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RECEIVED 21 February 2024

ACCEPTED 06 June 2024

PUBLISHED 04 July 2024

CITATION

Tang Y, Yang J, Hang F, Huang H and
Jiang L (2024) Unraveling the relationship
between gut microbiota and site-specific
endometriosis: a Mendelian randomization
analysis.

Front. Microbiol. 15:1363080.
doi: 10.3389/fmicb.2024.1363080

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Unraveling the relationship between gut microbiota and site-specific endometriosis: a Mendelian randomization analysis

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Background: Although numerous studies have illustrated the connection
between gut microbiota and endometriosis, a conspicuous gap exists in
research focusing on the pathogenesis of endometriosis at various sites and its
linkage with infertility.

Methods: In this study, we used a two-sample Mendelian randomization
analysis to investigate the effect of gut microbiota on the development of
endometriosis in different regions, including the uterus, ovary, fallopian tube,
pelvic peritoneum, vagina, and rectovaginal septum, as well as the intestine.
Additionally, we explored the correlation between gut microbiota and
endometriosis-induced infertility. Genetic associations with gut microbes were
obtained from genome-wide association study (GWAS) datasets provided by the
MiBioGen consortium, whereas endometriosis-related GWAS data were sourced
from the FinnGen dataset. In our analysis, single-nucleotide polymorphisms
were used as instrumental variables, with the primary estimation of the causal
effect performed via the inverse variance weighting method. Our sensitivity
analyses incorporated heterogeneity tests, pleiotropy tests, and the leave-one-
out method.

Results: We identified associations at the genus level between four bacterial
communities and endometriosis. Subsequently, several associations between
the gut microbiota and various subtypes of endometriosis at different
anatomical sites were recognized. Specifically, three genera were linked with
ovarian endometriosis, six genera were associated with tubal endometriosis,
four genera showed links with pelvic peritoneum endometriosis, five genera
were connected with vaginal and rectovaginal septum endometriosis, and
seven genera demonstrated linkages with intestinal endometriosis. Additionally,
one genus was associated with adenomyosis, and three genera exhibited
associations with endometriosis-induced infertility.

Conclusion: Our study elucidates associations between gut microbiota and
site-specific endometriosis, thereby augmenting our understanding of the
pathophysiology of endometriosis. Moreover, our findings pave the way for
potential therapeutic strategies targeting gut microbiota for individuals grappling
with endometriosis-related infertility.

KEYWORDS

Mendelian randomization, endometriosis, gut microbiota, infertility, single-nucleotide polymorphisms, pathogenesis

1 Introduction

Endometriosis, an estrogen-dependent chronic inflammatory disease (Ozkan et al., 2008), is characterized by functional endometrial glands and stroma outside the uterine cavity (Vercellini et al., 2014). It commonly affects adjacent tissues and organs, such as the ovaries, uterosacral ligaments, uterine wall muscles, and pelvic peritoneum. However, its presence in extrapelvic organs, such as the gastrointestinal tract, urinary tract, lungs, nasal mucosa, and brain, is rare (Machairiotis et al., 2013). According to the existing literature, endometriosis affects approximately 2–10% of women and has a prevalence of as high as 50% in women experiencing infertility (Becker et al., 2022). Its main clinical manifestations are chronic pelvic pain, dysmenorrhea, dyspareunia, dyschezia, and complications including infertility. These symptoms can affect patients' fertility, mental health, and social functioning. Given that endometriosis has an insidious onset, atypical clinical symptoms, and lacks specific diagnostic markers, its definitive diagnosis may be delayed for 4–11 years (Taylor et al., 2021). The high recurrence rates after surgery and the side effects of medication further complicate the treatment of this disease. Therefore, additional research is urgently needed to explore the pathogenesis of endometriosis to provide a theoretical basis and guidance for clinical treatment strategies.

Various hypotheses regarding the origin of ectopic endometrial-like tissue exist. They include coelomic metaplasia, induction, retrograde menstruation, and lymphatic spread theories (Talwar et al., 2022). Among these hypotheses, Sampson's theory of retrograde menstruation is widely accepted (Sampson, 1927, 1940). This theory proposes that viable fragments of endometrial tissue reflux through the fallopian tubes to the ovaries and peritoneal cavity during menstruation, leading to the development of ectopic endometrial lesions (Bulun et al., 2019). However, even though over 90% of women of reproductive age experience retrograde menstruation, only approximately 10% develop endometriosis. This situation suggests that Sampson's theory alone cannot fully explain the pathogenesis of endometriosis. Therefore, composite theories combining implantation and lymphatic spread have been proposed (Javert, 1949). Considering the varied locations of endometriotic tissue, endometriosis exhibits heterogeneity. All the above hypotheses are based on the understanding that different genetic, hormonal, inflammatory, immune, and environmental factors substantially contribute to endometriosis (Wang et al., 2020; Saunders and Horne, 2021). However, these factors alone cannot explain the pathogenesis of endometriosis at different ectopic sites (Lagana et al., 2017). The pathogenesis of this disease, such as its origin, remains poorly understood.

The human gut microbiome, often referred to as the "second human genome," encompasses approximately 10^{13} to 10^{14} microorganisms, primarily consisting of Bacteroidetes, Firmicutes, Actinobacteria, and Proteobacteria. Changes in the composition and function of the gut microbiota can influence intestinal permeability,

digestion, metabolism, and immune responses due to their role in immune regulation and metabolic processes. Recent studies have demonstrated a close association between gut microbiota and the onset and development of various diseases, including diabetes (Yang et al., 2021), irritable bowel syndrome (IBS) (Salmeri et al., 2023b), colon cancer (Novello et al., 2019), and polycystic ovary syndrome (Liang et al., 2021). Up to 90% of patients with endometriosis report gastrointestinal symptoms, such as bloating, nausea, constipation, diarrhea, and vomiting (Xholli et al., 2023), in addition to gynecological symptoms. This situation suggests a potential link between gut health and endometriosis. In recent years, the relationship between gut microbiota and endometriosis has attracted increasing attention from researchers (Chadchan et al., 2021; Talwar et al., 2022). The possible mechanisms through which gut microbiota imbalance influences the pathogenesis of endometriosis include the following: (1) Involvement of the gut microbiota in immune-mediated chronic inflammation regulation. Gut microbiota imbalance disrupts immune responses, leading to inflammation and the impaired immune clearance of endometrial fragments, thereby promoting the development of endometriotic lesions (Tang et al., 2024). (2) Bacterial contamination hypothesis (Khan et al., 2018): this hypothesis involves the lipopolysaccharide (LPS)/Toll-like receptor (TLR) 4 cascade. The gut microbiota is believed to influence the generation of serum LPS, triggering an inflammatory cascade reaction, stimulating the release of immune cells and inflammatory factors in the body, establishing a chronic inflammatory state, and accelerating the development of endometriosis (Anderson, 2019). (3) Involvement of the gut microbiota in estrogen metabolism changes: The collection of genes encoding estrogen-metabolizing enzymes in the gut microbiota is known as the "estrobolome" (Ervin et al., 2019). The gut microbiota secretes β -glucuronidase, which can deconjugate estrogen and increase the reabsorption of free estrogen, hence raising serum estrogen levels and promoting the growth of ectopic endometrial lesions (Kwa et al., 2016; Baker et al., 2017). Moreover, gut microbiota interacts with hormones, such as estrogen, androgen, and insulin, thus playing a crucial role in the female reproductive endocrine system (Qi et al., 2021). (4) The gut-brain axis of endometriosis. The gut microbiota regulates bidirectional communication with the central nervous system through the gut-brain axis (Salmeri et al., 2023b). The gut microbiota promotes central sensitization to chronic pain by regulating neuroinflammatory responses. This effect may also be the basis of chronic pain in endometriosis. Given that gut microbiota imbalance has numerous adverse effects on the body, correcting this imbalance to restore normal function may aid in the future treatment of endometriosis. However, the efficacy of gut microbiota preparations in treating endometriosis remains controversial. In observational studies, the relationship between gut microbiota and endometriosis can easily be confounded by factors, such as age, environment, and lifestyle (Rinninella et al., 2019). Therefore, further exploration and refinement are needed to identify characteristic microbial changes in the gut of patients with endometriosis and their causal relationships.

Mendelian randomization (MR) has recently gained considerable clinical relevance in assessing causal relationships between exposure factors and diseases (Grover et al., 2017). It serves as an effective methodology for determining causal associations between exposure and outcome by utilizing genetic variants that are strongly associated with exposure as instrumental variables (IVs). MR can be likened to a randomized controlled trial because it has a low susceptibility to confounding factors, providing a high level of evidence. Previous studies have applied MR to investigate the link between gut microbiota and endometriosis, identifying *Anaerotruncus*, *Olsenella*, and *Oscillospira* as potential risk factors for endometriosis (Liang et al., 2023). Despite the current understanding, no studies have yet elucidated the mechanisms underlying the association between gut microbiota and site-specific endometriosis. Consequently, this study was designed to investigate comprehensively the potential causal relationship between gut microbiota and endometriosis in different regions, including the uterus, ovary, fallopian tube, pelvic peritoneum, vagina, and rectovaginal septum, as well as the intestine. In addition, this research aims to explore the correlation between gut microbiota and endometriosis-related infertility using a two-sample MR analysis. The findings of this investigation are expected to enhance our understanding of the underlying mechanisms of endometriosis and provide innovative insights into the development of therapeutic strategies for treating infertility associated with endometriosis.

2 Materials and methods

2.1 Exposure to gut microbiota

We utilized single-nucleotide polymorphisms (SNPs) associated with human gut microbiota as IVs. The data from a comprehensive genome-wide association study (GWAS) dataset provided by the MiBioGen consortium were utilized. This GWAS dataset incorporated 16S fecal microbiome data and expansive genotype information from 24 study cohorts worldwide (Kurilshikov et al., 2021). The project included a total of 18,340 participants, comprising 16,632 adults and adolescents, as well as 1,708 children, representing diverse ethnic backgrounds, including European, Middle-Eastern, East Asian, American Hispanic/Latin, and African American backgrounds, with Europeans accounting for 72.3% of the cohort. The gut microbiota data consisted of 211 taxa with a relative abundance exceeding 1%, encompassing 9 phyla, 16 classes, 20 orders, 35 families, and 131 genera. After we excluded 15 taxa of unknown groups (12 genera and three families), 119 genera were included in the analysis of this study. The GWAS data relevant to gut microbiota were obtained from <http://www.mibiogen.org>.

2.2 GWAS summary data for endometriosis

We obtained data on endometriosis from the FinnGen cohort, accessed through the OPEN GWAS platform,¹ with the GWAS ID finn-b-N14-ENDOMETRIOSIS. The diagnosis of endometriosis in

patients was based on the International Classification of Diseases, 10th Edition. This dataset included 77,257 European female participants, consisting of 8,288 cases and 68,969 controls. Patients with endometriosis included those with adenomyosis (2,372 cases), ovarian endometriosis (3,231 cases), tubal endometriosis (116 cases), pelvic peritoneal endometriosis (2,953 cases), vaginal and rectovaginal septum endometriosis (1,360 cases), intestinal endometriosis (177 cases), endometriosis in cutaneous scars (unknown cases), and unspecified/other endometriosis (1,435 cases). We compiled GWAS data from the FinnGen cohort to investigate the potential causal relationship between gut microbiota and site-specific endometriosis. These data pertain to endometriosis occurring at diverse locations, including the uterus, ovary, fallopian tube, pelvic peritoneum, vagina, and rectovaginal septum, as well as the intestine. Furthermore, we sought to explore the role of gut microbiota in endometriosis-related infertility. Patients with endometriosis complicated by infertility included 1,593 cases and 70,651 controls. These data can be accessed through the GWAS IDs provided in the R package TwoSampleMR (version 0.5.7). The GWAS IDs are listed in the IEU OpenGWAS database. Refer to [Table 1](#) for detailed information.

2.3 IV selection

We examined the association between gut microbiota and endometriosis by considering gut microbiota as the exposure and endometriosis as the outcome. We implemented several quality control steps to screen the IVs to ensure the precision and stability of our analysis. First, we selected SNPs that showed a significant association with gut microbes as IVs ($p < 1 \times 10^{-5}$). In the second step, we applied a clumping window of 10,000 kb and an r^2 threshold of 0.001 to exclude SNPs in linkage disequilibrium. For the third step, we replaced missing SNPs with those with high linkage ($R^2 > 0.8$) and removed SNPs without sufficient substitution sites. In the fourth step, we set a screening threshold of 0.3 for minor allele frequency. In the fifth step, we excluded palindrome SNPs to avoid any misrepresentation in strand direction or allelic coding. Furthermore, we calculated the F -statistic by using the formula $F = R^2 \times (N - 2) / (1 - R^2)$ to assess the strength of IVs. A high F -statistic value indicates a low bias from weak IVs, and a value exceeding 10 is generally considered acceptable (Burgess and Thompson, 2011).

2.4 MR analysis

Our MR analysis adhered to three fundamental assumptions. First, the IVs should correlate significantly with the exposure variable (gut microbiota). Second, the effect of IVs on the outcome variable (endometriosis) should not be confounded by other variables. Third, the IVs should influence the outcome solely through the exposure variable, indicating the absence of horizontal pleiotropy. An overview of the study design is illustrated in [Figure 1](#). A total of 119 genera of gut microbiota were selected based on the GWAS data, and IVs were screened for each genus. Two-sample MR analyses were then conducted independently to assess the causal effect of gut microbiota on endometriosis at specific locations and endometriosis combined with infertility. The Wald ratio was used to estimate the association between the identified IVs and the outcome for a single IV (Burgess et al., 2017). The inverse-variance

¹ <https://gwas.mrcieu.ac.uk/>

TABLE 1 Summary of genome-wide association studies (GWAS) datasets in our study.

Phenotype	Source	Population	Sample Size	No. of cases	No. of controls	GWAS ID
Gut microbiome	MiBioGen	Multi-ancestry	18,340	—	—	—
Endometriosis	FinnGen	European	77,257	8,288	68,969	finn-b-N14_ENDOMETRIOSIS
Ovarian endometriosis	FinnGen	European	72,200	3,231	68,969	finn-b-N14_ENDOMETRIOSIS_OVARY
Tubal endometriosis	FinnGen	European	69,085	116	68,969	finn-b-N14_ENDOMETRIOSIS_FALLOPIAN_TUBE
Pelvic peritoneum endometriosis	FinnGen	European	71,922	2,953	68,969	finn-b-N14_ENDOMETRIOSIS_PELVICPERITONEUM
Vaginal and rectovaginal septum endometriosis	FinnGen	European	70,329	1,360	68,969	finn-b-N14_ENDOMETRIOSIS_RECTPVAGSEPT_VAGINA
Intestinal endometriosis	FinnGen	European	69,146	177	68,969	finn-b-N14_ENDOMETRIOSIS_INTESTINE
Adenomyosis	FinnGen	European	71,341	2,372	68,969	finn-b-N14_ENDOMETRIOSIS_UTERUS
Endometriosis-related infertility	FinnGen	European	72,244	1,593	70,651	finn-b-N14_ENDOMET_INFERT

weighted (IVW) method was predominantly used for multiple IVs. IVW combines the Wald ratios of outcomes for each SNP to obtain an estimate while accounting for excessive dispersion (Burgess et al., 2013). Additionally, supplemental verification methods, including weighted modal estimation (Hartwig et al., 2017), simple mode estimation (Hartwig et al., 2017), MR-Egger regression (Bowden et al., 2015), and weighted median estimation (Bowden et al., 2016) were used.

2.5 Sensitivity analysis

We conducted a sensitivity analysis to assess the robustness of the findings. This approach involved leave-one-out analysis, wherein we systematically evaluated one SNP at a time to evaluate its influence on results. Heterogeneity was assessed by using Cochran's Q statistic, with a p -value below 0.05 indicating the presence of significant heterogeneity (Greco et al., 2015). Furthermore, we used the MR-Egger intercept test to detect potential pleiotropy in the data, with a p -value below 0.05, suggesting the presence of pleiotropic effects (Burgess and Thompson, 2017).

2.6 Statistical analysis

All procedures linked to the selection and quality control of IVs were conducted using the R packages TwoSampleMR (version 0.5.7) and Mendelian Randomization Pleiotropy RESidual Sum and Outlier (MR-PRESSO) in R software (version 4.2.1). A p -value of less than 0.05 was considered to demonstrate a statistically significant correlation.

3 Results

3.1 SNP selection

Initially, we identified SNPs associated with gut microbiota at the genus level in consideration of significance levels of $p < 1 \times 10^{-5}$. A total of 1,213 SNPs were included as IVs. Among these SNPs, we observed 49 that were significantly associated with endometriosis. In the

subsequent phase, we focused on investigating the direct associations between intestinal flora and site-specific endometriosis. Our findings revealed 14, 20, 63, and 32 independent SNPs associated with adenomyosis, ovarian endometriosis, tubal endometriosis, and pelvic peritoneum endometriosis, respectively. Additionally, we identified 46, 69, and 34 independent SNPs associated with rectovaginal septum and vaginal endometriosis, intestinal endometriosis, and endometriosis accompanied by infertility, respectively (Supplementary Table S1). After we implemented rigorous quality control measures, all IVs exhibited F -statistics exceeding 10, indicating the absence of weak IVs. The MR-PRESSO global test did not detect any evidence of pleiotropy ($p > 0.05$). Finally, we identified pleiotropic SNPs using the MR-PRESSO outlier test and excluded them from further analysis, resulting in no indications of horizontal pleiotropy in the re-evaluated IVs (p -values > 0.05 for the MR-PRESSO global test and MR-Egger regression).

3.2 Gut microbiota and endometriosis

We conducted a two-sample MR analysis to investigate the potential role of gut microbiota in the development of endometriosis. Our findings revealed an association between endometriosis and four bacterial communities at the genus level. Specifically, we identified the *Eubacterium ruminantium* group as a protective factor against endometriosis [odds ratio (OR): 0.881, 95% confidence interval (CI): 0.795–0.976, $p = 0.0152$], whereas we identified the genera *Anaerotruncus* (OR: 1.252, 95% CI: 1.028–1.525, $p = 0.0253$), *Olsenella* (OR: 1.109, 95% CI: 1.007–1.223, $p = 0.0360$), and *Oscillospira* (OR: 1.215, 95% CI: 1.014–1.456, $p = 0.0351$) to be risk factors (Figure 2 and Table 2).

3.3 Gut microbiota and ovarian endometriosis

We performed additional analyses to explore the relationships between gut microbiota and specific subtypes of endometriosis and to understand the mechanisms underlying the development of endometriosis at different locations. For ovarian endometriosis, we identified the *Blautia* genus as detrimental (OR: 2.958, 95% CI:



1.399–6.253, $p=0.0045$), whereas the *Barnesiella* (OR: 0.741, 95% CI: 0.573–0.958, $p=0.0221$) and *Terrisporobacter* genera (OR: 0.708, 95% CI: 0.527–0.952, $p=0.0223$) were found to be protective.

3.4 Gut microbiota and fallopian tube endometriosis

Adlercreutzia and *Ruminococcus torques* (OR: 9.37, 95% CI: 1.51–57.99, $p=0.0161$) were associated with an increased risk for fallopian tube endometriosis. Conversely, *Barnesiella* (OR: 0.09, 95% CI: 0.01–0.45, $p=0.0002$), *Oscillibacter* (OR: 0.22, 95% CI: 0.07–0.66, $p=0.0069$), *Phascolarctobacterium* (OR: 0.19, 95% CI: 0.05–0.80, $p=0.0239$), and *Ruminococcaceae* UCG003 (OR: 0.27, 95% CI: 0.08–0.95, $p=0.0421$) were associated with a decreased risk for fallopian tube endometriosis.

3.5 Gut microbiota and pelvic peritoneum endometriosis

The *Escherichia-Shigella* genus was associated with an increased risk for pelvic peritoneum endometriosis (OR: 1.51, 95% CI: 1.10–2.07, $p=0.0116$). By contrast, *Catenibacterium* (OR: 0.76, 95% CI:

0.60–0.97, $p=0.0253$), *Eubacterium oxidoreducens* group (OR: 0.75, 95% CI: 0.58–0.98, $p=0.0384$), and *Paraprevotella* (OR: 0.81, 95% CI: 0.68–0.95, $p=0.0179$) were associated with a decreased risk for pelvic peritoneum endometriosis.

3.6 Gut microbiota and vaginal/rectovaginal septum endometriosis

We also identified associations between gut microbiota and vaginal/rectovaginal septum endometriosis. Specifically, *Tyzzereella3* was associated with an increased risk, whereas *Erysipelotrichaceae* UCG003, *Eubacterium oxidoreducens* group, *Lachnospiraceae* NK4A136 group, and *Ruminococcaceae* UCG003 were associated with a decreased risk for vaginal/rectovaginal septum endometriosis.

3.7 Gut microbiota and intestinal endometriosis

In the case of intestinal endometriosis, *Intestinimonas*, *Lachnospiraceae* NC2004 group, *Lactococcus*, *Oscillospira*, *Phascolarctobacterium*, and *Ruminococcaceae* UCG005 were associated

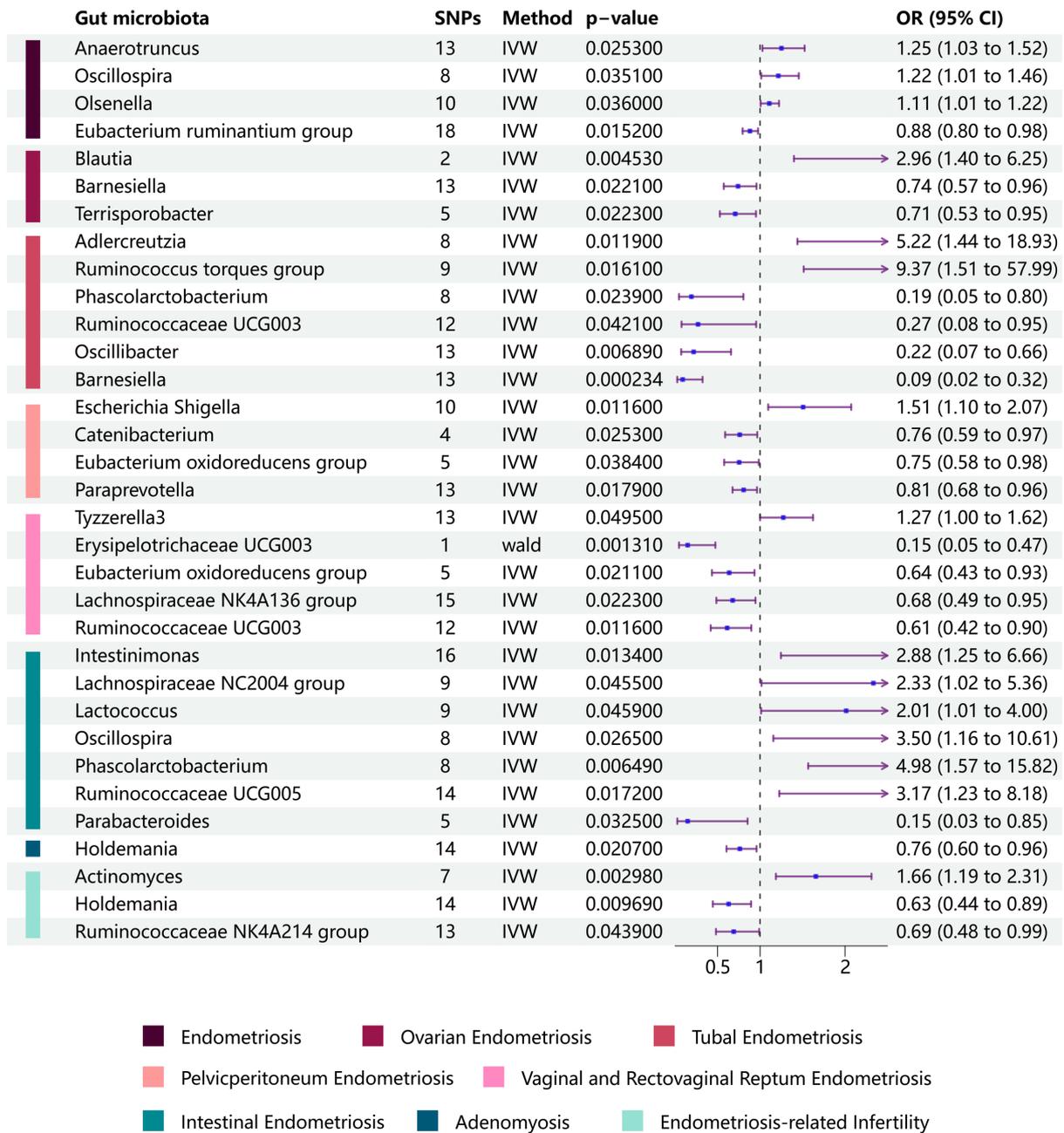


FIGURE 2

Forest plots of the Mendelian randomization analysis of site-specific endometriosis. Relationships between gut microbiota and site-specific endometriosis were estimated using the IVW method. The purple bars represent the 95% confidence interval of IVW estimates. An OR >1 indicates an increased risk, while an OR <1 indicates a decreased risk (IVW, inverse-variance weighting; OR, odds ratio).

with an increased risk, whereas *Parabacteroides* was associated with a decreased risk. These findings provide valuable insights into the potential role of gut microbiota in the development and progression of various forms of endometriosis (Figure 2 and Supplementary Table S2).

3.8 Gut microbiota and adenomyosis

We also examined adenomyosis, a specific type of endometriosis characterized by the infiltration of endometrial glands and stroma into

the myometrium. In our findings, *Holdemanina* genus emerged as a protective factor against adenomyosis (OR: 0.762, 95% CI: 0.605–0.959, $p=0.0207$).

3.9 Association between gut microbiota and endometriosis-related infertility

We further sought to investigate the associations between gut microbiota and co-occurrence of endometriosis and infertility.

TABLE 2 MR analysis and Sensitivity analysis of gut microbiome in endometriosis and endometriosis-related infertility ($p < 1 \times 10^{-5}$).

Outcomes	Bacterial genera (exposure)	nSNPs	Methods	OR (95% CI)	Beta	Se	p-value	Heterogeneity		MR-PRESSO	Horizontal pleiotropy		
								Cochran'sQ	p-value		p-value	Egger intercept	Se
Endometriosis	<i>Anaerotruncus</i>	13	MR Egger	0.85 (0.49–1.47)	−0.17	0.28	0.57	11.47	0.41	0.34	0.03	0.02	0.17
			IVW	1.25 (1.03–1.53)	0.23	0.10	0.03	13.76	0.32				
			Weighted median	1.24 (0.97–1.59)	0.22	0.13	0.08						
			Simple mode	1.21 (0.87–1.66)	0.19	0.17	0.28						
			Weighted mode	1.20 (0.87–1.66)	0.18	0.16	0.29						
	<i>Eubacterium ruminantium</i> group	18	MR Egger	0.93 (0.62–1.24)	−0.13	0.1	0.47	12.73	0.69	0.83	0.00	0.02	0.98
			IVW	0.88 (0.79–0.98)	−0.13	0.05	0.02	12.73	0.75				
			Weighted median	0.88 (0.80–1.07)	−0.08	0.07	0.31						
			Simple mode	0.96 (0.74–1.25)	−0.04	0.13	0.78						
			Weighted mode	0.96 (0.77–1.19)	−0.04	0.11	0.70						
	<i>Olsenella</i>	10	MR Egger	1.03 (0.75–1.40)	0.03	0.16	0.86	7.12	0.52	0.59	0.01	0.02	0.63
			IVW	1.11 (1.01–1.22)	0.10	0.05	0.04	7.37	0.60				
			Weighted median	1.11 (0.97–1.26)	0.10	0.07	0.13						
			Simple mode	1.12 (0.89–1.41)	0.12	0.12	0.35						
			Weighted mode	1.04 (0.86–1.26)	0.04	0.10	0.71						
	<i>Oscillospira</i>	8	MR Egger	0.96 (0.45–2.06)	−0.04	0.39	0.92	2.88	0.24	0.73	0.02	0.04	0.55
			IVW	1.21 (1.01–1.46)	0.19	0.09	0.04	3.27	0.86				
Weighted median			1.17 (0.93–1.47)	0.15	0.12	0.19							
Simple mode			1.14 (0.78–1.66)	0.13	0.19	0.53							
Weighted mode			1.12 (0.80–1.58)	0.11	0.17	0.54							
Adenomyosis	<i>Holdemania</i>	14	MR Egger	0.85 (0.43–1.67)	−0.16	0.35	0.64	9.72	0.64	0.858	−0.01	0.03	0.75
			IVW	0.76 (0.61–0.96)	−0.27	0.12	0.02	9.83	0.71				
			Weighted median	0.84 (0.60–1.16)	−0.18	0.17	0.28						
			Simple mode	0.83 (0.45–1.54)	−0.18	0.31	0.57						
			Weighted mode	0.87 (0.48–1.55)	−0.14	0.30	0.64						

(Continued)

TABLE 2 (Continued)

Outcomes	Bacterial genera (exposure)	nSNPs	Methods	OR (95% CI)	Beta	Se	p-value	Heterogeneity		MR-PRESSO	Horizontal pleiotropy		
								Cochran'sQ	p-value	p-value	Egger intercept	Se	p-value
Endometriosis-related infertility	<i>Actinomyces</i>	7	MR Egger	0.88 (0.39–1.99)	−0.12	0.42	0.78	0.97	0.96	0.79	0.08	0.05	0.16
			IVW	1.66 (1.19–2.31)	0.50	0.17	0.00	3.73	0.71				
			Weighted median	1.41 (0.89–2.24)	0.35	0.23	0.14						
			Simple mode	1.40 (0.66–2.99)	0.34	0.38	0.41						
			Weighted mode	1.30 (0.74–2.29)	0.27	0.29	0.39						
	<i>Holdemania</i>	14	MR Egger	0.36 (0.13–0.99)	−1.03	0.52	0.07	18.94	0.09	0.09	0.06	0.05	0.27
			IVW	0.63 (0.52–1.11)	−0.46	0.18	0.01	21.06	0.07				
			Weighted median	0.76 (0.44–0.89)	−0.27	0.19	0.16						
			Simple mode	0.75 (0.42–1.33)	−0.29	0.29	0.34						
			Weighted mode	0.75 (0.43–1.31)	−0.28	0.28	0.34						
	<i>Ruminococcaceae</i> NK4A214 group	13	MR Egger	0.31 (0.09–1.01)	−1.18	0.61	0.08	7.23	0.78	0.71	0.06	0.04	0.19
			IVW	0.69 (0.48–0.99)	−0.37	0.18	0.04	9.19	0.69				
			Weighted median	0.68 (0.41–1.10)	−0.39	0.25	0.12						
			Simple mode	0.71 (0.31–1.64)	−0.34	0.42	0.44						
			Weighted mode	0.68 (0.32–1.45)	−0.39	0.39	0.34						

Three significant associations at the genus level were identified. The *Actinomyces* genus was positively associated with the risk of endometriosis-related infertility (OR: 1.657, 95% CI: 1.187–2.312, $p=0.00298$). Conversely, the *Holdemania* genus (OR: 0.63, 95% CI: 0.444–0.894, $p=0.00969$) and *Ruminococcaceae* NK4A214 group (OR: 0.69, 95% CI: 0.481–0.99, $p=0.0439$) were negatively associated with the risk of the simultaneous diagnosis of endometriosis and infertility (Figure 2 and Table 2). The leave-one-out results further confirmed the robustness of our data (Figures 3, 4).

4 Discussion

Despite the growing research interest in the relationships between gut microbiota and endometriosis, the underlying causes and mechanisms of endometriosis remain unclear. Our study represents the first attempt to investigate the associations between gut microbiota and endometriosis at various sites, including the uterus, ovary, fallopian tubes, pelvic peritoneum, vagina, and rectovaginal septum, as well as the intestine, using the two-sample MR analysis. Moreover, our study is the first to explore the association between gut microbiota at the genus level and endometriosis in women with infertility. Several associations between gut microbiota and various subtypes of endometriosis at different anatomical sites were recognized. Specifically, three, six, four, five, and seven genera were associated with

ovarian, tubal, pelvic peritoneum, vaginal/rectovaginal septum, and intestinal endometriosis, respectively. Additionally, one genus was associated with adenomyosis, and three genera were linked with endometriosis-related infertility. This research offers novel insights into endometriosis mechanisms and potential directions for treating endometriosis in women with infertility.

Previous MR research has highlighted a relationship between the gut microbiome and endometriosis. Liang et al. (2023) identified *Anaerotruncus*, *Olsenella*, and *Oscillospira* genera as harmful factors in endometriosis this result is in accordance with our findings. The researchers proposed that *Olsenella* may be implicated in the pathogenesis of endometriosis through the regulation of Interleukin 10 (IL-10) levels. In addition, Liu et al. (2023) identified *Anaerotruncus* as a risk factor for ovarian endometriosis. Walther-Antonio et al. (2016) reported remarkable concentrations of *Anaerotruncus* in patients with endometrial cancer. Furthermore, *Anaerotruncus* was found to be positively correlated with glucose intolerance and increased intestinal permeability (Kong et al., 2019). However, the mechanism of its involvement in the development of endometriosis remains unknown.

Notably, our study indicates that the *Eubacterium ruminantium* group may act as a protective factor against endometriosis. Moreover, the *Eubacterium oxidoreducens* group has been identified as a protective factor against vaginal and rectovaginal septum endometriosis and pelvic peritoneal endometriosis at the genus level. The *Eubacterium ruminantium* group and *Eubacterium oxidoreducens*

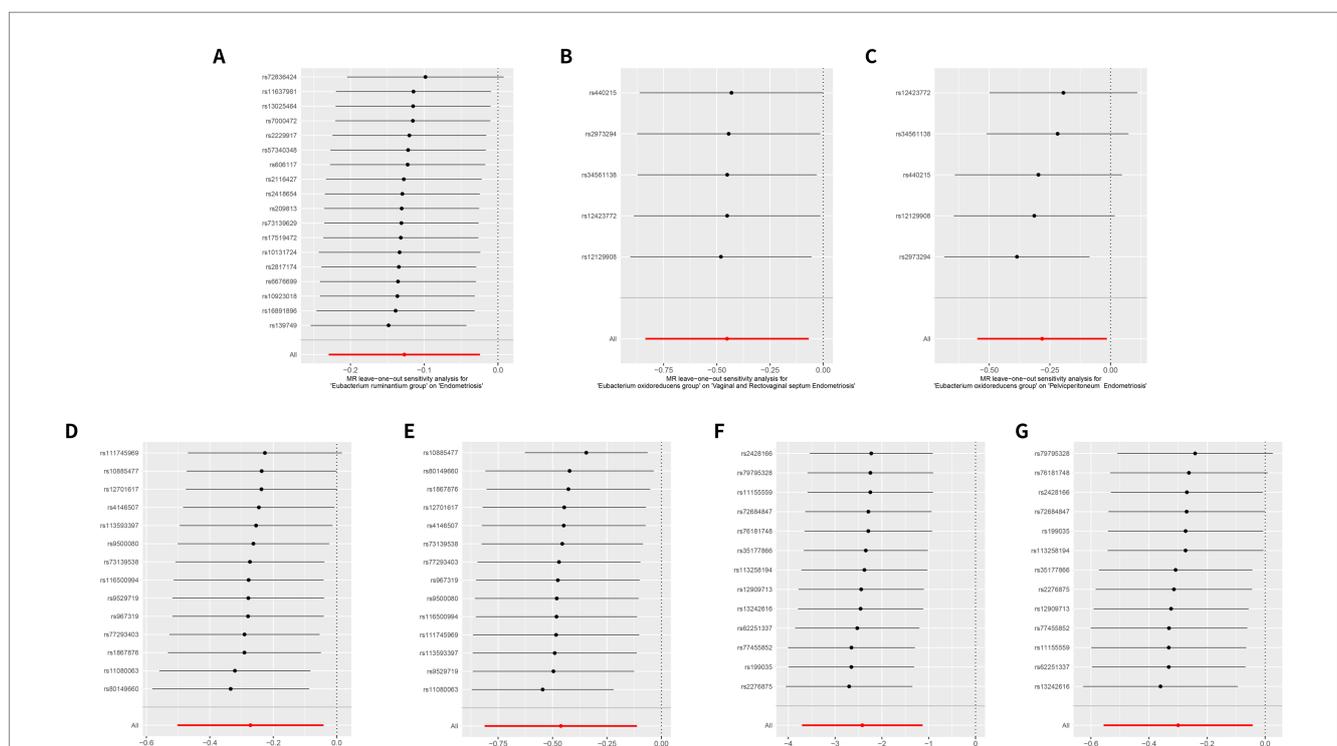


FIGURE 3

Leave-one-out sensitivity analysis reveals a consistent protective role of the gut microbiome at the same genus in different subtypes of endometriosis.

(A) MR leave-one-out sensitivity analysis for *Eubacterium ruminantium* group on endometriosis. (B) MR leave-one-out sensitivity analysis for *Eubacterium oxidoreducens* group on vaginal and rectovaginal septum endometriosis. (C) MR leave-one-out sensitivity analysis for *Eubacterium oxidoreducens* group on pelvic peritoneum endometriosis. (D) MR leave-one-out sensitivity analysis for *Holdemania* on adenomyosis. (E) MR leave-one-out sensitivity analysis for *Holdemania* on endometriosis-related infertility. (F) MR leave-one-out sensitivity analysis for *Barnesiella* on tubal endometriosis. (G) MR leave-one-out sensitivity analysis for *Barnesiella* on ovarian endometriosis.

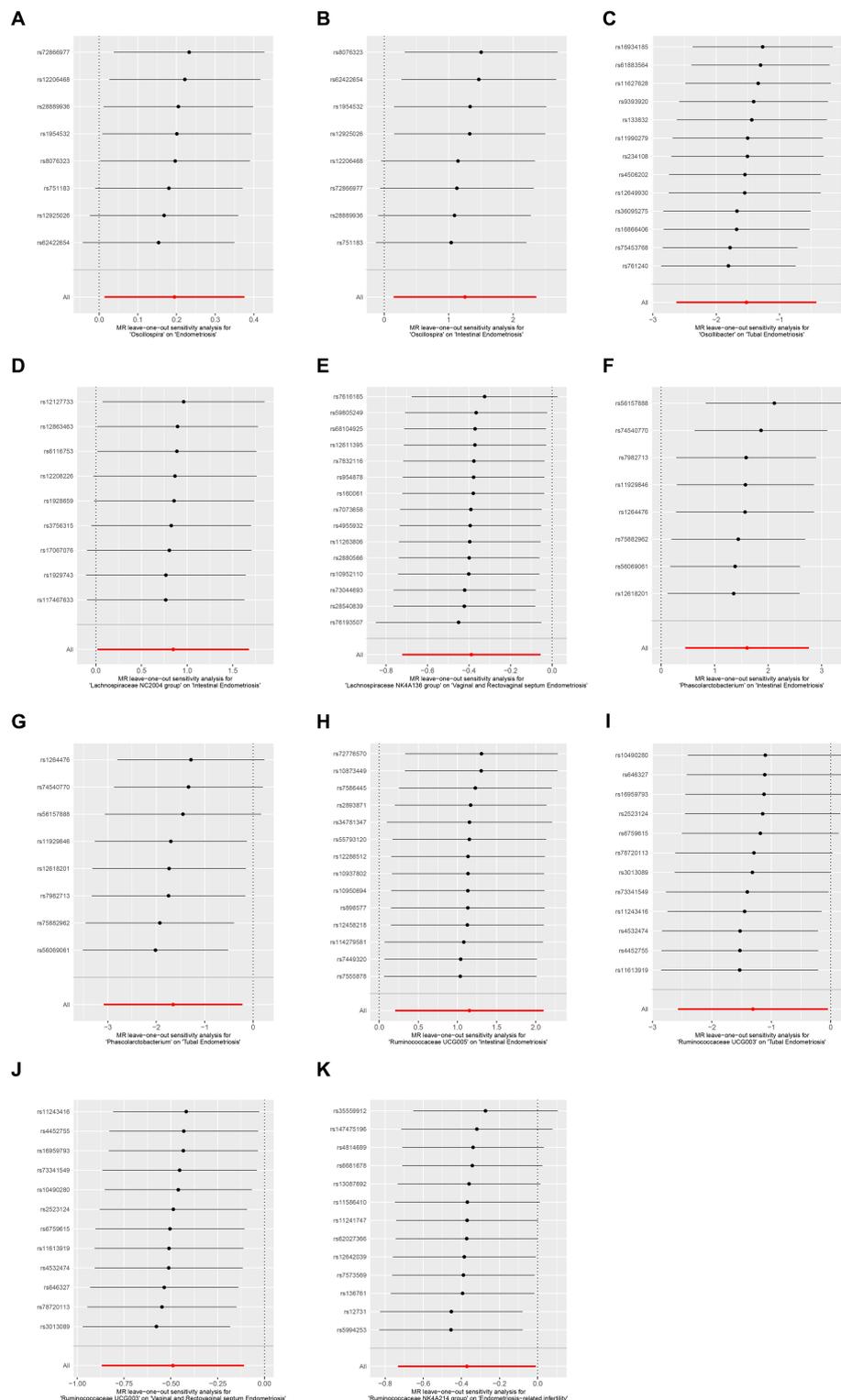


FIGURE 4

Leave-one-out sensitivity analysis demonstrated the opposite effect of the gut microbiome at the family level in different subtypes of endometriosis. (A) MR leave-one-out sensitivity analysis for *Oscillospira* on endometriosis. (B) MR leave-one-out sensitivity analysis for *Oscillospira* on intestinal endometriosis. (C) MR leave-one-out sensitivity analysis for *Oscillospira* on tubal endometriosis. (D) MR leave-one-out sensitivity analysis for *Lachnospiraceae NC2004* group on intestinal endometriosis. (E) MR leave-one-out sensitivity analysis for *Lachnospiraceae NK4A136* group on vaginal and rectovaginal septum endometriosis. (F) MR leave-one-out sensitivity analysis for *Phascolarctobacterium* on intestinal endometriosis. (G) MR leave-one-out sensitivity analysis for *Phascolarctobacterium* on tubal endometriosis. (H) MR leave-one-out sensitivity analysis for *Ruminococcaceae UCG005* on intestinal endometriosis. (I) MR leave-one-out sensitivity analysis for *Ruminococcaceae UCG003* on tubal endometriosis. (J) MR leave-one-out sensitivity analysis for *Ruminococcaceae UCG003* on vaginal and rectovaginal septum endometriosis. (K) MR leave-one-out sensitivity analysis for *Ruminococcaceae NK4A214* group on endometriosis-related infertility.

produce butyric acid, a short-chain fatty acid (SCFA) (Krumholz et al., 1987; Maia et al., 2007). SCFAs, encompassing butyrate, acetic acid, and propionate, are metabolic byproducts of undigested dietary fiber fermented by gut microbiota. They not only supply energy to the host and intestinal epithelial cells, but they also counteract the invasion of intestinal pathogens and safeguard the function of the intestinal mucosal barrier. A decrease in SCFA concentration in the human gut can precipitate disease onset. Previous research has demonstrated that in mice with endometriosis, fecal butyrate levels are diminished, and butyrate application curtails the growth of endometriotic lesions (Chadchan et al., 2021). SCFAs primarily exert anti-inflammatory effects through two signaling pathways (Guo and Zhang, 2024). First, they activate G protein-coupled receptors such as GPR41, GPR43, and GPR109a, inhibiting the activation of Nod-like receptor pyrin domain 3 inflammasomes and thus reducing the secretion of proinflammatory cytokines. Second, they can also inhibit histone deacetylases, reducing the production of the proinflammatory tumor necrosis factor, and leading to the inactivation of nuclear factor kappa B. Additionally, research has found that butyrate can modify the cytokines of helper T cells and foster the integrity of the intestinal epithelial barrier (Kau et al., 2011). Our results further suggest that the *Eubacterium* genus may stave off endometriosis by participating in the regulation of immune responses, providing fresh insights for the treatment of endometriosis.

Our research findings suggest that the *Holdemania* genus not only plays a protective role in adenomyosis but also in endometriosis-associated infertility. Our conclusion aligns with that of a previous study that identified *Holdemania* as a protective factor against female infertility (Xi et al., 2023), thus reinforcing the validity of our results. The frequent co-occurrence of infertility and endometriosis suggests a close correlation between these conditions. The mechanisms of inflammation and immune dysregulation are now widely accepted to negatively affect endometrial receptivity and embryonic viability at implantation sites. Current literature indicates that chronic inflammation associated with endometriosis can instigate alterations in the endometrial microenvironment and immune milieu (Vallve-Juanico et al., 2019). Immune-mediated events are crucial factors in endometriosis-associated infertility (Kolanska et al., 2021). The presence of either endometriosis or autoimmune diseases, or a combination thereof, can amplify inflammation and immune activity, potentially impairing oocyte maturation, embryo development, and endometrial receptivity; this effect disrupts endometrium-embryo crosstalk at the implantation site (Salmeri et al., 2023a). Gut microbiota can influence female fertility by fostering an inflammatory environment in the reproductive tract, abdominal cavity, and pelvis (Salliss et al., 2021). Prior research has identified the increased expression of *Holdemania* in other inflammatory diseases (Jang et al., 2020). Moreover, rodent studies have shown that the consumption of sucrose and fructose can alter microbiota composition, leading to the proliferation of *Holdemania* and the onset of colitis (Song et al., 2023). In a mouse model of carbapenem-resistant *Klebsiella pneumoniae*, the administration of *Lactobacillus LP1812* resulted in an increased abundance of *Holdemania*, underscoring its potential role as a gut probiotic (Yan et al., 2021). The *Lactobacillus* genus has been revealed to mitigate endometriotic lesions in mice by enhancing the levels of IL-12 and the activity of natural killer cells (Itoh et al., 2011).

Women with endometriosis are two to three times more likely to be diagnosed with IBS, with over 20% of patients with endometriosis

presenting IBS-like symptoms (Chiapparino et al., 2021). Extant research has found that gut microbiota contributes to the pathogenesis of IBS and endometriosis via similar mechanisms. Gut microbiota dysbiosis can affect intestinal permeability, initiate inflammatory responses, and provoke immune reactions. Furthermore, both conditions are linked to the central and enteric nervous systems via a bidirectional communication pathway known as the gut-brain axis, which regulates secretion, immune responses, intestinal motility, and visceral sensitivity (Salmeri et al., 2023b). Therefore, we propose that the *Holdemania* genus may be involved in the pathogenesis of adenomyosis and endometriosis-associated infertility through inflammatory and immune responses. Additionally, we identified *Barnesiella* as a protective factor against endometriosis affecting the ovaries and fallopian tubes. Previous studies have reported an association between *Barnesiella* and variation in the bilirubin reductase A locus (Ruhlemann et al., 2021). Biliverdin reductase A has been proven to suppress the expression of the TLR4 gene, a pattern recognition receptor that modulates intracellular immune and inflammatory responses and is ubiquitous on immune cell membranes. LPS can activate Nuclear Factor Kappa-B (NF- κ B) via TLR4 binding and MyD88-dependent and -independent pathways (Khan et al., 2018), thereby eliciting immune and inflammatory responses and promoting the transcription of genes associated with cell adhesion, proliferation, and antiapoptosis (Guo and Zhang, 2024). Therefore, we postulate that *Barnesiella* may exert a protective effect against endometriosis by modulating the LPS/TLR4/NF- κ B pathway to inhibit the initiation and progression of endometriosis.

Additionally, our study found that the same genus can play contrasting roles at different sites. Specifically, the *Phascolarctobacterium* genus, while identified as a protective factor against tubal endometriosis, posed a risk for intestinal endometriosis. Previous research has shown that *Phascolarctobacterium* can metabolize succinic acid to produce propionate and acetic acid. Succinic acid can activate immune cells via the specific surface receptor succinic acid receptor 1, thereby enhancing inflammatory responses (Connors et al., 2018). Propionic acid, on the other hand, is recognized as an effective immunomodulatory supplement that augments the function of intestinal T-regulatory cells associated with multiple sclerosis (Duscha et al., 2020). Propionate has also demonstrated anti-inflammatory effects in human subcutaneous adipose tissue (Al-Lahham and Rezaee, 2019). This dichotomy may originate from the complex balance between pro- and anti-inflammatory metabolic signals involving succinate as an intermediate and SCFAs. Therefore, the *Phascolarctobacterium* genus has diverse roles at various sites of endometriosis. Moreover, two members of the same family, namely, *Lachnospiraceae* NC2004 and *Lachnospiraceae* NK4A136, play contrasting roles. The former is a risk factor for intestinal endometriosis, whereas the latter is a protective factor against vaginal and rectovaginal septum endometriosis. *Lachnospiraceae* members are major producers of SCFAs and are implicated in various inflammatory diseases (Vacca et al., 2020). *Ruminococcaceae* UCG003, *Ruminococcaceae* NK4A214 group, and *Ruminococcaceae* UCG005 are all from the same family. The first two exert protective effects against the fallopian tube, vagina, and rectovaginal septum endometriosis, as well as endometriosis-related infertility, whereas the third poses a risk for intestinal endometriosis. Past research has established a causal link between *Ruminococcaceae* and endometriosis (Ji et al., 2023). *Ruminococcaceae* is known primarily for producing acetic acid (Chassard and Bernalier-Donadille, 2006), and some members follow a succinate pathway with succinate as the end product instead of

propionate (Louis and Flint, 2017). Ji et al. (2023) suggested that the divergent abilities of *Ruminococcaceae* members to produce butyrate might account for the contrasting causal relationships within the *Ruminococcaceae* family. Further investigation is required to explore these aspects in the future.

The association between gut microbiota and endometriosis has been the focus of extensive research. In our study, we used MR analysis to investigate the associations between gut microbiota and endometriosis in different regions based on GWAS summary statistics. Rigorous quality controls were used to eliminate IVs that could potentially bias the results. The MR approach offers an advantage over traditional observational studies because it is less susceptible to confounding factors than other approaches. However, our study has some limitations. The precise biological functions of numerous genetic variants remain unknown, and MR may be prone to errors if genetic variants are pleiotropic. Moreover, population stratification could bias the results. Our gut microbiota data included participants of multiple races and both sexes, whereas endometriosis datasets were collected from European women. However, due to the absence of demographic data in the original study, we could not circumvent this bias by performing gender or ethnic subgroup analyses. The GWAS dataset contains cases wherein multiple locations are concurrently afflicted by endometriosis. Owing to the absence of raw data for these cases, we were unable to conduct further analyses and exclusions. Furthermore, the smaller sample size per taxon in gut microbiota (GWAS) than that in complex diseases leads to the insufficient identification of IVs at the genus level. Finally, we did not conduct bidirectional MR analysis due to the limited number of SNP sites for outcomes, and bidirectional causality between exposure and outcome cannot be excluded.

In conclusion, our study elucidates relationships between gut microbiota and site-specific endometriosis, thereby augmenting our understanding of the pathophysiology of endometriosis. Moreover, our findings pave the way for potential therapeutic strategies targeting gut microbiota for individuals grappling with endometriosis-related infertility. In the future, further validation analyses will be performed on animal models of endometriosis and human gut microbiota samples.

Data availability statement

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

Ethics statement

The studies involving humans were approved by the Institutional Review Board. Data used in this study are publicly accessible,

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de-identified. Hence, no additional ethical approval was required. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

Author contributions

YT: Visualization, Data curation, Writing – review & editing, Writing – original draft. JY: Visualization, Data curation, Writing – review & editing, Writing – original draft. FH: Writing – review & editing, Writing – original draft, Visualization, Data curation. HH: Writing – review & editing, Writing – original draft, Visualization, Data curation. LJ: Writing – review & editing, Writing – original draft, Supervision, Data curation.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This study was funded by the Research Project of the Guangxi Zhuang Autonomous Region (S2019096 and Z-A20230332).

Conflict of interest

HH was employed by Guangxi Henbio Biotechnology Co., Ltd.

The remaining 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/fmicb.2024.1363080/full#supplementary-material>

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