Journal of Ovarian Research

RESEARCH

Open Access

# Exploring the causal associations of the gut microbiota and plasma metabolites with ovarian cancer: an approach of mendelian randomization analysis combined with network pharmacology and molecular docking

Junfeng Guo[1†], Chen Wang[1†], He Li[1*] and Chenhuan Ding[1,2*]

## Abstract

**Background** While increasing evidence suggests that alterations in the gut microbiota and metabolites are associated with ovarian cancer (OC) risk, whether these associations imply causation remains to be identified.

**Methods** We conducted a two-sample Mendelian randomization (MR) study utilizing a large-scale genome-wide association study (GWAS) to explore the causal effects of the gut microbiota of 196/220 individuals and 1,400 plasma metabolites on OC and epithelial ovarian cancer (EOC) subtypes. Data on the gut microbiota were obtained from the MiBioGen consortium of 18,340 subjects and the Dutch Microbiome Project of 7,738 volunteers. Data on plasma metabolites were derived from a GWAS of plasma metabolites in 8,299 participants. Ovarian cancer ($n=25,509$) and EOC subtypes were obtained from the Ovarian Cancer Association Consortium (OCAC). Metabolites and associated targets were analyzed via network pharmacology and molecular docking.

**Results** At the genus and species levels, we identified seven risk factors for the gut microbiota: the genus *Dialister* ($P=0.024$), genus *Ruminiclostridium5* ($P=0.0004$), genus *Phascolarctobacterium* ($P=0.0217$), species *Bacteroides massiliensis* ($P=0.011$), species *Phascolarctobacterium succinatutens* ($P=0.0212$), species *Paraprevotella clara* ($P=0.0247$) and species *Bacteroides dorei* ($P=0.0054$). In addition, five gut microbes at the genus and species levels were found to be protective: genus *Family XIII AD3011 group* ($P=0.006$), genus *Butyrivibrio* ($P=0.0095$), genus *Oscillibacter* ($P=0.0206$), species *Roseburia hominis* ($P=0.0241$), and species *Bifidobacterium bifidum* ($P=0.0224$). For plasma metabolites, we

†Junfeng Guo and Chen Wang contributed equally to this work and shared first authorship.

*Correspondence:
He Li
lihe1972@hotmail.com
Chenhuan Ding
dingchenhuan@sjtu.edu.cn

Full list of author information is available at the end of the article

revealed five positive and four negative correlations with OC. Among these, caffeic acid and caffeine metabolites and sphingomyelin and ceramide metabolites were identified as risk factors, whereas phenylalanine metabolites, butyric acid metabolites, and some lipid metabolites were recognized as protective factors. A series of sensitivity analyses revealed no abnormalities, including pleiotropy and heterogeneity analyses.

**Conclusion** Our MR analysis demonstrated that the gut microbiota and metabolites are causally associated with OC, which has significant potential for the early detection and diagnosis of OC and EOC subtypes, providing valuable insights into this area of research.

**Keywords** Ovarian cancer, Mendelian randomization, Gut microbiota, Metabolites, Network pharmacology

## Introduction

Ovarian cancer (OC), which accounted for 13,940 deaths in the United States in 2020, is the second most prevalent cause of gynecologic cancer death in females globally and is the deadliest gynecologic tumor [1, 2]. Approximately 50-70% of these cases are epithelial ovarian cancer (EOC). Lack of early diagnosis criteria leads to the majority of patients being in advanced stages upon diagnosis [3], with the 5-year survival rate of patients of EOC less than 30% [4]. Hence, urgent attention needs to be given to new ways of preventing and treating OC.

Increasing evidence suggests that the gut microbiota is associated with metabolism, immunity, and cancer [5, 6], and regulation of gut microbes can contribute to the disease treatment [7]. Recently, the communication pathway between the gut and ovarian axes has attracted our attention [8–10], with mechanisms of action that include sex hormone secretion and metabolism, regulation of inflammation and oxidative stress [11]. The gut microbiota hydrolyzes bound estrogen into biologically active free estrogen by producing β-glucuronidase. Free estrogen can activate estrogen receptors to trigger downstream signaling pathways, including MAPK, NF-κB, IGF-1, and EGF, which play important roles in regulating the proliferation and differentiation of ovarian epithelium [12]. Significant change of gut microbiota composition have also been observed in ovarian cancer patients [13–15]. For example, in EOC patients, the relative abundances of Bacteroidetes (especially *Bacteroides* and *Prevotella*) and Proteobacteria are increased, but the abundances of Firmicutes and Actinobacteria are decreased compared with those in healthy control people [14], indicating that the gut microbiota may serve as biomarker for the early identification and diagnosis of OC and provide evidence for potential therapeutic strategies. Nonetheless, traditional observational studies are susceptible to confounding factors in addition to the constraints of limited sample sizes. Ethical concerns further impede comprehensive randomized controlled studies involving all strains of bacteria, especially potentially detrimental bacteria. Further insights into the causal relationship between the gut microbiota and OC require more detailed evidence and research.

Metabolomics can reveal correlations between metabolites or metabolic pathways in relation to physiological and pathological changes, thus providing new insights into disease mechanisms [16, 17]. A varity of metabolic compounds including organic acids and their derivatives, ceramides and sphingolipids have been reported to be associated with the occurence of OC [18, 19]. For example, Zeleznik [18] reported that SMs were associated with an increased risk of ovarian cancer, especially in postmenopausal women. Pseudouridine is associated with an increased risk of overall OC and suggests serous/poorly differentiated tumors, whereas the C18:1 LPC and LPC: PC ratios are related to a reduced risk of endometrioid/clear cell tumors [19]. However, it is difficult to explore specific causal relationships in clinical practice because of the unavailability of large samples.

Although previous studies have identified associations between the gut microbiota and metabolites in OC, the exact causal relationships, especially between the subsets of EOC, remain unclear. Mendelian randomization (MR) can circumvent the drawbacks of traditional observational studies by using genetic variants as instrumental variables (IVs) to assess potential causal relationships between exposures and outcomes [20]. MR simulates a scene resembling that of a randomized controlled test, as single nucleotide polymorphisms (SNPs) are randomly assigned at embryo conception, thus reducing confounding factors [21]. Notably, there are no relevant MR studies investigating the correlation between the gut microbiota and metabolites and OC and EOC subtypes.

Therefore, we aimed to assess the genetic associations between the gut microbiota and plasma metabolites in patients with OC and EOC via a two-sample MR approach utilizing publicly available large-scale GWAS summary statistics, validated by network pharmacology and molecular docking, to provide practical and targeted guidance for the early detection, treatment and prevention of OC.

## Materials and methods

### Study design

A two-sample MR approach was employed to evaluate the potential causal relationships between the gut

microbiota and metabolites and between OC and EOC subtypes via summary statistics from GWASs. Ethical approval for each of the GWAS included in this study is available through the corresponding original article. The correctly designed MR study should rely on three assumptions: (1) genetic variants must be strongly correlated with the exposure factors (gut microbiota and metabolites in this study); (2) genetic variants are independent of confounders; and (3) genetic variants must not be directly related to the outcome (ovarian cancer in this study) but only influence the outcome through their effect on the exposure. The MR design flowchart for this study is shown in Fig. 1.

## Sources of data on exposure

Genetic instrumental variables for the gut microbiota were acquired from the MiBioGen consortium [22], which performs the most extensive genome-wide meta-analysis by combining human genome-wide genotypes with discriminating 16 S rRNA sequencing data. This comprehensive analytic study was conducted on 18,340 individuals from 24 cohorts, which were primarily of European ancestry. After excluding 15 unknown bacterial classes, 9 phyla, 16 orders, 20 orders, 32 families, and 119 genera were defined, but these GWAS data lacked species-level gut microbiota data. Therefore, we integrated data from the Dutch Microbiome Project (DMP) to enhance our analysis [23]. DMP initiated and reported a large-scale GWAS of the gut microbiota of 7,738 volunteers from the Netherlands by applying macrogenomic sequencing, which allowed bacterial identification at a species-level resolution. The GWAS summary data for DMP included 5 phyla, 10 orders, 13 orders, 26 families, 48 genera, and 105 species.

We obtained 1,400 metabolite-associated genome-wide summary data points from the IEU database (https://www.ebi.ac.uk/gwas/) with the IDs GCST90199621–GCST90201020, including 1,091 plasma metabolites and 309 metabolite ratios from 8,299 European individuals [24]. In this study, 850 known metabolites out of 1,091 plasma metabolites were classified into 8 major metabolic groups: lipids (395), amino acids (210), xenobiotics (130), nucleotides (33), cofactors and vitamins (31), carbohydrates (22), peptides (21), and energy (8); the remaining metabolites were partially characterized molecules (21) and unknown molecules (220).

## Outcome data sources

Summary data on OC and EOC typing contained in this study were obtained from a GWAS conducted by the Ovarian Cancer Association Consortium (OCAC, http://ocac.ccge.medschl.cam.ac.uk/). The consortium comprised 25,509 women with OC and 40,941 controls of European ancestry who had passed quality control [25]. The database includes 63 genotyping projects/case–control sets that represent participants of European ancestry

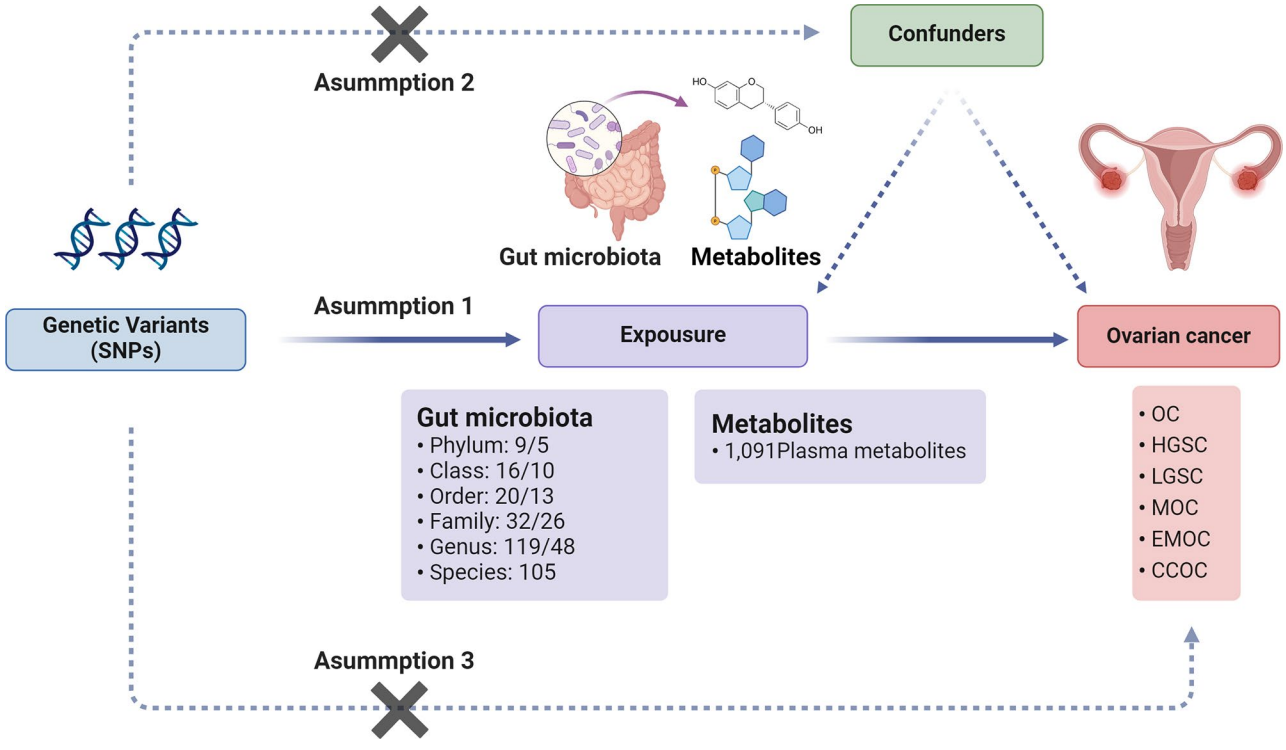


**Fig. 1** Flowchart of the MR study

recruited from 14 countries. We analyzed 40,941 controls and 22,406 patients with invasive EOC, including the following tissue types: high-grade serous carcinoma (HGSC, $n = 13,037$), low-grade serous carcinoma (LGSC, $n = 1,012$), mucinous ovarian cancer (MOC, $n = 1,417$), endometrioid ovarian cancer (EMOC, $n = 2,810$) and clear cell ovarian cancer (CCOC, $n = 1,366$). Ethical approval for all OCAC studies was granted by the relevant research ethics committees, and written informed consent was obtained from all participants in these studies.

## Pathway enrichment analysis of metabolites

Metabolic pathway analysis was performed via the web-based tool MetaboAnalyst 6.0 (https://www.metaboanalyst.ca/). The Kyoto Encyclopedia of Genes and Genomes (KEGG) database was used in this study, and the significance level was set at $P < 0.05$.

### Statistical analysis

Overall, data from 196/220 gut microbiota at six levels (phylum, order, order, family, genus, and species) and 1400 metabolites, including 1091 metabolites and 309 metabolite ratios, were used as the exposure data. To guarantee the correctness and accuracy of the study results on the causal relationship between exposure and OC and EOC subtypes, IVs were selected via the following procedures for quality control: (1) $P$ values less than the locus-wide significance level ($1 \times 10^{-5}$) were selected as IVs strongly associated with the gut microbiota and metabolites. (2) To ensure independence between the included IVs, linkage disequilibrium (LD) between the included SNPs was assessed via the clumping approach ($R2 < 0.001$ and clumping distance = 10,000 kb). (3) SNPs with palindromes and ambiguous alleles were removed. The final screened SNPs were used as IVs to harmonize with the outcome GWAS summary statistics. (4) The F statistic of each SNP was calculated to determine the statistical strength. SNPs with F statistics < 10 were ignored to avoid weak IV bias [26]. (5) SNPs that were highly linked with the result (defined as a p value for the outcome less than $1 \times 10 - 5$) were manually examined and eliminated from the harmonized data. (6) The MR-PRESSO test was applied to monitor potential horizontal pleiotropy and detect outliers. After removing outliers, the remaining SNP list was used for subsequent MR analysis. (7) To preserve the stability of our results, we retained only IVs for at least three SNPs.

In our study, the inverse variance weighting (IVW) method was used as the primary method for assessing the causal relationship between exposure and outcome, with the weighted median (WM) and MR–Egger methods used as supplements. IVW assumes that all IVs are valid without pleiotropy, and it performs a meta-analysis of Wald ratio estimates for each SNP [27, 28]. With at least 50% of the IVs being valid, the weighted median still provides a reliable causal estimate despite the presence of heterogeneous horizontal pleiotropy [29]. MR–Egger is based on regression modeling in a similar way to IVW but allows for the possibility of pleiotropy [30, 31]. Even if all selected IVs are invalid, the MR–Egger method still produces unbiased estimates, with an intercept that is also used to detect horizontal pleiotropy [30]. The results were considered reliable and were subjected to subsequent sensitivity analyses only if the results of the IVW method reached a threshold of $p < 0.05$ and if the three methods estimated the effect in the same direction [32].

We performed several sensitivity analyses to ensure the stability of the MR results. As mentioned previously, we used MR PRESSO as a sensitivity analysis technique for detecting horizontal pleiotropy [33]. The MR–Egger intercept was also calculated to identify potential horizontal pleiotropy [30, 31]. Close-to-zero MR–Egger intercepts indicated that none of the selected genetic variants exhibited pleiotropic effects. Cochran's Q test was used to evaluate the heterogeneity among available SNPs [27]. In addition, leave-one-out analyses were carried out to assess the validity of causal inferences and to determine whether the causal signal was driven by a single SNP or bias [34].

A rigorous Bonferroni correction was applied to address the problem of multiple comparisons of the gut microbiota. A significance threshold of $P_{IVW}$ values less than 0.025 (0.05/2, two MR analyses of the gut microbiota) was used to identify the prominence of the causal influence of the gut microbiota on OC and EOC typing. Given the high false-positive rate of the Bonferroni correction, we performed a false discovery rate (FDR) correction on the primary IVW results of 1400 metabolites via the Benjamini–Hochberg procedure [35]. A significance threshold of an $FDR < 0.2$ suggests a substantial association, whereas a $PIVW < 0.05$ and an $FDR > 0.2$ imply a suggestive association [36]. The overall statistical analysis was performed in the R program (version 4.1.3) with the two sample MR package (version 0.5.6).

### Network pharmacology analysis

We validated the metabolites via PubChem (https://pubchem.ncbi.nlm.nih.gov/). Swiss Target Prediction (http://www.swisstargetprediction.ch/) was further used to obtain relevant targets for the metabolites. The keyword "ovarian cancer" was obtained by searching the following databases: the GeneCards (https://www.genecards.org/), OMIM (https://omim.org/), TTD (https://db.idrblab.net/ttd/) and CTD (https://ctdbase.org/) databases for disease targets. Targets of protective and risky metabolites were crossed with disease targets and imported into the STRING database (https://cn.string-db.org/). The

obtained data were entered into Cytoscape software to construct the PPI network. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of the core targets was performed via the annotation, visualization and integrated discovery database DAVID (https://david.ncifcrf.gov/). A "metabolite-target-pathway" network was subsequently constructed via Cytoscape software for visualization and analysis.

## Molecular docking

Molecular docking of each metabolite to its corresponding core target. SDF structure files of metabolites were downloaded from the PubChem database, and PDB files of core protein receptors were downloaded from the RSCB PDB (https://www.rcsb.org/) database. Metabolite and protein structure files were processed via AutoDock software. AutoDock Vina was run to dock the processed metabolites to the target proteins 10 times, and the lowest binding energy of each docking was used as the final result. The results were also visualized and analyzed by PyMOL and software.

## Results

### Selection of instrumental variables

In this study, we set the suggested significance at $p < 1 \times 10^{-5}$. After LD clumping and harmonization, we identified 6,693 and 34,843 SNPs associated with the gut microbiota and metabolites, respectively. SNPs with ambiguous or palindromic alleles were removed. Moreover, SNPs that had substantial associations with outcomes and F statistics < 10 were eliminated. On the basis of the MR-PRESSO results, SNPs exhibiting pleiotropy were discarded. As shown in Supplementary Tables A1-A6 and B1-B6, 4,177 and 32,389 SNPs were selected for the MR analysis of the gut microbiota and metabolites in the OC and EOC subtypes, respectively. The results of the detailed MR analyses of all included gut microbiota and metabolites associated with OC and EOC subtyping are shown in Supplementary Tables C1-C3, respectively.

### Causal associations of the gut microbiota with OC and EOC subtypes

Drawing on the GWAS for gut microbiota data from the MiBioGen consortium and DMP, we identified 21 causal relationships of the gut microbiota with OC and EOC subtyping (Fig. 2 and Table C4), 13 of which were at the genus and species levels. The findings revealed suggestive correlations, identifying 9 negative correlations as potential protective factors. A greater abundance of the genus *Family XIII AD3011 group* (OR = 0.875, 95% CI = 0.755, 0.974; $P = 0.018$) was predicted to be adversely correlated with OC, with a notable protective effect. Similarly, the abundances of the genus *Family XIII AD3011 group* (OR = 0.809, 95% CI = 0.695, 0.941, $P = 0.006$) and species *Roseburia hominis* (OR = 0.828, 95% CI = 0.703, 0.976, $P = 0.024$) were negatively associated with the HGSC; the abundance of the genus *Collinsella* (OR = 0.517, 95% CI = 0.305, 0.876, $P = 0.014$) was inversely correlated with the LGSC; the abundance of the species *Bifidobacterium bifidum* (OR = 0.884, 95% CI = 0.796, 0.983, $P = 0.022$) was adversely associated with the EMOC; and the abundance of the genus *Oscillibacter* (OR = 0.715, 95% CI = 0.538,

| Exposure | Outcome | Method | Pval | OR(95% CI) | Source |
|---|---|---|---|---|---|
| phylum Firmicutes | OC | IVW | 0.009 | 1.186 (1.043 to 1.349) | DMP |
| class Clostridia | OC | IVW | 0.005 | 1.199 (1.055 to 1.362) | DMP |
| order Clostridiales | OC | IVW | 0.005 | 1.199 (1.055 to 1.362) | DMP |
| genus Dialister | OC | IVW | 0.024 | 1.115 (1.015 to 1.226) | DMP |
| genus Family XIII AD3011 group | OC | IVW | 0.018 | 0.857 (0.755 to 0.974) | MiBioGen |
| species Bacteroides massiliensis | OC | IVW | 0.011 | 1.154 (1.034 to 1.288) | DMP |
| phylum Firmicutes | HGSC | IVW | 0.007 | 1.235 (1.061 to 1.439) | DMP |
| class Betaproteobacteria | HGSC | IVW | 0.011 | 0.811 (0.689 to 0.953) | MiBioGen |
| genus Family XIII AD3011 group | HGSC | IVW | 0.006 | 0.809 (0.695 to 0.941) | MiBioGen |
| species Roseburia hominis | HGSC | IVW | 0.024 | 0.828 (0.703 to 0.976) | DMP |
| genus Collinsella | LGSC | IVW | 0.014 | 0.517 (0.305 to 0.876) | MiBioGen |
| genus Ruminiclostridium5 | LGSC | IVW | **<0.001** | 2.483 (1.503 to 4.101) | MiBioGen |
| species Paraprevotella clara | LGSC | IVW | 0.025 | 1.317 (1.036 to 1.674) | DMP |
| genus Phascolarctobacterium | MOC | IVW | 0.022 | 1.272 (1.036 to 1.562) | DMP |
| species Phascolarctobacterium succinatutens | MOC | IVW | 0.021 | 1.272 (1.037 to 1.562) | DMP |
| species Bacteroides dorei | MOC | IVW | 0.005 | 1.450 (1.116 to 1.884) | DMP |
| species Bifidobacterium bifidum | EMOC | IVW | 0.022 | 0.884 (0.796 to 0.983) | DMP |
| order Clostridiales | CCOC | IVW | 0.009 | 0.610 (0.422 to 0.882) | MiBioGen |
| genus Oscillibacter | CCOC | IVW | 0.021 | 0.715 (0.538 to 0.950) | MiBioGen |
| genus Collinsella | CCOC | IVW | 0.009 | 1.701 (1.145 to 2.529) | DMP |
| genus Butyrivibrio | CCOC | IVW | 0.009 | 0.788 (0.659 to 0.944) | DMP |




**Fig. 2** Forest plot of the causal relationships of the gut microbiota with OC and EOC subtypes estimated via the IVW method

0.950, $P = 0.021$) and the genus *Butyrivibrio* (OR = 0.788, 95% CI = 0.659, 0.944, $P = 0.009$) were negatively correlated with the CCOC.

Twelve positive causal associations were identified as potential risk factors, as shown in Fig. 2: the abundance of the phylum *Firmicutes* was strongly related to the risk of OC (OR = 1.186, 95% CI = 1.043, 1.349, $P = 0.009$) and HGSC (OR = 1.235, 95% CI = 1.061, 1.439, $P = 0.007$); the abundance of the genus *Dialister* (OR = 1.115, 95% CI = 1.015, 1.226, $P = 0.024$) and the abundance of the species Bacteroides massiliensis (OR = 1.154, 95% CI = 1.034, 1.288, $P = 0.011$) were positively correlated with OC; and the abundance of the genus *Ruminiclostridium5* (OR = 2.483, 95% CI = 1.503, 4.101, $P < 0.001$) and the abundance of the species *Paraprevotella clara* (OR = 1.317, 95% CI = 1.036, 1.674, $P = 0.0247$) were directly associated with the LGSC; the genus *Phascolarctobacterium* (OR = 1.272, 95% CI = 1.036, 1.562, $P = 0.022$), species *Phascolarctobacterium succinatutens* (OR = 1.272, 95% CI = 1.037, 1.562, $P = 0.021$) and species *Bacteroides dorei* (OR = 1.450, 95% CI = 1.116, 1.884, $P = 0.005$) were positively correlated with MOC; genus *Collinsella* (OR = 1.701, 95% CI = 1.145, 2.529, $P = 0.009$) was predictively associated with CCOC. Higher abundance of these gut microbes indicates a higher risk of developing OC and EOC typing. The scatterplot (Fig. S1) depicts the causal relationship between the gut microbiota and patient outcomes.

### Causal relationships of plasma metabolites with OC and EOC subtypes

Among all included metabolites, a total of 11 causal associations were established after FDR correction (Fig. 3 and Table C5). Butyric acid included caffeine and caffeic acid metabolites (5-acetylamino-6-amino-3-methyluracil (OR = 1.116, 95% CI = 1.060, 1.174, $P < 0.001$), 5-acetylamino-6-formylamino-3-methyluracil (OR = 1.071, 95% CI = 1.031, 1.113, $P < 0.001$), 3-(3-hydroxyphenyl) propionate (OR = 1.602, 95% CI = 1.243, 2.065, $P < 0.001$)), sphingolipid and ceramide metabolites (ceramide (d18:1/14:0, d16:1/16:0 (OR = 1.122, 95% CI = 1.055, 1.194, $P < 0.001$)), hydroxypalmitoyl sphingomyelin (d18:1/16:0(OH)) (OR = 1.122, 95% CI = 1.036, 1.160, $P = 0.001$)), phenylalanine metabolites (N-lactoyl phenylalanine (OR = 0.849, 95% CI = 0.769, 0.937, $P = 0.001$)), butyric acid metabolite (2R,3R-dihydroxybutyrate (OR = 0.905, 95% CI = 0.857, 0.955, $P < 0.001$)) and other lipid metabolites (Linolenoylcarnitine (C18:3) (OR = 0.876, 95% CI = 0.810, 0.947, $P < 0.001$) and 1-(1-enyl-palmitoyl)-GPC (p-16:0) (OR = 0.677, 95% CI = 0.554, 0.826, $P < 0.001$)).

Caffeic acid and caffeine metabolites as well as sphingomyelin and ceramide metabolites were recognized as risk factors, whereas phenylalanine metabolites, butyric acid metabolites, and other lipid metabolites were identified as protective factors. The scatter plot (Fig. S2) shows the causal relationships between metabolites and outcomes.

Given that HGSC, LGSC, MOC, and EMOC typing was inconclusive after FDR correction, we proceeded to analyze KEGG pathways for plasma metabolites with potential associations and integrate the results. As shown in Fig. 4, each subtyping method revealed interrelated metabolites as well as enrichment pathways. Among the top-ranked pathways were arginine biosynthesis, arginine and proline metabolism, glyoxylate and dicarboxylate metabolism, propanoate metabolism, and starch and sucrose metabolism.

### Sensitivity analysis

The F statistics of all the chosen IVs were greater than 10, indicating that there was no weak IV bias (Supplementary Tables A1–A6, B1–B6). Both the MR–PRESSO global test and the MR Egger intercept p values were greater than 0.05, suggesting the absence of horizontal pleiotropy and potential outlier IVs. No obvious evidence of general heterogeneity was shown by Cochran's Q test (all $p > 0.05$). Furthermore, as shown by the leave-one-out test (Supplementary Fig. S3 and S4), no single SNP significantly affected the MR estimate, which further supports the stability of our findings.

### Network pharmacology and molecular docking

A total of four metabolites were identified and successfully predicted as targets, namely, 2R,3R-dihydroxybutyrate and N-lactoyl phenylalanine, which have protective

| Exposure | Outcome | Method | Pval | FDR | OR(95% CI) |
|---|---|---|---|---|---|
| 5−acetylamino−6−amino−3−methyluracil levels | OC | IVW | <0.001 | 0.0381 | 1.116 (1.060 to 1.174) |
| 5−acetylamino−6−formylamino−3−methyluracil levels | OC | IVW | <0.001 | 0.1114 | 1.071 (1.031 to 1.113) |
| Ceramide (d18:1/14:0, d16:1/16:0) levels | OC | IVW | <0.001 | 0.1004 | 1.122 (1.055 to 1.194) |
| Hydroxypalmitoyl sphingomyelin (d18:1/16:0(OH)) levels | OC | IVW | 0.001 | 0.1956 | 1.096 (1.036 to 1.160) |
| 2R,3R−dihydroxybutyrate levels | OC | IVW | <0.001 | 0.1004 | 0.905 (0.857 to 0.955) |
| Linolenoylcarnitine (C18:3) levels | OC | IVW | <0.001 | 0.1861 | 0.876 (0.810 to 0.947) |
| N−lactoyl phenylalanine levels | OC | IVW | 0.001 | 0.1861 | 0.849 (0.769 to 0.937) |
| 3−(3−hydroxyphenyl)propionate levels | CCOC | IVW | <0.001 | 0.1916 | 1.602 (1.243 to 2.065) |
| 1−(1−enyl−palmitoyl)−GPC (p−16:0) levels | CCOC | IVW | <0.001 | 0.1774 | 0.677 (0.554 to 0.826) |




**Fig. 3** Forest plot of the causal relationships of plasma metabolites with OC and EOC subtypes estimated via the IVW method

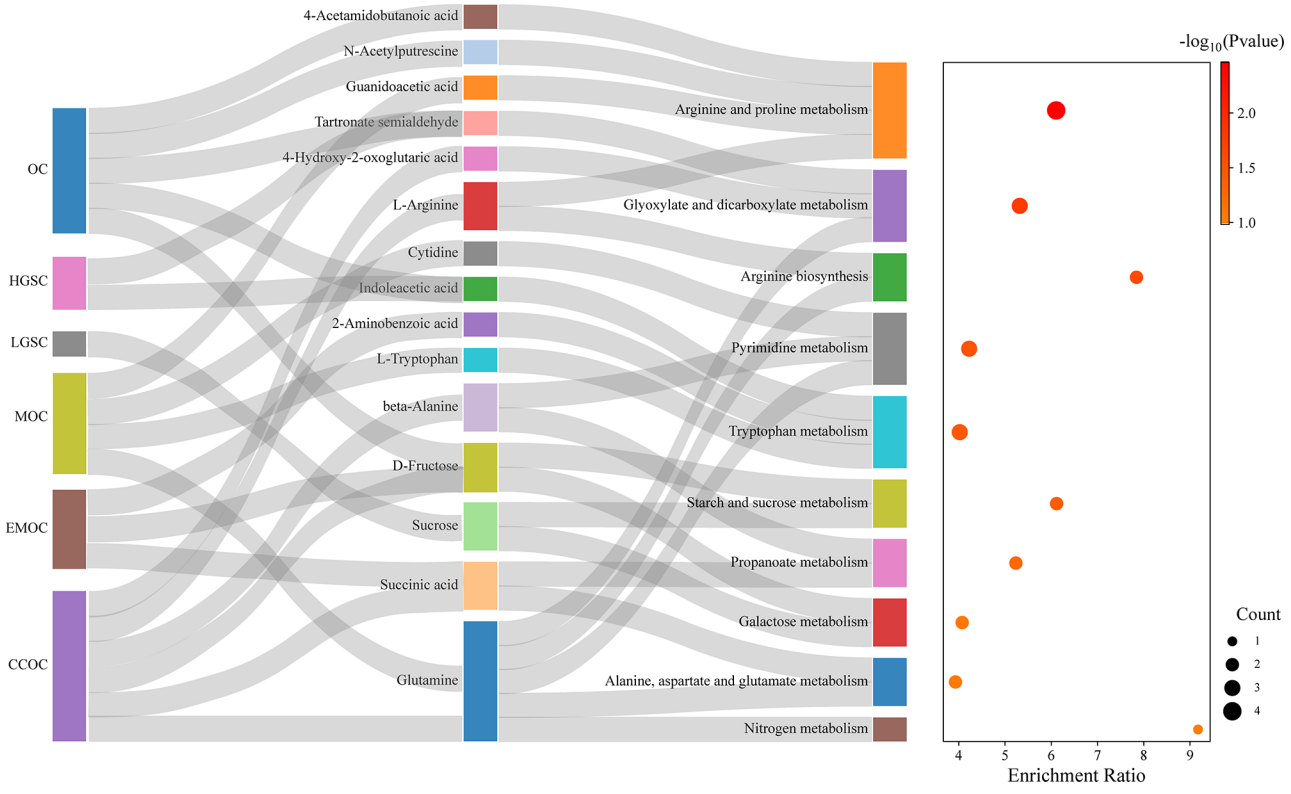


**Fig. 4** KEGG pathway enrichment analysis of the potentially associated metabolites in the OC and EOC subtypes

effects, and 5-acetylamino-6-amino-3-methyluracil and 3-(3-hydroxyphenyl) propionate, which are potentially at risk. A Venn analysis of the targets associated with metabolites and OC is shown in Fig. 5A, which revealed a total of 26 protective metabolite targets and 18 risk metabolite targets associated with OC. PPI analysis of these targets revealed that CTNNB1 and PTGS2 are located in the core region of the protective metabolite network, whereas PARP1 and EGFR are in the core region of the dangerous metabolite network (Fig. 5C, D), revealing that these core targets play important regulatory roles. KEGG analysis revealed that protective targets (Fig. 5E) were enriched mainly in cancer pathways, the NF−kappa B pathway, the adhesion plaque pathway and the TNF signaling pathway. The risk factors (Fig. 5F) were enriched in various cancers, including breast cancer, colorectal cancer and bladder cancer. The "metabolite-target-pathway" network allows for a more intuitive view of these connections (Fig. 5B). We then performed molecular docking of the metabolites with the corresponding relevant core targets (Fig. 6). Visualization of the receptor−ligand interactions revealed that all the docking complexes were able to form 1–3 hydrogen bonds while possessing low binding energies, which are essential for the formation of stable complexes. These findings demonstrate that metabolites may have protective or pathogenic effects on OC through relevant targets.

## Discussion

This study represents seminal MR research on the potential causal relationships between the gut microbiota and plasma metabolites and between OC and EOC subtypes on the basis of large-scale GWAS summary data. Our results demonstrated that specific gut microbes and metabolites exert potentially beneficial or deleterious effects on OC and EOC subtypes.

With technological advances and cost reductions in sequencing and metabolomics, interest in the role of the gut microbiota and metabolites in cancer has recently increased, which may provide prospective answers for cancer diagnostics, prevention, and treatment in the future [37, 38].

A high abundance of the *Family XIII AD3011 group* at the genus level was a protective factor against OC, and *Dialister* was a risk factor in our study. At the species level, the abundance of *Bacteroides massiliensis* from the genus *Bacteroides* was positively correlated with OC. The relative abundance of bacteria (*B. massiliensis*) with the β-glucuronidase gene has been reported to be greater in prostate cancer patients [39]. The uncoupling activity of β-glucuronidase leads to increased levels of free estrogen in the blood, resulting in dysregulation of endocrine homeostasis, which is one of the carcinogenic factors of OC [13, 40]. Free radicals activated by reactive metabolites from estrogen and cytochrome P450 enzymes can

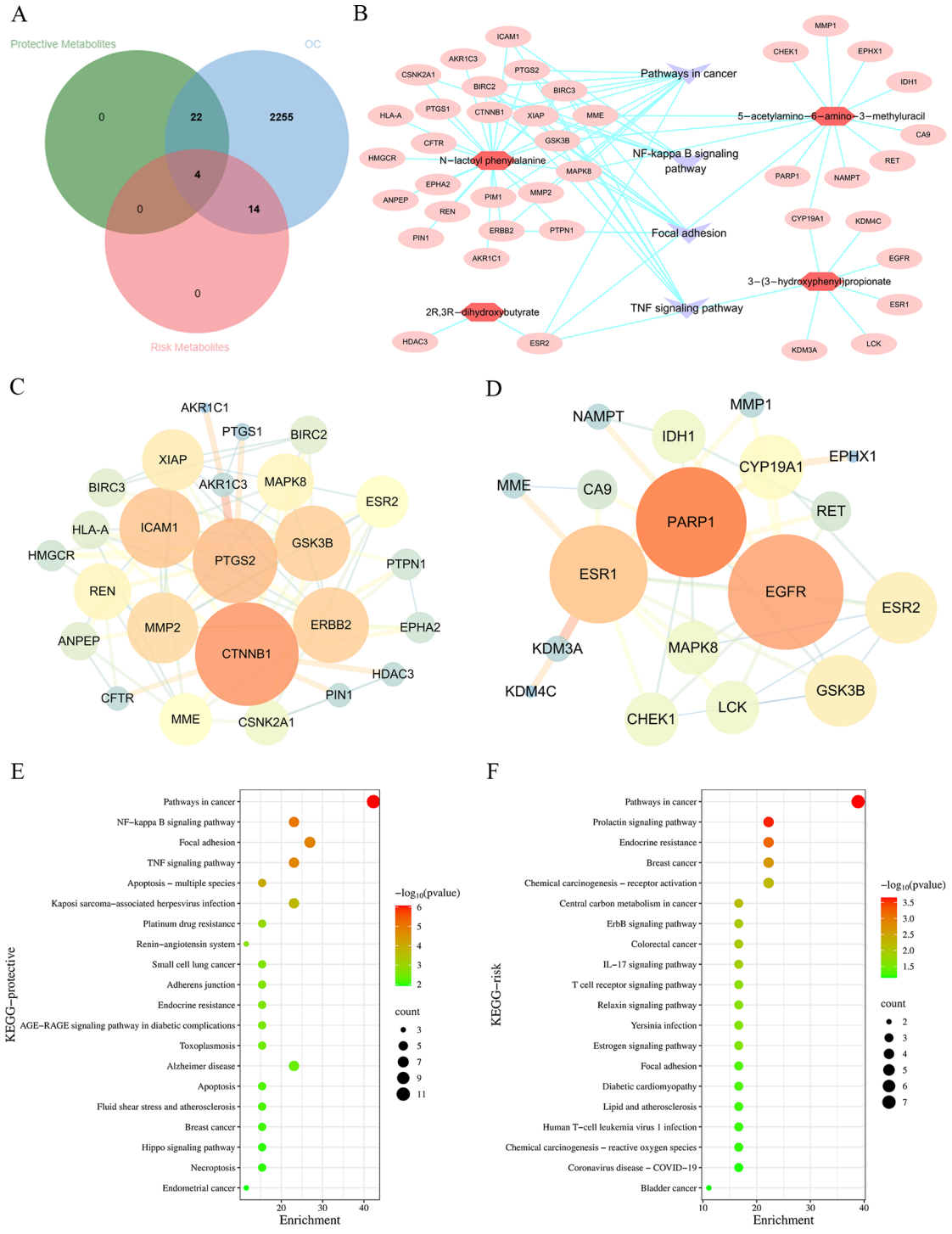


**Fig. 5** Venn analysis of protective metabolites, risk metabolites and ovarian cancer targets. **B** "Metabolite–target–pathway" network diagram. **C** PPI analysis of protective targets. **D** PPI analysis of risk targets. **E** KEGG pathway enrichment analysis of protective targets. **F** KEGG pathway enrichment analysis of risk targets

cause mutations. The accumulation of mutations leads to tumor transformation of proliferating cells [41, 42]. In cancer cells, estrogen induces the expression of extracellular signal-regulated kinase (ERK), phosphatidylinositol 3-kinase (PI3K), and epidermal growth factor receptor (EGFR), thus increasing cell proliferation. Estrogen also has the ability to produce purine sites in DNA to cause mutations and stimulate tumorigenesis [43]. In addition, the abundance of *B. massiliensis* was associated with a shorter progression-free survival zone in patients with

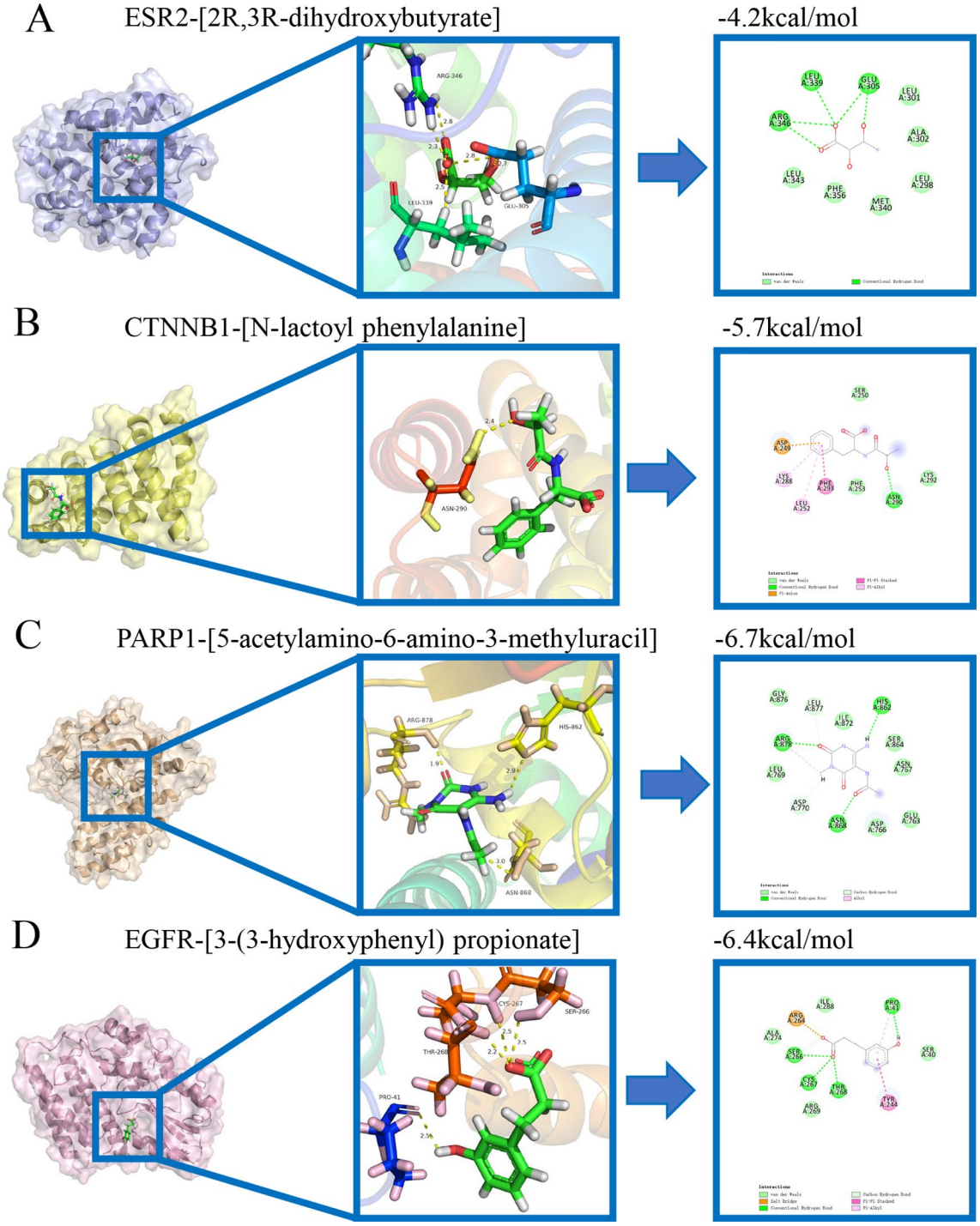


**Fig. 6** Diagram of the molecular docking patterns of metabolites with target proteins (2D and 3D)

melanoma [44] and was also a tumor tissue-specific bacterial biomarker for CRC [45]. The abundance of *B. massiliensis* was also found to be positively correlated with lipid metabolism biomarkers (e.g., TC, LDL, NEFA, and TG) and with insulin resistance [46], which could promote the proliferation of OC cells and reduce their sensitivity to cisplatin [47].

The high abundance of the *Family XIII AD3011 group* was a protective factor against HGSC at the genus level, and *Roseburia hominis* was identified at the species level. *R. hominis* acts as a major butyrate-producing bacterium [48], producing butyrate to maintain intestinal health and alleviate inflammatory bowel disease, diabetes, and colon cancer [49], as well as a potential preventive and

therapeutic agent for cancer by functioning as a histone deacetylase inhibitor [50]. Butyrate has been shown to enhance the antitumor effects of CD8 + T cells in experimental animal models of colorectal cancer and pancreatic cancer [51, 52]. At the genus level, a high abundance of *Collinsella* was a protective factor for LGSC, whereas *Ruminiclostridium5* was a risk factor. Moreover, the abundance of *Paraprevotella clara* at the species level was a risk factor for LGSC, but this genus has been less studied and has potential research value. Notably, the genus *Collinsella* was identified as having dual roles as a protective agent for LGSC and as a potential risk factor for CCOC. By inhibiting cytokine storm syndrome, *Collinsella* can produce ursodeoxycholate to prevent COVID-19 infection [53]. In contrast, another study suggested that *Collinsella* could lead to a loss of intestinal barrier integrity, which is associated with rheumatoid arthritis in humans [54]. It is possible that the difference in the category of the species led to this result. A high abundance of *Phascolarctobacterium succinatutens* and *Bacteroides dorei* are risk factors for MOC at the species level. Both are recently discovered species isolated from the human gut and are of potential interest for research [55, 56]. The findings of a large cohort study from Finland supported the association of *B. dorei* with the development of type 1 diabetes in children at high risk of autoimmunity [57]. At the species level, a high abundance of *Bifidobacterium bifidum* was a protective factor against EMOC. *B. bifidum*, the most common probiotic in the human body, helps maintain the balance of the intestinal flora, promotes immunity and is used in the food market worldwide [58]. Recently, research has revealed that *B. bifidum* is the second most predominant strain found in breastfed infants [59], and it has strong antitumor effects [60]. Furthermore, the administration of probiotic supplements consisting of *B. bifidum* and other probiotics had significant beneficial effects on mental health parameters, hormones, inflammation and oxidative stress in women with polycystic ovary syndrome [61]. It has been shown that *B. bifidum* produces indole compounds [62], improves fat metabolism and inflammation, and interacts with estrogen receptors to inhibit the growth of ovarian cancer [63]. Finally, at the genus level, high abundances of *Oscillibacter* and *Butyrivibrio* were protective factors against CCOC. *Oscillibacter* is an enigmatic genus of bacteria that has never been cultured; however, it has recently been hypothesized by researchers to be a producer of the short-chain fatty acid butyrate [64]. Butyrate not only improves the efficacy of anti-PD-1 against tumors by activating cytotoxic CD8 T + cells [51, 52], but also induces iron death in endometrial cancer cells [65]. However, *Byrivibrio* species are more important producers of butyrate, and *Butyrivibrio* species also produce acetic acid and lactic acid [66], all of which have antitumor effects. Acetate metabolism is an important metabolic pathway in many cancers and is controlled by acetyl coenzyme A synthase 2 (ACSS2). Targeting ACSS2 to inhibit acetate conversion promotes anti-tumor immune responses and enhances the efficacy of chemotherapy in preclinical breast cancer models [67]. Therefore, gut microbiota-derived metabolites are key hubs connecting the gut microbiome and cancer progression, and deciphering the specific link between the two is of great importance [68]. In one study, the gut microbiota-derived metabolites propionate, KYNA, and indole-3-carboxaldehyde were shown to protect patients from the side effects of radiotherapy, thereby improving survival [69]. 5-Fluorouracil (5-FU) plays an important role in the treatment of colon cancer, and the conversion of 5-FU to inactive dihydrofluorouracil by the enzyme dihydropyrimidine dehydrogenase in *Escherichia coli* significantly reduces the effectiveness of treatment [70]. Overall, the gut microbiota operates as a complex ecosystem, and further research is essential to reveal the exact role that each specific bacterium plays in these causal relationships.

To determine the potentially critical role of metabolites in OC and EOC subtypes, we performed MR analyses to investigate any potential links between them. Interestingly, higher levels of genetically predicted 2R,3R-dihydroxybutyrate, a form of butyrate, exhibited a protective effect against OC. Similarly, the KEGG pathway of metabolites was highly enriched in glyoxylate and dicarboxylic acid metabolism as well as propionate metabolism. In addition to these findings, short-chain fatty acids (SCFAs), especially acetate, propionate and butyrate [71], have shown powerful antitumor effects [51, 52, 72, 73]. SCFAs also play important physiological roles as intracellular signaling factors, maintaining metabolic homeostasis by binding to SCFA receptors [74]. The KEGG pathway was also highly enriched in arginine biosynthesis as well as arginine and proline metabolism, implying that these amino acids play important roles in the development of OC. Curiously, it has been reported [75] that hepatocellular carcinoma cells have an increased demand for and dependence on arginine. A large amount of arginine and its binding protein RBM39 can regulate the metabolic reprogramming of hepatocellular carcinoma cells to promote growth and proliferation by promoting the uptake of arginine through positive feedback. Similarly, proline metabolism is associated with ATP production, protein and nucleotide synthesis, and redox homeostasis in tumor cells [76]. Our study also revealed that high levels of N-lactoyl phenylalanine were a significant protective factor against OC. A recent paper published in *Nature* reported that N-lactoyl phenylalanine, a circulating signaling metabolite, selectively inhibited feeding and obesity in mice fed a high-fat diet [77],

whereas obesity and high-fat status are important risk factors for OC [78]. Thus, we believe that N-lactoyl phenylalanine has beneficial biological effects in the treatment of OC.

Furthermore, high levels of sphingomyelin (SM)- and ceramide (Cer)-related metabolites were found in our study to be correlated with a high risk of OC. Cer is a pro-apoptotic signaling molecule in OC [79, 80] with potential metastasis inhibitory properties [81]. Cer levels are very low in ovarian tumors [79, 82]' and the main source is SM metabolism, which appears to be greater in ovarian tumors than in normal tissues [83]. Cer and its metabolites, however, have dynamic opposite effects on cancer biology, a phenomenon known as "sphingolipid rheology" [84, 85]. Cer promotes apoptosis and inhibits the proliferation and migration of cancer cells, while its downstream metabolite sphingosine 1-phosphate promotes cell proliferation and angiogenesis [84, 85]. SM controls the balance between cell proliferation and apoptosis and often has cancer-specific effects [84].

In this study, we found that specific gut microbes and metabolites were associated with the risk of OC, as this may contribute to the development of new biomarkers for the early identification and diagnosis of OC, which is crucial for improving patient survival and treatment success since patients with ovarian cancer detected at an early stage have higher chances of being cured. It will also help develop targeted dietary or probiotic interventions to modulate the gut microbiota to reduce the risk of OC. The findings also identified potential targets of specific metabolites for the treatment of OC, as well as signaling pathways and biological processes associated with the metabolites, which could help in drug development. Although our study is the first two-sample MR analysis to explore the causal effects of the gut microbiota and metabolites between OC and EOC subtypes, several limitations must be considered in utilizing the largest available GWAS dataset. First, although most participants in the GWAS summary data were of European ancestry, a small portion of the gut microbiota data came from other ethnic groups, which may have biased our findings. Hence, the generalizability of our findings to different racial backgrounds may be limited. Second, despite the use of the largest available GWAS dataset of gut microbiota and metabolites, the sample size remains relatively small. Third, cancer patients tend to be more likely to be elderly, but our study did not take age differences into account. Finally, integrated multi-omics analysis including microbial gene and module analysis should likely better explain the potential link between gut microbes and plasma metabolites as well as their mechanisms of risk and protection for OC, which is a limitation of our work and the focus of our next steps.

## Conclusion

In summary, our study supports the potential causal influence of the gut microbiota and metabolites on the relationship between OC and EOC subtypes. The identification of beneficial and harmful gut microbes and metabolites associated with OC and EOC subtypes provides valuable insights for early identification and diagnosis. Deeper studies are needed to validate the causal effects of the gut microbiota and metabolites on OC and EOC subtyping, as well as the underlying mechanisms driving these relationships.

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13048-025-01610-9.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

### Acknowledgements

The authors express their gratitude to the participants and investigators of the Ovarian Cancer Association Consortium. The authors also appreciate the MiBioGen consortium and Dutch Microbiome Project for releasing the gut microbiota GWAS summary statistics.

### Author contributions

G.J. and D.C. conceived and designed the study. G.J. and W.C. wrote the manuscript. G.J., W.C. and L.H. collected and analyzed data. All authors contributed and approved the manuscript.

### Funding

This research was funded by the National Natural Science Foundation of China (82174135, 82374163) and the Specialized Discipline of Chinese Medicine and Liver Disease in Summit Plateau, Pudong New Area (YC-2023-0610) to H.L.

### Data availability

Data is provided within the manuscript or supplementary information files.

## Declarations

### Competing interests

The authors declare no competing interests.

### Author details

[1]Department of Traditional Chinese Medicine, Renji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai 200127, China
[2]Department of Traditional Chinese Medicine, School of Medicine, Pujiang Hospital, Minhang Campus of Renji Hospital, Shanghai Jiao Tong University, Shanghai 201112, China

Received: 7 October 2024 / Accepted: 24 January 2025
Published online: 13 February 2025

### References

1. Webb PM, Jordan SJ. Global epidemiology of epithelial ovarian cancer. Nat Rev Clin Oncol 2024.
2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin. 2020;70(1):7–30.

3. Lheureux S, Braunstein M, Oza AM. Epithelial ovarian cancer: evolution of management in the era of precision medicine. CA Cancer J Clin. 2019;69(4):280–304.
4. Peres LC, Cushing-Haugen KL, Köbel M, Harris HR, Berchuck A, Rossing MA, Schildkraut JM, Doherty JA. Invasive epithelial ovarian Cancer survival by Histotype and Disease Stage. J Natl Cancer Inst. 2019;111(1):60–8.
5. White M, Sears C. The microbial landscape of colorectal cancer. Nat Rev Microbiol 2023.
6. Routy B, Lenehan JG, Miller WH Jr., Jamal R, Messaoudene M, Daisley BA, Hes C, Al KF, Martinez-Gili L, Punčochář M, et al. Fecal microbiota transplantation plus anti-PD-1 immunotherapy in advanced melanoma: a phase I trial. Nat Med. 2023;29(8):2121–32.
7. Ling Y, Tian Z, Wu E, Ding L, Pei L. YINDARA-4 relieves visceral hypersensitivity in irritable bowel syndrome rats via regulation of gut microbiota and serotonin levels. Acupunct Herb Med. 2022;2(4):274–83. https://doi.org/10.1097/hm9.0000000000000042.
8. Ahmed SMH, Maldera JA, Krunic D, Paiva-Silva GO, Pénalva C, Teleman AA, Edgar BA. Fitness trade-offs incurred by ovary-to-gut steroid signalling in Drosophila. Nature. 2020;584(7821):415–9.
9. Baker JM, Al-Nakkash L, Herbst-Kralovetz MM. Estrogen-gut microbiome axis: physiological and clinical implications. Maturitas 2017, 103:45–53.
10. Prakash A, Nourianpour M, Senok A, Atiomo W. Polycystic Ovary Syndrome and Endometrial Cancer: A Scoping Review of the Literature on Gut Microbiota. Cells 2022, 11(19).
11. Liu X, Chen X, Wang C, Song J, Xu J, Gao Z, Huang Y, Suo H. Mechanisms of probiotic modulation of ovarian sex hormone production and metabolism: a review. Food Funct. 2024;15(6):2860–78.
12. Wang L, Tang L, Zhai D, Song M, Li W, Xu S, Jiang S, Meng H, Liang J, Wang Y, et al. The role of the sex hormone-gut microbiome axis in tumor immunotherapy. Gut Microbes. 2023;15(1):2185035.
13. Cross TL, Simpson AMR, Lin CY, Hottmann NM, Bhatt AP, Pellock SJ, Nelson ER, Loman BR, Wallig MA, Vivas EI, et al. Gut microbiome responds to alteration in female sex hormone status and exacerbates metabolic dysfunction. Gut Microbes. 2024;16(1):2295429.
14. Hu X, Xu X, Zeng X, Jin R, Wang S, Jiang H, Tang Y, Chen G, Wei J, Chen T, et al. Gut microbiota dysbiosis promotes the development of epithelial ovarian cancer via regulating hedgehog signaling pathway. Gut Microbes. 2023;15(1):2221093.
15. Chalif J, Wang H, Spakowicz D, Quick A, Arthur EK, O'Malley D, Chambers LM. The microbiome and gynecologic cancer: cellular mechanisms and clinical applications. Int J Gynecol Cancer 2023.
16. Johnson CH, Ivanisevic J, Siuzdak G. Metabolomics: beyond biomarkers and towards mechanisms. Nat Rev Mol Cell Biol. 2016;17(7):451–9.
17. Yang C, Lai C, Ru Y, Shen B, Wu X, Cui J, et al. Elucidating the mechanism of hepatotoxicity in Euodia rutaecarpa: insights from QSAR toxicity prediction and metabolomics. Acupunct Herb Med. 2024;4(2):257–70. https://doi.org/10.1097/HM9.0000000000000108.
18. Zeleznik OA, Clish CB, Kraft P, Avila-Pacheco J, Eliassen AH, Tworoger SS. Circulating lysophosphatidylcholines, Phosphatidylcholines, ceramides, and Sphingomyelins and Ovarian Cancer risk: a 23-Year prospective study. J Natl Cancer Inst. 2020;112(6):628–36.
19. Zeleznik OA, Eliassen AH, Kraft P, Poole EM, Rosner BA, Jeanfavre S, Deik AA, Bullock K, Hitchcock DS, Avila-Pacheco J, et al. A prospective analysis of circulating plasma metabolites Associated with Ovarian Cancer Risk. Cancer Res. 2020;80(6):1357–67.
20. Emdin CA, Khera AV, Kathiresan S. Mendelian randomization. JAMA. 2017;318(19):1925–6.
21. Richmond RC, Davey Smith G. Mendelian randomization: concepts and scope. Cold Spring Harb Perspect Med 2022, 12(1).
22. Kurilshikov A, Medina-Gomez C, Bacigalupe R, Radjabzadeh D, Wang J, Demirkan A, Le Roy CI, Raygoza Garay JA, Finnicum CT, Liu X, et al. Large-scale association analyses identify host factors influencing human gut microbiome composition. Nat Genet. 2021;53(2):156–65.
23. Lopera-Maya EA, Kurilshikov A, van der Graaf A, Hu S, Andreu-Sánchez S, Chen L, Vila AV, Gacesa R, Sinha T, Collij V, et al. Effect of host genetics on the gut microbiome in 7,738 participants of the Dutch Microbiome Project. Nat Genet. 2022;54(2):143–51.
24. Chen Y, Lu T, Pettersson-Kymmer U, Stewart ID, Butler-Laporte G, Nakanishi T, Cerani A, Liang KYH, Yoshiji S, Willett JDS, et al. Genomic atlas of the plasma metabolome prioritizes metabolites implicated in human diseases. Nat Genet. 2023;55(1):44–53.
25. Phelan CM, Kuchenbaecker KB, Tyrer JP, Kar SP, Lawrenson K, Winham SJ, Dennis J, Pirie A, Riggan MJ, Chornokur G, et al. Identification of 12 new susceptibility loci for different histotypes of epithelial ovarian cancer. Nat Genet. 2017;49(5):680–91.
26. Burgess S, Thompson SG. Avoiding bias from weak instruments in mendelian randomization studies. Int J Epidemiol. 2011;40(3):755–64.
27. Bowden J, Del Greco MF, Minelli C, Zhao Q, Lawlor DA, Sheehan NA, Thompson J, Davey Smith G. Improving the accuracy of two-sample summary-data mendelian randomization: moving beyond the NOME assumption. Int J Epidemiol. 2019;48(3):728–42.
28. Burgess S, Butterworth A, Thompson SG. Mendelian randomization analysis with multiple genetic variants using summarized data. Genet Epidemiol. 2013;37(7):658–65.
29. Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent estimation in mendelian randomization with some Invalid instruments using a weighted median estimator. Genet Epidemiol. 2016;40(4):304–14.
30. Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. Int J Epidemiol. 2015;44(2):512–25.
31. Burgess S, Thompson SG. Interpreting findings from mendelian randomization using the MR-Egger method. Eur J Epidemiol 2017, 32(5):377–89.
32. Jin Q, Ren F, Dai D, Sun N, Qian Y, Song P. The causality between intestinal flora and allergic diseases: insights from a bi-directional two-sample mendelian randomization analysis. Front Immunol. 2023;14:1121273.
33. Verbanck M, Chen CY, Neale B, Do R. Publisher correction: detection of widespread horizontal pleiotropy in causal relationships inferred from mendelian randomization between complex traits and diseases. Nat Genet. 2018;50(8):1196.
34. Hemani G, Tilling K, Davey Smith G. Correction: orienting the causal relationship between imprecisely measured traits using GWAS summary data. PLoS Genet. 2017;13(12):e1007149.
35. Benjamini Y, Hochberg Y. Controlling the false Discovery rate: a practical and powerful Approach to multiple testing. J Roy Stat Soc: Ser B (Methodol). 1995;57(1):289–300.
36. Hao X, Ren C, Zhou H, Li M, Zhang H, Liu X. Association between circulating immune cells and the risk of prostate cancer: a mendelian randomization study. Front Endocrinol (Lausanne) 2024, 15:1358416.
37. Zhang J, Wang P, Wang J, Wei X, Wang M. Unveiling intratumoral microbiota: an emerging force for colorectal cancer diagnosis and therapy. Pharmacol Res 2024:107185.
38. Cao B, Li Y, Lin M, Xu J, Li T, Fei X, et al. Integrated analysis of metabolomic and gut microbiota reveals idiosyncratic drug-induced liver injury resulting from the combined administration of bavachin and icariside II. Acupunct Herb Med. 2024;4(2):222–33. https://doi.org/10.1097/HM9.0000000000000099.
39. Garbas K, Zapała P, Zapała Ł, Radziszewski P. The role of microbial factors in prostate Cancer Development-An Up-to-date review. J Clin Med 2021, 10(20).
40. Li D, Sun T, Tong Y, Le J, Yao Q, Tao J, Liu H, Jiao W, Mei Y, Chen J, et al. Gut-microbiome-expressed 3β-hydroxysteroid dehydrogenase degrades estradiol and is linked to depression in premenopausal females. Cell Metab. 2023;35(4):685–e694685.
41. Chang CY, McDonnell DP. Molecular pathways: the metabolic regulator estrogen-related receptor α as a therapeutic target in cancer. Clin Cancer Res. 2012;18(22):6089–95.
42. Gajjar K, Martin-Hirsch PL, Martin FL. CYP1B1 and hormone-induced cancer. Cancer Lett. 2012;324(1):13–30.
43. Mungenast F, Thalhammer T. Estrogen biosynthesis and action in ovarian cancer. Frontiers in Endocrinology 2014, 5(NOV).
44. Peters BA, Wilson M, Moran U, Pavlick A, Izsak A, Wechter T, Weber JS, Osman I, Ahn J. Relating the gut metagenome and metatranscriptome to immunotherapy responses in melanoma patients. Genome Med. 2019;11(1):61.
45. Hasan R, Bose S, Roy R, Paul D, Rawat S, Nilwe P, Chauhan NK, Choudhury S. Tumor tissue-specific bacterial biomarker panel for colorectal cancer: Bacteroides massiliensis, Alistipes species, Alistipes onderdonkii, Bifidobacterium pseudocatenulatum, Corynebacterium appendicis. Arch Microbiol. 2022;204(6):348.
46. Ma X, Qiu Y, Mao M, Lu B, Zhao H, Pang Z, Li S. PuRenDan alleviates type 2 diabetes mellitus symptoms by modulating the gut microbiota and its metabolites. J Ethnopharmacol 2024, 322:117627.
47. Barczyński B, Frąszczak K, Kotarski J. Perspectives of metformin use in endometrial cancer and other gynaecological malignancies. J Drug Target. 2022;30(4):359–67.

48. Bhattacharya A, Majtorp L, Birgersson S, Wiemann M, Sreenivas K, Verbrugghe P, Van Aken O, Van Niel EWJ, Stålbrand H. Cross-feeding and enzymatic catabolism for Mannan-Oligosaccharide utilization by the butyrate-producing gut bacterium Roseburia hominis A2-183. Microorganisms 2022, 10(12).
49. Fung KY, Cosgrove L, Lockett T, Head R, Topping DL. A review of the potential mechanisms for the lowering of colorectal oncogenesis by butyrate. Br J Nutr. 2012;108(5):820–31.
50. Davie JR. Inhibition of histone deacetylase activity by butyrate. J Nutr. 2003;133(7 Suppl):s2485–93.
51. Tran NL, Lee IK, Choi J, Kim SH, Oh SJ. Acetate decreases PVR/CD155 expression via PI3K/AKT pathway in cancer cells. BMB Rep. 2021;54(8):431–6.
52. Luu M, Riester Z, Baldrich A, Reichardt N, Yuille S, Busetti A, Klein M, Wempe A, Leister H, Raifer H, et al. Microbial short-chain fatty acids modulate CD8(+) T cell responses and improve adoptive immunotherapy for cancer. Nat Commun. 2021;12(1):4077.
53. Hirayama M, Nishiwaki H, Hamaguchi T, Ito M, Ueyama J, Maeda T, Kashihara K, Tsuboi Y, Ohno K. Intestinal Collinsella may mitigate infection and exacerbation of COVID-19 by producing ursodeoxycholate. PLoS ONE. 2021;16(11):e0260451.
54. Tsetseri MN, Silman AJ, Keene DJ, Dakin SG. The role of the microbiome in rheumatoid arthritis: a review. Rheumatol Adv Pract. 2023;7(2):rkad034.
55. Watanabe Y, Nagai F, Morotomi M. Characterization of Phascolarctobacterium succinatutens sp. nov., an asaccharolytic, succinate-utilizing bacterium isolated from human feces. Appl Environ Microbiol. 2012;78(2):511–8.
56. Bakir MA, Sakamoto M, Kitahara M, Matsumoto M, Benno Y. Bacteroides dorei sp. nov., isolated from human faeces. Int J Syst Evol Microbiol. 2006;56(Pt 7):1639–43.
57. Davis-Richardson AG, Ardissone AN, Dias R, Simell V, Leonard MT, Kemppainen KM, Drew JC, Schatz D, Atkinson MA, Kolaczkowski B, et al. Bacteroides dorei dominates gut microbiome prior to autoimmunity in Finnish children at high risk for type 1 diabetes. Front Microbiol. 2014;5:678.
58. Ku S, Park MS, Ji GE, You HJ. Review on Bifidobacterium bifidum BGN4: functionality and nutraceutical applications as a probiotic microorganism. Int J Mol Sci 2016, 17(9).
59. Turroni F, Peano C, Pass DA, Foroni E, Severgnini M, Claesson MJ, Kerr C, Hourihane J, Murray D, Fuligni F, et al. Diversity of bifidobacteria within the infant gut microbiota. PLoS ONE. 2012;7(5):e36957.
60. de Roos NM, Katan MB. Effects of probiotic bacteria on diarrhea, lipid metabolism, and carcinogenesis: a review of papers published between 1988 and 1998. Am J Clin Nutr. 2000;71(2):405–11.
61. Ostadmohammadi V, Jamilian M, Bahmani F, Asemi Z. Vitamin D and probiotic co-supplementation affects mental health, hormonal, inflammatory and oxidative stress parameters in women with polycystic ovary syndrome. J Ovarian Res. 2019;12(1):5.
62. Min BH, Devi S, Kwon GH, Gupta H, Jeong JJ, Sharma SP, Won SM, Oh KK, Yoon SJ, Park HJ, et al. Gut microbiota-derived indole compounds attenuate metabolic dysfunction-associated steatotic liver disease by improving fat metabolism and inflammation. Gut Microbes. 2024;16(1):2307568.
63. Verardi L, Fiori J, Andrisano V, Locatelli A, Morigi R, Naldi M, Bertucci C, Strocchi E, Boga C, Micheletti G et al. Indole Derivative Interacts with Estrogen Receptor Beta and Inhibits Human Ovarian Cancer Cell Growth. Molecules: 2020, 25(19).
64. Konikoff T. U Gophna 2016 Oscillospira: a Central, enigmatic component of the human gut microbiota. Trends Microbiol 24 7 523–4.
65. Wang Z, Shu W, Zhao R, Liu Y, Wang H. Sodium butyrate induces ferroptosis in endometrial cancer cells via the RBM3/SLC7A11 axis. Apoptosis. 2023;28(7–8):1168–83.
66. Palevich N, Kelly WJ, Leahy SC, Denman S, Altermann E, Rakonjac J, Attwood GT. Comparative Genomics of Rumen Butyrivibrio spp. Uncovers a Continuum of Polysaccharide-Degrading capabilities. Appl Environ Microbiol 2019, 86(1).
67. Miller KD, O'Connor S, Pniewski KA, Kannan T, Acosta R, Mirji G, Papp S, Hulse M, Mukha D, Hlavaty SI, et al. Acetate acts as a metabolic immunomodulator by bolstering T-cell effector function and potentiating antitumor immunity in breast cancer. Nat Cancer. 2023;4(10):1491–507.
68. Yang Q, Wang B, Zheng Q, Li H, Meng X, Zhou F, Zhang L. A review of Gut Microbiota-Derived metabolites in Tumor Progression and Cancer Therapy. Adv Sci (Weinh). 2023;10(15):e2207366.
69. Shiao SL, Kershaw KM, Limon JJ, You S, Yoon J, Ko EY, Guarnerio J, Potdar AA, McGovern DPB, Bose S et al. Commensal bacteria and fungi differentially regulate tumor responses to radiation therapy. Cancer Cell 2021, 39(9):1202–e12131206.
70. Spanogiannopoulos P, Kyaw TS, Guthrie BGH, Bradley PH, Lee JV, Melamed J, Malig YNA, Lam KN, Gempis D, Sandy M, et al. Host and gut bacteria share metabolic pathways for anti-cancer drug metabolism. Nat Microbiol. 2022;7(10):1605–20.
71. Cummings JH, Pomare EW, Branch WJ, Naylor CP, Macfarlane GT. Short chain fatty acids in human large intestine, portal, hepatic and venous blood. Gut. 1987;28(10):1221–7.
72. Bose S, Ramesh V, Locasale JW. Acetate metabolism in Physiology, Cancer, and Beyond. Trends Cell Biol. 2019;29(9):695–703.
73. Gomes AP, Ilter D, Low V, Drapela S, Schild T, Mullarky E, Han J, Elia I, Broekaert D, Rosenzweig A, et al. Altered propionate metabolism contributes to tumour progression and aggressiveness. Nat Metab. 2022;4(4):435–43.
74. Hamer HM, Jonkers D, Venema K, Vanhoutvin S, Troost FJ, Brummer RJ. Review article: the role of butyrate on colonic function. Aliment Pharmacol Ther. 2008;27(2):104–19.
75. Mossmann D, Müller C, Park S, Ryback B, Colombi M, Ritter N, Weißenberger D, Dazert E, Coto-Llerena M, Nuciforo S et al. Arginine reprograms metabolism in liver cancer via RBM39. Cell 2023, 186(23):5068–e50835023.
76. Geng P, Qin W, Xu G. Proline metabolism in cancer. Amino Acids. 2021;53(12):1769–77.
77. Li VL, He Y, Contrepois K, Liu H, Kim JT, Wiggenhorn AL, Tanzo JT, Tung AS, Lyu X, Zushin PH, et al. An exercise-inducible metabolite that suppresses feeding and obesity. Nature. 2022;606(7915):785–90.
78. Bandera EV, Lee VS, Rodriguez-Rodriguez L, Powell CB, Kushi LH. Impact of Chemotherapy Dosing on Ovarian Cancer Survival according to body Mass Index. JAMA Oncol. 2015;1(6):737–45.
79. Morita Y, Perez GI, Paris F, Miranda SR, Ehleiter D, Haimovitz-Friedman A, Fuks Z, Xie Z, Reed JC, Schuchman EH, et al. Oocyte apoptosis is suppressed by disruption of the acid sphingomyelinase gene or by sphingosine-1-phosphate therapy. Nat Med. 2000;6(10):1109–14.
80. Prinetti A, Millimaggi D, D'Ascenzo S, Clarkson M, Bettiga A, Chigorno V, Sonnino S, Pavan A, Dolo V. Lack of ceramide generation and altered sphingolipid composition are associated with drug resistance in human ovarian carcinoma cells. Biochem J. 2006;395(2):311–8.
81. Kitatani K, Usui T, Sriraman SK, Toyoshima M, Ishibashi M, Shigeta S, Nagase S, Sakamoto M, Ogiso H, Okazaki T et al. Ceramide limits phosphatidylinositol-3-kinase C2β-controlled cell motility in ovarian cancer: potential of ceramide as a metastasis-suppressor lipid. Oncogene 2016, 35(21):2801–12.
82. Ogretmen B, Hannun YA. Biologically active sphingolipids in cancer pathogenesis and treatment. Nat Rev Cancer. 2004;4(8):604–16.
83. Fong MY, McDunn J, Kakar SS. Identification of metabolites in the normal ovary and their transformation in primary and metastatic ovarian cancer. PLoS ONE. 2011;6(5):e19963.
84. Tallima H, Azzazy HME, El Ridi R. Cell surface sphingomyelin: key role in cancer initiation, progression, and immune evasion. Lipids Health Dis. 2021;20(1):150.
85. Li RZ, Wang XR, Wang J, Xie C, Wang XX, Pan HD, Meng WY, Liang TL, Li JX, Yan PY, et al. The key role of sphingolipid metabolism in cancer: New therapeutic targets, diagnostic and prognostic values, and anti-tumor immunotherapy resistance. Front Oncol. 2022;12:941643.


## Publisher's note