Causal Relationship Between Gut Microbiota and Gynecological Tumor: A Two-Sample Mendelian Randomization Study

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Abstract

Background: Previous research has linked alterations in the composition of the gut microbiota to a variety of gynecologic tumors. Nevertheless, although the causal relationship between the gut microbiota and gynecologic tumors remains to be fully elucidated. Therefore, this study uses a two-sample Mendelian randomization analysis (MR) to explore the causal correlation between the gut microbiota community and prevalent gynecologic tumours. With the goal of identifying specific pathogenic bacterial communities that may be involved in gynecologic tumor development.

Materials and Methods: We utilized data from the MiBioGen consortium’s Genome-Wide Association Study (GWAS) on gut microbiota as the exposure variable. Four common gynecologic neoplasms including uterine fibroids (UF), endometrial cancer (EC), ovarian cancer (OC) and cervical cancer (CC) were selected as the outcome variables. Single-nucleotide polymorphisms (SNPs) significantly associated with exposure were selected as the instrumental variables (IVs). The inverse variance-weighted (IVW) method was used as the principal MR analysis to assess the causal relationship between gut microbiota and these tumors, with the goal of identifying microbial communities associated with gynecologic tumor development. An independent validation cohort was used for further validation. We conducted sensitivity analyses to ensure robustness of the findings. Lastly, we performed reverse MR analysis to assess the potential for reverse causation.

Results: Combining the results from the discovery and validation cohorts, we found that higher relative abundance of Lachnospiraceae is associated with lower risk of UF (OR: 0.882, 95% CI: 0.793-0.999, $P = 0.982$). Conversely, a higher incidence of OC is associated with a higher relative abundance of Lachnospiraceae (OR: 1.329, 95% CI: 1.019-1.732, $P = 0.036$). Sensitivity analyses confirmed the reliability of these results. Furthermore, the results of the reverse MR analysis showed no evidence of a reverse cause-and-effect relationship between UF, OC, and Lachnospiraceae.

Conclusion: In this study, a causal relationship between Lachnospiraceae and both UF and OC was established. This provides new insights into the role of gut microbiota in the mechanism of gynecological tumor development.

Introduction

In recent years, cancer has become the leading cause of death in urban areas and the second leading cause of death in rural areas. Incidence and mortality rates of gynecological tumors are on the rise and pose a threat to women's health. Among these tumors, UF are the most common. CC, EC, and OC are the three most common gynecologic malignancies and rank in the top 10 in female cancer incidence. [1]. The occurrence of gynecological tumors is influenced by a variety of factors, including genetics, hormonal imbalances, obesity, persistent HPV infection, and chronic conditions such as endometriosis.

The human microbiome is the microbiome that resides in specific areas of the body [2]. It plays a vital role in maintaining organismal balance and host health. It possesses many important functions, such as nutrient absorption, maintaining the integrity of epithelial barrier, removing harmful substances, regulating inflammation and immune response, and protecting against pathogen invasion [3]. In recent years, with the
development of next-generation sequencing technologies, we have gained a deeper understanding of the diversity of the human microbiome. Among them, the gut microbiome is widely studied. The normal gut microbiome consists mainly of the phyla Bacteroidetes and Firmicutes [4]. The dominant microbiota in the gut of healthy individuals produces short-chain fatty acids by fermenting carbohydrates, proteins and peptides. These SCFAs help maintain an acidic gut environment, promote the growth of beneficial bacteria and inhibit the colonization of potential pathogens. In addition, SCFA helps maintain gut barrier function, stimulates intestinal epithelial cell regeneration, and promotes the production of antimicrobial peptides and mucus. In summary, a healthy gut microbiome plays an important role in suppressing chronic inflammation, obesity, metabolic syndrome and cancer-related diseases[5].

Microbial communities exists in a symbiotically balanced manner within the host. However, several factors can influence the composition of the microbiome, such as medication, obesity, diet, exercise, race, geography, and genetics [5]. Intestinal dysbiosis refers to a decline in diversity and stability of the gut microbiota, leading to the overgrowth of opportunistic pathogens and the production of specific bacterial by-products, and immunological and metabolic disturbances. Studies have linked intestinal dysbiosis to inflammatory bowel disease, diabetes, obesity, metabolic syndrome, cancer, and more [5]. Recently, the relationship between gut microbiota and tumors has become a hot topic. Previous studies have confirmed the role of microbial dysbiosis in gastrointestinal tumors, such as colorectal and liver cancer. In addition to the digestive tract, the gut microbiota is also associated with skin, mouth, lung and reproductive cancers [7]. The mechanisms by which microbes drive cancer are multifaceted. In gynecological tumors, intestinal dysbiosis, in addition to regulating inflammatory responses, plays a critical role in gynecological tumors through DNA damage, effects on estrogen levels, and production of toxins and metabolites associated with gut bacteria[8].

The gut microbiome affects the carcinogenesis of gynecological tumors. By comparing the differences in gut microbiota composition between UF patients and healthy individuals, we found a decrease in the abundance of Bifidobacteria scardovii, Ligilactobacillus saerimneri, and Lactococcus raffinolactis, while the abundance of Pseudomonas stutzeri and Prevotella amnii were increased [9]. Zq Wang et al.’s study, using 16S rRNA sequencing analysis, showed a significant increase in the abundance of Proteobacteria in the gut microbiota of CC patients compared to the control, while the abundance of Firmicutes was relatively reduced [10]. After adjusting for age and race, Prevotella, Porphyromonas, and Dialister were significantly enriched, while Bacteroides, Alistipes, and Lachnospiraceae were decreased in CC[11]. Ss Zhao et al. identified the differences in gut microbiota between EC patients and healthy individuals. The study found Ruminococcus were enriched in EC and could serve as prognostic biomarkers, providing new targets for clinical treatment [12]. Some bacterial metabolites also impact the development of EC: butyrate exhibits anti-inflammatory and anti-tumor effects, while the overexpression of tryptophan decarboxylase is associated with poor prognosis in EC [13]. Proteobacteria and Veillonella were more abundant among breast cancer and ovarian cancer patients with cachexia [14]. Chambers et al.’s study demonstrated that dysbiosis of the gut microbiota in OC can lead to tumor progression and resistance to cisplatin chemotherapy [15].

However, these findings are mainly based on cross-sectional studies and cannot determine causation. Establishing causality can improve our understanding of gynecological tumor development mechanisms
and potentially guide the development of microbial interventions. Therefore, elucidating the causal relationship between gut microbiota and gynecological tumors is critical. Mendelian randomization explores causal relationships between risk factors and outcomes, often unaffected by confounding and reverse causality, by integrating aggregated data from GWAS and using genetic variation as IVs [16]. Hence, the purpose of this study is to explore the causal relationship between gut microbiota and gynecological tumors and to provide a theoretical basis for the development of potential therapeutic targets for gynecological tumors.

Method

Study design

In this study, we investigated the causal relationship between gut microbiota and four common gynecological tumors (UF, EC, OC, and CC) employing MR analysis. The gut microbiota was the exposure variable, SNPs were significantly associated with gut microbiota, and UF, EC, OC and CC were the outcome variables. The study included the discovery and validation cohorts of gynecological tumors samples. The selected genetic IVs in this study need to satisfy the following assumptions: (1) IVs significantly associated with gut microbiome; (2) IVs independent of all confounding factors except gut microbiome; (3) IVs influence outcomes only through gut microbiome. In addition, we performed reverse MR analysis to explore a causal relationship between these gynecological tumors and gut microbiota. The study flowchart is shown in Fig. 1.

Data sources

Summary genetic data for the gut microbiota were taken from MiBioGen (https://mibiogen.gcc.rug.nl), a large scale, multiethnic GWAS meta-analysis. In this study, 18,340 participants from 24 cohorts were included, with approximately 78% from Europe. Three different variable regions of the 16S rRNA gene were analyzed for microbial composition. 211 taxonomic groups were represented in the dataset, consisting of 9 phyla, 16 classes, 20 orders, 36 families and 131 genera. It was considered to be the smallest and most speciose taxonomic level of the genus. In order to accurately identify each microbial pathogen group, we analyzed only at the genus level, excluding 15 unknown bacterial taxonomic groups. A total of 196 bacterial taxonomic groups were included. Adjustments were made for age, sex, technical covariates and genetic principal components.

Genetic summary data for the UF, EC, OC and CC discovery and validation cohorts were downloaded from the IEU Open GWAS Project (https://gwas.mrcieu.ac.uk/). Details are shown in Table 1. The data used in this study is publicly available GWAS summary data and has been subject to ethical approval.
Table 1
Gynecological tumors GWAS samples used in this study.

<table>
<thead>
<tr>
<th>Discovery</th>
<th>UF</th>
<th>finn-b-CD2_BENIGN_LEIOMYOMA_UTERI</th>
<th>18,060</th>
<th>105,519</th>
<th>16,379,784</th>
<th>European</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC</td>
<td>ebi-a-GCST006464</td>
<td>12,906</td>
<td>108,979</td>
<td>9,470,555</td>
<td>European</td>
<td></td>
</tr>
<tr>
<td>OC</td>
<td>ieu-a-1229</td>
<td>2,966</td>
<td>40,941</td>
<td>/</td>
<td>European</td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>ieu-b-4876</td>
<td>563</td>
<td>198,523</td>
<td>8,506,261</td>
<td>European</td>
<td></td>
</tr>
<tr>
<td>Replication</td>
<td>UF</td>
<td>bbj-a-157</td>
<td>5,954</td>
<td>95,010</td>
<td>8,877,739</td>
<td>East Asian</td>
</tr>
<tr>
<td>EC</td>
<td>ukb-b-13545</td>
<td>1,151</td>
<td>461782</td>
<td>9851867</td>
<td>European</td>
<td></td>
</tr>
<tr>
<td>OC</td>
<td>ieu-b-4963</td>
<td>1,218</td>
<td>198,523</td>
<td>9,822,229</td>
<td>European</td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>ukb-b-8777</td>
<td>1,889</td>
<td>461,044</td>
<td>9,851,867</td>
<td>European</td>
<td></td>
</tr>
</tbody>
</table>

UF, uterine fibroids; EC, endometrial cancer; OC, ovarian cancer; CC, cervical cancer; GWAS, genome-wide association study.

Selection of Instrumental Variables

We applied the following quality control criteria to select SNPs meeting the conditions as IVs: (1) To ensure the authenticity and accuracy of the results given that only a few gene loci identified by GWAS of the gut microbiome reach genome-wide significance \((P < 5 \times 10^{-8})\), in order to select SNPs significantly associated with the exposure, we set the threshold to \(P < 1 \times 10^{-5}\) with the goal of obtaining more relevant results [17]. (2) To avoid bias caused by linkage disequilibrium (LD) between IVs, we set the LD coefficient to \(r^2 < 0.1\) and the region width to 500kb in order to ensure independence between SNPs, thereby ruling out the impact of pleiotropy on the results [18]. (3) Weak IVs could result in biased results. For this reason, we used the \(F\) test to assess whether SNPs are affected by weak instrument bias and excluded SNPs with an \(F\) statistic of less than 10 [18]. (4) SNPs that were directly associated with the outcome \((P < 1 \times 10^{-5})\) were excluded [20]. (5) We removed the palindrome SNPs as well as the incompatible SNPs.

MR analysis

To investigate causal relationships between the gut microbiome and UF, EC, OC, and CC, we ran three regression models, namely IVW, Weighted Median Estimation (WME), and MR-Egger regression, respectively. The IVW method was used as the primary method, with the other two methods acting as complementary methods. The IVW method utilized the inverse variance of each IV as weights to calculate the summary causal effect estimate. The WME method employed weighted median estimation, requiring at least 50% of valid IVs and ordering SNPs based on their weights before selecting the median as the result [21]. The MR-Egger regression estimated the general linear regression model by calculating the correlation coefficients between each SNP and the outcome, as well as between the SNP and the exposure.

We validated the significant bacterial genera identified in the discovery cohort and used the same MR analysis methods in the validation cohort in order to ensure consistency and reliability of the findings.
Pleiotropy Heterogeneity and Sensitivity Analysis

We first used Cochran's Q test to evaluate SNP heterogeneity. A statistically significant $P$ value from Cochran's Q test ($P < 0.05$) indicates a significant heterogeneity in the result[22]. To detect outliers, we employed MR-PRESSO. Secondly, in the present study we used the intercept term from MR-Egger regression to test for horizontal pleiotropy. If $P > 0.05$ it suggests that the difference between the intercept term of the MR-Egger model and zero is small, indicating the absence of significant horizontal pleiotropy. As a result, the IVW results are reliable[23]. Lastly, we conducted a leave-one-out sensitivity test to evaluate the robustness of the results. We sequentially removed individual SNPs and calculated MR scores with the remaining SNPs. The fact that the results of the analysis do not differ significantly from the overall results indicates the MR results are robust [24].

Reverse MR analysis

We performed inverse MR analysis to investigate the causal association between gynecologic tumors and significant bacterial genera identified. SNPs associated with these diseases were used as IVs, with UFs, ECs, OCs, and CCs as exposures, and bacterial pathogen genera identified as outcomes.

All statistical analyses were performed using R 4.0.3 software. We utilized the "TwoSampleMR" package to perform the IVW, WME, and MR-Egger regression method. We used the "MRPRESSO" package for MR-PRESSO detection.

Results

The Selection of Instrumental Variables

We found that, following a series of quality control steps, UF were associated with 47 SNPs from 5 bacterial genera. EC had 47 SNPs associated with 4 bacterial genera. OC showed an association with 55 SNPs from 5 bacterial genera, while CC had an association with 36 SNPs from 3 bacterial genera (Supplementary Table S1). The $F$ statistics for the IVs significantly correlated with gut microbiota ranged from 18.403 to 73.397, all exceeding 10. This suggests that the estimates are unlikely to be affected by weak instrument bias.

Causal Effects of Gut Microbiota on gynecological tumor

UF

The results of genetic prediction revealed that the risk of UF was positively associated with an increased relative abundance of Bacteroides (OR: 1.178, 95% CI: 1.003–1.383, $P = 0.046$) and Turicibacter (OR: 1.129, 95% CI: 1.012–1.259, $P = 0.029$). In contrast, increased genetic predicted abundance of Enterorhabdus (OR: 0.808, 95% CI: 0.688–0.948, $P = 0.009$), Lachnospiraceae(OR: 0.882, 95% CI: 0.793–0.982, $P = 0.022$), and Oscillospira (OR: 0.874, 95% CI: 0.774–0.988, $P = 0.031$) exhibited a protective effect against UF.

EC
The relative abundance of *Butyrivibrio* (OR: 1.083, 95% CI: 1.009–1.163, *P* = 0.022) was significantly increased and positively associated with the risk of EC. Conversely, higher genetic predicted abundances of *Dorea* (OR: 0.796, 95% CI: 0.657–0.964, *P* = 0.020), *Ruminococcaceae UCG014* (OR: 0.820, 95% CI: 0.686–0.979, *P* = 0.028), and *Turicibacter* (OR: 0.843, 95% CI: 0.735–0.966, *P* = 0.014) were negatively correlated with the risk of EC.

**OC**

The genetic prediction results indicated that higher abundances of *Barnesiella* (OR: 1.395, 95% CI: 1.041–1.869, *P* = 0.026), *Butyrivibrio* (OR: 1.219, 95% CI: 1.048–1.418, *P* = 0.010), and *Lachnospiraceae* (OR: 1.329, 95% CI: 1.019–1.732, *P* = 0.036) were associated with an increased risk of OC. Additionally, higher genetic predicted levels of *Coprobacter* (OR: 0.773, 95% CI: 0.616–0.970, *P* = 0.026) and *Ruminococcaceae UCG010* (OR: 0.644, 95% CI: 0.431–0.962, *P* = 0.032) were associated with a decreased risk of OC.

**CC**

The risk of CC was negatively correlated with the levels of *Roseburia* (OR: 0.998, 95% CI: 0.996–1.000, *P* = 0.038). However, two other genera, namely *Lachnospiraceae* (OR: 1.002, 95% CI: 1.000–1.004, *P* = 0.021) and *Ruminococcaceae UCG003* (OR: 1.002, 95% CI: 1.001–1.004, *P* = 0.009), were positively associated with the risk of CC ([Supplementary Table S2](#), Table 2).

Further validation of gut microbiota associated with the risk of gynecologic tumors in the discovery cohort was conducted. As shown in Table 3, the causal effect between *Lachnospiraceae* and both UF and OC was consistent with the findings of the discovery cohort, thereby enhancing the credibility of the true causal association ([Supplementary Table S3](#)).

**Sensitivity Analyses**

There is no evidence of heterogeneity among genetic SNPs in *Lachnospiraceae* ([Supplementary Table S4](#)). Neither the MR-Egger test nor the MR-PROSSO test provide evidence for horizontal pleiotropy among SNPs (*P* > 0.05) ([Supplementary Table S5 and S6](#)). Furthermore, the leave-out-analysis demonstrates that the causal association between *Lachnospiraceae* and both UF and OC is not driven by any single SNP ([Supplementary Table S7](#)). The reverse MR analysis shows no evidence for a causal association between both UF and OC and *Lachnospiraceae* (Table 4). Details of the IVs used in the reverse MR analysis are available in [Supplementary Table S8](#).
Table 2
Significant MR analysis results in the discovery samples.

<table>
<thead>
<tr>
<th>Gynecological cancer(outcome)</th>
<th>Bacterial taxa(exposure)</th>
<th>N.SNP</th>
<th>OR</th>
<th>95%CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>UF</td>
<td><em>Bacteroides</em></td>
<td>9</td>
<td>1.178</td>
<td>1.003–1.383</td>
<td>0.046</td>
</tr>
<tr>
<td></td>
<td><em>Enterorhabdus</em></td>
<td>6</td>
<td>0.808</td>
<td>0.688–0.948</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td><em>Lachnospiraceae</em></td>
<td>13</td>
<td>0.882</td>
<td>0.793–0.982</td>
<td>0.022</td>
</tr>
<tr>
<td></td>
<td><em>Oscillospira</em></td>
<td>9</td>
<td>0.874</td>
<td>0.774–0.988</td>
<td>0.031</td>
</tr>
<tr>
<td></td>
<td><em>Turicibacter</em></td>
<td>10</td>
<td>1.129</td>
<td>1.012–1.259</td>
<td>0.029</td>
</tr>
<tr>
<td>EC</td>
<td><em>Butyrivibrio</em></td>
<td>15</td>
<td>1.083</td>
<td>1.009–1.163</td>
<td>0.028</td>
</tr>
<tr>
<td></td>
<td><em>Dorea</em></td>
<td>11</td>
<td>0.796</td>
<td>0.657–0.964</td>
<td>0.020</td>
</tr>
<tr>
<td></td>
<td><em>RuminococcaceaeUCG014</em></td>
<td>11</td>
<td>0.820</td>
<td>0.686–0.979</td>
<td>0.028</td>
</tr>
<tr>
<td></td>
<td><em>Turicibacter</em></td>
<td>10</td>
<td>0.843</td>
<td>0.735–0.966</td>
<td>0.014</td>
</tr>
<tr>
<td>OC</td>
<td><em>Barnesiella</em></td>
<td>13</td>
<td>1.395</td>
<td>1.041–1.869</td>
<td>0.026</td>
</tr>
<tr>
<td></td>
<td><em>Butyrivibrio</em></td>
<td>15</td>
<td>1.219</td>
<td>1.048–1.418</td>
<td>0.010</td>
</tr>
<tr>
<td></td>
<td><em>Coprobacter</em></td>
<td>10</td>
<td>0.773</td>
<td>0.616–0.970</td>
<td>0.026</td>
</tr>
<tr>
<td></td>
<td><em>Lachnospiraceae</em></td>
<td>11</td>
<td>1.329</td>
<td>1.019–1.732</td>
<td>0.036</td>
</tr>
<tr>
<td></td>
<td><em>RuminococcaceaeUCG010</em></td>
<td>6</td>
<td>0.644</td>
<td>0.431–0.962</td>
<td>0.032</td>
</tr>
<tr>
<td>CC</td>
<td><em>Lachnospiraceae</em></td>
<td>9</td>
<td>1.002</td>
<td>1.000–1.004</td>
<td>0.021</td>
</tr>
<tr>
<td></td>
<td><em>Roseburia</em></td>
<td>14</td>
<td>0.998</td>
<td>0.996–1.000</td>
<td>0.038</td>
</tr>
<tr>
<td></td>
<td><em>RuminococcaceaeUCG003</em></td>
<td>13</td>
<td>1.002</td>
<td>1.001–1.004</td>
<td>0.009</td>
</tr>
</tbody>
</table>

N.SNP is the number of SNPs being used as IVs.
Table 3
Results of the identified bacterial taxa in the replication samples.

<table>
<thead>
<tr>
<th>Gynecological cancer(outcome)</th>
<th>Bacterial taxa(exposure)</th>
<th>N.SNP</th>
<th>OR</th>
<th>95%CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>fibrosis</td>
<td><em>Bacteroides</em></td>
<td>6</td>
<td>0.937</td>
<td>0.630–1.394</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td><em>Enterorhhabdus</em></td>
<td>5</td>
<td>0.987</td>
<td>0.822–1.185</td>
<td>0.89</td>
</tr>
<tr>
<td></td>
<td><em>Lachnospiraceae</em></td>
<td>11</td>
<td>0.793</td>
<td>0.679–0.924</td>
<td><strong>0.003</strong></td>
</tr>
<tr>
<td></td>
<td><em>Oscillospira</em></td>
<td>6</td>
<td>0.950</td>
<td>0.707–1.276</td>
<td>0.730</td>
</tr>
<tr>
<td></td>
<td><em>Turicibacter</em></td>
<td>7</td>
<td>1.071</td>
<td>0.871–1.318</td>
<td>0.520</td>
</tr>
<tr>
<td>EC</td>
<td><em>Butyrivibrio</em></td>
<td>6</td>
<td>1.000</td>
<td>0.999–1.001</td>
<td>0.540</td>
</tr>
<tr>
<td></td>
<td><em>Dorea</em></td>
<td>3</td>
<td>1.000</td>
<td>0.998–1.003</td>
<td>0.610</td>
</tr>
<tr>
<td></td>
<td><em>Turicibacter</em></td>
<td>10</td>
<td>0.843</td>
<td>0.735–0.966</td>
<td>0.014</td>
</tr>
<tr>
<td>OC</td>
<td><em>Barnesiella</em></td>
<td>14</td>
<td>1.002</td>
<td>1.000–1.004</td>
<td>0.070</td>
</tr>
<tr>
<td></td>
<td><em>Butyrivibrio</em></td>
<td>15</td>
<td>1.000</td>
<td>0.999–1.002</td>
<td>0.630</td>
</tr>
<tr>
<td></td>
<td><em>Coprobacter</em></td>
<td>11</td>
<td>1.001</td>
<td>0.999–1.003</td>
<td>0.290</td>
</tr>
<tr>
<td></td>
<td><em>Lachnospiraceae</em></td>
<td>14</td>
<td>1.003</td>
<td>1.000–1.006</td>
<td><strong>0.030</strong></td>
</tr>
<tr>
<td></td>
<td><em>RuminococcaceaeUCG010</em></td>
<td>5</td>
<td>1.000</td>
<td>0.996–1.004</td>
<td>0.970</td>
</tr>
<tr>
<td>CC</td>
<td><em>Lachnospiraceae</em></td>
<td>4</td>
<td>1.001</td>
<td>0.998–1.003</td>
<td>0.52</td>
</tr>
<tr>
<td></td>
<td><em>Roseburia</em></td>
<td>5</td>
<td>0.999</td>
<td>0.997–1.002</td>
<td>0.600</td>
</tr>
<tr>
<td></td>
<td><em>RuminococcaceaeUCG005</em></td>
<td>4</td>
<td>0.997</td>
<td>0.994–1.000</td>
<td><strong>0.045</strong></td>
</tr>
</tbody>
</table>

N.SNP is the number of SNPs being used as IVs.

MR, Mendelian randomization; SNP, single-nucleotide polymorphism; IVW, inverse-variance weighted; OR, odds ratio; CI, confidence interval; UF, uterine fibroids; EC, endometrial cancer; OC, ovarian cancer; CC, cervical cancer.
Table 4

Reverse causal association between gynecological tumors and gut microbiota.

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Outcome</th>
<th>N.SNP</th>
<th>OR</th>
<th>95%CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>UF</td>
<td><em>Lachnospiraceae</em></td>
<td>125</td>
<td>0.989</td>
<td>0.955–1.025</td>
<td>0.018</td>
</tr>
<tr>
<td>OC</td>
<td><em>Lachnospiraceae</em></td>
<td>21</td>
<td>0.973</td>
<td>0.934–1.013</td>
<td>0.185</td>
</tr>
</tbody>
</table>

N.SNP is the number of SNPs being used as IVs.

MR, Mendelian randomization; SNP, single-nucleotide polymorphism; IVW, inverse-variance weighted; OR, odds ratio; CI, confidence interval; UF, uterine fibroids; CC, cervical cancer.

**Discussion**

To investigate the causal relationship between the gut microbiota and four common gynecologic tumors (UF, EC, OC, and CC), we performed a two sample MR analysis. Combining the findings from the discovery and validation cohorts, we confirmed a significant association between the *Lachnospiraceae* and both UF and OC. In particular, we observed a negative correlation between *Lachnospiraceae* and UF risk, as well as a positive correlation with OC risk.

*Phyla Firmicutes* dominate the gut microbiota of healthy individuals, and *Lachnospiraceae* is a family of anaerobic bacteria belonging to the *Firmicutes phylum*. *Lachnospiraceae* has the potential to promote human health by converting primary bile acids into secondary bile acids and producing short-chain fatty acids (SCFAs) such as acetic acid and butyric acid [24]. A study conducted on melanoma patients receiving anti-PD-1 immunotherapy found that a high abundance of *Lachnospiraceae* was associated with enhanced systemic immune response and anti-tumor treatment response [26]. In colorectal cancer patients, the relative abundance of *Lachnospiraceae* is lower compared to control groups, suggesting *Lachnospiraceae* may reduce the risk of colorectal cancer and have potential implications for its prevention and control [27].

Research on gynecological diseases has shown significantly reduced relative abundance of *Lachnospiraceae* in patients with polycystic ovary syndrome (PCOS) [28]. Siddiqui et al. found a decreased quantity of *Lachnospiraceae* in CC patients [29], while another study demonstrated a positive correlation between *Lachnospiraceae* and persistent HPV infection in CC, and a negative correlation with HPV clearance [30]. *Lachnospiraceae* is also associated with the risk of BC, as BC patients have lower relative abundance of *Lachnospiraceae* compared to the control group [31]. However, there is currently limited research on the gut microbiota in relation to UF and OC, and no studies have indicated an association between *Lachnospiraceae* and these gynecological tumors.

Research suggests that dysbiosis of the gut microbiota may be associated with an increased risk of gynecological tumors. In particular, the presence and abundance of *Lachnospiraceae* are related to estrogen and its metabolites. They regulate β-glucuronidase, which is involved in endogenous estrogen metabolism, leading to estrogen deconjugation and reabsorption into the bloodstream, thereby affecting estrogen concentration and activity [32]. There is a significant positive correlation between *Lachnospiraceae* and circulating estrogen levels [33]. The risk of UF in women is related to estrogen levels as estrogen promotes...
the proliferation of uterine smooth muscle cells, thereby facilitating the growth of UF. On the other hand, the host's inflammatory status may be influenced by the gut microbiota and its metabolites. A study reported an association between the occurrence of UF and persistent inflammation and immune response [34]. Inflammatory mediators such as interleukin (IL)-1, IL-4, and tumor necrosis factor (TNF), as well as immune cells like CD4 + CD8 + T cells, regulatory T cells (Treg, CD4+), and follicular helper T cells (Tfh), are significantly elevated in patients with UF [35]. Short-chain fatty acids produced by Lachnospiraceae can exert anti-inflammatory and immune-modulatory effects through their interaction with the immune system and improvement of intestinal barrier integrity [36]. Our study results indicate that Lachnospiraceae reduces the risk of UF, suggesting Lachnospiraceae may primarily exert a protective role in UF development by influencing inflammation and immune response.

As mentioned, Lachnospiraceae can influence circulating estrogen levels, and the development of OC is also associated with abnormalities in estrogen synthesis and metabolism. A meta-analysis revealed the risk of OC is higher in individuals using hormone replacement therapy (HRT) compared to those who do not use HRT, which aligns with our research findings suggesting that Lachnospiraceae may increase the risk of OC by elevating estrogen levels. However, another study indicated that in terms of metabolomics, SCFAs produced by Lachnospiraceae exert anti-tumor effects in the progression of OC. Specifically, butyric acid can inhibit histone deacetylase (HDAC) in OC cells, leading to the transition of tumor cells from the S phase to the G0/G1 and/or G2/M phases, thereby increasing tumor cell apoptosis[37]. While Lachnospiraceae may play a role in the development of UF and OC, further research is needed to validate the specific mechanisms of Lachnospiraceae in these two types of tumors. This will contribute to a better understanding of the mechanisms underlying gynecological tumor development and provide more targeted strategies for the prevention, detection, and treatment of UF and OC.

This study has several advantages: Firstly, we conducted a comprehensive investigation of four common gynecological tumors. Secondly, we not only established causal relationships in the discovery cohort but also validated them in an independent validation cohort, enhancing the credibility of the identified causal associations. Thirdly, our MR analysis has identified valuable candidate microbial taxa for subsequent functional research, which can contribute to the development of novel approaches targeting specific gut microbiota for the prevention and treatment of gynecological tumors.

However, our study has some limitations. Firstly, our research primarily utilized GWAS summary data from European populations, with a small portion of gut microbiota data from other ethnic groups. The variability in data sources may impact the accuracy of our results. Secondly, our bacterial classification was analyzed only at the genus level. Thirdly, the development of gynecological tumors is the result of multifactorial interactions, with the composition of gut microbiota being influenced by both genetic and environmental factors. Therefore, we cannot exclude the potential influence of interactions between diet and genes, or genes and the environment, on the outcomes.

Our study has laid the theoretical foundation for exploring the role of gut microbiota in the occurrence and treatment of gynecological tumors, particularly UF and OC. However, further research is still needed to gain a deeper understanding of the relationship between gut microbiota and gynecological tumors. This includes
expanding the sample size, conducting human cohort studies, and undertaking functional research to provide more precise scientific evidence for the prevention and treatment of gynecological tumors.

Declarations

Ethics approval and consent to participate

Ethical approval was waived because this study used the data from publicly available databases.

Consent for publication

Not application.

Availability of data and materials


Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

YJX, XNZ and XYN have made contributions to the design of the work, the acquisition, analysis and interpretation of data, the drafting and revising of the article. LZ and JLJ have made contributions to the design, drafting, and revising of the article. AGX have made contributions to the design of the work, the acquisition of funding, the administration of the project and the drafting and revising of the article. All authors read and approved the final manuscript.

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References


**Figures**
Figure 1. The flowchart of the study. The whole workflow of MR analysis. MR, Mendelian randomization; UF, uterine fibroids; EC, endometrial cancer; OC, ovarian cancer; CC, cervical cancer.

See image above for figure legend

**Supplementary Files**

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- SupplementaryS1S8.xlsx