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Causal relationships of gut microbiota and blood metabolites with ovarian cancer and endometrial cancer: a Mendelian randomization study

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Abstract

Objectives The study aimed to investigate the causal relationships of gut microbiota (GM), ovarian cancer (OC), endometrial cancer (EC), and potential metabolite mediators using Mendelian randomization (MR) analysis.

Methods Bidirectional two-sample MR analysis and reverse MR analysis of GM on OC/EC were employed to determine the causal effects of GM on OC/EC and the mediating role of blood metabolites in the relationship between GM and OC/EC, with results validated through sensitivity analysis.

Results We identified 6 pathogenic bacterial taxa associated with OC, including *Euryarchaeota*, *Escherichia-Shigella*, *FamilyXIIIAD3011group*, *Prevotella9*, and two unknown genera. *Christensenellaceae R.7group*, *Tyzzerella3*, and *Victivallaceae* were found to be protective against OC. The increase in EC risk was positively associated with *Erysipelotrichia*, *Erysipelotrichaceae*, *Erysipelotrichales*, and *FamilyXI*. *Dorea*, *RuminococcaceaeUCG014*, and *Turicibacter* exhibited a negative correlation with the EC risk. A total of 26 and 19 blood metabolites related to GM were identified, showing significant correlations with OC and EC, respectively. Cytosine was found to be an intermediate metabolite greatly associated with EC and *FamilyXI*. In reverse MR analysis, the *FamilyXIIIAD3011group* exhibited a significant bidirectional causal relationship with OC.

Conclusion Our study revealed causal relationships of GM and intermediate metabolites with OC/EC, providing new avenues for understanding OC/EC and developing effective treatment strategies.

Keywords Mendelian randomization, Metabolites, Gut microbiota, Ovarian cancer, Endometrial cancer

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Introduction

Ovarian cancer (OC) and endometrial cancer (EC) are two common gynecologic malignancies affecting women globally [1, 2]. The International Agency for Research on Cancer released global cancer statistics for 2022, showing that there were 9.7 million cancer-related deaths and close to 20 million new cancer cases worldwide, with OC and EC accounting for 3.7% and 3.1% of all new cases and deaths [3]. OC is the most deadly malignant tumor in gynecologic cancers globally, and its incidence has been continuously increasing in recent years [4, 5]. Due to atypical early clinical manifestations, early diagnosis of OC is very difficult, with nearly 80% of patients being diagnosed at an advanced stage and a 5-year survival rate of lower than 40% [6]. In 2022, there were 420,242 new diagnoses of EC globally [3]. Like most cancers, the recent incidence of EC has uplifted, especially among young women [7–9]. Although the surgical treatment of EC has been improved and most patients can achieve a relatively good clinical outcome after treatment, about 14% of EC cases may recur, with a higher proportion in advanced-stage patients, severely affecting patients' quality of life and clinical outcomes [10, 11].

The gut microbiota (GM) is a multifaceted and dynamic entity that evolves with the host and constantly changes throughout our lives [12]. Currently, more than 22 million genes are identified from the GM, and certain microbial subgroups can be impactful on host physiology through direct cell-to-cell interactions and indirect regulation of their metabolites [13, 14]. Dysbiosis of GM can lead to increased intestinal permeability, allowing bacterial metabolites such as lipopolysaccharides to enter circulation. These metabolites act in the bloodstream and have systemic effects on humans, increasing inflammation, immune imbalance, DNA damage, abnormal estrogen levels, and ultimately resulting in carcinogenesis [15–17]. Previous studies using animal models have demonstrated that GM is implicated in tumor growth through multiple signaling pathways of metabolites [18–20]. Nandi et al. [21]. pointed out that dysbiosis of GM is a major factor in the occurrence, metastasis, and growth of breast cancer (BC). Wang et al. [22]. observed that the phylum Proteobacteria and the genus Parabacteroides may be potential biomarkers for cervical cancer. However, there is currently inadequate evidence from observational studies to establish a causal connection between GM and metabolite changes and the risk of cancer. Although randomized controlled trials (RCTs) are the gold standard for uncovering causal relationships, we cannot conclude the latent causal relationship between GM and related metabolites in OC/EC from RCTs due to the long latency period of some microbiotas from the exposures to tumor formation [23]. Therefore, innovating a new approach to measure the causal impact of GM on the risk of OC/EC is of urgent need.

Mendelian randomization (MR) is an analytical method commonly applied for causal inference, which uses genetic variants as instrumental variables (IVs) to mimic RCTs, thus enabling causal inference between risk factors and diseases [24]. The advantage of MR lies in its ability to effectively avoid the influence of common confounding factors and reverse causality in traditional bioinformatics analysis. Compared with traditional bioinformatics methods, genetic variations in MR are regarded as IVs, which are randomly assigned during fertilization and therefore not affected by environmental factors and disease progression [25]. In addition, MR methods do not require expensive and time-consuming RCTs but can infer causal relationships from observational data. These characteristics make MR an ideal tool for studying the causal effects of GM on OC/EC risk.

In this research, we intended to dig out the causal relationships of GM and blood metabolites with OC/EC by utilizing data from Genome-wide association studies (GWAS) as well as the method of two-sample MR. Furthermore, we intended to investigate the mediating effects of blood metabolites on OC and EC through a two-step MR analysis, shedding new insights for OC/EC early diagnosis and treatment.

Methods

Research design

In the present work, we applied the two-sample MR method to elucidate the causal relationships of GM and blood metabolites with the risk of OC/EC. In this MR study, we utilized 211 GMs as exposure variables and OC/EC as outcome variables. To figure out whether this causal connection can be modulated by metabolites, we designed a mediation analysis. In reverse analysis, OC/EC was selected as the exposure variable, while 211 GMs were as outcome variables. To meet the requirements of the MR method, independent genetic variations were used as IVs, which were required to comply with three key assumptions [26]: (1) IVs must be strongly associated with the exposure; (2) IVs cannot be related to confounders; (3) IVs only influence the outcome through the exposure variables. We extracted genetic data related to GM, blood metabolites, and OC/EC from separate GWAS datasets to eliminate the issue of sample overlap. Figure 1 outlines the comprehensive overview of this MR study.

Data source

GM data

The large-scale GWAS data on GM that we included was from the Mibiogen Consortium, including 18,340 individuals from 24 cohorts [27]. By utilizing three different regions of the 16SrRNA gene, we analyzed the

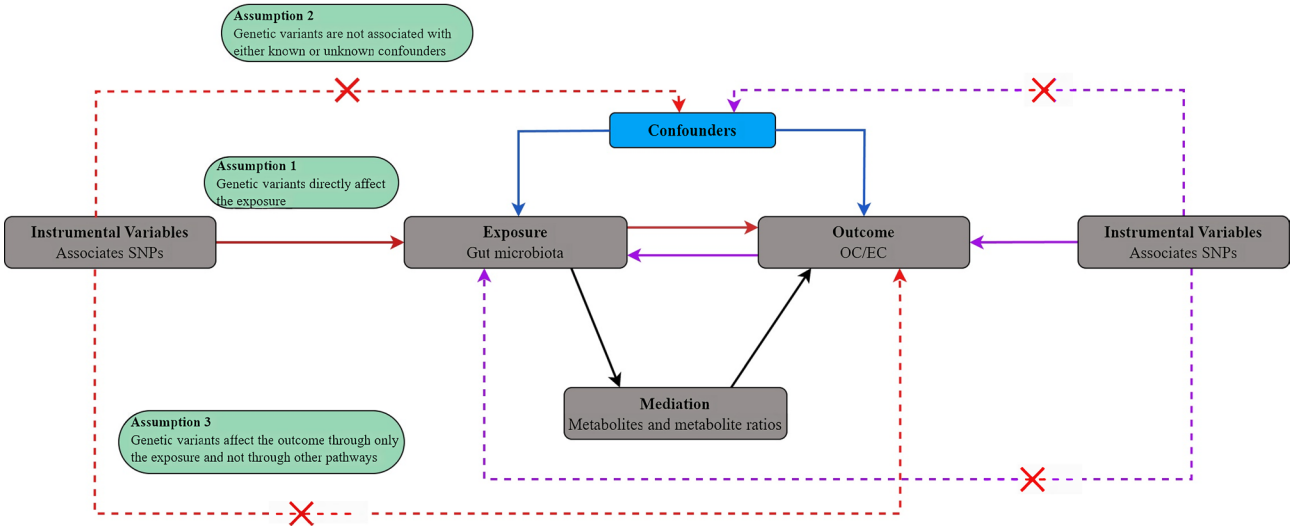

Fig. 1 Flow chart of the MR study

Table 1 Detailed information on the GWAS in our analysis

Disease	Year	ID	Population	Sample size	Control	Case
Gut Microbiota	2021	/	European	18,340	NA	NA
Ovarian cancer	2017	ieu-a-1120	European	66,450	40,941	25,509
Endometrial cancer	2018	ebi-a-GCST006464	European	121,885	108,979	12,906
Metabolites and metabolite ratios	2023	GCST90199621–GCST90201020	European	8299	NA	NA

composition of GM and identified genetic variants influencing the relative abundance of microbial taxa by applying microbial quantitative trait loci (mbQTL) mapping [27]. 211 units (9 phyla, 131 genera, 20 orders, 35 families, and 16 classes) were defined [28].

OC and EC data

We included GWAS data of 25,509 OC patients and 40,941 controls from the OC Association Consortium [29]. The GWAS data for EC came from 12,906 EC cases and 108,979 controls provided by O'Mara et al. [30]. (including 5624 new genotype data) [31].

Metabolism data

The blood metabolites and metabolite ratios included in this project were from 8,299 individuals in the Canadian Longitudinal Study on Aging cohort (1,091 metabolites and 309 metabolite ratios). We utilized European GWAS data from the GWAS Catalog: GCST90199621–GCST90201020 [32, 33]. Table S1 displays the IDs corresponding to the metabolite features of 1400 metabolites and metabolite ratios.

The information on GWAS is outlined in Table 1. All data were publicly available in the original studies, and each study within each GWAS obtained approval from the relevant institutional review board and informed consent from participants or caregivers, legal guardians, or other authorized representatives.

Selection of IVs

The criteria for selection of IVs were as follows: (1) We included whole genomic significant single nucleotide polymorphisms (SNPs) ($P < 5e-8$). If no whole genomic significant SNPs were available as IVs, SNPs with $P < 1e-5$ were utilized as candidate IVs (211 GMs and 1400 metabolite or metabolite ratio-associated SNPs with $P < 1e-5$ were considered as potential eligible IVs); (2) SNPs were subjected to clumping to exclude linkage disequilibrium ($r^2 = 0.001$, region length = 10000 kb); (3) A larger F-statistic implied stronger instrument strength and was employed to assess for weak IVs, which were excluded by calculating the F-statistic. All included SNPs had F-statistics greater than 10.

MR analysis and mediation MR analysis

We utilized five regression models for the two-sample MR analysis (Inverse variance weighted (IVW), MR-Egger regression, Weighted mode, Weighted median estimator, and Simple model). The SNPs were utilized as IVs. Given the limited number of meaningful loci of GM, a more lenient significance threshold ($P < 1e-05$) was applied to analyze exposure variables (GM) and outcome variables (OC and EC). The IVW method could directly examine causal effect values using combined data rather than individual-level data. The MR-Egger regression calculated the correlation of each SNP with OC and EC (Y) as well as the correlation of each SNP with metabolites

and metabolite ratios (X) and fitted a linear function. The weighted median estimation method calculated the causal impact estimate of the exposure-outcome for the No. j SNP (βj). In the second stage, we evaluated whether OC and EC as exposure variables causally affected GM, and employed the same workflow for MR analysis, with the significance threshold of exposed SNPs for IVs set at $P < 5e\text{-}08$.

We further launched a two-step MR analysis for mediation analysis to probe into whether metabolites mediate the causal pathways from GM to OC and EC outcomes. The overall effect was decomposed into indirect effects (through the mediator) and direct effects (without the mediator). The total effect of GM on OC and EC was decomposed into (1) the direct effects of GM on OC and EC and (2) the indirect effects of GM mediated by metabolites. By dividing the indirect impact by the total effect, we were able to determine the proportion of the mediated effect. Moreover, we calculated the 95% confidence interval (CI) by utilizing the delta method.

Sensitivity analysis

A sensitivity analysis was designed to make sure that the results were robust. Three methods were employed: leave-one-out method, horizontal pleiotropy test, and heterogeneity test. The heterogeneity of SNPs was examined by Cochran's Q test. The random-effects IVW model was applied when heterogeneity ($P < 0.05$) existed; otherwise, the fixed-effects IVW model was utilized. MR-PRESSO and MR-Egger regression were employed to determine the horizontal pleiotropy of IVs. When the intercept term of MR-Egger was statistically significant, the presence of horizontal pleiotropy was indicated. Additionally, we carried out the global test of MR-PRESSO to figure out if there was pleiotropy in this project. The sensitivity analysis was conducted by employing a "Leave-one-out" test to sequentially remove each SNP to assess the effects of individual SNP on the causal outcome.

Statistical analysis

Two-sample MR analysis was performed by utilizing R (version 4.3.1) software and the R package *Two Sample MR*. The causal relationship between exposure and outcome was assessed by the random-effect IVW analysis. MR-Egger regression, Simple mode, Weighted mode, and Weighted median were employed as auxiliary analytic methods. In MR analysis, $P < 0.05$ indicated a significant causal relationship between exposure and outcome.

Results

MR results

The results of the IVW model manifested that 9 of the 211 GMs were significantly causally related to OC, of which 2 were unknown genera. 7 GMs were significantly causally related to EC (Table 2). Specifically, at the phylum level, *Euryarchaeota* (OR = 0.914, 95%CI:0.852–0.980, $P = 0.012$) had a protective effect on OC. At the genus level, a total of 5 GMs were found to be protective factors for OC, including *Escherichia-Shigella* (OR: 0.875, 95%CI: 0.781–0.981, $P = 0.022$), *FamilyXIIIAD3011 group* (OR: 0.857, 95%CI: 0.755–0.974, $P = 0.018$), *Prevotella9* (OR: 0.885, 95% CI: 0.806–0.972, $P = 0.011$) and two unknown genera (OR < 1, $P < 0.05$). However, *ChristensenellaceaeR.7group* (OR: 1.256, 95%CI: 1.040–1.518, $P = 0.018$) and *Tyzzerella3* (OR: 1.084, 95% CI: 1.001–1.174, $P = 0.047$) were likely to elevate the risk of OC. In addition, at the family level, *Victivallaceae* (OR = 1.098, 95%CI: 1.023–1.177, $P = 0.009$) may be linked with a higher risk of OC.

Table 2 Results of causal association of IVW MR regression

Exposure	Outcome	Method	SNPs	Beta	SE	*P*-Value	OR (95%CI)
family.Victivallaceae.id.2255	OC	IVW	12	0.093	0.036	0.009	1.098 (1.023–1.177)
genus.ChristensenellaceaeR.7group.id.11,283	OC	IVW	11	0.228	0.096	0.018	1.256 (1.040–1.518)
genus.Escherichia.Shigella.id.3504	OC	IVW	15	-0.133	0.058	0.022	0.875 (0.781–0.981)
genus.FamilyXIIIAD3011group.id.11,293	OC	IVW	14	-0.154	0.065	0.018	0.857 (0.755–0.974)
genus.Prevotella9.id.11,183	OC	IVW	17	-0.122	0.048	0.011	0.885 (0.806–0.972)
genus.Tyzzerella3.id.11,335	OC	IVW	13	0.081	0.041	0.047	1.084 (1.001–1.174)
genus.unknowngenus.id.2041	OC	IVW	10	-0.111	0.056	0.048	0.895 (0.802–0.999)
genus.unknowngenus.id.2071	OC	IVW	17	-0.125	0.059	0.034	0.883 (0.786–0.991)
phylum.Euryarchaeota.id.55	OC	IVW	13	-0.090	0.036	0.012	0.914 (0.852–0.980)
class.Erysipelotrichia.id.2147	EC	IVW	13	0.202	0.089	0.024	1.224 (1.027–1.459)
family.Erysipelotrichaceae.id.2149	EC	IVW	13	0.202	0.089	0.024	1.224 (1.027–1.459)
family.FamilyXI.id.1936	EC	IVW	10	0.086	0.042	0.039	1.090 (1.004–1.182)
genus.Dorea.id.1997	EC	IVW	13	-0.205	0.087	0.018	0.810 (0.687–0.965)
genus.RuminococcaceaeUCG014.id.11,371	EC	IVW	18	-0.141	0.066	0.032	0.869 (0.763–0.988)
genus.Turicibacter.id.2162	EC	IVW	14	-0.121	0.059	0.042	0.886 (0.789–0.996)
order.Erysipelotrichales.id.2148	EC	IVW	13	0.202	0.089	0.024	1.224 (1.027–1.459)

In terms of EC, *Erysipelotrichia* (OR: 1.224, 95%CI:1.027–1.459, $P = 0.024$), *Erysipelotrichaceae* (OR: 1.224, 95%CI: 1.027–1.459, $P = 0.024$), *FamilyXI* (OR: 1.090, 95% CI: 1.004–1.182, $P = 0.039$) and *Erysipelotrichales* (OR: 1.224, 95% CI: 1.027–1.459, $P = 0.024$) were linked with an elevated risk of EC at the level of class, family, and order. At the genus level, *Dorea* (OR: 0.810, 95% CI: 0.687–0.965, $P = 0.018$), *RuminococcaceaeUCG014* (OR: 0.869, 95% CI: 0.763–0.988, $P = 0.032$), *Turicibacter* (OR: 0.886, 95% CI: 0.789-996, $P = 0.042$) were negatively linked with EC risk (Table 2).

The other four MR methods, MR-Egger regression, simple model, weighted model, and weighted median for causal analyses as well as MR forest plot are shown in Table S2 and Figure S1.

Sensitivity test

To ensure the reliability and robustness of the results, we conducted sensitivity analyses. To eliminate potential bias in IVs, we carried out the heterogeneity test and horizontal pleiotropy test in the MR study. In sensitivity analysis, the IVW method and MR-Egger method did not detect heterogeneity between IVs ($P > 0.05$) (Table 3). Furthermore, for the test of pleiotropy, both MR-PRESSO analysis and MR-Egger regression showed intercept P values > 0.05, implying no evidence of pleiotropy among the included SNPs. Scatter plots and funnel plots manifested that the distribution of all included SNPs was approximately symmetrical, implying that causal associations were unlikely to be influenced by potential bias (Figure S2-3).

According to the leave-one-out sensitivity analysis, the results with the remaining SNPs were similar to those including all SNPs after sequentially removing each GM SNP, with no SNP exerting a substantial influence on the estimated causal association values, exhibiting the robustness of the MR results in this work (Figure S4).

Mediation MR results

Preliminary screening of blood metabolites

To examine the function of metabolites in the exposure variables and outcome, we launched a preliminary screening of metabolites. The IVW model results in Table S3 indicated significant causal relationships of 9 GMs and 511 metabolites with OC, including 2 unknown genera. The IVW model results in Table S4 manifested significant causal relationships of 7 GMs and 198 metabolites with EC.

The IVW model results in Table S5 indicated 75 out of 1400 metabolites or metabolic ratios having significant causal relationships with OC, while the IVW model results in Table S6 indicated 105 metabolites or metabolic ratios having significant causal relationships with EC.

Preliminary IVW analysis uncovered that 26 metabolites possessed causal relationships with OC and 9 related GMs. 19 metabolites had causal relationships with EC and 7 relevant GMs (Fig. 2).

Associations between GM and blood metabolites related to OC/EC

We further included 9 OC-related GMs as exposure variables and 26 metabolites as outcomes. The IVW model results in Fig. 3 indicated significant causal relationships ($P < 0.05$) between 9 OC-related GMs and 26 metabolites. Neither heterogeneity nor horizontal pleiotropy was detected in the MR analysis (Table S7).

We included 7 EC-related GMs as exposure variables and 19 metabolites as outcomes. The results of the IVW model in Fig. 4 suggested a significant causal relationship between the 7 EC-related GMs and 19 metabolites ($P < 0.05$). Similar to OC, in the MR analysis, there was neither horizontal pleiotropy nor heterogeneity in the SNPs of EC, indicating that the causal association between the two was unlikely to be impacted by potential bias (Table S8).

Association of blood metabolites with OC/EC

In the analysis of the causal relationship between GM and OC, we launched IVW analysis on 26 specific metabolites. The IVW model results in Fig. 5 manifested a significant causal relationship between the 26 metabolites and OC ($P < 0.05$). MR analysis results demonstrated that, except for GCST90199945 ($P = 0.012$), the other metabolites did not exhibit horizontal pleiotropy ($P > 0.05$). However, results from Cochran's Q test and MR-Egger regression suggested that GCST90200424 metabolite had P values less than 0.05, implying potential bias or heterogeneity association between SNPs (Table S9).

The causal relationship between EC and 19 metabolites was analyzed (Fig. 6). Results from the IVW model uncovered a significant causal relationship between the 19 metabolites and EC ($P < 0.05$). In MR analysis, except for GCST90199835 ($P < 0.001$)and GCST90199855 ($P = 0.045$), we observed no horizontal pleiotropy in other results ($P > 0.05$). GCST90199842 and GCST90200097 had $P < 0.05$ in Cochran's Q test and MR-Egger regression, implying the presence of heterogeneity between SNPs (Table S10).

The heterogeneity in our analysis might be induced by differences in data from different analysis platforms, different experiments, or different populations. However, since the IVW default method was a random effects model, the presence of heterogeneity exerted no influence on the interpretation of the results. The results of the "Leave-one-out" sensitive analysis can be found in Figure S5-6.

Table 3 Tests for heterogeneity and Pleiotropy

Exposure ID	Outcome	Heterogeneity				Pleiotropy			
		MR Egger		Inverse variance weighted		Egger intercept	SE	Pval	MR-PRESSO Global
		Statistics Q	P-Value	Statistics Q	P-Value				Pval
family.Victivallaceae.id.2255	OC	6.269	0.792	7.482	0.759	0.030	0.028	0.297	0.773
genus.ChristensenellaceaeR.7group.id.11,283	OC	11.822	0.224	14.591	0.148	0.029	0.020	0.181	0.174
genus.Escherichia.Shigella.id.3504	OC	7.828	0.855	8.116	0.883	-0.008	0.014	0.600	0.868
genus.FamilyXIIIAD3011group.id.11,293	OC	9.115	0.693	9.668	0.721	0.020	0.026	0.471	0.758
genus.Prevotella9.id.11,183	OC	11.305	0.731	13.832	0.611	-0.019	0.012	0.133	0.645
genus.Tyzzerella3.id.11,335	OC	7.582	0.750	7.633	0.813	0.007	0.032	0.827	0.820
genus.unknowngenus.id.2041	OC	7.666	0.467	8.384	0.496	-0.014	0.016	0.422	0.509
genus.unknowngenus.id.2071	OC	16.662	0.339	17.100	0.379	-0.016	0.025	0.540	0.402
phylum.Euryarchaeota.id.55	OC	10.916	0.450	12.219	0.428	-0.025	0.022	0.278	0.437
class.Erysipelotrichia.id.2147	EC	5.778	0.888	7.793	0.801	0.034	0.024	0.183	0.807
family.Erysipelotrichaceae.id.2149	EC	5.778	0.888	7.793	0.801	0.034	0.024	0.183	0.819
family.FamilyXI.id.1936	EC	6.992	0.537	7.796	0.555	-0.026	0.029	0.396	0.590
genus.Dorea.id.1997	EC	12.422	0.333	12.527	0.404	0.006	0.018	0.767	0.416
genus.RuminococcaceaeUCG014.id.11,371	EC	14.383	0.570	14.383	0.640	1.0E-04	0.015	0.995	0.665
genus.Turicibacter.id.2162	EC	10.369	0.584	10.787	0.629	0.015	0.023	0.530	0.644
order.Erysipelotrichales.id.2148	EC	5.778	0.888	7.793	0.801	0.034	0.024	0.183	0.812

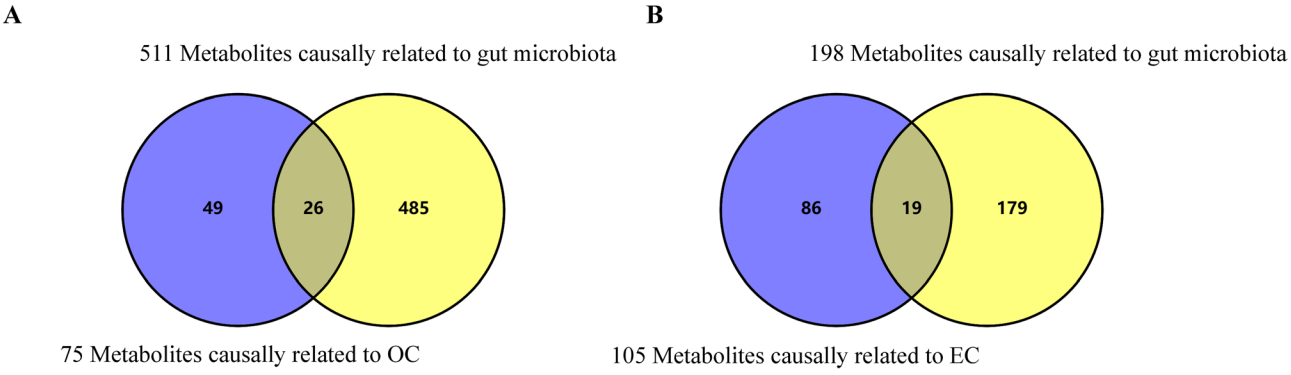

Fig. 2 Venn diagram (**A**) Metabolites that could mediate the relationship between GM and OC were screened. (**B**) Metabolites that could mediate the relationship between GM and EC were screened

exposure	outcome	nsnp	method	pval	OR(95% CI)
genus.unknowngenus.id.2071	GCST90200188	16	Inverse variance weighted	**0.007**	1.184 (1.047 to 1.340)
genus.unknowngenus.id.2041	GCST90200726	12	Inverse variance weighted	**0.031**	1.133 (1.012 to 1.269)
genus.unknowngenus.id.2041	GCST90199826	12	Inverse variance weighted	**0.004**	0.841 (0.747 to 0.946)
genus.unknowngenus.id.2041	GCST90199658	12	Inverse variance weighted	**0.015**	0.876 (0.788 to 0.975)
genus.Tyzzerella3.id.11335	GCST90199945	14	Inverse variance weighted	**0.029**	1.095 (1.009 to 1.188)
genus.Prevotella9.id.11183	GCST90200643	17	Inverse variance weighted	**0.009**	0.858 (0.765 to 0.963)
genus.Prevotella9.id.11183	GCST90200612	17	Inverse variance weighted	**0.006**	0.843 (0.746 to 0.953)
genus.Prevotella9.id.11183	GCST90200132	17	Inverse variance weighted	**0.013**	0.871 (0.782 to 0.971)
genus.FamilyXIIIAD3011group.id.11293	GCST90200759	14	Inverse variance weighted	**0.002**	1.265 (1.091 to 1.468)
genus.FamilyXIIIAD3011group.id.11293	GCST90200041	14	Inverse variance weighted	**<0.001**	1.368 (1.159 to 1.616)
phylum.Euryarchaeota.id.55	GCST90200794	13	Inverse variance weighted	**0.010**	0.898 (0.827 to 0.975)
genus.FamilyXIIIAD3011group.id.11293	GCST90199920	14	Inverse variance weighted	**0.048**	0.867 (0.752 to 0.999)
genus.FamilyXIIIAD3011group.id.11293	GCST90199915	14	Inverse variance weighted	**0.047**	0.837 (0.702 to 0.997)
genus.FamilyXIIIAD3011group.id.11293	GCST90199742	14	Inverse variance weighted	**0.023**	1.184 (1.024 to 1.370)
genus.FamilyXIIIAD3011group.id.11293	GCST90200443	14	Inverse variance weighted	**0.036**	0.849 (0.728 to 0.989)
genus.Escherichia.Shigella.id.3504	GCST90200993	15	Inverse variance weighted	**0.025**	0.863 (0.760 to 0.981)
genus.Escherichia.Shigella.id.3504	GCST90200478	15	Inverse variance weighted	**0.044**	1.182 (1.004 to 1.391)
genus.Escherichia.Shigella.id.3504	GCST90200443	15	Inverse variance weighted	**0.031**	1.139 (1.012 to 1.283)
genus.Escherichia.Shigella.id.3504	GCST90200424	15	Inverse variance weighted	**0.007**	1.185 (1.047 to 1.340)
genus.Escherichia.Shigella.id.3504	GCST90200188	15	Inverse variance weighted	**0.014**	1.164 (1.031 to 1.316)
genus.Escherichia.Shigella.id.3504	GCST90199828	15	Inverse variance weighted	**0.032**	1.140 (1.011 to 1.285)
phylum.Euryarchaeota.id.55	GCST90200692	13	Inverse variance weighted	**<0.001**	1.159 (1.070 to 1.254)
genus.ChristensenellaceaeR.7group.id.11283	GCST90199941	10	Inverse variance weighted	**0.026**	0.786 (0.636 to 0.972)
family.Victivallaceae.id.2255	GCST90200103	13	Inverse variance weighted	**0.034**	1.089 (1.006 to 1.179)
phylum.Euryarchaeota.id.55	GCST90200685	13	Inverse variance weighted	**<0.001**	1.160 (1.072 to 1.254)
phylum.Euryarchaeota.id.55	GCST90199826	13	Inverse variance weighted	**0.032**	0.897 (0.813 to 0.991)
phylum.Euryarchaeota.id.55	GCST90200443	13	Inverse variance weighted	**0.026**	0.920 (0.855 to 0.990)
phylum.Euryarchaeota.id.55	GCST90200103	13	Inverse variance weighted	**0.014**	1.109 (1.022 to 1.204)
genus.unknowngenus.id.2071	GCST90200537	16	Inverse variance weighted	**0.007**	1.203 (1.051 to 1.376)
genus.unknowngenus.id.2071	GCST90200095	16	Inverse variance weighted	**0.048**	0.880 (0.775 to 0.999)
phylum.Euryarchaeota.id.55	GCST90200983	13	Inverse variance weighted	**0.030**	0.913 (0.842 to 0.991)

Fig. 3 Causal association results from IVW MR Regression of 9 GMs with 26 metabolites

Association proportion of metabolite-mediated GM and OC/EC

Next, we carried out a mediation analysis to probe into potential causal chains and dissect potential metabolites that may mediate the relationship between GM and OC/EC (Fig. 7). The results of Tables 4 and 5 demonstrated that metabolites in the GM populations at the phylum, genus, and species levels might play a mediating part in the impact of GM on OC/EC. We identified a significant mediating function of cytosine levels on the causal relationship between *Family XI* and EC (B=-0.017, 95% CI: -0.034–0.000, $P = 0.046$), with a mediation proportion of 19.7% (Table 4). For OC, no metabolites with significant mediating effects were found (Table 5).

Reverse MR results

To determine whether the observed GMs were impacted by the risk of OC/EC, we launched reverse MR analysis, treating OC and EC as exposure variables and GM as the outcome. The IVW results in Fig. 8 manifested a reverse causal relationship between OC and 12 GMs. The detected OC had fewer causal effects on GMs identified in the forward MR analysis. Only *FamilyXIIIAD3011group* exhibited a bidirectional causal

exposure	outcome	nsnp	method	pval	OR(95% CI)
genus.RuminococcaceaeUCG014.id.11371	GCST90200023	17	Inverse variance weighted	**0.021**	1.203 (1.028 to 1.407)
genus.RuminococcaceaeUCG014.id.11371	GCST90200620	18	Inverse variance weighted	**0.047**	1.143 (1.002 to 1.303)
genus.RuminococcaceaeUCG014.id.11371	GCST90200475	17	Inverse variance weighted	**0.024**	1.188 (1.022 to 1.381)
genus.RuminococcaceaeUCG014.id.11371	GCST90200433	17	Inverse variance weighted	**0.021**	1.181 (1.025 to 1.360)
genus.Dorea.id.1997	GCST90200475	11	Inverse variance weighted	**0.047**	0.818 (0.670 to 0.998)
genus.Dorea.id.1997	GCST90200097	11	Inverse variance weighted	**0.033**	0.789 (0.635 to 0.981)
genus.Dorea.id.1997	GCST90200632	11	Inverse variance weighted	**0.012**	1.251 (1.050 to 1.491)
family.FamilyXI.id.1936	GCST90200433	10	Inverse variance weighted	**0.011**	0.891 (0.815 to 0.974)
family.FamilyXI.id.1936	GCST90200606	10	Inverse variance weighted	**0.034**	0.893 (0.805 to 0.991)
family.FamilyXI.id.1936	GCST90199842	10	Inverse variance weighted	**0.034**	1.083 (1.006 to 1.167)
genus.Turicibacter.id.2162	GCST90200567	13	Inverse variance weighted	**0.020**	1.150 (1.023 to 1.294)
family.FamilyXI.id.1936	GCST90199835	10	Inverse variance weighted	**0.033**	1.079 (1.006 to 1.156)
family.FamilyXI.id.1936	GCST90200179	10	Inverse variance weighted	**0.036**	0.910 (0.834 to 0.994)
family.Erysipelotrichaceae.id.2149	GCST90200693	11	Inverse variance weighted	**0.039**	1.206 (1.010 to 1.441)
class.Erysipelotrichia.id.2147	GCST90200693	11	Inverse variance weighted	**0.039**	1.206 (1.010 to 1.441)
genus.Turicibacter.id.2162	GCST90200552	13	Inverse variance weighted	**0.022**	1.163 (1.022 to 1.322)
genus.Turicibacter.id.2162	GCST90200921	13	Inverse variance weighted	**0.021**	1.152 (1.022 to 1.300)
genus.Turicibacter.id.2162	GCST90199645	13	Inverse variance weighted	**0.048**	1.130 (1.001 to 1.275)
genus.Turicibacter.id.2162	GCST90199693	13	Inverse variance weighted	**0.002**	1.201 (1.068 to 1.350)
genus.Turicibacter.id.2162	GCST90199855	13	Inverse variance weighted	**0.032**	0.876 (0.776 to 0.989)
genus.Turicibacter.id.2162	GCST90200435	13	Inverse variance weighted	**0.006**	1.183 (1.050 to 1.333)
genus.RuminococcaceaeUCG014.id.11371	GCST90199918	17	Inverse variance weighted	**0.039**	1.151 (1.007 to 1.315)
order.Erysipelotrichales.id.2148	GCST90200693	11	Inverse variance weighted	**0.039**	1.206 (1.010 to 1.441)

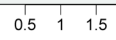

Fig. 4 Causal association results from IVW MR Regression of 7 GMs with 19 metabolites

id.exposure	outcome	nsnp	method	pval	OR(95% CI)
GCST90199945	OC	27	Inverse variance weighted	**0.036**	1.065 (1.004 to 1.129)
GCST90200643	OC	19	Inverse variance weighted	**0.034**	1.128 (1.009 to 1.262)
GCST90200612	OC	19	Inverse variance weighted	**0.008**	0.906 (0.842 to 0.974)
GCST90200132	OC	21	Inverse variance weighted	**<0.001**	0.876 (0.810 to 0.947)
GCST90200759	OC	21	Inverse variance weighted	**0.024**	1.102 (1.013 to 1.200)
GCST90200041	OC	27	Inverse variance weighted	**0.032**	0.922 (0.856 to 0.993)
GCST90199920	OC	14	Inverse variance weighted	**0.025**	1.134 (1.016 to 1.266)
GCST90199915	OC	22	Inverse variance weighted	**0.048**	0.920 (0.847 to 0.999)
GCST90199742	OC	28	Inverse variance weighted	**0.010**	1.098 (1.022 to 1.178)
GCST90200993	OC	18	Inverse variance weighted	**0.034**	0.908 (0.831 to 0.993)
GCST90200794	OC	24	Inverse variance weighted	**0.012**	1.058 (1.013 to 1.106)
GCST90200478	OC	23	Inverse variance weighted	**0.001**	1.130 (1.050 to 1.217)
GCST90200443	OC	22	Inverse variance weighted	**0.011**	0.895 (0.822 to 0.974)
GCST90200424	OC	29	Inverse variance weighted	**0.032**	1.079 (1.007 to 1.157)
GCST90200188	OC	24	Inverse variance weighted	**0.037**	1.087 (1.005 to 1.176)
GCST90199828	OC	25	Inverse variance weighted	**0.045**	0.923 (0.854 to 0.998)
GCST90199941	OC	30	Inverse variance weighted	**0.046**	1.056 (1.001 to 1.114)
GCST90200103	OC	19	Inverse variance weighted	**0.008**	0.928 (0.878 to 0.981)
GCST90200692	OC	27	Inverse variance weighted	**0.021**	0.956 (0.920 to 0.993)
GCST90200685	OC	34	Inverse variance weighted	**0.040**	0.963 (0.930 to 0.998)
GCST90200537	OC	23	Inverse variance weighted	**0.024**	1.070 (1.009 to 1.134)
GCST90200095	OC	22	Inverse variance weighted	**0.042**	1.067 (1.002 to 1.136)
GCST90200726	OC	33	Inverse variance weighted	**0.020**	1.048 (1.007 to 1.091)
GCST90199826	OC	18	Inverse variance weighted	**0.025**	0.935 (0.882 to 0.992)
GCST90199658	OC	21	Inverse variance weighted	**0.026**	1.105 (1.012 to 1.207)
GCST90200983	OC	22	Inverse variance weighted	**0.027**	1.071 (1.008 to 1.139)

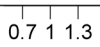

Fig. 5 Causal association results from IVW MR Regression of 26 metabolites with OC

id.exposure	outcome	nsnp	method	pval	OR(95% CI)
GCST90200620	EC	20	Inverse variance weighted	**0.041**	1.094 (1.004 to 1.192)
GCST90200475	EC	27	Inverse variance weighted	**0.012**	1.099 (1.021 to 1.183)
GCST90200097	EC	14	Inverse variance weighted	**0.050**	0.897 (0.805 to 1.000)
GCST90200632	EC	15	Inverse variance weighted	**0.044**	0.889 (0.794 to 0.997)
GCST90200433	EC	16	Inverse variance weighted	**0.005**	1.158 (1.045 to 1.284)
GCST90200606	EC	20	Inverse variance weighted	**<0.001**	1.153 (1.066 to 1.246)
GCST90199842	EC	27	Inverse variance weighted	**0.001**	1.174 (1.067 to 1.293)
GCST90199835	EC	29	Inverse variance weighted	**0.010**	1.125 (1.029 to 1.230)
GCST90200179	EC	23	Inverse variance weighted	**0.016**	0.914 (0.850 to 0.983)
GCST90200693	EC	24	Inverse variance weighted	**0.005**	1.070 (1.020 to 1.122)
GCST90200552	EC	18	Inverse variance weighted	**0.003**	0.865 (0.785 to 0.953)
GCST90200921	EC	20	Inverse variance weighted	**0.042**	1.126 (1.004 to 1.263)
GCST90199645	EC	17	Inverse variance weighted	**0.022**	0.882 (0.793 to 0.982)
GCST90199693	EC	13	Inverse variance weighted	**0.004**	1.158 (1.049 to 1.279)
GCST90199855	EC	28	Inverse variance weighted	**<0.001**	1.138 (1.062 to 1.219)
GCST90200435	EC	24	Inverse variance weighted	**0.044**	1.077 (1.002 to 1.158)
GCST90199918	EC	21	Inverse variance weighted	**0.011**	1.119 (1.026 to 1.221)
GCST90200023	EC	16	Inverse variance weighted	**0.041**	0.886 (0.790 to 0.995)
GCST90200567	EC	19	Inverse variance weighted	**0.018**	1.127 (1.021 to 1.243)

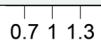

Fig. 6 Causal association results from IVW MR of 19 metabolites with EC

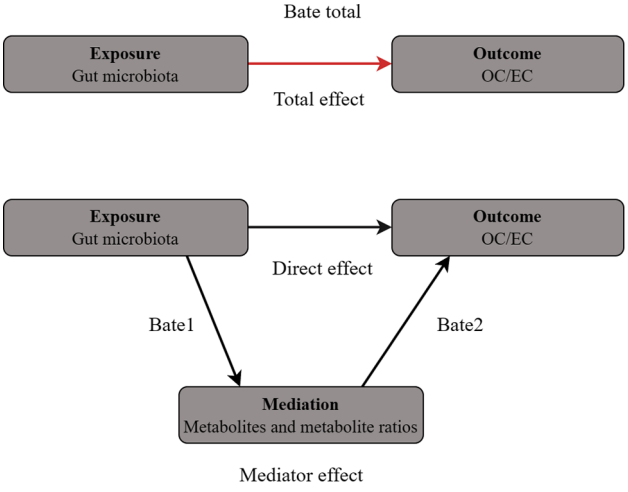

Fig. 7 Flow chart of mediation analysis

relationship with OC. OC was a risk factor for *FamilyXIIIAD3011group* (OR = 1.101, 95%CI: 1.011–1.198, *P* = 0.027).

In terms of EC, we observed no significant causal effects of EC on the GMs identified in the forward MR analysis. However, we observed that EC was significantly associated with two other types of GMs (Fig. 8), namely *Ruminiclostridium9* (OR: 1.083, 95%CI: 1.019–1.152, *P* = 0.011) and *Peptococcaceae* (OR: 1.102, 95%CI: 1.021–1.189, *P* = 0.013), with EC being a risk factor for both.

Furthermore, we also validated results using four other MR methods (Table S11). In the reverse MR analysis, neither heterogeneity nor horizontal pleiotropy was present, indicating that causal connections were less likely to be impacted by potential biases (Table S12). The results of the "Leave-one-out" sensitive analysis can be found in Figure S7.

Discussion

Herein, we designed MR analysis to probe into the causal relationships of GM and GM-derived metabolites with OC/EC. The summary data from the largest and most recent GWAS were utilized, we detected 9 GMs and 26 metabolites playing essential roles in OC, and 7 GMs and 19 metabolites associated with EC development. Reverse MR analysis results suggested a bidirectional causal relationship between *Family.XIIIAD3011group* and OC. By two-step MR analysis, we identified important blood metabolites that functioned as mediators in the causal relationship of cytosine levels in EC and *FamilyXI*.

In our study, we identified potential causal relationships of *Euryarchaeota*, *Escherichia-Shigella*, *FamilyXIIIAD3011group*, and *Prevotella9* with the reduced risk of OC, whereas *Christensenellaceae R.7group*, *Tyzzerella3*, and *Victivallaceae* did the opposite. *Methanobrevibacter smithii*, the main components of *Euryarchaeota*, is the main archaeal species in the human intestine [34], which can not only induce the growth of other microorganisms and maintain the stability and diversity of the intestinal microbial community but also significantly affect the host health through its specific metabolites (such as methane) or specific metabolic pathways [35, 36]. Studies have reported that variations in the abundance of

Table 4 Mediating role of metabolites in the causal relationship between GM and OC

Exposure	Mediation	Outcome	Mediated effect	Mediated proportion	Pvalue	Direct effect	All effect
genus.unknowngenus.id.2071	1-linoleoyl-2-linolenoyl-GPC (18:2/18:3) levels	OC	-0.008(-0.027, 0.010)	6.7% (21.3%, -7.9%)	0.369	-0.116	-0.125
genus.unknowngenus.id.2041	N-acetylputrescine to (N(1) + N(8))-acetylspermidine ratio	OC	0.006(-0.009, 0.021)	-5.3% (8.3%, -18.9%)	0.445	-0.117	-0.111
genus.unknowngenus.id.2041	N6-acetyllysine levels	OC	0.012(-0.010, 0.034)	-10.5% (9.3%, -30.3%)	0.300	-0.123	-0.111
genus.unknowngenus.id.2041	Alpha-hydroxyisocaproate levels	OC	-0.013(-0.031, 0.004)	11.9% (27.9%, -4.0%)	0.142	-0.098	-0.111
genus.Prevotella9.id.11,183	X-24,951 levels	OC	-0.019(-0.041, 0.004)	15.2% (33.6%, -3.3%)	0.107	-0.104	-0.122
genus.Prevotella9.id.11,183	X-23,678 levels	OC	0.017(-0.007, 0.041)	-13.8% (5.9%, -33.6%)	0.170	-0.139	-0.122
genus.Prevotella9.id.11,183	Linolenoylcarnitine (C18:3) levels	OC	0.018(-0.002, 0.039)	-14.9% (2.0%, -31.9%)	0.084	-0.140	-0.122
genus.FamilyXIIIAD3011group.id.11,293	Histidine to trans-urocanate ratio	OC	0.023(-0.015, 0.061)	-14.9% (9.6%, -39.4%)	0.234	-0.177	-0.154
genus.FamilyXIIIAD3011group.id.11,293	Pseudouridine levels	OC	0.018(-0.012, 0.049)	-11.8% (7.9%, -31.5%)	0.239	-0.172	-0.154
phylum.Euryarchaeota.id.55	Oleoyl-linoleoyl-glycerol (18:1 to 18:2) [2] to linoleoyl-arachidonoyl-glycerol (18:2 to 20:4) [1] ratio	OC	-0.006(-0.016, 0.004)	6.8% (17.9%, -4.4%)	0.234	-0.084	-0.090
genus.FamilyXIIIAD3011group.id.11,293	1-palmitoyl-2-stearoyl-gpc (16:0/18:0) levels	OC	-0.026(-0.080, 0.028)	16.6% (51.5%, -18.4%)	0.352	-0.129	-0.154
genus.FamilyXIIIAD3011group.id.11,293	Trimethylamine n-oxide levels	OC	-0.018(-0.045, 0.009)	11.7% (29.1%, -5.8%)	0.191	-0.136	-0.154
genus.FamilyXIIIAD3011group.id.11,293	Imidazole propionate levels	OC	0.015(-0.020, 0.050)	-9.7% (12.8%, -32.1%)	0.399	-0.169	-0.154
genus.FamilyXIIIAD3011group.id.11,293	1-linoleoyl-gpc (18:2) levels	OC	0.016(-0.012, 0.044)	-10.2% (8.0%, -28.4%)	0.272	-0.170	-0.154
genus.Escherichia.Shigella.id.3504	Salicylate to oxalate (ethanedioate) ratio	OC	0.014(-0.008, 0.037)	-10.6% (6.3%, -27.5%)	0.218	-0.147	-0.133
genus.Escherichia.Shigella.id.3504	X-12,221 levels	OC	0.021(-0.013, 0.054)	-15.4% (10.0%, -40.7%)	0.235	-0.154	-0.133
genus.Escherichia.Shigella.id.3504	Pseudouridine levels	OC	-0.015(-0.035, 0.006)	10.9% (26.1%, -4.4%)	0.161	-0.119	-0.133
genus.Escherichia.Shigella.id.3504	Dimethylglycine levels	OC	0.013(-0.010, 0.036)	-9.7% (7.5%, -26.9%)	0.269	-0.146	-0.133
genus.Escherichia.Shigella.id.3504	3-amino-2-piperidone levels	OC	0.013(-0.008, 0.034)	-9.6% (6.4%, -25.5%)	0.239	-0.146	-0.133
genus.Escherichia.Shigella.id.3504	N-acetylserine levels	OC	-0.010(-0.029, 0.008)	7.8% (21.6%, -6.0%)	0.266	-0.123	-0.133
phylum.Euryarchaeota.id.55	1-palmitoyl-2-arachidonoyl-gpc (16:0/20:4n6) levels	OC	-0.007(-0.019, 0.006)	7.4% (21.0%, -6.2%)	0.286	-0.083	-0.090
genus.ChristensenellaceaeR.7group.id.11,283	Gamma-CEHC glucuronide levels	OC	-0.013(-0.065, 0.039)	-5.8% (-28.7%, 17.2%)	0.623	0.241	0.228

Table 4 (continued)

Exposure	Mediation	Outcome	Mediated effect	Mediated proportion	Pvalue	Direct effect	All effect
family.Victivallaceae.id.2255	Linoleoyl-arachidonoyl-glycerol (18:2/20:4) [1] levels	OC	-0.006(-0.015, 0.003)	-6.9% (-16.6%, 2.8%)	0.163	0.099	0.093
phylum.Euryarchaeota.id.55	1-stearoyl-2-arachidonoyl-gpc (18:0/20:4) levels	OC	-0.006(-0.018, 0.006)	6.2% (19.5%, -7.2%)	0.366	-0.084	-0.090
phylum.Euryarchaeota.id.55	Pseudouridine levels	OC	0.009(-0.001, 0.020)	-10.3% (1.1%, -21.6%)	0.076	-0.099	-0.090
phylum.Euryarchaeota.id.55	Linoleoyl-arachidonoyl-glycerol (18:2/20:4) [1] levels	OC	-0.008(-0.018, 0.003)	8.6% (20.3%, -3.1%)	0.148	-0.082	-0.090
phylum.Euryarchaeota.id.55	N6-acetyllysine levels	OC	0.007(-0.005, 0.020)	-8.1% (5.9%, -22.0%)	0.257	-0.097	-0.090
genus.unknowngenus.id.2071	X-16,087 levels	OC	0.012(-0.014, 0.039)	-10.0% (11.3%, -31.2%)	0.358	-0.137	-0.125
genus.unknowngenus.id.2071	3-amino-2-piperidone levels	OC	0.014(-0.009, 0.038)	-11.3% (7.4%, -30.0%)	0.234	-0.139	-0.125
phylum.Euryarchaeota.id.55	Cholesterol to linoleoyl-arachidonoyl-glycerol (18:2 to 20:4) [1] ratio	OC	-0.006(-0.016, 0.003)	6.9% (17.3%, -3.4%)	0.188	-0.084	-0.090

methanogenic archaea are linked to changes in pro-inflammatory pathways in inflammatory bowel disease [37, 38]. A large number of former studies and epidemiological data have emphasized the function of chronic inflammation in the development of OC [39, 40]. Thus, the modulation of the metabolic activity of archaea, and in particular the uplift in the population of beneficial archaea, may aid in reducing inflammation, thus favoring the treatment of OC.

Prevotella is part of the Bacteroidetes, a group of Gram-negative bacteria that are widely considered to be foundational members of the human GM, participating in polysaccharide fermentation, thereby contributing to the production of short-chain fatty acids to maintain the integrity of the host's gut barrier and modulate immune responses [41, 42]. Currently, *Prevotella 9* is a protective factor in autoimmune diseases [43, 44]. However, its function in the development of cancer seems to vary depending on the type of cancer. For example, *Prevotella 9* levels are often elevated in patients with unresectable liver cancer (LC) [45] but are negatively correlated with the occurrence and development of esophageal cancer [46]. The mechanisms by which *Prevotella 9* influences specific cancers merit further exploration.

Escherichia and *Shigella* are Gram-negative rod-shaped bacteria belonging to the Enterobacteriaceae family, which are part of the Proteobacteria and are considered conditioned pathogens [47]. *Escherichia* and *Shigella* are considered separate genera in many categorization systems. However, because of the high sequence similarity between their genomes and the inaccuracies of current metagenomic sequencing technologies in distinguishing members of the two genera, they are often classified together into one genus in bioinformatics analysis, especially in clinical microbiology testing [48]. A study on the identification of related bacterial communities in OC samples uncovered that the Proteobacteria was dominant when comparing OC sample pathologies with adjacent tissues of tumors [49]. The high abundance of Proteobacteria in post-treatment patients with adrenocortical carcinoma was also associated with better prognosis [50], both of which are consistent with our findings that the Proteobacteria in the GM of OC patients is a protective factor. However, as a common pathogenic bacteria in the human gut, Proteobacteria is generally considered to be associated with various inflammatory and chronic diseases, exerting pro-inflammatory effects by protecting infections [51]. Most results also indicated that tumor patients have more deformed bacterial members in their bodies compared to normal tissues and feces [52–54]. The appearance of this seemingly contradictory result may be related to the complex relationship between GM and host health. Therefore, further study on the potential connection mechanism between such bacteria and OC is necessary. Of course, this result cannot rule out the reason that a lack of precision in describing the names of specific bacteria is caused by the minimum classification of MR studies that is only limited to genera.

Christensenellaceae and Tyzzerella are both members of the Firmicutes, participating in the favorable

Table 5 Mediating role of metabolites in the causal relationship between GM and EC

Exposure	Mediation	Outcome	Mediated effect	Mediated proportion	Pvalue	Direct effect	All effect
genus.RuminococcaceaeUCG014.id.11,371	X-11,858 levels	EC	0.016(-0.013, 0.046)	-11.5% (9.4%, -32.5%)	0.280	-0.157	-0.141
genus.RuminococcaceaeUCG014.id.11,371	Cytosine levels	EC	0.024(-0.007, 0.056)	-17.3% (4.9%, -39.6%)	0.126	-0.165	-0.141
genus.RuminococcaceaeUCG014.id.11,371	Trans 3,4-methyleneheptanoate levels	EC	-0.022(-0.057, 0.012)	15.8% (40.3%, -8.7%)	0.207	-0.119	-0.141
genus.RuminococcaceaeUCG014.id.11,371	Carboxyethyl-gaba levels	EC	0.016(-0.008, 0.040)	-11.2% (5.8%, -28.2%)	0.197	-0.157	-0.141
genus.Dorea.id.1997	X-24,307 levels	EC	-0.026(-0.071, 0.018)	12.8% (34.4%, -8.8%)	0.245	-0.179	-0.205
genus.Dorea.id.1997	X-11,858 levels	EC	-0.019(-0.063, 0.025)	9.3% (30.8%, -12.3%)	0.400	-0.186	-0.205
genus.Dorea.id.1997	Furaneol sulfate levels	EC	0.026(-0.031, 0.082)	-12.5% (15.0%, -40.0%)	0.373	-0.231	-0.205
genus.Turicibacter.id.2162	Mannose to mannitol to sorbitol ratio	EC	0.017(-0.005, 0.039)	-14.0% (4.5%, -32.4%)	0.138	-0.138	-0.121
family.FamilyXI.id.1936	X-22,509 levels	EC	-0.016(-0.035, 0.003)	-18.7% (-40.7%, 3.3%)	0.096	0.102	0.086
family.FamilyXI.id.1936	Cytosine levels	EC	-0.017(-0.034, -0.000)	-19.7% (-39.1%, -0.4%)	0.046	0.103	0.086
family.FamilyXI.id.1936	Glyco-beta-muricholate levels	EC	0.008(-0.003, 0.020)	9.8% (-3.5%, 23.1%)	0.148	0.077	0.086
family.FamilyXI.id.1936	Androstenediol (3beta,17beta) disulfate (1) levels	EC	0.013(-0.000, 0.026)	15.0% (-0.5%, 30.5%)	0.058	0.073	0.086
family.Erysipelotrichaceae.id.2149	Glycochenodeoxycholate glucuronide (1) levels	EC	0.013(-0.023, 0.048)	6.3% (-11.3%, 23.8%)	0.484	0.190	0.202
class.Erysipelotrichia.id.2147	Glycochenodeoxycholate glucuronide (1) levels	EC	0.013(-0.023, 0.048)	6.3% (-11.3%, 23.8%)	0.484	0.190	0.202
genus.Turicibacter.id.2162	X-21,353 levels	EC	0.017(-0.005, 0.038)	-13.8% (4.1%, -31.8%)	0.130	-0.137	-0.121
genus.Turicibacter.id.2162	X-18,887 levels	EC	-0.022(-0.049, 0.005)	18.1% (40.5%, -4.2%)	0.111	-0.099	-0.121
genus.Turicibacter.id.2162	Mannose levels	EC	0.013(-0.009, 0.034)	-10.4% (7.8%, -28.5%)	0.262	-0.133	-0.121
genus.Turicibacter.id.2162	3-hydroxydecanoate levels	EC	0.027(-0.001, 0.054)	-22.3% (0.5%, -45.1%)	0.055	-0.148	-0.121
genus.Turicibacter.id.2162	Quinate levels	EC	-0.015(-0.036, 0.006)	12.6% (30.1%, -4.9%)	0.157	-0.105	-0.121
genus.RuminococcaceaeUCG014.id.11,371	X-24,243 levels	EC	0.012(-0.009, 0.033)	-8.5% (6.5%, -23.5%)	0.267	-0.153	-0.141
order.Erysipelotrichales.id.2148	Glycochenodeoxycholate glucuronide (1) levels	EC	0.013(-0.023, 0.048)	6.3% (-11.3%, 23.8%)	0.484	0.190	0.202
genus.RuminococcaceaeUCG014.id.11,371	X-11,858 levels	EC	0.016(-0.013, 0.046)	-11.5% (9.4%, -32.5%)	0.280	-0.157	-0.141
genus.RuminococcaceaeUCG014.id.11,371	Cytosine levels	EC	0.024(-0.007, 0.056)	-17.3% (4.9%, -39.6%)	0.126	-0.165	-0.141

Table 5 (continued)

Exposure	Mediation	Outcome	Mediated effect	Mediated proportion	Pvalue	Direct effect	All effect
genus.RuminococcaceaeUCG014.id.11,371	Trans 3,4-methy-leneheptanoate levels	EC	-0.022(-0.057, 0.012)	15.8% (40.3%, -8.7%)	0.207	-0.119	-0.141
genus.RuminococcaceaeUCG014.id.11,371	Carboxyethyl-gaba levels	EC	0.016(-0.008, 0.040)	-11.2% (5.8%, -28.2%)	0.197	-0.157	-0.141

exposure	outcome	nsnp	method	pval	OR(95% CI)
OC	family.Oxalobacteraceae.id.2966	8	Inverse variance weighted	**0.019**	1.198 (1.030 to 1.393)
OC	family.Bifidobacteriaceae.id.433	9	Inverse variance weighted	**0.026**	0.910 (0.838 to 0.989)
OC	class.Actinobacteria.id.419	9	Inverse variance weighted	**0.013**	0.905 (0.837 to 0.979)
EC	genus.Ruminiclostridium9.id.11357	11	Inverse variance weighted	**0.011**	1.083 (1.019 to 1.152)
EC	family.Peptococcaceae.id.2024	11	Inverse variance weighted	**0.013**	1.102 (1.021 to 1.189)
OC	order.Bifidobacteriales.id.432	9	Inverse variance weighted	**0.026**	0.910 (0.838 to 0.989)
OC	genus.Slackia.id.825	8	Inverse variance weighted	**0.007**	1.219 (1.056 to 1.408)
OC	genus.Senegalimassilia.id.11160	9	Inverse variance weighted	**0.043**	1.130 (1.004 to 1.273)
OC	genus.RuminococcaceaeUCG013.id.11370	9	Inverse variance weighted	**0.045**	1.082 (1.002 to 1.169)
OC	genus.FamilyXIIIAD3011group.id.11293	9	Inverse variance weighted	**0.027**	1.101 (1.011 to 1.198)
OC	genus.Coprococcus1.id.11301	9	Inverse variance weighted	**0.018**	0.911 (0.843 to 0.984)
OC	genus.Bifidobacterium.id.436	9	Inverse variance weighted	**0.040**	0.917 (0.844 to 0.996)
OC	family.Pasteurellaceae.id.3689	9	Inverse variance weighted	**0.045**	0.899 (0.810 to 0.998)
OC	order.Pasteurellales.id.3688	9	Inverse variance weighted	**0.045**	0.899 (0.810 to 0.998)

Fig. 8 Causal association results from IVW MR Regression of OC/EC with GM

regulation of the intestinal environment and immune regulation and health homeostasis in the host, playing a pivotal part in human gut health [55–57]. *Christensenellaceae R-7 group* is linked with an elevated risk of prostate cancer [58]. In another study on the risk of BC, a negative connection between the *Christensenellaceae R-7 group* and the risk of BC is detected [59]. In our work, *ChristensenellaceaeR-7group* was positively linked to OC risk, and its effect was different in different cancers. The reports exhibiting the association between *Christensenellaceae* and various cancer risks further confirmed the important role of this microbiota in the occurrence and development of cancer. The current literature about *Tyzzerella3* is very limited, but increased *Tyzzerella3* abundance has been reported in gestational diabetes mellitus patients and patients at increased risk of spinal pain, as well as in mice with neuroblastoma-induced tumors [60, 61]. This is in line with our study, where we observed a positive connection between *Tyzzerella3* and OC risk. However, the mechanism of *Christensenellaceae* and *Tyzzerella* in the occurrence and development of OC is still blank, awaiting rigorous experiments in the future.

Moreover, we also detected a genetic causality of the interaction between *FamilyXIIIAD3011group* and OC. *FamilyXIIIAD3011group* represents a type that is less easy to identify in the Firmicutes. Research on *FamilyXIIIAD3011group* is very limited. The *Victivallaceae* family belonging to the Verrucomicrobia is a normal flora in the human intestine, functioning as beneficial bacteria in the human body in most cases [62]. However, in this study, it is positively correlated with the occurrence of OC, as a taxon that has not been well studied in clinical practice. The study of *Victivallaceae* as a harmful bacteria needs to be carried out in the future.

The taxa with significantly increased risk of EC at the class, family, and order levels were *Erysipelotrichia*, *Erysipelotrichaceae*, and *Erysipelotrichales*, all of which belong to Firmicutes. The elevated *Erysipelotrichaceae* levels are linked with intestinal inflammation [63, 64] and have been utilized as a biomarker for experimental autoimmune encephalitis in animal models [65]. The abundance of *Erysipelotrichaceae* is found to be elevated in colorectal cancer (CRC) and oral cancer [66, 67]. A study also manifested that *Erysipelotrichaceae-Erysipelothrix* is highly immunogenic, exhibiting a positive correlation with tumor necrosis factor [68]. Therefore, there is a positive correlation between *Erysipelotrichaceae* and inflammation, which may elevate the risk of OC patients by increasing the risk of inflammation. As a member of the phylum Firmicutes, class *Erysipelotrichia*, order *Erysipelotrichales*, and family *Turicibacteraceae*, *Turicibacter* is a Gram-positive obligately anaerobic bacteria [69]. Although the causal relationship between *Turicibacter* and EC has not yet been determined, some studies have demonstrated that *Turicibacter* may be a beneficial intestinal bacterium with anti-inflammatory properties [70, 71]. In cancers of the digestive system, *Turicibacter* is a protective bacterium and negatively lined with LC [72], and our study supported the idea that *Turicibacter* may serve as a protective bacterial species for EC. However,

it is interesting that different metabolites have different effects on the causal relationship with EC, but the exact reasons and underlying mechanisms causing this difference are currently unclear, thus requiring further clarification in future prospective studies.

In a previous study predicting the response of CRC patients receiving GM-assisted chemoradiotherapy, the microbe *Dorea* associated with butyrate production is overrepresented in responders at baseline samples [73]. Butyrate is a common short-chain fatty acid in gut fermentation products and plays a crucial role in host health, being capable of repairing intestinal mucosal damage, increasing the expression of ZO-1 protein, enhancing intestinal barrier function, reducing endotoxin levels in the gut, suppressing inflammatory responses, improving tumor microenvironment, and hindering tumor growth [74, 75]. This may explain why we identified *Dorea* as a protective factor for EC in our study. However, since no conclusive evidence was found in the reverse MR analysis to prove that EC influenced the nature of *Dorea*, further research is required for validation. Similarly, in this investigation, we also observed a positive effect of *RuminococcaceaeUCG014* on EC. As a member of the Firmicutes phylum and Clostridium class, *Ruminococcaceae* is known for its anaerobic nature in the gut, playing a dominant part in fermenting complex carbohydrates and amino acids into short-chain fatty acids, which can be utilized for energy metabolism and gut health enhancement [76]. Existing studies suggested that short-chain fatty acids may have a preventive and therapeutic effect on cancer, and it has been proposed that Clostridium may influence cancer occurrence and development by generating short-chain fatty acids [52, 77, 78]. However, whether the protective effect of *RuminococcaceaeUCG014* on EC is directly mediated by the generation of short-chain fatty acids remains to be determined. Further research is instrumental in uncovering the exact mechanisms behind these associations.

This work uncovered that *Family XI* elevated the risk of EC occurrence. Furthermore, we found that changes in cytosine levels were effective intermediate metabolites influencing the two. The research seems to be the first investigation to experimentally establish an association among these three. DNA methylation is an epigenetic alteration that alters gene expression without changing the DNA sequence by adding methyl covalently to cytosine under the CpG sequence [79]. Changes in cytosine methylation are associated with cancer etiology in two distinct ways. Firstly, aberrant methylation patterns can lead to genomic instability, oncogene expression, and tumor suppressor gene silence. Second, C > T transition mutations occurring in CpG predominate in mutations in human tumors, which are often associated with cancer-associated mutational hotspots and are the most common single-base changes in human tumors [80]. Therefore, *Family XI* may affect EC risk by regulating cytosine levels in the host. However, the current literature is limited in this direction, necessitating detailed investigation to clarify its role. The discovery of cytosine levels as a mediating metabolite in the relationship between *FamilyXI* and EC provides a new perspective for understanding the complex interactions between GM and cancer and further suggests that there may be different patterns of association between microbiota and cancer risk in different studies. Further in-depth research is needed to elucidate the reasons and mechanisms behind these differences.

Over the past five years, microbial therapy has emerged as a treatment approach that is different from traditional anti-cancer treatments, with potential benefits in the treatment of diseases [81]. A previous animal experiment revealed that overexpression of β-glucuronidase and glycyrrhetinic acid in Escherichia coli for targeted therapy in colon cancer mice exhibited a great tumor suppression rate and low toxicity [82]. Zhang et al. [83]. also pointed out that liposomal paclitaxels encapsulated in electroporated Escherichia coli or Lactobacillus plantarum formed LP-in-E. coli or LP-in-L. casei, which, when administered by inhalation, accumulates in the lungs and effectively combats cancer with fewer side effects. In addition, a study has designed a non-pathogenic Escherichia coli that can specifically lyse in the tumor microenvironment and generate a nano antagonist targeting CD47, which can activate tumor-infiltrating T cells, activate rapid tumor regression, and prevent tumor metastasis [84]. Engineered bacteria can be utilized for safe and local delivery of the payload of immunotherapy, thereby achieving systemic anti-tumor immunity. GM is both a driver of cancer and a potential therapeutic target. However, given the intricate connection between treatment modalities, GM, and cancer, more studies are needed to elucidate specific GM and mechanisms in individual cancers, which may facilitate the advancement of clinical translation.

This study utilized the MR analysis to effectively reduce confounding bias, thereby more accurately determining the causal relationship between GM and OC/EC. Compared to traditional observational studies, MR analysis uses genetic variation as IVs to simulate RCTs, reducing the influence of reverse causality and confounding factors, and thereby improving the credibility of results. Our study provided new insights into the prevention, diagnosis, and treatment of OC/EC by summarizing the clear microbiome profile of OC/EC. Specifically, the specific GM and blood metabolites associated with OC/EC risk were identified in this study. The work exhibited potential biomarkers for early diagnosis, helping to identify high-risk populations earlier and achieve early intervention.

At the same time, these findings also provided a theoretical basis for developing therapeutic strategies based on regulating the GM, such as promoting the growth of beneficial bacteria through probiotic preparations or dietary fiber supplements or reducing the abundance of harmful bacteria through antibiotics and other means, thereby curbing the development of tumors. In addition, this study guided the development of prevention strategies in OC/EC, emphasizing the importance of adjusting dietary structure and avoiding disruption of GM balance. In summary, this study is not only innovative in methodology but also has important guiding significance in the clinical application. It can open up new ideas and directions for the prevention and treatment of OC/EC in the future.

This work shares similar limitations with most current MR studies. Firstly, GM's GWAS data mainly represent individuals of European ancestry, with considerably limited data on non-European ancestry, which may limit the applicability of results to other races and populations. There are significant differences among different races in terms of genetic background, lifestyle, and environmental factors, which may affect the composition and function of GM and thus affect its association with OC/EC. Therefore, caution should be exercised when extending the conclusions of this study to other races. It is recommended that future studies include more data from different races to more comprehensively reveal the differences and commonalities in the relationship between GM and these diseases. Secondly, since the lowest classification level for exposure data is at the genus level, a more detailed causal analysis at the species or strain level was conducted. This may lead to our inability to identify the specific impact of specific species or strains on OC/EC risk, thereby limiting our in-depth understanding of the relationship between GM and cancer. Lastly, there is considerable variation in sample collection and management due to the lack of a standardized GM measurement method and criteria in current studies. The differences in sequencing platforms and analysis methods used in different studies may lead to inconsistent and incomparable results, which may affect the accuracy and reliability of the relationship between GM and OC/EC, as well as the comparison and validation with other research results.

Conclusion

Our investigation summarized 9 GMs with causal effects on OC from genetic analysis, among which 4 may be pathogenic risk factors, while the other 5 may reduce the risk of OC. There are 4 pathogenic GMs and 3 beneficial bacteria for EC. Furthermore, OC/EC also alters the composition of GM. Among them, a significant bidirectional causal relationship is detected between the *FamilyXII-IAD3011group* and OC, and the level of cytosine is found to be a significant intermediate metabolite between the two, providing valuable insights for the subsequent GM-mediated pathogenic mechanisms of OC and the development of preventive and therapeutic strategies for the disease.

Supplementary Information

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Supplementary Material 1
Supplementary Material 2
Supplementary Material 3
Supplementary Material 4
Supplementary Material 5
Supplementary Material 6
Supplementary Material 7
Supplementary Material 8
Supplementary Material 9
Supplementary Material 10
Supplementary Material 11
Supplementary Material 12
Supplementary Material 13
Supplementary Material 14
Supplementary Material 15
Supplementary Material 16
Supplementary Material 17
Supplementary Material 18
Supplementary Material 19
Supplementary Material 20

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Author contributions
Jinyan Chen conceived and designed the study. Jinyan Chen and Xuejun Chen performed the experiments. Jiong Ma wrote the paper. Jinyan Chen reviewed and edited the manuscript. All authors read and approved the manuscript.

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Data availability
The data and materials in the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Competing interests
The authors declare no competing interests.

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