022	China	Cross-sectional study	Rotterdam criteria	16S rRNA gene sequencing	PCOS	31	24.35 ± 4.48
						Healthy control	27	27.0 ± 4.90

NIH (National Institutes of Health); PCOS (polycystic ovarian syndrome); PCOM (polycystic ovarian morphology); PCOS-LB (normal body mass index (BMI) PCOS); PCOS-HB (high body mass index (BMI) PCOS).

Age, years, mean ± standard deviation, median (range), or age lower limit–age upper limit.

“–” indicates that the literature did not provide this information.

None of the included studies reported differential gut microbiota taxa between PCOS patients and controls at the class and order levels. The groups for comparison were those with PCOS and those without PCOS, with or without involved subgroups. Three studies reported a significant increase in the abundance of the following phyla: *Proteobacteria*, *Actinobacteria*, and *Fusobacteria* in PCOS patients (35, 39, 43). Moreover, the following phyla were decreased in PCOS patients in three studies: *Bacteroidetes*, *Firmicutes*, *Tenericutes*, *Proteobacteria*, and *Actinobacteria* (11, 35, 39). At the family taxonomic level, *Lactobacillaceae*, *Streptococcaceae*, *Erysipelotrichaceae*, and *Enterobacteriaceae* were significantly elevated in patients with PCOS in three studies (35, 43, 44), while *S24-7*, *Prevotellaceae*, *Porphyromonadaceae*, *Barnesiellaceae*, *Christensenellaceae*, *Aerococcaceae*, and *Pasteurellaceae* were significantly less abundant in five studies (11, 31, 35, 42, 44). Changes in the abundance of some families of gut microbiota in PCOS patients, including *Bacteroidaceae*,

Ruminococcaceae, and *Lachnospiraceae*, were inconsistent in different studies (29, 31, 35, 36, 38, 44). Many significant differences were found at the genus level. The genus *Bacteroides* differed in abundance between PCOS and controls in six studies, with opposite results (12, 29, 33, 35, 37, 41). Moreover, the following genera were significantly increased in the PCOS group: *Ruminococcaceae* UCG-002, *Subdoligranulum*, *Lactobacillus*, *Oscillibacter*, *Catenibacterium*, *Kandleria*, *Coproccoccus*, *Lactococcus*, *Megamonas*, *Eubacterium*, *Escherichia*, *Klebsiella*, and *Bifidobacterium* (29, 30, 33, 34, 36, 40, 41, 44). The following genera were significantly reduced in the PCOS group: *Prevotella*, *Akkermansia*, *Faecalibacterium*, *Ruminococcus*, *Blautia*, *Roseburia*, *Lachnospira*, *Fusicatenibacter*, *Erysipelatoclostridium*, *Abiotrophi*, and *Haemophilus* (29, 33, 37, 39, 42–44). Three genera, namely, *Parabacteroides*, *Clostridium*, and *Bifidobacterium*, showed opposite abundance changes in different studies (33, 35, 37, 40).

In addition, four studies examined changes in abundance at the species or genus subgroup taxa level. Among them, genus *Bacteroides coprophilus*, genus *Bacteroides fragilis*, genus *Ruminococcus gnavus*, species *Blautia* spp., and genus *Collinsella aerofaciens* had significant increases in PCOS patients (32, 33, 42). Moreover, the significantly reduced genera or species taxa included species *Odoribacter* spp., genus *Ruminococcus bromii*, genus *Clostridium cluster XVII*, genus *Clostridium sensu stricto*, species *Anaerococcus* spp., and species *Bifidobacterium* spp. (29, 30, 43). Table 2 summarizes the gut microbiota taxa at the four levels with significant differences among the included studies.

The dietary patterns of populations from different regions vary, which can lead to differences in the species and abundance of gut microbiota. To compare the changes in gut microbiota of PCOS populations in different regions, the abundances of gut microbiota in populations from various countries were summarized (Supplementary Table 1).

3.3 Risk of bias assessment

The quality of the included studies was assessed using the RoBANS tool and is summarized in Supplementary Table 2. The included studies did not show a risk of exposure measurement bias, and they reported complete outcome data. Six studies had a high risk of bias in the selection of participants, and three studies involved confounding variables. In addition, 14 studies did not assess the risk of outcome blinding.

3.4 MR analysis results

In total, 2832 SNPs were identified as gut microbiota IVs. All SNPs had F-statistics greater than 10 (F ranged between 11.43 and 98.47), which indicated that there were no weak IVs. Additional information on the selected IVs is provided in Supplementary Table 3. In the forward MR Analysis, eight gut microbiota taxa were identified as having a significant causal relationship with PCOS using the IVM method, including one gut microbiota taxon at the family level and seven gut microbiota taxa at the genus level. Among them, four taxa had protective effects on PCOS, and four taxa were risk factors for PCOS.

The MR estimates with the IVW method showed that some genera, including *Sellimonas* [odds ratio (OR) 0.69, 95% confidence interval (CI) 0.58 - 0.84, $P = 1.22 \times 10^{-4}$], *Coprococcus2* (OR 0.58, 95% CI 0.41 - 0.83, $P = 3.20 \times 10^{-3}$), *Ruminococcaceae UCG011* (OR 0.75, 95% CI 0.60 - 0.95, $P = 1.62 \times 10^{-2}$), and *Ruminococcus (Gauvreauii group)* (OR 0.72, 95% CI 0.53 - 0.98, $P = 3.78 \times 10^{-2}$), had protective effects on PCOS. IVM analysis showed that the risk gut microbiota taxa for PCOS were genus *Streptococcus* (OR 1.53, 95% CI 1.13 - 2.07, $P = 5.93 \times 10^{-3}$), family *Streptococcaceae* (OR 1.51, 95% CI 1.11 - 2.05, $P = 8.76 \times 10^{-3}$), genus *Ruminococcaceae UCG005* (OR 1.39, 95% CI 1.04 - 1.86, $P = 2.79 \times 10^{-2}$), and genus *Actinomyces* (OR, 1.37; 95% CI, 1.03 - 1.82; $P = 3.19 \times 10^{-2}$). After Bonferroni correction, genus *Sellimonas* remained significant in the MR analysis results (adjusted $P = 2.57 \times 10^{-2}$). Figure 3 shows the MR analysis results of the causal

effects of the eight gut microbiota taxa on PCOS. The results of the MR analysis on the causal relationship between the 211 gut microbiota taxa and PCOS are shown in Supplementary Table 4.

Cochran's Q test did not identify significant heterogeneity in the IVs in the forward MR Analysis (Supplementary Table 5). Visual inspection of the scatter plots and leave-one-out plots of the IVs revealed no potential outliers for the gut microbiota taxa (Figures 4, 5). Additionally, no horizontal pleiotropy was observed in the MR-Egger regression intercept analysis (Supplementary Table 6), and no significant outliers were found in the MR-PRESSO analysis (global test, $P > 0.05$) (Supplementary Table 6). Therefore, there was insufficient evidence for horizontal pleiotropy in the causal relationship between the eight gut microbiota taxa and PCOS.

In the reverse MR analysis, a significant causal relationship was not observed between PCOS and any of the eight gut microbiota taxa (Supplementary Tables 7, 8). Cochran's Q test revealed no significant heterogeneity in the IVs of patients with PCOS (Supplementary Table 9). In the reverse MR analysis, MR-Egger regression did not show significant results for horizontal pleiotropy (Supplementary Table 10).

4 Discussion

The human gut contains trillions of microbes, which have been recognized as significant environmental factors impacting human physiology and pathology (45). Following the discovery by Poretzky et al. that gut microbiota disorders are closely associated with metabolic abnormalities in PCOS patients, numerous studies have been conducted to investigate the connection between gut microbiota and PCOS (46–49). Regardless of the level of gut microbiota taxa, various observational studies have arrived at different and even opposite findings, as shown in Table 2. The significant variations in results can be attributed to the diversity among different observational studies, including differences in sample sizes, grouping criteria, and microbiological identification methods. It is important to consider that populations from different regions have varying dietary patterns, which can contribute to the inconsistent findings (50). Future research should prioritize the development of more effective methods to compare the gut microbiota composition among individuals from different geographical locations.

Observational studies make it challenging to determine whether these changes are a cause or consequence of PCOS. However, the utilization of MR and publicly available GWAS data has facilitated the identification of causal relationships between gut microbiota and PCOS (51, 52). In the present study, the IVM analysis results for the genus *Sellimonas* remained significant after applying the Bonferroni correction. Genus *Sellimonas* belongs to the family *Lachnospiraceae*, which is part of the phylum *Firmicutes*. Genus *Sellimonas* is an anaerobic gram-positive microbe that has received relatively little attention in previous research (53). Muñoz et al. found that the genus *Sellimonas* may be a marker of intestinal homeostasis, and its abundance gradually increases with the restoration of intestinal barrier function (54, 55). Genus *Coprococcus*, an important member of the phylum *Firmicutes*, is

TABLE 2 Summary of representative taxa in PCOS patients compared to healthy controls in the included observational studies.

Phylum	Increase	Decrease	Family	Increase	Decrease	Genus	Increase	Decrease	Species or Genus Subgroups	Increase	Decrease
Bacteroidetes		Jobira2020	<i>Bacteroidaceae</i>	zeng 2018	Jobira2020	<i>Bacteroides</i>	Liu 2017	Jobira 2020	<i>Bacteroides coprophilus</i>	Torres 2018	
		Chen 2021					Qi 2019	Chu 2020	<i>Bacteroides fragilis</i>	Dong 2021	
							Zhang 2019		<i>Dialister succinatiphilus</i>	Dong 2021	
							Liang 2021				
			S24-7		Lindheim 2017						
			<i>Prevotellaceae</i>		Zeng 2018	<i>Prevotella</i>		Chu 2020	<i>Prevotella stercora</i>	Dong 2021	
					Li 2022			Chen 2021			
			<i>Porphyromonadaceae</i>		Jobira2020	<i>Porphyromonas</i>			<i>Porphyromonas</i> spp.	Torres 2018	
			<i>Odoribacteraceae</i>			<i>Odoribacter</i>			<i>Odoribacter</i> spp.		Torres 2018
			<i>Tannerellaceae</i>			<i>Parabacteroides</i>	Zhang 2019	Jobira 2020			
		<i>Barnesiellaceae</i>		Dong 2021							
Verrucomicrobia			<i>Akkermansiaceae</i>			<i>Akkermansia</i>		Liu 2017			
Firmicutes		Chen 2021	<i>Ruminococcaceae</i>	Zeng 2018	Liu2017	<i>Faecalibacterium</i>		Zhang 2019	<i>Faecalibacterium prausnitzii</i>	Torres 2018	Zhang 2019
				Eyupoglu 2020	Li 2022	<i>Ruminococcus</i>		Liu 2017	<i>Ruminococcus bromii</i> .		Torres 2018
									<i>Ruminococcus gnavus</i>	Dong 2021	
						<i>Ruminococcaceae</i> UCG002	Lüll 2021				
						<i>Subdoligranulum</i>	Liang 2020				
			<i>Lactobacillaceae</i>	Jobira 2020		<i>Lactobacillus</i>	Zhang 2019				

(Continued)

TABLE 2 Continued

Phylum	Increase	Decrease	Family	Increase	Decrease	Genus	Increase	Decrease	Species or Genus Subgroups subgsfdgssdfssu-bSubgroup	Increase	Decrease
			<i>Oscillospiraceae</i>			<i>Oscillibacter</i>	Zhang 2019				
			<i>Clostridiaceae</i>			<i>Clostridium</i>	Zhang 2019	Chu 2020	<i>Clostridium cluster XVII</i>		Mammadova 2021
									<i>Clostridium sensu stricto</i>		Mammadova 2021
			<i>Erysipelotrichaceae</i>			<i>Catenibacterium</i>	Insenser 2018				
						<i>Kandleria</i>	Insenser 2018				
			<i>Lachnospiraceae</i>	Zeng 2018	Liang2020	<i>Blautia</i>		Zhang 2019	<i>Blautia</i> spp.	Torres 2018	
						<i>Roseburia</i>		Mammadova 2021	<i>Roseburia</i> spp.	Dong 2021	Torres 2018
						<i>Lachnospira</i>		Zhang 2019			
						<i>Coprococcus</i>	Zhou 2020				
			<i>Peptoniphilaceae</i>			<i>Anaerococcus</i>			<i>Anaerococcus</i> spp.		Torres 2018
			<i>Streptococcaceae</i>	Jobira 2020		<i>Lactococcus</i>	Zhou 2020				
			<i>Selenomonadaceae</i>			<i>Megamonas</i>	Liang 2020				
			<i>Eubacteriaceae</i>			<i>Eubacterium</i>	Lüll 2021				
			<i>Christensenellaceae</i>		Dong 2021						
						<i>Fusicatenibacter</i>		Dong 2021			
			<i>Erysipelotrichaceae</i>	Mammadova 2021		<i>Erysipelatoclostridium</i>		Li 2022			
			<i>Aerococcaceae</i>		Li 2022	<i>Abiotrophia</i>		Li 2022			
			<i>Peptostreptococcaceae</i>								
Tenericutes		Lindheim 2017									

(Continued)

TABLE 2 Continued

Phylum	Increase	Decrease	Family	Increase	Decrease	Genus	Increase	Decrease	Species or Genus Subgroups subgsfdgssdfssu- bSubgroup	Increase	Decrease
Proteobacteria	Mammadova 2021 d2021202122021 2021	Chen 2021	<i>Enterobacteriaceae</i>	Mammadova 2021		<i>Escherichia</i>	Zhang 2019				
							Liu 2017				
							Liang 2021				
						<i>Klebsiella</i>	Li 2022				
						<i>Pasteurellaceae</i>		Dong 2021	<i>Haemophilus</i>		Dong 2021
Actinobacteria	Jobira 2020	Chen 2021	<i>Bifidobacteriaceae</i>			<i>Bifidobacterium</i>	Lüll 2021	Zhang 2019	Bifidobacterium spp.		Zhang 2019
						<i>Coriobacteriaceae</i>			<i>Collinsella</i>		
Verrucomicrobia			<i>Akkermansiaceae</i>			<i>Akkermansia</i>		Liu 2017			
Fusobacteria	Chen 2021										

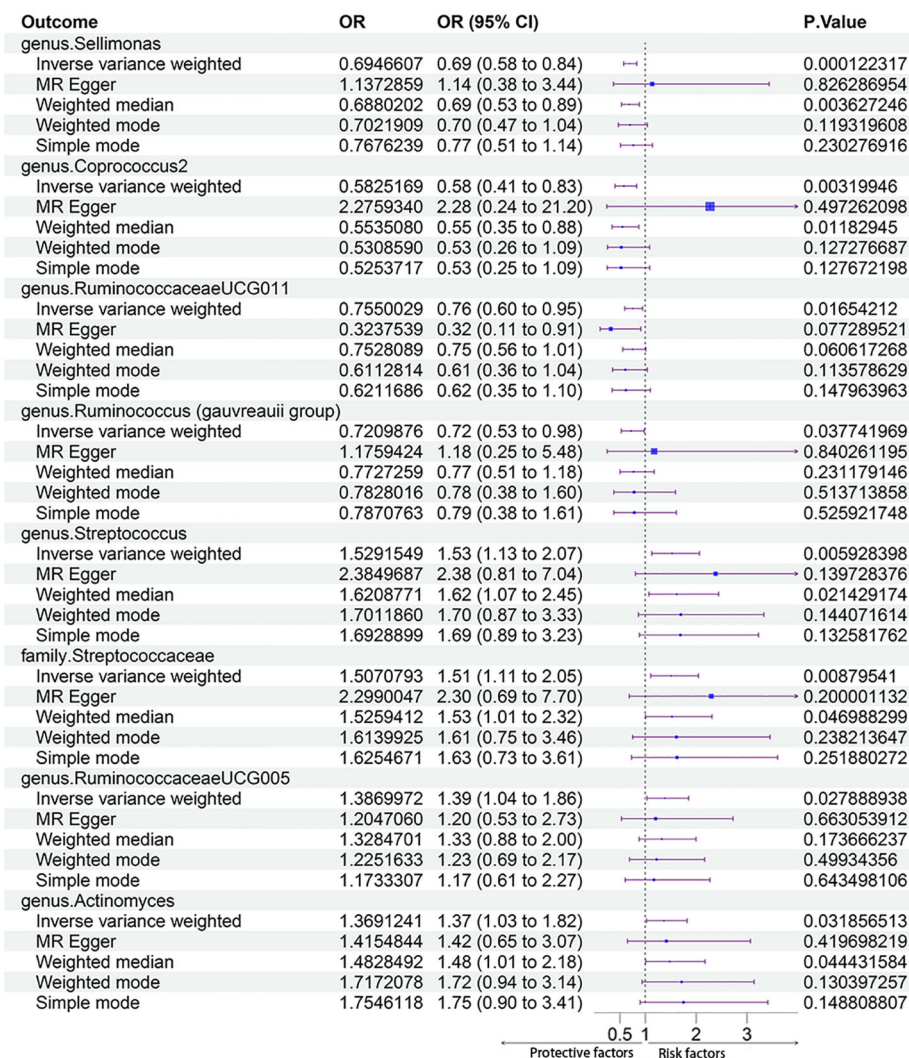
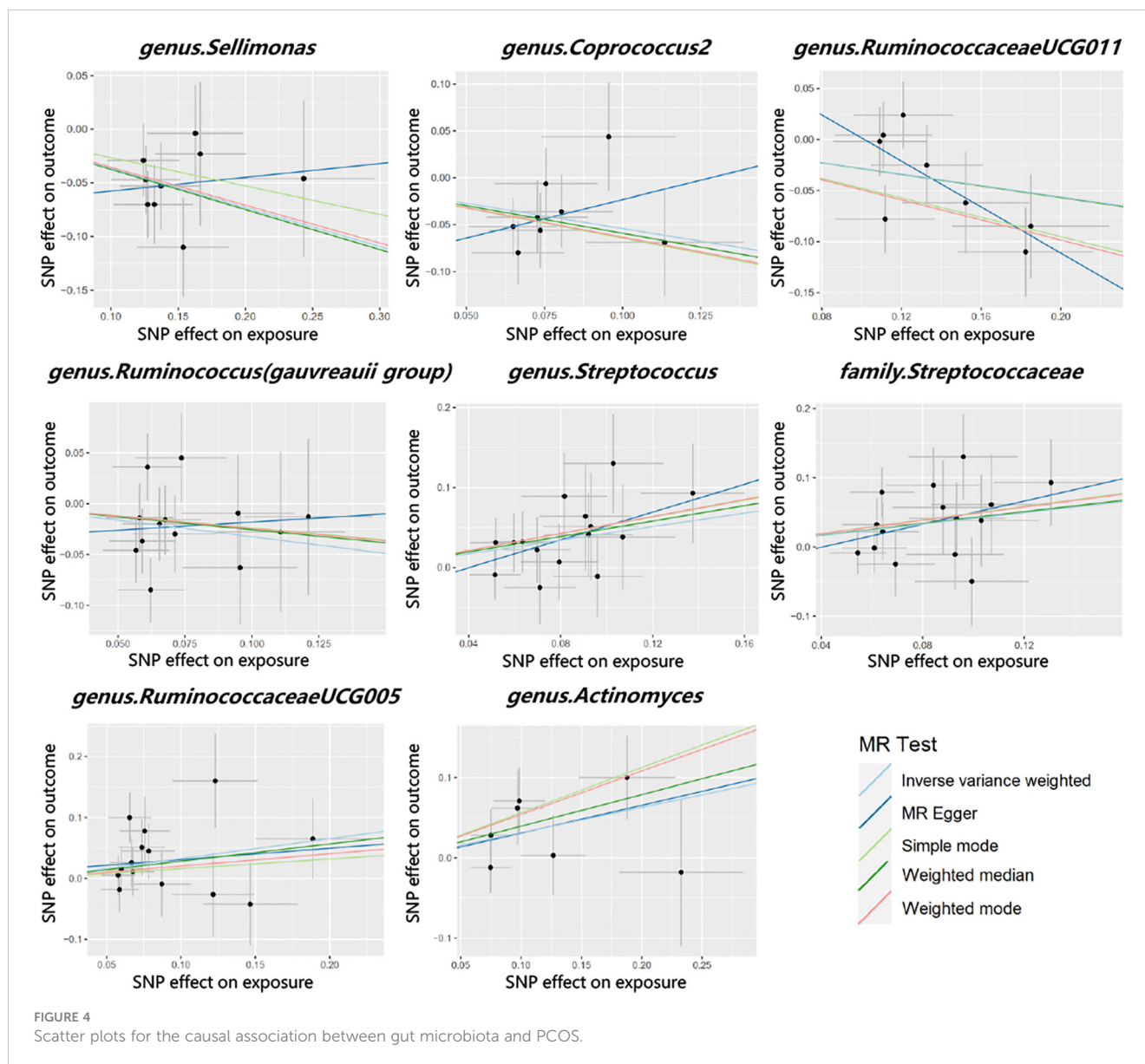


FIGURE 3 The results of MR analysis on the causal effect of the eight gut microbiota taxa on PCOS.

known for its production of butyric acid (56). Zhou et al. proposed that genus *Coprococcus2* is a distinguishing gut microbiota in obese PCOS patients (34). However, the present IVM analysis did not support this observation, which may be attributed to the distinct group specificity of gut microbiota (57). The genus *Ruminococcus*, belonging to the family *Ruminococcaceae*, has a potential role in the protective effect against PCOS. One possible explanation for this effect is that an increase in the genus *Ruminococcus* (*Gavreauii* group) is strongly associated with improved insulin sensitivity in obese patients (58). Interestingly, genera *Streptococcus* and *Actinomyces* are significantly increased in the saliva and vagina, but not in the intestine, of PCOS patients (59, 60). The present MR study suggested that the observed relationship between gut microbiota and PCOS may not necessarily be causal. One possible explanation is that the gut microbiota, which has a causal relationship with PCOS, may not be detected due to its low abundance, as well as limitations in current detection and statistical methods. Nevertheless, the findings obtained through the MR method are noteworthy and warrant further investigation.

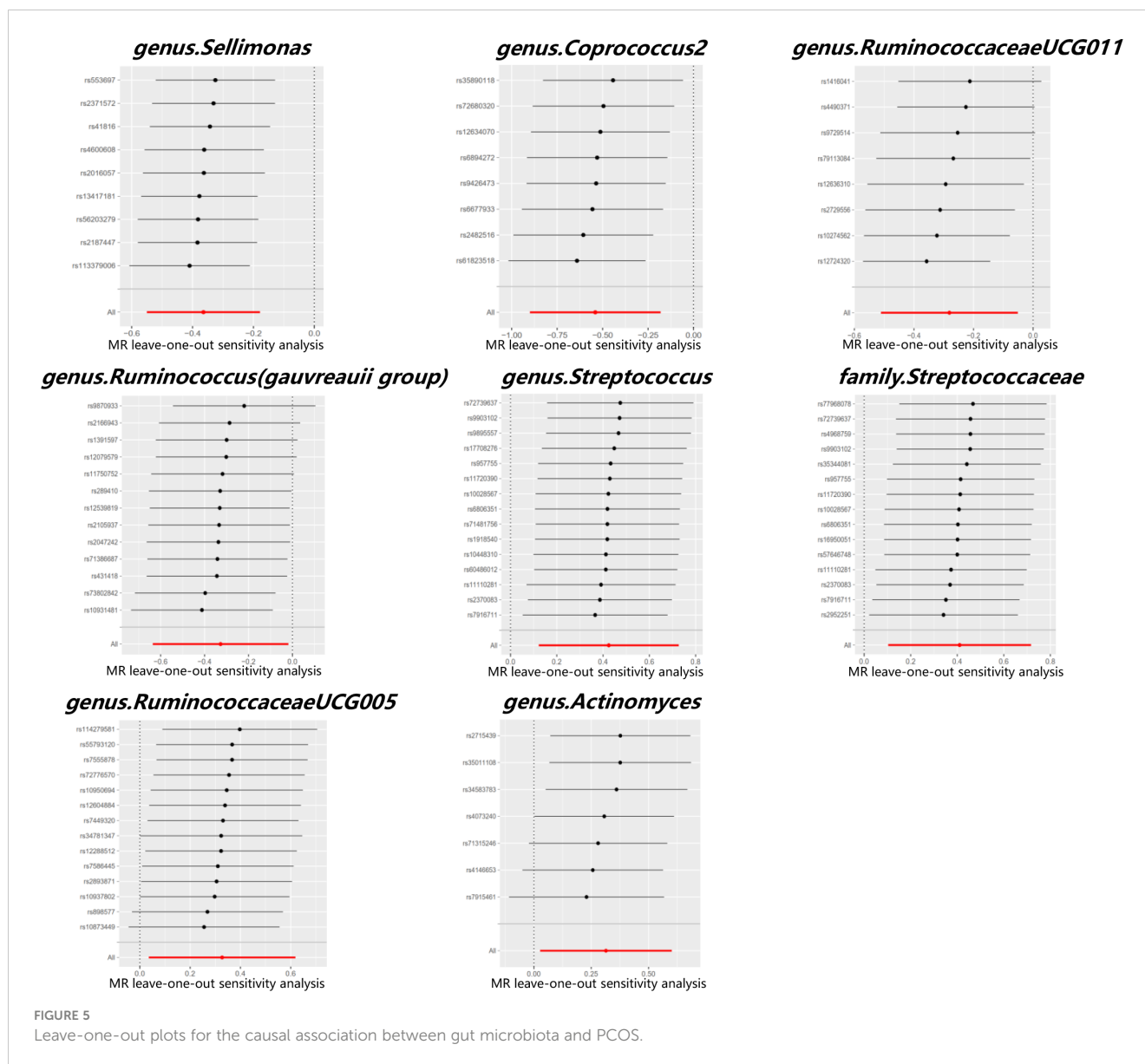
The gut microbiota/bile acid/interleukin-22 axis has been investigated as one of the possible potential mechanisms for this causal relationship (12, 61). Transplantation of fecal microbiota from women with PCOS or *B. vulgatus*-colonized recipient mice results in various negative effects on ovarian functions, insulin resistance, bile acid metabolism, interleukin-22 secretion, and fertility. IL-22 improves the PCOS phenotype. Some gut microbiota taxa are capable of inducing oxidative stress and an inflammatory response from mononuclear cells of women with PCOS by influencing the dietary trigger (62, 63). Oxidative stress and inflammation also damage the structure and function of intestinal permeability, leading to an increase in gram-negative bacteria in the blood. This activation of the immune system hampers the function of insulin receptors, which results in elevated insulin levels, subsequently leading to high levels of androgens and abnormal follicular development (64). Energy absorption may be a mechanism of gut microbiota leading to PCOS (65). Studies have indicated that more than half of PCOS patients are overweight or obese (11). Researchers have confirmed



that the richness and phylogenetic diversity of gut microbiota in patients with PCOS are significantly reduced, and multiple meta-analyses have confirmed that this reduction is closely related to the obesity phenotype of PCOS (66, 67). Other possible mechanisms include the lipopolysaccharide metabolic pathway, short-chain fatty acid (SCFA) metabolic pathway, and choline pathway (68–70).

The present study of the relationship between gut microbiota and PCOS holds potential clinical significance. Firstly, changes in gut microbiota may serve as an indicator for the occurrence of PCOS, suggesting that gut microbiota may potentially be used for diagnostic purposes in PCOS. A functional predictive analysis conducted by Zhou et al. revealed significant differences in fecal microbes among obese PCOS patients, non-obese PCOS patients, and healthy individuals (34), highlighting significant differences in the gut microbiota and its predicted functions in obese and non-obese women with PCOS. The positive bacteria identified in the

present MR study have promising potential as diagnostic bacteria for the occurrence and post-treatment effects of PCOS in the future. Moreover, alterations in gut microbiota have been found to be closely associated with various PCOS-related clinical parameters, including metabolic factors, sex hormones, and mediators of the brain-gut axis (29). Secondly, treatment of PCOS based on gut microbiota has attracted increasing attention. Animal experiments involving fecal microbiota transplantation (FMT) have demonstrated significant improvements in PCOS-related symptoms after PCOS mice receive feces from healthy mice. The use of FMT as a potential approach to treat PCOS holds promise for the future. Additionally, microbial agents, such as probiotics, prebiotics, and synbiotics, have shown potential therapeutic value in PCOS treatment (71, 72). The protective bacteria identified in the present MR study should be considered as potential sources of microbial agents in future studies. Moreover, studies have indicated



that specific drugs used for PCOS treatment may achieve their intended effects by influencing changes in gut microbiota. These drugs include metformin, thiazolidinediones, and lipid-lowering drugs (73–75). Thus, the mediating role played by gut microbiota deserves attention.

5 Limitations

In reviewing observational studies, a major challenge is the difficulty in adjusting for potential confounding factors and other variables due to the significant heterogeneity among the studies. The complexity of the included population groups limited our ability to conduct a more rigorous quantitative systematic review of these studies. Additionally, the various gut microbiota patterns influenced by diet habits in different regions made it challenging to

interpret the summarized results using MR analysis. Moreover, the GWAS data used in the present MR analysis was obtained from 24 cohorts in different countries. Due to the polymorphism observed among different human populations, the outcomes of GWAS may vary across these populations (76). Furthermore, the presence of genetic heterogeneity within each population can also impact the reliability of GWAS results. This limitation may affect the interpretation of the GWAS findings. In MR Studies, it is challenging to completely avoid LD. In addition, when using genes as IVs, biases may arise due to weak instruments, population stratification, and developmental compensation (77). Bonferroni correction was used as a multiple test in the present MR analysis. One advantage of this correction method is its effectiveness in controlling type I errors, but it may be overly conservative, which can increase type II error rates (78). Additionally, the strictness of Bonferroni may filter out potentially meaningful results.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/[Supplementary Material](#).

Ethics statement

Ethical approval was not required for the study involving humans in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and the institutional requirements.

Author contributions

JS: Data curation, Formal analysis, Writing – original draft. MW: Methodology, Resources, Software, Writing – original draft. ZK: Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fendo.2024.1280983/full#supplementary-material>

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