Anxiety Disturbs Metabolome of Blood Plasma in Coronary Heart Disease Patients

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Research Article

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Abstract

Coronary heart disease (CHD) is the result of complex metabolic disorder caused by various environmental and genetic factors, and often comorbidity with anxiety. Anxiety was known to an independent risk factor for the adverse cardiovascular events and mortality in patients with CHD, while how stress-induced anxiety behavior impacts the metabolome of blood plasma in CHD patients and contributes to CHD worse is unclear. So this study aimed to investigate the effect of anxiety on metabolome of plasma in CHD patients. According to the inclusion and exclusion standard the blood plasma of CHD patients and CHD comorbidity with anxiety were collected after ethics approval. Metabolome analysis of blood plasma using liquid chromatography mass spectrometry (LC/MS) was performed, then multivariate data analysis was applied to evaluate the data. Disturbance of 39 plasma metabolites were altered in CHD patients accompany by anxiety patients as compared to control group. These disturbed metabolites were mainly involved in tryptophan metabolism, pyrimidine metabolism, glycerophospholipid metabolism, pentose phosphate metabolism, phenylalanine metabolism, pentose and glucuronate interconversions. The most significant pathway was tryptophan metabolism including the down-regulation of tryptophan and serotonin. In addition to tryptophan metabolism, the glycerophospholipids metabolism, pentose and glucuronate interconversions and pentose phosphate pathway were also greatly affected in this study. These results suggest that anxiety may further disturb CHD three translation of material metabolome. Besides theses metabolism pathway pyrimidine metabolism disturbed significantly which can aggravate the disease in patients with CHD. From these resoults the plasma metabolites monitoring was suggested to be recommended and may be conducive to early detect biomarkers to personalized treatment anxiety in patients with CHD in future.

Introduction

Coronary heart disease (CHD) is the leading cause of all health loss globally, as well as in each world region[1]. CHD is the result of complex metabolic disorder caused by various environmental and genetic factors[2], and often accompany by psychological disease in patients' lifelong time, such as anxiety[3,4]**.** Increasing data from clinics showed anxiety is an independent risk factor for the adverse cardiovascular events and mortality in patients with acute coronary syndrome (ACS)[5]. Anxiety correlated with elevated risks for quality of life, adverse outcomes and medical expenditure in patients with ACS. Anxiety may predict 12-month non-fatal myocardial infarction and cardiac rehospitalization[6]**.**

The mechanism of CHD comorbidity anxiety is complex and unclear. Previous evidences suggest that anxiety influence on neuroendocrine factors, platelet activation, inflammation, vascular endothelial dysfunction and so on[7]. Recent research found that changes in several metabolites, including certain amino acids, products of pyrimidine metabolism and the pentose phosphate pathway, were observed in ACS patients[2,8–10]. There are also metabolic disorders in anxiety[11,12] which aggravate coronary artery disease.

Metabonomics is an important part of system biology. Its research methods are systematic, dynamic and sensitive. It provides an effective research method for exploring the pathogenesis of diseases. Among the analytical platforms of metabolomics, liquid chromatography mass spectrometry (LC/MS) is supplied as a potential tool for identifying biomarkers for better risk classification and for understanding the pathophysiological of CHD and anxiety[13,14]. Meanwhile, LC-MS has increased the number of lipid classes that can be analyzed, separated and identified trace components of complex[15]. This study will reveal the whole functional state of the organism and the response rule to external stimulation by LC-MS detection of the changes of endogenous substances in patients, so as to search for biomarkers for coronary heart disease comorbidity anxiety disorder.

Materials And Methods

Study design and participants

The data were extracted from electronic medical record of Hunan Provincial People's Hospital according to inclusion criteria and exclusion criteria in 2019. This study's protocol was established according to the ethical guidelines of the Helsinki Declaration and was approved by the Human Ethics Committee of the First Affiliated Hospital of Hunan Normal University (approval NO.2018-20). The inclusion criteria for patients with coronary heart disease were (1) The diagnosis of coronary heart disease was in accordance with WHO diagnostic standard in 1999, and was confirmed as coronary heart disease by electrocardiogram, color doppler echocardiography and coronary angiography; (2) There was no anti-anxiety and depression drugs taking before; (3) The patients have normal reading and cognitive ability, and can cooperate to fill out the Hamilton Anxiety Scale (HAMA); (4) Hamilton Anxiety Scale-17 (HAMD-17) score ≤14; (5) Age ranges from ≥ 18 to ≤ 80 years old. (6) Resting blood pressure value ≤180/120 mmHg. The exclusion criteria were (1) Cardiac function grading of New York Heart Association(NYHA) grade ≥ Ⅲ grade; (2) Comorbidity with severe arrhythmia or severe cardiac dilatation; (3) Resting blood pressure value above 180/120 mmHg; (4) Comorbidity with diabetes or blood sugar still has not been improved after treatment; (5) Complicated with chronic infectious diseases, serious liver, brain, kidney and lung related diseases; (6) History of depression or anxiety, and used psychotropic drugs, alternative drugs or psychotherapy in the first four weeks or included in the electric shock treatment (ECT) eight weeks ago, substance abuse or dependence in the

first three months. (7) Severe anxiety or depression, cognitive impairment, nervous system disease, or other mental illness. (8) In recent 3 months patients have had or will have traumatic heart surgery. (9) Tumor patients. (10) Abnormal thyroid function.

In addition to the above diagnostic criteria for CHD, patients with CHD comorbid anxiety also met the criteria diagnostic criteria of Chinese classification and diagnostic criteria for mental disorders (3rd Edition), and the international HAMD-17 score≥14.

The data of patients contained detailed information including age, gender, diagnosis, body mass index(BMI), chronic diseases including hypertension (no/yes), or hyperlipidemia (no/yes), medical insurance (with/without) and employment status (employment/retirement), left ventricular ejection fraction(LVEF), N terminal pro B type natriuretic peptide(NT-proBNP), fasting blood glucose(FBS), triglyceride(TG), cholesterol(TC), low density cholesterol(LDL-C), routine blood test, hepatic and renal functions. These above data were extracted as baseline demographic characteristics, and were divided into two groups: CHD and CHD comorbidity anxiety group (HAMD-17 score≥14). The informed consent was obtained from all patients.

Collection, treatment and analysis of blood samples

Biochemical Assay

Blood was collected from veins, kept in heparinized tubes, and centrifuged at 3,000 × g at 4◦C for 5min to obtain plasma. Plasma neuropeptide Y(NPY) levels were measured by enzymelinked immunosorbent assay (CUSABIO, USA) according to instruction.

Chemicals

LC/MS-grade acetonitrile and HPLC-grade methanol were purchased from Merck (Darmstadt, Germany). Methanoic acid was purchased from CNW Company (Germany). All other chemicals were of analytical grade and were purchased from Sigma (St. Louis, MO, USA). Watson's distilled water was used.

Metabolome Analysis Using UPLC-QTOF-MS

Blood was collected as above, and the supernatant plasma was stored at −80◦C. All samples were then thawed for 15min and vortexed for 5s prior to analysis. A total of 200 µL of plasma sample was mixed with 600 µL of methanol, vortexed for 40s, and left undisturbed for 20min. After centrifugation at 12,000 × g for 15min at 4◦C, 600 µL supernatant dried in vacuum at room temperature, then the residue dissolved in 200 µL 50% acetonitrile, vortexed for 40 s, and centrifuged at low temperature and high speed (4 ℃, 12000 RPM / min) for 10 min. 100 µL supernatant was extracted for ultra performance liquid chromatography coupled with quadrupole time-of-flight tandem mass spetrometry (UPLC-QTOF-MS) assay.

UPLC-QTOF-MS Analysis (Impact II, Bruker) were used to analyze the samples. The chromatographic separation conditions and quality control (QC) procedures referred to the experimental method of Ren Yu et al[33]. The details were as follows:each sample (10µL) was injected into a Acclaim TM 120 C18 column (Themo Fisher, USA, 100 × 2.1mm, 2.2 µm) at 4◦C, and the following mobile phases were used at a flow-rate of 0.2mL/min at 30◦C: H_2O with 0.1% methane acid (A) and acetonitrile with 0.1% methane acid (B). Mass spectrometry analysis was performed in V flight tube detection mode with nitrogen as the atomization cone gas in both positive and negative ion modes. The source temperature was set at 200◦C, the extraction cone was at 4v, and a cone gas flow of 8.0 L/min was used in both modes. Capillary voltage was set at 4.0 and 3.5 kV, the sampling cone was 35 and 50 kV, the desolvation temperature was 350◦C and 300◦C, respectively, and the desolvation gas flow was set at 600 and 700 L/h, respectively. Mass spectrometric data was collected in centroid mode from 20 to 1,000 m/z.

The peak height for the internal standard was continuously monitored during analysis to ensure signal stability. Quality control (QC) procedures were used to validate the methods and to ensure stability. 50 µL QC samples prepared by pooling identical volumes of individual plasma sample. To ensure that the system was suitable for use, 6 pooled QC samples were run prior to analysis in each ion mode. Six ions (min_m/z) were selected to evaluate the relative standard deviation (RSD) of retention time, m/z, and peak area.

The scatter plot of the first principal component is shown in Figure 5. The results show that all QC samples are distributed within the scope of 2SD , indicating that the consistency of experimental operation and the stability of the instrument system are within the controllable range.

Identification of Metabolites

The UPLC-QTOF-MS data were imported into the Metaboscape version 3.0 analysis software (Bruker company), and the positive and negative Brooke matrix tables were established respectively to detect and align the peaks of all samples. The parameters were set as follows: the chromatographic peaks with retention time of 0-30min were intercepted, the peak intensity threshold was 1000, the minimum peak length was 5 spectra, the peaks were screened according to the 80% rule, and the mass spectrum data were corrected with sodium formate. After being identified and aligned, we normalized the strength of each ion with the total strength of the total ions in each chromatogram. We used the standard database, HMDB database and online search database of Bruker company to identify the secondary mass spectra and get the corresponding compounds. Finally, the three-dimensional matrix information includes retention time(RT), mass charge ratio (M/Z), ionic strength information (variables) and compound name. In addition, the Kyoto encyclopedia of genes and genomes (KEGG, http://www.genome.jp/kegg/) biochemical database was used to interpret possible pathways involving the identified metabolites.

Statistical Analysis

The continuous variables were expressed as means ± standard errors of the mean, compared by Student's t-test before the Levene test to ensure the equality of variances, otherwise using wilcoxon rank-sunm test. Categorical variables were expressed as numbers and percentages, compared by Chi-square test. All statistical analysis was performed with SPSS 24.0 software; significant differences were indicated by $p < 0.05$.

Mass Profile software was used for peak extraction, retention time (RT) alignment, peak alignment, and deconvolution analysis. Finally, data were imported into SIMCA-P software (v14.0, Umetric, Umea, Sweden) for principal component analysis(PCA) and orthogonal partial least squares discriminant analysis(OPLS-DA). Variable Importance in Projection (VIP)> 1 and $p < 0.05$ were considered statistically significant.

Results

Baseline characteristics of enrolled patients

A total of the 260 patients in cardiology department have been screened, and 47 cases met the enrolled criteria. According to the inclusion and exclusion standard CHD comorbidity with anxiety group was enrolled 21 patients, and CHD group was enrolled 26 patients. There were no statistically significant difference (P value≥0.05) except HAMD-17 score in baseline data between the two groups. Demographic information about all participants was shown in Table 1.

Metabonomics results about UPLC-QTOF-MS

Base peak chromatogram (BPC) under positive and negative ion conditions

The original data were drawn with Origin 2017, and the chromatogram as shown in Figure 1 was obtained. It can be roughly seen that the characteristics of metabolites under the two modes are basically similar, but the responsivity is different. A total of 415 positive ions and 420 negative ions were identified by HMDB database and PubChem database.

Principal Component Analysis(PCA)

Unsupervised principal component analysis (PCA) was performed to provide an overview of the LC-MS data. PCA score plots are shown in Figure 2. Significant differences are observed between the CHD and anxiety group in both ion modes for plasma samples.

Orthogonal partial least squares discriminant analysis(OPLS-DA)

The orthogonal partial least squares-discriminate analysis (OPLS-DA) model was constructed. The key model parameters are summarized in Figure 3(A,B). Results for different groups were visualized as score plots to show group clusters. S-plots were used to identify variables that contributed to the classification. Good separation between the groups was observed. Measures of the quality of the resulting discrimination, including the values of R2X, R2Y, and Q2, are shown in Figure 3(C,D). The values of R2 and Q2 were > 0.5, indicating good fitness and prediction.

Anxiety Disturbs Metabolome in CHD

Differentially-expressed metabolites were identified based on the variable VIP with a threshold of 1.0 from the OPLS-DA model and p-value < 0.05 in the t-test. Metabolites were identified based on mass assignment and identified ion (m/z), retention time (RT), and were then compared

with authentic standards or database resources, such as KEGG (http://www.genome.jp/kegg/) and METLIN (http:// metlin.scripps.edu). Among thousands of metabolites, 39 molecules in plasma were significantly correlated with depression in CHD(Table 2). Specifically, in the plasma of anxiety comorbidity with CHD group, levels of 3-alpha-Androstanediol glucuronide, serotonin, 3-hydroxycapric acid, 25-hydroxyvitamin D2, androsterone sulfate, 5a-tetrahydrocorticosterone, 5-androstenetriol, beta-alanine, 4-hydroxycyclohexylcarboxylic acid, tryptophan, gamma-glutamylthreonine, 5-hydroxydantrolene, 3-carbamoyl-2-phenylpropionaldehyde, 4-hydroxynonenal, alpha-carboxyethyl hydroxychroman(alpha-CEHC), aminoethoxyacetic acid, isobutyryl-L-carnitine, phosphoribosyl pyrophosphate and phosphorylcholine were decreased, meanwhile levels of lysoPC, oleoylcarnitine, 19-hydroxyandrost-4-ene-3,17-dione, tetrahydrocortisone, 17-hydroxypregnenolone sulfate, 11-oxo-androsterone glucuronide, 7-methylguanine, deoxycholic acid 3-glucuronide, glycerophosphocholine, hippuric acid, L-tryptophan, thromboxane B2, trans-aconitic acid were increased. These data suggest that changes mainly occurred in the following 7 metabolic pathways in anxiety comorbidity with CHD group (Figure 4): tryptophan metabolism, glycerophospholipid metabolism, pentose phosphate pathway, pyrimidine metabolism, and pentose and glucuronate interconversion.

Discussion

The baseline of the two groups included in this study were basically the same(shown in Table 1). In consideration of physiological roles of Neuropeptide Y(NPY) on blood pressure, atherogenic processes and anxiety[16,17], the level of NPY was detected. Our results found the concentration of NPY were increased slightly in coronary heart disease with anxiety group. However, there was no significant difference between the two groups. From this point we conclude neuropeptide Y can not distinguish anxiety disorder patients from coronary heart disease patients [18] .

By LC-MS metabonomics and OPLS-DA analysis 39 metabolites disturbed in plasma between two groups(shown in Table 2), involving in multiple pathways(shown as Figure 4). The most significant pathway was tryptophan metabolism including the down-regulation of tryptophan and serotonin, which were closely related. The starting point of this pathway is tryptophan. As the only precursor of serotonin, when its consumption increases in the central nervous system, the serotonin level and activity in the brain will enhance accordingly[19]. However, the abnormal decrease of plasma tryptophan content, such as the occurrence of acute tryptophan depletion (ATD), will lead to anxiety behavior. ATD can cause the increase of sympathetic nerve activity, while the decrease of parasympathetic nervous system activity. These effects are positively correlated with the anxiety score of patients[20]. Serotonin is a monoamine that acts as a neurotransmitter and neuromodulator, affecting cognitive and emotional abilities[21]. By the study of gene expression and transporter activity, it has been proved that the destruction of serotonin system is related to a variety of mental diseases, and serotonin is the key component of anxiety performance[22].

Inflammation increases platelet activation, which has been shown to play an important role in thrombosis and myocardial ischemia[23] . The 5-hydroxytryptamine has been shown to increase platelet aggregation, because anxiety has been associated with 5-hydroxytryptamine system abnormalities[24], and increased cardiac events. Serotonin binds 5-hydroxytryptamine-2 (5ht-2) receptor on platelets and precipitates factors that enhance platelet aggregation. In healthy blood vessels, nitrous oxide prevents thrombosis by releasing into the endothelium and subsequent vasodilation. However, when atherosclerotic diseases damage endothelial cells, blood vessels are unable expand properly, and exposure to 5-hydroxytryptamine will resulted in vasoconstriction. This may be the underlying mechanism of the association between increased serotonin blood levels and cardiac events in coronary heart disease[11]. In this study thromboxane B2 metabolism was increased in anxiety group. This suggests that there may be abnormalities of platelet serotonin (5-HT) receptor, which enhances the response of platelets to 5-HT, and the release of 5-HT promotes platelet aggregation. In addition, the imbalance of thromboxane A2 prostacyclin can cause vasoconstriction, which can further promote the occurrence of heart thrombosis.

In addition to tryptophan metabolism, the metabolism of glycerophospholipids were also greatly affected in this study. It was found that phosphatidylcholine was down-regulated, but glycerophosphate choline was up-regulated in CHD comorbidity with anxiety group. Our study shows severn compounds belongs to glycerophospholipid metabolism pathway. These substance including lysoPC (16:0), lysoPC (15:0), lysoPC (20:5 (5Z, 8Z, 11Z, 14Z, 17Z), lysoPC(22:6(4Z,7Z,10Z,13Z,16Z,19Z)), lysoPC(18:0), lysoPC(18:1(11Z)), and lysoPC (22:5 (4Z, 7Z, 10Z, 13Z, 16Z)) were all up-regulated. In mice, dogs and other animal experiments, phospholipid metabolism disorders are also common. Puurunen and other non-target LC-QTMF-MS were used to analyze the whole blood samples of frightened and anxious dogs. It was found that under the same diet control, 6 glycerophospholipid metabolism disorders decreased in the anxiety group [25,26]. Robert et al. analyzed the plasma phospholipid spectrum of 31 mice models with LC and hydrophilic interaction liquid chromatography and high-resolution mass spectrometry, and found that the most obvious up-regulation was phosphatidylinositol (PE) and lysophosphatidylethanolamine (LPE) [11]. Although these phospholipids are different from the phospholipid compounds in this study, they all provide theoretical support for the hypothesis that membrane lipids play a key role in anxiety related diseases at the animal. As a component of cell membrane, lipids determine the location and function of various receptors, which may be useful biomarkers in the analysis of emotions. However, in the metabonomics study of coronary heart disease diagnosis, it was also observed that phosphatidylethanolamine ceramide was the biomarker of its diagnosis[27]. Therefore, more

clinical studies are still needed to determine more accurate phospholipid compounds, which may be helpful to identify anxiety patients with coronary heart disease. The lipid metabonomics may also be a valuable direction for anxiety research in the future.

Hyperlipidemia is closely related to stress and anxiety[28]. Sympathetic activation in generalized anxiety disorder increases lipoprotein lipase activity through the release of adrenaline and corticosteroids[11]. This hyperactivity in lipoprotein lipase results in an increase in free fatty acids, which can be converted into cholesterol and triglycerides. However this results indicate that 19-hydroxy-androsterol-4-ene-3, 17-dione and tetrahydrocortisone were down-regulated, which means steroid hormone biosynthesis and cortisone-metabolites decreased in CHD comorbidity with anxiety. The low baseline cortisol has been reported in people at risk for developing Post-Traumatic Stress Disorder (PTSD), and low corticosterone response to stressful and anxiogenic stimuli[29]. Our study also proved that anxiety caused by long-term stress can reduce the corticosterone response in patients with coronary heart disease.

In this study phosphoribosyl pyrophosphate and β-alanine were down- regulated, while 7-methylguanine and hippuric acid were up-regulated. Phosphoribosyl pyrophosphate is an important metabolic intermediate in the pentose phosphate pathway. The pentose phosphate pathway is a vital pathway for oxidative decomposition of glucose. Its function is not to produce ATP, but to produce special substances with important physiological functions, such as NADPH and 5-ribose phosphate. The pentose phosphate pathway effects on the metabolism in ischemic heart disease[30]. It is also involved in the de novo and remedial synthesis of purine, pyrimidine nucleotides, and some amino acids such as tryptophan. These substances all promote progression of atherosclerosis .

In addition to tryptophan metabolism, phospholipid metabolism, pentose phosphate and steroid hormone biosynthesis disturbance related compounds, 25-hydroxy vitamin D2 showed down-regulated 1.82 times. The greatly decline of vitamin D2 has been reported to be related to anxiety. Supplementation of vitamin D to rats helps regulate and protect dopamine system, and plays the role in anti-anxiety[31,32]. Therefore, 25-hydroxy vitamin D2 supplementation maybe help to reduce anxiety. Of course, this study was an exploratory study based on metabonomics research of CHD comorbidity with anxiety in a small sample size, and these differential metabolites between two groups need to be further verified in vivo and in vitro.

Conclusions

There are notable metabolic differences in patients comorbid anxiety with CHD compared with CHD patients alone in our study. These small-molecule metabolites mainly attribute to tryptophan metabolism, steroid hormone biosynthesis, pyrimidine metabolism, phenylalanine, the glycerophospholipids and pentose phosphate metabolism pathways. Identification of these biomarkers and pathay will help to unravel the molecular mechanism of comorbidity anxiety of coronary heart disease, facilitate the early diagnosis, accurate disease classification and personalized treatment of these patients.

Declarations

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Author contributions statement

Hongyan Wei and Boyu Tan conceived and designed the projects. Yimin Zhu, Nan Deng, Junyuan Gu, HonglianZou, Jing Wu and Xueyao Jiang performed the experiments and carried out data analysis. Boyu Tan and Junyuan Gu drafted the article, Hongyan Wei and Yimin Zhu finalized the paper and provided suggestions to improve it. All authors reviewed the manuscript.

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Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethics statement

The experiment protocol was approved by the Medical ethics committee of Hunan Provincial People's Hospital / First Affiliated Hospital of Hunan Normal University (Experiment License: 2018-20).

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Tables

Table 1. Demographic information about all enrolled participants between CHD and angxiety comorbidity CHD group.

Groups	Angxiety comorbidity CHD(21)	CHD group(26)	*P-value*
Age(year),mean(SD)	62.73(10.45)	62.4(8.23)	0.5
Male, n(%)	13(59.09	12(48.00)	0.57
Medical insurance, n(%)	12(54.54	13(52.00	1.00
Long-term smoking, n(%)	11(52.38)	10(38.46)	0.56
Being employed, n(%)	9(40.91)	14(56.00)	0.48
Hypertension, n(%)	17(77.27)	18(72.00)	0.75
Hyperlipidemia, n(%)	6(27.27)	8(32.00)	0.76
BMI, mean(SD)	18.95(9.33)	17.37(11.43)	0.11
LVEF, mean(SD)	60.43(14.43	53.25(14.33)	0.27
NT-proBNP, mean(SD)	850(192.20)	699.3(109.30)	0.08
WBC($\times 10^9$/L), mean(SD)	4.83(2.37)	12.04(22.05)	0.05
HB(g/L), mean(SD)	119.33(40.00)	112.57(38.65)	0.57
PLT($\times 10^9$/L), mean(SD)	177.73(65.01)	219(71.92)	0.61
FBS (mmol/L), mean(SD)	5.50(2.06)	4.91(3.01)	0.08
TC(mmol/L), mean(SD)	3.45(1.99)	4.17(1.48)	0.42
TG(mmol/L), mean(SD)	2.13(3.49	1.60(1.14	0.08
LDL-C(mmol/L), mean(SD)	1.83(1.05	2.89(1.16	0.62
Bun(mmol/L), mean(SD)	6.11(3.42)	5.16(1.65)	0.21
Cr(umol/L), mean(SD)	86.60(49.08)	71.43(24.58)	0.24
ALT(u/L), mean(SD)	21.48(9.58)	34.94(8.30)	0.22
TP(g/L), mean(SD)	60.96(5.17)	61.98(4.76)	0.59
ALB(g/L), mean(SD)	38.318(2.43)	38.53(3.12)	0.36
NPY(ng/mL), mean(SD)	10.36(2.63)	9.45(2.45)	0.18
HAMD-17 score, mean(SD)	22.93(5.28)	6.82(3.12)	0.00

SD, standard deviation

Table 2 Metabolites and pathways in plasma samples that differed between the anxiety comorbidity with CHD and CHD group

Metabolites	Ionization mode	VIP	RT(min)	m/z	Fold change (A/C)	*p*-value	Metabolic pathway	KEGG ID
3-alpha-Androstanediol glucuronide	ESI-	1.06	24	467.27	-2.41	0.00		
Serotonin	ESI-	2.14	15.85	175.28	-2.19	0.00	Tryptophan metabolism	C00780
3-Hydroxycapric acid	ESI-	1.37	22.75	187.15	-2.02	0.00		
25-Hydroxyvitamin D2	ESI-	1.87	22.39	411.33	-1.82	0.00		
Androsterone sulfate	ESI-	1.51	22.16	367.18	-1.80	0.04		
5a-Tetrahydrocorticosterone	ESI-	1.42	22.36	349.22	-1.54	0.00		
5-Androstenetriol	ESI-	3.16	24.27	305.20	-1.31	0.00		
beta-Alanine	ESI-	1.22	1.60	88.04	-1.13	0.01	Pyrimidine metabolism	C00099
4-Hydroxycyclohexylcarboxylic acid	ESI-	1.73	1.61	143.08	-1.12	0.03		C04404
Tryptophan	ESI-	1.94	1.61	101.05	-1.09	0.03	Tryptophan metabolism	C00078
gamma-Glutamylthreonine	ESI-	4.33	1.61	123.04	-1.08	0.01		
5-Hydroxydantrolene	ESI-	3.45	1.63	164.01	-1.049	0.00		
[a]LysoPC(20:5(5Z,8Z,11Z,14Z,17Z))	ESI-	1.74	20.74	540.33	0.82	0.00	Glycerophospholipid metabolism	C04230
Oleoylcarnitine	ESI-	5.00	31.49	424.33	0.75	0.01		
19-Hydroxyandrost-4-ene-3,17-dione	ESI-	1.08	22.43	301.1661	0.73	0.01	Steroid hormone biosynthesis	C05290
Tetrahydrocortisone	ESI-	1.63	24.32	363.20	0.69	0.01		
[a]LysoPC(16:0)	ESI-	1.18	21.01	478.29	0.64	0.00	Glycerophospholipid metabolism	C04230
[a]LysoPC(15:0)	ESI-	1.16	23.02	480.3096	0.64	0.00	Glycerophospholipid metabolism	C04230
[a]LysoPC(22:6(4Z,7Z,10Z,13Z,16Z,19Z))	ESI-	1.61	21.4	566.35	0.60	0.00	Glycerophospholipid metabolism	C04230
17-Hydroxypregnenolone sulfate	ESI-	1.38	23.92	411.21	0.45	0.00		
11-Oxo-androsterone glucuronide	ESI+	3.79	9.95	481.26	0.10	0.02		
3-Carbamoyl-2-phenylpropionaldehyde	ESI+	1.14	0.27	194.12	-3.40	0.00		
4-Hydroxynonenal	ESI+	2.99	9.25	139.08	-1.88	0.02		
7-Methylguanine	ESI+	1.45	1.54	83.54	0.48	0.03		C02242
alpha-Carboxyethyl hydroxychroman	ESI+	2.93	22.4	301.14	-1.43	0.00		
Aminoethoxyacetic acid	ESI+	1.53	6.89	120.08	-2.06	0.00		
beta-Alanine	ESI+	1.15	25.74	90.09	-1.36	0.03	Pyrimidine metabolism	C00099
Deoxycholic acid 3-glucuronide	ESI+	3.28	10.34	569.31	0.08	0.03	Pentose and glucuronate interconversions	C03033
Glycerophosphocholine	ESI+	2.12	1.76	280.10	0.735	0.01	Glycerophospholipid metabolism	C00670
Hippuric acid	ESI+	1.65	1.52	90.52	0.70	0.01	Phenylalanine metabolism	C01586
Isobutyryl-L-carnitine	ESI+	1.10	7.2	232.15	-2.26	0.04		
L-Tryptophan	ESI+	1.63	12.56	205.13	0.66	0.00	Glycine, serine and	C00078

							threonine metabolism	
[a]LysoPC(18:0)	ESI+	2.41	23.63	524.37	0.39	0.00	Glycerophospholipid metabolism	C04230
[a]LysoPC(18:1(11Z))	ESI+	2.92	21.39	544.34	0.81	0.01	Glycerophospholipid metabolism	C04230
[a]LysoPC(22:5(4Z,7Z,10Z,13Z,16Z))	ESI+	1.14	20.3	592.34	0.69	0.01	Glycerophospholipid metabolism	C04230
Phosphoribosyl pyrophosphate	ESI+	1.06	1.62	390.99	-1.74	0.00	Pentose phosphate pathway	C00119
Phosphorylcholine	ESI+	2.23	8.19	207.11	-1.49	0.00	Glycerophospholipid metabolism	C00588
Thromboxane B2	ESI+	3.71	9.46	393.21	0.12	0.03		C05963
trans-Aconitic acid	ESI+	1.85	0.08	88.02	0.23	0.03	C5-Branched dibasic acid metabolism	C02341

[a]*LysoPC* lysophosphatidylcholine

Figures

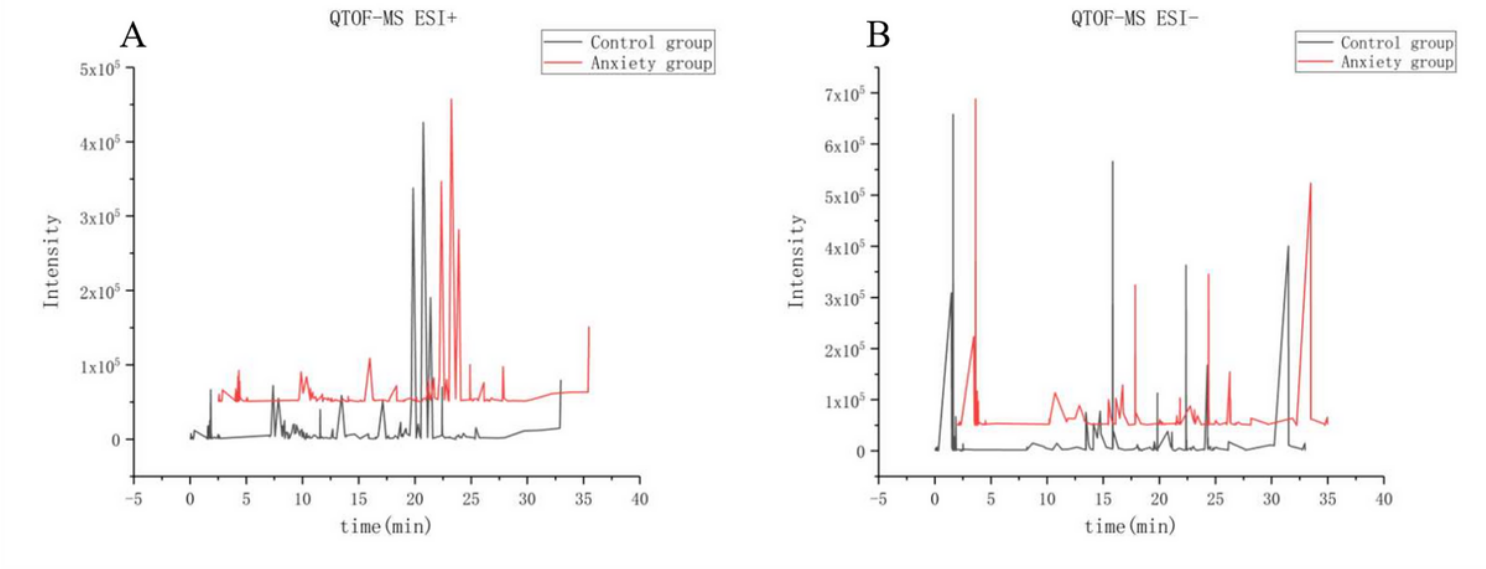

Figure 1

Representative LC-Q/TOF-MS analysis of total ion chromatograms for plasma. (A,B) Plasma samples in positive and negative mode.

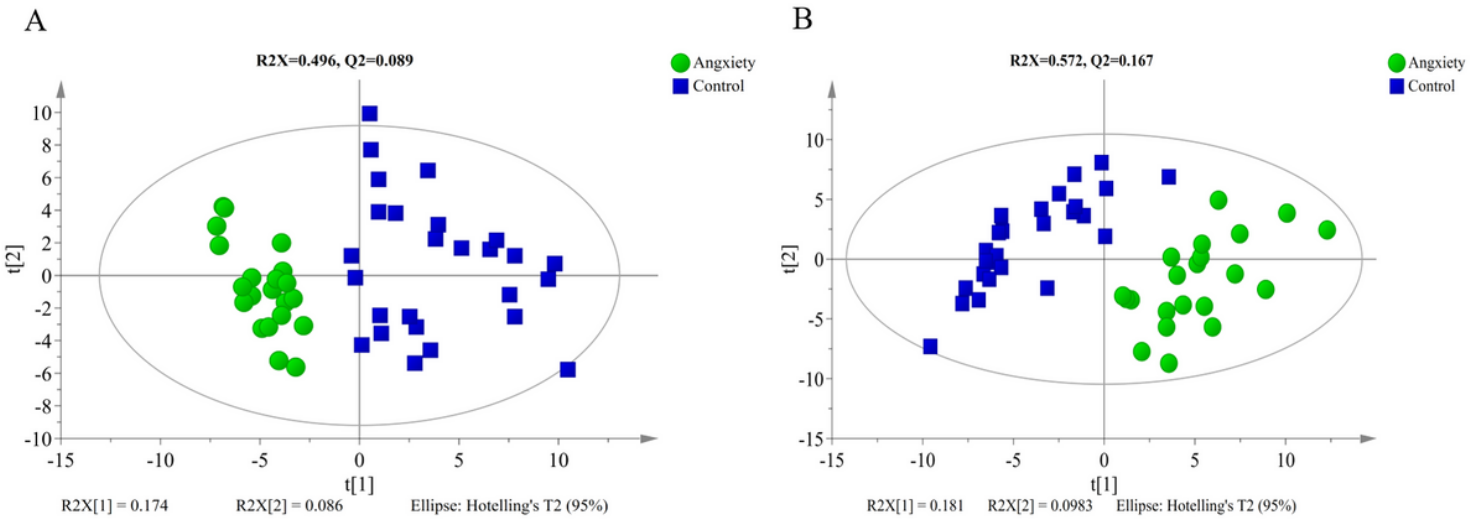

Figure 2

PCA score plots derived from LC-MS analysis of plasma from angxiety and control group . (A) Plasma in positive ion mode, (B) plasma in negative ion mode.

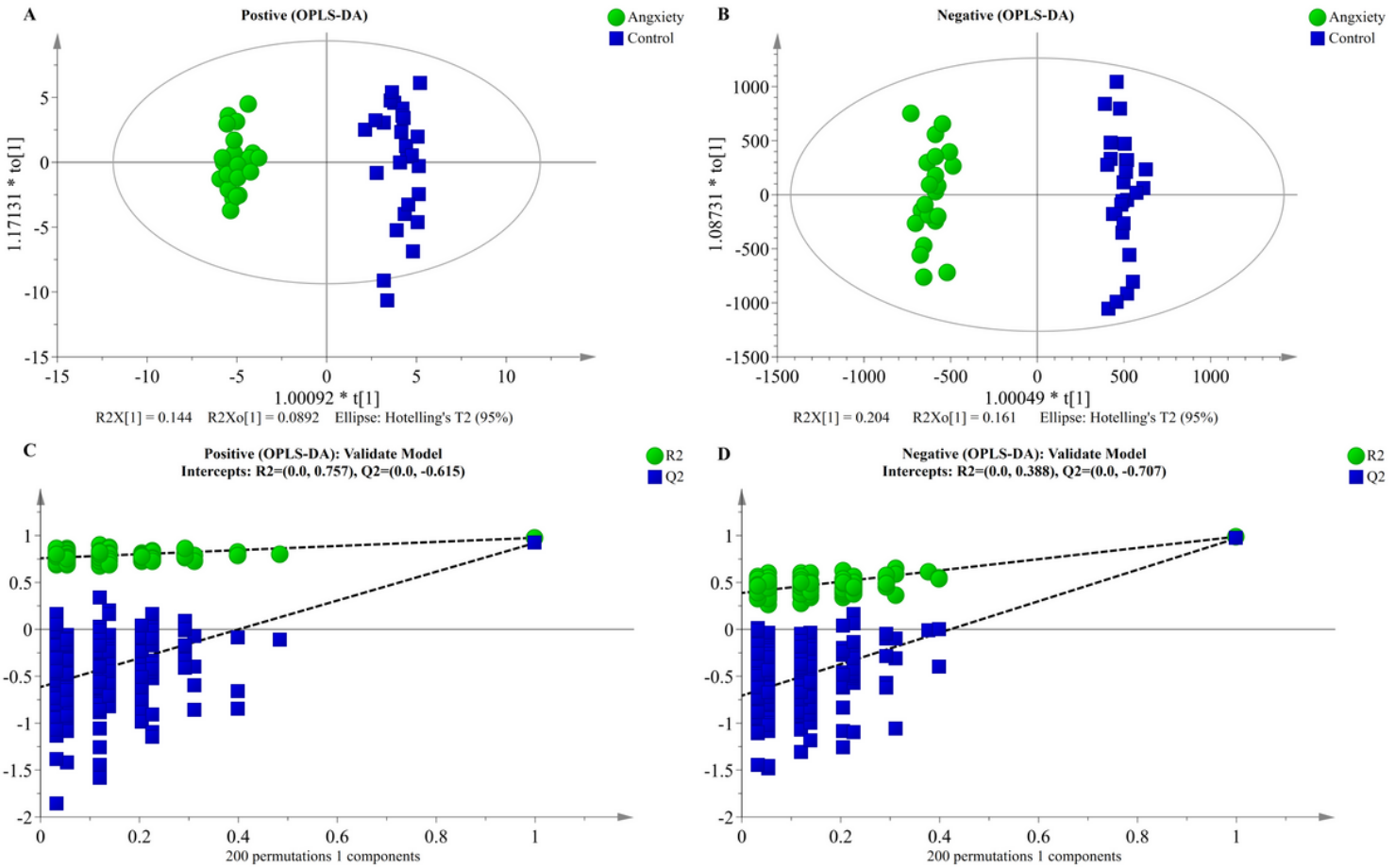

Figure 3

The OPLS-DA score plots obtained from the two groups in positive ion mode with R2Y=0.951, Q2=0.873 (A)in positive ion mode with R2Y=0.99, Q2=0.956 (B).

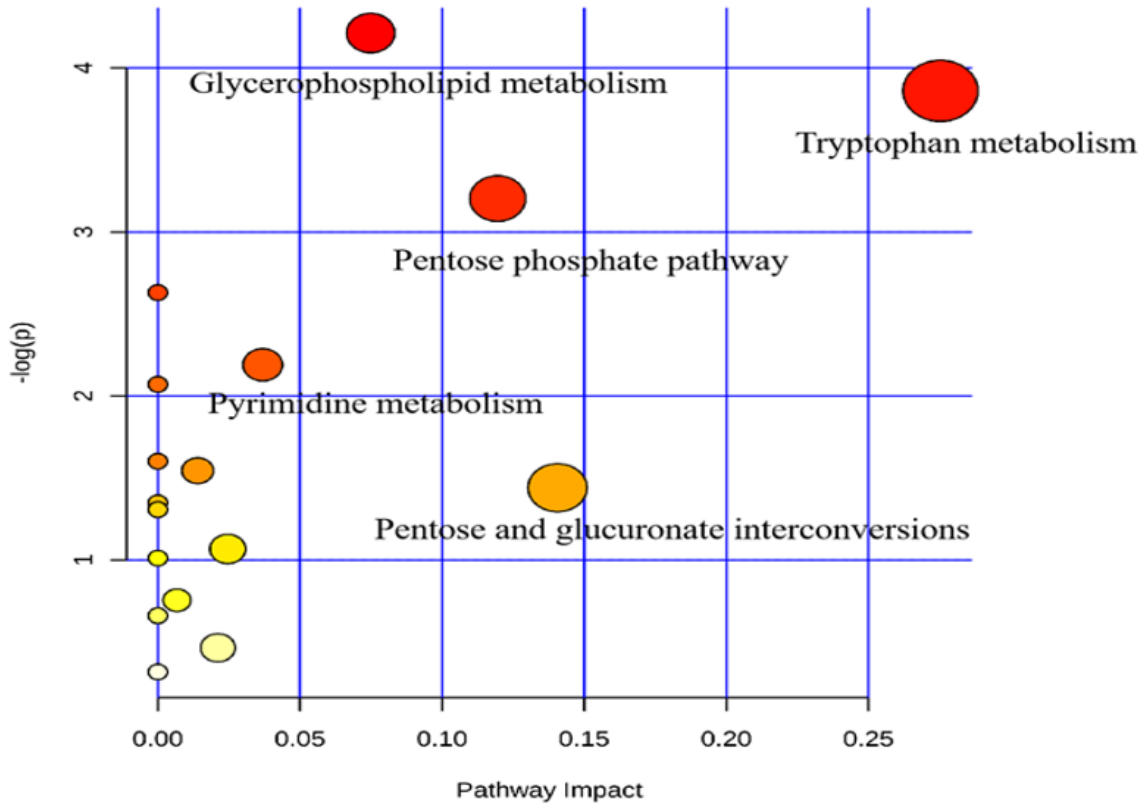

Figure 4

The differential metabolites of plasma involved in main pathways between two groups.

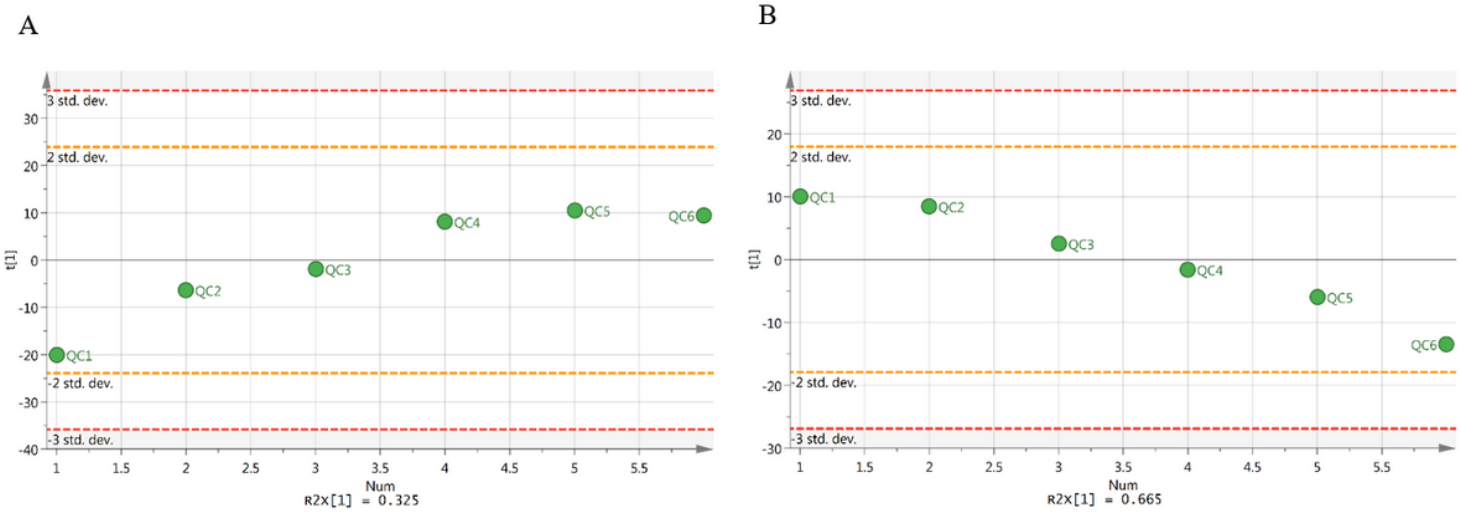

Figure 5

The first main dispersion point diagram of QC sample using UPLC-QTOF-MS method.（A. positive mode; B. negative mode)