ORIGINAL RESEARCH

Serum Metabolomics Analysis Revealed Metabolic Pathways Related to AECOPD Complicated with Anxiety and Depression

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Background: Anxiety and depression are two of the most common comorbidities of COPD, which can directly lead to the number of acute exacerbations and hospitalizations of COPD patients and reduce their quality of life. At present, there are many studies on anxiety and depression in stable COPD, but few studies on anxiety and depression in acute exacerbation of chronic obstructive pulmonary disease (AECOPD) patients.

Objective: We aim to explore the changes of serum metabolomics in AECOPD complicated with anxiety and depression and to provide some clues for further understanding its pathogenesis.

Methods: This is an observational high-throughput experimental study based on retrospective data extraction. Twenty-one AECOPD with anxiety and depressive patients and 17 healthy controls (HCs) were retrospectively enrolled in the Second Affiliated Hospital of Anhui Medical University. Hamilton anxiety scale (HAMA) and Hamilton depression scale (HAMD) for anxiety and depression were used to assess the patients with AECOPD. Untargeted metabolomics analysis was carried out to investigate different molecules in the serum of all participants. General information of all participants, baseline data and clinical measurement data of AECOPD patients were collected. Statistical analysis and bioinformatics analysis were performed to reveal different metabolites and perturbed metabolic pathways.

Results: A total of 724 metabolites in positive ionization mode and 555 metabolites in negative ionization mode were different in AECOPD patients with anxiety and depression. The 1,279 serum metabolites could be divided into 77 categories. Based on multivariate and univariate analysis, 74 metabolites were detected in positive ionization mode, and 60 metabolites were detected in negative ionization as differential metabolites. The 134 metabolites were enriched in 18 pathways, including biosynthesis of unsaturated fatty acids, aldosterone synthesis and secretion, protein digestion and absorption, ovarian steroidogenesis, long−term depression, retrograde endocannabinoid signaling, and so on.

Conclusion: This work highlights the key metabolites and metabolic pathways disturbed in AECOPD patients with anxiety and depression. These findings support the use of metabolomics to understand the pathogenic mechanisms involved in AECOPD patients with anxiety and depression.

Keywords: metabolomics, AECOPD, anxiety, depression, exacerbation

Introduction

Chronic obstructive pulmonary disease (COPD) is a common respiratory disease that seriously endangers human health. It is characterized by persistent respiratory symptoms and limited airflow, which is usually related to airway and/or alveolar abnormalities caused by significant exposure to harmful particles or gases.[1] Chronic cough, expectoration and

dyspnea are the clinical manifestations of COPD, and it is often associated with a variety of extrapulmonary diseases, such as cardiovascular disease, osteoporosis, anxiety and depression, which bring heavy burdens to patients, their families and society. With its high morbidity and mortality, COPD has become one of the important global public health problems.[2]

The incidence of anxiety and depression in COPD patients is higher than that in the general population, and it is also higher than that in patients with hypertension, diabetes, tumors and other chronic diseases. Compared with the general population, patients with COPD have a higher risk of comorbidity, such as anxiety and/or depression, which in turn increases their symptom burden and readmission rate.[3] The relationship between mood disorders such as anxiety and depression and COPD is reciprocal, which means that mood disorders can adversely affect the prognosis of COPD, while COPD increases the risk of developing anxiety and depression. Major mechanisms leading to COPD with anxiety and depressive symptoms are increased dyspnea and reduced exercise capacity. COPD patients with moderate-to-severe dyspnea had an increased risk of new-onset depression in a 3-year follow-up study.[4] On the other hand, depression has been shown to be an important risk factor for disabling dyspnea in COPD patients. COPD can lead to feelings of hopelessness, social isolation, reduced physical function and a sedentary lifestyle, all of which are associated with increased depressive symptoms.

The pathogenesis of COPD complicated with anxiety and depression is not completely clear, which may be the result of the interaction of many risk factors. Previous studies have shown that education, smoking, hypoxia, and some common drugs for COPD, such as glucocorticoid and systemic inflammatory response, may be related to the occurrence of COPD complicated with anxiety and depression.[5–8] In particular, smoking is one of the important risk factors for COPD, and it is also a risk factor for anxiety and depression.[9] Meanwhile, persons with depression are more likely to smoke cigarettes.[10] With the advancement of high-throughput "omics" technologies such as genomics, transcriptomics, proteomics, and metabolomics, the occurrence and progress of anxiety and depression in COPD patients are studied by using these omics methods, so as to better understand the potential mechanism of this disease and identify new biomarkers. Metabolic profiling is an excellent method for characterizing small-molecule metabolites, the end products of genetic, transcriptional and translational alterations, which can be used for more accurate and precise diagnosis.[11] Compared with genomics, transcriptomics or protein genomics, metabolomics can reflect the metabolic state of organisms more accurately.[12] Now, it has been widely used in the diagnosis of neuropsychiatric diseases,[13] liver fibrosis[14] and other diseases. LC-MS,[15] GC-MS[16] and NMR[17] are the main technologies of metabolomics. Metabolomics based on LC-MS has been widely applied in the study of mechanism and biomarker screening of COPD patients using blood/serum/plasma,[18] exhaled breath condensate,[19] urine or lung tissue samples,[20] and so on. This is due to their high coverage of metabolites and reliable analytical performance. The most relevant metabolomics research in COPD emphasizes the importance of metabolites directly related to protein (peptides and amino acids), nucleic acids (nitrogenous bases and nucleosides), lipids and their derivatives (especially fatty acids, phospholipids, ceramides and eicosanoids). These findings indicate the correlation between inflammatory immune process, oxidative stress, increased catabolism and changes in energy production.[21] This provides a promising method for further revealing the metabolic pathways related to anxiety and depression in COPD patients, but most of the current studies are aimed at stable COPD patients, and there are few metabolomics studies on acute exacerbation of chronic obstructive pulmonary disease (AECOPD) patients complicated with anxiety and depression.

In this study, we aimed to explore the metabolic changes in AECOPD patients with anxiety and depression by performing a comprehensive LC-MS-based metabolomics analysis of serum samples. The results of this study provide clues for the pathological research of AECOPD with anxiety and depression.

Materials and Methods

This study is based on the principles of the Helsinki Declaration. The Research Ethics Committee of the Second Affiliated Hospital of Anhui Medical University reviewed and approved this research. The approval number of the Ethics Committee is YX2021-149.

The informed consent of all participants was obtained.

Study Setting and Participants

This is an observational high-throughput experimental study based on retrospective data extraction. Initially, a total of 38 subjects, including 21 AECOPD with anxiety and depressive patients and 17 healthy controls (HCs), were retrospectively enrolled in the Second Affiliated Hospital of Anhui Medical University from March 2022 to November 2022 (Figure 1).

Inclusion and Exclusion Criteria

Inclusion and exclusion criteria for the patient group included the following: ①Patients with COPD were diagnosed according to GOLD criteria based[22] on COPD risk factors, symptoms, and post-bronchodilator FEV1/FVC less than 70%. Exacerbation of COPD was defined as an acute worsening of respiratory symptoms leading to additional therapy. All the patients admitted to the hospital for acute exacerbation of COPD were included in this study. ②Patients can understand the research process, participate voluntarily and sign written informed consent; ③Patients can complete relevant scale tests; ④All AECOPD patients were evaluated with Hamilton anxiety scale (HAMA) and Hamilton depression scale (HAMD) for anxiety and depression;[23,24] HAMA score ≥7 was diagnosed with anxiety. HAMD score ≥8 was diagnosed with depressive disorder. Those who had illnesses with serious liver, cardiovascular, metabolic or renal kidney failure or psychiatric disorders were excluded from the study. HCs were from a population who self-reported no disease and had no abnormal physical examination results, normal lung function, and the assessment of HAMA and HAMD scales excluded anxiety and depression. The baseline data of 21 AECOPD with anxiety and depressive patients were collected. The contents include age, gender, BMI, smoking index, the number of acute exacerbations in the previous year, whether there was comorbidity, whether glucocorticoid was inhaled, whether home oxygen therapy was
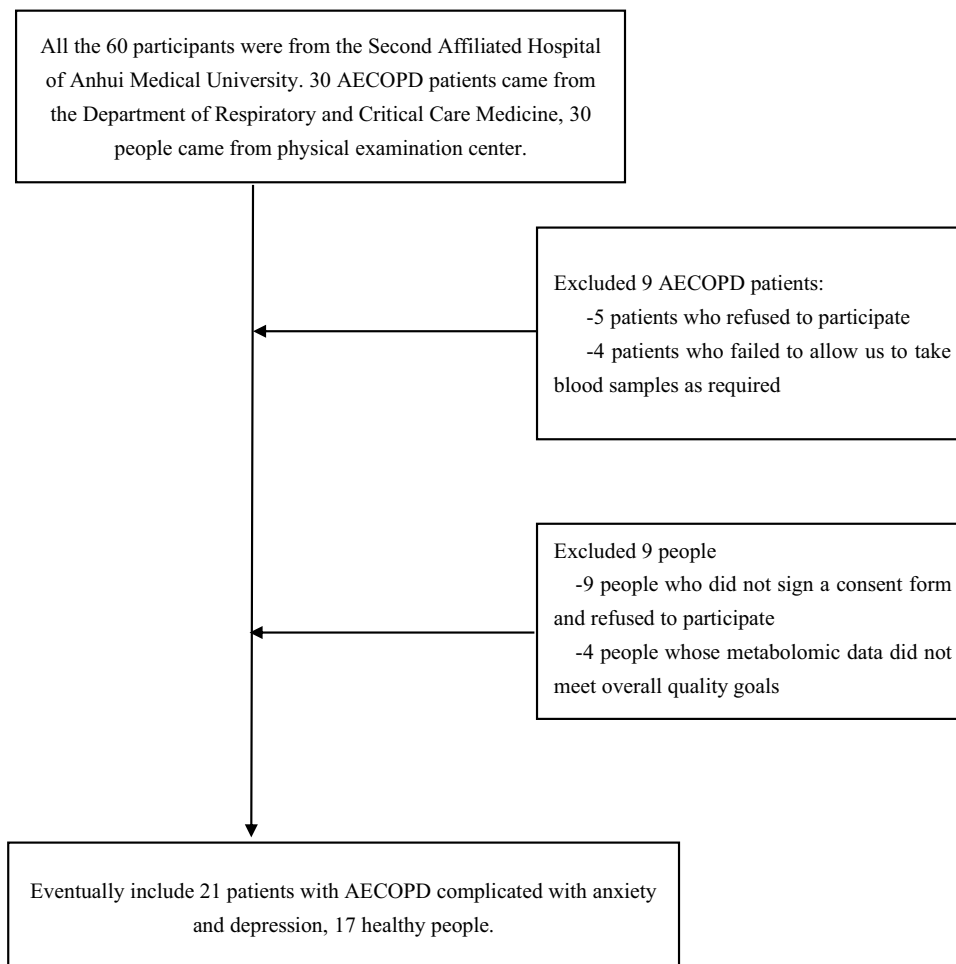
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│ All the 60 participants were from the    │
│ Second Affiliated Hospital of Anhui      │
│ Medical University. 30 AECOPD patients   │
│ came from the Department of Respiratory  │
│ and Critical Care Medicine, 30 people    │
│ came from physical examination center.   │
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                  │
                  │         ┌──────────────────────────────────┐
                  │         │ Excluded 9 AECOPD patients:      │
                  │◄────────│   -5 patients who refused to     │
                  │         │ participate                      │
                  │         │   -4 patients who failed to      │
                  │         │ allow us to take blood samples   │
                  │         │ as required                      │
                  │         └──────────────────────────────────┘
                  │
                  │         ┌──────────────────────────────────┐
                  │         │ Excluded 9 people                │
                  │◄────────│   -9 people who did not sign a   │
                  │         │ consent form and refused to      │
                  │         │ participate                      │
                  │         │   -4 people whose metabolomic    │
                  │         │ data did not meet overall        │
                  │         │ quality goals                    │
                  │         └──────────────────────────────────┘
                  ▼
┌─────────────────────────────────────────┐
│ Eventually include 21 patients with      │
│ AECOPD complicated with anxiety and      │
│ depression, 17 healthy people.           │
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Figure 1 The consort diagram of the study.

carried out, the chronic obstructive pulmonary disease assessment test (CAT) score, the mMRC score, the GOLD rating and whether there was respiratory failure. The information collected by the HCs was limited to age, gender, BMI and smoking index. The smoking index was determined by multiplying the number of cigarettes smoked per day by the number of years of smoking.[25] The participants were all active smokers and did not use electronic cigarettes. Two groups of serum samples were included in the metabolomics analysis, namely the patient group (AECOPD combined with anxiety and depression group, n = 21) and the healthy control group (HCs, n = 17).

Data Collection
General information of all participants was used to collect, including age, gender, BMI and smoking index. Other clinical baseline data of AECOPD patients with anxiety and depression also included the number of acute exacerbations in the previous year, CAT score, mMRC score, GOLD stages, comorbidities, inhaled glucocorticoids, home oxygen therapy, respiratory failure, HAMA score and HAMD score. The severity of airflow restriction was rated as GOLD I, II, III or IV, depending on FEV1/FVC after the bronchodilator described in GOLD (2019). Respiratory failure in AECOPD patients was defined as, according to the results of arterial blood gas analysis, the partial oxygen pressure of patients in an anaerobic state was lower than 60mmHg when they breathe quietly.

Experimental Instruments and Reagents
The low-temperature high-speed centrifuge uses Eppendorf 5430R. Ammonium acetate was purchased from Sigma-Aldrich. Acetonitrile, methanol and ammonia were purchased from Fisher Scientific.

Sample Preparation
Collection 5 mL of morning fasting blood from all participants was collected into EDTA tubes and kept on ice. The serum was separated by centrifugation at 3000 g for 10 min and stored at −80°C. All serum samples for metabolomic analysis were processed as a batch for metabolite extraction. After the sample was slowly thawed at 4°C, each serum sample was extracted by adding 400ul of pre-cooled methanol/acetonitrile/water solution (2:2:1, v/v). After 30 minutes of vortex centrifugation and cryogenic ultrasonic treatment, the mixtures were incubated at −20°C for 10 min and centrifuged at 14,000 g at 4°C for 20 min. The supernatant was transferred to a new Eppendorf tube, dried in a vacuum, and then dissolved in 100 μL acetonitrile/water solution (1:1, v/v). After vortex centrifugation at 14,000 g for 15 min (4°C), the final supernatant was collected for analysis.

UPLC-Q-TOF/MS Data Acquisition
For global metabolite analysis of serum samples, an Agilent 1290 Infinity LC system (Agilent Technologies, Santa-Clara, California, USA) equipped with an AB SCIEX Triple TOF 6600 system (AB SCIEX, Framingham, MA, USA) was used. Chromatographic separation was performed on an ACQUITY BEH Amide 1.7 μm (2.1 mm × 100 mm) column (waters, Ireland). The injection volume of each sample was 2 μL, the column temperature was 25°C, and the flow rate was 0.3 mL/min. The mobile phase included solvent A (25 mm ammonium acetate and 25 mm ammonia in water) and solvent B (acetonitrile). The gradient elution parameters were set as follows: 0–1.5min, 98%B; 1.5–12min, B varies linearly from 98% to 2%; 12–14min, B maintained at 2%; 14–14.1min, B varies linearly from 2% to 98%; 14.1–17min, B maintained at 98%. Mass spectrometry was performed using electrospray ionization (ESI) in positive and negative ion modes, respectively. Atomization auxiliary heating gases 1 and 2 were both 60PSI, while curtain gas was 30PSI. The ion source temperature was 600°C.

Metabolomics Data Analysis
Raw mass spectrometer files were converted into mzXML files using Proteo Wizard MS converter and XCMS software, which were then subjected to peak alignment, retention time correction, and peak area extraction. The metabolites were identified by accurate mass spectrometry, compared with the standard database established by Shanghai Applied Protein Technology Co., Ltd., and the mass spectrometry data with grade 2 and above were identified.[26] To monitor the stability and reproducibility of the platform, pooled quality control (QC) samples were inserted and analyzed every 7 samples. In

addition, we use multivariate statistical methods (PCA, PLS-DA, OPLS-DA) to reduce the dimension of the collected multidimensional data on the basis of retaining the original information to the greatest extent. Multivariate statistical analysis was performed using the MetaboAnalyst (www.metaboanalyst.ca) web system. After Pareto scaling, QC samples were tightly clustered in the principal component analysis (PCA) score plot (Figure S1), indicating good reproducibility. All metabolites (including unidentified metabolites) detected in positive and negative ion modes were differentially analyzed based on univariate analysis. FC > 1.5 or FC < 0.67, p-value <0.05 were identified as differential metabolites. The statistical significance thresholds for variable influence on projection (VIP) values derived from the orthogonal partial least squares discrimination analysis (OPLS-DA) model and two-tailed Student's t-test (p-value) on raw data to identify metabolites with significant differences, OPLS-DA VIP values >1.0, p-value <0.05 was considered a significant metabolite. To evaluate the rationality of candidate metabolites and more comprehensively and intuitively display the relationship between samples and the differences in the expression patterns of metabolites in different samples, we employ hierarchical clustering for significance differential metabolite. Based on the Pearson correlation analysis method, the correlation between the significantly different metabolites was analyzed. Hierarchical clustering was conducted to identify different metabolic patterns between the two groups. Kyoto Encyclopedia of Genes and Genomes (http://www. genome.jp/kegg/) database was used for pathway analysis.[27]

Results

Demographic and Clinical Characteristics
The baseline characteristics of the participants included in the metabolomics analysis were presented in Table 1. There was no significant difference in sex, age, BMI and smoking index between AECOPD patients with anxiety and depression and HCs. T test was used for age and BMI, and chi-square test was used for sex and smoking index. According to the results of lung function, COPD patients with GLOD I–IV grades were included. All patients had at least one acute exacerbation. According to the patients' symptoms and history of acute exacerbation, there were 4 patients in group A, 6 patients in group B and 11 patients in group E.[28]

Serum Metabolic Differences Between AECOPD Complicated with Anxiety and Depressive Patients Subjects and HCs
A non-targeted metabolomics technique UHPLC-Q-TOF-MS was used to analyze the metabolism of 38 serum samples from 21 AECOPD patients with anxiety and depression and 17 healthy controls. A total of 724 metabolites were detected in positive ionization mode and 555 metabolites in negative ionization mode (Table S1). The differential metabolites under the combination of the two models were classified. Excluding metabolites without chemical classification, the categories accounting for more than 5% include lipids and lipid-like molecules, organic acids and derivatives, organo-heterocyclic compounds, benzoids and organic oxygen compounds (Figure 2).

As shown in the PCA score (Figure 3), the clustering of QC samples in the combined mode of positive and negative ionization showed that the metabonomics platform had acceptable stability and repeatability. Twenty-one serum samples of AECOPD were obviously separated from the control group in PCA score chart, which indicated that there were obvious differences between the two groups in serum metabolism.

The differences in metabolic profiles that were clearly separated on the PLS-DA score map were analyzed using supervised multidimensional statistical methods (Figure 4). The results showed that the PLS-DA model could distinguish two groups of samples. To avoid overfitting of the supervised model during the modeling process, the permutation test was used to test the model to ensure the validity of the model. Figure 4 shows the permutation test chart of the PLS-DA model in the comparison group. The evaluation parameters of the PLS-DA model in AECOPD patients with anxiety and depression group were calculated. As the permutation retention gradually decreases, indicating that the original model does not have an overfitting phenomenon and the model is robust.

Supervised OPLS-DA statistical analysis was performed to determine the metabolites leading to the separation (Figure 5). Based on multivariate and univariate analysis, all metabolites (including unidentified metabolites, VIP ≥ 1 and $p \leq 0.05$) detected in positive and negative ion modes were analyzed. Excluding the differential metabolites that have

Table 1 Baseline Characteristics of the Participants

	HCs	AECOPD	*P* value
Number	17	21	–
Sex [n(%)]			
Male	14(82.35%)	17(80.95%)	0.678
Female	3(17.65%)	4(19.05%)	
Age ($\bar{X} \pm SEM$)	67.12±4.19	72.43±1.67	0.213
BMI ($\bar{X} \pm SEM$)	21.80±0.59	23.19±0.71	0.154
Smoking Index	7(41.18%)	18(85.71%)	0.006
≥400 [n(%)]			
<400 [n(%)]	10(58.82%)	3(14.29%)	
Number of AEs in the previous year (n) [M(P$_{25}$-P$_{75}$)]	–	2(1–2)	–
CAT score ($\bar{X} \pm SEM$)	–	20.24±1.58	–
mMRC score ($\bar{X} \pm SEM$)	–	2.43±0.29	–
GOLD stages [n(%)]	–		–
I		5(23.81%)	
II		7(33.33%)	
III		6(28.57%)	
IV		3(14.29%)	
Comorbidities [n(%)]	–		–
Yes		14(66.67%)	
No		7(33.33%)	
Inhaled glucocorticoids [n(%)]	–		–
Yes		14(66.67%)	
No		7(33.33%)	
Home oxygen therapy [n(%)]	–		–
Yes		11(52.38%)	
No		10(47.62%)	
Respiratory failure [n(%)]	–		–
Yes		9(42.85%)	
No		12(57.14%)	
HAMA score ($\bar{X} \pm SEM$)	–	15.57±1.34	–
HAMD score ($\bar{X} \pm SEM$)	–	11.76±0.81	–

Notes: The measurement data were presented as mean±standard error ($\bar{X} \pm SEM$) deviation for the normally distributed variables or as median and interquartile range [M(P25-P75)] for the non-normally distributed variables. The categorical data were described by rate and percentage. **Abbreviations**: BMI, body mass index; HCs, healthy controls; AECOPD, acute exacerbation of chronic obstructive pulmonary disease; AEs, acute exacerbations; CAT, the COPD assessment test; mMRC, modified Medical Research Council; GOLD, Global Initiative for Chronic Obstructive Lung Disease. SEM, Standard Error of Mean.

not been characterized, 74 metabolites were detected in the positive ion mode, and 60 metabolites were detected in the negative ion mode between the AECOPD combined with anxiety and depression group and the control group (Figure 6A and B, Tables S2 and S3).

Under the combined mode of positive and negative ions, compared with the control group, in the serum of AECOPD complicated with anxiety and depression, 101 metabolites increased and 45 metabolites decreased (Figure 7). All the metabolites were super-classified (Figure 8). Except for the undefined metabolites, most of the up-regulated metabolites belonged to Lipids and lipid-like molecules, organic acids and derivatives. However, the down-regulated metabolites were mainly concentrated in Lipids and lipid-like molecules, organoheterocyclic compounds and benzoids. The hierarchical clustering results of significantly different metabolites in the combined mode of positive and negative ions are shown in Figure 9.
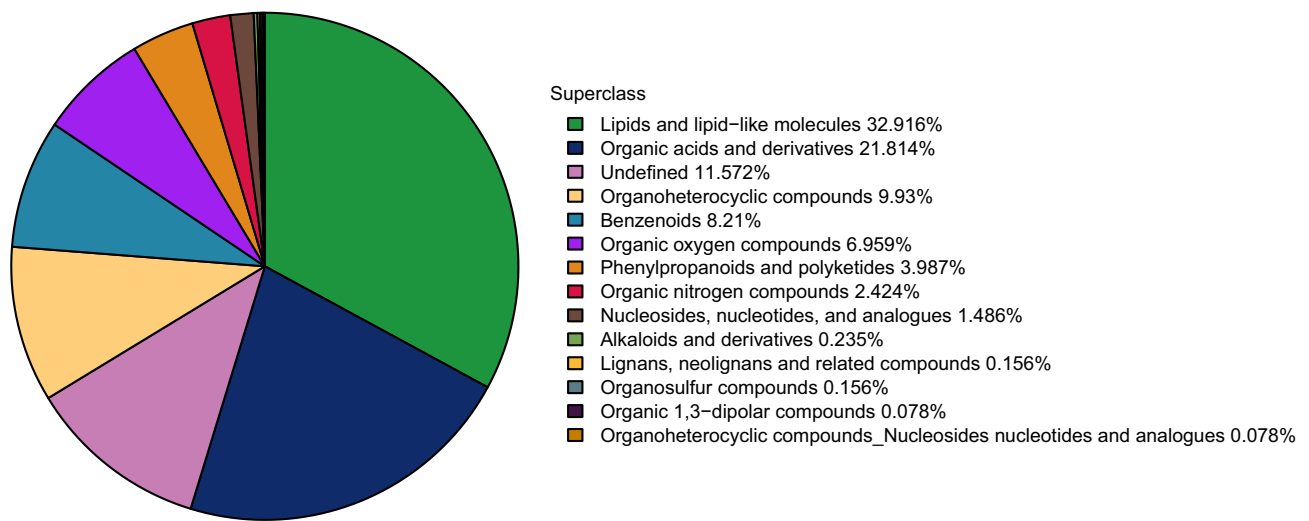
Figure 2 Quantitative proportion of identified metabolites in each chemical class. The different color blocks in the figure represent different chemical classification attribution entries, and the percentage represents the percentage of the number of metabolites in the chemical classification attribution entry to all identified metabolites. Metabolites without chemical class assignments were defined as undefined.
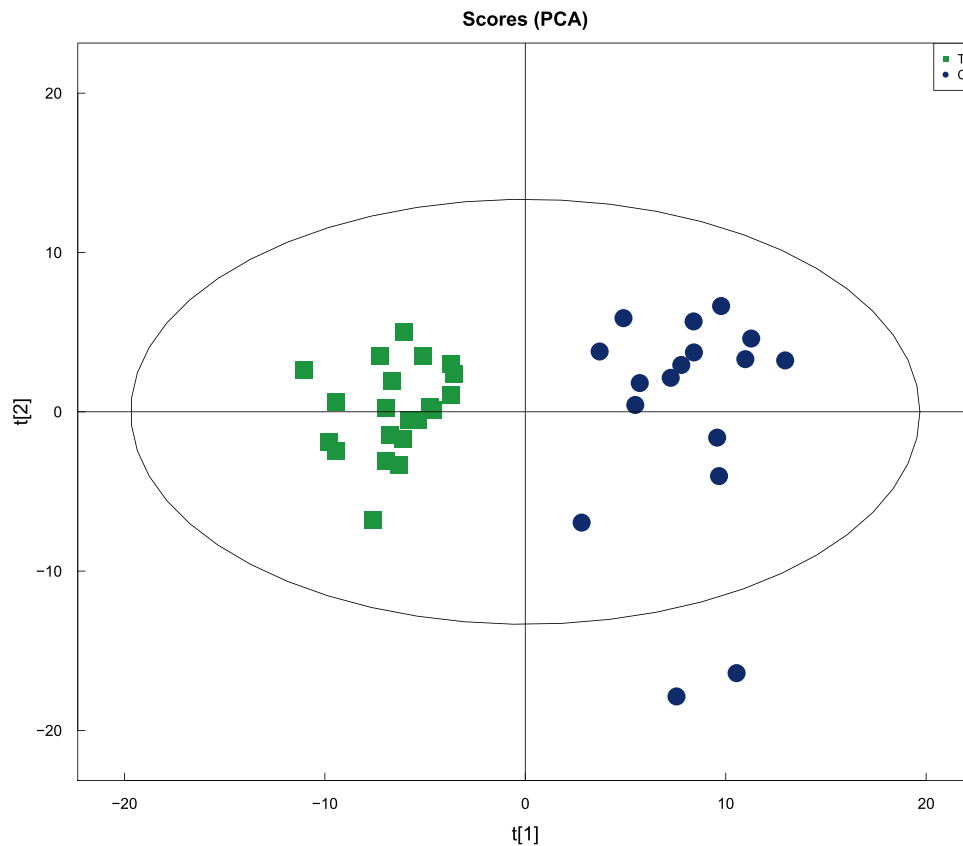

Figure 3 The PCA score in the combined mode of positive and negative ionization. C: the healthy control group; T: AECOPD combined with anxiety and depression group.

KEGG Metabolic Pathway Analysis

Kyoto encyclopedia of genes and genomes (KEGG) metabolic pathway enrichment analysis was performed on the metabolites in the samples. Fisher's exact test was used to calculate the significance level of metabolite enrichment in each
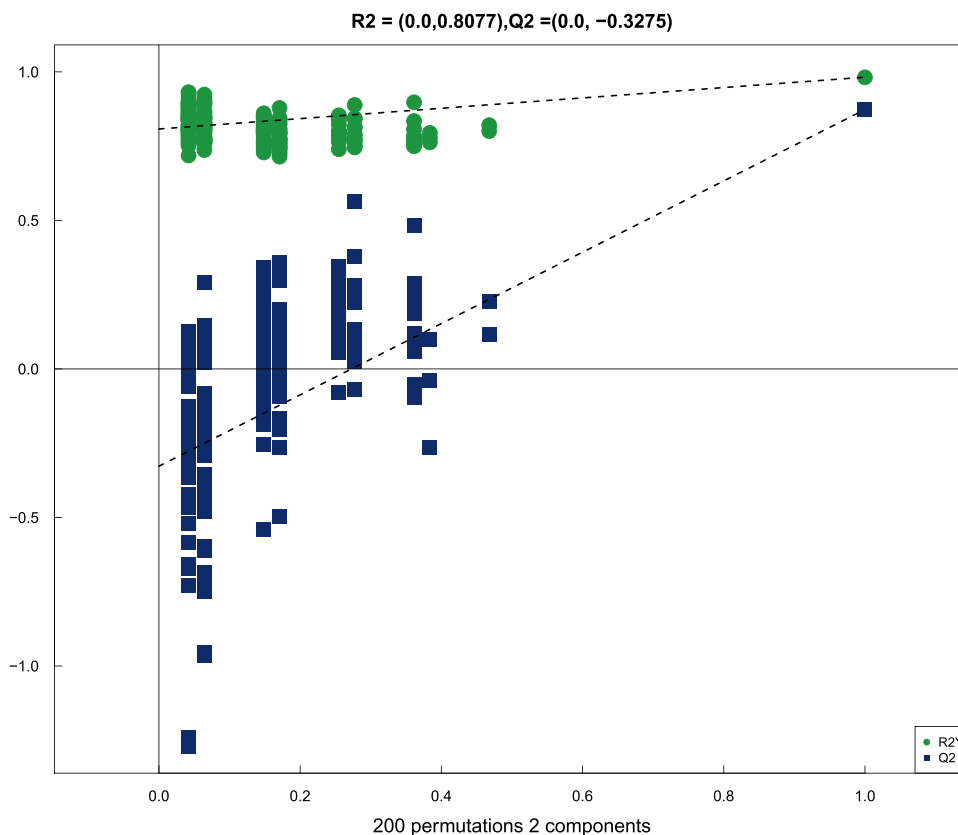
Figure 4 The permutation test chart of the PLS-DA model.

pathway, so as to determine the significantly affected metabolic and signal transduction pathways. KEGG pathway enrichment analysis was performed on these 134 metabolites, and 18 pathways were found to be significantly enriched (p < 0.05). The results in Figure 10 showed that the top 10 metabolic pathways with significant differences included central carbon metabolism in cancer, biosynthesis of unsaturated fatty acids, aldosterone synthesis and secretion, protein digestion and absorption, ovarian steroidogenesis, long-term depression, necroptosis, aldosterone synthesis and secretion, amoebiasis and basal cell carcinoma.

Discussion

In COPD patients, dyspnea and depression seem to be interrelated through various complicated pathways.[29] The pathogenesis of COPD complicated with anxiety and depression is not clear. More and more studies show that a variety of serum metabolites and metabolic pathways are related to the onset or progress of chronic obstructive pulmonary disease. It is well known that smoking is one of the main causes of COPD, and smoking cessation will also have a certain impact on patients' metabolic function and mood. Smoking cessation can improve the respiratory function parameters of COPD patients in a short time, including symptoms and obstruction parameters. It also affects lipid metabolism leading to a decrease in total cholesterol, and at the same time, it brings about an increase in HDL cholesterol level.[30] Smoking abstinence expectancies are beliefs about negative and positive short-term psychological and physiological consequences of not smoking.[31] The disorder pathway involved in COPD provides clues for further study of the pathological mechanism of COPD.[18] On the other hand, there are clear metabolic changes in anxiety and depression, mainly lipids.[32,33] However, there is no report on the metabolic changes of AECOPD complicated with anxiety and depression at present. The purpose of this study is to explore the metabolic profile of serum in this population and provide new clues for the study of pathological mechanism.
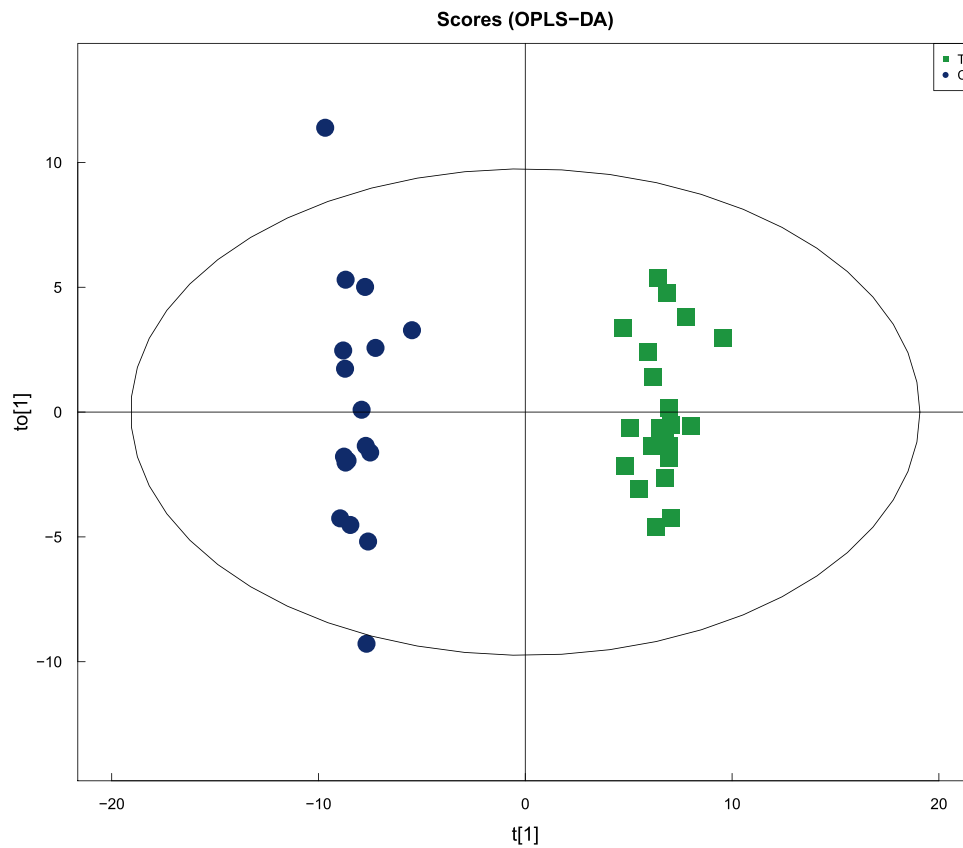
Figure 5 Score plot in orthogonal partial least squares discrimination analysis (OPLS-DA) between serum samples from AECOPD patients with anxiety and depression and control group.C: the healthy control group; T: AECOPD combined with anxiety and depression group.
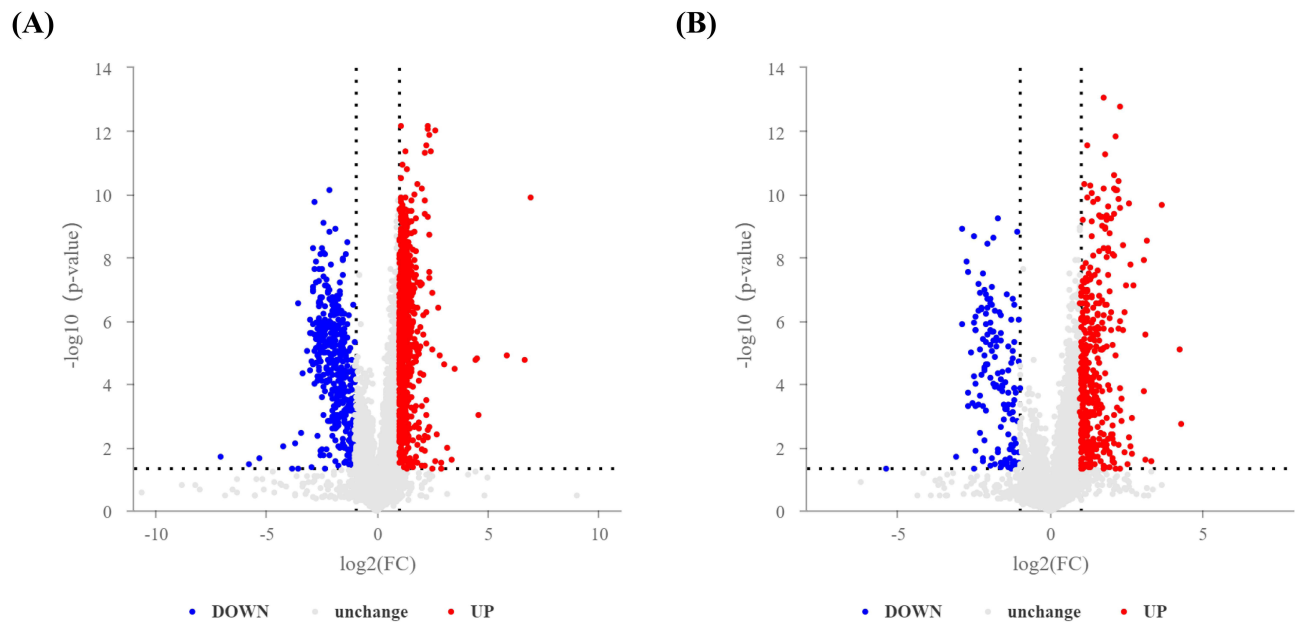
(A)

(B)

Figure 6 Volcano plot for the ratios of serum metabolites between AECOPD patients with anxiety and depression and the healthy control group in (**A**) Positive ion mode and (**B**) Negative ion mode.C: the healthy control group; T: AECOPD combined with anxiety and depression group.
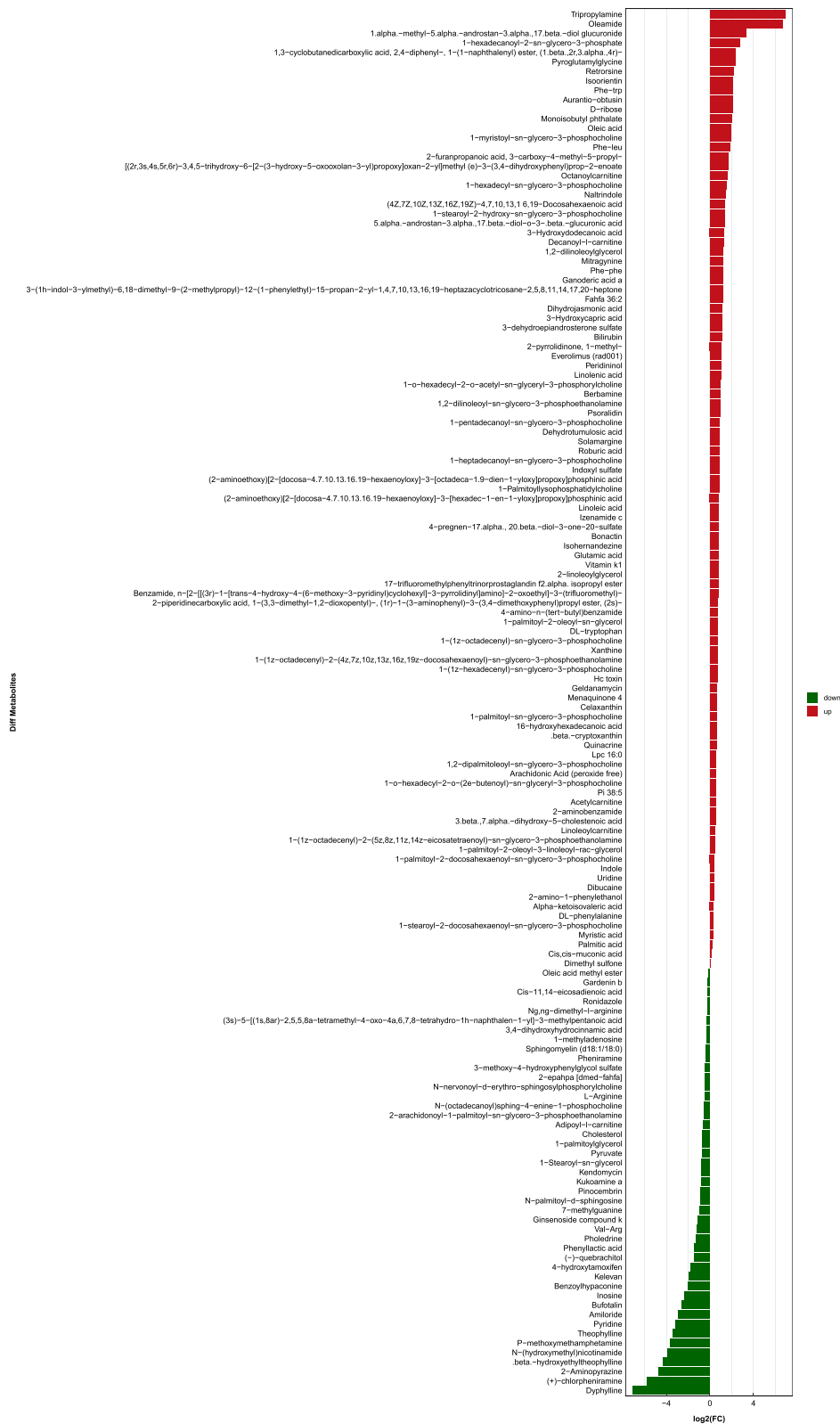
Figure 7 Significant expression analysis of differential metabolites (AECOPD combined with anxiety and depression group vs HCs group).
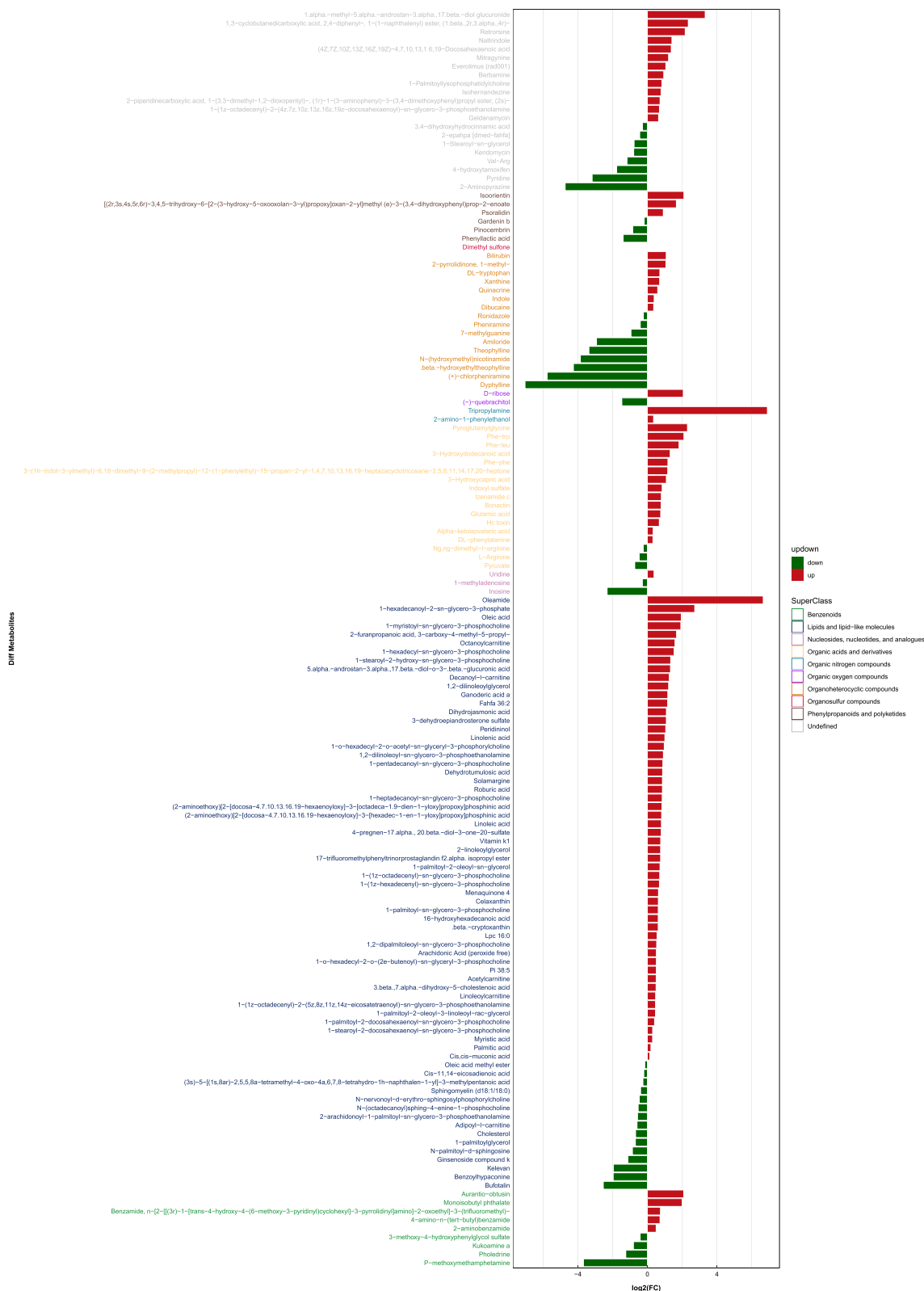
Figure 8 Super-classification of up-regulated and down-regulated differential metabolites under the combined mode of positive and negative ions.
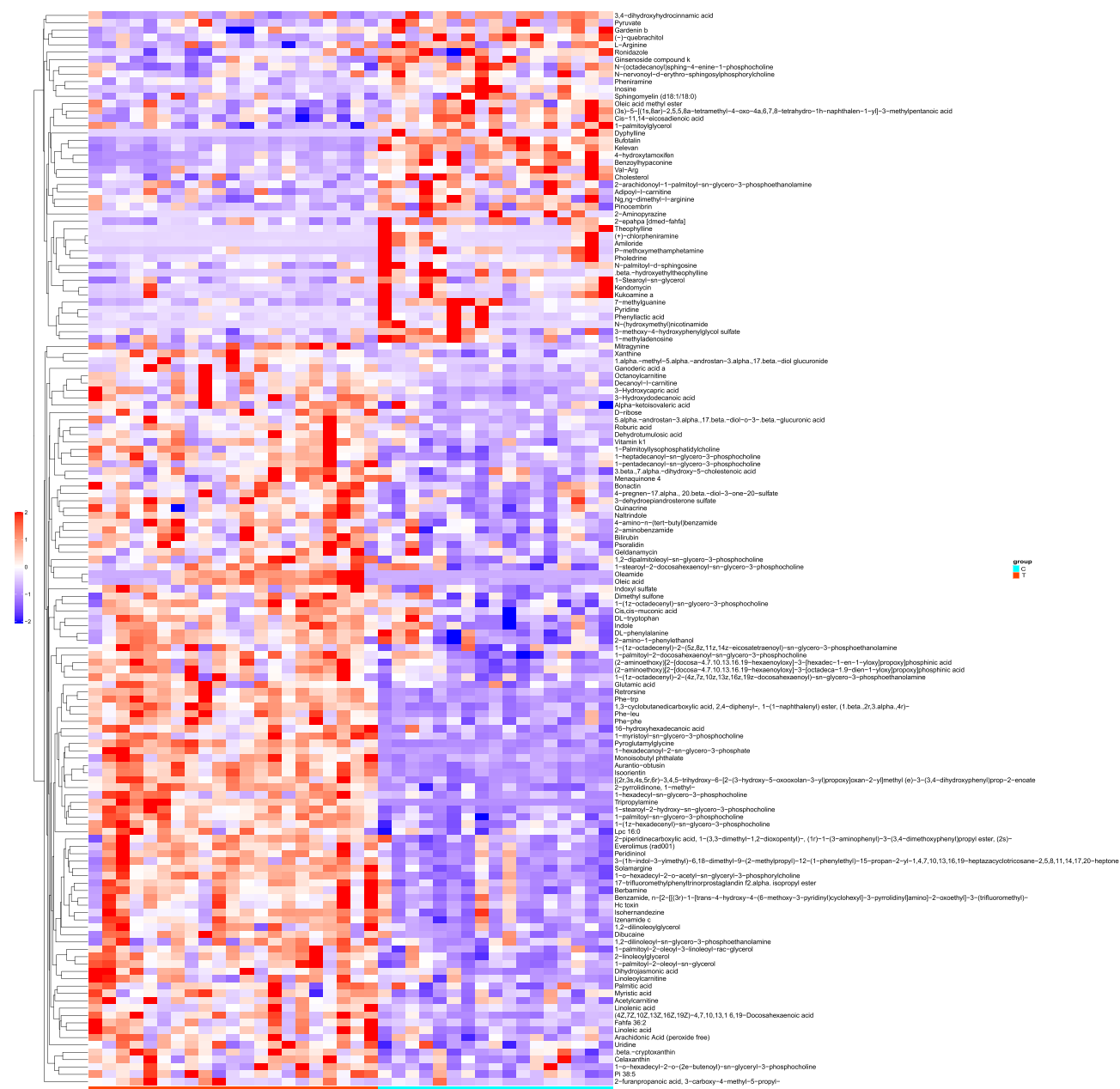
Figure 9 Significant difference metabolite hierarchical clustering results between AECOPD combined with anxiety and depression group and the healthy control group. The ordinate in the figure is the metabolites with significant differential expression, and the abscissa is the sample information. The color blocks at different positions represent the relative expression of metabolites at the position, red represents relatively high expression, blue represents relatively low expression, and metabolites with similar expression patterns are gathered under the same Cluster on the left. The red area on the horizontal axis is marked as T, which indicates the AECOPD group with anxiety and depression. The blue area on the horizontal axis is marked as C, indicating the healthy control group.

Most of the up-regulated metabolites belonged to lipids and lipid-like molecules, such as Oleamide (cis-9,10-octadecenamide, OLE), oleic acid (OA), 1-myristoyl-sn-glycero-3-phosphocholine and so on. OLE was belonged to the family of long-chain fatty acid amides, which was the most significant up-regulation substance.[34] It as an endogenous bioactive lipid signal molecule acts on endogenous cannabinoids, serotonin and GABAergic systems. Previous studies have shown that OLE has been found in the cerebrospinal fluid of sleep-deprived cats and rats, and it has been proven that it can enhance the signal transduction mediated by human serotonin 2A receptor (5-$HT_{2A}R$).[35] The abnormal sensitivity of 5-HT2AR to OLE and other endogenous fatty acids may be the cause of some aspects of mental disorders,
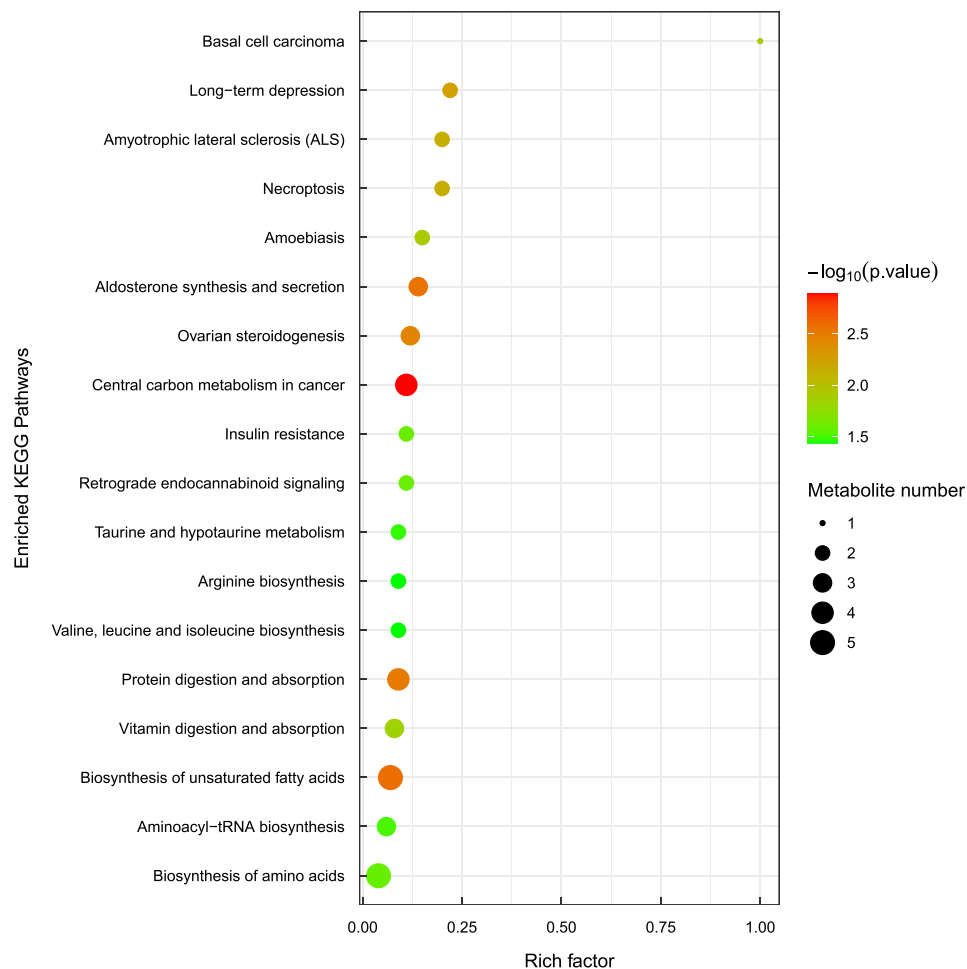
Figure 10 KEGG pathway enrichment analysis of significantly changed metabolites in serum samples from AECOPD complicated with anxiety and depression.
Notes: Each bubble in the diagram represents a metabolic pathway. The abscissa and bubble size indicate the influence factor of the path in topology analysis and the larger the bubble, the greater the influence factor. The vertical coordinate of the bubble and the color of the bubble indicate the P value of enrichment analysis. The deeper the color, the smaller the P value and the more significant the enrichment degree. Rich factor represents the proportion of the number of different metabolites in the pathway to the number of metabolites annotated in the pathway.

such as anxiety and depression.[36] This provides some reference value for designing anti-anxiety and depression drugs for AECOPD patients in the future. OA as one of fatty acids exists in human adipose tissue, plasma and cell membrane. It is known that it participates in membrane fluidity, docking and activity of G-protein coupled receptors.[37] Previous research reports on OA levels in patients with COPD have both increased and decreased. However, study has shown that higher levels of OA may increase the risk of depression.[38] The results of this study are consistent with the above result. OA synthesized and released by astrocytes in the presence of albumin causes neuronal differentiation by promoting axonal growth and neuronal clustering.[39] In the past, patients with COPD often advocated a high-protein diet to reduce respiratory quotient, besides, we speculate that patients with AECOPD need to reduce OA intake to reduce the risk of depression. In addition to lipids and lipid-like molecules, other up-regulated substances included tripropylamine, retrorsine (RST), isoorientin (ISO) and D-ribose (RIB). RST is a phytotoxin in the pyrrolizidine alkaloid family, which can damage the proliferation of liver cells. Previous studies have shown that RST causes comparable deoxygenation states in red blood cells, and the formation of pyrrole-hemoglobin adduct may lead to the decrease of oxygen carrying capacity of red blood cells. However, the decrease of oxygen carrying capacity and the resulting hypoxia can lead to the development of pulmonary hypertension.[40] Whether this is meaningful in the pathogenesis of AECOPD complicated with anxiety and depression needs further discussion. ISO is a natural c-glucosylflavone, which has strong antioxidant and anti-inflammatory properties. The existing evidence emphasizes the main role of systemic inflammation in the occurrence

of AECOPD complicated with anxiety and depression, as well as chronic diseases, heredity, consequences of smoking, hypoxemia, oxidative stress and intestinal microflora. ISO has an inhibitory effect on monoamine oxidase in vitro, which indicates its antidepressant mechanism.[41] Further study on the role of ISO in AECOPD complicated with depression deserves attention. D-ribose (RIB) has often been used as a food additive or nutritional supplement. RIB is a natural pentose monosaccharide existing in all living cells. Supplementing RIB in a daily diet may be beneficial to maintain the necessary ATP level during high-intensity exercise. A high dose of RIB can induce depression, anxiety-like behavior and spatial memory impairment in mice.[42] It can be seen that high-dose RIB is potentially harmful to AECOPD patients as a long-term energy source.

Except undefined metabolites, the down-regulated metabolites are mainly concentrated in lipids and lipid-like molecules and Organoheterocyclic compounds. In addition to the above two types of substances, we found that inosine and pinocembrin have antidepressant effects. Inosine is an endogenous purine nucleoside formed by the decomposition of adenosine. Some studies have shown an antidepressant-like effect of adenosine administrated systemically or centrally, while inosine has an antidepressant effect in adult rodents.[43,44] Pinocembrin is a natural flavonoid with anti-inflammatory, antioxidant and neuroprotective effects, which may alleviate the harmful and depressive behavior of depressed rats by inhibiting P2X4 receptor–mediated pyroptosis in the hippocampus.[45] However, the above research is limited to animal experiments, and what kind of influence mechanism exists in human body is still unclear.

Through KEGG pathway enrichment analysis of differential metabolites, this study found that three pathways, biosynthesis of unsaturated fatty acids, long-term depression (LTD) and retrograde endocannabinoid signaling may be involved in the occurrence and development of AECOPD complicated with anxiety and depression. Metabolomics analysis highlights a range of metabolites, including oleic acid, palmitic acid, linolenic acid, arachidonic acid (peroxide free) and Linoleic acid. These differential metabolites are mainly involved in the biosynthesis of unsaturated fatty acids. Evidence shows that unsaturated fatty acids are related to anti-inflammatory status. Nutritional research on COPD patients advocates omega-3 polyunsaturated fatty acid supplementation.[46] For patients with AECOPD complicated with anxiety and depression, we suspect that unsaturated fatty acids may be more needed. LTD was first found in hippocampus, a form of synaptic plasticity. LTD has two forms: NMDA receptor-dependent and NMDA receptor-independent, but the ultimate mechanism is to activate intracellular signal pathways and cause AMPA receptor endocytosis, thus reducing synaptic transmission efficiency.[47] More and more evidence shows that the hippocampal function of patients with depression has changed, and its ultimate pathway may be NMDA receptor-dependent synaptic plasticity abnormality.[48] On the other hand, retrograde endogenous cannabinoid signaling may also be involved. Retrograde endogenous cannabinoid signaling system is a widely existing neuroregulatory system in the brain, and it is also widely used in the peripheral nervous system, which is very important for the regulation of brain function, metabolism and immune system. Preclinical data show that it is an important stress buffer and regulates mood and cognitive function. These data suggest that the endogenous cannabinoid signal may be particularly involved in the influence of stress on the risk of depression, and the change of the endogenous cannabinoid signal balance between cortical and subcortical regions may lead to depression.[49] The metabolites involved in this pathway detected in this study are mainly glutamic acid and nonoxidized arachidonic acid. Glutamate is an excitatory neurotransmitter in the central nervous system and plays an important role in the termination of neurotransmitter signals in excitatory synapses. Previous studies have shown that stress can promote the outbreak of glutamate and then lead to the activation of glutamate receptors, thus causing depression.[50] Arachidonic acid is a degradation product of 2- arachidonoyl glycerol (2-AG), which has been widely studied as an endogenous ligand of cannabinoid receptors in the brain and other mammalian tissues.[51] The results of this study suggest that the changes of metabolites, especially amino acid metabolism, may play an important role in the pathogenesis of AECOPD complicated with depression.

Strengths and Limitations

The strength of this study is that the metabolic changes of AECOPD patients with anxiety and depression and healthy control group are preliminarily explored through the serum metabonomics analysis, which is completely novel. This helps us to understand the pathogenesis of anxiety and depression in patients with AECOPD from a new perspective.

This study had several limitations. (1) This study is a single-center small sample study (n = 38), which has some limitations. Retrospective or prospective multicenter studies are needed in the future. (2) In this study, there are only two groups: AECOPD with anxiety and depression group and healthy control group. If we can include the relevant data of AECOPD without anxiety and depression group or stable COPD group, we can discuss the metabolic process of such patients more comprehensively. (3) The expression level of metabolites in serum is a comprehensive reflection of body metabolism. Considering that cell metabolism is highly flexible and varies with tissue of origin, environmental factors and diet, we cannot directly evaluate the exact metabolic changes in lung tissue and brain tissue from the results of serum analysis. (4) Our research aims to provide clues for the metabolic changes related to the occurrence and development of AECOPD complicated with anxiety and depression. The detailed molecular regulative mechanism in lung tissue and brain tissue needs to be further verified by more biological experiments before a final conclusion can be drawn.

Conclusion

Using an untargeted metabolomics approach based on comprehensive LC-MS, the present study demonstrated the differential expression of serum metabolites and several metabolic pathways in AECOPD patients with anxiety and depression in China population. These metabolites may be involved in the occurrence and development of AECOPD patients with anxiety and depression. The metabolic pathways with significant differences, including biosynthesis of unsaturated fatty acids, long-term depression and retrograde endocannabinoid signaling, may provide some clues for further study of the pathological mechanism of AECOPD patients with anxiety and depression.

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Disclosure

The authors declare that they do not possess any known conflicting financial interests or personal relationships that could have influenced the work reported in this manuscript.

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