

Mendelian Randomization Analysis of Gut Microbiota and Premature Ovarian Failure

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Abstract:

Premature ovarian failure (POF) refers to the abnormal decline of ovarian function in women before the age of 40, characterized by decreased serum estrogen (E2) levels and increased follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels. Studies have indicated an association between gut microbiota and the occurrence and progression of premature ovarian failure (POF); however, the causality between gut microbiota and POF remains unclear. Therefore, Mendelian randomization (MR) analysis was employed to explore the causal relationship between gut microbiota and premature ovarian failure. Using summary statistics from genome-wide association studies (GWAS), gut microbiota was considered as the exposure factor, utilizing GWAS data from 18,340 participants as instrumental variables and GWAS data from 469 POF patients from Europe as the outcome variable, MR analysis was conducted against 200,581 controls. Initially, a two-sample MR analysis was performed to identify beneficial and pathogenic bacterial taxa associated with POF in the discovery sample. Subsequently, sensitivity analysis was conducted to assess the robustness of the results, excluding the influence of confounding factors, and the robustness of the MR analysis results was evaluated through leave-one-out analysis and funnel plots. Finally, reverse MR analysis was conducted to assess the possibility of reverse causality. Gut microbiota (GM) was found to be associated with POF in the inverse variance weighted (IVW) method. Elevations in NB1n genus [OR=1.69, 95% CI (1.02, 2.80), P=0.040], unknown genus [OR=1.69, 95% CI (1.03, 2.79), P=0.039], Intestinibacter genus [OR=1.90, 95% CI (1.11, 3.24), P=0.019], Parasutterella genus [OR=1.69, 95% CI (1.02, 2.80), P=0.040], Intestinimonas genus [OR=1.79, 95% CI (1.07, 3.00), P=0.027], and Adlercreutzia genus [OR=2.47, 95% CI (1.14, 5.37), P=0.022] were positively correlated with an increased risk of premature ovarian failure, while elevations in Methanobacteriaceae family [OR=0.66, 95% CI (0.44, 0.99), P=0.047], Methanobacteriales order [OR=0.66, 95% CI (0.44, 0.99), P=0.047], Methanobacteriaceae family [OR=0.66, 95% CI (0.44, 0.99), P=0.047], Eubacteriumventriosumgroup genus [OR=0.49, 95% CI (0.27, 0.91), P=0.023], Eubacteriumhalliigroup genus [OR=0.47, 95% CI (0.26, 0.85), P=0.012], and Actinomyces genus [OR=0.47, 95% CI (0.26, 0.85), P=0.013] were negatively correlated with a decreased risk of premature ovarian failure. Sensitivity analysis showed no evidence of reverse causality, pleiotropy, or heterogeneity. The results indicate an association between gut microbiota and POF, with the unknown genus,

Intestinibacter, *Parasutterella*, *Intestinimonas*, and *Adlercreutzia* genera potentially acting as risk factors for POF, while *Methanobacteria*, *Methanobacteriales*, *Methanobacteriaceae*, *Eubacteriumventriosum* group, *Eubacteriumhallii* group, and *Actinomyces* genera may serve as protective factors against POF. Gut microbiota plays an important role in the pathogenesis of premature ovarian failure, providing a new perspective on how gut microbiota influences the development of premature ovarian failure.

Keywords:

Premature Ovarian Failure, Gut Microbiota, Mendelian Randomization, Single Nucleotide Polymorphism, Estrogen Levels, Gut-ovary Axis

1. Introduction

Premature ovarian failure (POF), also known as premature ovarian insufficiency (POI), represents the end stage of early ovarian dysfunction characterized by elevated levels of follicle-stimulating hormone (FSH) and decreased levels of estradiol (E2) in serum, resulting in hormonal imbalance, amenorrhea, and decreased fertility or even infertility in women under 40 years old [1]. Under the influence of high pressure and fast-paced lifestyles, the incidence of POF has been increasing annually and tends to occur at younger ages [2]. Statistics indicate that among women under 35 years old, 1 in every 250 is affected by POF, while among women under 40 years old, 1 in every 100 is affected [3,4]. Known etiological factors of POF include iatrogenic, metabolic, infectious, chromosomal abnormalities, and autoimmune diseases [5], yet many causative factors remain unknown, making it difficult to prevent its occurrence at the source.

The gut microbiota (GM) constitutes a widely distributed and complex microbial community, playing crucial roles in maintaining normal physiological functions and immune responses in the gastrointestinal tract [6]. Emerging research on gut microbiota has revealed its significant impact on female reproductive health [7], as it can influence the occurrence of various endocrine diseases, such as polycystic ovary syndrome, endometriosis, and POF, by regulating estrogen, producing inflammatory factors, affecting immunity, and stabilizing metabolism. This provides new insights into the prevention and treatment of premature ovarian failure. However, the causal relationship between premature ovarian failure and gut microbiota remains incompletely understood.

Mendelian randomization (MR) is a statistical method that utilizes genetic variants strongly associated with the exposure factor as instrumental variables (IVs) to assess causality between exposure factors and outcomes. Compared to observational studies such as case-control studies, MR is less affected by confounding factors [8]. To date, MR has been widely used to investigate the associations between genetic factors and various diseases, including autoimmune diseases [9], metabolic diseases [10], psychiatric disorders [11], and pregnancy complications [12]. In this study, to explore the potential causal relationship between gut microbiota and premature ovarian failure, we conducted comprehensive bidirectional two-sample MR analysis of premature ovarian failure.

2. Research Methods and Materials

2.1. Study Design

In this study, TwoSampleMR analysis was conducted using publicly available summary statistics from genome-wide association studies (GWAS) [13]. A total of 211 taxonomic groups (131 genera, 35 families, 20 orders, 16 classes, and 9 phyla) were included as exposure factors, with premature ovarian failure (POF) as the outcome, to assess the causal relationship between them. Figure 1 illustrates the workflow of this MR study. Throughout the entire analysis process, we strictly adhered to the MRSTROBE guidelines for reporting [14].

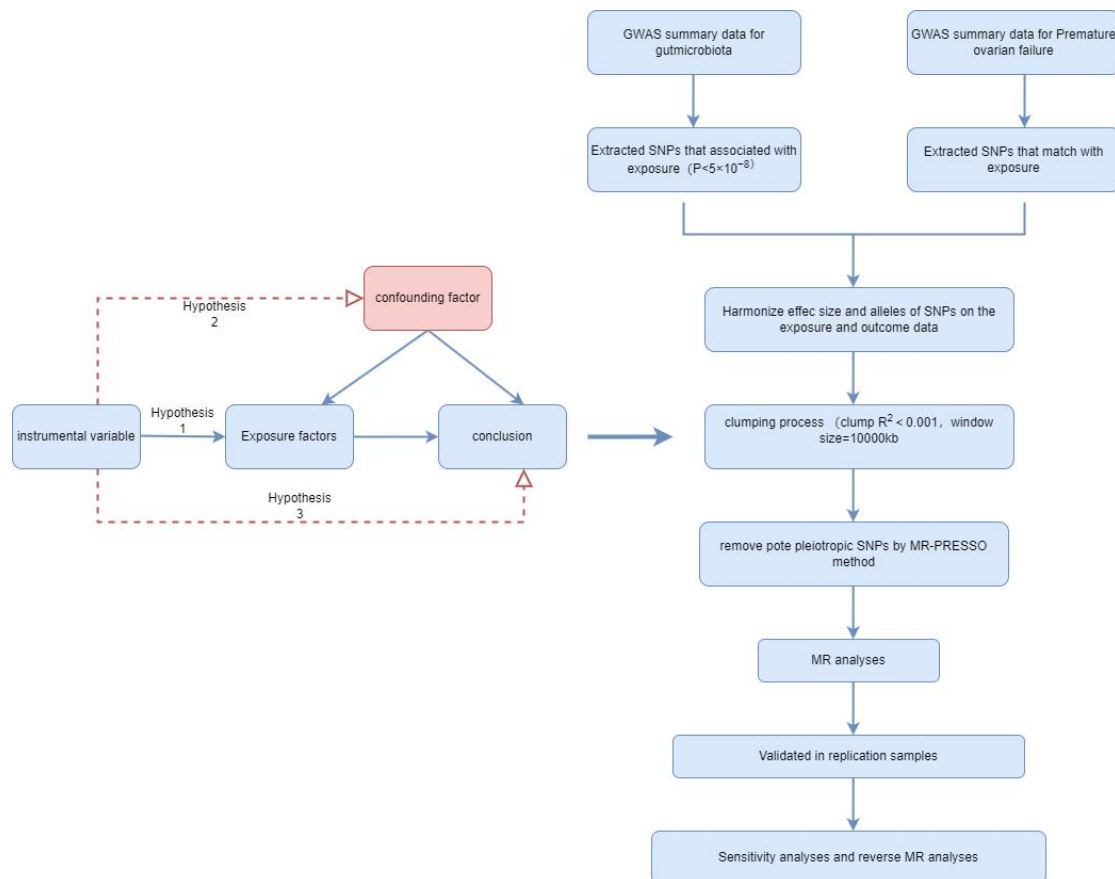


Figure 1. Mendelian Randomization Analysis Model.

Note: ① IVs are associated with exposure; ② IVs are independent of confounding factors; ③ IVs affect outcomes only through exposure.

2.2. Data Sources (Table 1)

The gut microbiota data used in the study were obtained from the MiBioGen database [15] which comprises sequences from 24 populations across the United States, Canada, Israel, South Korea, Germany, Denmark, the Netherlands, Belgium, Sweden, Finland, and the United Kingdom, totaling 18,340 individuals. The GWAS data for premature ovarian failure were downloaded from the FinnGen database, selecting the most recent release, which included 496 patients and 200,581 healthy controls. Refer to Table 1. As this study utilized publicly available summary data, no additional ethical approval or participant consent was required.

Table 1. Details of GWAS Research Data and Datasets Used in the Analysis.

Exposure or outcome	Sample size	Ancestry	Links for data download
GM POF	18340 participants 496 cases, 200581 controls	Mixed European ancestry	http://mibiogen.gcc.rug.nl https://storage.googleapis.com/finngen-public

2.3. Selection of Instrumental Variables

The MR analysis included 211 bacterial taxa, spanning 5 taxonomic levels, including 9 phyla, 16 classes, 20 orders, 35 families, and 131 genera. Subsequently, a series of quality control steps were carried out to select appropriate instrumental variables (IVs). To ensure the authenticity and accuracy of the research data, the following quality control procedures were adopted in selecting the optimal IVs: (1) SNPs with significance levels of association with POF reaching genome-wide significance ($P < 1.0 \times 10^{-5}$) were selected as IVs; (2) SNPs in linkage disequilibrium (LD) ($R^2 < 0.001$, distance set at 10,000 kb) were excluded to ensure the independence of IVs, thus reducing bias caused by residual genetic variation [16,17]. SNPs with the lowest P-values associated with gut microbiota were chosen. (3) Genetic variants were independent of confounding factors. (4) Genetic variants were unrelated to the outcome variable.

The statistical strength of the correlation between SNPs and exposure was evaluated using the F-statistic, calculated by the formula $F = R^2 / (1 - R^2) \times (N - K - 1) / K$, where N is the sample size of the gut microbiota database, K is the number of SNPs, R^2 represents the proportion of variation in gut microbiota explained by SNPs, calculated by the formula $R^2 = 2 \times (1 - EAF) \times EAF \times \beta^2$, where EAF is the effect allele frequency of each SNP, and β is the effect size of the allele. An F-value < 10 was considered indicative of weak correlation between SNPs and exposure. SNPs with palindromic structure were automatically excluded during the analysis process.

2.4. Mendelian Randomization Analysis

Utilizing a bidirectional sample approach, gut microbiota (GM) and premature ovarian failure (POF) data were separated from GWAS to conduct MR analysis on the causal relationship between the gut microbiota and the outcome of premature ovarian failure. The inverse-variance weighted method (IVW) was employed as the primary analytical method [18,19,20], along with MR-Egger regression and weighted median methods, to ascertain the correlation between GMs and POF.

2.5. Sensitivity Analysis

To ensure the accuracy of MR results, Cochran's Q test was utilized to assess heterogeneity among the effect estimates of instrumental variables (IVs) [21]. If the p-value of Cochran's Q test was < 0.05 , it was considered indicative of heterogeneity among the effect estimates of IVs. Sensitivity analysis was conducted to ensure the stability of IVW results and to avoid bias caused by ineffective IVs. MR-Egger intercept test and Mendelian randomization pleiotropy residual sum and outlier (MR-PRESSO) [22] method were used to detect whether the causal association estimates were affected by pleiotropy. When the p-value of the MR-Egger intercept test was < 0.05 , it indicated a significant difference from the numerical zero, suggesting the presence of pleiotropic effects within the IVs set. MR-PRESSO was employed for detecting and removing pleiotropic SNPs. Leave-one-out analysis was employed to

evaluate whether significant results were determined by individual SNPs. The risk relationship between human gut microbiota and premature ovarian failure was expressed as odds ratio (OR) and its 95% confidence interval (CI). Evidence supporting a potential causal relationship was considered if $p \leq 0.05$.

2.6. Statistical Software Versions and Names

MR analysis was conducted using R (version 4.3.1) with the TwoSampleMR package and MR-PRESSO package.

3. Results

3.1. Primary Results of Gut Microbiota Associated with Premature Ovarian Failure Risk (Table 2)

According to the specified selection criteria,a total of 2,818 independent SNPs ($P < 1 \times 10^{-5}$) were found to be associated with premature ovarian failure at the phylum, class, order, family, and genus levels. After excluding IVs with $P < 10$, a final selection of 142 SNPs was made, all with F-statistics > 10 ; see Table 2.

Through MR analysis using the IVW method, we identified 12 gut microbiota taxa associated with premature ovarian failure out of 211 taxa (Figure 2). The F-statistics of gut microbiota ranged from 14.58 to 88.42, with an average of 21.69, all exceeding the threshold of 10, indicating minimal potential for bias.

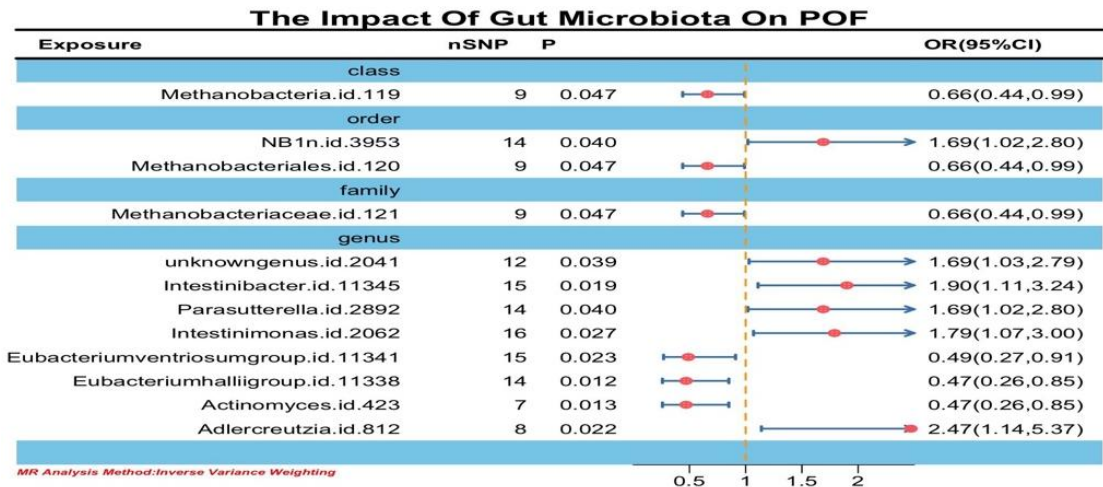


Figure 2. MR forest plot analysis of the causal effect of gut microbiota on premature ovarian failure.

3.2. Two-Sample Mendelian Randomization Analysis (Table 2)

The IVW method analysis results showed positive associations between premature ovarian failure and the following taxa: NB1n order [OR=1.69,95%CI (1.02,2.80), $P=0.040$], unknown genus [OR=1.69,95%CI (1.03,2.79), $P=0.039$], Intestinibacter genus [OR=1.90,95%CI (1.11,3.24), $P=0.019$], Parasutterella genus [OR=1.69,95%CI (1.02,2.80), $P=0.040$], Intestinimonas genus [OR=1.79,95%CI (1.07,3.00), $P=0.027$], and Adlercreutzia genus [OR=2.47,95%CI (1.14,5.37), $P=0.022$]. Conversely, negative associations were observed between premature ovarian failure and the following taxa: Methanobacteria phylum [OR=0.66,95%CI (0.44,0.99), $P=0.047$], Methanobacteriales order [OR=0.66,95%CI (0.44,0.99), $P=0.047$], Methanobacteriaceae family [OR=0.66,95%CI (0.44,0.99), $P=0.047$], Eubacterium

ventriosum group genus [OR=0.49,95%CI (0.27,0.91), P=0.023], Eubacterium hallii group genus [OR=0.47,95%CI (0.26,0.85), P=0.012], and Actinomyces genus [OR=0.47,95%CI (0.26,0.85), P=0.013]; see Table 2.

Table 2. Results of MR analysis with GM as a whole.

GM	method	SNP	OR	95%CI	P
Methanobacteria	IVW	9	0.66	0.44,0.99	0.047
	MR Egger	9	0.72	0.17,4.23	0.844
NB1n	IVW	14	1.69	1.02,2.80	0.040
	MR Egger	14	1.31	0.31,5.49	0.713
Methanobacteriales	IVW	9	0.66	0.44,0.99	0.047
	MR Egger	9	0.84	0.17,4.23	0.844
Methanobacteriaceae	IVW	9	0.66	0.44,0.99	0.047
	MR Egger	9	0.84	0.17,4.23	0.844
unknowngenus	IVW	12	1.69	1.03,2.79	0.039
	MR Egger	12	0.63	0.13,3.17	0.592
Intestinibacter	IVW	15	1.90	1.03,2.79	0.019
	MR Egger	15	3.18	0.56,18.0	0.213
Parasutterella	IVW	14	1.69	1.02,2.80	0.040
	MR Egger	14	1.31	0.31,5.49	0.713
Intestinimonas	IVW	16	1.79	1.07,3.00	0.027
	MR Egger	16	3.54	0.84,14.9	0.105
Eubacteriumventriosumgroup	IVW	15	0.49	0.27,0.91	0.023
	MR Egger	15	1.23	0.07,19.0	0.883
Eubacteriumhalliigroup	IVW	14	0.47	0.26,0.85	0.012
	MR Egger	14	0.46	0.13,1.56	0.238
Actinomyces	IVW	7	0.47	0.26,.085	0.013
	MR Egger	7	0.24	0.06,1.07	0.121
Adlercreutzia	IVW	8	2.47	1.14,5.37	0.022
	MR Egger	8	0.12	0.006,2.02	0.190

3.3. Sensitivity Analysis (Table 3)

Level pleiotropy between SNPs and outcomes was assessed using MR-Egger regression. The MR-Egger intercepts for NB1n order (intercept P=0.40), unknown genus (intercept P=0.35), Intestinibacter genus (intercept P=0.70), Parasutterella genus (intercept P=0.40), Intestinimonas genus (intercept P=0.82), Adlercreutzia genus (intercept P=0.51), Methanobacteria phylum (intercept P=0.79), Methanobacteriales order (intercept P=0.79), Methanobacteriaceae family (intercept P=0.79), Eubacterium ventriosum group genus (intercept P=0.93), Eubacterium hallii group (intercept P=0.44), and Actinomyces genus (intercept P=0.69) were all >0.05, indicating no evidence of horizontal pleiotropy.

To assess whether SNPs selected by Cochran's Q test exhibited heterogeneity, the following results were obtained: NB1n order Q-PIVW=0.47, Q-PMR-Egger=0.40; unknown genus Q-PIVW=0.53, Q-PMR-Egger=0.59; Intestinibacter genus Q-PIVW=0.74, Q-PMR-Egger=0.70; Parasutterella genus Q-PIVW=0.47, Q-PMR-Egger=0.40; Intestinimonas genus Q-PIVW=0.80, Q-PMR-Egger=0.82; Adlercreutzia genus Q-PIVW=0.19, Q-PMR-Egger=0.51; Methanobacteria phylum Q-PIVW=0.86, Q-PMR-Egger=0.79; Methanobacteriales order Q-PIVW=0.86, Q-PMR-Egger=0.79; Methanobacteriaceae family Q-PIVW=0.86, Q-PMR-Egger=0.79; Eubacterium ventriosum group genus Q-PIVW=0.94, Q-PMR-Egger=0.93; Eubacterium hallii group genus Q-PIVW=0.52, Q-PMR-Egger=0.44; Actinomyces genus Q-PIVW=0.68,

Q-PMR-Egger=0.69. Both IVW and MR-Egger's Q_pval were >0.05, indicating no heterogeneity. MR-PRESSO did not detect any outliers (P>0.05); see Table 3.

Table 3. Results of sensitivity analysis of intestinal flora and POF.

GM	Method	MR Egger Intercept	Intercept SE	Intercept P	Q	Q_df	Q_pval	MR PRESS O
Methano bacteria	MR Egger	-0.04	0.13	0.76	3.87	7.00	0.79	4.73
	IVW				3.96	8.00	0.86	
NBln	MR Egger	0.02	0.06	0.72	12.54	12.00	0.40	5.37
	IVW				12.68	13.00	0.47	
Methano bacterial es	MR Egger	-0.04	0.13	0.76	3.87	7.00	0.79	4.73
	IVW				3.96	8.00	0.86	
Methano bacteriac eae	MR Egger	-0.04	0.13	0.76	3.87	7.00	0.79	4.73
	IVW				3.96	8.00	0.86	
unknown genus	MR Egger	-0.05	0.15	0.74	21.22	13.00	0.07	24.6
	IVW				21.41	14.00	0.09	
Intestini bacter	MR Egger	-0.04	0.07	0.55	9.89	13.00	0.70	4.82
	IVW				10.26	14.00	0.74	
Parasutte rella	MR Egger	0.02	0.02	0.06	0.72	12.54	12.00	14.4
	IVW				12.68	13.00	0.47	
Intestini monas	MR Egger	-0.06	0.06	0.33	9.16	14.00	0.82	11.7
	IVW				10.17	15.00	0.81	
Eubacter iumventr iosumgr oup	MR Egger	-0.07	0.10	0.51	6.33	13.00	0.93	7.79
	IVW				6.78	14.00	0.94	
Eubacter iumhallii group	MR Egger	0.001	0.00	0.05	0.97	11.99	12.00	13.5
	IVW				11.99	13.00	0.53	
Actinom yces	MR Egger	0.08	0.08	0.08	0.39	3.04	5.00	5.38
	IVW				3.91	6.00	0.69	
Adlercre utzia	MR Egger	0.28	0.28	0.13	0.07	5.19	6.00	6.22
	IVW				9.83	7.00	0.20	

4. Discussion

In this study, utilizing public databases containing gut microbiota and premature ovarian failure (POF) data, we employed a two-sample Mendelian randomization (MR) approach to investigate the causal relationship between 211 gut microbiota and POF. We identified 12 gut microbiota significantly associated with POF, including NB1n order, unknown genus, *Intestinibacter* genus, *Parasutterella* genus, *Intestinimonas* genus, *Adlercreutzia* genus, *Methanobacteria* phylum, *Methanobacteriales* order, *Methanobacteriaceae* family, *Eubacterium ventriosum* group genus, *Eubacterium hallii* group genus, and *Actinomyces* genus. Our findings suggest that *Methanobacteria* phylum, *Methanobacteriales* order, *Methanobacteriaceae* family, *Eubacterium ventriosum* group genus, *Eubacterium hallii* group genus, and *Actinomyces* genus have protective effects, while NB1n order, unknown genus, *Intestinibacter* genus, *Parasutterella* genus, *Intestinimonas* genus, and *Adlercreutzia* genus are detrimental to POF.

The gut microbiota is considered crucial for maintaining human health due to its abundance, diversity, and rich genetic information, often referred to as the “second genome.” These genetically modified organisms form a symbiotic relationship with the host, influencing normal physiological functions and disease susceptibility [23,24], including Alzheimer’s disease (AD) [25], Parkinson’s disease (PD) [26] and POF. [27]. Among them, the gut microbiota and ovaries establish a novel and explicit interaction known as the ovary-gut axis. [28] The central nervous system plays a significant role in this axis, termed the “gut-brain-ovary axis” [29] or “gut-brain-ovary axis,” which consists of a complex communication network involving hypothalamic neurons synthesizing gonadotropin-releasing hormone (GnRH) and pituitary neurons secreting gonadotropins (Gn) and their target organs. This axis plays a crucial role in the female reproductive system, regulating female hormone levels, correcting endocrine disorders, promoting ovarian repair, and influencing follicle growth and maturation [30].

Recent studies have demonstrated a close correlation between the gut microbiota and the onset of Premature Ovarian Failure (POF). The gut microbiota exerts a significant influence on ovarian function by modulating female hormone levels, thereby playing a pivotal role in the manifestation of POF-related symptoms. [19,31,32], Estrogen synthesis primarily occurs within the ovaries, and circulating estrogen exerts its effects via an endocrine mechanism on various target tissues, including the skeletal system, brain, and immune system [33]. Additionally, the gut microbiota serves as a crucial regulator of circulating estrogen [34]. Studies have suggested that alterations in the gut microbiota during the menopausal transition may be associated with reductions in estrogen levels, loss of ovarian function, and dysfunction of hormone receptors, leading to menstrual cessation [35]. Furthermore, the elevated activity of gut microbiota and gut microbiota β -glucuronidase (gmGUS) contributes to the circulation or activation of endogenous and exogenous estrogen-like compounds, thereby contributing to the development of estrogen-driven diseases and menopause-related disorders. Notably [36] gmGUS can convert estrogen from an inactive to an active form, consequently influencing estrogen levels within the host [37,38]. Moreover, in postmenopausal women, the relative relationship between estradiol levels and the composition of the gut microbiota has been identified. Specifically, certain bacterial taxa within the Gammaproteobacteria class and Mixococcales order are positively correlated with estradiol levels, whereas the Prevotellaceae family is negatively correlated. Comparatively [39,40], in healthy

female gut microbiota, there are elevated levels of Firmicutes, Clostridia, and Clostridiales, whereas POF patients exhibit increased abundances of Bacteroidetes, Desulfovibrionaceae, Prevotellaceae, and Sutterellaceae, accompanied by changes in hormone levels such as decreased E2 and AMH levels and increased FSH and LH levels. [41] Consequently, differential levels of gut microbiota can modulate hormone levels through the “gut-brain-ovary axis,” thereby exerting a significant impact on the reproductive health of POF patients [42,43,44].

Although some experimental studies have suggested a correlation between gut microbiota dysbiosis and Premature Ovarian Failure (POF), they have not definitively demonstrated a causal relationship between gut microbiota and POF. Firstly, Randomized Controlled Trials (RCTs) may be susceptible to confounding factors and reverse causality, and most research findings are based on animal experiments, which may differ in gut microbiota composition from that of humans. Secondly, this analysis included participants and controls of European descent, which may limit the generalizability of our study to other ethnicities. Lastly, we did not conduct a specific analysis of gut microbiota and subtypes of POF, indicating the need for further research.

5. Conclusions

In conclusion, this study assessed the potential causal role of gut microbiota in POF and identified detrimental effects of increased levels of NB1n order, unknown genus, Intestinibacter genus, Parasutterella genus, Intestinimonas genus, and Adlercreutzia genus on POF, while increased levels of Methanobacteria family, Methanobacteriales order, Methanobacteriaceae family, Eubacterium ventriosum group, Eubacterium hallii group, and Actinomyces genus had protective effects against POF. Further research is required to elucidate the potential protective or detrimental effects of gut microbiota on POF and its underlying mechanisms. This could lead to the development of therapeutic interventions aimed at increasing beneficial bacterial levels in the body to treat Premature Ovarian Failure, thus providing a novel direction for pharmaceutical research in the treatment of POF.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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