Metagenomic analysis identified microbiome alterations and pathological association between intestinal microbiota and polycystic ovary syndrome

Weiwei Chu, M.D.,[a,b] Qixin Han, M.D.,[a,b] Jieying Xu, Ph.D.,[a,b] Juan Wang, M.D.,[a,b] Yun Sun, Ph.D.,[a,b] Weiping Li, Ph.D.,[a,b] Zi-Jiang Chen, Ph.D.,[a,b,c] and Yanzhi Du, Ph.D.[a,b]

[a] Center for Reproductive Medicine, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai; [b] Shanghai Key Laboratory for Assisted Reproduction and Reproductive Genetics, Shanghai; [c] National Research Center for Assisted Reproductive Technology and Reproductive Genetics, Key Laboratory for Reproductive Endocrinology of Ministry of Education, Center for Reproductive Medicine, Shandong University, Jinan, People's Republic of China

Objective: To identify different microbial species in women with polycystic ovary syndrome (PCOS) and reveal a possible relationship between gut dysbiosis and pathological changes.
Design: Cross-sectional study.
Setting: Academic institution.
Patient(s): Reproductive-aged women with PCOS (n = 14) and controls (n = 14) from the Centre for Reproductive Medicine.
Intervention(s): Shotgun metagenomic sequencing on fecal samples from patients, and clinical parameters (including body mass index, endocrine hormone levels, and glycemia level) gathered for correlation analysis.
Main Outcome Measure(s): Identification of different gut microbial strains and relativity between microbiota and clinical parameters.
Result(s): We found several microbial strains were statistically significantly more abundant in the PCOS group, including *Parabacteroides merdae*, *Bacteroides fragilis*, and strains of *Escherichia* and *Shigella*, whereas *Faecalibacterium prausnitzii* was enriched in the control group. Metagenomic species (MGS) analysis revealed that the microbes of the PCOS group were negatively correlated with those of the control group. Of note, we observed a positive correlation between MGS relevant to PCOS and endocrine disorders, including body mass index and elevated levels of serum testosterone, luteinizing hormone, and antimüllerian hormone. Functional alterations, reflected by Kyoto Encyclopedia of Genes and Genomes orthologues, could imply potential mechanisms of microbial involvement in the developmental progress of PCOS.
Conclusion(s): Our findings suggest an intimate association and potential mechanisms linking microbial dysbiosis and the pathophysiologic changes of PCOS. We address the importance of monitoring and modulating microbial composition and functional shifts in future clinical practice. (Fertil Steril® 2020;113:1286–98. ©2020 by American Society for Reproductive Medicine.)
El resumen está disponible en Español al final del artículo.

Key Words: Gut microbiota, KEGG orthologue, metagenomic sequencing, pathophysiologic progress, polycystic ovary syndrome

Received September 27, 2019; revised December 25, 2019; accepted January 21, 2020.
W.C. has nothing to disclose. Q.H. has nothing to disclose. J.X. has nothing to disclose. J.W. has nothing to disclose. Y.S. has nothing to disclose. W.L. has nothing to disclose. Z.-J.C. has nothing to disclose. Y.D. has nothing to disclose.
W.C., Q.H., and J.X. should be considered similar in author order.
Supported in part by grants from the National Key Research and Development Program of China (2017YFC1001002 and 2018YFC1003202), the National Natural Science Foundation (81671414, 81671413, and 81971343), and Shanghai Commission of Science and Technology (17DZ2271100).
Reprint requests: Yanzhi Du, Ph.D., 845 Lingshan Road, Shanghai 200135, People's Republic of China (E-mail: duyz@sjtu.edu.cn).

Fertility and Sterility® Vol. 113, No. 6, June 2020 0015-0282

https://doi.org/10.1016/j.fertnstert.2020.01.027

Polycystic ovary syndrome (PCOS) is an endocrinal and metabolic disorder in women of reproductive age, with a prevalence of about 5% to 20% (1). Clinically, PCOS is characterized by hyperandrogenism, oligo-anovulation, and polycystic ovarian morphology (2). The pathologic state of PCOS is a life-long condition and leads to an increased risk of hyperlipidemia, cardiovascular disease, hypertension, the metabolic syndrome,

and endometrial cancer (3–5). Polycystic ovary syndrome affects not only women's physical health but also their mental health (6). Although the pathogenesis of PCOS remains unknown, the interplay between genetic and environmental factors has been suggested to play a key role in its development (7).

As one of the environmental factors, gut microbiota has attracted increasing attention owing to its vital role in the metabolic and immune systems. The composition of gut microbiota depends not only on disease states but also on age, gender, ethnicity, dietary habits, and medication (8–11). Gut microbial dysbiosis is associated with the development of chronic metabolic conditions such as obesity, diabetes, and nonalcoholic fatty liver disease (12–14). Bacteria dysbiosis is also linked to altered energy homeostasis, increased inflammation, and choline and bile acid metabolism, all of which are considered important to the development of a metabolic disorder (15).

Many studies using 16S rRNA sequencing techniques to compare women with PCOS and control groups have found distinctive differences in the diversity and composition of gut microbiota (16–19). They have observed altered microbial taxa associated with clinical parameters, including the levels of serum total testosterone and luteinizing hormone (LH), and insulin resistance (17–20). Moreover, research conducted by Qi et al. (21) to investigate the impact of the gut microbiota on the regulation of PCOS-associated ovarian dysfunction and insulin resistance found the application of probiotics improved steroid hormone levels in PCOS patients (22) and murine models (23). These findings suggested that modifying the gut microbiota and altering bile acid metabolism could be of value for the treatment of PCOS.

However, despite the correlations between microbial dysbiosis and clinical parameters in PCOS patients, some investigations have had inconsistent results regarding the altered gut microbial taxon. Although 16S rRNA sequencing allows reliable taxa resolution up to the genus level, it has not provided information about the functional characteristics. Shotgun metagenome studies go far beyond conventional 16S rRNA microbiome sequencing, and add a functional component to the microbiome analysis (24, 25). Thus, we used multiomics technologies to explore the taxonomic structure of specific strains and identify the microbial function and metabolic mechanisms of strain-specific involvement in the developmental progress of PCOS.

In addition, we conducted a correlation analysis between the clinical parameters and microbial abundance. Although many researchers have found gut microbiologic dysbiosis in PCOS, few have explained the pathologic changes these microbiota promote. Understanding this pathologic progression would further our knowledge of how gut microbiota apply to PCOS. By comparing the functional Kyoto Encyclopedia of Genes and Genomes (KEGG) orthologues (KOs) enriched in women with PCOS compared with controls, we profiled the functional differences of the corresponding microbiomes and propose possible pathologic mechanisms of PCOS development. Our study provides novel prospects for microbial investigation into the pathogenesis of PCOS as well as suggests the value of monitoring microbiomes in future clinical treatments for PCOS.

MATERIALS AND METHODS

Study Participants

The participants in our study were recruited from female patients during their first appointment with the Centre for Reproductive Medicine at Ren Ji Hospital, part of the School of Medicine at Shanghai Jiao Tong University. The medical information from all the participants was reviewed, and we recorded data on the age of menarche, duration of menstrual periods, menstrual cycle, fertility desires, and history of infertility and treatments. The inclusion criteria for this study were [1] 20 to 35 years of age and [2] Han ethnicity. The exclusion criteria were [1] hyperprolactinemia, hypo- or hyperthyroidism, or abnormal liver, kidney, or heart function; [2] gastrointestinal disease, active infections, thyroid dysfunction, hyperprolactinemia, hypertension, or diabetes mellitus; [3] oral contraceptives, glucocorticoids, antiandrogen agents, ovulation induction agents, diabetic drugs, or other steroid agents taken within 3 months; or [4] antibiotic therapy received within at least 3 months before the study.

Fourteen PCOS patients fulfilled all three of the Rotterdam criteria (2), and 14 non-PCOS patients were selected who had tubal factor infertility with regular menstrual cycles and normal ovarian morphologies. All the participants were defined as either not overweight (<24 kg/m^2) or overweight (≥ 24 kg/m^2) by the cutoff points for body mass index (BMI) in the Chinese BMI reference from the Group of China Obesity Task Force (26). All participants were asked to record their daily dietary habits, including the different types and total amount of carbohydrate ingestion per day. Their daily intake of desserts, beverages, yogurt, vegetables, and fruit as well as their defecation routine and preferred method of exercise were also collected via written questionnaire.

This study was approved by the Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University, and all participants gave their written, informed consent about the use of clinical data and fecal samples. All experimental procedures were reviewed and approved by the institutional review board, and signed, informed consent was obtained from each patient (number 2015030307).

Laboratory Measurements

Baseline blood samples were collected on day 3 of a natural cycle for hormone and metabolic measurements. The plasma and serum aliquots were stored at −80°C before the tests. The levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), total testosterone, fasting blood glucose, thyroid-stimulating hormone (TSH), and antimüllerian hormone (AMH) were measured by chemiluminescent assay (Roche Diagnostics) or enzyme-linked immunosorbent assay (Kangrun) kits.

Sample Collection and DNA Extraction

Fresh fecal samples were collected from the participants on their first visit and before any pharmacotherapy treatment

at the Centre for Reproductive Medicine. These samples were then transported to the laboratory with an ice pack within 2 hours. All samples were then snap frozen and stored at −80°C before analyses. We extracted DNA from each fecal sample using an improved protocol based on the QIAamp Fast DNA Stool Mini Kit instructions (Qiagen). In more detail, 1 mL of InhibitEX buffer and a sufficient number of glass beads (0.5 mm diameter; Qiagen) were added to each 200 mg of feces. The mixture was homogenized and beaten with 60 Hz for 1 minute, twice with a homogeneous instrument (FASTPREP-24; Aosheng Biotech). We subsequently performed DNA purification according to the manufacturer's instructions.

Metagenomic Sequencing

Following the Illumina TruSeq DNA Sample Prep v2 Guide (Illumina), we constructed the DNA libraries with approximately 5,000-base pair (bp) insert sizes for the 28 fecal samples (14 from PCOS patients and 14 from the controls). The quality of all libraries was evaluated using an Agilent 2100 Bioanalyzer (Agilent Technologies) and the DNA LabChip 1000 kit. All samples were subjected to 150-bp paired-end sequencing on an Illumina HiSeq 4000 platform (Illumina). Reads containing three ambiguous N bases were removed, and those containing lower-quality (Q $<$20) bases were trimmed. Reads containing less than 60% of high-quality bases (Phred score $\geq$20) were deleted and then mapped to the human genome based on alignment with SOAPaligner 2.21) (27). Consequently, an average of 97.88% high-quality reads were obtained from all samples. The sequence data we report have been deposited in the NCBI database (accession no. PRJNA549764 [metagenomic sequencing data]).

De Novo Assembly and Construction of the Gene Catalogue

To construct the gene catalogue, filtered high-quality reads of all samples were assembled into contigs using SOAPdenovo (version 1.05). In total, 935,241 contigs (minimum length of 500 bp) were generated. These contigs had an average N50 length of 12,568 bp and ranged from 3,813 to 43,826 bp. Prediction of open reading frames (ORFs) in contigs was realized by MetaGeneMark (version 2.10). The nonredundant gene catalogue set was constructed by pairwise comparison of predicted ORFs (gene length $>$100 bp) using CD-HIT (version 4.5.7; identity $>$95%, and coverage $>$90%). The final nonredundant gene catalogue contained 842,737 ORFs with an average length of 843 bp. The nonredundant genes were annotated against the KEGG database by BLAST version 2.2.28+ (high-scoring segment pair scoring $>$60). We accumulated the relative abundance of all orthologous genes to generate the relative abundance of each KO.

Taxonomic and Gene Profiling

We used SOAPaligner 2.21 to align clean reads to the microbial reference genomes downloaded from the U.S. National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov). The taxonomic relative abundance profile was generated according to the procedure described by Qin et al. (28). Reads aligned to multiple taxa were allocated proportionally to read counts uniquely mapped to these taxa (normalized by genome length). The same strategy was used for the gene abundance profile.

Taxonomic and KO Marker Identification

To identify species that were differentially abundant among the two groups, we applied Wilcoxon tests—along with Benjamini–Hochberg multiple test correction—to calculate the probabilities that frequency profiles did not differ between the two groups. Based on a threshold of $P<.05$, we found 506 species. A similar P value and group enrichment method was also computed for the genes and KOs.

Metagenomic Species Analysis

Genes were clustered into metagenomic species (MGS) as follows. Briefly, based on the relative abundance across all samples, the differentially abundant genes ($P<.05$ in the Wilcoxon test) were clustered using single-linkage clustering (Pearson correlation coefficient, $P>.8$). Subsequently, the mean relative abundance of each cluster with more than 25 genes was computed separately for controls and PCOS patients. Clusters with $P>.9$ were fused to produce MGS. We generated 52 and 34 clusters from 8,780 "control" enriched genes and 10,494 "PCOS" enriched genes, respectively. They were annotated to a taxonomical assignment from strain to super kingdom level. The relative abundance of the 86 MGS was computed using the 25 tracer genes.

Correlation Network of MGS

The co-occurrence pattern was constructed between the MGS using the method described by Zhang et al. (29). Each MGS was calculated using the mean value from all samples in each group. Statistical P values were corrected using the false discovery rate (FDR) method of the p.adjust package in R (https://www.r-project.org). Correlations have an absolute Pearson correlation above 0.6 with an FDR-corrected statistical significance level under 0.1. These correlations were transformed into links between two MGS in the co-occurrence network. The co-occurrence networks were then visualized using Cytoscape 3.0.2 (https://cytoscape.org).

Statistical Analyses

To analyze the statistical significance of the different taxonomic (phylum, genus, and species) levels, genes, and KOs, we used the nonparametric Wilcoxon test (wilcox.test in R). The Benjamini-Hochberg method correction was used in multiple comparisons. Enriched features with $P<.05$ were identified, and the enrichment group was then determined according to a higher rank-sum value. The linear discriminant analysis (LDA) effect size (LEfSe) method was used to determine the organisms most capable of explaining differences between the two groups. Different organisms with an LDA score cutoff of 2.0 were identified. For continuous variables,

the differences between groups were analyzed with independent-samples *t*-tests, and the mean ± standard deviation in each group is reported. For categorical variables, Fisher's exact test was used at a two-sided significance level of .05.

RESULTS

Clinical, Hormone, and Metabolic Variables of Participants

All the participants were at their first visit to our reproductive center, and none had undergone any antibiotic or hormone therapy (such as clomiphene, letrozole, or progestins) for at least 3 months before this study. Age did not differ among the groups. We summarize the clinical, hormone, and metabolic data of the recruited participants in Table 1.

To examine the characterization of PCOS patients and effect of BMI, we also subdivided the women with PCOS and the control group by weight, and found BMI to be statistically significantly higher in the overweight group compared with the nonoverweight group. Besides longer menstrual cycles, women with PCOS had higher levels of AMH, LH, and total testosterone compared with the control group. When excluding the effect of BMI, the AMH, LH, and total testosterone levels were increased in the overweight women with PCOS, whereas only AMH and LH levels were increased in the normal weight women with PCOS. Notably, the concentration of FSH was obviously lower in the overweight group compared with the nonoverweight group. To distinguish the influence of disease, we then compared the FSH level between the normal weight and overweight controls, and the normal weight and overweight women with PCOS. The baseline FSH level was statistically significantly lower in the overweight control group ($P=.047$) and PCOS group ($P=.026$) (data not shown). The levels of TSH and fasting blood glucose were not different among the groups.

We also obtained information on the daily dietary habits of each participant via a questionnaire (Supplemental Table 1, available online). The eating habits, including dietary frequency, dessert and soda beverage intake, and yogurt and fruit consumption, were not different among the groups. This excluded the effect of daily diet on gut microbiota and suggested that any attribution was from disease.

Microbial Composition and Diversity of Gut Microbiota

A total of 587,358,019 reads were identified as clean reads through analysis of the high-throughput sequencing results. An average of 6.3 Gb clean reads was generated per sample. These reads were aligned to the reference genomes from the U.S. National Center for Biotechnology Information and Human Microbiome Project. Accumulation and rarefaction curves were used to evaluate the adequacy of sample size. As indicated in Supplemental Figure 1A (available online), the interquartile range became smaller, and the number of genera detected was closer to 1,000, as the sample size increased. The curve began to plateau when the sample size reached 28, indicating that the sample size selected for sequencing was adequate. The rarefaction curve also implicated suitable experimental data for further analysis (see Supplemental Fig. 1B).

To find out the composition of intestinal microbiota of women enrolled in this study, we compared the variety and relative abundance of taxon in the two groups. The major microbial taxa in samples of two groups were *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* at the phylum level (Supplemental Fig. 2A, available online). At the genus level, the intestinal flora of the two groups was dominated by some microbial genera, such as *Bacteroides*, *Prevotella*, *Alistipes*, and *Parabacteroides* (see Supplemental Fig. 2B). Based on abundance detection at the species level, most of the microbial species that composed the gut microbiota are currently unknown (see Supplemental Fig. 2C). Similar results were obtained in the dominant species analysis. For the top 20 species that enriched the control group, 14 belonged to the genus *Bacteroides*, three to the genus *Prevotella*, two to the genus *Clostridium*, and one to the species *Alistipes putredinis* (see Supplemental Fig. 1C). As for the dominant species in the PCOS group, 15 belonged to the genus *Bacteroides*, two to the genus *Parabacteroides*, two to the genus *Prevotella*, and one to the species *Alistipes putredinis* (see Supplemental Fig. 1D).

Next, to determine the differences in the community membership among the different groups, we performed principle component analysis (PCA) and analysis of variance (ANOVA). There was no difference in the gut microbiota between the control group and PCOS group (see Supplemental Fig. 2D; $P>.05$). The differences between the nonoverweight control group versus the nonoverweight PCOS group and the overweight control group versus the overweight PCOS group were also not statistically significant (see Supplemental Fig. 2E and 2F, respectively; $P>.05$). Briefly, independent of BMI, the community construction did not differ drastically between the PCOS and control groups.

Microbial Species Differed between the PCOS and Control Groups

To further investigate the microbial difference between the PCOS and control groups, we used the LDA effect size (LEfSe) method by coupling standard tests for statistical significance with additional analyses that examined biological consistency and effect relevance. Compared with controls, women with PCOS had a greater abundance of *Bacteroides* spp., *Escherichia* spp., *Shigella* spp., *Enterobacteria* phage SfV, *Parabacteroides merdae*, and *Comamonas kerstersii* at the species level. Yet the bacteria *Bacteroides* spp., species *Blautia hydrogenotrophica*, *Tannerella sp 6 1 58FAA CT1*, *Klebsiella pneumoniae*, *Faecalibacterium prausnitzii,* and *Alistipes obesi* were associated with the control group (see Fig. 1). Similar results were obtained with the analysis of differentially abundant phylotypes via the Wilcoxon rank sum test (Supplemental Fig. 3, available online). These results together disclosed the statistically significantly different microbial species between the PCOS and control groups.

TABLE 1

Clinical parameters of polycystic ovary syndrome and control participants in the study.

	Control group		PCOS group		*P* value			
Parameters	Nonoverweight (n = 7)	Overweight (n = 7)	Nonoverweight (n = 7)	Overweight (n = 7)	Control vs. PCOS	Overweight vs. Nonoverweight	CN vs. PN	CO vs. PO
Age, y	30.29 ± 3.90	28.57 ± 2.72	27.14 ± 4.56	29.14 ± 2.87	.339	.916	.744	.157
BMI, kg/m^2	20.58 ± 4.46	27.06 ± 3.92	20.98 ± 0.89	27.93 ± 4.23	.703	<.001[a]	.668	.505
MC, d	28.79 ± 0.53	29.71 ± 2.25	74.38 ± 17.89	68.79 ± 26.21	<.001[a]	.724	.011[b]	.022[b]
AMH, ng/mL	4.06 ± 1.41	4.65 ± 1.32	12.72 ± 5.14	14.41 ± 5.68	<.001[a]	.987	.011[b]	.005[c]
FSH, IU/L	7.57 ± 0.74	5.61 ± 2.05	6.79 ± 1.28	5.17 ± 0.88	.343	.003[c]	.647	.216
LH, IU/L	5.09 ± 1.18	4.30 ± 0.92	18.04 ± 7.67	9.05 ± 3.93	.001[c]	.073	.025[b]	.006[c]
T, nmol/L	1.06 ± 0.28	0.72 ± 0.32	1.84 ± 0.81	1.41 ± 0.51	.003[c]	.13	.015[b]	.058
FBG, mmol/L	5.25 ± 0.52	4.90 ± 0.59	5.20 ± 0.22	5.31 ± 0.44	.076	.293	.426	.078
TSH, μIU/mL	1.91 ± 0.45	1.88 ± 1.04	2.20 ± 0.90	1.73 ± 0.62	.496	.603	.309	.808

Note: Data are shown as mean ± standard deviation. AMH = antimüllerian hormone; BMI = body mass index; CN = nonoverweight control group; CO = overweight control group; FBG = fasting blood glucose; FSH = follicle-stimulating hormone; LH = luteinizing hormone; MC = menstrual cycle; PCOS = polycystic ovary syndrome; PN = nonoverweight PCOS group; PO = overweight PCOS group; T = total testosterone; TSH = thyroid-stimulating hormone.

[a] *P*< .001.

[b] *P*< .05.

[c] *P*< .01.

Chu. Microbial metagenomic analysis in PCOS. Fertil Steril 2020.

MGS Reassembly and Correlation Analysis

To further explore the differential intestinal microbes at the strain level, we binned the shotgun metagenomic sequencing data into coabundance gene groups and reassembled them into MGS (28). Finally, 86 MGS were clustered, and 46 MGS were assigned specific taxonomic levels (Supplemental Table 2, available online). Among them, we observed that 34 MGS, including *Faecalibacterium prausnitzii L2–6*, *Parabacteroides goldsteinii CL02T12C30*, *Parabacteroides goldsteinii CL02T12C30*, *Bacteroidales*, *Alistipes indistinctus YIT 12060*, and *Bacteroides intestinalis DSM 17393*, were enriched in the control group, whereas 52 MGS, including *Parabacteroides merdae*, *Clostridiales*, *Enterobacteriaceae*, *Bacteroides fragilis*, *Bacteroides,* and *Bilophila*, were enriched in the PCOS group. This finding was in accordance with that of the LEfSe analysis (see Fig. 1).

To decipher the relationship between the MGS of the PCOS and control groups, we performed a correlation analysis. It is interesting that we found the MGS enriched in the PCOS group were completely different (Fig. 2A). These MGS were subsequently used to construct a network for further detecting intragroup and intergroup correlations of microbial taxon (see Fig. 2B, Spearman's rank correlation coefficient >0.6, adjusted *P*< .05). A notable characteristic in our study was that taxonomically related microbes tended to cluster. This was consistent with a previous report (28). We found that the intragroup associations were completely positive, with species *Parabacteroides merdae*, *Bacteroides fragilis*, order Clostridiales, and family Enterobacteriaceae positively correlated with each other in the PCOS group, yet negatively associated with control-enriched MGS, including strains *Faecalibacterium prausnitzii L2–6*, *Parabacteroides goldsteinii CL02T12C30*, *Bacteroides intestinalis DSM 17393*, and *Alistipes indistinctus YIT 12060*. Although some of these MGS are yet to be annotated to specific taxonomy, the intragroup and intergroup correlation results revealed that some microbes possibly tend to cluster and constitute a different community. The microbes that correlated positively in PCOS patients may function together under specific pathologic conditions, and their negative correlation with control group suggested physiological ecological damage in the same time

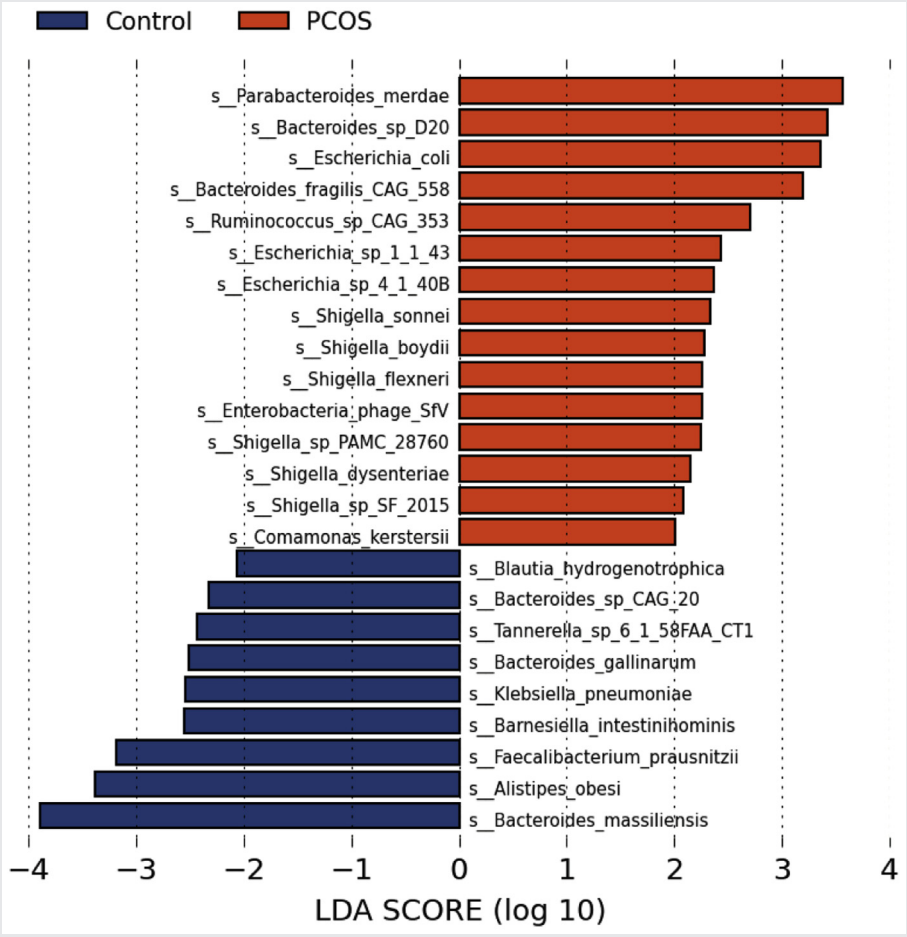
MGS Association with Clinical Parameters

We further explored the correlation between MGS and clinical manifestations of PCOS. Thirty-nine MGS showed a statistically significant correlation with reproductive hormones. Three were associated with glucose metabolism (Fig. 3A). Among the MGS relevant to PCOS, nine were annotated to the Enterobacteriaceae family and positively correlated with testosterone, AMH, and LH. Additionally, MGS annotated to species *Parabacteroides merdae* and *Bacteroides fragilis* had a statistically significant positive association with LH and AMH, respectively. Furthermore, the species *Bacteroides fragilis* was found to increase as BMI increased in the PCOS group. By contrast, the MGS related to the control group were entirely negatively correlated with the serum levels of testosterone, AMH, and TSH. The Enterobacteriaceae family had a positive association with the fasting blood glucose level. Limited annotation information revealed that only one MGS belonged to the microbial order Bacteroidales, and it was negatively correlated with the AMH level (see Fig. 3B; Supplemental Table 2). This could have been a result of the finite investigation on gut microbiota under the current physiologic conditions, so more studies on gut microbial properties and functions are needed in the future.

DISCUSSION

In this study, we recruited outpatient participants, divided them into control and PCOS groups, and collected their clinical indices. In accordance with the current literature (30), we found LH, AMH, and testosterone levels were much higher in the PCOS group compared with the control group. It is interesting that we found the baseline FSH abundance to be

FIGURE 1



Linear discrimination analysis (LDA) effect size (LEfSe). Histogram of the LDA scores computed for differentially abundant species between the polycystic ovary syndrome (PCOS) and control groups. The LDA scores (log 10) > 2 are listed. The s_ indicates microorganisms in species level (n = 14/group).

Chu. Microbial metagenomic analysis in PCOS. Fertil Steril 2020.

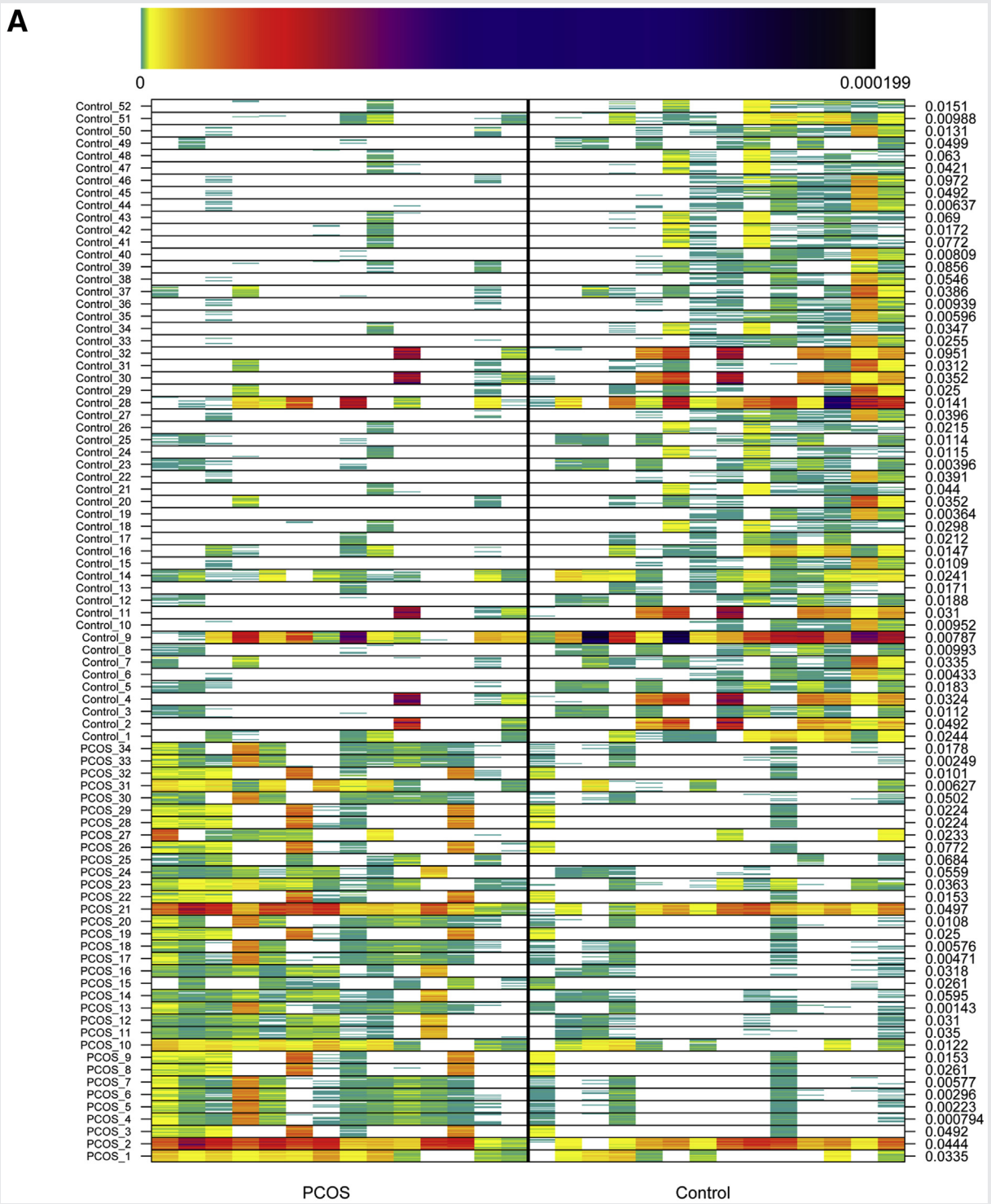
higher in the nonoverweight participants, which was in agreement with a previous study conducted by Xu et al. (31). Other studies have found this abnormality and lower serum estradiol (E_2) level at baseline in normal weight women (32). We hypothesize that this is associated with the fact that overweight women have peripheral conversion of androgens to estrogens; the negative feedback at the hypothalamus level thus suppresses FSH release by the pituitary (33).

Because differences in daily food intake could have a considerable effect on gut microbiota, we collected the participants' diet information and found no differences between the groups. We could exclude the effect of daily diet on gut microbiota, which suggests the attribution was related to disease. Through deep shotgun metagenomic sequencing of the participants' fecal samples followed by the LEfSe method and top abundance analysis by Wilcoxon rank sum test, we found that many gut microbial species differed between the PCOS and control groups.

The microbes enriched in the control group were critical to stable, beneficial gut microbiota constitution. For example, *Faecalibacterium prausnitzii* produces formate and butyrate, and requires acetate for growth. *Blautia hydrogenotrophica* can grow on carbon dioxide, hydrogen gas, glucose, and fructose and generates acetate. This kind of system thus constitutes a rare example of two strain pairs that simultaneously compete and mutually cross-feed (34). In addition, both microbial species, which were enriched in the control group, can produce butyrate, a beneficial short fatty acid that is an important energy source for gut epithelial cells (34).

By contrast, many kinds of opportunistic pathogens were enriched in PCOS group, including *Bacteroides* spp., *Escherichia* spp., *Shigella* spp., *Enterobacteria* phage SfV, *Parabacteroides merdae,* and *Comamonas kerstersii,* which was in accordance with the findings of Liu et al. (18). Based on previous studies, these pathogens could be associated with metabolic disorders and severe inflammation. In particular, *Parabacteroides merdae*, which could be associated with

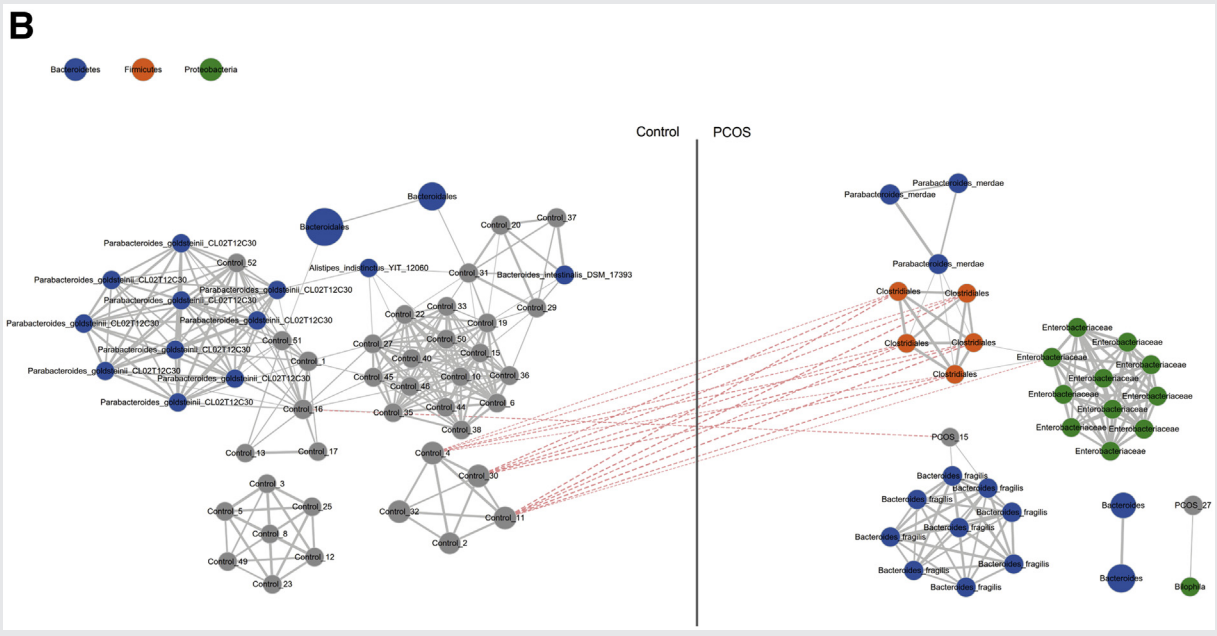
FIGURE 2



Distinct metagenomic species (MGS) between the polycystic ovary syndrome (PCOS) and control groups and correlations among MGS. (**A**) Heat map of the 86 MGS associated with PCOS. Each MGS was shown by the 25 "tracer" genes abundance. The enrichment statistical significance is listed on the right with *P* value by Wilcoxon test. The MGS identification number is shown on the left. Subjects are arranged along the horizontal axis. Gene abundance is represented by color gradient (white = not detected). (**B**) SparCC network plot of MGS. Each node represents one species, and two nodes are linked if the correlation was statistically significant (Spearman's rank correlation coefficient >0.6, adjusted $P<.05$). Solid lines indicate a positive correlation, and dashed lines indicate a negative correlation. The width of the lines indicates the association strength. The size of the nodes represents the mean relative abundance of species in each group.

Chu. Microbial metagenomic analysis in PCOS. Fertil Steril 2020.

FIGURE 2 Continued



Chu. Microbial metagenomic analysis in PCOS. Fertil Steril 2020.

developing antimicrobial drug resistance (35), has been related to a ketogenic diet through decreasing gamma-glutamyl transpeptidase activity as well as inhibiting gamma-glutamylation (36). Also, *Escherichia* could readily use ethanolamine, a common intestinal carbon and nitrogen source in the lumen during intestinal inflammation, to outcompete the host microbiota (37). Their ability to transform the metabolism profile in vivo indicates the possible mechanism underlying pathologic metabolic disorders.

In addition, we discovered that *Shigella flexneri, Shigella sonnei, Shigella dysenteriae, Shigella boydii, Shigella sp PAMC 28760, Shigella sp SF 2015*, and *Enterobacteria phage SfV*, a temperate serotype-converting phage of *Shigella flexneri,* were enriched in the PCOS group. These are believed to cause disease by secreting virulence factors, inducing severe inflammation, and mediating enterotoxic effects on the colon. They inject virulence effectors into epithelial cells to allow invasion of the epithelial cells and produce effectors to down-regulate inflammation (38). *Comamonas kerstersii,* another pathogen we found to be enriched in PCOS patients, has been associated with inflammation in peritonitis and urinary tract infections (39, 40). The different properties and metabolic functions of these pathogens possibly make the host more susceptible to metabolic disorders and inflammation, thus contributing to the development of PCOS.

In addition to species differences between the PCOS and control groups, we reassembled coabundance gene groups into MGS and investigated their associations. As expected, the MSG that were enriched in the PCOS group tended to positively associate with each other, whereas the intergroup correlations seemed to be almost negative (28). This indicates that gut microbes could possibly mutually promote each other and take roles together in vivo. Different bacterial communities and properties might function distinctively in PCOS patients. Afterward we made a correlation analysis to reveal the relationship between microbiota and PCOS. We found that the MGS annotated to the family Enterobacteriaceae and species *Parabacteroides merdae* and *Bacteroides fragilis* were positively correlated with reproductive hormones such as testosterone, AMH, and LH. Furthermore, species *Bacteroides fragilis* was found to increase as BMI increased in the PCOS group. Accordingly, previous research conducted by Liu et al. (18) revealed a positive correlation among Bacteroide, testosterone, and BMI.

However, in women of the control group enriched MGS were entirely negatively correlated with serum levels of reproductive hormones. Surprisingly, in our finding, one MGS annotated to the microbial order Bacteroidales negatively correlated with the AMH level. As one dominant Gram-negative bacteria in the healthy human gut microbiome, order Bacteroidales could produce antagonistic forms of lipopolysaccharides (LPS) and drive immune silencing for the entire microbial community (41). In PCOS patients, the intestinal LPS composition could be shifted away from anti-inflammatory subtypes in favor of inflammatory LPS subtypes. In addition, intrafollicular dysregulation of the transforming growth factor-β superfamily, such as AMH, was affected by LPS concentration (42). We hypothesize that without the protection of order Bacteroidales, higher levels of an inflammatory form of intrafollicular LPS could be

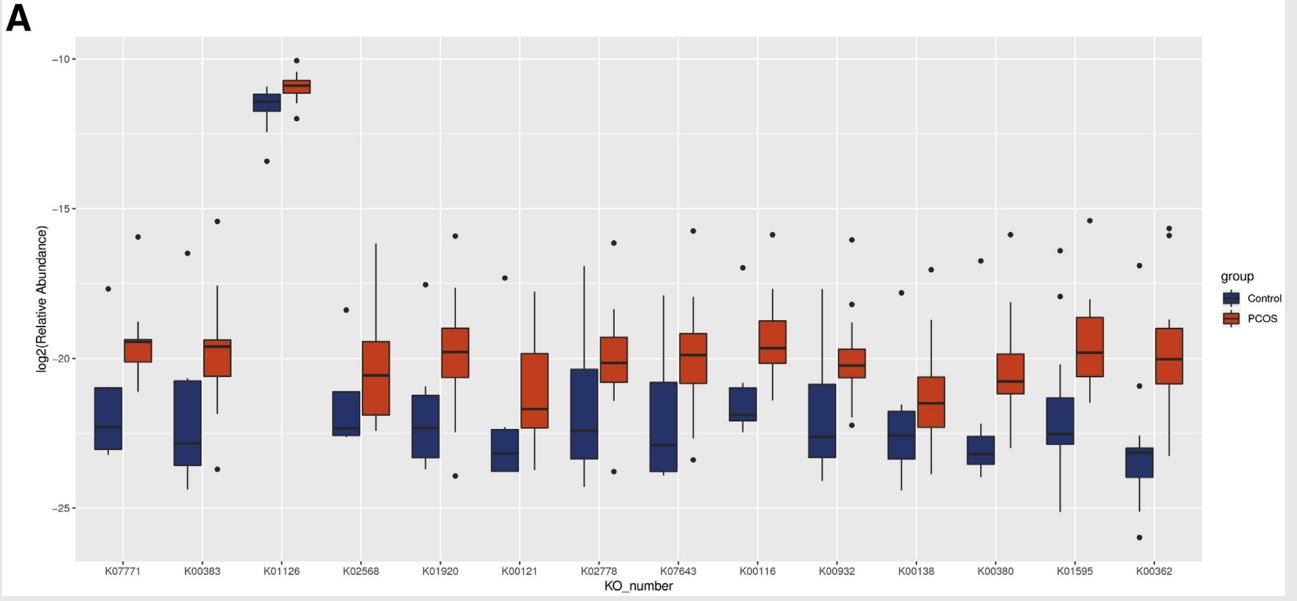
linked to poorer follicular development and recruitment, and higher AMH content in PCOS patients. These findings suggest a close relationship between flora and reproduction under different conditions, and indicate an intimate association between gut microbiota and endocrine disorder in PCOS. However, the underlying mechanism involved in this process needs further identification.

To investigate the functional role of the gut microbiota in PCOS, we identified 389 KOs associated with the disease. KOs represent functional orthologs in the context of KEGG pathway maps and are defined by extending experimental knowledge of specific organisms to other organisms (43). Collectively they could form KEGG molecular networks and represent certain aspects of molecular interaction, reaction, and relation networks. In our study, the most abundant KOs in PCOS patients and controls belonged to enzyme families (see Fig. 3A). At the module or pathway level, the PCOS associated KOs referred to cationic antimicrobial peptide resistance, phosphotransferase system (PTS), pyruvate metabolism, glutathione metabolism, short-chain fatty acids metabolism, glycolysis/gluconeogenesis, glycerophospholipid metabolism, and lipopolysaccharide biosynthesis.

With the numerous results of KOs, we propose a hypothesis of potential mechanisms regarding how imbalanced microbiota assume a role in the pathologic progress of PCOS (Supplemental Fig. 4, available online). According to this hypothesis, enriched KOs and MGS suggest that PCOS patients harbor disrupted intestinal tight junctions and increased gut permeability. Acetaldehyde, hydrogen sulfide, and ammonia, whose metabolic enzymes were enriched in the PCOS group, are known to be related to reduced gut lining integrity (44–46). Additionally, MGS annotated to *Parabacteroides goldsteinii*, which was enriched in the PCOS group, has also been found to be associated with enhanced intestinal integrity (47). In addition, the enriched *Bacteroides fragilis* and *Shigella flexneri* we found in PCOS patients have been reported to play a role in degrading and inhibiting mucin production (48, 49). Once mucin production is impaired, the mucus layer protection against pathogens is lost, and a more permeable gut barrier could trigger local or distant inflammation (48). All these results suggest the flora in the PCOS group could probably harm intestinal gut permeability and cause barrier dysfunction in intestinal tract.

Second, overgrowth of different microbes could lead to flourishing of pathogens due to a higher reactive oxygen species (ROS) environment. In our experiment, the opportunistic pathogen Enterobacteriaceae was found to be enriched in the PCOS group. This pathogen has been reported to overgrow in circumstances when it has acquired an adaptive advantage (50). Accumulation of these proinflammatory Enterobacteriaceae suggests a higher tolerance to an increased reactive oxygen species (ROS) level due to the respiratory flexibility of the enriched facultative bacteria (50, 51). In addition, ROS promote selective growth of bacterial groups through nitrate

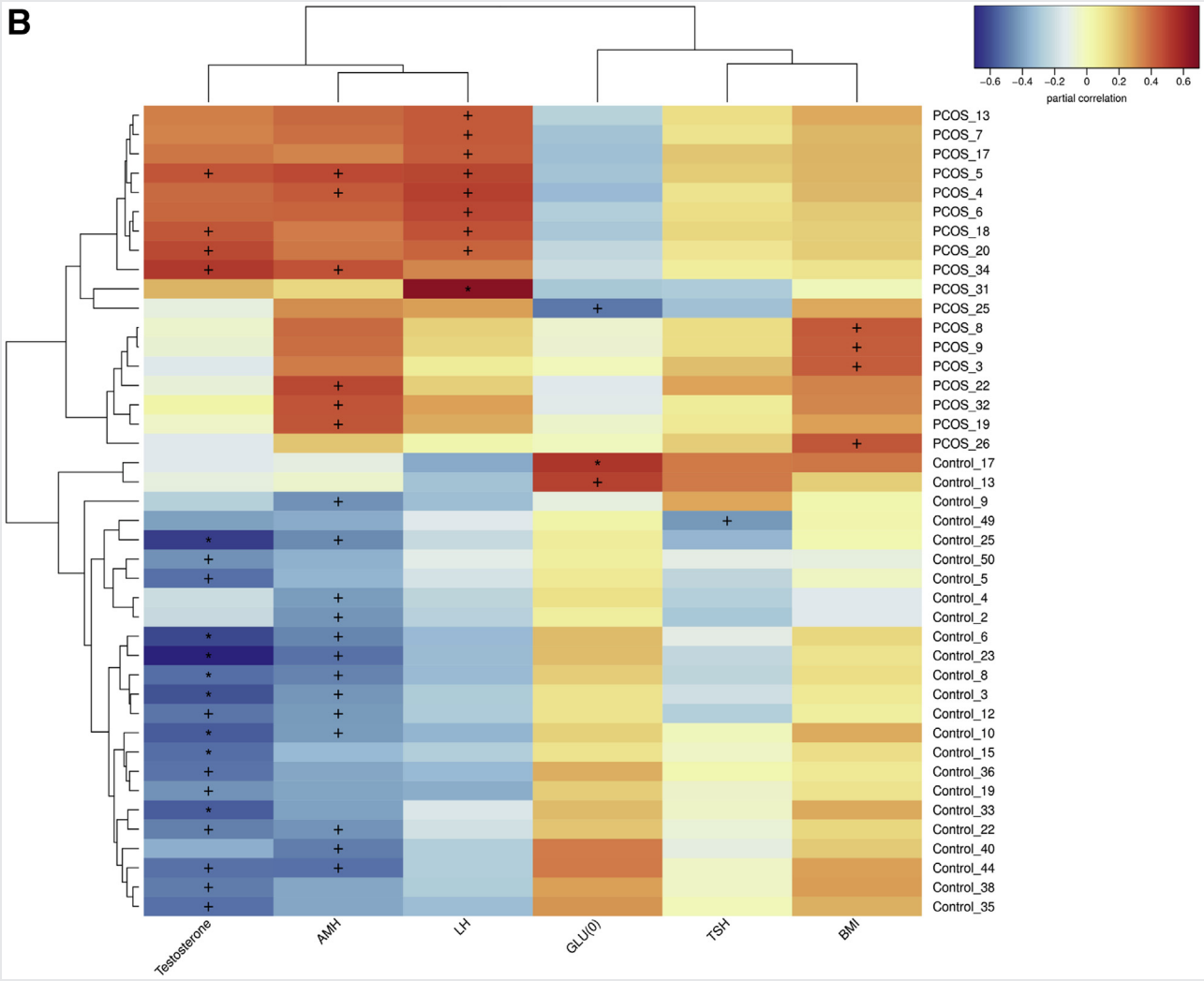
FIGURE 3



Functional characteristics of polycystic ovary syndrome (PCOS) microbiome and metagenomic species (MGS) association with clinical parameters. (**A**) Boxplot of KOs significantly differed in abundance between the PCOS and control groups. Definitions of the Kyoto Encyclopedia of Genes and Genomes (KEGG) orthologues: K07771, response regulator BasR; K00383, glutathione reductase; K01126, glycerophosphoryl diester phosphodiesterase; K02568, cytochrome c-type protein NapB; K01920, glutathione synthase; K00121, alcohol dehydrogenase; K02778, glucose-specific IIB component; K07643, sensor histidine kinase BasS; K00116, malate dehydrogenase; K00932, propionate kinase; K00138, aldehyde dehydrogenase; K00380, sulfite reductase flavoprotein alpha-component; K01595, phosphoenolpyruvate carboxylase; K00362, nitrite reductase large subunit. (**B**) Heat map of the correlation between MGS and clinical parameters of PCOS. Spearman's rank correlation coefficient is indicated using a color gradient. Red indicates positive correlation, and blue indicates negative correlation. $^{+}P<.05$; $^{*}P<.01$.

Chu. Microbial metagenomic analysis in PCOS. Fertil Steril 2020.

FIGURE 3 Continued



Chu. Microbial metagenomic analysis in PCOS. Fertil Steril 2020.

and tetrathionate respiration (52). Therefore, *Escherichia coli* and other commensal Enterobacteriaceae are likely to profit and flourish under the inflammatory environment (51, 53).

Additionally, the microbes enriched in the PCOS group were mostly Gram-negative bacteria, which were abundant with LPS. As one of the most potent inducers of inflammatory signaling, LPS was reportedly increased in a variety of metabolic diseases (54, 55). In our experiment, endotoxin excess along with a permeable gut barrier may have resulted in a greater release of LPS into the circulation. In spite of the relationship with metabolic disorders, some scientists have found that intraperitoneal (56) or intracerebroventricular (57) injection of LPS into mice reduces the expression and activity of the steroidogenesis enzyme Cyp3a11, the homologue of CYP3A4 in human livers. CYP3A4 is a member of the cytochrome P450 family. As the major form of CYP enzyme expressed in enterocytes (58), CYP3A4 undertakes metabolizing xenobiotics, including inactivation of testosterone, by hydroxylation (59). Briefly, the association between gut microbiota and endocrine disorders indicates the crucial role of bacteria, which, together with previous findings, suggests a possible mechanism underlying this pathologic progress in PCOS.

Gut microbiota and KO enriched in the PCOS group were associated with cardiovascular diseases. *Parabacteroides merdae* has been found to be higher in hypertensive individuals (60). The pathway of phosphatidylcholine dissimilation was enriched in PCOS patients, and the degradation products, such as choline, can be transformed into trimethylamine (TMA), the precursor to trimethylamine oxide (TMAO), by gut microbiota, which is a vital risk factor for cardiovascular diseases such as atherosclerosis (61).

Among the limitations of our study was the limited sample size, which likely accounts for the unchanged diversity in

gut microbiota. Moreover, when our findings are taken together with those of previous studies, it remains unclear how gut microbiota influences reproduction and metabolism in PCOS. More underlying mechanical investigations in animal models are needed to decipher the related pathologic progress. In addition, due to our limited knowledge of the gut microbiota, as well as the difficulty in distinguishing each strain, we currently cannot annotate every MGS into species or strain level, especially in the women of the control group. Another limitation was that we did not record the frequency, intensity, and duration of the participants' physical activity or include their sleeping habits in our research. Data on exercise and circadian rhythm would help to clarify the relationship between environment and PCOS.

CONCLUSION

Our study demonstrates microbiota dysbiosis and represents the microbiome alterations at the species and strain level in women with PCOS. It used shotgun metagenomic sequencing, a technology that clarifies distinctions with high sensitivity to study the potential functions of the microbiome underlying the pathologic progress of PCOS. Modulation of gut microbes toward microbiota dysbiosis, such as probiotic and prebiotic agents, may influence the degree of pathology, so this could lead to potential treatment options for women with PCOS. Our study suggests the importance of the gut microbiota in the occurrence and developing progress of PCOS, indicating that modulation of the gut microbiome could be a potential treatment option for PCOS patients.

Our study revealed alterations in the microbiome of PCOS patients in the southeast area of the People's Republic of China, pointing to an intimate association between gut microbiota and clinical parameters. We propose that the interplay between gut dysbiosis and inflammation plays a vital role in the development of endocrinal and metabolic disorders as well as complications in PCOS. It reveals the value of monitoring intestinal microbiota as an indicator and modulation of microbial dysbiosis in the clinical practice of PCOS in the future.

Acknowledgments: We thank all sample donors and the clinicians and embryologists of the Reproduction Center of Ren Ji Hospital for their excellent assistance. We are deeply grateful to Realbio Technology for the help with sample sequencing and bioinformatics analysis.

REFERENCES

1. Azziz R, Carmina E, Chen Z, Dunaif A, Laven JS, Legro RS, et al. Polycystic ovary syndrome. Nat Rev Dis Primers 2016;2:16057.
2. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil Steril 2004;81:19–25.
3. Yao K, Bian C, Zhao X. Association of polycystic ovary syndrome with metabolic syndrome and gestational diabetes: aggravated complication of pregnancy. Exp Ther Med 2017;14:1271–6.
4. Chiu WL, Boyle J, Vincent A, Teede H, Moran LJ. Cardiometabolic risks in polycystic ovary syndrome: non-traditional risk factors and the impact of obesity. Neuroendocrinology 2017;104:412–24.
5. Behboudi-Gandevani S, Amiri M, Bidhendi Yarandi R, Noroozzadeh M, Farahmand M, Rostami Dovom M, et al. The risk of metabolic syndrome in polycystic ovary syndrome: A systematic review and meta-analysis. Clin Endocrinol 2018;88:169–84.
6. Barnard L, Ferriday D, Guenther N, Strauss B, Balen AH, Dye L. Quality of life and psychological well being in polycystic ovary syndrome. Hum Reprod 2007;22:2279–86.
7. Goodarzi MO, Dumesic DA, Chazenbalk G, Azziz R. Polycystic ovary syndrome: etiology, pathogenesis and diagnosis. Nat Rev Endocrinol 2011;7:219–31.
8. Biagi E, Franceschi C, Rampelli S, Severgnini M, Ostan R, Turroni S, et al. Gut microbiota and extreme longevity. Curr Biol 2016;26:1480–5.
9. Rizzetto L, Fava F, Tuohy KM, Selmi C. Connecting the immune system, systemic chronic inflammation and the gut microbiome: the role of sex. J Autoimmun 2018;92:12–34.
10. Deschasaux M, Bouter KE, Prodan A, Levin E, Groen AK, Herrema H, et al. Depicting the composition of gut microbiota in a population with varied ethnic origins but shared geography. Nat Med 2018;24:1526–31.
11. David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, et al. Diet rapidly and reproducibly alters the human gut microbiome. Nature 2014;505:559–63.
12. Smith SB, Ravel J. The vaginal microbiota, host defence and reproductive physiology. J Physiol 2017;595:451–63.
13. Gangarapu V, Yildiz K, Ince AT, Baysal B. Role of gut microbiota: obesity and NAFLD. Turk J Gastroenterol 2014;25:133–40.
14. Gulden E, Wong FS, Wen L. The gut microbiota and type 1 diabetes. Clin Immunol 2015;159:143–53.
15. Cani PD, Bibiloni R, Knauf C, Waget A, Neyrinck AM, Delzenne NM, et al. Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. Diabetes 2008;57:1470–81.
16. Torres PJ, Siakowska M, Banaszewska B, Pawelczyk L, Duleba AJ, Kelley ST, et al. Gut microbial diversity in women with polycystic ovary syndrome correlates with hyperandrogenism. J Clin Endocrinol Metab 2018;103:1502–11.
17. Lindheim L, Bashir M, Munzker J, Trummer C, Zachhuber V, Leber B, et al. Alterations in gut microbiome composition and barrier function are associated with reproductive and metabolic defects in women with polycystic ovary syndrome (PCOS): a pilot study. PLoS One 2017;12:e0168390.
18. Liu R, Zhang C, Shi Y, Zhang F, Li L, Wang X, et al. Dysbiosis of gut microbiota associated with clinical parameters in polycystic ovary syndrome. Front Microbiol 2017;8:324.
19. Insenser M, Murri M, Del Campo R, Martinez-Garcia MA, Fernandez-Duran E, Escobar-Morreale HF. Gut Microbiota and the polycystic ovary syndrome: influence of sex, sex hormones, and obesity. J Clin Endocrinol Metab 2018;103:2552–62.
20. Zeng B, Lai Z, Sun L, Zhang Z, Yang J, Li Z, et al. Structural and functional profiles of the gut microbial community in polycystic ovary syndrome with insulin resistance (IR-PCOS): a pilot study. Res Microbiol 2019;170:43–52.
21. Qi X, Yun C, Sun L, Xia J, Wu Q, Wang Y, et al. Gut microbiota-bile acid-interleukin-22 axis orchestrates polycystic ovary syndrome. Nat Med 2019;25:1225–33.
22. Zhang J, Sun Z, Jiang S, Bai X, Ma C, Peng Q, et al. Probiotic *Bifidobacterium lactis* V9 regulates the secretion of sex hormones in polycystic ovary syndrome patients through the gut-brain axis. mSystems 2019;4:00017–19.
23. Guo Y, Qi Y, Yang X, Zhao L, Wen S, Liu Y, et al. Association between polycystic ovary syndrome and gut microbiota. PLoS One 2016;11:e0153196.
24. Mack A, Bobardt JS, Hass A, Nichols KB, Schmid RM, Stein-Thoeringer CK. Changes in gut microbial metagenomic pathways associated with clinical outcomes after the elimination of malabsorbed sugars in an IBS cohort. Gut Microbes. Published online December 6, 2019. Available at: https://doi.org/10.1080/19490976.2019.1686322.
25. Tai N, Wong FS, Wen L. The role of gut microbiota in the development of type 1, type 2 diabetes mellitus and obesity. Rev Endocr Metab Disord 2015;16:55–65.
26. Group of China Obesity Task Force. Body mass index reference norm for screening overweight and obesity in Chinese children and adolescents (article in Chinese). Zhonghua Liu Xing Bing Xue Za Zhi 2004;25:97–102.
27. Li R, Li Y, Kristiansen K, Wang J. SOAP: short oligonucleotide alignment program. Bioinformatics 2008;24:713–4.

28. Qin N, Yang F, Li A, Prifti E, Chen Y, Shao L, et al. Alterations of the human gut microbiome in liver cirrhosis. Nature 2014;513:59–64.
29. Zhang Z, Geng J, Tang X, Fan H, Xu J, Wen X, et al. Spatial heterogeneity and co-occurrence patterns of human mucosal-associated intestinal microbiota. ISME J 2014;8:881–93.
30. Zhao Y, Ruan X, Mueck AO. Clinical and laboratory indicators of polycystic ovary syndrome in Chinese Han nationality with different Rotterdam criteria-based phenotypes. Gynecol Endocrinol 2016;32:151–6.
31. Xu P, Huang BY, Zhan JH, Liu MT, Fu Y, Su YQ, et al. Insulin reduces reaction of follicular granulosa cells to FSH stimulation in women with obesity-related infertility during IVF. J Clin Endocrinol Metab 2019;104:2547–60.
32. Buyuk E, Asemota OA, Merhi Z, Charron MJ, Berger DS, Zapantis A, et al. Serum and follicular fluid monocyte chemotactic protein-1 levels are elevated in obese women and are associated with poorer clinical pregnancy rate after in vitro fertilization: a pilot study. Fertil Steril 2017;107:632–40.e633.
33. Freeman EW, Sammel MD, Lin H, Gracia CR. Obesity and reproductive hormone levels in the transition to menopause. Menopause 2010;17:718–26.
34. D'Hoe K, Vet S, Faust K, Moens F, Falony G, Gonze D, et al. Integrated culturing, modeling and transcriptomics uncovers complex interactions and emergent behavior in a three-species synthetic gut community. eLife 2018;7:e37090.
35. Boente RF, Ferreira LQ, Falcao LS, Miranda KR, Guimaraes PL, Santos-Filho J, et al. Detection of resistance genes and susceptibility patterns in Bacteroides and Parabacteroides strains. Anaerobe 2010;16:190–4.
36. Olson CA, Vuong HE, Yano JM, Liang QY, Nusbaum DJ, Hsiao EY. The gut microbiota mediates the anti-seizure effects of the ketogenic diet. Cell 2018;173:1728–41.e1713.
37. Ormsby MJ, Logan M, Johnson SA, McIntosh A, Fallata G, Papadopoulou R, et al. Inflammation associated ethanolamine facilitates infection by Crohn's disease-linked adherent-invasive *Escherichia coli*. EBioMedicine 2019;43:325–32.
38. Mattock E, Blocker AJ. How do the virulence factors of shigella work together to cause disease? Front Cell Infect Microbiol 2017;7:64.
39. Jiang X, Liu W, Zheng B. Complete genome sequencing of *Comamonas kerstersii* 8943, a causative agent for peritonitis. Sci Data 2018;5:180222.
40. Almuzara M, Cittadini R, Estraviz ML, Ellis A, Vay C. First report of *Comamonas kerstersii* causing urinary tract infection. New Microbes New Infect 2018;24:4–7.
41. D'Hennezel E, Abubucker S, Murphy LO, Cullen TW. Total lipopolysaccharide from the human gut microbiome silences Toll-like receptor signaling. mSystems 2017;2, e00046-17.
42. Shalom-Paz E, Weill S, Ginzberg Y, Khatib N, Anabusi S, Klorin G, et al. IUGR induced by maternal chronic inflammation: long-term effect on offspring's ovaries in rat model-a preliminary report. J Endocrinol Invest 2017;40:1125–31.
43. Kanehisa M, Sato Y, Kawashima M, Furumichi M, Tanabe M. KEGG as a reference resource for gene and protein annotation. Nucleic Acids Res 2016;44:D457–62.
44. Szabo G. Gut-liver axis in alcoholic liver disease. Gastroenterology 2015;148:30–6.
45. Le Chatelier E, Nielsen T, Qin J, Prifti E, Hildebrand F, Falony G, et al. Richness of human gut microbiome correlates with metabolic markers. Nature 2013;500:541–6.
46. Ramezani A, Massy ZA, Meijers B, Evenepoel P, Vanholder R, Raj DS. Role of the gut microbiome in uremia: a potential therapeutic target. Am J Kidney Dis 2016;67:483–98.
47. Wu TR, Lin CS, Chang CJ, Lin TL, Martel J, Ko YF, et al. Gut commensal Parabacteroides goldsteinii plays a predominant role in the anti-obesity effects of polysaccharides isolated from *Hirsutella sinensis*. Gut 2019;68:248–62.
48. Sicard JF, Le Bihan G, Vogeleer P, Jacques M, Harel J. Interactions of intestinal bacteria with components of the intestinal mucus. Front Cell Infect Microbiol 2017;7:387.
49. Huang JY, Lee SM, Mazmanian SK. The human commensal *Bacteroides fragilis* binds intestinal mucin. Anaerobe 2011;17:137–41.
50. Zeng MY, Inohara N, Nunez G. Mechanisms of inflammation-driven bacterial dysbiosis in the gut. Mucosal Immunol 2017;10:18–26.
51. Dubinkina VB, Tyakht AV, Odintsova VY, Yarygin KS, Kovarsky BA, Pavlenko AV, et al. Links of gut microbiota composition with alcohol dependence syndrome and alcoholic liver disease. Microbiome 2017;5:141.
52. Winter SE, Thiennimitr P, Winter MG, Butler BP, Huseby DL, Crawford RW, et al. Gut inflammation provides a respiratory electron acceptor for *Salmonella*. Nature 2010;467:426–9.
53. Weiss GA, Hennet T. Mechanisms and consequences of intestinal dysbiosis. Cell Mol Life Sci 2017;74:2959–77.
54. Faraj TA, McLaughlin CL, Erridge C. Host defenses against metabolic endotoxaemia and their impact on lipopolysaccharide detection. Int Rev Immunol 2017;36:125–44.
55. Janssen AW, Kersten S. The role of the gut microbiota in metabolic health. FASEB J 2015;29:3111–23.
56. Gandhi AS, Guo T, Shah P, Moorthy B, Chow DS, Hu M, et al. CYP3A-dependent drug metabolism is reduced in bacterial inflammation in mice. Br J Pharmacol 2012;166:2176–87.
57. Goralski KB, Abdulla D, Sinal CJ, Arsenault A, Renton KW. Toll-like receptor-4 regulation of hepatic Cyp3a11 metabolism in a mouse model of LPS-induced CNS inflammation. Am J Physiol Gastrointest Liver Physiol 2005;289:G434–43.
58. Obach RS, Zhang QY, Dunbar D, Kaminsky LS. Metabolic characterization of the major human small intestinal cytochrome p450s. Drug Metab Dispos 2001;29:347–52.
59. Schiffer L, Arlt W, Storbeck KH. Intracrine androgen biosynthesis, metabolism and action revisited. Mol Cell Endocrinol 2018;465:4–26.
60. Yan Q, Gu Y, Li X, Yang W, Jia L, Chen C, et al. Alterations of the gut microbiome in hypertension. Front Cell Infect Microbiol 2017;7:381.
61. Wang Z, Klipfell E, Bennett BJ, Koeth R, Levison BS, Dugar B, et al. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. Nature 2011;472:57–63.

Alteraciones del microbioma en el análisis metagenómico y asociación patológica entre la microbiota intestinal y síndrome de ovario poliquístico

Objetivo: Identificar las diferentes especies microbianas en mujeres con ovario poliquístico (PCOS) y relevar una posible relación entre la disbiosis intestinal y los cambios patológicos.

Diseño: Estudio transversal.

Ámbito: Institución académica.

Pacientes: Mujeres en edad reproductiva con PCOS (n=14) y controles (n=14) del Centro de Medicina Reproductiva.

Intervención (es): Secuenciación metagenómica aleatoria en muestras fecales de pacientes y los parámetros clínicos (incluyendo índice de masa corporal, niveles hormonales endocrinos, y glicemia) recogidos para análisis de correlación.

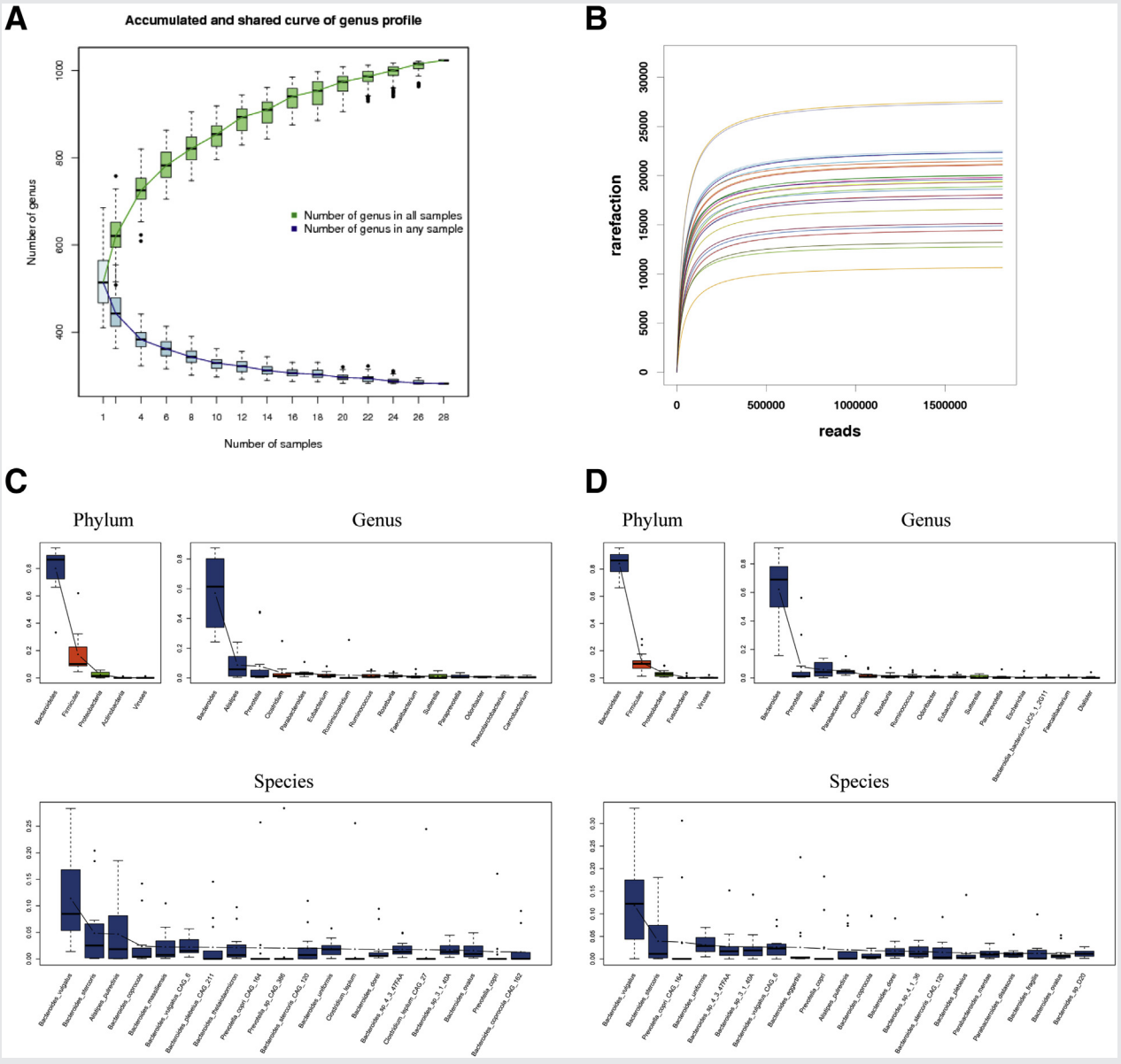
Principales medidas de resultado(s): Identificación de diferentes cadenas microbianas y la relación entre microbiota y parámetros clínicos.

Resultados: Las cadenas microbianas de *Parabacteroides merdae, Bacteroides fragilis, Escherichia* y *Shigella*, fueron significativamente más abundantes en pacientes con PCOS, mientras *Faecalibacterium prausnitzii* lo fue en el grupo control. El análisis metagenómico de especies (MGS) reveló, que los miroorganismos del grupo PCOS estaban negativamente correlacionados con los del grupo control. Es de destacar que observamos correlación positiva entre MGS relevantes en PCOS y desórdenes endocrinos, incluyendo índice de masa corporal, y niveles séricos elevados de testosterona, hormona luteinizante y hormona anti-Mülleriana. Las alteraciones funcionales reflejadas en la enciclopedia de Kyoto de genes y genomas ortólogos, podrían implicar mecanismos potenciales de la implicación microbiana en el desarrollo y progreso del PCOS.

Conclusión (es): Nuestros hallazgos sugieren una asociación estrecha y potenciales mecanismos entre la disbiosis microbiana y los cambios fisiopatológicos del PCOS. Destacamos la importancia de monitorizar y modular la composición microbiana y los cambios funcionales práctica clínica futura.

Palabras clave: Microbiota intestinal, KEGG ortólogo, secuenciación metagenómica, evolución fisiopatológica, síndrome de ovario poliquístico.

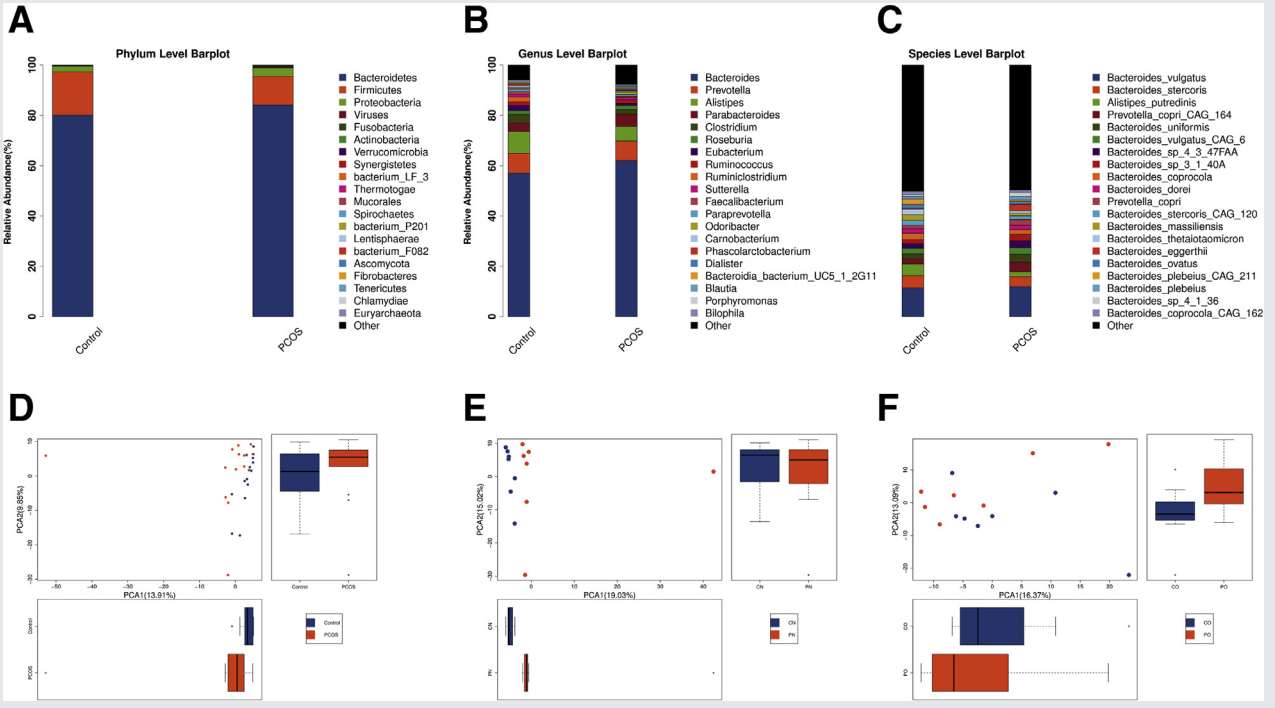
SUPPLEMENTAL FIGURE 1



(**A**) Accumulation curve of genus profile. (**B**) Rarefaction of reads in the experiment. (**C**) The top 5, top 15, and top 20 dominant microbes of control group in phylum (left), genus (right), and species (below) level, respectively. (**D**) The top 5, top 15, and top 20 dominant microbes of PCOS group in phylum (left), genus (right), and species (below) level, respectively (n = 14/group).

Chu. Microbial metagenomic analysis in PCOS. Fertil Steril 2020.

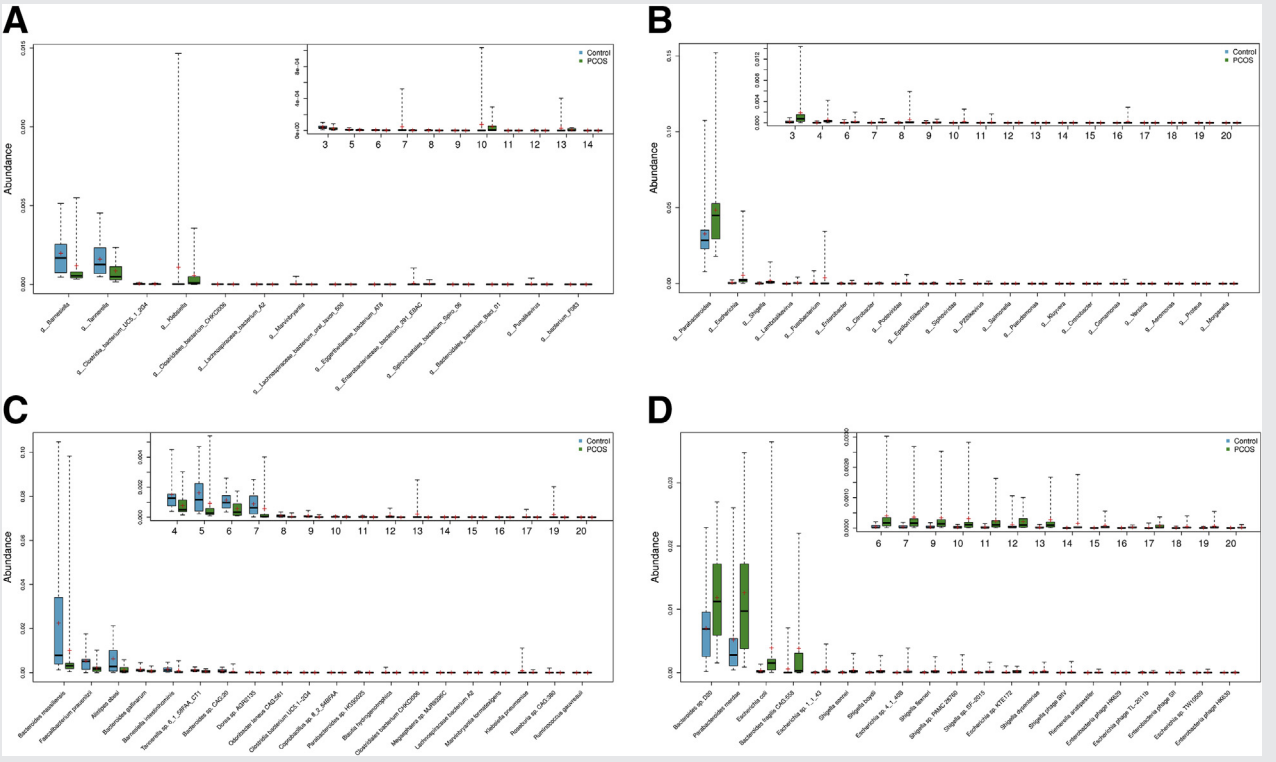
SUPPLEMENTAL FIGURE 2



Relative abundance of microbes at (**A**) phylum level, (**B**) genus level, and (**C**) species level in the control and polycystic ovary syndrome (PCOS) groups (n = 14/group). (**D**) Principal component analysis (PCA) between control and PCOS groups. (**E**) PCA of bacterial communities of CN and PN groups (n = 7/group). (**F**) Principal component analysis of bacterial communities of CO and PO groups (n = 7/group). CN = nonoverweight control group; CO = overweight control group; PN = nonoverweight PCOS group; PO = overweight PCOS group.

Chu. Microbial metagenomic analysis in PCOS. Fertil Steril 2020.

SUPPLEMENTAL FIGURE 3



(**A–B**) Comparison between the PCOS-enriched and control-enriched relative abundance of genus level microbes ranked by descending order in control group and PCOS group, respectively. (**C–D**) Comparison between the PCOS-enriched and control-enriched relative abundance of species level microbes ranked by descending order in control group and PCOS group, respectively (n = 14/group). Blue and green represent groups of controls and patients with PCOS, respectively. The top 20 statistically significantly different genera or species between two groups are shown for clarity. The phylotypes with median relative abundances greater than 0.01% of total abundance in either the healthy group or PCOS group are included (false discovery rate <.01, Wilcoxon rank sum test corrected by the Benjamini-Hochberg method). The boxes represent the interquartile range (IQR), from the first and third quartiles, and the inside line represents the median. The whiskers denote the lowest and highest values within 1.5 IQR from the first and third quartiles.

Chu. Microbial metagenomic analysis in PCOS. Fertil Steril 2020.

SUPPLEMENTAL FIGURE 4

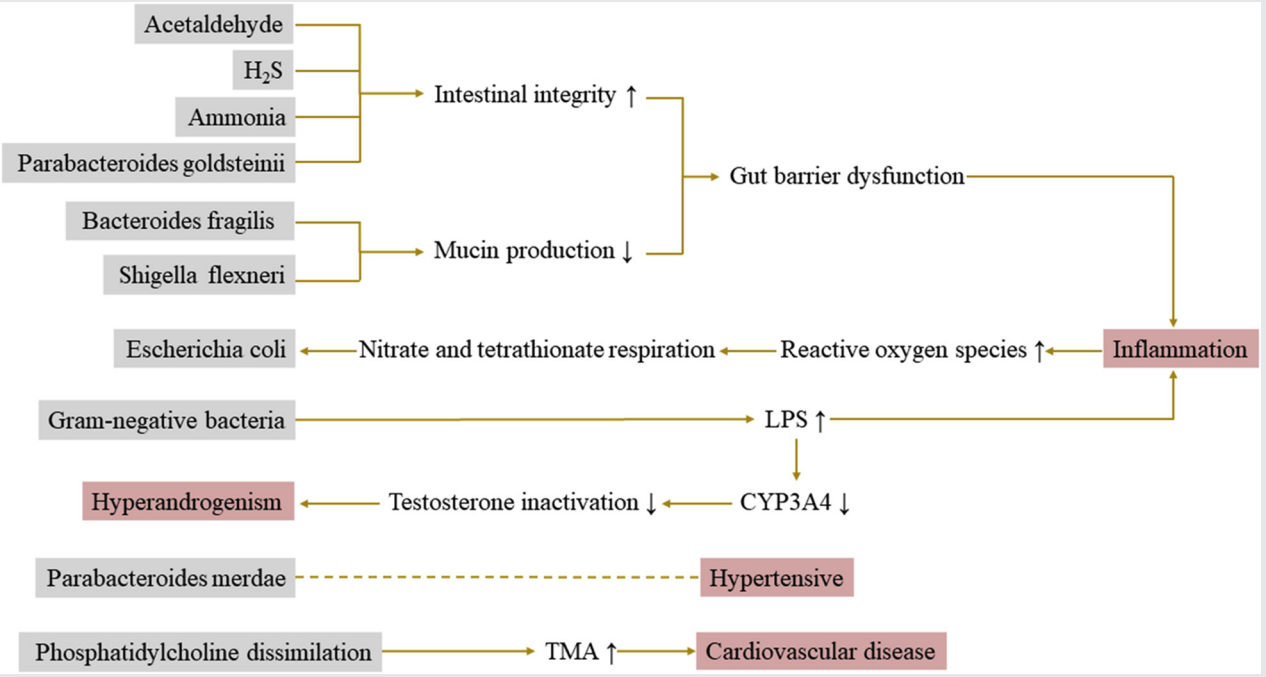


Diagram for a hypothesis regarding the possible mechanisms underlying relationship between gut bacteria abundance and pathologic changes of polycystic ovary syndrome (PCOS). Gray text boxes denote enriched microbes, product, or pathway in PCOS patients. Red text boxes denote the pathologic changes and complications in PCOS patients. H_2S = hydrogen sulfide; LPS = lipopolysaccharides; TMA = trimethylamine.

Chu. Microbial metagenomic analysis in PCOS. Fertil Steril 2020.