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Characteristics and potential diagnostic value of gut microbiota in ovarian tumor patients

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The gut microbiota is closely related to the occurrence and development of cancer. However, the characteristics of gut microbiota associated with ovarian tumors remain elusive. In this study, fecal samples were collected from healthy control (HC) group and patients with ovarian tumor (OT) or with other benign tumor (OBT) for 16s rRNA sequencing to determine differential flora in gut microbiota. The composition of gut microbiota in the OT group, including bacterial abundance and diversity, was significantly different form HC and OBT groups. In the OT group, *Escherichia_Shigella* was markedly higher than in the HC group, while *Coprococcus*, *Fusicatenibacter*, *Butyricicoccus* and *Oscillibacter* were significantly lower than in HCs. The abundance of *Fusicatenibacter*, *Butyricicoccus*, *Coprococcus Parasutterella*, and *Anaerotruncus* in the OBT group was distinctly higher than that in the OT group, while the *Lachnospiracae_ND3007_group* was significantly lower. In addition, in OT patients, ovarian cancer (OC) and benign ovarian tumor (BOT) patients also showed a unique composition of gut microbiota. The random forest model was designed using different bacteria. Compared with HCs, area under curve (AUC) values for BOT and OC groups were 0.77 and 0.86, respectively. These findings suggest that some gut microbiota such as *Escherichia_Shigella* show a certain ability to distinguish between healthy individuals and patients with OT.

Keywords Ovarian tumors, 16s RNA sequencing, *Escherichia_Shigella*, Gut microbiota

Ovarian cancer (OC) is a malignancy which poses grave threats to female health, and has the highest mortality rates affecting the female reproductive system[1]. Due to hidden disease locations and a lack of good screening methods, most cases are at advanced stages at initial diagnosis, with tumors often showing primary or secondary resistance to chemotherapeutic drugs, and Critically, 5-year survival rates in patients with OC are between 30 and 45%[2]. Currently, the main OC treatments include radical surgery, platinum-based combined chemotherapy, and poly ADP-ribose polymerase inhibitor maintenance therapy, but due to low response rates, toxicity, and drug resistance, many patients fail to benefit from such treatments[3]. Therefore, more convenient, non-invasive, and highly sensitive OC screening methods are required.

Known as a "super organism", billions of symbiotic bacteria called the "gut microbiota" live in the human body, with the intestinal tract numbering approximately 10^{14} microorganism species[4,5]. Due to a two-way influence between sex hormone levels and the microflora, gut microbiota composition in females is significantly different to that of males; *Bacteroides* abundance in females is lower, but α-diversity indices are higher[4,5].

Intestinal microbiome disorders are associated with several cancers, including colorectal, gastric, and liver cancers[6–8], and have been observed in various female malignant tumors[9,10]. Significant differences in α- and β-diversity indices have been reported between patients with cervical cancer and healthy controls. *Prevotella, Porphyromonas,* and *Dialister* levels were higher in patients with cervical cancer, while *Bacteroides, Alistipes,* and *Lachnospiracea* levels in healthy controls were higher[10]. Some studies have reported that gut microbiota diversity in breast cancer patients was lower than that in healthy controls, while *Clostridium* abundance was increased[11]. However, few studies have explored relationships between the intestinal microbiota and OC. Jacobson et al.[12] reported that the abundance of *Prevotella* bacteria were significantly increased in OC patients compared with BOTs, regardless of their response to platinum chemotherapy.

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We explored gut microbiota differences between patients with ovarian tumors (OTs) and HCs, patients with benign ovarian tumors (BOTs) and patients with OC, and patients with OTs and other benign tumors (OBTs). Critically, our research may benefit early OT diagnoses and/or screening strategies.

Materials and methods

The study population

From May 2018 to January 2022, we collected fecal samples from 382 female individuals from Zhejiang Cancer Hospital in China, including 239 patients with OTs (148 patients with OC and 91 with BOTs), 90 patients with OBTs, and 53 with HCs. This study was investigated in compliance with the Declaration of Helsinki. All subjects provided written informed consent, and the study was approved by our local ethics committee (Approval No. IRB-2023-417). The following patients were excluded: Patients who have been exposed to antibiotics, patients who have not signed consent forms and other patients with malignant tumors in the past eight weeks. Healthy individuals excluded people with severe cardiopulmonary diseases and other tumors, and were recruited by the health examination center of our hospital. Clinical data were collected by consulting medical records. Stool samples were freshly collected and immediately frozen at − 80 °C for follow-up analysis. In order to avoid the influence of medication as much as possible, we collected samples from the patients when they were just admitted to the hospital and had not received treatment. Subject clinical data were collected by consulting medical records (Table 1), including factor such as age, FIGO stage, body mass index (BMI), medication history and personal cancer history. Tumor staging was performed according to World Health Organization histological classification criteria and the International Federation of Gynecology and Obstetrics (FIGO) staging criteria. We confirm that all experiments are carried out in accordance with the relevant guidelines and regulations.

DNA extraction

Total bacterial genomic DNA was extracted from fecal samples using DNA isolation kits (GUhe Laboratories, Hangzhou, China). DNA concentrations and purity were tested on a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

16S rDNA amplicon pyrosequencing

The V4 region of bacterial 16s rRNA was amplified using forward (515F 5′-GTGCCAGCMGCCGCGGTAA-3′) and reverse primers (806R 5′-GGACTACHVGGGTWTCTAAT-3′). We also used specific 6-bp sequences to incorporate bar codes into TrueSeq adapters for multiple sequencing. Amplification included a pre-denaturation step at 98 °C for 30 s and then 25 cycles including denaturation at 98 °C for 15 s, annealing at 58 °C for 15 s, extension at 72 °C for 15 s, and a final extension at 72 °C for 1 min. Amplicons were purified and quantified using Agencourt AMPure XP Beads (Beckman Coulter, Indianapolis, IN, USA) and a PicoGreen dsDNA assay kit (Invitrogen, Carlsbad, CA, USA). In further analyses, GUHE Info Technology Co., Ltd (Hangzhou, China) used the Illumina NovaSeq6000 platform (Illumina, San Diego, CA, USA) for pairwise 2 × 150 bp sequencing, after amplifier quantification and pooling. After individual quantification steps, amplicons were pooled in equal amounts, and pair-end 2 × 150 bp sequencing was performed using the Illumina HiSeq4000 platform at Guhe Info Technology Co. Ltd (Hangzhou, China).

Sequence analysis

Operational taxonomic unit (OTU) picking using VSEARCH v2.22.1. Exact matches with bar codes were assigned to corresponding samples and identified as valid sequences. The average sequencing reads of the samples was 129,726, and the lowest sequencing depth was 81,116. The criteria for screening low-quality sequences were sequence length < 150 bp, average Phred scores of < 20, the sequence containing ambiguous bases, and the single nucleotide repeat sequence containing > 8 bp. Using VSearch, we selected amplified sequence variants (ASVs) for included dereplication (–derep_fulllength), cluster (–cluster_fast, –id 0.97), and detection of chimeras (–

Characteristics	HC[a]	BOT[b]	OC[c]	OBT[d]
Female	53	91	148	90
Age, years	52.54 ± 8.68	45.38 ± 14.41	58.62 ± 10.77	49.27 ± 11.72
FIGO stage				
I–II	–	–	14	–
III–IV	–	–	134	–
Body mass index (BMI), kg/m^2	23.28 ± 4.75	22.59 ± 3.21	22.74 ± 4.47	23.01 ± 2.94
Medication History				
No	–	82	110	70
Yes	–	9	38	20
Personal Cancer History				
No	–	90	143	89
Yes	–	1	5	1

Table 1. Clinical features of participants enrolled in this study. Measurement data are expressed as mean ± SEM. [a]Health control. [b]Benign ovarian tumor. [c]Ovarian cancer. [d]Other benign tumor.

uchime_ref)[13]. ASV sequence data in the ASV table were normalized to minimize sequencing depth differences between samples. A normalized value of 1 indicated relative abundance. A representative sequence (REP–SEQS) was selected from each ASV using default parameters. REP–SEQs and ASV table files were then imported into QIIME2 (V2022.2)[14]. QIIME2 removed ASVs with < 0.001 of total sequences. Resulting classifications were collapsed using the QIIME taxa collapse command.

Bioinformatics and statistical analysis

Sequence data analysis was primarily conducted using QIIME2 and R packages (V3.6.3). Alpha-diversity was indicated by the Shannon diversity index. Because the data do not conform to normal distribution, the statistical differences between groups were determined using Kruskal–Wallis tests. The UniFrac distance metric[15] was used for β-diversity analysis to examine structural changes in microbial communities in samples, and principal coordinate analysis (PCoA) was used for visualization[16]. Phylum, class, order, family, genus, and species abundance levels in groups were statistically compared. Besides β-diversity, the differences among samples were also analyzed by linear discriminant analysis (LDA) effect size (LEfSe). We used Kruskal–Wallis or Tukey tests to test taxa abundance differences between groups. Box charts were used for visualization. $P < 0.05$ values indicated statistical significance.

Using the R package "random Forest" with 1000 trees and default settings, random forest analyses were used to distinguish samples from different groups[17]. We used 10 × cross-validation to estimate generalization errors. The expected "baseline" error was also included, which was generated using classifiers that predicted the most common category tags. We also used the CatBoost and XGBoost algorithms to construct and test models to distinguish between the HC and OC groups. A tenfold cross-validation strategy was employed for model training and evaluation. Output files were further analyzed using the STAMP software package (V2.1.3)[18]. The R package and Microbiome Analyst (https://www.microbiomeanalyst.ca/) were used for data visualization.

Results

Intestinal microbial diversity differences between HCs and patients with OTs

To determine gut microbiota differences between patients with OTs and HCs, gut microbiota structures in groups were compared and analyzed. Microbial community phyla and genera were examined and described (Fig. 1A and B) to show the relatively higher phyla and genera abundance, while remaining phyla were merged under "other". Fecal microorganisms were mainly composed of Bacteroidota, Firmicutes, and Proteobacteria at the phylum level (Fig. 1A). Genus levels (Fig. 1B) were dominated by *Bacteroides, Faecalibacterium, Prevotella, Escherichia_Shigella, Megamonas,* and *Phascolarctobacterium*. At phylum and genus levels, no significant differences in gut microbiota composition were identified between OT and HC groups, but differences were recorded in the proportion of gut microbiota composition. At the phylum level, average Bacteroidota and Proteobacteria abundance in the OT group was higher than in HCs, while average *Firmicutes* abundance in OTs was lower than that in HCs. At genus levels, when compared to HCs, average *Bacteroides, Prevotella,* and *Escherichia_Shigella* abundance in the OT group increased, while average *Faecalibacterium, Megamonas,* and *Phascolarctobacterium* abundance decreased.

To evaluate gut microbiota diversity in OT and HC groups, α- and β-diversity indices were evaluated. The different alpha diversity indexes (Chao 1, ACE, Shannon and Simpson) were measured. Chao1 and ACE indexes were used to determine community abundance, the Shannon and Simpson indexes were used to determine community diversity. In this study, the ACE and Chao1 indices of the OT group were both higher than those of the HC group ($P < 0.001$), while the Shannon and Simpson indices were both lower than those of the HC group ($P < 0.001$). These data indicate that compared with the control group, the diversity of the gut microbiota in OT patients was significantly decreased, while the abundance was significantly increased (Fig. 1C). Additionally, PCoA based on weighted UniFrac distances was used to show compositional microflora differences. A significant difference in gut microbiota between HC and OT groups was observed ($P = 0.001$) (Fig. 1D). It was worth noting that when compared to HCs, gut microbiota in the OT group showed different composition and diversity.

We next used univariate analysis (in Microbiome Analyst) to compare specific gut microbiota between OT and HC groups. At genus levels, significant differences in 14 gut microbiota between groups were recorded (Table 2), including *Escherichia_Shigella, Coprococcus, Fusicatenibacter, Butyricicoccus, Oscillibacter, Blautia, Bilophila, Enterbacter, Alistipes, Lachnospira, Bacteroides, Parasutterella, Lachnospiraceae_ND3007_group,* and *Ruminococcus*. From an analysis of the first five flora by False Discovery Rate (FDR), *Escherichia_Shigella* in the OT group was significantly higher when compared with HCs, while *Coprococcus, Fusicatenibacter, Butyricicoccus,* and *Oscillibacter* were significantly lower than in HCs (Fig. S1). To comprehensively consider the biological consistency and effect size, taxonomic analysis using the linear discriminant analysis effect size (LEfSe) was carried out. Different classifications at the genus level were extracted and displayed as a bar chart. The results showed that 12 genera including *Lachnospira* and *Faecalibacterium* were increased and enriched in the healthy control group, while 3 genera including *Bacteroides, Escherichia_Shigella* and *Prevotella* were highly enriched in the OT group (Fig. S4).

Intestinal microbial diversity differences between patients with BOTs and OC

Next, we divided OTs into two groups: patients with BOTs and patients with OC, and compared gut microbiota levels between groups. Fecal microorganisms were mainly composed of Bacteroidota, Firmicutes, and Proteobacteria at the phylum level (Fig. 2A). Genus levels (Fig. 2B) were dominated by *Bacteroides, Prevotella, Faecalibacterium, Escherichia_Shigella, Phascolarctobacterium,* and *Parabacteroides*. At the phylum level, no significant differences in gut microbiota composition and proportions were identified between BOT and OC groups. However, differences in the proportions of gut microbiota were identified at genus levels. When

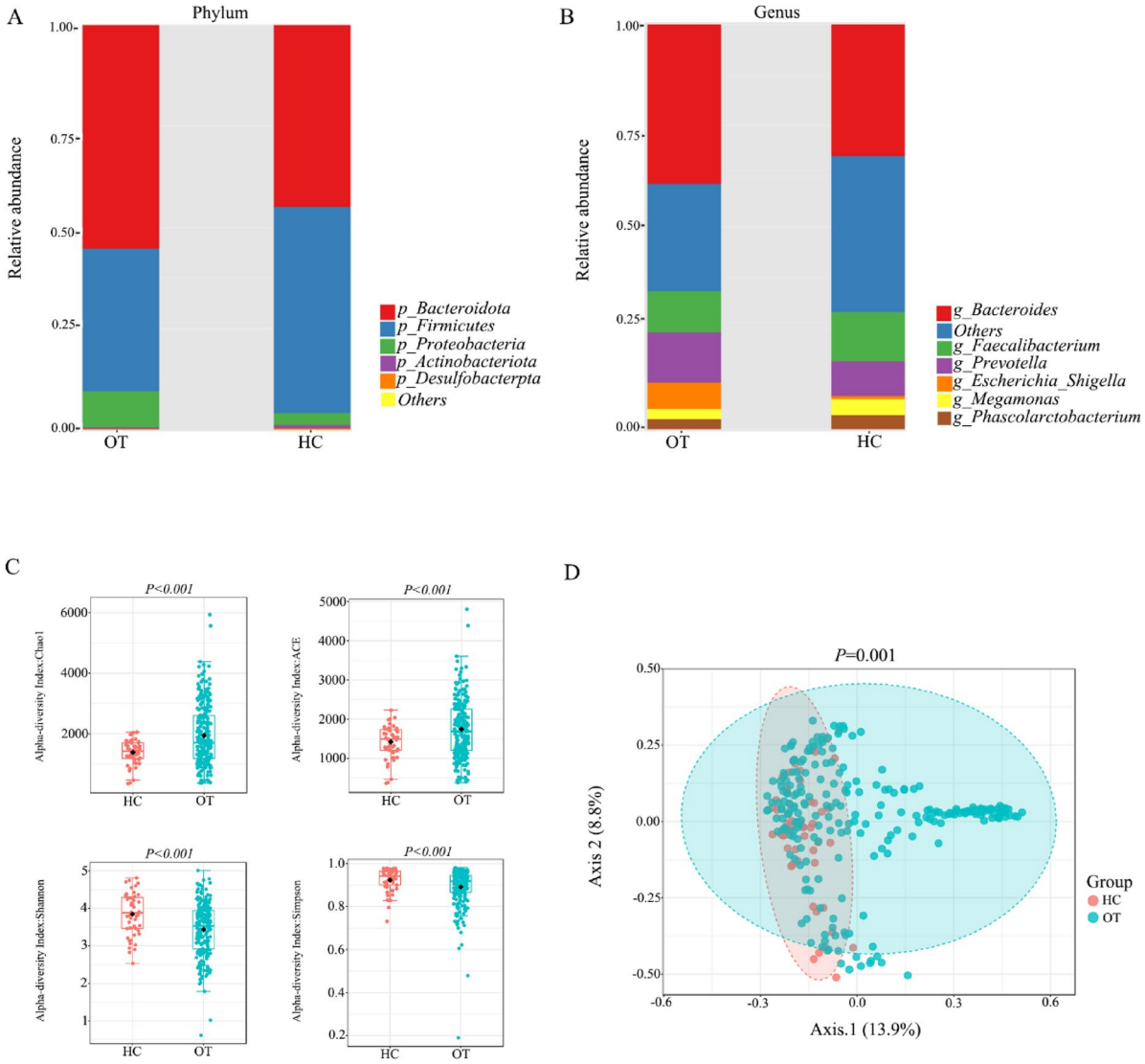

Fig. 1. The gut microbiota profile differs between ovarian tumor patients and healthy individuals. The top ten microbial communities are presented at the phylum (**A**) and genus level (**B**) in the ovarian tumor and healthy groups. α-diversity was estimated by Chao1, ACE, Shannon , and Simpson Index (**C**). Principal component analysis (PCoA) was used to display the microbiome space between groups, indicating significant differences in the gut microbiota between the HC and OT groups (**D**). *OT* Ovarian tumor, *HC* Healthy control.

compared to HCs, average *Bacteroides, Escherichia_Shigella, Phascolarctobacterium,* and *Parabacteroides* abundance increased in the OC group, while *Prevotella* and *Faecalibacterium* decreased.

Gut microbiota diversity in OC and BOT groups was also evaluated. In the OC and BOT groups, the Shannon and Simpson indices (Fig. 2C) showed no significant difference in the diversity of gut microbiota between the two groups of patients ($P=0.967$, $P=0.177$). According to the Chao1 index, there was no significant difference in the abundance of gut microbiota between the two groups of patients ($P=0.128$), but according to the ACE index, there was a significant difference in the abundance of gut microbiota between the two groups of patients ($P=0.046$). Additionally, weighted PCoA results showed a significant difference in gut microbiota composition between BOT and OC groups ($P=0.002$) (Fig. 2D). Univariate analysis showed eight gut microbiota differences between BOT and OC groups at genus levels (Table 3), including *Flavonifractor, Ruminococcus_gnavus_group, Prevotella, Anaerotruncus, Veillonella, Bacteroides,* and *Parabacteroides*. According to FDR, the first five flora were analyzed, of which, *Flavonifractor, Ruminococcus_gnavus_group,* and *Anaerotruncus* in the OC group were significantly higher than in BOT, while *Prevotella* and *Veillonella* were significantly decreased (Fig. S2). The classification analysis results of LEfSe showed that 12 genera including *Prevotella* and *Agathobacter* were increased and enriched in the BOT group, while 3 genera including *Bacteroides*, *Escherichia_Shigella* and *Ruminococcus_gnavus_group* were highly enriched in the OC group (Fig. S4).

Name	FDR[a]	Mean HC[b]	MeanBOT[c]	Mean OC[d]	Se[e]	P-Valve
Escherichia_Shigella	6.45E−09	0.006	0.046	0.050	2.386	8.71E−11
Coprococcus	0.001	0.006	0.004	0.003	0.252	1.07E−05
Fusicatenibacter	0.003	0.006	0.001	0.001	0.237	1.14E−04
Butyricicoccus	0.005	0.005	0.003	0.002	0.220	2.76E−04
Oscillibacter	0.005	0.002	0.001	0.002	0.349	4.40E−04
Blautia	0.007	0.007	0.004	0.005	1.379	6.74E−04
Bilophila	0.008	0.003	0.001	0.002	0.002	0.001
Enterobacter	0.008	0.002	0.012	0.020	0.481	0.001
Alistipes	0.011	0.016	0.011	0.011	1.735	0.002
Lachnospira	0.013	0.026	0.017	0.015	1.992	0.002
Bacteroides	0.017	0.294	0.358	0.358	1.554	0.003
Parasutterella	0.021	0.011	0.008	0.007	0.452	0.004
Lachnospiraceae_ND3007_group	0.021	0.004	0.002	0.001	0.237	0.004
Ruminococcus	0.021	0.011	0.006	0.002	0.328	0.005

Table 2. Differences in gut microbiota between ovarian tumor patients and healthy control group at the genus level. [a]False Discovery Rate. [b]Health control. [c]Benign ovarian tumor. [d]Ovarian cancer. [e]Standard Error.

Intestinal microbial diversity differences between patients with OBTs and those with OTs

We also compared gut microbiota differences between patients with OBTs and those with OTs. Microbial composition in feces was mainly comprised of Bacteroidota, Firmicutes and Proteobacteria at the phylum level (Fig. 3A). Genus levels (Fig. 3B) were dominated by *Bacteroides, Prevotella, Faccalibacterium, Escherichia_Shigella,* and *Megamonas.* At phylum and genus levels, no significant differences in gut microbiota composition were identified between OBT and OT groups, but differences in the proportion of gut microbiota composition were recorded. At phylum levels, average Bacteroidota and Proteobacteria abundance in the OT group was higher than in the OBT group, while average Firmicutes abundance in the OBT group was lower than in the OBT group. At genus levels, when compared with the OBT group, average *Bacteroides, Prevotella,* and *Escherichia_Shigella* abundance in the OT group increased, while average *Faccalibacterium* and *Megamonas* abundance decreased. The Chao1 and ACE indices of the OT group were significantly higher than those of the OBT group ($P < 0.001$, $P = 0.012$), while the Shannon and Simpson indices were lower than those of the OBT group ($P = 0.020$, $P = 0.122$). These data indicate that compared with the OBT group, the diversity of the gut microbiota in OT patients was significantly decreased, while the abundance was significantly increased (Fig. 3C). Also, weighted PCoA results showed a significant difference in gut microbiota between OBT and OT groups ($P = 0.001$) (Fig. 3D).

Microbiome Analyst univariate analysis was next used to compare specific gut microbiota in OBT and OT groups. At genus levels, significant differences in seven gut microbiota were identified between groups (Table 4), including *Fusicatenibacter, Butyricicoccus, Lachnospiraceae_ND3007_group, Coprococcus, Parasutterella,* and *Blautia.* According to FDR, the first five flora were analyzed, in which *Fusicatenibacter, Butyricicoccus, Coprococcus Parasutterella,* and *Anaerotruncus* in the OBT group were significantly higher than the OT group, while the *Lachnospiraceae_ND3007_group* was significantly decreased (Fig. S3). The classification analysis results of LEfSe showed that 13 genera including *Lachnospira* and *Roseburia* were increased and enriched in the OBT group, while 2 genera including *Prevotella* and *Streptococcus* were highly enriched in the OT group (Fig. S4).

The value of detecting gut microbiota for OT diagnoses

To evaluate gut microbiota potential to distinguish cancer populations, we established and tested a random forest classifier model. Gut microbiota diagnostic effects were evaluated using ROC analysis; when compared with HCs, the AUC value of the BOT group was 0.77 (Fig. 4A). From top to bottom, the main bacteria responsible for distinguishing patients with BOTs from HCs are shown (Fig. 4C), with an error rate of 31.6%. When compared with HCs, the AUC value of the OC group was 0.86 (Fig. 4B). The performance measured by the AUC for the CatBoost and XGBoost models of the HC and OC groups was 0.859 for both (Fig. S5). The main bacteria responsible for distinguishing patients with OC from HCs are shown (Fig. 4D), with an error rate of 34.1%. When compared with the OC group, the AUC value of the BOT group was 0.72 (Fig. 4E). The main bacteria responsible for distinguishing patients with OC from those with BOTs are shown (Fig. 4G), with an error rate of 42.51%. When compared with the OBT group, the AUC value of the OT group was 0.70 (Fig. 4F). The main bacteria responsible for distinguishing patients with OTs from those with OBTs are shown (Fig. 4H), with an error rate of 43.94%.

Discussion

In this study, fecal samples were collected from HCs (n = 53), 239 patients with OTs (patients with OC (n = 148) and with BOTs (n = 91)), and patients with OBTs (n = 90). Through the analysis of the gut microbiota of these patients, we observed that the gut microbiota composition, including bacterial abundance and diversity of OT group significantly differs from that of HC group and OBT group. Also, a significant difference was noted in the gut microbiota between OT and BOT patients. Moreover, among patients with OTs, OC and BOT patients showed distinctive gut microbiota compositions. Via the analysis of the microbiota in the patient sample, unique

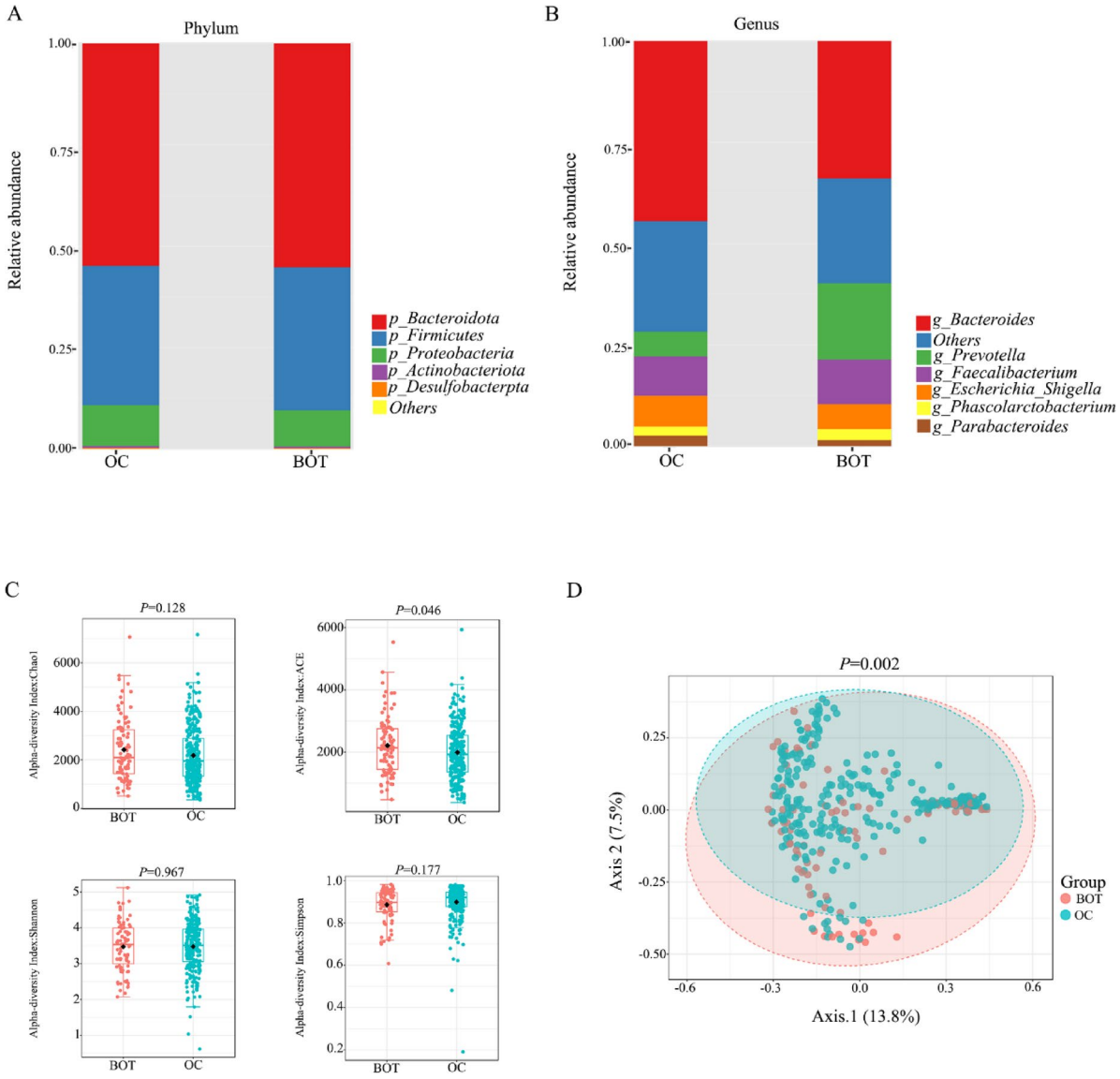

Fig. 2. The gut microbiota profile differs between ovarian cancer patients and benign ovarian tumor patients. The top ten microbial communities are presented at the phylum (**A**) and genus level (**B**) in the ovarian tumor and healthy groups. α-diversity was estimated by Chao1, ACE, Shannon , and Simpson Index (**C**). Principal component analysis (PCoA) was used to display the microbiome space between groups, indicating significant differences in the gut microbiota between the OC and BOT groups (**D**). *OC* Ovarian cancer, *BOT* Benign ovarian tumor.

Name	*P*-values	FDR[a]	Se[b]	mean BOT[c]	mean OC[d]
Flavonifractor	1.66E−05	0.001	0.003	0.001	0.002
Ruminococcus_gnavus_group	3.53E−05	0.001	0.347	0.001	0.004
Prevotella	5.76E−05	0.001	0.239	0.190	0.069
Anaerotruncus	8.03E−05	0.001	3.119	0.190	0.069
Veillonella	0.000424	0.006	2.807	0.004	0.015
Bacteroides	0.00054	0.006	1.649	0.284	0.360
Parabacteroides	0.00469	0.042	1.815	0.024	0.026

Table 3. Differences in gut microbiota between Benign ovarian tumors and ovarian cancer at the genus level. [a]False Discovery Rate. [b]Standard Error. [c]Benign ovarian tumor. [d]Ovarian cancer.

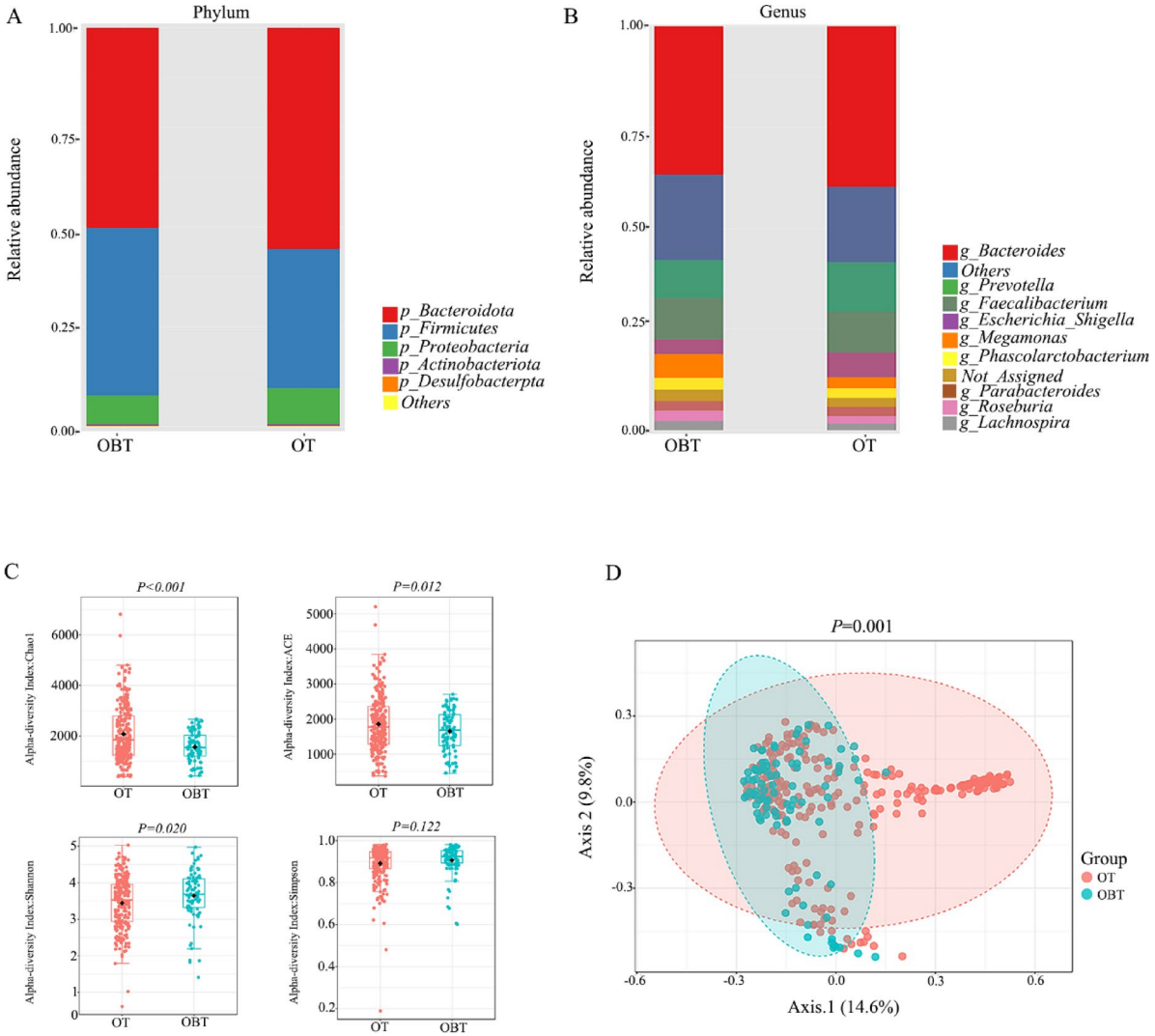

Fig. 3. The gut microbiota profile differs between ovarian tumor patients and other benign tumor patients. The top ten microbial communities are presented at the phylum (**A**) and genus level (**B**) in the ovarian tumor and other benign tumor patients. α-diversity was estimated by Chao1, ACE, Shannon , and Simpson Index (**C**). Principal component analysis (PCoA) was used to display the microbiome space between groups, indicating significant differences in the gut microbiota between the HC and OT groups (**D**). *OT* Ovarian tumor, *OBT* Other benign tumor.

Name	*P*-values	FDR[a]	Se[b]	mean OT[c]	mean OBT[d]
Fusicatenibacter	5.18E−05	0.004	0.002	0.001	0.003
Butyricicoccus	4.19E−04	0.011	0.004	0.002	0.003
Lachnospiraceae_ND3007_group	5.37E−04	0.011	0.003	0.002	0.002
Coprococcus	5.685E−04	0.011	0.008	0.003	0.005
Parasutterella	9.725E−04	0.015	0.011	0.007	0.010
Blautia	1.533E−03	0.020	0.005	0.005	0.005

Table 4. Differences in gut microbiota between ovarian tumor and other benign tumor at the genus level. [a]False discovery rate. [b]Standard Error. [c]Ovarian tumor. [d]Other benign tumor.

intestinal microbe species were discovered. We speculate that we could distinguish OT patients from HCs through these microbes.

Because of the close relationship between estrogen and intestinal microorganisms, differences in intestinal microorganisms in female tumors (cervical and breast cancer) have been extensively studied, Patients with cervical cancer and breast cancer have their own unique gut microbiota[19–21]. Kang et al.[19] reported that the

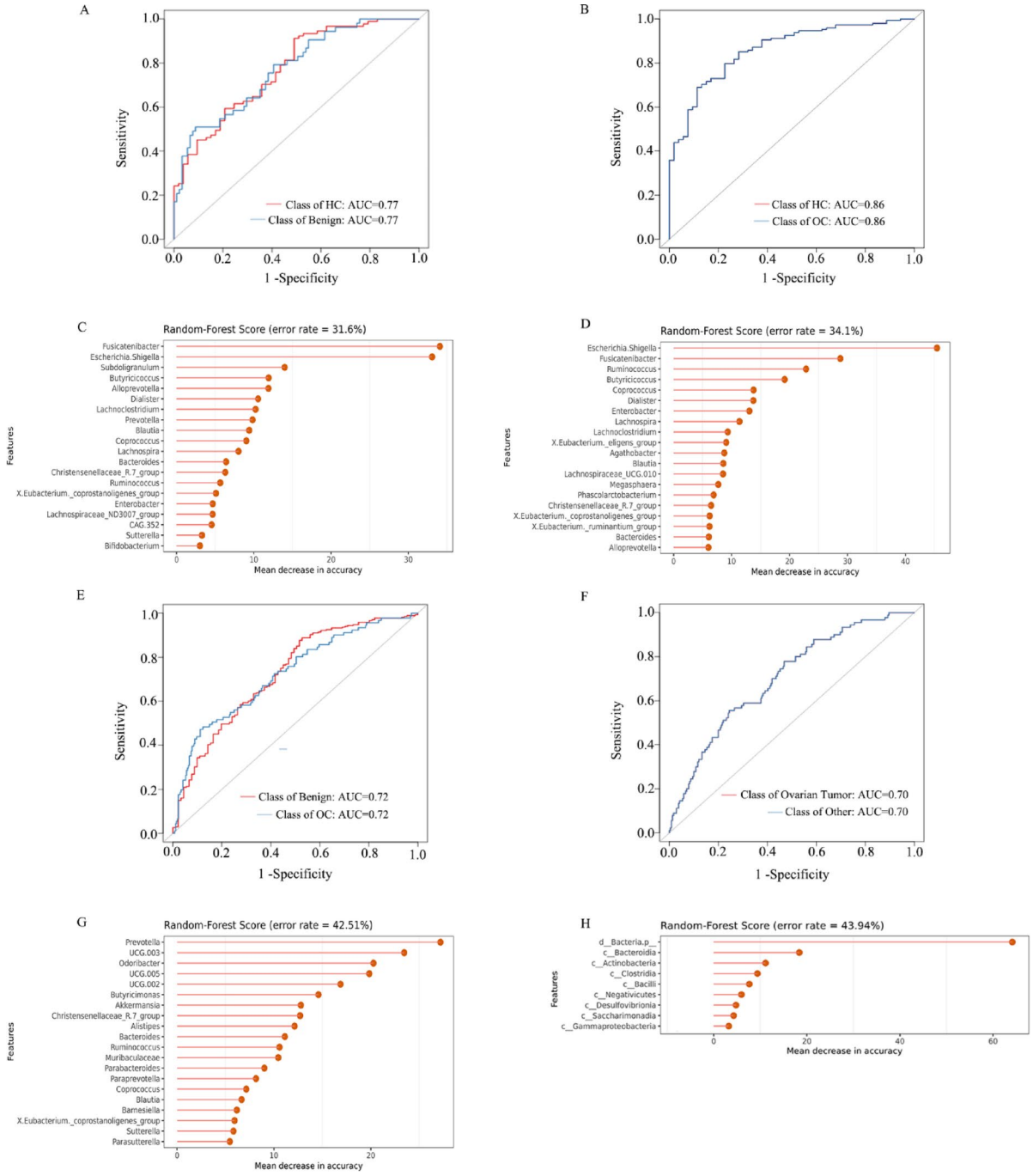

Fig. 4. The diagnostic efficacy of microbiota was evaluated by subject operating characteristics (ROC) analysis. The area under the curve (AUC) of the patients with BOT was 0.77 (**A**) compared with the healthy group. Compared with the healthy group, the AUC of patients with OC was 0.86 (**B**). The AUC of OC patients was 0.72 (**E**) compared with BOT patients. Compared with OBT, AUC of patients with OT was 0.70 (**F**). The error rate represents the error rate of finding characteristic microbiota to predict classification by the random forest method (**C**, **D**, **G**, **H**). *OT* Ovarian tumor, *OC* Ovarian cancer, *BOT* Benign ovarian tumor, *OBT* Other benign tumor.

abundance of *Prevotella* in fecal samples of early cervical cancer patients was higher than that in healthy control group. Additionally, cervical cancer stage was most significantly and negatively correlated with *Ruminococcus 2*, which was posited as a potential biomarker in predicting cervical cancer development[20]. High *Bacteroides* abundance was also found in fecal samples from patients with cervical cancer, with *Bacteroides* identified as a dominant bacteria related to estrogen metabolism. Thus, cervical cancer occurrence and development may be related to estrogen metabolism mediated by intestinal microorganisms[20]. When compared with healthy individuals, breast cancer patients usually have lower microbial diversity and microbial composition

alterations; relative *Streptomyces* and *Bacteroides* abundance in feces from breast cancer patients was lower, while *Verrucous* and *Proteus* abundance was higher[21]. The bacterial metabolites secreted by gut microbiota, similar to the role of hormones, are also involved in estrogen metabolism regulation in cancer cells[22,23]. Since 80% of breast cancer cases are estrogen receptor positive[22], the occurrence and development of breast cancer may be related to estrogen metabolism. In our study, when compared with HCs, *Escherichia_Shigella* abundance was significantly increased, while *Coprococcus, Fusicatenibacter, Butyricicoccus,* and *Oscillibacter* abundance was significantly decreased in patients with OTs. Some *E. coli* and *Shigella* strains may cause intestinal infections and diarrhea[23,24]. Current evidence also suggest that patients with non-HBV/ non-HCV hepatocellular carcinoma have intestinal ecological disorders characterized by excessive amounts of pro-inflammatory bacteria such as *Escherichia coli Shigella* and *enterococci* and a decrease in anti-inflammatory bacteria[25]. Studies have shown that *E. coli* and *Shigella* are *Enterobacteria* that generate lactic acid which promotes tumor growth and development by providing energy for tumor cells and immune defense evasion[26–28]. *Escherichia_Shigella* may potentially promote OT development, although mechanisms remain unclear. *Coprococcus* is an important member of the *Pleurococcus* genus, which mainly colonizes the intestines of healthy individuals[29] and *Butyricicoccus* is a known "probiotic", both of which are important butyric acid producers[30]. Some studies have reported that butyric acid exerts protective effects in patients with colorectal cancer by inhibiting tumor cell proliferation and inducing tumor cell apoptosis[31]. It was previously reported that when compared with fecal microbiota data in healthy women, relative *Butyricimonas* and *Coprococcus* abundance in patients with early breast cancer had decreased[32]. A study revealed that *Fusicatenibacter* can produce short-chain fatty acids SCFAs (i.e., butyrate, propionate, and acetate). SCFAs is essential for the integrity of the intestinal barrier and can also affect the intestinal nervous system and stimulate systemic anti-inflammatory properties[33]. OTs are also associated with abnormal estrogen levels, but whether unique intestinal microorganism levels in OTs are implicated in disease occurrence and development via estrogen metabolism requires investigation.

In patients with OTs, when compared with those with BOTs, *Flavonifractor, Ruminococcus_gnavus_group,* and *Anaerotruncus* in malignant OTs were significantly increased, while *Prevotella* was significantly decreased. *Ruminococcus gnavus* has been implicated in Crohn's disease; its relative abundance is increased in patients with the disease and is associated with severe disease symptoms[34]. *R. gnavus* abundance was also increased in patients with viral Hepatocellular carcinoma, which eventually induced tumor necrosis factor-α in dendritic cells and led to hepatocyte carcinogenesis[8]. Jacobson et al.[12] reported that *Prevotella* abundance increased significantly in patients with OC when compared with benign controls. The possible reason is that they included only five Native American female patients with BOTs. After being included in the study and treated for OC, intestinal microbes may alter after therapy. *Prevotella* is generally associated with healthy plant diets and has "probiotic" roles in the body, but too much *Prevotella* can stimulate intestinal epithelial cells to produce IL-8 and IL-6, thus promoting intestinal mucosal auxiliary Th17 immune responses, neutrophil recruitment, and chronic inflammation[35]. Similar to the gut microbiota of healthy controls included in this study, *Fusicatenibacter* was also significantly reduced in OTs when compared with females with OBTs.

In recent years, the gut microbiota has been widely investigated as early diagnostic markers in some cancers (e.g., gastric, colorectal, and liver cancers)[6,7,36]. Zhang et al. and other authors reported that Lactic Acid Bacteria and *Macrococci* abundance in patients with gastric cancer was significantly higher than in healthy individuals; Different bacteria were used to generate a random forest model, which provided an area under the curve (AUC) value of 0.91. Verification samples achieved a true positive rate of 0.83 in gastric cancer[7]. It was also reported that the combined observation of gut bacteria and metabolic biomarkers (such as branched chain amino acids, aromatic amino acids, and amino acyl tRNA organisms) may improve the diagnostic performance of colorectal cancer. The AUC value of colorectal cancer patients and healthy individuals is 0.94, indicating the possibility of early diagnosis of colon cancer[7]. Another study reported that in eight intestinal bacterial genus classification models with an average abundance of more than 0.1%, high diagnostic accuracy was achieved when classifying liver cancer types in the verification cohort[36]. In our study, gut microbiota diagnostic effects were evaluated using ROC analysis; when compared with HCs, AUC values in BOT and OC groups were 0.77 and 0.86, respectively. These findings suggest that some gut microbiota such as *Escherichia_Shigella* show a certain ability to distinguish between healthy individuals and patients with OT. But this is only a preliminary study and large-scale clinical verification is needed.

Our research also had some limitations. Sample size was small and conclusions were based on single-center data. Therefore, more samples and FIGO stages must be considered in future studies. Moreover, gut microbiome is dynamic, affected by multiple factors, including genetics, lifestyle, and environmental exposure. We also did not fully consider the relationship among menstruation, estrogen metabolism, and gut microbiota, and there was no validation through an independent cohort. These factors will be fully considered in future research. To conclude, our work has demonstrated characteristic changes in gut microbiota in OT patients and possible key genera in the identification of HCs and OT patients. In the future, we will further construct and verify the predictive model of OT based on gut microbiota in clinic.

Data availability

Sequence data that support the findings of this study have been uploaded on China National GeneBank DataBase with the primary accession code CNP0005514. https://db.cngb.org/search/?q=CNP0005514. Please contact the corresponding author for further information if necessary.

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Author contributions

WGG wrote the manuscript. MWN and SYM participated in the data collection. GLJ provided technical and material support and data analysis. YJB, QL, and JJW provided the samples and clinical data. YLZ and ZGZ conceived and designed the study. All authors read and approved the final manuscript.

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Declarations

Competing interests
The authors declare no competing interests.

Ethics approval and consent to participate
The study was approved by the Ethics Committee of Zhejiang Cancer Hospital (Approval No. IRB-2023-417).

Additional information
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