How to Screen and Prevent Metabolic Syndrome in Patients of PCOS Early: Implications From Metabolomics

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Abstract

Background: Polycystic ovary syndrome (PCOS) is a complex reproductive endocrine disorder with an increased risk of type 2 diabetes mellitus and cardiovascular disease, of which metabolic syndrome (MS) is an indispensable springboard to communicate PCOS and various comlications. Our aim was to study the potential metabolic characteristics of PCOS-MS, and identify sensitive biomarkers so as to provide targets for clinical screening, diagnosis and treatment.

Methods: 44 PCOS patients with MS, 34 PCOS patients without MS and 32 healthy controls were studied. Plasma samples of subjects were tested by ultra performance liquid chromatography (UPLC) system combined with LTQ-orbi-trap mass spectrometry. The changes of metabolic characteristics from PCOS to PCOS-MS were systematically analyzed. Correlations between differential metabolites and clinical characteristics of PCOS-MS were assessed. Differential metabolites with high correlation were further evaluated by the receiver operating characteristic (ROC) curve to identify their sensitivity as screening indicators.

Results: There were significant difference in general characteristics, reproductive hormone and metabolic parameters in PCOS-MS group when compared with PCOS group and healthy controls. We found 30 differential metabolites which were involved in 23 pathways when compared with PCOS group. The metabolic network further reflects the metabolic environment, including the interaction between metabolic pathways, modules, enzymes, reactions and metabolites.In the correlation analysis, 17 pairs of correlation coefficient between differential metabolites and clincal parameters were greater than 0.4, involving 11 metabolites that has the potential to be a marker for clinical diagnosis. They were assessed by ROC whose area under curve (AUC) were all greater than 0.7, with a good sensitivity. Furthermore, combinational metabolic biomarkers, such as glutamic acid+leucine+phenylalanine and carnitine C 4: 0+carnitine C18:1+carnitine C5:0 are expected to be sensitive combinational biomarkers in clinical practice.

Conclusion: Our study provides a new insight to understand the pathogenesis mechanism, and the discriminating metabolites may help screen high-risk of MS in patients with PCOS and provide sensitive biomarkers for clinical diagnosis.

Background

Polycystic ovary syndrome is a common endocrine-metabolic disorder affecting 12-18% of women, depending on the diagnostic criteria used[1]. Its main clinical manifestations include hirsutism, hypoovulation and polycystic ovary morphology, etc. [2]. Patients with PCOS are at high risk for metabolic diseases. Bhattacharya confirmed that Indian girls with PCOS were 4.2 times more likely to develop MS than girls without PCOS[3]. Moreover, a meta-analysis of 107 studies by Jamal Hallajzadeh et al. showed a significant relationship between PCOS and MS (OR=2.09), ranging from 0.31 to 4.69 when taking into account the definition of MS used[4]. Therefore, it was suggested that PCOS should no longer be considered a purely gynaecological disease. Besides, women with MS show an increased long-term risk of type 2 diabetes (T2DM), cardiovascular disease (CVD) and certain types of cancer, such as breast and gynaecological cancers[5]. This indicates that the occurrence of PCOS and MS exert a significant public health impact, so it is worthy of scholars' close attention. Longitudinal, in-depth and dynamic understanding of abnormal metabolic characteristics from health to PCOS, even to MS can help us to find and intervene abnormalities earlier and improve the prognosis. Current clinical predictors,

such as body mass index (BMI), fasting insulin (FINS) and high density lipoprotein (HDL), while useful in determining MS risk, represent established diseases and contribute little to our understanding of disease pathology. Therefore, it is necessary to screen sensitive biomarkers of PCOS with MS before the occurrence of obvious symptoms so as to predict and prevent the disease. With the development of Medical Sciences, an increasing number of people emphasize the importance of forecasting, individuation and prevention in the diagnosis and treatment of diseases by integrating various biological data to better define each person's health, predict the transition to disease, and determine the direction of medical interventions[6]. From a clinical perspective, the implementation of metabonomics will help to create predictive and personalized models that take full account of the heterogeneity of response to treatment and disease stratification. As the farthest downstream biochemical events in biological system, metabonomics reflects the final result of upstream or the disturbance of environment, and reveals the authentic biological activity in the organism, which is closely related to traditional biological and clinical endpoints. Therefore it is a novel and useful tool in pharmacology and personalized diagnosis[7]. In this studies, we recruited three types of volunteers including healthy control women, patients of PCOS and PCOS-MS, then mainly analyzed differential metabolites between PCOS and PCOS-MS, and explored the relationship between differential metabolites and clinical characterization so as to help identify biomarkers for diagnosis and prediction of disease. Besides, it is also conducive to providing insight into the underlying pathology, thus discovering novel targets for new treatments. The flowchart of study strategy is shown in Figure 1.Considering that the current metabolomics studies on PCOS mainly focus on the differences between PCOS and healthy control or concern the differences between different phenotypes of PCOS, such as hyperandrogen and non- hyperandrogen, fat and thin, etc. , there is still a lack of metabonomics studies to vertically explore the development of PCOS to MS. Thus, our experiment makes up for the drawback of current researches, and provides directions for the prevention and truncation of disease course.

Methods

Subjects

The subjects in this study were composed of 44 PCOS patients with MS (PCOS -MS group), 34 PCOS patients without MS risk factors (PCOS group), and 32 healthy women with age matching (healthy control group, HC group). They all visited the gynecological outpatient department or physical examination center of the First Affiliated Hospital of Heilongjiang University of Chinese Medicine from March 2018 to March 2020. All the subjects were between the ages of 18 and 35, with a two-year menstrual history. According to the Rotterdam criteria of 2003, the diagnosis of PCOS should conform to two or more of the following conditions: hypoovulation and/or anovulation, clinical and/or biochemical signs of hyperandrogen, and exclusion of secondary etiologies[8]. The diagnosis of MS must be in accordance with the Third guidelines for the Adult Treatment Group of the National Cholesterol Education Program (NCEP ATP)[9]: (1) central obesity: waist circumference(WC) ≥80 cm; (2) triglyceride (TG)>1.7mmol/L; (3) HDL<1.3mmol/L; (4) systolic blood pressure (SBP)≥130mmHg or diastolic blood pressure (DBP) ≥85mmHg, or having been diagnosed with hypertension; (5) fasting blood glucose (FBG) ≥5.6mmol/L, or having been diagnosed with diabetes. The healthy control group had regular menstrual cycles (27-35 days) without clinical or biochemical manifestations of hyperandrogen. Besides, Women with adrenal, thyroid and pituitary dysfunction, or who used oral contraceptives, androgen preparations, insulin sensitizers, iron supplements, or drugs that interfered with blood pressure, lipid or carbohydrate metabolism during the first six months were excluded from the study. This study

complied with the Declaration of Helsinki in clinical research and was approved by the Ethics Committee of Heilongjiang University of Traditional Chinese Medicine (NO. HZYLLKT201500401). Besides, informed consent was obtained from all participants.

Collection of serum samples and clinical data

After three days of 300 g carbohydrate diet and 12 hours of fasting at night, 20 ml of fasting blood samples were collected at 08:00-09:00 a. m. on the 2nd-4th day of natural menstrual cycle or withdrawal bleeding by taking 10 mg of oral dydrogesterone for 7 days in a row. The samples were placed at room temperature for 30 minutes, centrifuged at 3000r / min for 10 minutes, and then 8 ml of serum was separated and subpacked in 1.5 ml EP tubes. And part of the serum was stored at - 80 ℃ for metabonomics test. The other part was sent to the biochemistry and isotope departments of the First Affiliated Hospital of Heilongjiang University of traditional Chinese medicine for the determination of reproductive hormone tests and biochemical examination. The specific test items are as follows. Reproductive hormone was determined by chemiluminescence including blood follicle stimulating hormone (FSH), luteinizing hormone (LH), total testosterone (TT), dehydroepiandrosterone sulfate (DHEAS), androstenedione (A2), sex hormone binding globulin (SHBG). Glucose metabolism indicators were measured by chemiluminescence including FBG, FINS, homeostatic model index of insulin resistance (HOMA-IR), HOMA-β. Lipid metabolism was assessed by chemiluminescence, including total cholesterol (TC), TG, low density lipoprotein cholesterol (LDL-C), HDL-C, apolipoprotein A 1 (ApoA1), apolipoprotein B (ApoB). The calculation formula was shown as follows: HOMA-IR= FBG (mmol/L) × FINS (μIU/ml)/ 22. 5[10], free androgen index (FAI)= TT (nmol/L) × 100/SHBG (nmol/L), atherosclerotic index (AI)= TC-HDL-C/ HDL-C[11].

In the morning of the day when the blood samples were collected, the subjects were fasting for physical examination and relevant data were recorded, including blood pressure, height, weight, WC, hip circumference, Ferriman- Gallwey (F-G) score. And BMI, waist to hip ratio (WHR) were calculated.

Serum Metabolomic Analysis

Metabolomics analysis was performed in the Key Laboratory of Separation Science for Analytical Chemistry of Dalian Institute of Chemical Physics, and the specific operation process was as follows. Serum was thawing at 4 °C and 400 μL acetonitrile containing 11 internal standards were added to every 100 μL serum samples to deproteinize. And the 11 internal standards were choline-d4, carnitinen C2: 0-d3, carnitine C10: 0-d3, carnitine C16: 0-d3, cholic acid-d4, chenodeoxycholic acid-d4, LPC 19: 0, phenylalanie-d5, tryptophand5, Palmitic acid-d3 and stearic acid-d3 (Sigma–Aldrich, St. Louis, MO, USA). After oscillation, the supernatant was separated by centrifugation at 14, 000 g centrifugal force for 12 min at 4℃ . The supernatant was evenly divided into two parts for freeze-drying and stored in a refrigerator at -80℃. Before injection, the sample was redissolved in acetonitrile- water by 1:4 volume. After the oscillation, the samples were centrifuged at 14000g centrifugal force for 10min at 4 ℃, and the supernatant was taken for sample injection. Then 3 μL aliquot of each sample was injected onto the column in positive ion mode and 4μL in negative ion mode.

Ultra performance liquid chromatography (UPLC) system (Waters Corporation Milford, MA, USA) combined with LTQ-orbi-trap mass spectrometry (Thermo Fisher Scientific, Waltham, MA, USA) was applied to analyze the metabolic profiling in both electrospray ionization (ESI) positive and negative ion modes. For the ESI+ mode chromatographic separation was performed on a Waters ACQUITY C18 (100×2. 1 mm, 1.7 μm) (Waters, Ireland), and the mobile phase was contained in A (H2O/HCOOH=100:1) and B (CH3CN/HCOOH=100:1) with the flow

rate of 0. 35mL/min. The gradient program was as follows: 0-1min 5% B; 1-15min 5-100%B; 15-18min 100%; 18-18. 5min 100-5% B; 18. 5-20min 5%B. For the ESI- negative mode, chromatographic separation was performed on a Waters ACQUITYTM T3 (2.1 × 100 mm, 1.8 μm) (Waters, Ireland), and the mobile phase was contained in A (6. 5 mmol/L ammonium bicarbonate water solution) and B(6.5 mmol/L ammonium bicarbonate in 98% methanol and water) (95:5, v/v. The gradient program was 2% A for 1 min, changed to 100% B linearly within 18 min and held for 3 min, and finally back to 2% B. Flow rate was 0.35 mL/min, and the column temperature was kept at 50 °C both in positive ion mode and negative ion mode.

The mass spectrometry detection settings are as follows either in the positive or negative ion mode: mass spectrometry scan with mass range of m/z 80-1000 Da, with a desolvation gas temperature of 350 °C, drying gas flow rate at 11 L/min nitrogen, capillary voltage at 4.0 kV, and fragmentor voltage at 230 V.

Data Processing and Multivariate Analysis

SIEVE 1.2 version Workstation (Thermo Fisher Scientific, Waltham, MA, USA) was utilized to identify and match the peaks of the original data. After removing zero value with 80% rule[12], the intensity of each reserved peak was corrected with an internal standard to reduce the system error. SIMCA-P 11.0 version (Umetrics, Umea, Sweden) was used for multivariate statistical analysis where the data were preprocessed by unit variance (UV) scaling and mean centring before performing principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA). Variable importance in the projection (VIP) value were used to select the target metabolites which significant changed between compared groups with the standard of VIP>5 and P<0. 05. And the target metabolites was determined by MS/MS fragment spectra. Internal verification of 7-fold cross validation and response permutation test were adopted to evaluate the prediction ability of the model. All the results of metabolomics analysis were computed by IBM SPSS software (23. 0 version, SPSS inc, USA).

Statistics analysis

IBM SPSS 23.0 (SPSS, Chicago, IL, USA), statistical software was used for statistical analysis. One-way analysis of Variance (ANOVA) was used for multiple comparisons of clinical indicators in HC, PCOS, and PCOS-MS groups, and the adjustment methods was the Bonferroni correction. Data were described by mean ± SD. Spearman correlation analysis was used to analyze the correlation between different metabolites and clinical indicators. And the difference was statistically significant at 0. 05.

Results And Discussion

Comparison of clinical features and biochemical indicators

The general characteristics, reproductive hormone and metabolic parameters of the subjects were summarized in Table 1. We found that only F-G score, reproductive endocrine hormone (FSH, LH, LH/FSH, TT, A2) and LDL-C were significant changed in PCOS group when compared with HC group (P<0.05). It was obvious that the changes of hypothalamus-pituitary-ovarian (H-P-O) axis rather than glucolipid metabolism were remarkable in PCOS patients. Moreover, even eliminating the diagnosis of MS, there was still a significant increase in the level of LDL-C in PCOS group when compared with HC group. LDL-C is a lipoprotein particle that carries cholesterol into peripheral tissue cells, which can be changed into oxidative modification of LDL (OX - LDL). When LDL, especially the OX - LDL is too much, the level of cholesterol can be accumulated in arterial walls, leading to an

increased risk of CVD[13]. Our study was consistent with the research of Zhao et al. [14], suggesting that clinicians should also pay attention to the screening of lipid metabolism indicators in the diagnosis of lean PCOS patients, so as to find the susceptibility of metabolism disorder early, and take prevention measures for the development of the disease. It was interesting that we did not find IR in subjects of PCOS group, which also suggested that though IR was very common in PCOS[15], it was not universal, reflecting the metabolic heterogeneity of PCOS[15]. Of course, these patients may develop IR in the future as the course of the disease progresses, but we could discover that abnormalities in lipid metabolism were initiated before disorder of glucose metabolism occurred. Therefore, the mechanism between abnormal lipid metabolism and reproductive hormones in PCOS still deserves our in-depth attention.

There were differences in general characteristics, reproductive hormone (except for LH and DHEAS) and metabolic parameters between groups of PCOS-MS and HC. Besides, LH did not increase as much in the PCOS-MS group as in the PCOS group. And studies showed that LH tended to be more normal, not worse, in obese women with PCOS[16]. The LH pulse amplitude was the highest in lean PCOS, but it was relatively normal in the overweight PCOS patients, whose serum LH tended to increase when they lost weight for more than 3 to 6 months, which indicated that some metabolic factors related to weight might be the driving factors for the change of LH secretion[17]. In this table, we could find that there was no significant increase in DHEAS, an adrenal precursor androgen (APA) marker, between groups of HC and PCOS with or without MS. Data showed that about 20-30% of patients with PCOS showed excessive secretion of APA[18]. It indicated that elevated level of androgen levels in PCOS, especially in PCOS-MS group was mainly due to the abnormality of H-P-O axis, which matched with the result that TT and A2 of PCOS-MS were respectively 2 and 4 times of those in HC group.

There were differences in menarche age, SBP, DBP, BMI, WC and WHR between PCOS group and PCOS-MS group. We found that the age of menarche in PCOS-MS group was earlier than that in PCOS group. Studies found that the age of menarche was affected by various factors, including heredity, diet habits, natural environment, etc. We analyzed that patients in PCOS-MS group might be more likely to have more sources and reserves of fat at an early age due to the influence of heredity or diet, which may be a vital information for the secretion of leptin, stimulating the hypothalamus, and leading to the over secretion of GnRH. In addition, GnRH stimulates the pituitary-ovarian axis and initiates the acceleration of puberty[19]. A study by Kazem Mohamad et al. including 488 girls between 11 to 17 years in southern Iran showed that higher BMI was associated with lower menarche age[20]. This particular genetic and dietary factor may also underlie their later development into MS. Besides, TT and FAI were higher in PCOS-MS than in PCOS (P<0.05), we speculated that the abnormal metabolism of reproductive hormone would be aggravated after the onset of MS. Studies have shown that obesity can lead to changes in endocrine and/or metabolic patterns, hormone transport and/or its interaction with the target tissue, resulting in an abnormal concentration of androgen in peripheral blood[21]. In table 1, each glycolipid metabolism indicators in group PCOS-MS is different from that in group PCOS, one of which may be related to the increase of androgen level. Studies have shown that the increase of androgen and decrease of SHBG may play an important role in promoting the development of MS and T2DM[22]. And hyperactive androgen is the main reason for the increase of visceral adipose tissue [23]. Thus, it can be concluded that glycolipid metabolism and reproductive hormones interact to promote the development of the disease. This is consistent with our results, for example, in our investigation, the increased level of indicators related to glycolipid metabolism was accompanied by the increase of TT and FAI in PCOS-MS group. In addition, we also found an interesting phenomenon that the decreased level of LH in PCOS-MS was contrary to the

increased trend of TT when compared with PCOS group. Our result was consistent with the researches by Mu et al. [24] and Li et al. [25] considering that there was no significant difference in DHEAS, an adrenal gland-derived androgen, between the two groups, we speculated that the increased androgen may be due to the following aspects: 1). the increase level of follicular atresia in PCOS-MS patients due to chronic inflammation leads to the decrease of aromatase activity in the body and the increase of testosterone. 2). The enzyme system required for the synthesis of various ovarian steroids is dysregulated in PCOS patients, especially in PCOS-MS[26]. For example, the active function of 17- hydroxysteroid dehydrogenase lead to the increased conversion of androsterone to testosterone. 3). Since 50% of testosterone comes from the transformation of peripheral tissues, and adipose tissue, skin and liver are important sites involved in peripheral transformation, the increase of adipose tissue and abnormal liver metabolism in patients of PCOS-MS may be important sources of testosterone[27]. Specific intracellular mechanisms in female allow each cell to control androgen availability according to its own needs, regardless of the influence of the rest of the body. This mechanism is completely different from the male endocrinology, and this highly complex mechanism needs further exploration.

Analysis of the serum metabolic profiling by UPLC LTQ-Orbi-trap Mass Spectrometry

Pattern recognition of the comparisons of PCOS vs. HC, PCOS vs. PCOS-MS and PCOS-MS vs. HC

A series of multivariate variable pattern recognition analysis were carried out. Firstly, PCA was performed to reveal the internal structure of the data so as to better interpret the variables, as shown in Figure 2a, 2d and 2g. Furthermore, the supervised PLS-DA was carried out to maximize the separation among the observed groups. as shown in Figure 2b, 2e, 2h. Through OPLS-DA analysis, we could filter out the orthogonal variables in metabolites that were not related to categorical variables, so as to obtain more reliable differential metabolites between groups. Besides, the permutation test was carried out. The corresponding OPLS-DA model was established for several times (n= 200) by randomly changing the ranking order of Y to obtain the R^2 and Q^2 values of the random model, as shown in Figure 2c, 2f, 2i. And the figures showed that the model established accorded with the real situation of the sample data, with good robustness and no over fitting phenomenon. The score scatter plot of OPLS-DA model showed a significant difference in PCOS -MS whether compared to HC or PCOS group. However, there was no significant difference between PCOS and HC, which was then verified by further univariate analysis. This suggested that the occurrence of PCOS was mainly due to the abnormality of H-P-O axis rather than metabolism changes. Therefore, we focus on the analysis of metabolic differences in PCOS-MS when compared to PCOS or HC groups, especially the former comparison, so as to explore the metabolic changes from PCOS to PCOS-MS. Such research helps us predict the development of diseases and find out sensitivity screening indicators for screening high-risk population for MS in patients with PCOS at early stage and cutting off the development of the disease course.

Differential metabolites in groups of PCOS vs. PCOS-MS and groups of PCOS-MS vs. HC

After the differential metabolites between groups were obtained, various databases were searched according to their exact quality and other specific information for further analysis and identification, including METLIN (https://metlin.scripps.edu/), Kyoto Encyclopedia of Genes and Genomes (KEGG) (http://www.kegg.jp/kegg/pathway.html), Human Metabolome Database (HMDB) (http://www.hmdb.ca/), and PubChem Database (https://pubchem.ncbi.nlm.nih.gov/). The result was shown in Table 2 and 3. In addition,

after sorting out the substance names and classification, the Venn diagram was obtained by TB tools, as shown in Figure 3.

Differential metabolites between PCOS group and PCOS-MS group

A total of 40 differential metabolites were obtained, as shown in Table 2. The results of differential metabolites were visualized in the form of volcano plot, as shown in Figure 4a. Besides, the differential metabolites were clustered and shown by heatmap diagram (Figure 4b). The differential metabolites can be divided into the following 5 categories, including free fatty acids (FFAs), lysophosphatidylcholine, amino acids, purine metabolites and bile acids. FFA, a non-esterified fatty acid, is not only an important energy substance for human body, but also an important biomedical indicator of abnormal lipid metabolism, which has a significant impact on cells, leading to changes in metabolism, cell growth and differentiation[28]. This study showed that the level of FFA 14:0, FFA 16:0, FFA 18:1, FFA 18:2, FFA 18:3, FFA 20:0, FFA 20:4, and FFA 22:6 were significantly higher in PCOS-MS group than in PCOS group, indicating that these kinds of FFAs may be involved in the onset of MS in PCOS patients. Considering that previous studies have proved that FFA is closely related to IR[29], we believe that the increase of FFA is assosiated with the significant increase of IR in PCOS-MS patients. Melanie Cree-Green et al. confirmed that obese girls with PCOS had similar metabolic characteristics with diabetes, showing a close correlation between IR and FFA[30], which also verified our suspicion. The mechanism that the inhibition of FFA release by insulin in patients with hyperinsulinemiais is impaired, resulting in increased FFA concentrations in plasma. Moreover, the enhancement of FFA oxidation hinders glucose to enter tissue cells by changing the REDOX potential of the cells and inhibiting some key enzymes responsible of glycolysis and citric acid cycle. Thus, long-term increase of FFAs has been found to have cytotoxic and pro-apoptotic effects on human islet cells, inhibit the activity of phosphoinositi-3 kinase, interfere with the insulin receptor after signal transmission system, and make glucose transporter function decline, finally resulting in cell dysfunction and IR[31]. Therefore, high level of FFA and IR interact together to promote the occurrence and development of MS. Studies have found that level of FFA 16:0 (palmitoleic acid) in plasma and follicular fluid of obese PCOS patients was higher than that of control group and non-obese PCOS patients. Ni et al. demonstrated that FFA18:3 can predict the future development of metabolic syndrome (MS) in obese people[32]. These studies are consistent with our findings. Therefore, clinical screening of the above FFAs helps us to predict the risk of MS in PCOS patients in the early stage.

Carnitine is a vital biofactor in fatty acid (FA) oxidation, which takes charge of the transportation of long-chain FAs from cytoplasm to mitochondria. Acylcarnitine is transported to the mitochondria by acylbase and carnitine esters derived from the long chain FA, where acylcarnitines are changed into acylCoA, and act as the substrate for β-oxidation. Moreover, it eliminates intracellular acyl-compounds, regulating the ratio of coenzyme A (CoA) to acyl-CoA[33]. Thus serum carnitine may be an important indicator of metabolic dysfunction. A recent study conducted by Mihalik et al. [34] showed that the increase and accumulation of acylcoa intermediates in T2DM and diets-induced obesity, which exceeded the ability of mitochondria to complete FA β-oxidation, resulting in high carnitine ester flow, and was significantly correlated with long-chain acylcarnitine levels. Our study showed that patients with PCOS-MS presented mainly with an increase in long-chain carnitine, suggesting a decrease in the oxidation of FAs, which was in line with the increase in a variety of FFAs in our study. Besides, long-chain carnitine is also associated with abnormal glucose metabolism. Melanie Cree-Gree et al. found that C16:1 and C18:1 were strongly associated with IR in obese girls with PCOS (r=0.73 and 0.53, P<0.01)[35]. Long chain acylcarnitine induces the secretion of proinflammatory gene product COX-2 and various cytokines such as MCP-

1 and TNF -α in a concentration-dependent manner in mice. Meanwhile, long chain acylcarnitine activates toll-like receptor on the cell surface and nucleotide binding oligomerization domain proteins, which can activate inflammation by the NF- κB and the C-Jun N-terminal kinase (JNK)/ extracellular regulated protein Kinases (ERK) pathways. And these inflammatory signaling pathways inhibit IRS tyrosine phosphorylation, thereby preventing insulin signal transduction, reducing insulin sensitivity, and promoting systemic IR[36]. Of course, we also saw an increase in short chain carnitine C4:0 and C5:0, which were the main carnitine components that rose when hungry [37], which may be related to our sample collection at fastng state.

Besides, our study showed that the serum lysophosphatidylcholine (LPC) level was significantly higher in PCOS-MS group than that in PCOS group. LPC is not only the vital components of cell structure but also a very important medium for cellular communication, which can activate specific membrane receptors and/or nuclear receptors, involved in the development of various diseases, such as diabetes, obesity, atherosclerosis and cancer[38]. LPC can induce inflammation, increase oxidative stress, and interfere with vascular endothelial function. Meanwhile, studies have displayed that LPC 16:0, 18:0 and 18:1 can induce mitochondria ROS (mtROS) in human aortic endothelial cell. In addition, studies found that mtROS is involved in LPC-induced endothelial cell activation[39]. This oxidative stress and endothelial activation are also associated with PCOS and IR. Victor et al. conducted a prospective study recruiting 101 PCOS and 105 control subjects. The results showed that ROS and myeloperoxidase levels were generally increased in PCOS, especially in patients with IR[40]. Moreover, they further confirmed that LPC, as a signal activator, mediated NLRP3 inflammatory activation of adipose cells induced by homocysteine, and mediated IR[41]. In our study, patients with PCOS-MS had high level of IR, which may also be related to high level of LPC. Dagmar Drogan et al. [42] also found that LPC was clearly related to T2DM. Therefore, LPC possesses a good predictive and diagnostic value for PCOS combined with MS, and the pathological mechanism of LPC's involvement in the development of MS in patients with PCOS remains to be further explored.

In addition, we also found that PCOS-MS group showed a significant increased level of branched chain amino acids (BCAAs) and aromatic amino acids (AAAs) This is consistent with the metabolomics study in follicular fluid by Zhang et al. who found that the level of BCAAs, glutamate and phenylalanine were increased with BMI (P<0. 05). Furthermore, leucine, valine and glutamate levels were higher in PCOS with IR group than in non-IR PCOS patients and healthy controls (all P<0. 05)[43]. And Tang, et al. [44] found that the decreased level of BCAAs, AAAs (phenylalanine and tyrosine) and lysine in PCOS was accompanied by an improvement in weight and and insulin sensitivity. Thus, BCAAs and AAAs tend to be closely related to the occurrence of metabolic diseases. A study involving 1302, people aged 40-79 showed that higher levels of BCAA tracked with MS and the degree of obesity, dyslipidemia, hypertension and uric acid[45]. Therefore, focusing on the amino acid metabolism of PCOS patients, especially the metabolism of BCAAs and AAAs, enables us to find the risk of MS in PCOS patients in advance and prevent various complications.

Differential metabolites between PCOS-MS group and HC group

86 differential metabolites were identified in PCOS+MS group when compared with HC group, as shown in Table 3. The volcano plot and heatmap diagram were respectly shown in Figure. 4c and Figure. 4d. Carnitine, phospholipids and sphingomyelins were found in this comparison, but they showed no statistical difference in the comparison between HC and PCOS or PCOS vs.PCOS-MS. It can be seen that these types of substances show a cumulative effect in the development of the disease, from health to PCOS-MS, which are worth our early

attention.Phospholipid is the main component of biofilm. In eukaryotic cells, phosphatidy lethanolamine (PE) generates PC under the action of PE- methyl transferase (PE - methyltransferase, PEMT)[46]. LPC is an intermediate product of PC, which is hydrolyzed by PC under the action of PLA2 or Lecithin-cholesterol acyltransferase (LCAT). LPC can react with acylCoA transferase in liver microparticles to produce PC[47]. Besides, PC/LPC ratio in serum reflects inflammatory or infectious diseases of the liver. Studies have found that LPC levels are lower in patients with drug-induced liver injury, viral hepatitis and non-alcoholic steatohepatitis[48]. Therefore, the observation of characteristics of phospholipid spectrum in patients with PCOS- MS may reflect the inflammatory state of the liver, which is consistent with the study of Li et al. [49].

Sphingolipids are also part of membrane lipids (2-20%) and are involved in a variety of eukaryotic biological processes, such as cell proliferation, differentiation, apoptosis, and migration, cell-cell interactions and so on[50]. Sphingolipids can be hydrolysed into ceramide under the action of sphingomyelinase, and ceramide plays an important role in IR[51]. A large amount of ceramide can reduce insulin sensitivity, and induce apoptosis of islet cells in DM rats[52]. In addition, ceramide can promote the release of inflammatory substances such as arachidonic acid, prostaglandin and leukotriene by activating phospholipase A2, induce the expression of various inflammation-related proteins and promote the production of ROS at the same time[53]. Therefore, high levels of sphingomyelin in PCOS patients may be associated with the pathomechanism of IR, chronic inflammation and oxidative stress, leading to a high risk of obesity, fatty liver and CVD[54]. Therefore, the changes of SM 34:1, SM 36:2 and SM(D18:1/14:0) in PCOS patients are of positive significance for preventing the occurrence of the above complications.

KEGG annotation of differential metabolites and pathway analysis

Considering that complex metabolism in organisms is often regulated by different genes and proteins, which together lead to systematic changes in the metabolome, we annotated all pathways for differential metabolites in PCOS-MS group when compared with PCOS group and HC group respectively, with the help of the KEGG Pathway database. And “Homo sapiens (human) ” was chosen. The results were shown in Supplementary Table 1**.** These differential metabolites participate in a wide range of pathways, including energy metabolism, substance transport, signaling, cell cycle regulation, and so on. In addition, through comprehensive analysis including enrichment analysis and topological analysis, we further found the core pathways with the highest correlation with the metabolites. The results are shown in Supplementary Table 2. There were respectively 23 and 27 core pathways in PCOS-MS group when compared with PCOS group and HC group. The first 12 pathways are presented in the bubble diagram, as shown in Figure 5A and Figure 5B.

According to our findings, some signaling pathways were found in the comparison of HC vs. PCOS-MS group (as shown in Figure 5B) as well as in the comparison of PCOS vs. PCOS-MS (as shown in Figure 5A), such as aminoyl-tRNA biosynthesis, nitrogen metabolism, phenylalanine, tyrosine and tryptophan biosynthesis, etc. , but not in the comparison between PCOS vs. HC group. Thus, we consider that these pathways contribute to the occurrence of MS. The most significant signaling pathway is aminoacyl-tRNA biosynthesis. Under the action of aminoacyl- tRNA synthase, the carboxyl of amino acids and the hydroxyl of the 2 'or 3' end of homologous tRNA combine together to form ester bonds to synthesize aminoacyl-tRNA, which plays an important role in the transport of amino acids to ribosomes for protein synthesis[55]. Kyle Mohler et al. found that the aminoacylation state of the tRNA pool regulates the targets of the general amino acid control (GAAC) and targets of Rapamycin (TOR) response pathway in the yeast, playing a central role in determining the accuracy and sensitivity of the

stress response[56]. In addition, with the progress of genomics, proteomics and functionology, other biological functions of aminoacyl-tRNA biosynthesis have been gradually revealed and used as targets for drug intervention in the treatment of a variety of diseases, including cancer, neuropathology, autoimmune diseases and metabolic disorders [57]. For example, et al. found that mitochondrial leucine tRNA synthase (Mito-LRS) was accumulated in diabetic patients and was closely related to the occurrence of the disease[58]. And the aminoacyl-tRNA synthase interaction factor (AIMP1) exerts a significant effects on regulating glucose homeostasis[59]. These evidences have proved the regulatory role of aminoacyl biosynthesis in glucose metabolism. In our study, we found that the aminoacyl tRNA biosynthetic pathway was abnormal in PCOS-MS patients whether it was compared with PCOS or HC group, which may be the reason for the further development of MS in PCOS patients. In addition, considering that aminoacyl tRNA biosynthesis has been found to be closely related to the occurrence of cancer, it may also increase the risk of tumor disease in PCOS-MS patients. Thus, our findings provide implications for future research. Clarifying the mechanism of aminoacyl- tRNA biosynthesis during the development of PCOS into MS is expected to find accurate markers for disease screening and provide directions for drug intervention.

In addition, in these significantly enriched pathways, we also found that PCOS-MS patients showed significantly abnormal amino acid metabolism when compared with PCOS patients, and 6 of the 12 pathways presented in the bubble diagram were related to amino acids, suggesting that they play a core part in the occurrence and development of MS in PCOS. A cross-sectional analyses of large prospective cohort studies have shown that BCAAs and AAAs are positively correlated with BMI, WC, visceral fat, SBP, DBP, FBG, FINS and TG, while negatively correlated with HDL-C[60]. Moreover, accumulated evidence shows that the abundant bacteria responsible for amino acid fermentation in the large intestine have a huge impact on host metabolism, immunity, reproduction and other functions. Zeng et al. confirmed that MS mice displayed a significant increase in relative abundance of Firmicutes and higher Firmicutes-to-Bacteroidetes ratio, which was consistent with the occurrence of obesity, hyperlipidemia, IR and other phenotypes[61]. This also suggests that we can conduct multi-omics analysis in the future to reveal the association between amino acid metabolism and MS in PCOS from multiple perspectives. Due to significantly increased levels of various types of amino acids and their abnormal metabolism in PCOS-MS, We can pay special attention to guiding patients to change their diet structure and reduce the intake of certain amino acids. Meanwhile, probiotics can be appropriately supplemented to improve the composition of gut microbiota and amino acid metabolism. For example, bacteroides by gavage can reduce the serum BCAA concentration in mice and improve obesity caused by diet[62]. Thus, pathway analysis provides us new direction for treatment.

Network analysis for group PCOS+MS vs. PCOS

We used the differential metabolites as input compound and constructed a network analysis for group PCOS-MS vs. PCOS with the help of "FELLA " package in R language. The figure includes metabolic pathways, modules, enzymes, reactions and metabolites, which can reflect the intersection of metabolic pathways and potential enzymes and metabolites, as shown in Figure 6. We obtained five pathways, including the mTOR signaling pathway, renin- angiotensin system, protein digestion and absorption, mineral absorption, central carbon metabolism in cancer and Shigellosis. They interacted with a variety of enzymes and were involved in a wide range of biological processes in PCOS-MS. mTOR signaling pathway, for example, is a mammalian target of rapamycin, a serine-threonine protein kinase. This pathway can regulate cell growth, proliferation and survival by activating ribosomal kinase [63]. The figure showed that it could interact with BCAA transaminase and

participated in L-Leucine: 2-oxoglutarate aminotransferase reaction. BCAAs belong to essential amino acids, and must be obtained from dietary sources. Studies have proved that high level of BCAAs in plasma can continue to activate the mTOR signaling pathway, making the insulin receptor unhook from insulin receptor substrate 1, which is closely related to the occurrence of diabetes and obesity[64].A study showed that ketoisocaproic acid, a metabolite of leucine, suppressed insulin-stimulated glucose transport in skeletal muscle cells[65]. Conversely, deprivation of any BCAAs in normal weekly diet, or restriction of all the three BCAAs in genetically diabetic Zucker rats with isocaloric and isonitrogen diets could significantly improve insulin sensitivity and glycemic control[66]. We hypothesized that the occurrence of IR in PCOS-MS may be related to the increase of BCAAs and the overactivation of mTOR. Song et al. demonstrated that mTORC1 was activated in the PCOS mice induced by DHEA, leading to IR in the whole body and skeletal muscle, and the expression of GLUT4 in skeletal muscle was decreased[67]. These studies are consistent with our predictions. In addition, we also found that caspase-4 and caspade-11, which were related to apoptosis, were correlated with mTOR. Various studies have reported that autophagy and apoptosis are two forms of cell death and their relationship is complex and subtle. On one hand, autophagy can inhibit apoptosis under certain environmental stress. However, excessive depletion of intracellular substance caused by autophagy can lead to cell apoptosis[68]. mTOR is a pivotal molecule that regulates autophagy, and the overactivation of mTOR in PCOS-MS patients may affect the balance between autophagy and apoptosis, thus leading to complex pathological mechanism in patients with PCOS-MS. Last but not least, the figure also showed other metabolic pathways and regulatory mechanisms, which were complementary and mutually corroborated with the results of our previous pathway analysis. Furthermore, this network is helpful to provide targets for drug intervention and is conducive to the development of new treatments.

Clinical implications from metabonomics analysis

Correlation analysis of differential metabolites and differential clinical indicators between PCOS-MS and PCOS groups

Spearman correlation was calculated for the differential metabolites and clinical indicators between PCOS-MS and PCOS groups. The correlation coefficient (Corr) matrix and the correlation P value were obtained, and the matrix was used for subsequent analysis and production of heatmap. In the correlation analysis, we found 397 pairs of significantly correlated differential metabolites and clinical indications (P<0.05), among which correlation coefficient of 17 pairs were greater than 0. 4, involving 11 metabolites that tend to be biomarkers in clinical practice, as shown in Supplementary Table 3 and Figure 7. Carnitine in patients with PCOS-MS are closely related to glycolipid metabolism and reproductive hormones. For example, carnitine C18:1 (0.4310) are closely related to glucose metabolism indicators such as HOMA-IR (0.4310), FINS (0.4221), and carnitine C4:0 are closely associated with TG (0.426). The results are consistent with our previous analysis. Besides, the close correlation of carnitine and reproductive hormone metabolism is also discovered. For example, Carnitine C5:0 is negatively correlated with SHBG and positively correlated with free androgen index (FAI). This is consistent with the Vigerust and others' research, and they also found that total and free carnitines were negatively correlated with SHBG in PCOS patients [69].

Validation of the Combinational Metabolic Biomarkers

We evaluated the diagnostic value of 11 metabolites that were closely related to the clinical presentation of PCOS-MS by using “pPOC” package in R language, as shown in Figure 8. Biomarkers with high diagnostic value have been suggested, centred on leucine, carnitine C5:0、Phenylalanine、lactamide、LPC 20:3,etc., as shown in Figure 8a. To further improve the sensitivity of the diagnosis, we combined multiple biomarkers as a sensitive screening method for the disease. After the data were handled with binary logistic regression model, the AUC of glutamic acid+leucine+phenylalanine and carnitine C 4:0+carnitine C18:1+carnitine C5:0 were respectively 0.874 and 0.873, as shown in Figure 8b and Figure 8c. These two combinations seem to hold most promise as biomarkers of MS in patients with PCOS.

Conclusion

PCOS is a long-term challenge in clinical and basic research. Its purpose is to clarify the origin, pathological mechanism and development trend of PCOS. In this study, we compare the clinical characteristics of healthy control, PCOS and PCOS-MS patients, and then analyze metabolic characteristics of PCOS-MS, compared with normal people and PCOS respectively. KEGG annotation, pathway analysis and metabolic network analysis of the differential metabolites are of great significance for our further understanding of the direct metabolic changes of MS in PCOS. In addition, we also analyze the correlation between differential and clinical indicators, and evaluated their diagnostic value of closely related metabolites with ROC curve, so as to find sensitive combinational biomarkers in clinical practice. On considering that techniques utilized in metabolomic studies have matured, attempts have been made to look beyond the potential for biomarker identification and begin to understand the role metabolites might have in disease pathogenesis. So in our next research project, we expect to combine metabolomics with genomics to further explore the significance of differential metabolites in pathogenesis of PCOS-MS. This method- triangulation of genetic, metabolomic, and phenotypic data applied to the MS state in PCOS- is expected to be a potent technique for determining the contribution of metabolite biomarkers to disease. Besides, further effort should be made to replicate and validate these biomarkers in additional population cohorts, so as to serve in the clinical work of screening at-risk population, assessing drug efficacy, lifestyle interventions, as well as potentially discovering novel therapeutic targets.

Declarations

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Conflict of Interest/Competing interests

On behalf of all authors, the corresponding author states that there are no conflicts of interest.

Ethics approval and consent to participate

This study complied with the Declaration of Helsinki in clinical research and was approved by the Ethics Committee of Heilongjiang University of Traditional Chinese Medicine (NO. HZYLLKT201500401). Written

informed consent was obtained from individual or guardian participants.

Consent for publication

Not applicable

Availability of data and material

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Code availability

SIEVE 1.2 version Workstation (Thermo Fisher Scientific, Waltham, MA, USA); SIMCA-P 11.0 version (Umetrics, Umea, Sweden); IBM SPSS 23.0 (SPSS, Chicago, IL, USA)

Authors' contribution

Xiaoxuan Zhao, Xiaoling Feng data analyses, figure preparation and manuscript preparation. Xinjie Zhao, Yuepeng Jiang and Xianna Li interpretation, data analyses, and manuscript submission. Jingyun Niu and Xiaoyu Meng data interpretation, figure preparation. Jing Wu and Guowang Xu critically reviewed the manuscript. Lihui Hou, Ying Wang critically reviewed the manuscript and study initiation. All authors approved the final version of the manuscript.

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Tables

Table 1. Pair comparison of general characteristics, reproductive hormone and metabolic parameters among groups of HC, PCOS and PCOS-MS

Clinical features	Control group (n=32)	PCOS control group (n=34)	PCOS-MetS (n=44)	P-value Control vs PCOS control	P-value Control vs PCOS-MetS	P-value PCOS control vs PCOS-MetS
General Characteristics						
Age (years)	25.84±2.71	25.06±3.25	26.30±4.51	0.303	0.616	0.181
Menarche age (years)	13.50±1.19	14.09±1.74	13.02±1.30#	0.132	0.107	0.003
SBP (mmHg)	101.66±9.73	104.97±9.55	111.14±13.29*#	0.262	0.001	0.025
DBP (mmHg)	70.94±7.72	72.06±6.30	76.97±10.62*#	0.657	0.008	0.019
BMI (kg/m^2)	19.50±1.33	19.61±1.64	28.99±3.04*#	0.820	0.000	0.000
Waist circumference (cm)	72.47±3.78	72.97±4.79	95.46±7.22*#	0.634	0.000	0.000
Waist-to-hip ratio	0.80±0.03	0.81±0.03	0.92±0.04*#	0.142	0.000	0.000
Ferriman-Gallwey score	0.31±0.47	3.91±3.49*	4.84±2.93*	0.036	0.000	0.000
Reproductive hormone parameter						
FSH (mIU/ml)	6.94±2.30	4.48±1.03*	4.17±1.28*	0.000	0.000	0.247
LH (mIU/ml)	6.10±2.50	11.23±9.14*	7.66±4.75#	0.004	0.095	0.029
LH /FSH	0.90±0.32	2.57±2.21*	1.88±1.12*	0.000	0.000	0.076
Testosterone (ng/dl)	28.03±13.07	43.59±22.08*	58.79±26.28*#	0.001	0.000	0.008
DHEAS(ug/dl)	192.26±73.41	238.57±99.96	217.08±115.41	0.063	0.353	0.390
Androstenedione (ng/ml)	2.20±0.96	6.89±4.70*	8.50±5.72*	0.000	0.000	0.190
SHBG (nmol/l)	50.49±31.38	49.82±26.80	18.61±10.32*#	0.902	0.000	0.000
FAI	1.33±1.30	1.68±1.20	6.24±4.06*#	0.271	0.000	0.000

Metabolic parameter						
Fasting glucose (mmol/l)	4.62±0.33	4.74±0.34	5.60±1.12*#	0.116	0.000	0.000
Fasting insulin (μlU/ml)	7.68±3.21	9.26±3.74	24.14±11.38*#	0.077	0.000	0.000
HOMA-IR	1.59±0.69	1.96±0.85	6.05±3.09*#	0.064	0.000	0.000
HOMA-β	143.89±70.70.24	167.50±101.36	273.33±155.49*#	0.317	0.000	0.001
Total cholesterol (mmol/l)	3.86±0.54	4.08±0.54	4.98±0.86*#	0.211	0.000	0.000
LDL-C (mmol/L)	2.05±0.46	2.57±0.61*	3.16±0.79*#	0.005	0.000	0.000
Triglycerides (mmol/l)	0.80±0.23	0.90±0.35	2.39±1.16*#	0.174	0.000	0.000
HDL-C (mmol/l)	1.39±0.10	1.44±0.12	1.08±0.17*#	0.005	0.000	0.000
Atherogenic index	1.79±0.35	1.83±0.45	3.67±0.88*#	0.777	0.000	0.000
TG /HDL-C	0.58±0.18	0.63±0.31	2.30±1.30*#	0.436	0.000	0.000
Apolipoprotein B/A1	0.53±0.12	0.53±0.13	0.87±0.21*#	0.786	0.000	0.000

Table 2. Differential metabolites between PCOS group and PCOS-MS group

Metabolite	PCOS-MS and PCOS	Class	Pathway
FFA 16:0	↑*	free fatty acid	lipid metabolism
FFA 162	↑*	free fatty acid	lipid metabolism
FFA 16:3	↑*	free fatty acid	lipid metabolism
FFA 17:1	↑*	free fatty acid	lipid metabolism
FFA 20:3	↑*	free fatty acid	lipid metabolism
FFA 20:5-1	↑*	free fatty acid	lipid metabolism
FFA 22:5	↑*	free fatty acid	lipid metabolism
FFA 22:6	↑*	free fatty acid	lipid metabolism
Aliphatic amine C16:0	↑*	free fatty acid	lipid metabolism
Carnitine C4:0	↑*	carnitine	fatty acid β oxidation
Carnitine C5:0	↑*	carnitine	fatty acid β oxidation
Carnitine C18:1	↑*	carnitine	fatty acid β oxidation
LPC 14:0	↑*	lysophosphatidyl choline	phospholipid metabolism
LPC 16:0	↑*	lysophosphatidyl choline	phospholipid metabolism
LPC 16:1	↑*	lysophosphatidyl choline	phospholipid metabolism
LPC 20:3	↑*	lysophosphatidyl choline	phospholipid metabolism
LPC 22:4	↑*	lysophosphatidyl choline	phospholipid metabolism
LPC 22:5	↑*	lysophosphatidyl choline	phospholipid metabolism
glutamic acid	↑*	amino acid	amino acid metabolism
N -acetyl-L-lysine	↑*	amino acid	amino acid metabolism
γ- glut-leucine	↑*	amino acid	amino acid metabolism
Phenylalanine- -Phenylalanine	↑*	amino acid	amino acid metabolism
gly-phe	↑*	amino acid	amino acid metabolism
DL-Proline	↑*	amino acid	amino acid metabolism
L-kynurenine	↑*	amino acid	amino acid metabolism
Leucine	↑*	amino acid	amino acid metabolism

phenylalanine	↑*	amino acid	amino acid metabolism
Ornithine	↑*	amino acid	amino acid metabolism
tryptophane	↑*	amino acid	amino acid metabolism
valine	↑*	amino acid	amino acid metabolism
tyrosine	↑*	amino acid	amino acid metabolism
Glutamic acid	↑*	amino acid	amino acid metabolism
Uric Acid	↑*	Purine end products	purine metabolism
Hippuric acid	↓*	purine derivative	purine metabolism
Glycoursodeoxycholic acid	↑*	bile acid	bile acid metabolism
GUDCA	↑*	ursodexycholic acid	bile acid metabolism
lactamide	↑*	lactamide	Glycolysis metabolism

Table 3. Differential metabolites between HC group and PCOS-MS group

Metabolite	PCOS-MS and HC	Class	Pathway
carnitine	↑*	carnitine	fatty acid β oxidation
carnitine 4:0	↑*	carnitine	fatty acid β oxidation
carnitine C14:1	↑*	carnitine	fatty acid β oxidation
carnitine C18:1	↑*	carnitine	fatty acid β oxidation
carnitine C18:2	↑*	carnitine	fatty acid β oxidation
carnitine C2:0	↑*	carnitine	fatty acid β oxidation
carnitine C5:0	↑*	carnitine	fatty acid β oxidation
carnitine C6:0	↑*	carnitine	fatty acid β oxidation
FFA 14:0	↑*	free fatty acid	lipid metabolism
FFA 15:0	↑*	free fatty acid	lipid metabolism
FFA 16:0	↑*	free fatty acid	lipid metabolism
FFA 16:1	↑*	free fatty acid	lipid metabolism
FFA 16:2	↑*	free fatty acid	lipid metabolism
FFA 16:3	↑*	free fatty acid	lipid metabolism
FFA 17:0	↑*	free fatty acid	lipid metabolism
FFA 17:1	↑*	free fatty acid	lipid metabolism
FFA 18:1	↑*	free fatty acid	lipid metabolism
FFA 18:2	↑*	free fatty acid	lipid metabolism
FFA 18:3	↑*	free fatty acid	lipid metabolism
FFA 18:4	↑*	free fatty acid	lipid metabolism
FFA 19:1	↑*	free fatty acid	lipid metabolism
FFA 20:1	↑*	free fatty acid	lipid metabolism
FFA 20:2	↑*	free fatty acid	lipid metabolism
FFA 20:3	↑*	free fatty acid	lipid metabolism
FFA 20:4	↑*	free fatty acid	lipid metabolism
FFA 20:5-1	↑*	free fatty acid	lipid metabolism
FFA 20:5-2	↑*	free fatty acid	lipid metabolism
FFA 22:2	↑*	free fatty acid	lipid metabolism
FFA 22:5	↑*	free fatty acid	lipid metabolism
FFA 22:6	↑*	free fatty acid	lipid metabolism

FFA 24:1	↑*	free fatty acid	lipid metabolism
LPC 14:0	↑*	lysophosphatidyl choline	phospholipid metabolism
LPC 15:0	↑*	lysophosphatidyl choline	phospholipid metabolism
LPC 16:0	↑*	lysophosphatidyl choline	phospholipid metabolism
LPC 16:1	↑*	lysophosphatidyl choline	phospholipid metabolism
LPC 18:0	↑*	lysophosphatidyl choline	phospholipid metabolism
LPC 20:3	↑*	lysophosphatidyl choline	phospholipid metabolism
LPC O-16:0	↑*	lysophosphatidyl choline	phospholipid metabolism
LPC18:0	↑*	lysophosphatidyl choline	phospholipid metabolism
PC 30:0	↑*	lysophosphatidyl choline	phospholipid metabolism
PC 34:1	↑*	lysophosphatidyl choline	phospholipid metabolism
PC 34:2	↑*	lysophosphatidyl choline	phospholipid metabolism
PC 36:5	↑*	lysophosphatidyl choline	phospholipid metabolism
PC 38:5	↑*	lysophosphatidyl choline	phospholipid metabolism
SM 34:1	↑*	Sphingo myexin	phospholipid metabolism
SM 36:2	↑*	Sphingo myexin	phospholipid metabolism
SM(d18:1/14:0)	↑*	Sphingo myexin	phospholipid metabolism
SPHINGOSINE-1-PHOSPHATE	↑*	Sphingosine phosphate	
Asp-Phe	↑*	Aspartate - phenylalanine	amino acid metabolism
DL-Proline	↑*	proline	amino acid metabolism
Glutamic acid	↑*	glutamic acid	amino acid metabolism
gly-phe	↑*	alanine	amino acid metabolism
leucine	↑*	leucine	amino acid metabolism
L-Isoleucine	↑*	isoleucine	amino acid metabolism
L-Lysine	↑*	lysine	amino acid metabolism
Ornithine	↑*	ornithine	amino acid metabolism
Phenylalanine	↑*	phenylalanine	amino acid metabolism
Tryptophan	↑*	tryptophane	amino acid metabolism
Tyrosine	↑*	tyrosine	amino acid metabolism
Valine	↑*	valine	amino acid metabolism
bilirubin	↑*	bilirubin	bile acid metabolism

GUDCA	↑*	ursodexycholic acid	bile acid metabolism
GUDCS	↑*	Oxycholic acid salt	bile acid metabolism
glycodeoxycholate	↓*	glycodeoxycholate	bile acid metabolism
uric acid	↑*	uric acid	purine metabolism

Figures

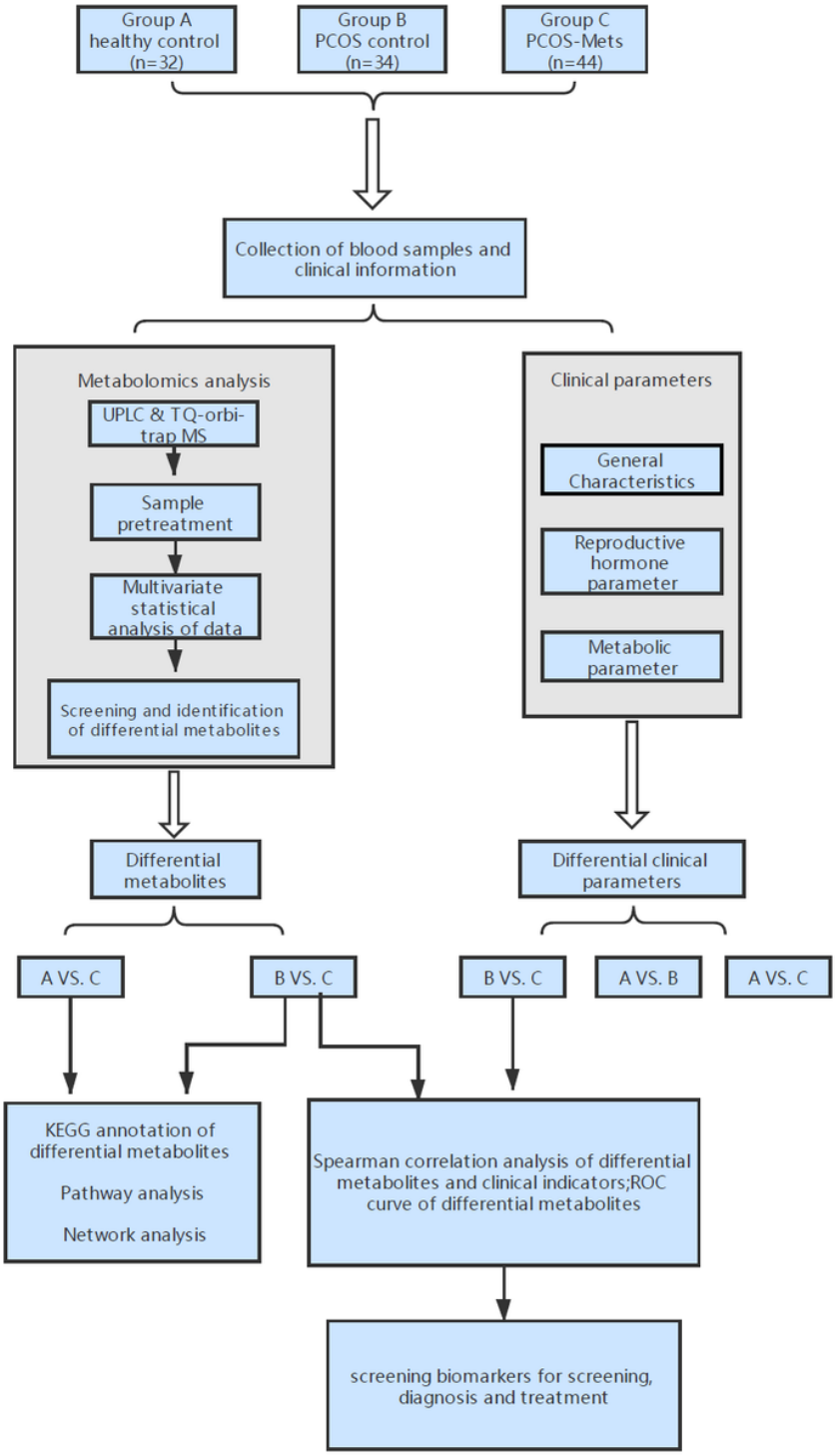

Figure 1

Flowchart of study strategy in this study

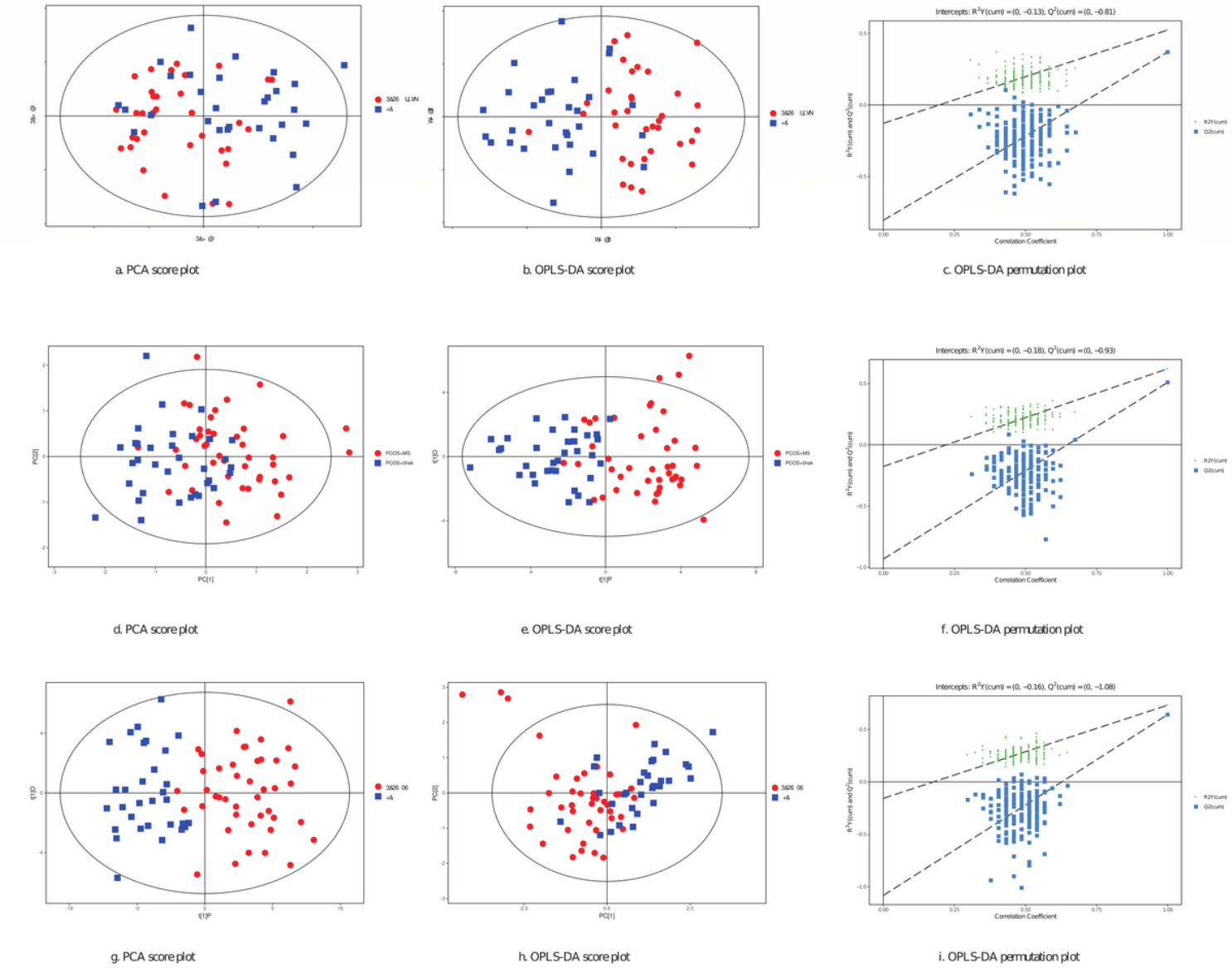

Figure 2

PCA (a, d, g), PLS-DA (b, e, h) score plots and permutation test (c, f, i) of plasma samples from HC, PCOS, PCOS-MS. HC：healthy control

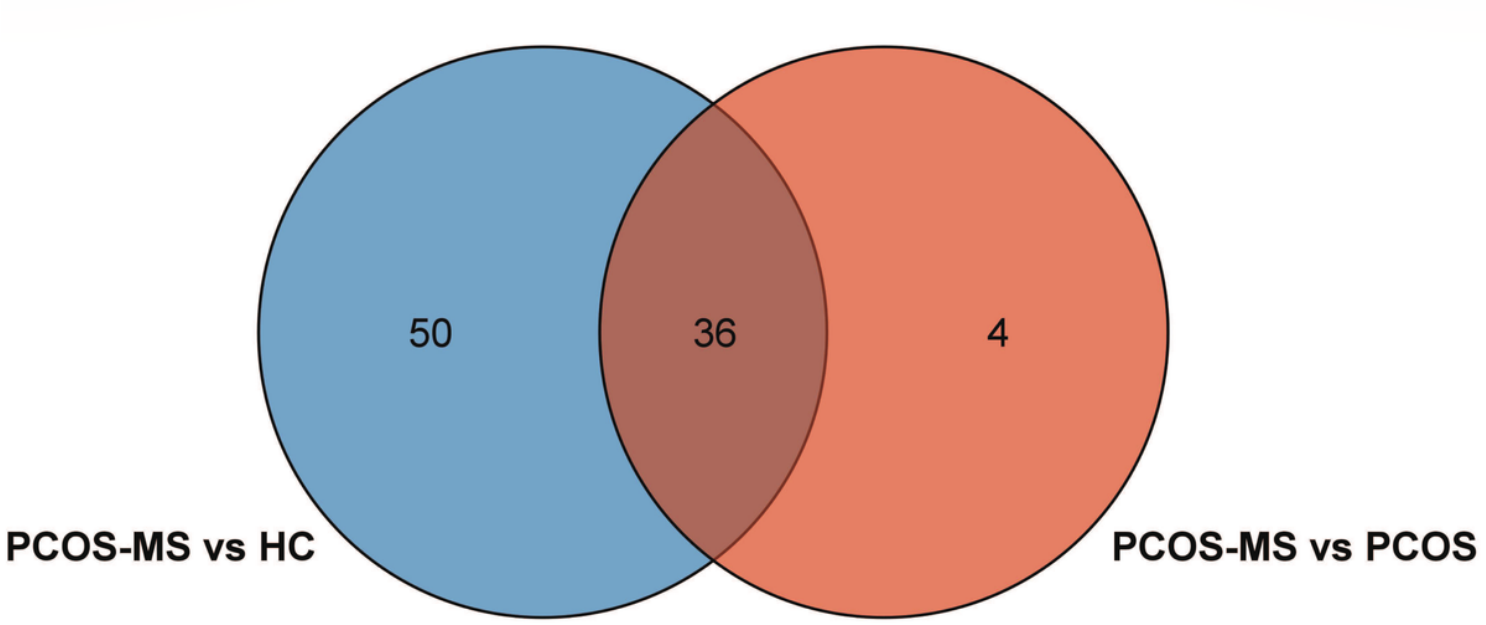

Figure 3

Venn diagram of differential metabolites

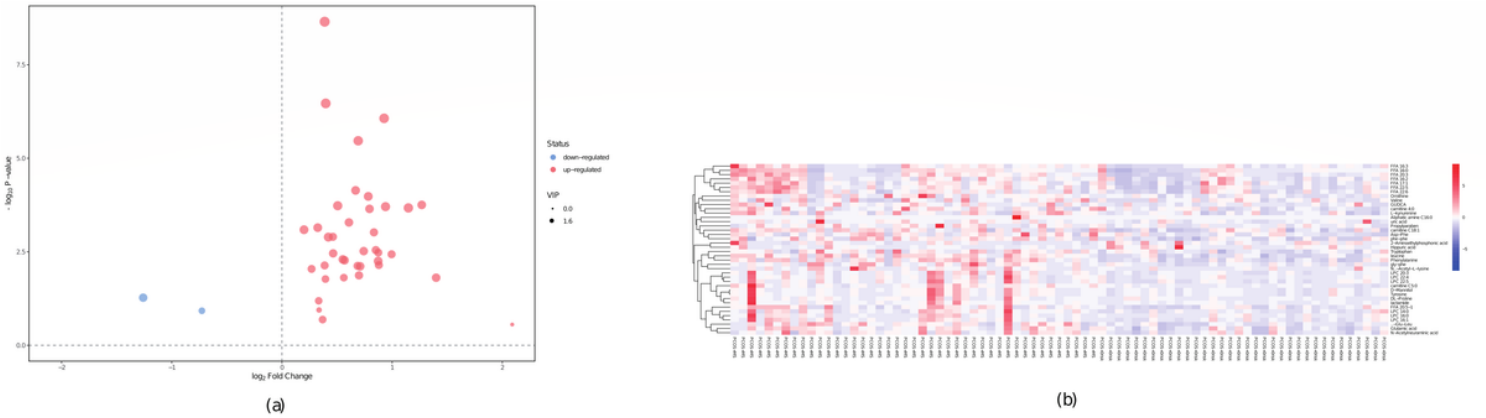

FIG. 4A.Volcano plot(a) and heatmap of hierarchical clustering analysis (b)for group PCOS vs. PCOS-MS

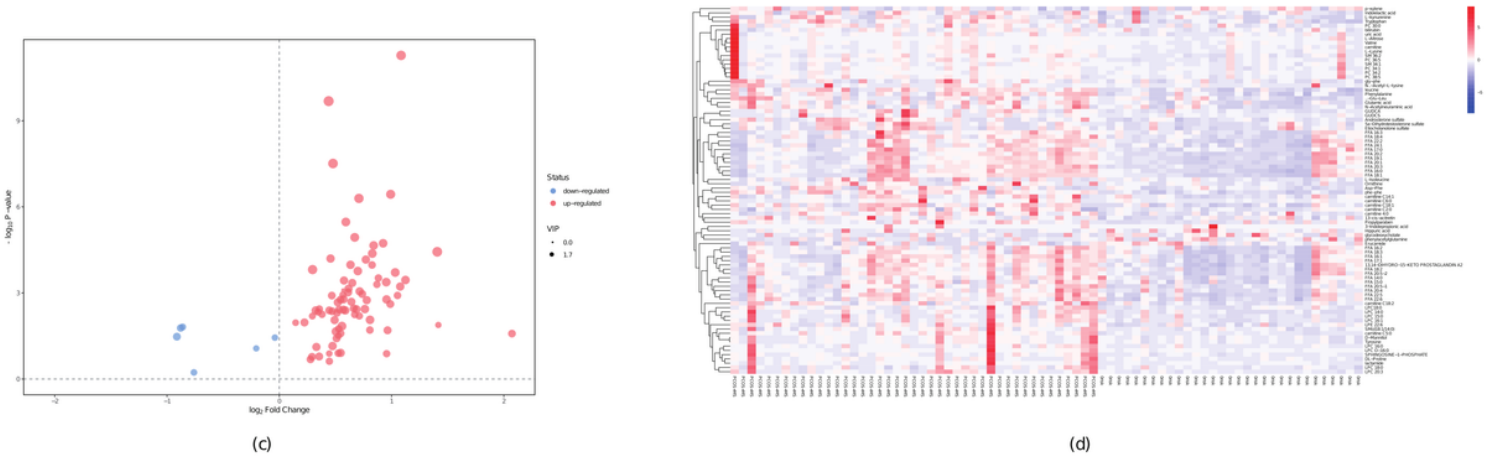

Figure 4

A.Volcano plot(a) and heatmap of hierarchical clustering analysis (b)for group PCOS vs. PCOS-MS B. Volcano plot(c) and heatmap of hierarchical clustering analysis (d) for group HC vs. PCOS-MS

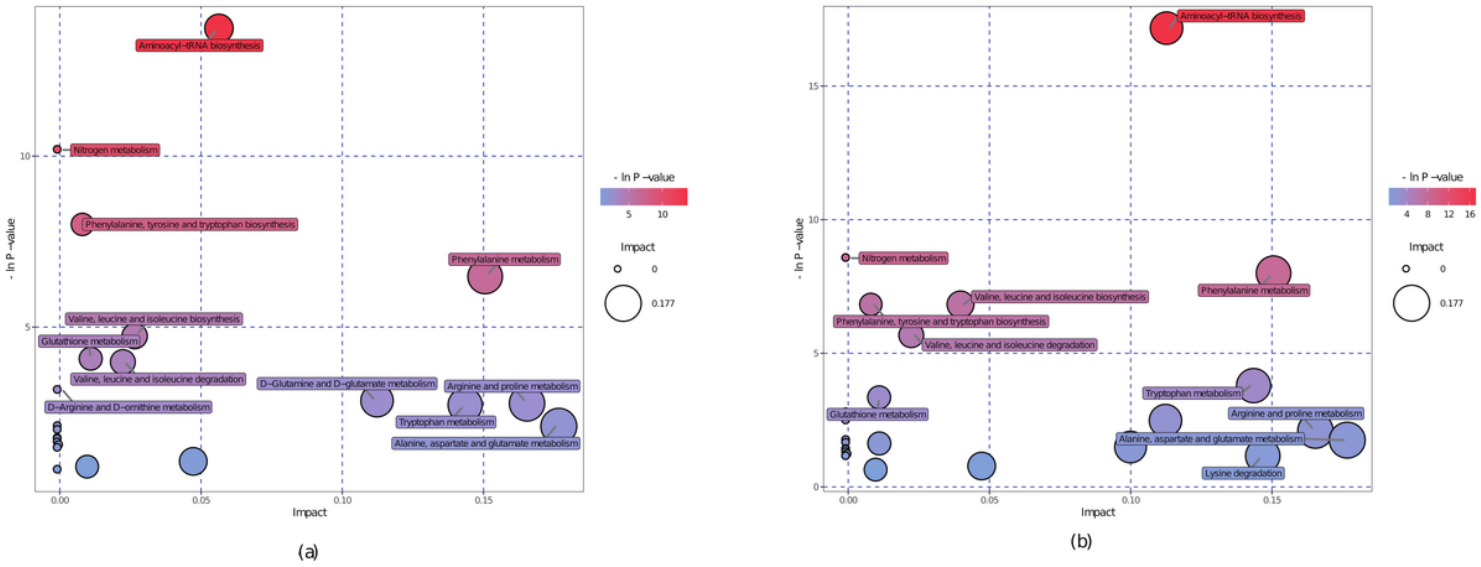

Figure 5

Pathway analysis for group PCOS vs. PCOS-MS (a) and group HC vs. PCOS-MS (b)

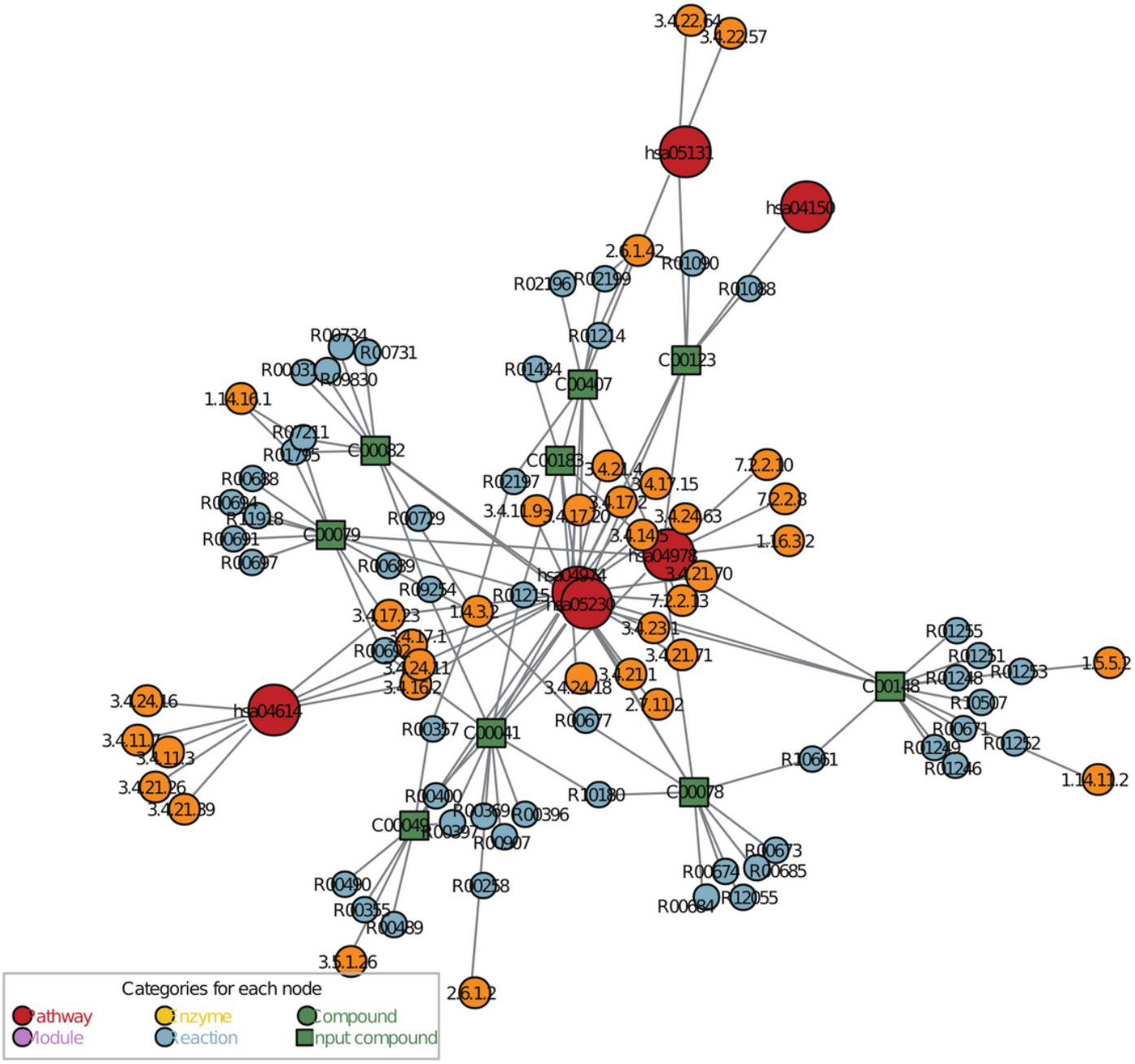

Figure 6

The metabolic network diagram of common differential metabolites of PCOS vs. PCOS-MS and PCOS-MS vs. HC In this diagram, the red dot represented a metabolic pathways, yellow dot represents enzyme, green dot represents background material, a blue dot represents a chemical reaction, and a green square represents the difference of materials.

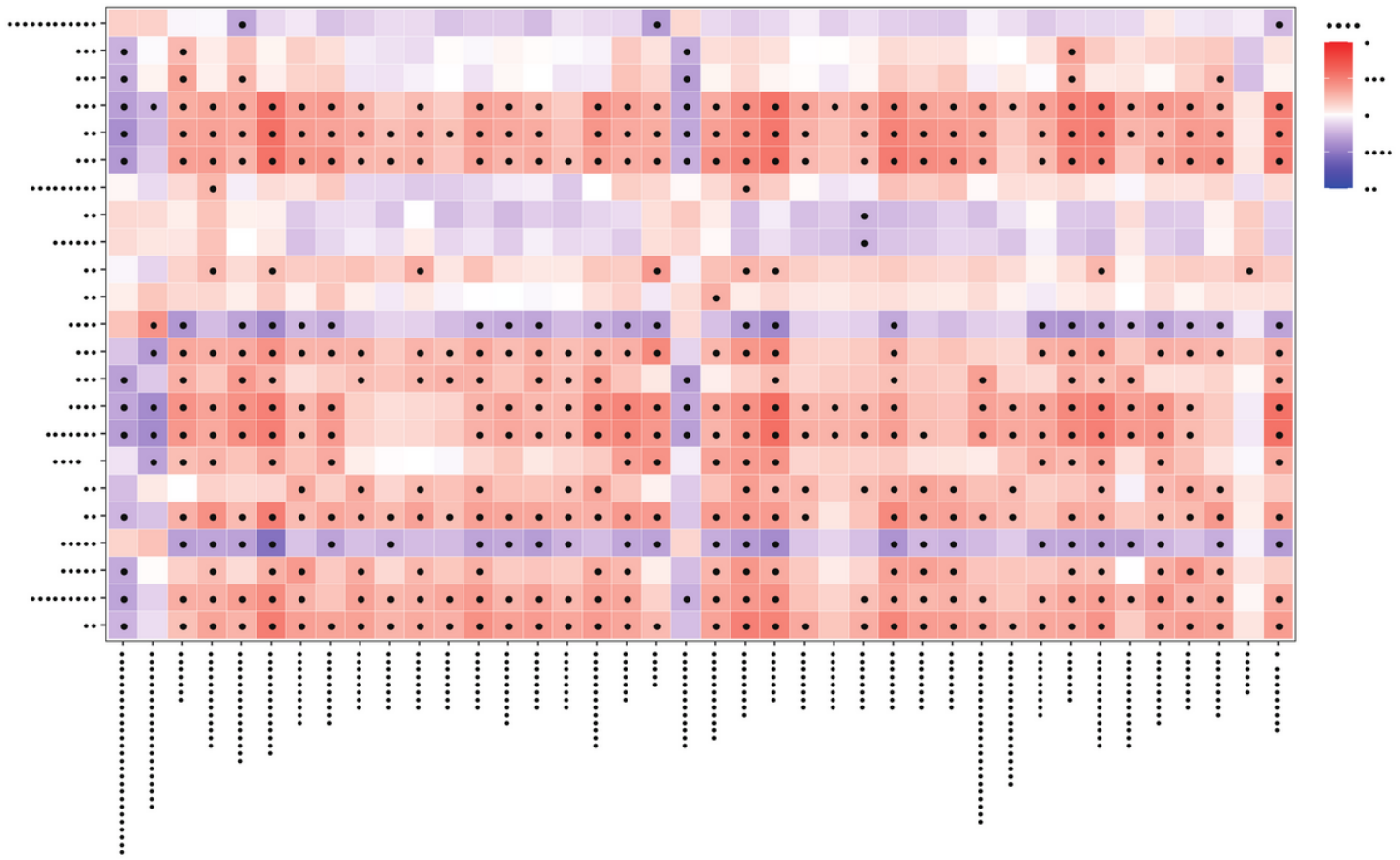

Figure 7

Correlation heat map of differential metabolites of PCOS vs. PCOS-MS and clinical indicators In this picture, red represents corr= 1, blue represents corr= -1, and white represents corr= 0. P value less than 0.05 for correlation are marked with "*" in the graph. The x-axis stands for metabolomics and the y-axis stands for clinical data.

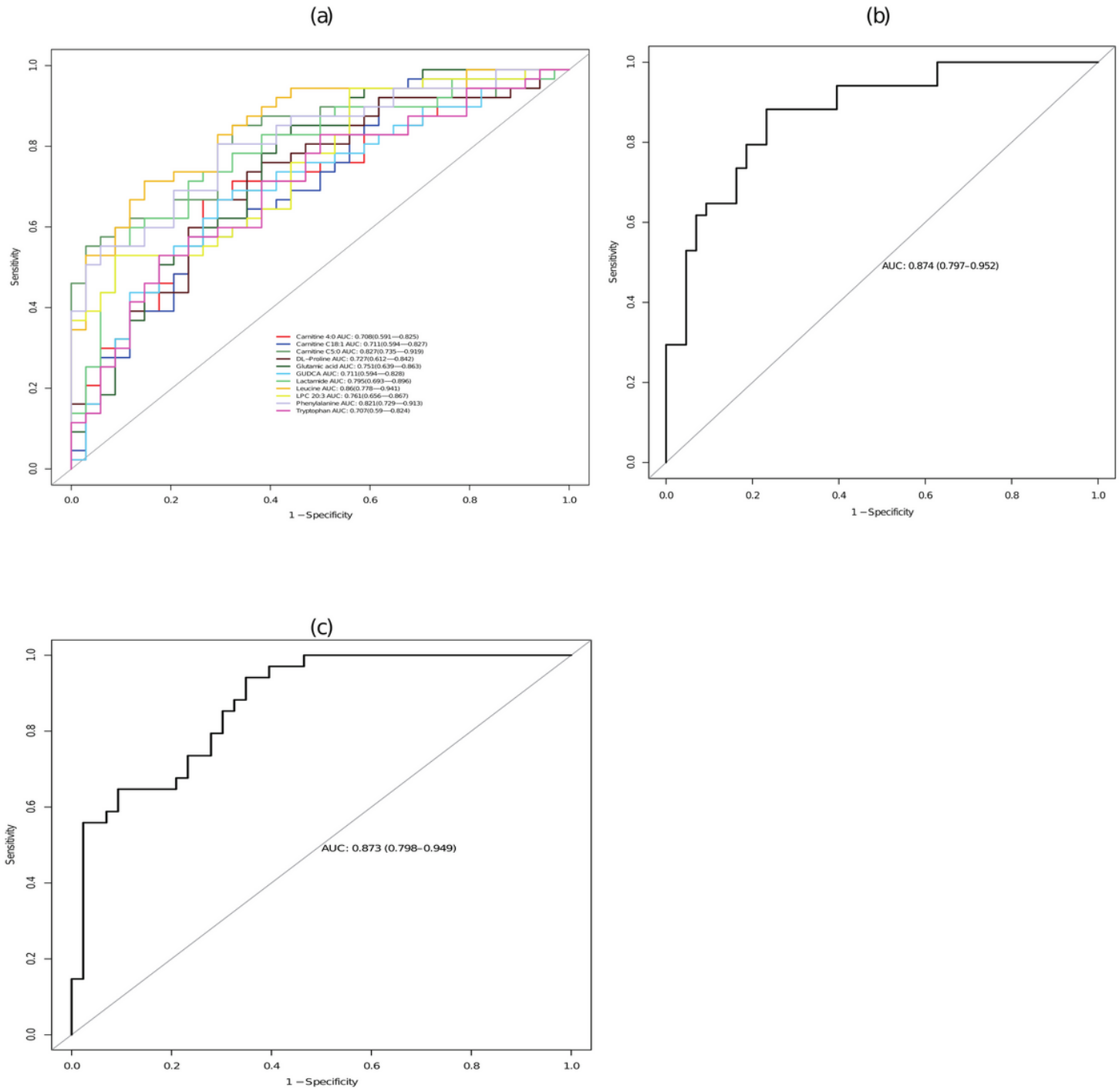

Figure 8

The ROC curve of potential biomarkers for the diagnosis of MS in patients with PCOS

Supplementary Files

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

- SupplementTable1.doc
- SupplementTable2.doc

- SupplementTable3.doc