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Causal association between gut microbiota and endometrial cancer in European and East Asian populations: a two-sample Mendelian randomization study

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Abstract

Background Endometrial cancer (EC) is a significant global health concern. While observational epidemiological studies suggest a potential link between gut microbiota dysbiosis and the development of EC, the direction and causality of this association remain uncertain.

Methods We performed Mendelian randomization (MR) analysis to investigate the causal relationship between gut microbiota and EC. Exposure data were obtained from the MiBioGen study consortium ($N=18,340$), and outcome data were sourced from the IEU OpenGWAS database, specifically datasets "ebi-a-GCST006464" ($N=121,885$) and "bbj-a-113" ($N=90,730$). The inverse variance-weighted(IVW) method was applied to evaluate the association between gut microbiota composition and EC risk. Sensitivity analyses were conducted to ensure the robustness of the findings.

Results Our study identified several microbial taxa linked to EC risk. In Europeans, genera such as *Marvinbryantia*, *RuminococcaceaeUCG014*, and *Dorea* exhibited protective effects, while family *Erysipelotrichaceae* (OR:1.224) and *FamilyXI* (OR:1.090) were significantly correlated with high EC risk. In East Asians, genera *Lachnospira* (OR:3.561) and family *Bifidobacteriaceae* (OR:1.715) were found associated with EC risk. Genera *Lachnoclostridium* and *ErysipelotrichaceaeUCG003*, family *Coriobacteriaceae* positively served as protective factors. Sensitivity analyses confirmed the reliability of our results, and there was no evidence of pleiotropy or heterogeneity. Our analysis identified several microbial taxa associated with EC risk. In Europeans, genera such as *Marvinbryantia*, *Ruminococcaceae UCG014*, and Dorea demonstrated protective effects, while the families *Erysipelotrichaceae* (OR: 1.224) and *FamilyXI* (OR: 1.090) were significantly associated with increased EC risk. In East Asians, the genus *Lachnospira* (OR: 3.561) and the family *Bifidobacteriaceae* (OR: 1.715) were linked to higher EC risk, whereas the genera *Lachnoclostridium* and *Erysipelotrichaceae UCG003* and the family *Coriobacteriaceae* were identified as protective factors. Sensitivity analyses confirmed the reliability of these results, with no evidence of pleiotropy or heterogeneity.

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Conclusion This study highlights a relationship between gut microbiota and EC, emphasizing the potential of gut microbiota as therapeutic targets and biomarkers for assessing EC prognosis and treatment efficacy. These findings provide novel insights into the role of gut microbiota in the development and progression of EC.

Keywords Gut microbiota, Endometrial cancer, Mendelian randomization, Causal relationship, Instrumental variables

Introduction

Endometrial cancer (EC) is the sixth most common cancer among women, with over 400,000 new cases diagnosed globally in 2020, accounting for 4.5% of all female malignancies. It is the second most prevalent gynecological cancer after cervical cancer [1]. The risk of EC increases with age and predominantly affects postmenopausal women, particularly in high-income regions such as North America, Central Europe, and Eastern Europe [2]. EC is classified into two main types: type I and type II. Type I, the most common subtype, has a five-year survival rate of 97.5% [3, 4]. However, despite this relatively high survival rate, the global incidence of EC has been steadily rising over the past two decades, while overall survival rates have declined [4, 5], highlighting the need for greater attention. Type I EC is strongly linked to metabolic syndrome, with obesity as its primary risk factor, alongside other factors such as diabetes, hypertension, and elevated estrogen levels [6].

Over 97% of the human microbiome resides in the gastrointestinal tract, collectively referred to as the gut microbiome, which consists of a diverse array of microorganisms, including bacteria, fungi, archaea, and viruses [7]. The gut microbiota plays a vital role in maintaining physiological homeostasis, regulating immune responses, and synthesizing essential nutrients [8]. Dysbiosis of the gut microbiome, characterized by alterations in microbial composition, metabolites, and secretory functions, has been associated with various diseases, including cancers such as colorectal, hepatic, pulmonary, and breast cancer [9–12]. Recent studies have highlighted the complex relationship between the gut microbiome and EC. The gut microbiota contributes to the pathogenesis of EC through mechanisms such as metabolite production, local inflammation, and estrogen level modulation. Dysregulation of intestinal flora affects estrogen metabolism in the gut [13]. It also triggers local inflammatory responses by stimulating toll-like receptor 4 (TLR-4) and its ligand LPS, which in turn promotes ovarian estrogen synthesis [14]. Elevated systemic estrogen levels disrupt the endometrial microbiota and drive excessive endometrial proliferation [15].

Mendelian randomization (MR) analysis is a method used to evaluate whether risk factors have a causal effect on disease outcomes in observational settings by using genetic variants as instrumental variables (IVs) [16, 17]. This approach takes advantage of the random allocation of genetic variants during conception to assess the impact of exposures on target outcomes, thereby reducing confounding biases inherent in traditional observational studies. Researchers can use publicly available genome-wide association study (GWAS) datasets for analysis, avoiding the need for expensive and time-consuming data collection. A key advantage of two-sample MR analysis is that genetic variants and exposure outcomes are obtained from independent datasets, addressing limitations of single-sample MR, such as weak instrument bias and pleiotropy. In summary, two-sample MR is a robust and efficient method that offers valuable insights into causal relationships between exposures and outcomes.

Although the causal relationship between gut flora and EC remains unclear, observational epidemiological studies have identified an association. A major limitation in this field is the absence of reliable randomized controlled trials. To address this gap and further explore the association, we utilized large-scale GWAS data from the MiBioGen consortium and the IEU Open GWAS database. Using a two-sample MR approach, we investigated the influence of gut microbiota on EC development in East Asian and European populations.

Materials and methods

Study design

The schema depicted in Fig. 1A illustrates the research design employed in this study. To thoroughly explore the causal relationship between gut microbiota composition and EC, an extensive two-sample MR analysis was conducted. The selection of suitable single nucleotide polymorphisms (SNPs) as IVs was paramount in rigorously examining the potential causal influence of the exposure (gut microbiota) on the outcome (EC). Within the framework of the MR study, the chosen IVs must satisfy three critical criteria: Firstly, Relevance Assumption: The IVs must exhibit strong associations with the exposure (gut microbiota). This was ensured by selecting SNPs with a locus-wide significance threshold ($P < 1 \times 10^{-5}$) and calculating the F-statistic to confirm instrument strength. Secondly, Independence Assumption: The IVs must not be associated with any confounding factors that could influence both the exposure and the outcome. Covariates such as gender, age, genotyping batch, and the top ten principal components were adjusted in the GWAS data to minimize confounding effects. Sensitivity analyses, including MR-Egger regression and Cochran's Q test, were conducted to detect potential violations of this assumption. Finally, Exclusion Restriction Assumption:

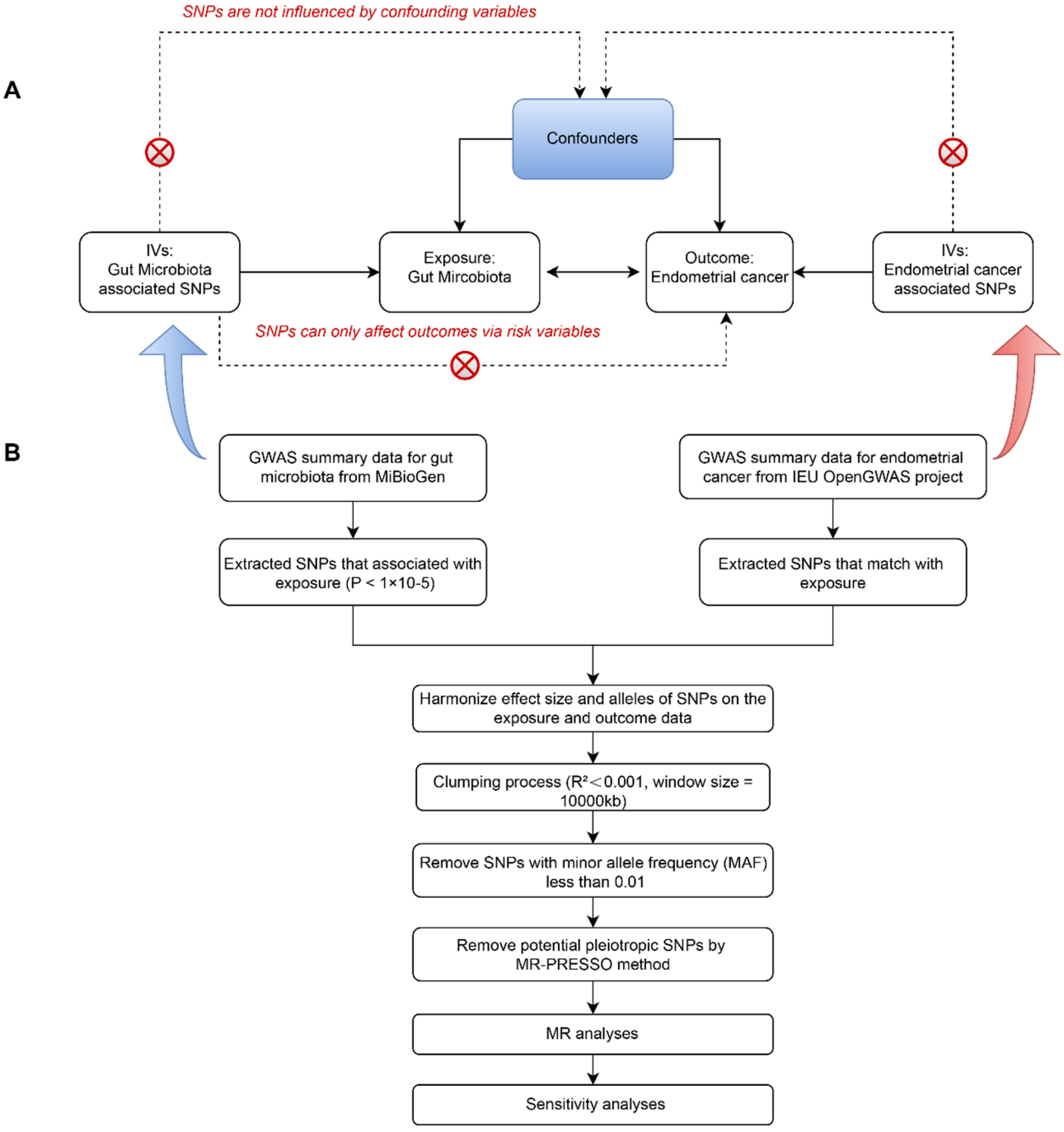

Fig. 1 Design and flowchart (**A**) The basic schema of Mendelian randomization (MR) analysis. We set gut microbiota as the exposure and EC as the outcome. The instrumental variables (IVs) must meet the following three criteria: (i) significantly associated with the exposure (ii) not affected by possible confounding factors (iii) did not affect outcomes through any other pathway. (**B**) The overall workflow

The IVs must influence the outcome (EC) exclusively through the exposure (gut microbiota) and not through any alternative pathways. This was validated using MR-Egger regression and MR-PRESSO to detect and correct for horizontal pleiotropy [17].

Exposure data

The GWAS data on gut microbiota were obtained from the MiBioGen research study, which is currently the largest study of transgenic genetics in the human microbiome. This investigation comprised samples from 18,340 participants across 24 cohorts, analyzing both the participants' whole-genome genotypes and 16 S rRNA fecal

microbiota gene sequencing data. A total of 211 bacterial taxa and 122,110 associated SNPs were identified. Covariates such as gender, age, genotyping batch, and the top ten principal components were adjusted in this study to satisfy the independence assumption. After excluding unnamed microbial taxa, we included 196 gut microbial taxa for analysis (9 phyla, 16 classes, 20 orders, 32 families, and 119 genera).

Outcome data

The GWAS summary data of EC were retrieved from the IEU OpenGWAS database. As for European populations, dataset ID "ebi-a-GCST006464" contained 121,885 participants, including 12,906 EC patients and 108,979 controls. Dataset ID "bbj-a-113" includes 90,730 Japanese participants, comprising 999 cases and 89,731 controls. For detailed descriptions of the database, please visit the website [18, 19].

Instrumental variable selection

The overall workflow of the study is presented in Fig. 1B. First, considering the limited number of SNPs available, we utilized the locus-wide significance threshold ($P < 1 \times 10^{-5}$) to filter SNPs related to the exposure, ensuring the relevance assumption. Second, for the reason of the biased results might caused by the presence of strong linkage disequilibrium (LD), the clumping process ($r^2 < 0.001$, clumping distance < 10,000 kb) was conducted to evaluate the LD between the included SNPs. Third, we calculated the F statistic to assess the strength of SNPs. The F statistic was calculated using a well-recognized formula: $F = R^2\ (n - k - 1)/k\ (1 - R^2)$, where R^2 and n represent the cumulative explained variance of selected SNPs and sample size, k represents the number of IVs. R^2 was calculated using the formula: $R^2 = 2\ MAF\ (1 - MAF)\ \beta^2$, where MAF represents the minor allele frequency. After calculation, we excluded weak IVs with $F < 10$, retaining the remaining variables as final IVs.

Statistical analysis

After instrumental variable selection, we merged the exposure data with outcome data and extracted IVs for MR analysis. The MR analyses were conducted separately for the European and East Asian populations to account for potential population stratification and differences in genetic architecture, microbiota composition, and environmental factors. A meta-analysis was not performed to preserve the validity of population-specific findings. In this study, we utilized six MR methods: IVW test, weighted mode, MR-Egger, weighted median, simple model, and MR-PRESSO. The IVW method is widely recognized as the most efficient approach under the assumption that all IVs are valid. Therefore, we used the IVW method as the primary statistical tool, with the other five methods serving as complements to ensure robustness. To address the exclusion restriction assumption, the MR-Egger method was used to identify the horizontal pleiotropy of selected IVs based on the intercept terms of the MR-Egger regression model. SNPs with horizontal pleiotropy were removed ($P < 0.05$). The MR-PRESSO method was used to detect SNP pleiotropic outliers and repeat MR analysis after eliminating abnormal SNPs. The following two methods, including weighted median and MR-Egger, were performed to conduct sensitivity analysis. The results of the weight median method are reliable if over 50% of the weights come from valid IVs. The MR-Egger method was able to identify the horizontal pleiotropy of selected IVs, according to the intercept terms of the MR-Egger regression model. SNPs with horizontal pleiotropy were removed ($P < 0.05$). The MR-PRESSO method was used to detect SNP pleiotropic outliers and repeat MR analysis after eliminating abnormal SNPs. We quantified heterogeneity by computing Cochran's Q statistic and the I^2 statistic. Heterogeneity was deemed to exist if the *p*-value of the Q statistic was less than 0.05. Low heterogeneity ($I^2 < 25\%$) among SNPs indicates that the results based on the IVW method are reliable. Scatter plots and funnel plots were drawn to visualize the magnitude of heterogeneity and identify outliers. Leave-one-out analyses were also performed to assess the model's stability.

All MR analyses were conducted utilizing the TwoSampleMR (version 0.5.9), VariantAnnotation, and gwasglue package in R software (version 4.3.2) [20, 21].

Result

Selection of instrumental variables

13,321 SNPs at the locus-wide significance level ($P < 1 \times 10^{-5}$) were selected based on 196 microbiota features in the MiBioGen study. After a series of quality control procedures, 2601 SNPs were selected as IVs. The F-statistics of the selected IVs were all greater than 10, excluding weak ones. All IVs passed the MR-PRESSO outlier test and MR-Egger regression showed that there did not exist the evidence of horizontal pleiotropy ($p > 0.05$).

Causal effects of gut microbiota on the development of EC

We finally selected a total of 196 taxa for MR analysis (9 phyla, 16 classes, 20 orders, 32 families, and 119 genera). Figure 2a and b present the preliminary MR estimates for the associations between gut microbiota and the risk of EC. Supplementary Tables S2 and S3 show IVs employed in selected gut microbes.

In Europeans, genera *Marvinbryantia*, *Ruminococcaceae UCG014*, and *Dorea* were identified as protective factors, while the Family *Erysipelotrichaceae* and *FamilyXI* was positively associated with EC risk. In East Asians, genera *Lachnospira* and family *Bifidobacteriaceae*

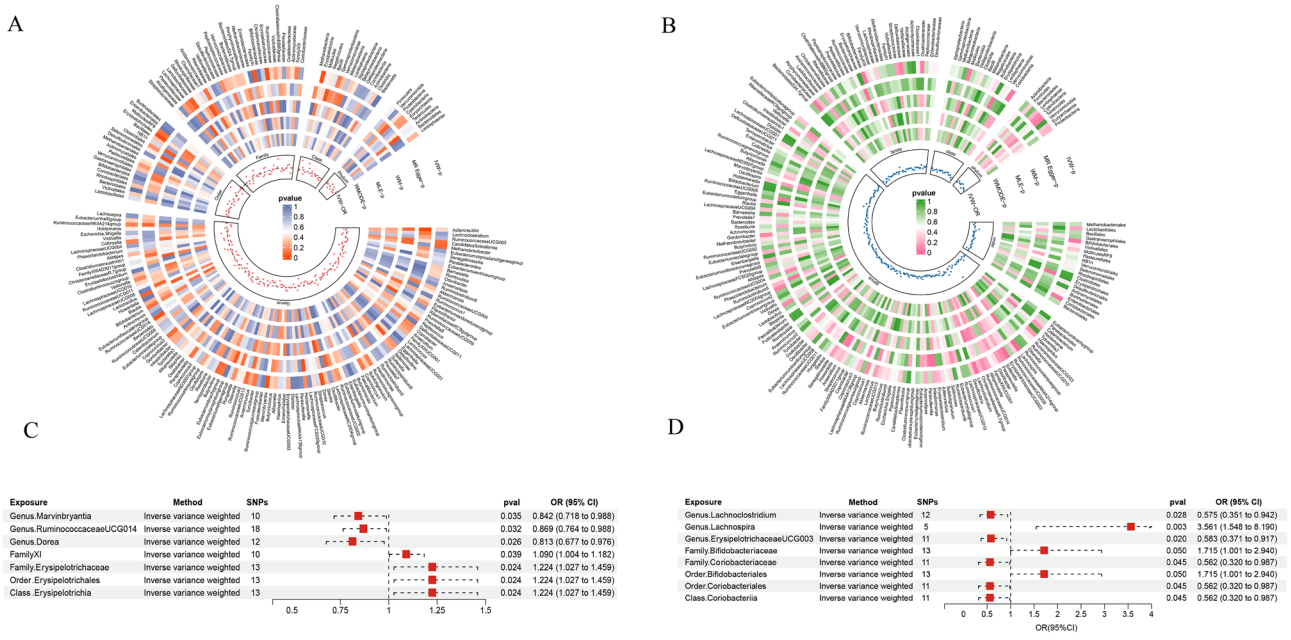

Fig. 2 MR analysis results (**A-B**) Preliminary MR estimates for the associations between gut microbiota and the risk of endometrial cancer in Europeans (**A**) and East Asians (**B**). From the outer to inner circles, the *P* values of IVW, MR Egger, WM, MLE, and WMODE analyses are represented, respectively. The shades of color reflect the magnitude of the value of p. (**C-D**) Forest plot of MR results based on IVW methods in Europeans (**C**) and East Asians (**D**)

Table 1 Significant gut microbes associated with endometrial cancer based on the EBI database and biobank Japan

Exposure	NSNP	Method	β	SE	*P*-value	OR	95%CI
In Europeans							
Genus.Marvinbryantia	10	Inverse variance weighted	0.172	0.082	0.035	0.842	(0.718, 0.988)
Genus.RuminococcaceaeUCG014	18	Inverse variance weighted	0.141	0.066	0.032	0.869	(0.764, 0.988)
Genus.Dorea	12	Inverse variance weighted	0.208	0.093	0.026	0.813	(0.677, 0.976)
FamilyXI	10	Inverse variance weighted	0.086	0.042	0.039	1.090	(1.004, 1.182)
Family.Erysipelotrichaceae	13	Inverse variance weighted	0.202	0.089	0.024	1.224	(1.027, 1.459)
Family.Erysipelotrichaceae	13	Inverse variance weighted	0.202	0.089	0.024	1.224	(1.027, 1.459)
Order.Erysipelotrichia	13	Inverse variance weighted	0.202	0.089	0.024	1.224	(1.027, 1.459)
In East Asians							
Genus.Lachnoclostridium	12	Inverse variance weighted	0.553	0.252	0.028	0.575	(0.351,0.942)
Genus.Lachnospira	5	Inverse variance weighted	1.270	0.425	0.003	3.561	(1.548,8.190)
Family.Bifidobacteriaceae	11	Inverse variance weighted	0.539	0.231	0.020	0.583	(0.371,0.917)
Family.Coriobacteriaceae	13	Inverse variance weighted	0.540	0.275	0.050	1.715	(1.001,2.940)
Genus.ErysipelotrichaceaeUCG003	11	Inverse variance weighted	0.576	0.287	0.045	0.562	(0.320,0.987)
Order.Bifidobacteriales	13	Inverse variance weighted	0.540	0.275	0.050	1.715	(1.001,2.940)
Order.Coriobacteriales	11	Inverse variance weighted	0.576	0.287	0.045	0.562	(0.320,0.987)
Class.Coriobacteriia	11	Inverse variance weighted	0.576	0.287	0.045	0.562	(0.320,0.987)

SE, Standard error; MR, Mendelian randomization; NSNP, number of single nucleotide polymorphisms; OR, odds ratio; CI, confidence interval

were associated with increased EC risk, while genera *Lachnoclostridium* and *ErysipelotrichaceaeUCG00*, family *Coriobacteriaceae* were protective factors.

Among Europeans, Seven gut microbiota were identified from the EBI database after MR analysis, including three generas, two families, and two classes of microorganisms (Table 1; Fig. 2c; Supplementary Table S1). The results of the IVW analysis showed that genera *Marvinbryantia* (OR = 0.842, 95%CI 0.718–0.988, *P* = 0.035), *RuminococcaceaeUCG014* (OR = 0.869, 95%CI 0.764–0.988, *P* = 0.032), and *Dorea* (OR = 0.813, 95%CI 0.677–0.976, *P* = 0.026) positively served as protective factors. Family *Erysipelotrichaceae* (OR = 1.224, 95%CI 1.027–1.459, *P* = 0.024) and *FamilyXI* (OR = 1.090, 95%CI 1.004–1.182, *P* = 0.039) exhibited significant causal associations with EC risk. Order *Erysipelotrichales* and *Erysipelotrichia* are actually the same ones. Each of them shares the same IVs with Family *Erysipelotrichaceae*, so the results of the MR analysis are the same.

In the case of East Asians, eight gut microbiota were identified from the BBJ database after MR analysis (Table 1; Fig. 2d; Supplementary Table S1). Among these taxa, *family.Coriobacteriaceae*, *Order.Coriobacteriales*, *Class.Coriobacteriia* share the same SNPs, similar to what Family. *Bifidobacteriaceae* and *Order.Bifidobacteriales* do. The results of the IVW analysis showed that genera *Lachnospira* (OR = 3.561, 95%CI 1.548–8.190, *P* = 0.028) and family *Bifidobacteriaceae* (OR = 1.715, 95%CI 1.001–2.940, *P* = 0.050) exhibited significant causal associations with EC risk. While genera *Lachnoclostridium* (OR = 0.575, 95%CI 0.351–0.942, *P* = 0.028) and *ErysipelotrichaceaeUCG003* (OR = 0.583, 95%CI 0.371–0.917, *P* = 0.020), family *Coriobacteriaceae* (OR = 0.562, 95%CI 0.320–0.987, *P* = 0.045) positively served as protective factors.

Sensitivity analysis

The intercept terms of the MR-Egger regression model exhibited no significant deviation from 0, and all *P*-values exceeded 0.05, thereby suggesting the absence of pleiotropy (Table 2). Additionally, the significance level ($p < 0.05$) observed in the MR-PRESSO global test affirmed the conclusion mentioned above, with no IVs identified as potential outliers. Notably, both the results of Cochrane Q statistics and I^2 statistics showed no significant heterogeneity, with *p*-values exceeding 0.05, indicating the robustness of our findings. Leave-one-out sensitivity analysis and funnel plots provided further assurance regarding the reliability and bias of the inferred causal effects across the four identified associations. In summary, these findings collectively suggest a compelling causal relationship between the identified flora and the corresponding risk of EC. To sum up, the results of our MR analysis demonstrate a high degree of reliability and robustness.

Data visualization

We generated scatter plots to depict the effects of individual single nucleotide SNPs with exposure factors and outcomes, complemented by regression curves illustrating causal estimates. Funnel plots were used to evaluate the data distribution, providing a visual representation of the magnitude of heterogeneity. Additionally, forest plots were constructed to present the final analysis results in a visually comprehensive manner (see Supplementary Fig. S1-S8 online).

Discussion

In this study, we performed a two-sample MR analysis using GWAS data on gut microbiota and EC. We systematically examined the potential relationship between gut microbiota and the risk of EC in both European and East Asian populations.

Table 2 Evaluation of heterogeneity and directional Pleiotropy using different methods

	Exposure	Heterogeneity			Horizontal pleiotropy			
		I^2 (%)	Cochran's Q	*P*-value	Egger intercept	SE	*P*-value	MR-PRESSO global test p
Europeans	*Genus.Dorea*	19	12.421	0.258	0.005	0.02	0.788	0.358
	Genus.Marvinbryantia	0	6.749	0.564	-0.035	0.027	0.23	0.506
	Genus.RuminococcaceaeUCG014	0	14.385	0.57	0	0.015	0.994	0.663
	FamilyXI	0	6.992	0.537	-0.026	0.029	0.396	0.56
	Family.Erysipelotrichaceae	0	5.778	0.888	0.034	0.024	0.183	0.824
	Order.Erysipelotrichales	0	5.778	0.888	0.034	0.024	0.183	0.816
	Order.Erysipelotrichia	0	5.778	0.888	0.034	0.024	0.183	0.816
East Asians	*Genus.Lachnoclostridium*	0	9.753	0.462	0.025	0.070	0.733	0.596
	Genus.Lachnospira	0	1.409	0.703	-0.019	0.148	0.907	0.877
	Genus.ErysipelotrichaceaeUCG003	0	7.869	0.547	0.005	0.058	0.935	0.696
	Family.Bifidobacteriaceae	32	16.191	0.134	0.009	0.076	0.910	0.241
	Family.Coriobacteriaceae	0	7.138	0.623	-0.044	0.073	0.563	0.722
	Order.Bifidobacteriales	32	16.191	0.134	0.009	0.076	0.910	0.244
	Order.Coriobacteriales	0	7.138	0.623	-0.044	0.073	0.563	0.728
	Class.Coriobacteriia	0	7.138	0.623	-0.044	0.073	0.563	0.719

I^2, I-squared percentage; Cochran's Q, Cochran's Q test; Egger intercept: Egger's regression intercept; MR-PRESSO: Mendelian Randomization Pleiotropy Residual Sum and Outlier

In Europeans, our study identified the genera *Marvinbryantia*, *Ruminococcaceae UCG014*, and *Dorea* as protective factors, whereas the family *Erysipelotrichaceae* was positively associated with an increased risk of EC. Additionally, the unclassified *Family XI* may also contribute to a higher EC risk. In East Asians, the genus *Lachnospira* and the family *Bifidobacteriaceae* exhibited significant causal associations with an increased EC risk, while the genera *Lachnoclostridium* and *Erysipelotrichaceae UCG003* and the family *Coriobacteriaceae* were identified as protective factors. Most of these bacteria belong to the Firmicutes phylum, except for *Bifidobacteriaceae* and *Coriobacteriaceae*. These differences may be attributed to variations in dietary habits, cultural practices, and genetic backgrounds between Europeans and East Asians. For example, the higher consumption of fermented foods in East Asian diets may influence the abundance and activity of specific gut microbiota, such as *Bifidobacteriaceae* [22, 23]. Additionally, genetic differences may affect host-microbiota interactions, leading to population-specific associations [24]. These findings highlight the importance of considering population-specific factors in microbiota-based interventions for EC prevention and treatment. Precision medicine programs should account for differences in gut microbiota composition and its interaction with the host across populations to develop tailored strategies that maximize efficacy and minimize risks.

As for Europeans, our study results indicate that genera *Marvinbryantia*, *RuminococcaceaeUCG014*, and Dorea are identified as protective factors, whereas the *Erysipelotrichaceae* family is positively correlated with EC risk. Additionally, the unclassified *Family XI* may potentially increase EC risk. In East Asians, genera *Lachnospira* and family *Bifidobacteriaceae* exhibited significant causal associations with EC risk. While genera *Lachnoclostridium* and *ErysipelotrichaceaeUCG003*, family *Coriobacteriaceae* positively served as protective factors. These bacteria all belong to the Firmicutes phylum except family *Bifidobacteriaceae* and *Coriobacteriaceae*.

The gut microbiota constitutes the largest microbial community in the human body, comprising diverse microorganisms. The composition and functionality of gut microbiota significantly influence the host's homeostasis. Dysbiosis in the gut microbiota has been associated with various diseases, including obesity, cancer, and metabolic disorders [25]. Normal gut microbiota colonization directly inhibits the growth of pathogenic bacteria and maintains the integrity of the intestinal mucosal barrier, preventing harmful substances and microbes from entering the bloodstream. Gut microbiota dysbiosis can lead to mucosal barrier damage, local inflammation, and systemic chronic inflammation through toll-like receptors and NF-kB pathways [26, 27]. Furthermore, gut microbiota can promote the development of distant cancers through their metabolic byproducts. Lipoteichoic acid and deoxycholic acid, components of Gram-positive gut bacteria, promote the development of liver cancer by circulating through the enterohepatic circulation [28, 29].

EC is more common in postmenopausal women, and studies have shown that the gut microbiota of perimenopausal women undergoes dysbiosis, characterized by an imbalance between the Firmicutes and Bacteroidetes phyla [30, 31]. his imbalance in the Firmicutes to Bacteroidetes ratio can trigger inflammatory reactions, negatively impacting human health [32]. atients exhibit significant differences in gut microbiota composition compared to healthy individuals. For instance, González et al. reported a lower proportion of Firmicutes in the gut of EC patients compared to Lynch syndrome patients without cancer [33]. Another study found a decrease in Firmicutes and Ruminococcaceae in the feces of EC patients [34]. In addition, Firmicutes and Ruminococcaceae play a key role in producing short-chain fatty acids (SCFAs), such as butyrate, which possess anti-inflammatory and anti-carcinogenic properties by inhibiting NF-κB signaling and promoting regulatory T cell differentiation [35, 36].

Therefore, the reduction of *Firmicutes* and *Ruminococcaceae* may increase the risk of EC, consistent with the relative abundance changes observed in *Marvinbryantia*, *Ruminococcaceae UCG014*, and *Dorea* in our findings.

Previous research has suggested an association between estrogen and EC risk, with higher estrogen levels increasing the risk of EC [15]. There exists a specialized group of gut bacteria known as the estrobolome, which modulates estrogen metabolism by releasing β-glucuronidase [37]. Circulating estrogen is primarily metabolized in the liver, where conjugated estrogens are deconjugated by β-glucuronidase upon entering the intestine, subsequently reabsorbed in the free form, and recirculated via the enterohepatic circulation [38]. Dysbiosis in the gut microbiota may disrupt the balance between estrogen reabsorption and excretion, leading to fluctuating estrogen levels and subsequent development of endometrial cancer. Studies have shown that alterations in the gut microbiota composition, particularly within *Firmicutes* and *Bacteroidetes*, significantly affect systemic non-ovarian estrogen levels, with changes in *Firmicutes* and *Ruminococcaceae* having the greatest impact [39]. *Firmicutes* to *Bacteroides* ratio is also related to systemic estrogen levels [29]. Several microbial taxa carry the β-glucuronidase gene, including *Firmicutes*, and *Bacteroidetes* [40]. Almost all the microbial taxa identified in our MR study belong to the estrobolome, classified as *Firmicutes*, indicating that gut microbiota may influence the occurrence and development of EC through estrogen. In East Asians, family *Bifidobacteriaceae* was found to

increase EC risk, which can also produce β-glucuronidase [41]. Hydroxysteroid dehydrogenases are also ubiquitously present in the human gut and participate in the partial reduction process of estrogen synthesis from cholesterol precursors [42]. Studies have demonstrated positive correlations between urinary [43] and serum [44] estrogen levels and gut microbial diversity. Our study also found Family *Coriobacteriaceae* was inversely related to EC risk. Previous research has revealed a close correlation between intestinal *Coriobacteriaceae* and levels of hepatic triglycerides, glucose, glycogen, and exogenous metabolism [45]. It may exert anticancer effects through the modulation of intra-body fat content, thereby regulating levels of endogenous estrogen. Additionally, gut microbiota confer biological activity to exogenous estrogen-like compounds [46, 47]. For instance, they possess the capability to metabolize daidzein into equol or O-desmethylangolensin [48]. The beneficial effects of lignans are also contingent upon the activity of gut microbial metabolites such as enterodiol and enterolactone [49]. Therefore, beyond the direct effects of inherent female genetic variations, altered physiological conditions, and environmental estrogen exposure [50] on hormone levels, the dysbiosis of gut microbiota resulting from poor lifestyle choices, dietary habits, and antibiotic misuse also indirectly influences estrogen levels. Subsequently, this dysregulation of estrogen levels promotes endometrial carcinogenesis. Furthermore, estrogen levels are influenced by the inflammatory status of the gut. Dysbiosis in the gut microbiota can trigger microbial pattern recognition receptors, such as TLR-4 and its ligand LPS, leading to the upregulation of pro-inflammatory cytokines (IL17, TNF-α, and IFN-γ) in the bloodstream, thereby increasing ovarian estrogen production [14].

Short-chain fatty acids (SCFAs) are fermentation products of dietary fiber by gut microbiota, primarily including acetate, butyrate, and propionate [51]. SCFAs play various physiological roles and contribute to multiple metabolic pathways [52–54]. In the gut, SCFAs maintain a low pH and promote the growth of probiotic bacteria such as Lactobacillus and Bifidobacterium [55]. SCFAs also exhibit immunomodulatory effects by influencing T cell metabolism and gene expression, regulating immune cell recruitment, and alleviating local inflammation [56]. Butyrate, an important SCFA, induces the expression of tight junction proteins and antimicrobial peptides, thereby protecting the intestinal barrier and preventing the translocation of pathogens and harmful metabolites [56, 57]. At the systemic level, SCFAs have been shown to have anti-inflammatory and anti-tumor effects [58–60]. At the systemic level, SCFAs have been shown to have anti-inflammatory and anti-tumor effects [61]. SCFAs inhibit histone deacetylase, activate apoptosis, and inhibit cancer cell growth and migration [62]. Butyrate, in particular, activates the expression of the tumor-suppressor gene like sodium-coupled monocarboxylate transporter [63]. Seventy-four bacterial species in the gut can produce SCFAs, including *Lactobacillus*, *Bifidobacterium*, and *Clostridium*, with Clostridium being the primary SCFA-producing bacterium [64]. Studies have measured the gut microbiota composition in menopausal women and found a significant reduction in SCFA-producing bacteria at the genus and species levels [31, 44], which may be associated with an increased risk of EC after menopause. However, there is currently no detailed research on the effects of SCFAs on endometrial cancer. In Europeans, our study found that the genus *Ruminococcaceae UCG014* (OR: 0.869), *Marvinbryantia* (OR: 0.842), and *Dorea* (OR: 0.813), belonging to the *Clostridium* class, may act as protective factors against EC by producing SCFAs, thereby regulating systemic inflammation levels and exerting anticancer effects. In East Asians, we found that the genus *Lachnoclostridium* (OR:0.575) and *ErysipelotrichaceaeUCG003* (OR:0.583) can play the same role. However, other SCFAs-producing bacteria such as Family *Erysipelotrichaceae* in Europeans and genus *Lachnospira* were identified as pathogenic factors for EC, offering a new perspective for future research.

Although previous studies have indicated differences in gut microbiota between EC patients and healthy women, the results of different studies vary due to factors such as ethnicity and sampling methods. Moreover, these studies are observational and based on small sample sizes, thus the specific mechanisms by which gut microbiota affects EC remain unclear. Our study utilized the largest sample size to date and employed MR analysis to mitigate confounding biases inherent in traditional epidemiological studies. The level of evidence from MR analysis is similar to that of randomized controlled trials. Additionally, sensitivity analysis demonstrated that our findings were free from pleiotropy or heterogeneity and held statistical significance. However, the GWAS data used in this study were sourced from European and East Asian populations, while the composition of gut microbiota is influenced by factors such as geography and dietary habits. Consequently, the composition of gut microbiota may vary among different countries and ethnicities [65]. Thus, the generalizability of our study results to other populations is limited. Future research should focus on larger sample sizes and multicenter randomized controlled trials to further investigate the causal relationship between gut microbiota and EC. Another limitation of this study is the potential for misclassification bias in the microbiome data, which arises from the use of relative abundance rather than absolute abundance. Relative abundance, while commonly employed in microbiome research, does not reflect the total microbial load and may lead to biased interpretations, particularly for low-abundance taxa [66,

67]. Additionally, the inherent limitations of 16 S rRNA gene amplicon sequencing, such as its lower resolution compared to shotgun sequencing, may further contribute to this bias. Future research utilizing absolute abundance measurements and higher-resolution sequencing methods, such as shotgun metagenomics, could provide a more accurate and comprehensive understanding of the causal roles of microbial taxa.

Conclusion

We employed the MR method to analyze the association between EC and gut microbiota, identifying six microbial taxa associated with EC risk. Increasing evidence suggests that alterations in gut microbiota play a pivotal role in the development of EC and may represent potential therapeutic targets. Therefore, further investigation into specific microbial communities in the gut of EC patients is needed to identify potential biomarkers. These biomarkers could serve as valuable references for assessing treatment efficacy and prognosis in EC patients in the future.

Abbreviations

EC	Endometrial cancer
MR	Mendelian randomization
GWAS	Genome-wide association study
IV	Inverse variance-weighted
SNP	Single nucleotide polymorphism
LD	Linkage disequilibrium
IVW	Inverse variance-weighted
SCFA	Short-chain fatty acids

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

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Author contributions

ZYC, XLH and JQC designed this study. XRL, HT, XX, HQL, XLH, HZ, YYL, and PL conducted this study, including data collection, and data analysis. JQC drafted the manuscript writing and ZYC revised the manuscript. All authors contributed to the final approval of the manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

Our analysis used publicly available genome-wide association study (GWAS) summary statistics. No new data were collected, and no new ethical approval was required.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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