Changes in the vaginal microbiota associated with primary ovarian failure

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Abstract

Background

Primary ovary failure (POF) is defined as follicular failure in women of reproductive age. Although many factors are speculated to contribute to the occurrence of POF, the exact aetiology is unclear. Alterations in the microbiome of women with POF are poorly studied;

Methods

This study investigated the vaginal microbiota of 22 patients with POF and 29 healthy individuals. High-throughput Illumina Miseq sequencing targeting the V3-V4 region of the 16S ribosomal RNA (rRNA) gene was used to evaluate the relationships between vaginal flora and clinical characteristics of POF.

Results

Different from before, we found the diversity and richness of the vaginal flora of patients with POF was significantly different to that of healthy controls. Comparison of the flora of patients POF with that of menopausal women revealed that the relative abundance of Lactobacillus was significantly reduced in the latter, and women with reduced relative abundance of Lactobacillus in microbiota community decreased the pregnancy success rate at term. This study confirms that vaginal microbiota dysbiosis occurs in patients with POF. Additionally, the vaginal microbiota is closely related to clinical characteristics of POF and the inclusion of a disease verification model could reveal more accurate information related to the composition of the microbiota and its functions.

Conclusion

The result suggests the present study enabled identification of microbiota associated with POF, further investigations of differences in the microbiota in the context of POF will improve our understanding of the pathogenesis of the disease which involve modification of the vaginal microbiota.

Introduction

Primary ovarian failure (POF) is defined as failure of ovarian function in women below the age of 40. Clinically, the condition is characterised by amenorrhea lasting 4 months or more, accompanied by oestrogen deficiency and elevated levels of gonadotropin[1, 2]. The prevalence of POF is 1% in the women before age 40[3, 4], and the incidence is increasing. Development of POF is associated with particular autosomal gene defects, autoimmune dysfunction, infection, and iatrogenic factors. Smoking, drinking, and nutritional factors may influence the age at which menopause occurs[2]. However, in nearly half the cases of POF, the causal factors are not clear. Furthermore, the clinical manifestation differs between individuals, the most serious being gonadal dysplasia secondary to infertility—only 5% of women with POF can conceive naturally. Patients with POF can also experience comorbidities including osteoporosis, dyslipidemia, blood pressure fluctuations, and cardiovascular disease[5]. The clinical recommendations

are to undergo in vitro fertilisation-embryo transfer (IVF-ET) with egg donation on the basis of hormone-replacement therapy (HRT)[6]. The complexity of POF and the decreasing age of patients experiencing POF means that research into the underlying mechanism of the disease is of high importance.

There is emerging evidence indicating that the vaginal microbiota of women of childbearing age mainly comprises Lactobacillus species[7]. Prevotella, Atopobium, and Gardnerella spp. are associated with bacterial vaginosis (BV) and their presence in the vaginal microbiota may lead to preterm birth[8]. A number of studies have found that a high incidence of BV in women undergoing IVF, and reported this condition to be associated with infertility[9, 10]. Women with a high relative abundance of Gardnerella and Atopobium spp in the vaginal microbiota have been reported to have poor IVF outcomes in terms of pregnancy[11]. When the vaginal microbiota is altered, the production of lactic acid will be changed, potentially leading to an increase in the secretion of inflammatory factors such as interleukin (IL)-6, IL-8, and tumour necrosis factor (TNF)-α which activate the immune system and cause the body to be in a chronic inflammatory state. This affects the success rate of pregnancy[12, 13], highlighting the important roles of vaginal microbiota in the reproductive tract microbiome and maintenance of reproductive tract health in females[14].

Primary ovarian failure has been shown to have an autoimmune component. Proposed mechanisms of POF have suggested that viral, genetic, or other environmental stimuli may induce the expression of major histocompatibility complex (MHC) Class 1 (I) and class II (II) antigens in granulosa cells. These antigens are recognised by ovarian T cells which respond by secreting lymphokines to stimulate macrophages to secrete more interferon (INF)-γ which further increases expression of MHC in ovarian granulosa cells triggering humoral and cell-mediated autoimmune responses including secretion of IL-1 from macrophages and lymphocytes and acceleration of follicular atresia[15]. However, the vaginal epithelium has many innate immune protection mechanisms including the presence of tight junctions, antimicrobial peptides (AMPs), and mucus. In addition, immune cells such as γ- and δ-T cells, dendritic cells (DC), and macrophages are present below and between the epithelial cell layer of the vagina[16].

Class II antigen expression can be induced in patients with POF, and the in vitro expression of these antigens in granulosa cells is enhanced by the addition of IFN-γ to cell culture[17]. Vaginal microbiota has also been linked to female infertility via its effect on the concentration of various inflammatory factors in plasma. Compared with women with normal fertility, the vaginal lavage fluid of infertile women was found to have increased levels of inflammatory factors such as TNF-α and IFN-γ, and decreased levels of IL-6 and IL-10[18]. A number of studies have also shown that the proliferation of Gardnerella vaginalis associated with inflammatory response can be inhibited by Lactobacillus.12 By activating TLR-2 on the surface of monocytic THP-1 cells, G. vaginalis activates NF-kB to induce the secretion of large amounts of TNF-α[19, 20]. Similar increases in TNF-α levels have also been reported from a study on a transgenic rat model of POF[21]. Therefore, it is important to study the vaginal microbiota of women with POF during the development of the disease

We present the first study to use 16S rRNA gene sequencing to investigate the microbiota communities of the vaginal microbiota of patients with POF in comparison with healthy women. Furthermore, we analysed the relationship between vaginal microbiota and clinical characteristics of POF.

Methods

Study cohort and sample collection

We recruited 22 patients aged 20 – 40 years who visited the Reproductive Hospital affiliated to Shandong University for premature ovarian failure from June to August 2018. According to the diagnostic criteria for POF[22], patients reported a previously regular menstrual cycle with cessation of menstruation for at least four menstrual cycles and blood follicle-stimulating hormone (FSH) level of 40 IU/L for more than one month. All patients with POF have received hormone replacement therapy for more than three consecutive months, but serum hormone levels still meet the diagnostic criteria for POF. We also recruited 29 healthy volunteers for the control group. These participants were selected according to normal menstrual cycles and regulatory factors (FSH of ≤ 10 IU/L, anti-Mullerian hormone (AMH) of ≥ 2 IU/L). Exclusion criteria for both groups were antibiotic treatment within three months prior to enrollment, liver or kidney dysfunction, surgical resection of one side of the ovary, previous smoking history, and vaginal medication in the past three days. In addition, the post-menopausal data is referred which we recruited 50 women, the standard is at least one year after menopause and excludes other organic lesions. This study and all its protocol were approved by the Reproductive Ethics Committee of Ren Ji Hospital affiliated to Shanghai Jiao Tong University School of Medicine (approval number: 2018072610). All participants gave informed consent and voluntarily signed informed consent.

The outpatient doctor at the department of gynaecology collected vaginal secretions from the vaginal posterior fornix using a sterile cotton swab according to standard clinical practice. Samples were treated by adding 750 μl of PowerSoil®-htp Bead Solution (MO BIO Laboratories, Inc. Carlsbad, CA, USA; catalogue number 12955-12-BS), then stored at − 80 °C until analysis. Samples were collected from the posterior vaginal fornix and were stored in duplicate.

Laboratory measurements

Baseline blood samples were collected and stored at − 80 ℃ until measurement. Serum AMH, FSH, luteinising hormone (LH), estradiol (E2), and thyroid stimulating hormone (TSH) were tested used enzyme-linked immunosorbent assay (ELISA) in the laboratory at the Affiliated Reproductive Hospital of Shandong University.

Extraction of DNA and 16S rRNA amplicon sequencing

We isolated DNA from vaginal samples and assessed the DNA quality using a Thermo NanoDrop 2000 UV spectrophotometer and electrophoresis on 1% agarose gel to assess integrity and size. The 16S rRNA gene was amplified using the universal primers U341F 5'- CCTACGGGRSGCAGCAG − 3' and U806R 5'- GGACTACVVGGGTATCTAATC − 3' targeting the V3-V4 hypervariable regions. All quantified amplicons

were pooled to equalise concentrations prior to sequencing using the Illumina MiSeq system (Illumina Inc., CA, USA). Library construction and sequencing were conducted at the Realbio Genomics Institute (Shanghai, China). Reads obtained by paired-end sequencing were spliced into a sequence by the Pandaseq software to obtain long reads of the hypervariable region. Reads length range is 220 ~ 500nt. To facilitate downstream microbial diversity analysis, long reads were clustered into operational taxonomic units (OTUs). Usearch was used to cluster reads at a similarity of 0.97, then the clustered sequences were chimericly filtered to obtain OTUs, each of which was considered to represent a single taxon. The number of reads corresponding to different samples varies widely, and we will perform a random flattening process on each sample when the sample reaches a sufficient sequencing depth, the downsize is 27420. The longest sequence was selected from each OTU as a representative sequence for that OTU and was submitted to the Ribosomal Database Project database to obtain the annotation. The annotated sequences were classified for each OTU and the composition of flora analysed at the level of the phylum, class, order, family, and genus. The bioinformatics pipeline QIIME was used to calculate alpha diversity, carry out beta diversity analysis and to create the corresponding dilution curves. Alpha diversity represents the richness and evenness of microbial communities, including the observed species, Chao1, Shannon, and Simpson indices, as well as the phylogenetic diversity whole tree index. Beta diversity analysis is used to identify differences between different samples. To further investigate differences in taxon diversity between samples, we calculated the unweighted and weighted UniFrac distances for beta diversity analysis using the OTU table and phylogenetic tree. We generated ordination plots using principal coordinate analysis as implemented in R software (the “vegan” package). Principal coordinate analysis (PCoA) was then performed, and linear discriminant analysis (LDA) effect size (LEfSe) analysis (which is often used to identify the presence and effect size of region-specific OTUs among different groups was used to determine the microbiota associated with POF by comparing the flora of the POF and control groups[23, 24]. The organisms which demonstrated the differences between groups most comprehensively were identified different organisms using an LDA score cut-off of 2.0.

Redundancy analysis

Redundancy analysis (RDA) is a multivariate direct gradient analysis method based on the development of corresponding analysis. Corresponding analysis is combined with multiple regression analysis and each step is calculated considering environmental factors. This analysis is based on a linear model and is mainly used to investigate the relationship between microflora and environmental factors.

Random forest classification

Random forest classification is a tree-based algorithm which requires simulation and iteration, and is utilised in machine learning. In general, random forests randomly generate hundreds to thousands of classification trees, and select the tree with the highest degree of repetition as the final result. Based on the real category and prediction probability of the sample, receiver operating characteristic (ROC) curves can be generated, and the area under the curve (AUC) calculated to evaluate the model.

Functional inference of 16S data

Functional annotation analysis was performed based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. This database integrates information on genomic, chemical, and systemic functions and contains information on metabolic pathways. The bioinformatics software PICRUSt (version 1.0.0) was used to evaluate the relative abundance of functional categories based on 16S rRNA data. The input of PICRUSt is an OTU table built using a closed-reference out-picking strategy, which involves comparison with an existing reference (Greengenes v13.5). The output of PICRUSt is a count table of functional categories such as KEGG pathways, constructed based on the functional content of each OTU.

Statistical analyses

Data analysis was performed using SPSS statistical software. Normally distributed data sets were compared using an independent-samples t test. Non-normally distributed data were compared using the Wilcoxon signed-ran test function of the R language stats package. Continuous data are presented as mean ± standard deviation. Differences were considered statistically significant when $P < 0.05$. Correlation analysis was carried out using Spearman's rank correlation coefficient.

Results

Demographic and clinical characteristics of the study population

We enrolled 22 women with POF and 29 healthy controls for analysis. The clinical characteristics of the two groups are shown in Table 1. Among patients with POF, the mean age was 30.50 ± 3.17 years, body mass index (BMI) was 22.34 ± 3.32, waist-to-hip ratio was 0.83 ± 0.04. Among the healthy control group, the mean age was 29.79 ± 3.99 years, BMI was 23.47 ± 3.51, and waist-to-hip ratio was 0.84 ± 0.06. The age, BMI, and waist-to-hip ratio were not significantly different between the two groups ($P > 0.05$), while AMH and E2 were significantly lower in the POF group ($P < 0.001$). Levels of FSH and LH were higher among patients with POF compared with control ($P < 0.001$ and $P < 0.01$, respectively). Among menopausal women, the mean age was 57.96 ± 6.57 years, BMI was 23.57 ± 3.21, and menopause had been experienced for at least 1 year.

Microbial profiling

The mean community diversity indexes (alpha diversity, including Chao1, observed species, Shannon, and Simpson indices) were significantly higher in the POF group compared with the control group (Fig. 1A, Supplementary Fig. 2, $P < 0.01$). Beta diversity was also significantly different between groups according to the weighted UniFrac phylogenetic distance matrices (analysis of similarities, $R = 0.175$, $P = 0.002$), and showed in PCoA plots (Fig. 1B and C). Thus, the vaginal microbiota of the POF group was significantly different to that of the control group. The detailed 16S rRNA raw sequence data were avaliable in the NCBI Sequence Read Archive (SRA) under accession number SRP594533

Abundance of taxa in the two groups

By LEfSe analysis, we identified 51 genera-discriminative features (Fig. 2A, LDA > 2, P < 0.05). Comparison of vaginal microbiota by the Mann-Whitney U test revealed 51 taxa that were differentially abundant between the groups (P < 0.05); the species of the top 20 are shown in Fig. 2B. The agreement of results of the two analytical methods indicates the stability of the vaginal microbiological data.

At the level of the phyla, Actinobacteria and Bacteroidetes was enriched in the POF group, whereas Firmicutes was enriched in the control group. The relative abundance of bacterial taxonomic groups at the genus level showed that ten genera including Gardnerella, Prevotella, Bacteroides, Sneathia, Dialister, and Anaerococcus were abundant in the POF group (Fig. 2C). Only Lactobacillus was found to be abundant in the control group (Fig. 2D).

A Spearman heatmap was constructed to identify correlations among the above-mentioned genera, which revealed Lactobacillus to be negatively correlated with other genera (Fig. 2E). Lactobacillus is a probiotic which plays an important role in human health[25, 26]. Thus, studying the interactions of this genus with other genera could provide insight into the functions of these species in the development of POF.

Analyses of correlations between reproductive-related clinical indicators and vaginal flora

Redundancy analysis was used to produce a two-dimensional sorting map relating vaginal flora to reproductive-related clinical indicators. Serum FSH and LH levels showed the greatest association with female vaginal flora, and E2 had a significant effect. Gardnerella and Prevotella were positively correlated with serum FSH and LH, and negatively correlated with E2. Lactobacillus in the vagina were positively correlated with E2 and negatively correlated with FSH and LH. Similar to E2, AMH was positively correlated with Lactobacillus and negatively correlated with Gardnerella and Prevotella (Fig. 3).

Next, the predictive model from the random forests system was based on the vaginal flora profile including the taxon, taxon that exhibited significantly different abundances at the genus level from Wilcoxon rank-sum test. We identified 34 genera that could be used to predict occurrence of POF with the random forests model (Fig. 4A). A mean classification error of 0.382 was achieved, and the AUC was 0.841 (95% confidence interval [CI]: 0.618–1, sensitivity: 71.4%, specificity: 100%, cut-off rate: 43.2%; Fig. 4B).

Functional alterations in the vaginal flora

Then, we analyse the metabolic pathways of the two groups of subjects. The predicted KEGG pathways that were significantly enriched in POF were amino acid metabolism, metabolism of cofactors and vitamins, genetic information processing, energy metabolism, biosynthesis of other secondary metabolites, cell motility, endocrine system, transport and catabolism, metabolism of terpenoids and polyketides, and immune system (Fig. 5A).

Figure 5B shows the results of LEfSe, as well as correlation analysis of microbiota and predictive functions performed using PICRUSt. Lactobacillus was enriched in the control group and was negatively correlated with metabolic pathways such as amino acid metabolism, genetic information processing, energy metabolism, cell motility, and so on. However, other genera such as Gardnerella and Prevotella that were enriched in the POF group were mostly positively correlated with the above-mentioned metabolic pathways. Further metabolic pathway analysis revealed Lactobacillus to be positively associated with cell growth and death such as apoptosis, cellular processes and signalling including signal transduction mechanisms, immune system disease including primary immunodeficiency, infectious disease including Staphylococcus aureus infection, and lipid metabolism including fatty acid biosynthesis. These metabolic pathways were negatively correlated with Gardnerella, Prevotella, and other genera. (Supplementary Fig. 3).

Comparison of vaginal flora in the case of premature ovarian failure or menopause

Finally, we compared the vaginal microbial composition of patients with POF and menopausal women. The high abundance of Lactobacillus, Gardnerella, and Prevotella was confirmed from comparison of vaginal micribiota of women with POF and menopausal individuals. However, the flora of menopausal women exhibited increased diversity (Fig. 6A and B). Differential species analysis showed Lactobacillus to be less abundant among menopausal women than those with POF (Fig. 6C).

Discussion

In recent years, a wealth of evidence has been published supporting the significant contribution of cervicovaginal microbiota to genitourinary and reproductive health outcomes[27]. It was first found that the microbial taxonomic composition differs between women with POF and healthy individuals. We found the vaginal microbiota to be increasingly diverse with increased species richness in the case of POF, and a significant shift in overall microbial diversity was observed. However, a previous cross-sectional study of microbiota failed to identify obvious differences between individuals in terms of vaginal microbiota diversity[28, 29]. The strength of our study lies in the comprehensive description of microbial communities associated with POF achieved through the use of 16S rRNA sequencing; particularly, the association with clinical characteristics of POF; and the utilisation of predictive models to identify bacterial taxa that are differentially expressed in POF.

Previous studies on the vaginal microbiota in the case of POF have mainly involved amine test (or the Whiff test)[28], whereas our study focused on differences at the level of the genus. One of the most attractive features of 16S rRNA gene sequence informatics is the potential for genus identification[30]. Dysbiosis of the vaginal microbiota was characterised by the altered abundance of 34 genera in POF. The combination of these 34 associated taxa were able to discriminate patients with POF from the control group with high accuracy. We noted that vaginal-microbiota-based analysis displayed a similar predictive ability for the disease as the classifier based on POF-associated genetic variants (which had an AUC of

0.841, sensitivity of 71.4%, specificity of 100%, and cut-off rate of 43.2%), implying that the microbial signature that we identified could represent a powerful tool for the prediction of POF. Our results of the changes in relative abundance of particular genera in the context of POF confirm that species of the dominant vaginal genus, Their dominance in the vaginal niche indicate that Lactobacillus are the dominant facultative anaerobes of the genital tract, supported by their presence in most women[31, 32].

In women with higher levels of basal FSH and lower levels of basal E2, there were fewer Lactobacillus in the vagina than the control group. Eade et al. evaluated the presence of Lactobacillus spp in confluent monolayers of endocervical, ectocervical, and vaginal epithelial cells and they found that the majority of Lactobacillus caused a significant decrease in the expression of AMPs, although Lactobacillus increased in the vaginal[33]. The expression of AMPs, which include cathelicidins, defensins, can also promote IL-22 secretion and thus prevent autoimmune disease[34]. A previous study has suggested that Lactobacillus can reinforce the mononuclear phagocytic response by inducing production of the autophagy-promoting factors[35]. Studies have also shown that inflammatory ageing and the autoimmune response are closely related to POF [15, 36]. Our results suggest that reduced colonization of Lactobacillus may accelerate the development of POF disease through the induction of immune responses by some inflammatory factors.

Moreover, it was our first found that the relative abundance of Lactobacillus and some Anaerobic bacteria in the vagina were correlated with the FSH level and AMH level. FAH level and AMH level had previouely been deemed determined the ovarian reserve[37, 38].

As previously studied, hormonal change caused menstruation and menopause, result in a drastic decreases in Lactobacillus in the vaginal microbiota. In this case, infections by Gardnerella vaginalis (GV) are increased 8. GV has a significant importance in vaginal immunity. In fact, the overgrowth of anaerobic species during menopause can increase the immunity molecules release such as NF-κB, TNFα□COX-2, iNOS[39]. Our result also found that the higher level of genera Prevotella, Gardnerella. Abnormal vaginal microbiota may have adverse effect on the Pregnancy. And we observed the negative correlation between anaerobic species and Lactobacillus. These bacteria exploit the same class of environmental resources in a similar way and are defined as an ecological “guild”. Guild members do not necessarily share taxonomic similarities, but they adapt to the changing environment to co-exist to affect female reproductive function by altering the concentration of inflammatory factors.

Our functional analysis showed that the pathways involved in metabolism, immune and apoptosis were related to POF[40]. However, the 16S rRNA gene sequencing does not clear the functionally important changes of the microbiota due to the technical flaw. Because little is known about the relationship between POF and vaginal microbiota, further research is required to deepen our understanding of this subject.

When we compared the vaginal microbiota of menopausal women with that of women with POF, we found that although the three most abundant genera were the same, the relative abundance of Lactobacillus is reduced in the case of menopause, which supports previous research[41]. It is well known

that menopause and POF have similar clinical manifestations. Menopause is a natural physiological phenomenon caused by age, but POF is mostly related to genetics and immunity. Our results further validate the important role of relative abundance in Lactobacillus during the development of ovarian insufficiency.

In conclusion, our study provides novel insights into the potential dysbiosis of vaginal microbiota that occurs in patients with POF. Future treatments for POF may, therefore, target the reproductive tract microbiota and involve probiotic treatment to slow follicular atresia, which may improve the success rate of IVF. However, the present study had several limitations which should be addressed in future studies. First, the sample size was small and we couldn't trace the date of the POF diagnosed. Second, we could not clarify detailed roles of specific constituents of the vaginal microbiota in the pathogenesis of POF. Third, the inclusion of a disease verification model could reveal more accurate information related to the composition of the microbiome and its functions. Therefore, future multicenter studies involving larger study populations and animal models are needed in order to explore potential mechanisms underlying the association of the vaginal microbiota with POF. Genomics represents a potential approach to elucidate associations between the vaginal microbiota and disease, and analysis of the gut microbiota may help to explain other pathologies and improve many aspects of prevention and treatment.

Conclusion:

The result reveals for the first time that there are differences in the reproductive tract flora of women with premature ovarian failure, confirming that Lactobacillus plays a vital role in female reproductive health. We suggest that Lactobacillus may affect women's ovarian function through immunity, endocrine, etc. Its in-depth research in the future provides new possibilities for the treatment of Primary ovarian insufficiency.

Declarations

Ethics approval and consent to participate

This study and all its protocol were approved by the Reproductive Ethics Committee of Ren Ji Hospital affiliated to Shanghai Jiao Tong University School of Medicine (approval number: 2018072610).

Consent for publication

Not applicable.

Availability of data and materials

The detailed 16S rRNA raw sequence data were avaliable in the NCBI Sequence Read Archive (SRA) under accession number SRP594533.

Competing interests

The authors have no conflict of interest to disclose.

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Author contributions

Juan Wang and Yanzhi Du conceived and designed the study. Juan Wang, jieying Xu and Yingying Qin collected and provided patient specimens and related information. Juan Wang, jieying Xu, Qixin Han, Gang Lu, Wai-Yee Chan,Weiwei Chu, and Yanzhi Du analyzed the data. Juan Wang and Yanzhi Du drafted and revised the paper. All authors reviewed the results and approved the final version of the manuscript.

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Authors' Information

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Supplementary Figure Captions

Supplementary Figure 1. Diagram of the number of clean reads randomly selected from a sample, showing species diversity within a single sample. One curve represents the sample, The curve tends to be flat, indicating an adequate amount of sequencing data.

Supplementary Figure 2. The abscissa indicates sample grouping, and the ordinate indicates the alpha diversity index value under different groupings. **a**: The Chao1 index was used to estimate the total number of OTUs contained within a sample. **b**: Observed_ indicates the actual number of OUT observations. **c:** A greater Simpson value higher microbial diversity. Key: *0.01<p<0.05 indicates a significant difference, **p<0.01 indicates an extremely significant difference; “NS” indicates no significant difference.

Supplementary Figure 3. The area above the horizontal axis represents the predicted metabolic pathway. The figure below shows the type of metabolic pathway, and the area on the left corresponds to the different species. Red indicates a positive correlation and blue a negative correlation. The red box indicates extremely significant correlations.

Table

Table 1 Clinical information of patients

	Primary ovary failure	Control group	P value
Number of subjects	22	29	
Age(year)	30.50±3.17	29.79±3.99	>0.05
Waist to hip ratio(cm)	0.83±0.04	0.84±0.06	>0.05
BMI(kg/m^2)	22.34±3.32	23.47±3.51	<0.001
AMH(pmol/L)	0.06±0.03	4.31±2.25	<0.001
FSH(IU/L)	75.28±27.60	5.82±1.22	<0.001
LH(IU/L)	42.23±16.00	4.68±1.51	<0.001
E2(pg/ml)	17.47±19.75	35.56±17.19	<0.01

Data shown as mean±SD. BMI, body mass index(kg/m2); Calculated using two independent samples T test

Figures

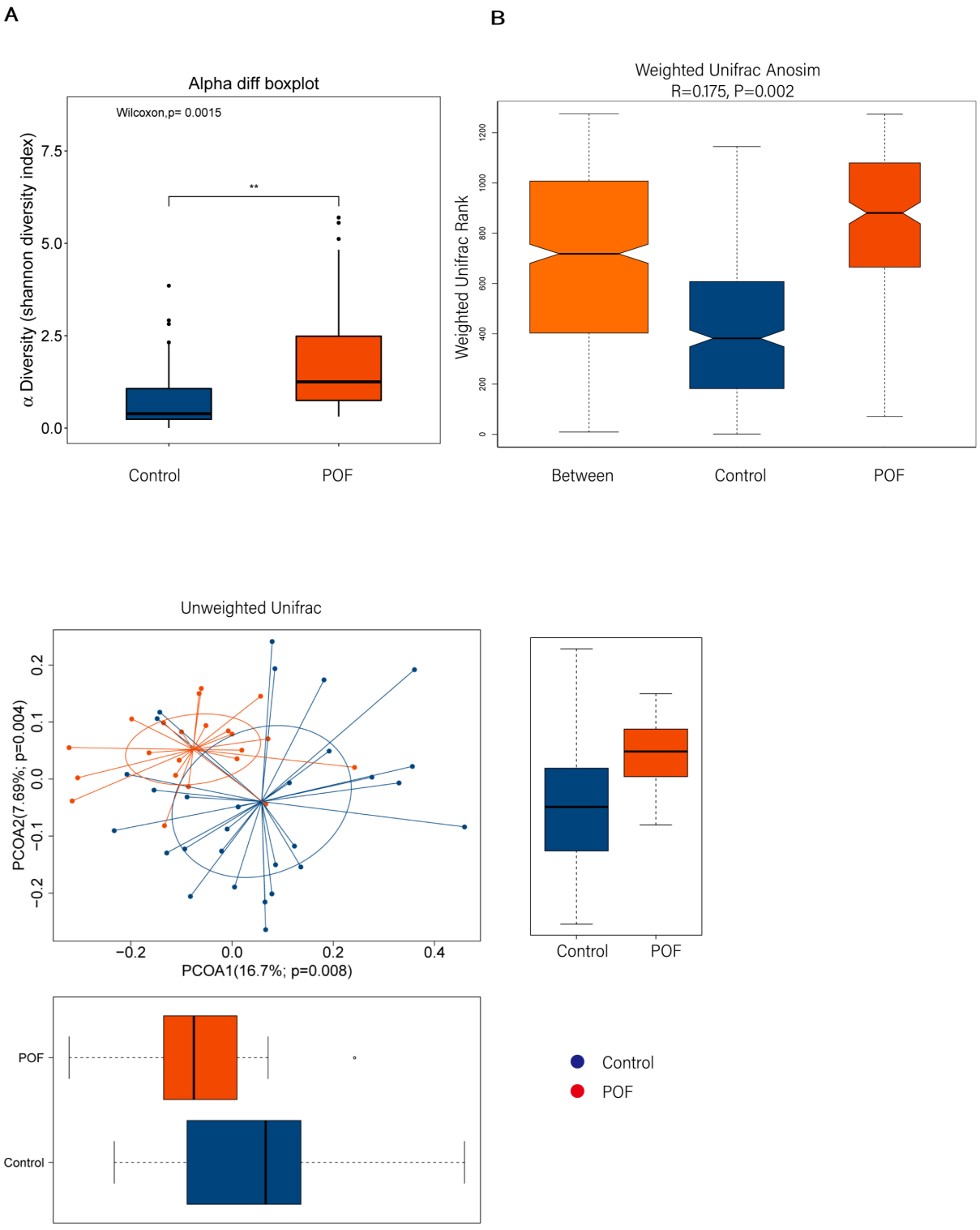

Figure 1

Comparison of diversity and shift of vaginal flora composition of females with POF and healthy controls. A:The abscissa indicates the sample grouping, and the ordinate indicates the alpha diversity index value under different groupings. A greater Shannon value indicates higher diversity. B: Beta diversity analysis is used to compare species diversity between each sample. The abscissa represents all samples (between) and each group, and the ordinate represents the rank of the Unifrac distance. R>0 indicats that the

between-group difference is greater than the within-group difference; R<0 indicates that within-group difference is greater than the between- group difference. P < 0.05 was considered as statistically significant C:Horizontal and vertical coordinates represent the first and second main coordinates, respectively. Percentages indicate the contribution rate of the corresponding main coordinate to the sample difference, and the P value is the test p value of the corresponding main coordinate. The points represent the respective samples, different colours represent different groups. The horizontal box diagram illustrates the distribution of values of different groups on the first principal coordinate; the vertical box diagram illustrates the distribution of values of different groups on the second principal coordinate.

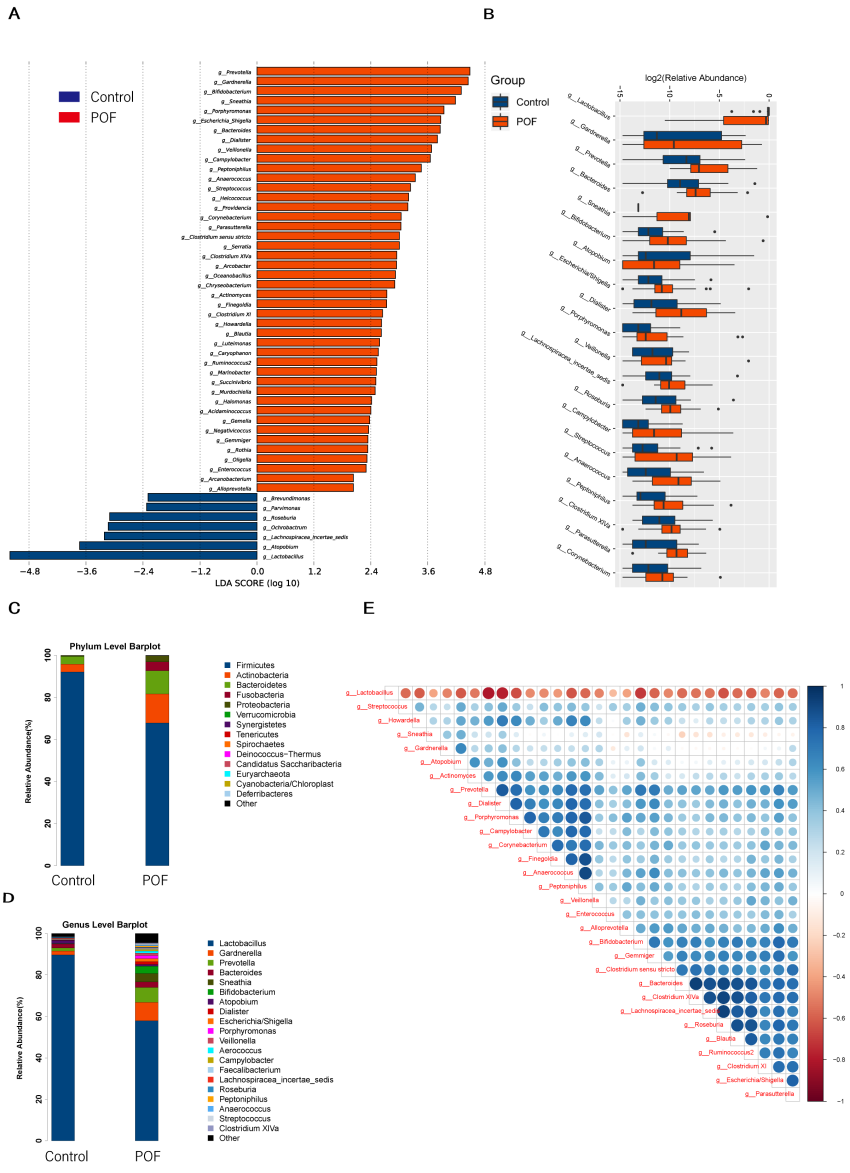

Figure 2

Comparison vaginal flora phylotype between groups A: Species that were able to classify significant differences between patients and groups. B: The abscissa is the name, the ordinate is the value of the log of relative abundance, and different colours represent different groups. Species that were abundant in at

least one group are not displayed. C,D: Species abundance map of the two groups. E: The correlation coefficient between top 30 most abundant species at all levels of classification. Right blue indicates a positive correlation and red indicates a negative correlation. Darker colour indicates a stronger correlation between the species. The species prefixes "k__", "p__", "c__", "o__", and "f__" on the left indicate that the species are annotated to the boundaries, gates, classes, orders, and subjects.

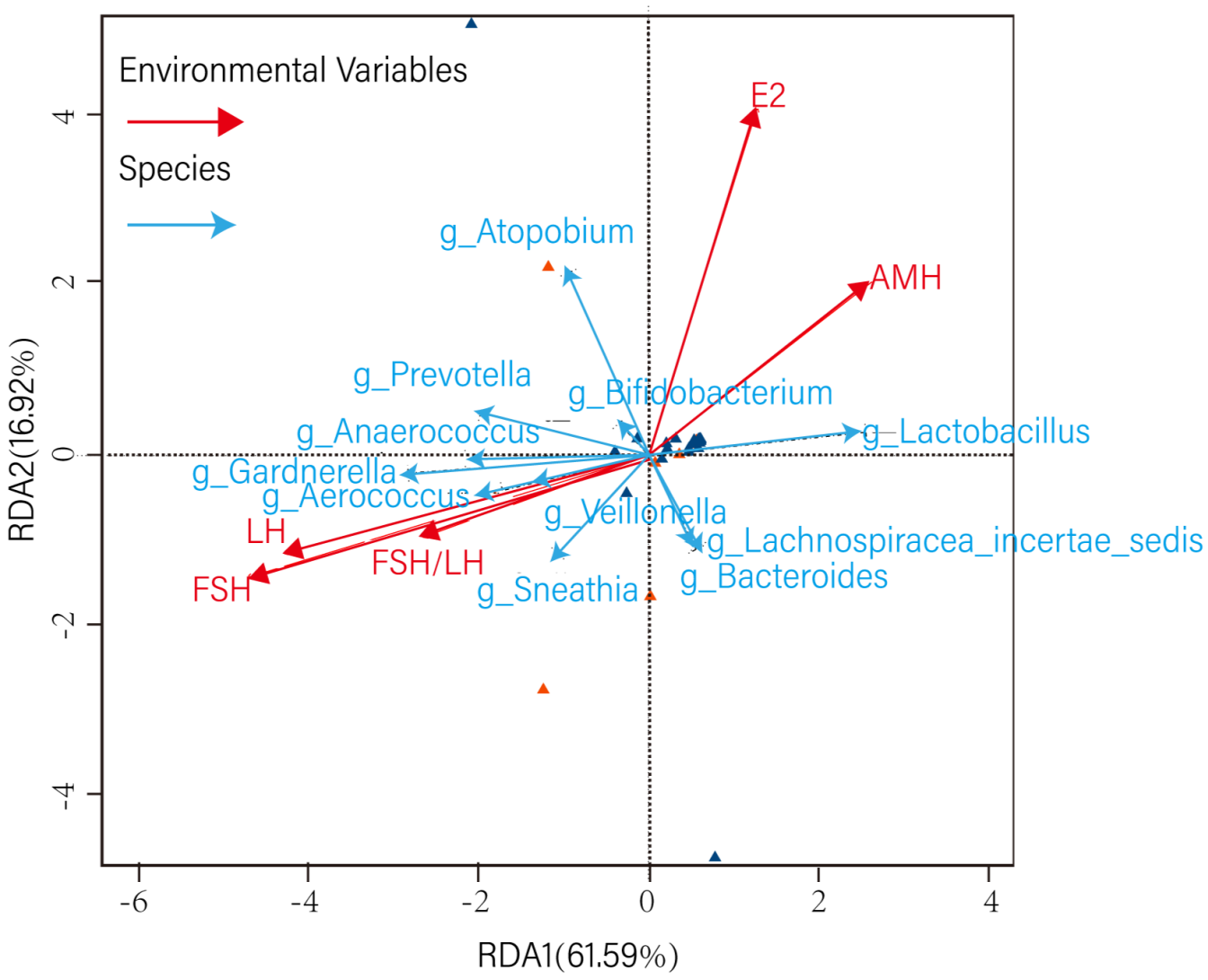

Figure 3

Coloured triangles represent sample groups in different environments or under different conditions. red: POF group, blue: control group; arrows represent different environmental factors; an acute angle between arrows indicates a positive correlation, a negative correlation is indicated by an obtuse angle. The length of the solid line of the environmental factor indicates the impact of the factor. Dotted lines pointing to the type of bacteria indicate the corresponding genus level.

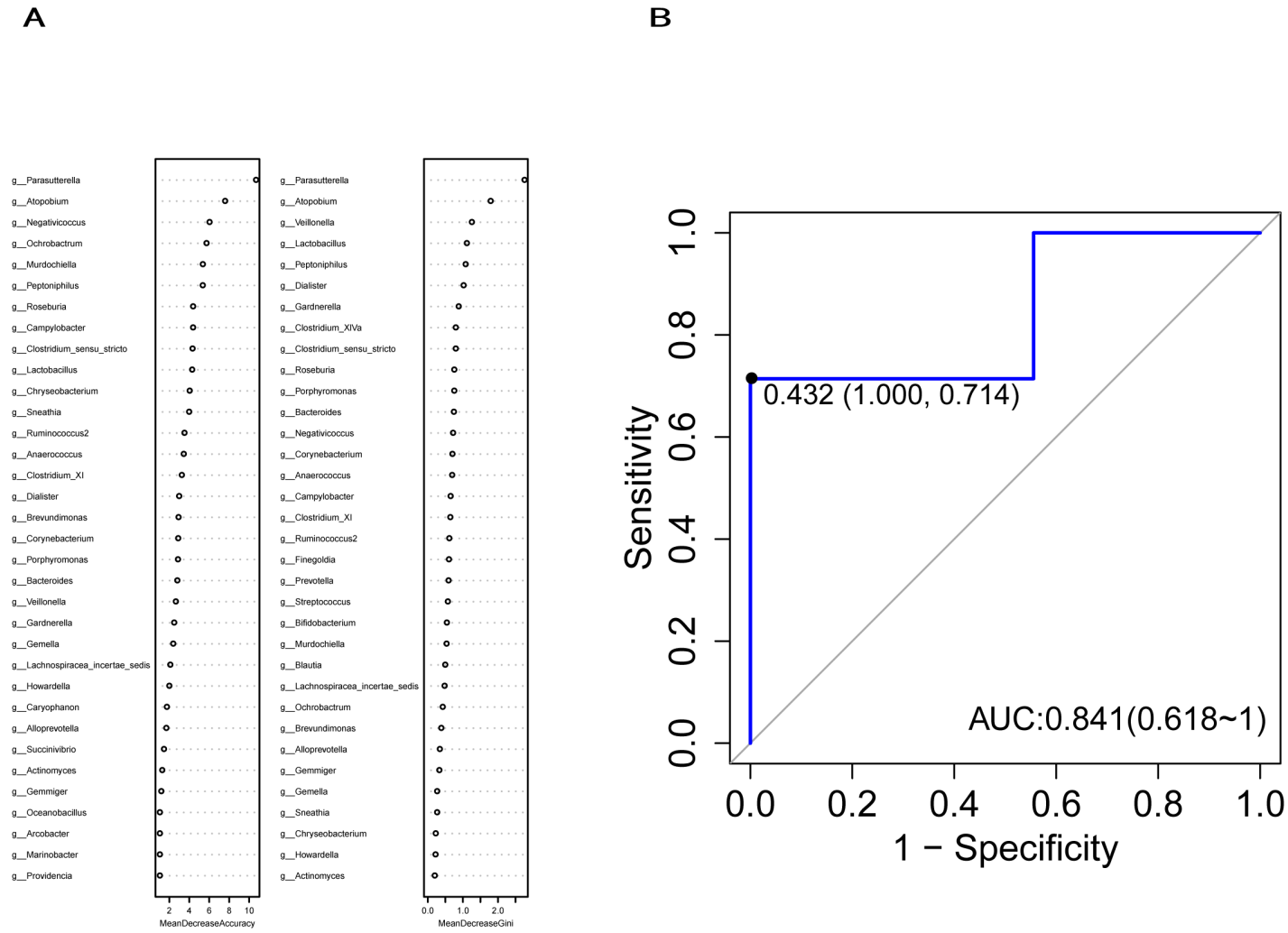

Figure 4

The predictive model based on genus-level abundance taxa using a random forests model. A: The difference in contributions of different species enabled groups A and B to be distinguished; B The ROC curve of a random forest model was constructed based on the sorted different species, where the abscissa is 1-specificity and the ordinate is sensitivity. When the area under the curve (AUC is 0.5-0.7, the accuracy is low; when AUC is 0.7-0.9, there is certain accuracy; when AUC is above 0.9, the accuracy is high). Larger AUC indicates better model prediction effect.

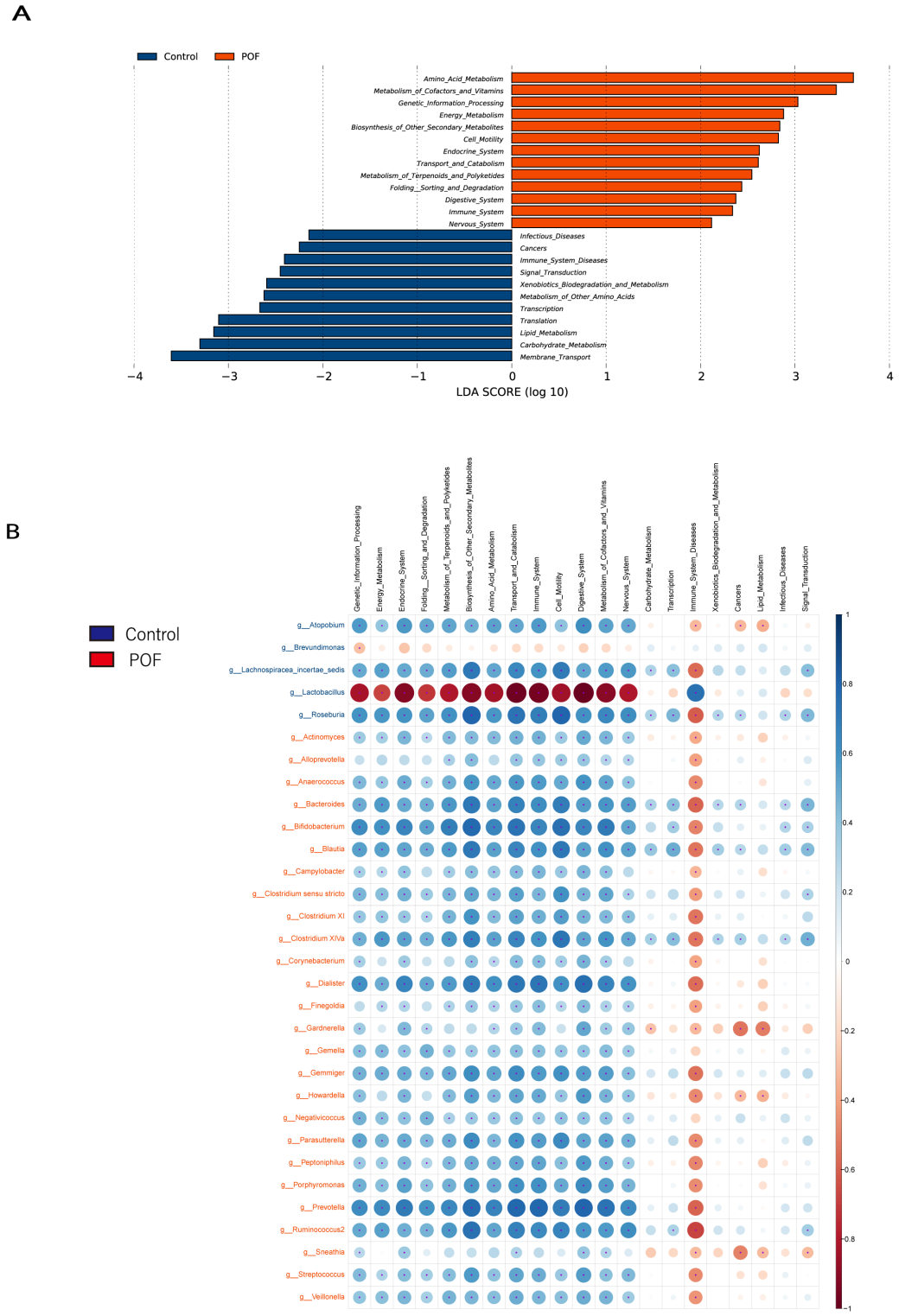

Figure 5

Functional predictions of vaginal flora of the POF and control groups. A: The LDA scores obtained by LDA (linear regression analysis) of the microbial populations identified to have significant effects in different groups were counted. The threshold for LDA was 2.B: The area above the horizontal axis represents the predicted metabolic pathway. The figure below shows the type of metabolic pathway, and the left

corresponds to the particular species. Red indicates a negative correlation and blue a positive correlation. The red box highlights extremely significant correlations.

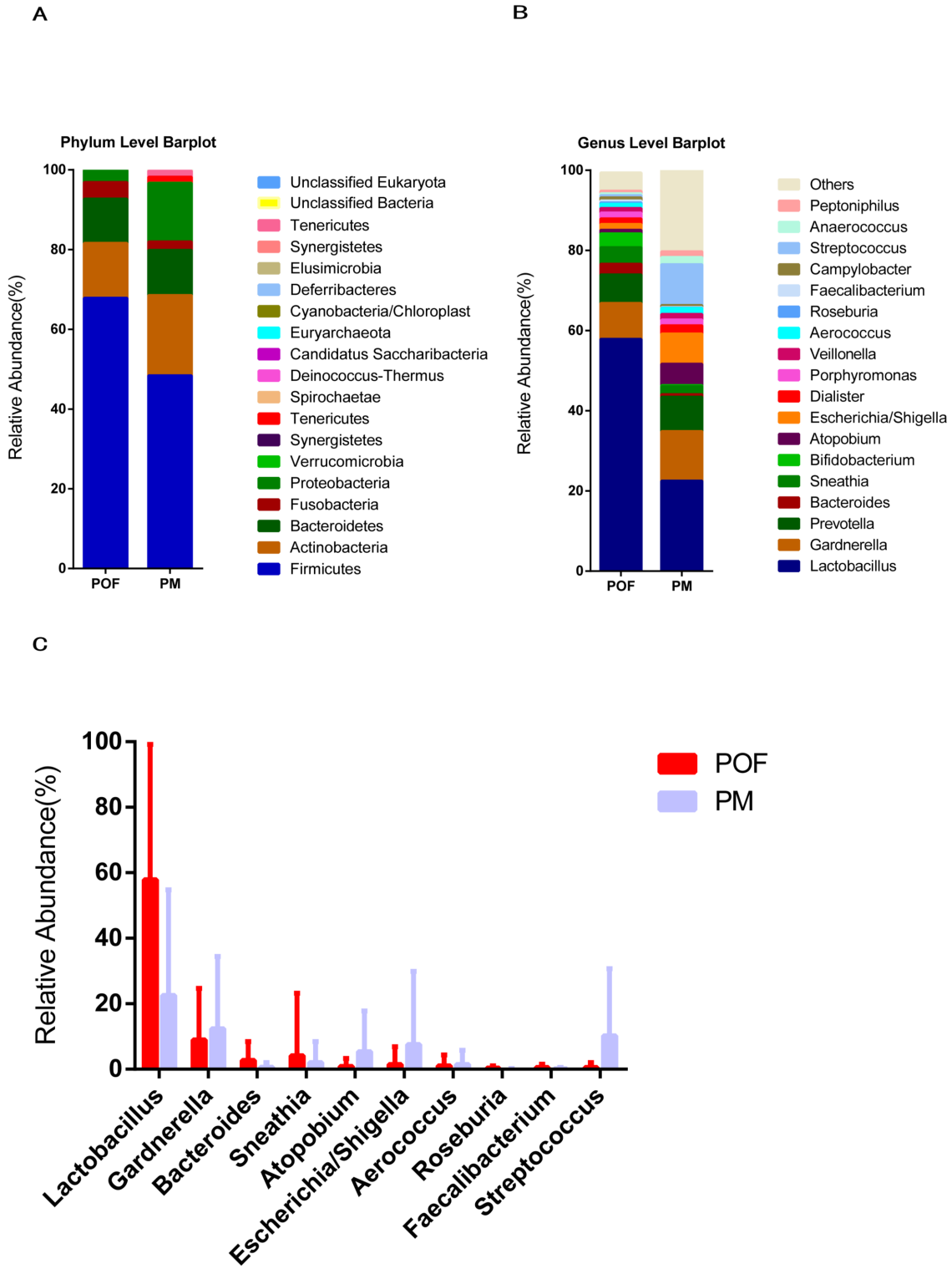

Figure 6

Species abundance map between POF with menopause. A:Phylum level barplot; B: Genus level barplot; C: Species of significant differences between POF and menopause.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementryFigure1.tif
- SupplementaryFigure2.tif
- SupplementaryFigure3.tif