

Serum metabolomics study of end-stage renal disease with depression: potential biomarkers for diagnosis and promising targets for therapy

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Research article

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Abstract

Background End-stage renal disease (ESRD) is the most severe stage during the development of the renal failure. And depression is the most common psychological disorder in patients with ESRD, which in turn aggravates the progression of renal failure and seriously reduce the quality of life in ESRD patients with depression, but its underlying mechanism remains unclear. This study aimed to reveal the pathogenesis and discover novel peripheral biomarkers for ESRD with depression through metabolomics analysis. **Methods** Ultra-high-performance liquid chromatography tandem quadrupole time-of-flight mass spectrometry (UPLC-QTOF-MS) was used to explore changes of serum metabolites among healthy controls (n = 12), ESRD patients (n = 17), and ESRD patients with depression (n = 17). Also, the differential metabolites between groups were subjected to clustering analysis, pathway analysis, receiver operating characteristic (ROC) curve analysis. **Results** A total of 57 significant serum differential metabolites were identified between the ESRD without depression group and the ESRD with depression group, which were involved in 19 metabolic pathways, such as energy metabolism, glycerolipid metabolism and glutamate-centered metabolism. Moreover, the area under the ROC curve of Gentisic acid, Uric acid, 5-HT, 2-Phosphoglyceric acid, Leucyl-phenylalanine, Propenoyl carnitine, Malaixon, Pregnenolone, 6-Thioxanthine 5'-monophosphate, Hydroxyl ansoprazole, Zileuton O-glucuronide, Cabergoline, PA (16:0/18:2(9Z,12Z)), PG (18:0/18:1(11Z)), probucol, etc. and their combination was greater than 0.90. **Conclusions** Inflammation, oxidative stress and metabolic abnormalities in energy metabolism, glycerolipid metabolism and glutamate-centered metabolism may be associated with the pathogenesis of ESRD with depression, which may be promising targets for therapy. Furthermore, the identified differential metabolites may serve as biomarkers for the diagnosis of ESRD patients with depression.

Background

End-stage renal disease (ESRD) is the most sever stage of the acute and chronic renal failure, with massively accumulated metabolic end products and toxic substances, disordered electrolytes and acid-base balance, as well as some endocrine dysfunction, resulting in a series of autotoxic symptoms [1]. In patients with ESRD, depression is the most common psychological disorder, with a prevalence rate as high as 20% - 25% by some contemporary estimates [2, 3]. Depression has been identified as a complicating comorbid diagnosis in ESRD, shown as low mood, slow thinking, cognitive impairment, physical symptoms and even suicide [3-5]. There is overwhelming evidence that chronic illness with comorbid depression is associated with increased symptom burden and functional impairment, poor quality of life, non-adherence to treatment, and worse clinical outcomes [6-8]. Nevertheless, the pathogenesis of ESRD with depression remains unclear.

The kidney is a metabolically-active organ involved in the handling of biochemical classes of metabolites [9]. One of the hallmarks of progression to ESRD is the plasma accumulation of certain metabolites, uremic solutes [10, 11]. Therefore, the metabolomic profiling of ESRD patients may be a promising method to identify new biomarkers for the prognoses of ESRD patients. Ultra-performance liquid

chromatography tandem mass spectrometry (UPLC-MS) has high selectivity, high sensitivity and good time-retention reproducibility, thereby suitable for metabolome analysis, especially for non-targeted metabolome studies [12]. Therefore, recently, metabolomics on chronic kidney disease (chronic kidney disease, CKD) has become a global hotspot. Previous studies[9] have found that there are significant statistical differences between the levels of various metabolic products of the CKD group. While other studies have revealed that the levels of fatty acid metabolism, particularly the polyunsaturated fatty acids (PUFAs) metabolism, and purine metabolism, are significantly different between depressed and non-depressed patients[13]. However, there is no metabolomic study focusing on ESRD with depression to our best knowledge. However, there is no metabolomic study focusing on ESRD with depression to our best knowledge.

In this study, we aimed to perform a metabolomics analysis to discover novel peripheral biomarkers for ESRD with depression as well as to elucidate the underlying mechanisms that initiate this progression. Our results may provide new theoretical basis for the complicated metabolic disorder and pathogenesis of ESRD with depression.

Methods

Study populations

All participants had signed written informed consent papers before study. The procedures of this study were approved by Ethics Committee of Chongqing Medical University. From January 2016 to July 2017, 17 ESRD patients, and 17 ESRD patients with depression in Department of Nephrology in Second Affiliated Hospital of Chongqing Medical University were enrolled in this study. The determination of depression was according to Hamilton Depression Rating Scale for Depression (HAMD) [39]: depression group with total score > 17 points, and non-depression group with total score ≤ 17 points. Additionally, 12 healthy subjects with matched age, gender, and body mass index (BMI) recruited from physical examination center were included as control group.

Inclusion criteria were as follows: (1) Confirmed as chronic renal failure uremia; (2) Serum creatinine > 707 μmol/L and endogenous creatinine clearance < 15 ml/(min·1.73m²); (3) The diagnosis and treatment of the disease were informed; (4) With normal liver function and blood glucose fluctuation range of 5.4 to 11.2 mmol/L; (5) Brain computed tomography and magnetic resonance imaging showed no new lesions; (6) Without previous history of drug poisoning and psychosis. Patients with severe complications or severely impaired organs except for kidney were excluded.

Sample and clinical data collection

All subjects underwent venous blood collection 10 h after fasting. Collected blood was stored at 4°C for 30-60 min followed by centrifugation at 3000 g/min for 10 min, and the supernatant was stored. The samples were stored at -80°C with dry ice as cold chain during transport. All subjects underwent routine

(height, weight, blood pressure, and BMI) and biochemical examination. Plasma biochemical indicators for subjects are shown in Table 1.

Sample preparation

Before analysis, every frozen plasma sample was thawed and dissolved at 4°C. A mixture of acetonitrile/methanol (75:25 v/v, 300 µl) (Merck, Germany) was added to plasma (100 µl) to precipitate proteins. After vortexing for 60 s, the mixture was stood for 10 min and then centrifuged at 12000 g/min for 10 min at 4°C. The supernatant was filtered through 0.22 µm syringe filters (Jinteng, China) and then analyzed by UPLC-MS. Samples were subjected to quality control (QC) by pooling equal volumes of different individual serum samples to assess the reproducibility and reliability of the UPLC-MS system. QC of mixed samples was interspersed at the start, middle, and end of the test.

Liquid chromatography-mass spectrometry (LC-MS) analysis

Liquid chromatography (LC) separation was performed on ZORBAX Eclipse Plus C18 column (2.1 × 100 mm, 3.5 µm; Agilent, USA). The column was maintained at 45°C. A 10 µl sample was injected into the column for each run in the full loop injection mode. The flow rate of the mobile phase was 0.5 ml/min. In reversed-phase liquid chromatographic (RPLC) mode, gradient elution was performed with the following solvent system: (A) 0.1% formic acid-water (Merck, Germany), (B) acetonitrile with 0.1% formic acid. The gradient started with 98% A and decreased to 10% A in 13 min, holding at 10% A for 3 min, and then turned to 98% A immediately, holding at 98% A for 4 min. Mass spectrometry experiments were performed on Triple TOF 5600+, an orthogonal accelerated time of flight mass spectrometer (AB SCIEX, USA) equipped with an electrospray ion source. Data were acquired in positive and negative-V-geometry mode for each chromatography separation technique LC-MS analysis. The mass spectrum parameters were as follows: the capillary voltages 2500 V and 3000 V, cone gas 50 L/h, desolvation gas 600 L/h, source temperature 120°C and desolvation temperature 500°C. The scan range was from 50 to 1500 m/z in the full scan mode and data were collected in centroid mode. Data were centralized during acquisition using independent reference lock-mass ions via the Analyst TF 1.6 and Marker View 1.2.1.

Metabolites were identified by searching the free databases of Human Metabolome Database (HMDB) (<http://www.hmdb.ca/spectra/ms/search>) [40]. The mass tolerance for the HMDB database search was set at 0.05 Da. The chromatographic retention behavior was used to reduce false-positive matches.

Statistical analysis

The multivariate analyses, including unsupervised principal component analysis (PCA), supervised partial least squares discriminant analysis (PLS-DA) and orthogonal projections to latent structures discriminant analysis (OPLS-DA), were conducted to determine the distributions and identify the metabolic difference in two or three groups using the MetaboAnalyst 4.0 (<http://www.metaboanalyst.ca/MetaboAnalyst/>) [41, 42]. The parameter R² was used to evaluate the fitting condition of the PLS-DA models, and Q² was used to assess the predictive ability. These parameters ranged from 0 to 1, where 1 indicated a perfect fit.

When the values of R² and Q² were > 0.5, the model considered to be successful. To avoid overfitting, 7-fold cross-validation and response permutation testing (RPT) were used for model validation[43]. In the PLS-DA model, variables with variable important in projection (VIP) > 1 considered to be potentially differential metabolites. Meanwhile, single variable statistical analysis was performed on the identified metabolites.

Welch's t-test was used to compare the two groups that were correlated with the intensities of the integrated regions using MetaboAnalyst 4.0, and p-value < 0.05 was considered statistically significant. Moreover, peaks with consistently upregulated or downregulated were identified, the regional intensity data of which were used in hierarchical cluster analysis and metabolic pathway analysis.

Pathway analysis

The differential chemical metabolites were subjected to pathway analysis with Metaboanalyst web portal (<http://www.metaboanalyst.ca/>) followed by visualization. Additional powerful pathway enrichment analysis was conducted by Metabolite Set Enrichment Analysis (MSEA) (<http://www.msea.ca/MSEA/faces/Home.jsp>). Pearson's r correlation was calculated to evaluate the relations among the biomarkers (p < 0.05, impact > 0.01).

Receiver operating characteristic (ROC) curve analysis

ROC curve was used to investigate the diagnostic value of differential metabolites. The area under the ROC curve (AUC) indicates the overall ability of the test. A test with an AUC greater than 0.9 has high accuracy, while 0.7-0.9 indicates moderate accuracy, 0.5-0.7, low accuracy and 0.5 a chance result. ROC curve was obtained using the SPSS 25.0 software.

Results

Biochemical characteristics

The demographics and clinical characteristics of the subjects are shown in Table 1. There was no significant difference in age, gender and BMI between the ESRD patients (with or without depression) and the healthy controls. Additionally, compared with the control group, the Neutrophilic granulocyte% (N%), Systolic blood pressure (SBP), Diastolic blood pressure (DBP), Triglyceride (TG), Creatinine (Cr), Blood urea nitrogen (BUN), Potassium ion (K⁺), phosphorus and depression scores were significantly higher (p < 0.05), while Total cholesterol (TC), Hemoglobin (HB) and albumin were lower (p < 0.05) in the ESRD group (with or without depression). Moreover, compared with the ESRD without depression group, the N%, anxiety score and depression score were significantly higher in the ESRD with depression group (p < 0.05).

Multivariate analysis of UPLC-QTOF-MS data

The distribution of samples, rationality of the experimental design, and homogeneity of biological replicates were determined by PCA. As shown in the score plot in the positive or negative ion mode, the ESRD and control groups were separated, but the ESRD without depression group and with depression group were not separated (Figure S1).

To improve the separation of the three groups, PLS-DA and OPLS-DA were performed to visualize their metabolic differences. The score plots of PLS-DA are shown in Figure 1. After response replacement test of these score plots, there was no overfitting, indicating that the PLS-DA model was successfully constructed. Moreover, the three groups were well separated in the OPLS-DA score plot, in both positive and negative ions (Figure 2). All models were cross-validated and no overfitting was identified. These results indicated that the ESRD patients and ESRD patients with depression had unique metabolic characteristics, making them to be separated.

Differential metabolite analysis and identification

According to the VIP values of characteristic variables obtained from the cross-validated OPLS-DA model, the potential markers were further screened. Variables with $FC > 5$ or $FC < 0.1$, $VIP > 1$ and $p < 0.05$ were considered as potential markers and were structurally identified. A total of 643 characteristic compounds that were differential between ESRD and healthy control groups, 459 ones in ESRD with depression group vs. healthy control group, 57 in ESRD without depression group vs. ESRD with depression group were identified. The screened differential metabolites were mapped to HMDB to identify specific substances. Qualitative results and related information of differential metabolites were screened in positive and negative ion modes. Results of ESRD without depression group vs. ESRD with depression group are shown in Table 2.

Clustering analysis

Bidirectional clustering of samples and metabolites was performed on all metabolites using the hierarchical clustering analysis. The heatmaps of the differential metabolites in each sample are shown in Figure 4, which provide an intuitive understanding of the relative content of each metabolite.

Pathway analysis

Pathway analysis of differential compounds was performed using the KEGG database. The relevant influence scores ($-\log(p) > 0.5$, $\text{impact} > 0.01$) of metabolic pathways enriched by the differential compound in ESRD without depression group vs. ESRD with depression group are shown in Table 3. The metabolic pathways of ESRD without depression group vs. healthy controls shown in Table S1. For simplicity, the metabolic pathways were converted into a pathway overview map, each point representing a pathway (Figure 5). There were 19 differential serum metabolite pathways in the ESRD without depression group vs. ESRD with depression group, and 16 of them were key pathways. The metabolic

pathway overview maps of ESRD without depression group vs. healthy control group are shown in Figure S2.

ROC analysis

To obtain a simple metabolite combination that can separate ESRD and ESRD with depression in clinical practice, we further analyzed the 57 differential metabolites in the main metabolic pathway. To investigate the diagnostic value of these differential metabolites, ROC curve analysis was conducted to assess the sensitivity and specificity of these metabolites. The compounds with AUC > 0.90 are shown in Table 4.

Discussion

This study examined serum metabolite differences between ESRD/ ESRD with depression and healthy controls, and between ESRD and ESRD with depression. Based on the pattern recognition method and the recognition model (PLS-DA, OPLS-DA), metabolite changes between groups were distinguished, and satisfactory model parameters were obtained. Through multivariate and univariate statistical analyses, the unique metabolic patterns related to ESRD were also obtained. The differential metabolites in ESRD group and healthy controls were involved in metabolic pathways such as alanine, aspartate and glutamate metabolism, phenylalanine metabolism, glutathione metabolism, and cysteine and methionine metabolism. ESRD with depression group was significantly different from ESRD without depression group in metabolic pathways, such as energy metabolism, glycerolipid metabolism and glutamate-centered metabolism (Figure 6). Additionally, differential metabolites with high diagnostic performance may serve as potential diagnostic markers for distinguishing ESRD and ESRD with depression patients.

Metabolic disorders in ESRD patients

Clinical biochemical characteristics of ESRD patients

Compared with the healthy control group, the SBP, DBP, TG, Cr, BUN, K⁺, phosphorus and depression scores of the ESRD without depression group were increased, while HB and albumin were significantly decreased. These biochemical changes are consistent with hypertension, azotemia, and anemia in ESRD patients, and suggest a high inflammatory response in ESRD patients.

Reduction of antioxidants

Glutathione, taurine and hypotaurine metabolism, cysteine and methionine metabolic pathways are abnormal in ESRD patients, and metabolites in these pathways such as: S-adenosyl methionine (SAM), glutathione (GSH) and taurocholic acid, etc. antioxidants were lower than that of the healthy control group. The GSH structure contains an active sulfhydryl group (-SH) that is easily oxo-dehydrogenated; studies have shown that SAM inhibits the strong inflammatory and oxidative stress processes that occur

in patients[14, 15]. Therefore, ESRD patients have reduced antioxidant capacity and may have oxidative stress damage in vivo, which is consistent with the findings of Kalender B et al. [16].

Metabolic disorders of aromatic amino acids

Phenylalanine, tyrosine and tryptophan belong to aromatic amino acids, in which phenylalanine is catalyzed by phenylalanine hydroxylase to form tyrosine, and tyrosine is further metabolized to produce catecholamine (dopamine, norepinephrine and adrenaline). Compared with the healthy control group, the tyrosine content of the ESRD without depression group was decreased, which was consistent with previous studies[17, 18], and the decrease of tyrosine was also observed in patients with renal failure and diabetes[19]. In addition, patients with ESRD had a lower Kynurenine (KYN) and a higher 3-Hydroxyanthranilic acid (3-HANA) than the healthy controls. Tryptophan is mainly metabolized by the kynurenine pathway (KP) and the 5-HT metabolic pathway, the former being more than 95% in mammals; KYN can inhibit antigen presentation, suppress immune response, and ultimately reduce inflammation[20]. However, 3-HANA is neurotoxic and induces the formation of free radicals such as hydroxyl radicals and hydrogen peroxide, and raises the level of oxidative stress[21]. It is concluded that patients with ESRD may be in a state of high inflammatory response and oxidative stress.

ESRD with metabolic disorders in patients with depression

Clinical biochemical characteristics of ESRD patients with depression

In our study, the neutrophil percentage of the ESRD with depression group was higher than that of the ESRD without depression group ($p < 0.05$). The occurrence of depression is highly related to inflammation[22], which is shown obviously in ESRD patients, with neutrophils as the indicator of inflammatory response[23]. Turkmen et al.[24] reported that the presence of inflammatory factors (such as TNF- α , IL-4, IL-6) in ESRD patients may be effectively regulated by the hypothalamic-pituitary-adrenal axis (HPA); Inflammatory factors can also directly stimulate HPA to cause abnormalities. Studies have shown that HPA abnormalities are one of the main causes of depression. Therefore, high inflammatory response in ESRD patients with depression may be the pathological basis of depression.

Energy metabolism

Compared with the ESRD without depression group, the ESRD with depression group had higher N-acetyl-L-aspartic acid (NAA) and gentisic acid, and lower 5-HT and thiamine pyrophosphate (TPP). KEGG analysis showed that these metabolites were involved in the tricarboxylic acid cycle (TCA cycle). TCA cycle is the ultimate and hub metabolic pathway of three major nutrients (sugars, lipids and amino acids). TCA metabolic abnormality has been reported in ESRD with depression[13]. Disorder of nutrient metabolism is common in ESRD patients, leading to insufficient energy supply for biochemical reactions.

NAA as a biomarker of neuronal damage severity, only exists in neurons, which is one of the most concentrated metabolites in the human brain, and is not detected in the blood[25, 26]. This study revealed that the NAA level in the ESRD with depression group was higher than that in the ESRD without depression group, which may be due to neuronal apoptosis and necrosis, indicating that neuronal activity was reduced or functional damage in ESRD patients with depression. 5-HT is an important neurotransmitter, and the lack of 5-HT in the central nervous system can result in depression. Reduction of 5-HT function and activity is closely related to depression, loss of appetite, and endocrine dysfunction[27]. This phenomenon can be observed in patients with major depression [29]. In accordance with the findings above, our result showed that the 5-HT level in ESRD patients with depression was lower than that in ESRD patients. We speculated that the factors affecting the metabolism of tryptophan to 5-HT and further metabolism to melatonin or acetyl-CoA in ESRD with depression patients may be one of the causes of depression.

KP in the inflammatory hypothesis of depression [30]: the emergence of inflammatory depression is caused by immune function and neurotransmitter changes in the activation of Indoleamine 2,3-dioxygenase (IDO), which not only leads to tyrosine failure also causes an increase in neurotoxic products through KP, resulting depression. There have been KP disorders in ESRD patients in this study, which may be one of the causes of depression with the prolongation of ESRD.

Thiamine, the vitamin B1, whose biologically active form is TPP[28]. TPP is an important cofactor for pyruvate dehydrogenase complex (PDHC), α -ketoglutarate dehydrogenase complex (KGDHC) and branched-chain α -keto acid dehydrogenase (BCKDH) [29]. PDHC and KGDHC are important components of cells using glucose to produce adenosine triphosphate (ATP) pathways, and BCKDH is a key enzyme for gluconeogenesis [30]. Consequently, thiamine plays an important role in maintaining the balance of oxidative metabolism in the brain. In patients with ESRD, the accumulation of toxins results in gastrointestinal reactions such as nausea, vomiting, and loss of appetite, leading to insufficient intake of thiamine, meanwhile, water-soluble thiamine is lost during dialysis, which together lead to thiamine deficiency. Deficiency of TPP can lead to metabolic disorders of sugar, lipids and amino acids, resulting in the reduction of ATP synthesis in the brain; moreover, TPP deficiency leads to cytotoxic edema, exacerbation of excitotoxic damage, oxidative stress damage, induction of inflammatory reaction, and destruction of blood brain barrier; all above considered to be potential mechanisms of depression [30, 31]. Specially, TPP catalyzes the conversion of tryptamine to 5-HT by increasing the activity of decarboxylation enzyme, thereby affecting the secretion of 5-HT[32]. In this study, ESRD patients with depression presented simultaneous descending of 5-HT and TPP, which may jointly promote the occurrence and deterioration of depression. Therefore, we speculated that serotonin reuptake inhibitors combined with thiamine may have a good anti-depressant effect on ESRD patients with depression.

Glycerolipid metabolism

In this study, we also found abnormalities in the glycerolipid metabolism, with elevated LysoPC (18:1 (9Z)), PG (18:0/18:1 (11Z)) and PA (16:0/18: 2 (9Z, 12Z)) in ESRD with depression compared to the ESRD

without depression group. Phospholipids that account for 60% of the brain weight, is critical for brain neuronal structures, especially synaptic structures. The three phospholipids of PA, PG and LysoPC play important roles in signal transduction of dopamine, serotonin, glutamate and acetylcholine[33]. Study has reported that PA, PG and LysoPC are important signaling molecules with various biological functions involved in cell proliferation and inflammatory processes[34], but the specific mechanism remains to be further studied.

Glutamate-centered metabolism

The glutamate-centered metabolism was also a main pathway enriched by the differential metabolites between the ESRD with depression and ESRD without depression groups, such as L-glutamic acid (decreased in the ESRD with depression group), urocanic acid and creatine (elevated in the ESRD with depression group). Glutamate is an excitatory neurotransmitter with the highest content, the widest distribution and the strongest effect in central nervous system[35]. Glutamate can be recycled in brain cells by two conversion mechanisms. First, glutamate is the starting material for GABA biosynthesis, catalyzed by Glutamic acid decarboxylase (GAD), which is thought to be associated with mood disorders and schizophrenia[36]. In addition, glutamate is also the starting material for the synthesis of glutamine by Glutamine synthetase (GS) in astrocytes and neutralizes ammonia during biochemical metabolism[37]. Cryan et al.[38] have reported the reduction in glutamate-glutamine cycle in plasma and cerebrospinal fluid in ESRD patients with depression. In the present study, L-glutamate deficiency may be one of the causes of depression in patients. L-arginine is a downstream metabolite of L-glutamic acid as well as the sole provider of guanyl that in turn synthesizes creatine. Creatine can rapidly re-synthesize ATP for energy supply. In this study, the creatine may be increase in response to energy metabolism disorder.

Biomarkers for diagnosis

Based on the ROC curve analysis, 20 differential compounds have high diagnostic value ($AUC \geq 0.9$) and can be used as a biomarker for predicting ESRD with depression. Besides, the combined analysis of biomarkers with high diagnostic potency obtained an ROC curve ($AUC = 0.945$), which may be of diagnostic value superior to a single differential compound.

Limitations

There are some limitations in this study. First, this is a cross-sectional study with a relatively small sample size which is insufficient to fully reflect all metabolic changes in ESRD with depression patients. Additionally, other biological samples, such as urine and cerebrospinal fluid are not tested to obtain more differential metabolites. Therefore, a cohort study with a larger sample size is needed to further confirm the findings and reveal the pathogenesis of ESRD and ESRD with depression.

Conclusions

Our study applied non-targeted metabolomics methods to study the metabolic characteristics of ESRD and ESRD with depression. Inflammation, oxidative stress and metabolic abnormalities in energy metabolism, glycerolipid metabolism and glutamate-centered metabolism may be associated with the pathogenesis of ESRD with depression, which may be promising targets for therapy. Furthermore, several novel biomarkers were identified, which can distinguish ESRD patients with or without depression.

Abbreviations

ESRD End stage renal disease UPLC-QTOF-MS Ultra-high performance liquid chromatography tandem quadrupole time-of-flight mass spectrometry CKD Chronic kidney disease PUFAs polyunsaturated fatty acids PCA Principal component analysis PLS-DA Partial least squares-discriminant analysis RPT Response permutation testing HAMD Hamilton Depression Rating Scale for Depression OPLS-DA Orthogonal partial least square-discriminate analysis VIP Variable important in projection ROC Receiver operating curve AUC Area under the curve WBC White blood cell RBC Red blood cell Ca²⁺ Calcium ion K⁺ Potassium ion N% Neutrophilic granulocyte% DBP Diastolic blood pressure SBP Systolic blood pressure TG Triglyceride Cr Creatinine BUN Blood urea nitrogen TC Total Cholesterol HB Hemoglobin PDHC Pyruvate dehydrogenase complex α -KGDHC alpha-Ketoglutarate dehydrogenase complex BCKDH Branched-chain alpha-keto acid dehydrogenase complex GAD Glutamic acid decarboxylase HMDB The Human Metabolome Database FC Fold change KEGG Kyoto Encyclopedia of Genes and Genomes IL-4 Interleukin-4 IL-6 Interleukin-6 HPA The hypothalamic-pituitary-adrenal axis SSRIs Selective serotonin reuptake inhibitors TCA-cycle Tricarboxylic acid cycle NAA N-Acetyl-L-aspartic acid TPP Thiamine pyrophosphate ATP Adenosine triphosphate 5-HT 5-Hydroxytryptamine

Declarations

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Authors' Contributions

Ran J.H. and Li J.F. as well as Hu J. and Yuan D.Z. contributed to study design. Hu J., Yuan D.Z. and Tian K. contributed to data analysis and writing the paper. Zhao Q.Y., Wang H.K., Feng L.P., Liao X.H., Jiang Q.H., Wang X.F., Zhang X.T., Hu L., Luo H.R., Zhou P., Guo X.X.

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Availability of data and materials

The study data can be accessed from the corresponding author Ran J.H. or Li J.F. by request.

Ethics approval and consent to participate

The study protocol was approved by the Second Affiliated Hospital of Chongqing Medical University (Approved ID: 2019-Research No. 279–01).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interest.

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Tables

Table 1. Demographic and clinical characteristics of the three groups

Indicators	Control group	ESRD without depression group	ESRD with depression group
Age (year)	57.00 ± 11.51	50.18 ± 10.65	50.82 ± 13.45
Hypertension history (month)	0.00 ± 0.00	73.82±61.47**	66.76±85.80*
Diabetes history (month)	0.00 ± 0.00	52.24±88.69	43.06±98.23
History of nephropathy (month)	0.00 ± 0.00	42.00±38.29**	30.71±43.41*
Heart disease history (month)	0.00 ± 0.00	0.29±1.21	7.76±16.40
SBP (mmHg)	126.33 ± 4.50	163.53±23.15**	171.12±33.06**
DBP (mmHg)	80.33± 2.67	94.24±9.75**	101.59±16.19**
Dialysis frequency (times/week)	0.00 ± 0.00	2.53±1.01**	2.12±1.11**
WBC (× 10 ⁹ /L)	5.79±1.55	7.22±2.53	6.62±3.48
N (%)	58.56±8.17	69.23±9.82*	78.24±11.20** [△]
RBC (× 10 ¹² /L)	4.67±0.35	3.73±0.63**	3.17±1.13**
Hb (g/L)	142.50±13.36	109.71±17.09**	92.29±25.84**
TC (mmol/L)	4.72±0.92	3.25±1.57*	4.30±1.68
TG (mmol/L)	1.25±0.68	3.13±1.50**	2.02±1.46
Albumin (g/L)	44.88±3.66	38.39±2.92**	124.34±280.64
Cr (μmol/L)	59.10±12.43	985.87±342.62**	915.14±213.44**
BUN (mmol/L)	5.38±1.43	21.37±5.85**	28.72±7.23** ^{△△}
K ⁺ (mmol/L)	4.04±0.51	4.78±0.56**	4.67±0.81*
Phosphoru (mmol/L)	1.09±0.12	1.82±0.47**	1.97±0.82**
Ca ²⁺ (mmol/L)	2.32±0.15	2.15±0.32	2.07±0.33*
Anxiety scores	4.33±5.66	7.41±3.40	21.29±4.91** ^{△△}
Depression scores	3.75±3.11	9.00±3.06**	22.88±4.20** ^{△△}

SBP: systolic blood pressure; DBP: diastolic blood pressure; WBC: white blood cells; N: neutrophils; RBC: red blood cells; Hb: hemoglobin; TC: cholesterol; TG: triglycerides; Cr: serum creatinine; BUN: blood urea nitrogen.

Compared with the control group, *: $p < 0.05$, **: $p < 0.01$; compared with ESRD with depression group, [△]: $p < 0.05$, ^{△△}: $p < 0.01$.

Table 2. Characteristic compounds of ESRD without depression group vs. ESRD with depression group

Compound name	ESI+/-	FC	raw.pval	vip	Trend
5'-Deoxy-5-fluorocytidine	+	5.444	0.0263	2.0509	↑
Dehydrogenated ticlopidine	+	31.16	0.019107	2.1539	↑
N-Desmethylpromazine	+	0.0012086	0.015404	2.2204	↓
Leucyl-phenylalanine	+	0.0397	0.036056	1.9432	↓
Cladribine	+	7.958	0.048751	1.8341	↑
Pregnenolone	+	0.0029882	0.045939	1.8561	↓
Bevantolol	+	0.03225	0.046476	1.8518	↓
Tetracosanoic acid	+	9619	0.022889	2.0964	↑
6-Thioguanosine monophosphate	+	23.301	0.049064	1.8318	↑
Farnesyl pyrophosphate	+	6.3943	0.038448	1.9205	↑
Alfuzosin	+	0.018852	0.006956	2.4468	↓
Cephapirin	+	15.773	0.022201	2.1062	↑
Thiamine pyrophosphate	+	8.7289	0.021436	2.1175	↑
Cabergoline	+	0.011239	0.023683	2.0853	↓
Almitrine	+	0.098922	0.046332	1.853	↓
Probucof	+	0.026526	0.021199	2.121	↓
LysoPC(22:4(7Z,10Z,13Z,16Z))	+	0.040511	0.045457	1.86	↓
PA (16:0/18:2(9Z,12Z))	+	0.022735	0.003310	2.6358	↓
PG (18:0/18:1(11Z))	+	0.0014305	0.041451	1.8936	↓
CL (i-14:0/i-12:0/i-14:0/18:2(9Z,11Z))	+	0.067084	0.007024	2.4442	↓
Phosphoric acid	-	0.04634	0.023768	1.893	↓
4-Methylcatechol	-	0.041521	0.029016	1.8329	↓
Dihydrothymine	-	29.157	0.000168	2.9428	↑
3-Methyl-2-oxovaleric acid	-	0.092461	0.006130	2.2528	↓
Urocanic acid	-	0.096113	0.048671	1.6664	↓
5-ethyl-5-methyl-2,4-oxazolidinedione	-	4.65E-09	0.036618	1.76	↓
L-Glutamic acid	-	24.417	0.000410	2.7978	↑
Gentisic acid	-	2.57E-09	0.046875	1.6791	↓
2,5-Furandicarboxylic acid	-	7.19E-09	0.032056	1.8021	↓
L-3-Phenyllactic acid	-	7.2911	0.048732	1.666	↑
Uric acid	-	1.42E-09	0.040158	1.7302	↓
N-Acetyl-L-aspartic acid	-	0.023715	0.004395	2.3306	↓
Serotonin	-	0.022792	0.017415	1.9826	↓
Vanylglycol	-	0.0047169	0.027679	1.8473	↓
2-Phosphoglyceric acid	-	0.056671	0.002838	2.4274	↓
Homocitrulline	-	6.0653	0.002780	2.4319	↑
Hydroxyphenylacetyl glycine	-	6.8042	0.007710	2.1971	↑
1-Hydroxylorcaserin	-	1.32E-09	0.026271	1.8631	↓
Propenoylcarnitine	-	2.18E-09	0.042833	1.7091	↓
cyclic 6-Hydroxymelatonin	-	32.34	0.000443	2.7843	↑
Desacetyl-nitazoxanide	-	10.564	0.01959	1.9492	↑
Deoxyartemisinin	-	17.749	0.000381	2.8099	↑
Malaoxon	-	0.056423	0.015184	2.0206	↓
5-HEPE	-	0.074764	0.019045	1.9573	↓
15(S)-Hydroxyeicosatrienoic acid	-	0.019982	0.023266	1.8993	↓
5'-O-Desmethyl omeprazole	-	0.040628	0.000461	2.7776	↓
17-HDoHE	-	0.086085	0.012912	2.0645	↓
5-(4'-Hydroxyphenyl)-gamma-valerolactone-4'-O-glucuronide	-	8.9677	0.023437	1.8971	↑
Hydromorphone-3-sulphate	-	5.4727	0.045925	1.686	↑
6-Thioxanthine 5'-monophosphate	-	0.082069	0.007364	2.2084	↓
Hydroxylansoprazole	-	8.92E-09	0.046771	1.6799	↓
Eletriptan N-oxide	-	0.060055	0.02196	1.9162	↓
Carboxycelecoxib	-	0.060337	0.033844	1.7851	↓
Zileuton O-glucuronide	-	2.53E-09	0.014994	2.0241	↓
Hesperetin 3'-O-glucuronide	-	7.9491	0.037187	1.7551	↑
DG (14:0/16:1(9Z)/0:0)	-	7.0082	0.008436	2.1747	↑
Enkephalin L	-	10.077	0.035126	1.7733	↑

Table 3. Different metabolite Pathway in ESRD without depression group vs. ESRD with depression group

	Total	Expected	Hits	Raw <i>p</i>	Holm adjust	DR	Impact
Alanine, aspartate and glutamate metabolism	24	0.2393	2	0.02301	1	1	0.17664
Tyrosine metabolism	76	0.75779	3	0.038024	1	1	0.01825
Glycerophospholipid metabolism	39	0.38887	2	0.056434	1	1	0.10429
Histidine metabolism	44	0.43872	2	0.069917	1	1	0.06816
D-Glutamine and D-glutamate metabolism	11	0.10968	1	0.10458	1	1	0.1123
Citrate cycle (TCA cycle)	20	0.19942	1	0.18227	1	1	0.04844
Thiamine metabolism	24	0.2393	1	0.21468	1	1	0.02865
Glycolysis or Gluconeogenesis	31	0.3091	1	0.26847	1	1	0.00068
Pyruvate metabolism	32	0.31907	1	0.27586	1	1	0.00459
Glycerolipid metabolism	32	0.31907	1	0.27586	1	1	0.01247
Terpenoid backbone biosynthesis	33	0.32904	1	0.28317	1	1	0.05141
Glutathione metabolism	38	0.37889	1	0.31871	1	1	0.01095
Valine, leucine and isoleucine degradation	40	0.39884	1	0.33245	1	1	0.03781
Pyrimidine metabolism	60	0.59826	1	0.45599	1	1	0.01492
Aminoacyl-tRNA biosynthesis	75	0.74782	1	0.53393	1	1	0.05634
Arginine and proline metabolism	77	0.76776	1	0.54348	1	1	0.03582
Tryptophan metabolism	79	0.7877	1	0.55284	1	1	0.05734
Purine metabolism	92	0.91732	1	0.60933	1	1	0.00969
Steroid hormone biosynthesis	99	0.98712	1	0.63685	1	1	0.02542

Table 4 The metabolites for the diagnosis of ESRD with depression (AUC \geq 0.90)

Compound	AUC	TPR	FPR	95% CI
5-ethyl-5-methyl-2,4-oxazolidinedione	0.934	0.9	0.8	0.834-0.986
Gentisic acid	0.965	0.9	0.9	0.896-1
2,5-Furandicarboxylic acid	0.931	0.9	0.8	0.829-0.986
Uric acid	0.931	0.9	0.8	0.834-0.99
Serotonin	0.931	0.9	0.9	0.822-0.986
2-Phosphoglyceric acid	0.924	0.8	0.9	0.811-0.986
N-Desmethylpromazine	0.965	0.9	0.9	0.9-1
Leucyl-phenylalanine	0.938	0.9	0.9	0.834-0.99
Propenoyl carnitine	0.934	0.9	0.8	0.832-0.99
Malaoxon	0.927	0.9	0.8	0.803-0.99
Pregnenolone	0.976	0.9	0.9	0.917-1
Bevantolol	0.948	0.9	0.9	0.861-1
6-Thioxanthine 5'-monophosphate	0.924	0.9	0.9	0.789-1
Hydroxyl ansoprazole	0.924	0.9	0.8	0.824-0.986
Alfuzosin	0.931	0.8	0.9	0.823-0.986
Zileuton O-glucuronide	0.958	0.9	0.9	0.867-1
Cabergoline	0.952	0.9	0.8	0.849-0.997
Probucol	0.931	0.9	0.8	0.815-0.986
PA (16:0/18:2(9Z,12Z))	0.945	0.9	0.9	0.839-1
PG (18:0/18:1(11Z))	0.955	0.9	0.9	0.875-1

TPR: True positive rate; FPR: False positive rate; 95% CI: 95% Confidence interval

Figures

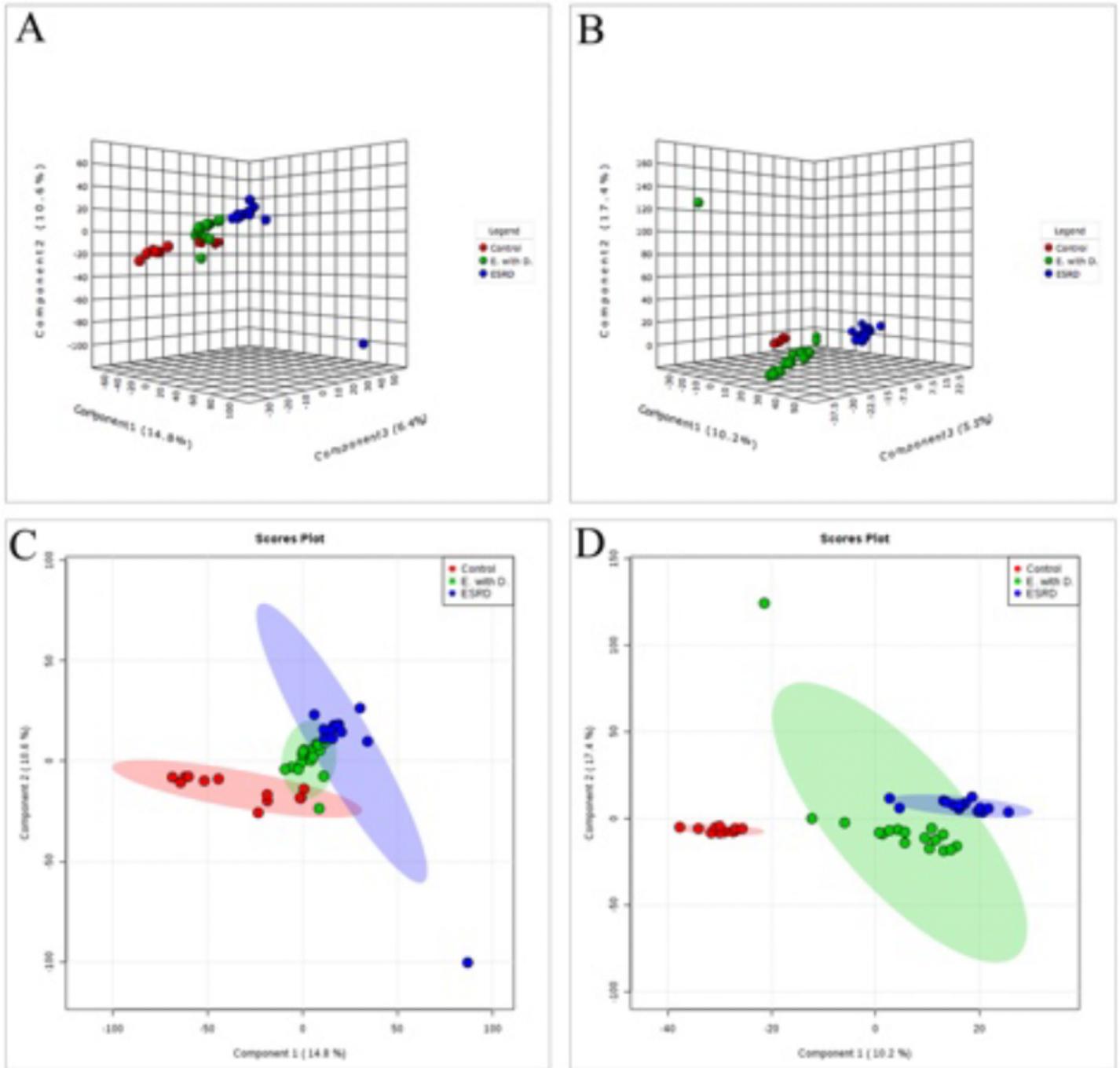


Figure 1

The score plots of the three groups of PLS-DA model in positive ion mode (A, C) and negative ion mode (B, D)

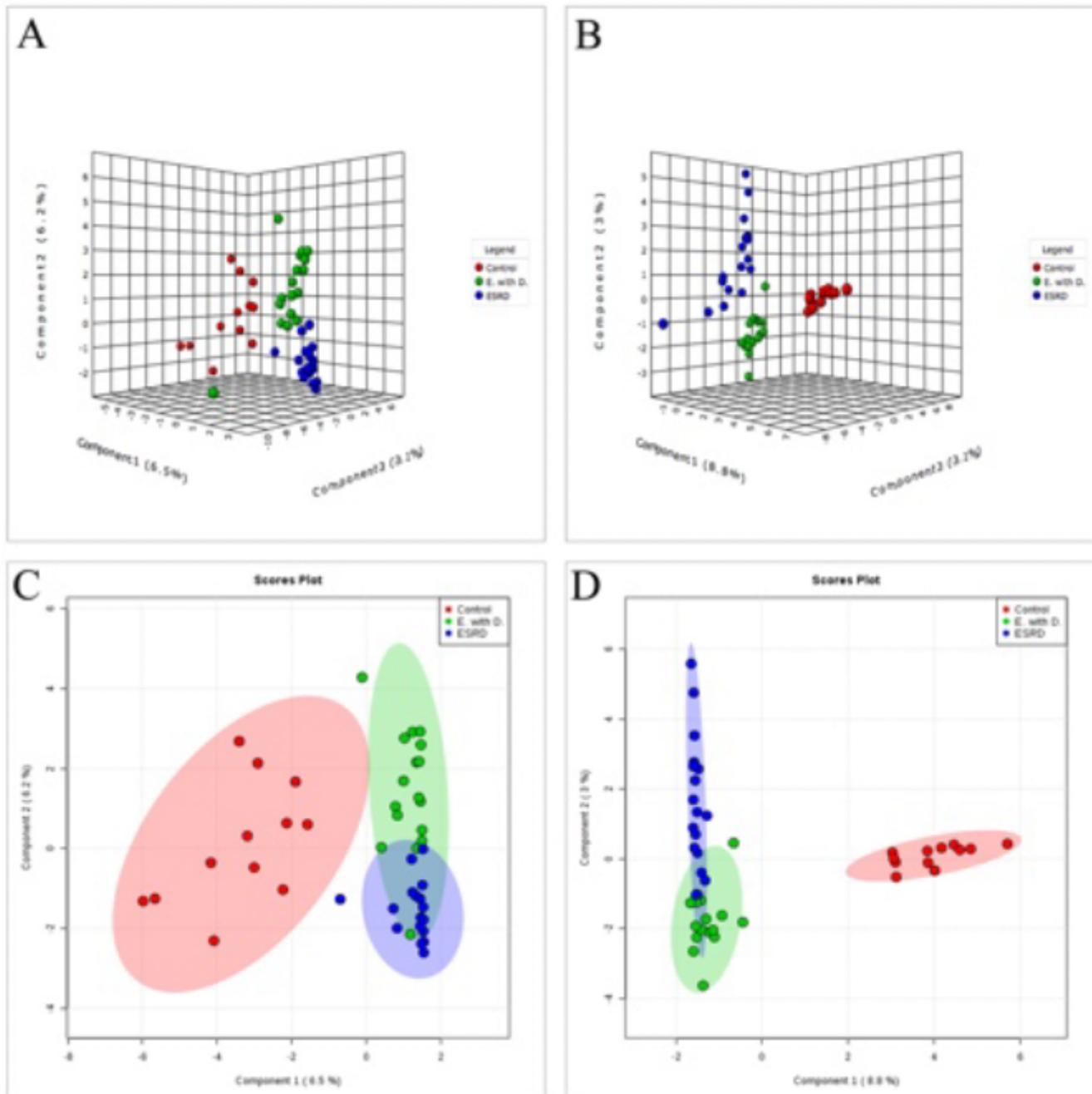


Figure 2

The score plots of the three groups of OPLS-DA model in positive ion mode (A, C) and negative ion mode (B, D)

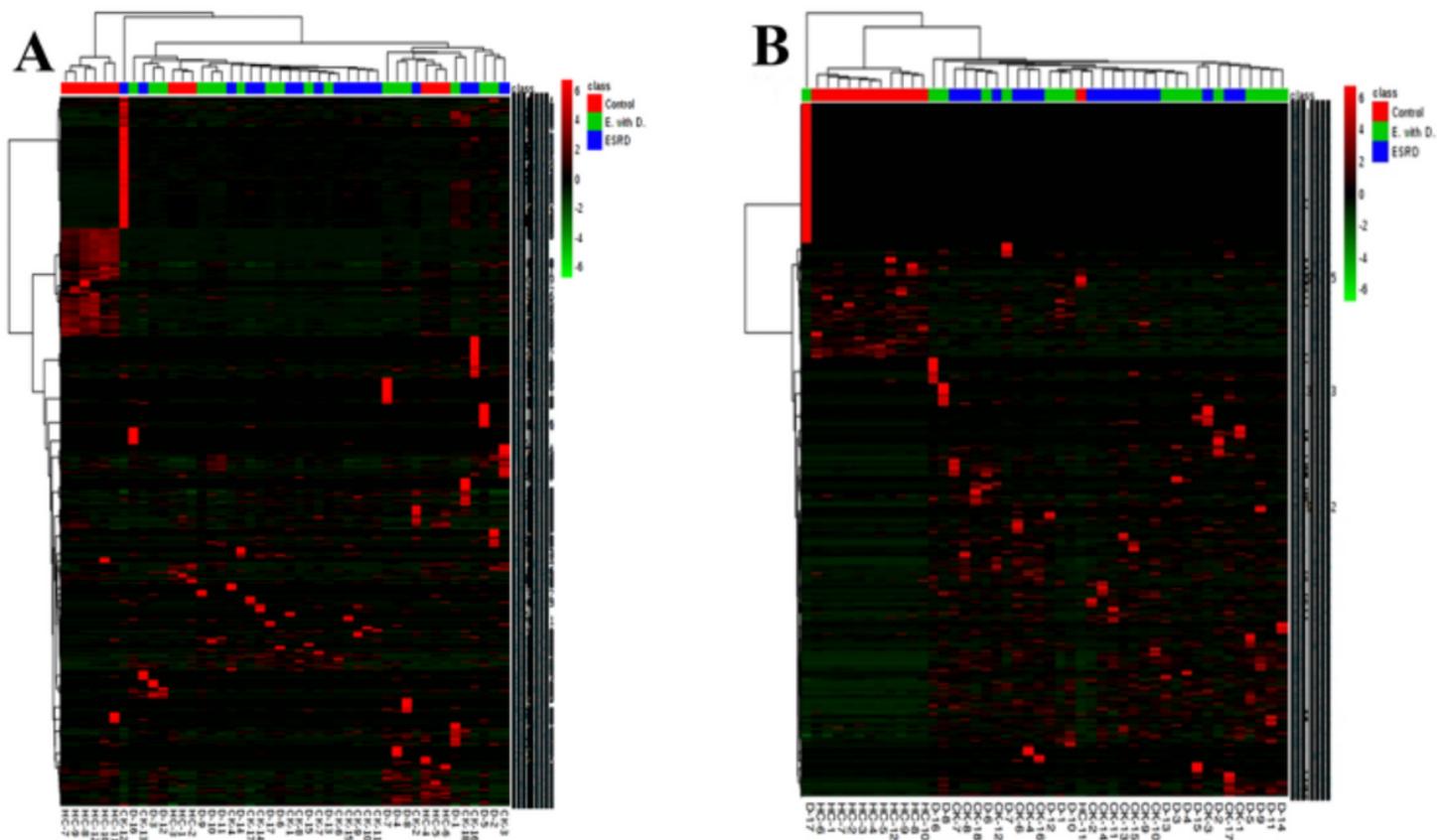


Figure 3

Thermogram of the relative content of differential metabolites: A is in Positive ion mode, B is in negative ion mode

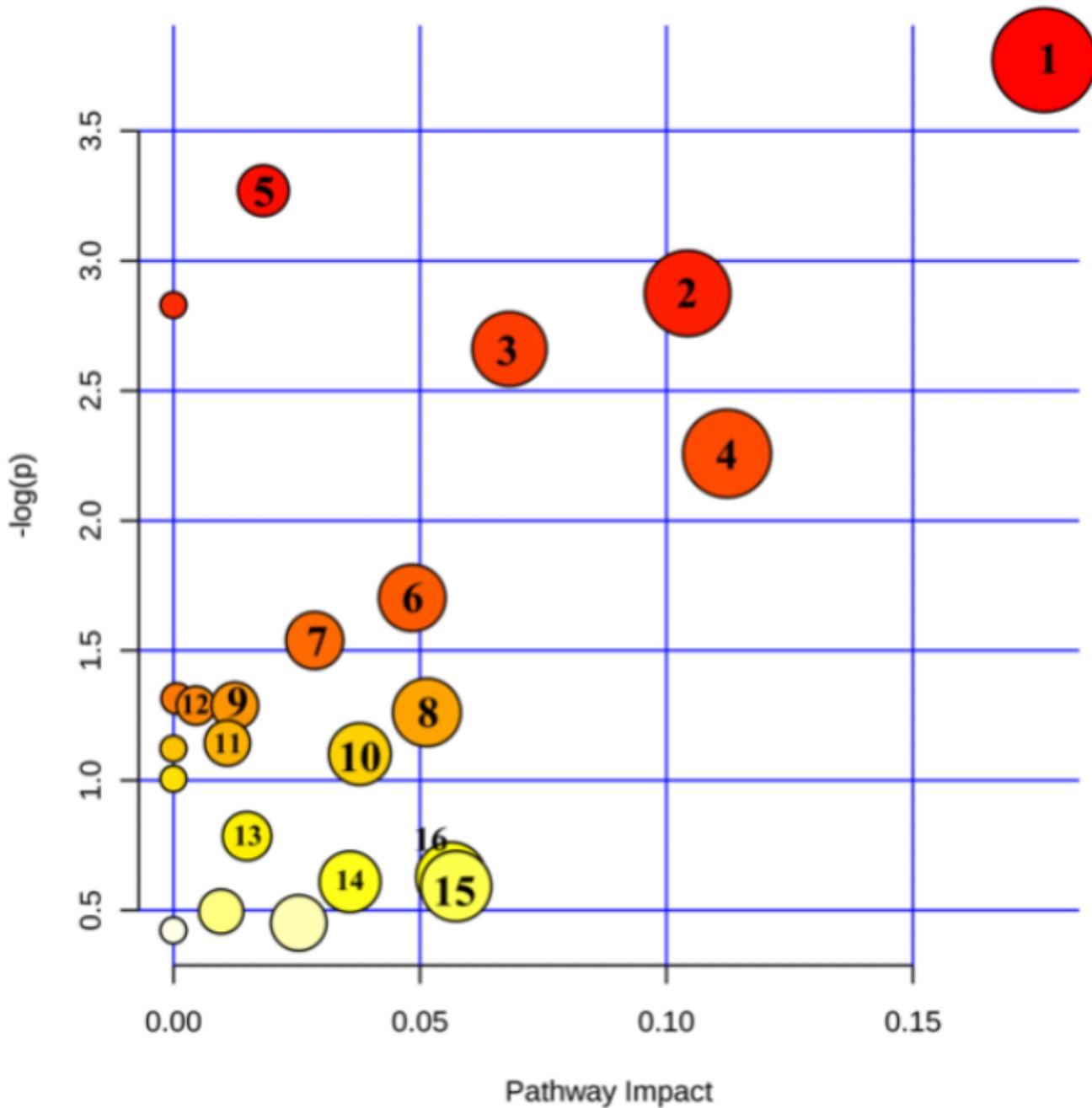


Figure 4

Scores of metabolite pathways involved by differential metabolites in ESRD without depression group vs. ESRD with depression group. The abscissa is the importance value of the compound in the pathway, and the ordinate is the negative logarithm of the p-value log with a base of 10. The closer to the upper right corner, the more significant the enrichment represents and the more important the role of the compound plays in the pathway. 1: Alanine, aspartate and glutamate metabolism; 2: Glycerophospholipid metabolism, 3: Histidine metabolism; 4: D-Glutamine and D-glutamate metabolism; 5: Tyrosine metabolism; 6: Citrate cycle (TCA cycle); 7: Thiamine metabolism; 8: Terpenoid backbone biosynthesis; 9: Valine, leucine and isoleucine degradation; 10: Glycerophospholipid metabolism; 11: Glutathione

metabolism; 12: Pyruvate metabolism; 13: Pyrimidine metabolism; 14: Arginine and proline metabolism; 15: Tryptophan metabolism; 16: Aminoacyl-tRNA biosynthesis

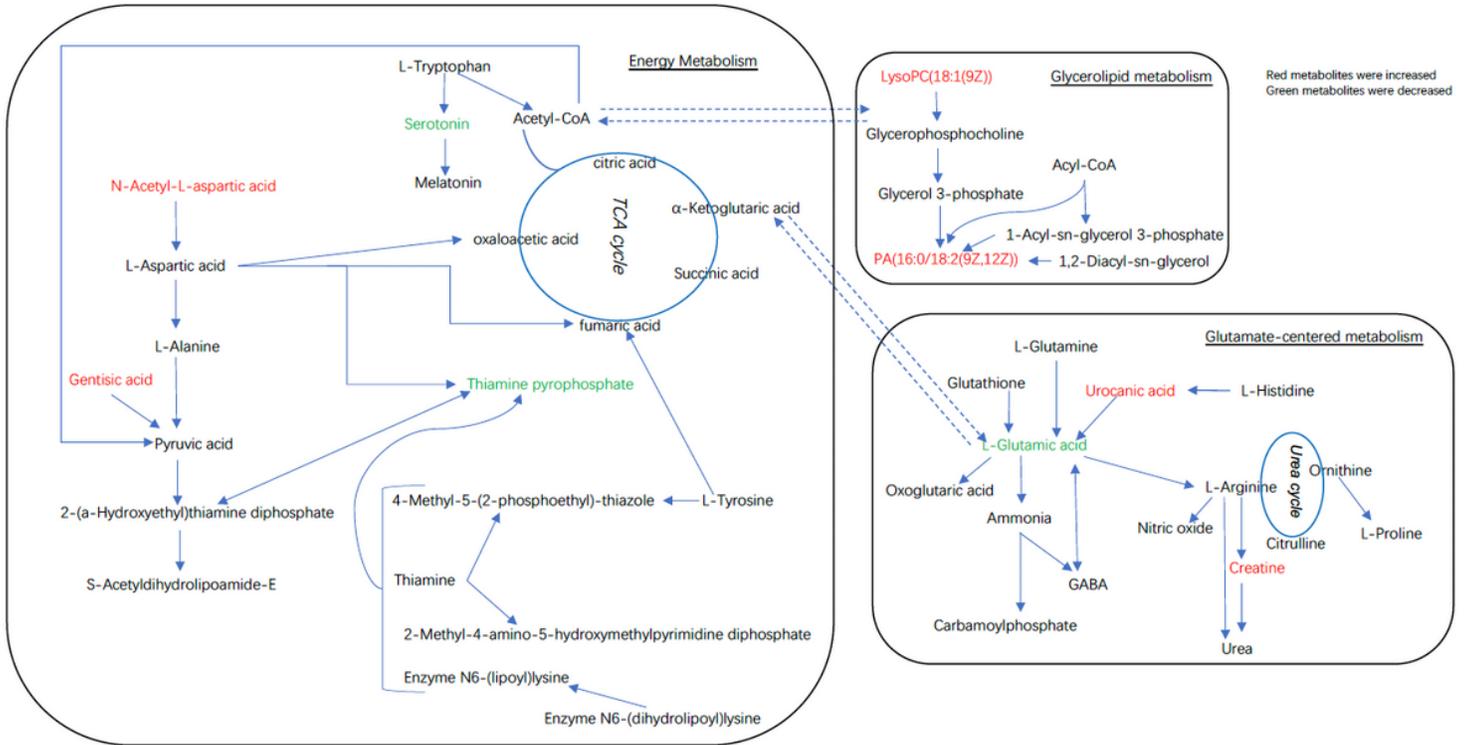


Figure 5

Biochemical transformation of differential metabolites Note: ESRD patients with depression have increased red metabolites and decreased green metabolites compared with ESRD patients without depression.