Characteristics of Gut Microbiota and Its Relationship With Serum Sex Hormones in Non-obese Polycystic Ovary Syndrome Patients With Insulin Resistance

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Abstract

Background: Insulin resistance (IR) is a common symptom in obese polycystic ovary syndrome (PCOS) patients, as well as in non-obese PCOS patients. Gut microbiota is closely related to IR. However, the relationship between gut microbiota and IR remains unclear in non-obese PCOS patients.

Objective: To analyze the composition of the gut microbiota population in non-obese PCOS-IR patients compared to PCOS alone and healthy women.

Methods: In the present study, fecal samples from non-obese PCOS patients with and without IR and healthy women were analyzed by 16S rRNA gene sequencing. Moreover, serum hormone levels were detected on the third day of menstruation, and the correlation between gut microbiota and serum hormone was analyzed using R language.

Results: We found that the gut microbiota character differed in each group, and the gut microbiota abundance of PCOS-IR group was remarkably reduced. The abundance of *Ruminococcaceae_UCG-004* and *Acetanaerobacterium* in the PCOS-IR group was significantly lower than that of the PCOS-NIR group. Furthermore, the comparison between PCOS-IR and control group revealed that *g_Fusobacterium* and *g_Faecalibaculum* were the unique genus of the PCOS-IR group. Moreover, the relationship between gut microbiota, IR, and serum sex hormones was determined. In PCOS with IR group, the relationship between gut microbiota and androgen was more significant. In PCOS without IR group, the relationship between gut microbiota and androgen was no significant. Its mechanism may be related to the different abundance in enzymes (futalosine hydrolase, arogenate dehydrogenase (NADP+), and L-lysine 6-transaminase) and metabolic pathways (isoquinoline alkaloid biosynthesis, styrene degradation, and atrazine degradation), but it needs to be further explored in subsequent experiments.

Conclusion: The gut microbiota composition of non-obese PCOS patients with insulin resistance is different from that of PCOS alone and healthy women. The difference is correlated with the clinical characteristics of PCOS, with regards to insulin resistance and sex hormone. The abnormality of gut microbiota may lead to the occurrence and development of PCOS-IR. All in all, this study not only provided supplements for the existing studies, but also revealed the potential pathogenic bacteria genus, thus providing a new idea for the treatment of PCOS with IR.

1. Introduction

Polycystic ovary syndrome (PCOS) is a common gynecologic endocrine disease; its incidence was 6%–10% in women and 9%–18% in childbearing age women [1,2]. PCOS is characterized by hyperandrogenemia, continuous anovulation, polycystic ovarian changes, and IR [3]. IR is closely related to PCOS, and 50%–70% of PCOS patients experience IR [4]. IR can cause metabolic abnormalities and increase the risk of type 2 diabetes, metabolic syndrome [5], and the risk of endometrial cancer. In addition, IR will aggravate the symptoms of hyperandrogenemia, thus promoting the development of PCOS [3]. Moreover, the incidence of ovulation disorders, abortion and preeclampsia in PCOS with IR patients is significantly higher than that in normal women [6,7]. Therefore, IR should be studied for preventing the long-term complications, improving the hormone level, and restoring the reproductive function of PCOS patients.

Gut microbiota is an essential microbial organ, which affects the metabolism and immune function and plays a very important role in endogenous secretion metabolic diseases. The incidence of PCOS is closely related to gut microbiota. Notably, the gut microbiota disorder is also an early factor of IR [8-10]. Therefore, gut microbiota plays a crucial role in the occurrence and development of PCOS with IR. The preliminary study of our research group showed that traditional Chinese medicine treatment could improve the IR of patients, and the abundance of *[Eubacterium]_rectale_group*, *Escherichia-Shigella*, *Fusicatenibacter*, and *Megamonas* also changed significantly [11]. Therefore, the gut microbiota is closely related to PCOS-IR. In addition, our research showed that *Lachnospirillum*, *Fusobacterium*, *Coproccoccus_2*, and *Tyzzerella4* are the characteristic genus of the gut microbiota of obese PCOS patients [12]. *Lactococcus* is the characteristic genus of the gut microbiota of non-obese PCOS patients [13]. However, the gut microbiota characteristics of non-obese PCOS patients with IR have not been studied.

Although IR is more common in obese PCOS patients, non-obese PCOS patients are also accompanied IR. The relationship between IR and gut microbiota in non-obese PCOS patients remains unclear, and the relationship between sex hormones and gut microbiota remains to be explored. Exploring the relationship between gut microbiota and non-obese PCOS-IR can reveal the pathogenesis of PCOS and provides new ideas for probiotic drug research and development. Association analysis between gut microbiota and hormone can explain the change of PCOS sex hormone and IR. In the present study, 16S rRNA sequencing and R language were used to explore the gut microbiota characteristic in non-obese PCOS patients with IR and the correlation between gut microbiota and hormone levels. The results aim to determine the characteristic gut microbiota in non-obese PCOS-IR patients and provide a supplement for the current research on the gut microbiota of PCOS.

2. Results

2.1. Analysis of OTU level difference of gut microbiota

Based on PLS-DA analysis, we found significant differences in gut microbiota among the three groups (Fig. 1a). OTU level difference analysis results showed that PCOS-IR group rank-bank curve was steeper than the control group, revealing that the abundance and diversity of gut microbiota decreased (Fig. 1b). No significant difference was observed between the PCOS-NIR and control group (Fig. 1c). In addition, in comparison with the control group, the sobs index of PCOS-IR group was significantly lower ($P < 0.05$), and the ace index of PCOS-IR group was lower without statistical difference ($P > 0.05$), thus proving the above results. No significant differences were observed in the sobs and ace index between the PCOS-NIR and control group ($P > 0.05$, Figs. 1d, e).

To provide reference for the microbial diagnosis of PCOS, we used ROC curve to evaluate the accuracy of the model. The larger the area under the curve, the higher the diagnostic accuracy. Species with the top 3 OTU level abundance were selected for the analysis, and we found that the AUC value of the PCOS-IR

and control group was 0.66 (Fig. 2a), and the AUC value of PCOS-NIR and control group was 0.66 (Fig. 2b). AUC was greater than 0.65, indicating that this model has a potential value for clinical diagnosis, but this result needs to be verified by expanding the sample capacity.

2.2. Genus level analysis of gut microbiota

We found 168 same genera in three groups, five characteristic genus in PCOS-IR group, six characteristic genus in PCOS-NIR group, and 26 characteristic genus in control group (Fig. 3a). Further, we analyzed the proportion of gut microbiota in three groups. The top five bacteria in the PCOS-IR group were *Bacteroides* (26.02%), *Faecalibacterium* (13.15%), *Prevotella_9* (9.55%), *Phascolarctobacterium* (4.08%), and *Lachnospiraceae* (2.94%, Fig. 3b). The top five bacteria in the PCOS-NIR group were *Bacteroides* (22.53%), *Faecalibacterium* (14.52%), *Prevotella_9* (7.72%), *Blautia* (4.26%), and *Phascolarctobacterium* (3.67%, Fig. 3c). The top five bacteria in the control group were *Bacteroides* (20.83%), *Faecalibacterium* (15.12%), *Prevotella_9* (11.98%), *Agathobacter* (3.29%), and *[Eubacterium]_ellgens_group* (2.68%, Fig. 3d). The proportion of bacteria in each group differ. Further, we obtained the bacteria with statistical difference among the three groups. The bacteria with statistical difference were *Ruminococcaceae_UCG-014*, *Lachnospiraceae_ND3007_group*, *norank_f_Ruminococcaceae*, *norank_o_Mollicutes_RF39*, and *Coprococcus_1* ($P < 0.05$, Fig. 3e). We also compared the genus level of gut microbiota in the PCOS-IR and PCOS-NIR group. The results showed that the abundance of *Ruminococcaceae_UCG-004* and *Acetanaerobacterium* in the PCOS-IR group was significantly lower than that of the PCOS-NIR group ($P < 0.05$, Fig. 3f).

Furthermore, Lefse analysis was performed on the gut microbiota of the three groups. The comparison between PCOS-IR and control group revealed that *g_Fusobacterium* and *g_Faecalibaculum* were the unique genus of the PCOS-IR group (Figs. 4a, b). Comparison between the PCOS-NIR and control group showed that *g_Tyzzeraella*, *g_norank_o_Gastranaerophilales*, *g_unclassified_p_Firmicutes*, *g_Morganella*, *g_Phocaea*, *g_Faecalibaculum*, and *g_unclassified_f_Desul* the unique genus of the PCOS-NIR group (Figs. 4c, d). In conclusion, differences were observed in the genus level abundance among the three groups.

2.3. Analysis of gut microbiota function and enzyme abundance

We used PICRUST to analyze the KEGG pathway abundance of gut microbiota. The abundance of each pathway can be calculated according to the gut microbiota. KEGG pathway included organismal systems, metabolism, genetic information processing, environmental information processing, and cellular processes. In these pathways, no significant difference was observed in the functional abundance among the three groups (Figs. 5a–e). Considering that IR is closely related to metabolism, we further analyzed the attached pathway of metabolism. The results showed the functional abundance of isoquinoline alkaloid biosynthesis, styrene degradation, and atrazine degradation with statistical difference ($P < 0.05$, Figs. 5f, g).

Further, we analyzed the enzyme abundance of gut microbiota among the three groups. The results showed that the top 10 enzymes with significantly difference in abundance were fucosyltransferase ($P < 0.01$), arginase ($P < 0.01$), L-lysine 6-transaminase ($P < 0.05$), homoserine O-acetyltransferase ($P < 0.05$), aldehyde dehydrogenase (FAD-independent, $P < 0.05$), kdsA ($P < 0.05$), lauroyltransferase ($P < 0.05$), scyllo-inositol 2-dehydrogenase ($P < 0.05$), serratins ($P < 0.05$), and 3-aminobutyryl-CoA ammonia-lyase ($P < 0.05$, Fig. 6).

2.4. Correlation analysis between gut microbiota and serum sex hormones

After clarifying the level and function differences of gut microbiota in the three groups, we further explored the relationship between gut microbiota and sex hormones. We first compared the hormone levels of the three groups, luteinizing hormone (LH) was significantly higher in the PCOS-IR and PCOS-NIR groups than in the control group ($P < 0.001$, $P < 0.01$), and LH/follicle-stimulating hormone (FSH) was significantly higher ($P < 0.001$, $P < 0.0001$). In addition, FINS and HOMA-IR significantly increased in the PCOS-IR group compared with PCOS-NIR group ($P < 0.0001$, $P < 0.0001$). No significant differences in FSH, estradiol (E2), dehydroepiandrosterone (DHEA), prolactin (PRL), testosterone (T), and FBS were observed among the three groups ($P > 0.05$, Figs. 7a–j). Although T and DHEA in PCOS group had no significant difference compared with the control group, they were higher than the clinical androgen threshold in normal women, and this result is consistent with the biochemical hyper androgen manifestations of PCOS [15].

Based on correlation analysis, the LH level was negatively correlated with *Blautia* and *Agathobacter* abundance ($P < 0.05$), LH/FSH was positively correlated with *norank_f_Prevotellaceae* abundance ($P < 0.05$), T level was negatively correlated with *norank_f_Prevotellaceae* and *Roseburia* abundance ($P < 0.01$), DHEA level was positively correlated with *Ruminococcus_torques_group*, *norank_f_Lachnospiraceae* and *Parabacteroides* abundance ($P < 0.05$), and HOMA-IR was positively correlated with *Anaerostipes* and *norank_f_Lachnospiraceae* abundance ($P < 0.05$) in PCOS-IR patients (Fig. 8a). FINS level was positively correlated with *Faecalibacterium* abundance ($P < 0.001$), HOMA-IR was positively correlated with *Faecalibacterium* abundance ($P < 0.01$) and negatively correlated with *Klebsiella*, *Lachnospira*, and *Prevotella_2* abundance ($P < 0.01$, $P < 0.05$), and LH/FSH was positively correlated with *Bacteroides* and *Lachnospiraceae* abundance ($P < 0.05$) in PCOS-NIR patients (Fig. 8b).

3. Discussion

In this study, we analyzed the characteristics of gut microbiota at different levels in the PCOS-IR, PCOS-NIR, and control group and explored the correlation among gut microbiota, IR, and sex hormones. It provides supplements for the current PCOS gut microbiota research.

Our results showed no significant difference between the PCOS-NIR and control group at the OTU level, while the diversity and abundance of gut microbiota in PCOS-IR group were significantly lower than the control group, suggesting that IR may be closely related to gut microbiota. Pre-diabetes and type 2 diabetes patients have abnormal gut microbiota [14,15], which support our study. In addition, the ROC area under the curve of PCOS-IR and control group, PCOS-NIR, and control group were all greater than 0.65, suggesting that the gut microbiota may have potential value as the discrimination of PCOS subtypes.

Further, the bacterial genera with statistical difference among the three groups were *Ruminococcaceae_UCG-014*, *Lachnospiraceae_ND3007_group*, *norank_f_Ruminococcaceae*. Liu R *et al.* found that the abundance of *Ruminococcaceae* in PCOS patients remarkably decreased [16]. Krete Lüll *et al.* found that *Ruminococcaceae_UCG-002* in PCOS patients was significantly different from normal women [17]. Moreover, the abundance of *Ruminococcaceae_UCG-003* in rats significantly increased after TCM treatment [18]. However, *Ruminococcaceae_UCG-014* has not been reported. In addition, differences between *Lachnospiraceae_UCG-008* and *Lachnospiraceae_NC2004_group* were also revealed at the animal level [19,20]. Therefore, a certain correlation exists between the current research and our results, and the latter can be used as a supplement to study the gut microbiota of PCOS.

To further explore the relationship between IR and gut microbiota, we compared the genus level of gut microbiota in the PCOS-IR and PCOS-NIR group. The results showed that the abundance of *Ruminococcaceae_UCG-004* and *Acetanaerobacterium* in the PCOS-IR group was obviously lower than that of the PCOS-NIR group. Bo Zeng *et al.* also found a significant difference in the abundance of *Ruminococcaceae* between PCOS-IR and PCOS-NIR [21]. However, the difference in abundance of *Acetanaerobacterium* has not been reported. In addition, Lefse analysis was performed on the gut microbiota of the three groups. Based on the comparison of the PCOS-IR and control group, *g_Fusobacterium* and *g_Faecalibaculum* were the endemic genus in the PCOS-IR group. The abundance of *Faecalibacterium prausnitzii* and *Fusobacterium nucleatum* in patients with metabolic syndrome is significantly lower than that of the control group [22]. The relationship between *Faecalibacterium* and metabolism has been verified in mouse model [23,24]. In addition, the application of traditional Chinese medicine extract can remarkably reduce the abundance of *Fusobacterium* in type 2 diabetic rats [25]. Our results are in line with the current research, and it can be used as a supplement to the related research on gut microbiota and IR.

Based on the analysis of pathway abundance, we found that isoquinoline alkaloid biosynthesis remarkably differed in the three groups. Isoquinoline alkaloid is a tyrosine derived plant alkaloid with isoquinoline skeleton. Berberine is an alkaloid isoquinoline derivative that has been used as an insulin sensitizer. Clinical studies have shown that berberine can improve IR, reduce serum androgen level, and regulate lipid metabolism and chronic inflammation [26,27]. We hypothesized that the functional turbulence of gut microbiota in patients with PCOS-IR may result in the decrease isoquinoline alkaloid synthesis, further resulting in IR and chronic inflammation. IR can further affect serum androgen level, thus forming a vicious cycle.

Our study also showed that the abundance of styrene degradation remarkably differed in the three groups. The absorption of chemical solvents and active metabolic intermediates can increase oxidative stress and cytokine levels, leading to changes in glucose metabolism and inducing IR [28]. Animal experimental evidence also shows that oxidative stress, inflammatory factors, cell viability, and glucose-metabolism-related genes are sensitive to styrene [29]. The oral administration of monodisperse polystyrene nanoplastics remarkably increases ROS, and the high level of ROS disrupts the PI3K/Akt pathway, leading to IR and high plasma glucose in mice [30]. Similarly, Atrazine is a chemical solvent, and long-term exposure to atrazine can cause IR [31,32]. We hypothesized that the occurrence of PCOS-IR may caused by the dysfunction of gut microbiota, causing the decrease in futasoline hydrolase and arogenate dehydrogenase (NADP (+)) enzyme function. Finally, it causes styrene degradation and atrazine degradation disorders.

Moreover, the abundance of L-lysine 6-transaminase, homoserine O-acetyltransferase, and aldehyde dehydrogenase (FAD-independent) substantially differed. Among these enzymes, aldehyde dehydrogenase (FAD-independent) and IR are related [33,34]. L-lysine 6-transaminase and homoserine O-acetyltransferase are related to the metabolism of amino acids. Gut microbiota can cause abnormal secretion of bile acids, which may promote the growth of *Clostridium*. The increase in *Clostridium* abundance can induce protein decomposition to the amino acids, and the amino acids and IR are correlated [35]. Therefore, gut microbiota also plays an important role between IR and amino acid.

Finally, we found a certain relationship between gut microbiota and hormones through association analysis, and different studies have also confirmed the correlation between gut microbiota and hormones [36-39]. However, whether the imbalance of gut microbiota leads to IR or IR leads to the imbalance of gut microbiota has not been determined. The causal relationship between gut microbiota and serum sex hormones remains unclear. The gut microbiota of the PCOS-IR group was associated with androgen, whereas that of the PCOS-NIR group was not. Therefore, IR can aggravate the hyper androgen state, further promoting the development of PCOS. In conclusion, we analyzed the effect of gut microbiota on IR and sex hormones and found the correlation between gut microbiota, IR and sex hormones, but the causal relationship still needs to be verified by subsequent experiments.

The deficiencies of this study are as follows: (1) The sample size is small, which is related to our strict control of the inclusion criteria and the low patient compliance. However, our study has important supplementary significance to the existing literature, and we will increase the sample size in the following study. (2) The present study found pathogenic bacteria genus that has not been reported. Although it can provide a supplement for the current research, it still needs to be verified by subsequent transplantation of animal fecal bacteria. (3) The results show a relationship among gut microbiota, IR, and sex hormones, but the causal relationship between them still needs to be further explored *in vitro* and *in vivo* experiments.

4. Materials And Methods

4.1. Participants

We recruited 29 patients without obesity suffering from PCOS (BMI< 28 kg/m², WHR≤0.85, 16–35 years old) from the TCM Gynecology Clinic of Changhai Hospital in Shanghai between June 2016 and November 2017. The diagnostic criteria for non-obesity PCOS were based on the 2003 Rotterdam Conference revisions and the criteria set by Li *et al.* [40], respectively. The exclusion criteria are as follows: history of the use of oral contraceptives, antiandrogens, and insulin sensitizers in the past 3 months before the experiment; being pregnant; presence of other known hyperandrogenemia and ovulation disorders, such as 21-hydroxylase deficiency, congenital adrenal hyperplasia, Cushing's syndrome, androgen-secreting tumors, thyroid diseases, and hyperprolactinemia; history of mental disorders or organic diseases; history of the use of corticosteroids or sex steroids; history of drug and alcohol abuse in the past 2 years before the experiment; history of the use of antibiotics, probiotics, or prebiotics in the past 3 months before the experiment; and ulcerative colitis, Crohn's disease, and other diseases that would cause dysbacteriosis. The Chinese Ethics Committee of Registering Clinical Trials approved our study (No. ChiCTRCTEC2016050), and each subject voluntarily signed the informed consent form before the trial.

4.2. Sample collection

The samples were examined on the third day of the menstrual cycle, the levels of sex hormones such as, LH, FSH, E2, T, DHEA, and PRL in the peripheral blood were detected immediately by chemiluminescence by using a UniCel DxI 800 Access Immunoassay System (Beckman Coulter, Brea, United States) in the laboratory diagnostics department of the hospital. Fasting blood glucose (FBG) and fasting insulin (FINS) were detected in the morning after 8 h of fasting. Homeostatic model assessment for insulin resistance (HOMA-IR) was calculated as $HOMA-IR = FBG \times FINS / 22.5$, $HOMA-IR \geq 1.66$ indicated IR [11]. Fecal samples were collected 3–5 days after menstruation, but 3 days before sampling, the patients received guidance for carbohydrate-based diet (300 g/day). Approximately 10 g of fresh fecal samples was collected from each patient by using a sterile plastic spoon and test tube. Before defecation, the patients needed to clear their urine. After spreading the disposable sterile nursing pad, each patient defecated and used a sterile plastic spoon to collect the central part of the feces and transfer it into a sterile tube. The sterile tube was then refrigerated while waiting for a laboratory personnel to obtain the sample. The samples were transported to the laboratory in an ice box within 2 h from sampling and stored at 80 °C. Subsequently, the structure of the gut microbiota was analyzed by 16S rRNA gene sequencing, and the fecal metabolites were examined using non-targeted metabolomics.

4.3 DNA Extraction and 16S rRNA Gene Amplicon Sequencing

Sample DNA extraction, PCR amplification, Illumina MiSeq sequencing, and post-processing of data were performed as previously described [11–13]. The gut microbiota diversity and changes in the abundance were analyzed using the OTU abundance data, and the community composition of each sample at different classification levels was obtained. The alpha diversity was also assessed using Mothur software (v.1.30.1, <https://mothur.org/>), including sobs and ace. By using the R language, we generated a bar map of the community structure at the genus level. The differences in the species between the three groups at the genus level were analyzed via Wilcoxon rank-sum test. Furthermore, the difference of species abundance in the gut microbiota between the three groups was evaluated by linear discriminant analysis (LDA). Finally, we used PICRUST to analyze the abundance of KEGG pathways and enzymes. The correlation between serum hormones and gut microbiota was analyzed by R language (Heatmap package), indicating the correlation between serum hormones and gut microbiota through color gradient in the heat map.

4.4. Data Processing and Statistical Analysis

All statistical data were analyzed using SPSS software (version 21.0). The clinical data with normal distribution were analyzed by paired t-test, while those with normal distribution and homogeneity of variance were analyzed by variance analysis. The data are expressed as mean with standard deviation. We employed Wilcoxon rank-sum test to analyze the quantitative sequencing data with non-normal distribution and Benjamini and Hochberg false discovery rate to check the p values for several times. A double-tailed $p < 0.05$ indicated significant difference.

5. Conclusion

In the present study, 16S rRNA sequencing and R language were used to explore the characteristics of gut microbiota and its correlation with serum in non-obese PCOS-IR, PCOS-NIR, and normal women. Results show that the diversity and abundance of gut microbiota in the PCOS-IR group were reduced. In comparison with normal women, *g__Fusobacterium* and *g__Faecalibaculum* are endemic genera of the PCOS-IR group, and *g__Tyzzerella*, *g__norank_o__Gastraerophilales*, *g__unclassified_p__Firmicutes*, *g__Morganella*, *g__Phoceia*, *g__Faecalibaculum*, and *g__unclassified_f__Desulfovibrionaceae* are endemic genera of the PCOS-NIR group. In addition, a relationship was observed among gut microbiota, IR and serum sex hormones. The relationship between gut microbiota disorder and androgen in PCOS-IR group was significant, which may be related to the abundance of enzymes and metabolic pathways. This study not only complements the existing research on the gut microbiota of PCOS, but also reveals the potential pathogenic bacteria, thus providing new information for the treatment of PCOS-IR.

Abbreviations

PCOS: Polycystic ovary syndrome; IR: Insulin resistance; P: Progesterone; PRL: Prolactin; T: Testosterone; LH: Luteinizing hormone; FSH: Follicle-stimulating hormone; E2: Oestradiol; DHEA: Dehydroisoandrosterone; FBG: Fasting blood glucose; FINS: fasting insulin; HOMA-IR: Homeostatic model assessment for insulin resistance.

Declarations

Ethics Approval and Consent to Participate: The experiments were approved by the Chinese Ethics Committee of Registering Clinical Trials (Approval No. ChiCTRCTE2016050). 2016-09-13, retrospectively registered. All subjects gave written, informed consent before inclusion in the study.

Informed Consent Statement: Written informed consent was obtained from each subject.

Conflicts of Interest: The authors declare that they have no conflict of interest.

Author Contributions: D.Z. and L.Z. designed the research. M.C., Z.N., and Y.Y. performed this study. M.C. analyzed the data. M.C., Z.N. and Y.Y. wrote the paper. Z.N., J.Y. and C.Y. was responsible for the critical revision of the paper. M.C., Z.N. and Y.Y. contributed equally to this work. All authors read and approved the final manuscript.

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Institutional Review Board Statement: The experiments were approved by the Chinese Ethics Committee of Registering Clinical Trials (Approval No. ChiCTRCTE2016050).

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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Figures

Figure 1

Analysis of OTU level difference of gut microbiota. (a) PLS-DA analysis of differences between groups. (b, c) The OTU levels differences of gut microbiota in control group, PCOS-IR group and PCOS-NIR group. The X-axis represents OTU level rank, and the Y-axis represents the relative abundance. The X-axis of the extension end of the sample curve is the number of species in the sample. If the curve drops sharply, it indicates that the diversity of bacteria in the sample is low. (d, e) Alpha diversity analysis of gut microbiota in three groups. (d) Sobs index, (e) Gace index. *represent compared with the control group, *P<0.05. Control represent healthy women group. PCOS-NIR represent PCOS patients without IR. PCOS-IR represent PCOS patients with IR

Figure 2

PCOS-NIR group and PCOS-IR group gut microbiota model accuracy prediction. The X-axis represents 1-specificity. Y-axis represents sensitivity. AUC represents the area under the curve. The larger the area under the curve, the better the diagnostic effect. (a) ROC curves between PCOS-IR group and control

group. (b) ROC curves between PCOS-NIR group and control group. Control represents healthy women group. PCOS-NIR represent PCOS patients without IR. PCOS-IR represent PCOS patients with IR

Figure 3

Analysis of genus level difference of gut microbiota. (a) Intersection diagram of gut microbiota. (b) PCOS-IR group gut microbiota distribution. (c) PCOS-NIR group gut microbiota distribution. (d)Control group gut microbiota distribution. (e, f) Analysis of differences in the abundance of gut microbiota, X-axis represents the average abundance of different groups in genus level, Y-axis represents the genus name. (e) Analysis of differences in the abundance of gut microbiota among three groups. (f) Analysis of differences in the abundance of gut microbiota between PCOS-NIR group and PCOS-IR group. *P<0.05. Control represents healthy women group. PCOS-NIR represent PCOS patients without IR. PCOS-IR represent PCOS patients with IR

Figure 4

Lefse analysis of gut microbiota (from phylum level to genus level). Yellow represents no difference, the hierarchy tree is divided into phylum, class, order, family and genus levels from inside to outside. (a, c) Hierarchy tree diagram. (a) PCOS-IR group and control group. (c) PCOS-NIR group and control group. (b, d) LDA discriminant barplot, the greater the LDA score, the greater the impact of species abundance on the differential effect. (b) PCOS-IR group and control group. (d) PCOS-NIR group and control group. Control represents healthy women group. PCOS-NIR represent PCOS patients without IR. PCOS-IR represent PCOS patients with IR

Figure 5

Analysis of functional abundance spectrum of gut microbiota. X-axis represented abundance value; Y-axis represented pathway name. (a-e) Statistical map of KEGG pathway function abundance. (a) Organismal systems, (b) Metabolism, (c) Genetic information Processing, (d) Environmental information processing, (e) Cellular processes. (f, g) Statistical map of metabolic pathway function abundance. (f) Biosynthesis of other secondary metabolites, (g) Xenobiotics biodegradation and metabolism. *P<0.05. Control represents healthy women group. PCOS-NIR represent PCOS patients without IR. PCOS-IR represent PCOS patients with IR

Figure 6

Analysis of enzyme abundance of gut microbiota. The X-axis represents the name of the enzyme, Y-axis represents the abundance value. The top 10 enzymes with statistically different abundances are shown in the figure. * $P < 0.05$, ** $P < 0.01$. Control represents healthy women group. PCOS-NIR represent PCOS patients without IR. PCOS-IR represent PCOS patients with IR

Figure 7

Hormone levels in three groups. (a) LH, (b) FSH, (c) LH/FSH, (d) E₂, (e) DHEA, (f) PRL, (g) T, (h) FBS, (i) FINS, (j) HOMA-IR. *represent compared with the control group, **P<0.01, ***P<0.001, ****P<0.0001. ####, compared with the PCOS-NIR group, P<0.0001

Figure 8

The correlation analysis between serum hormone (LH, LH/FSH, FINS, HOMA-IR, T, DHEA) and gut microbiota (top 30 genus). (a) PCOS-IR group, (b) PCOS-NIR group. Red is positive, blue is negative. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. PCOS-NIR represent PCOS patients without IR. PCOS-IR represent PCOS patients with IR