Transformations in Plasma Metabolic Profiles of Patients with Major Depression During Treatment: A Pilot Metabolomics Study

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Abstract

Background Major depression disorder (MDD) is a mental disease that seriously endangers human physical and mental health. The purpose of present study is to detect the differences of plasma metabolic profiles between MDD patients and healthy controls. Moreover, the hospitalization process of MDD patients was followed to explore the reversal of metabolic abnormalities in MDD patients by conventional treatment in the form of self-control.

Methods Ultra-Performance Liquid Chromatography- Mass Spectrometry (UPLC-MS) was used to detect the metabolic profiles in 47 plasma samples from 12 controls and 12 MDD patients. Multivariate statistical analysis and K-means clustering were operated to search for significantly different metabolites (SDMs) between pair-comparison groups and specific metabolites (SMs) with ideal variation trend in relative content. Finally, the metabolites were integrated into Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways to preliminarily explore the potential mechanism of MDD disrupting the metabolic process.

Results There were significant differences in plasma metabolic profiles between healthy controls and MDD patients. A total of 14 SDMs between untreated MDD patients and healthy controls were classified into the top ten KEGG pathways enrichment, among which the relative contents of 4 SMs, 9-HPODE, imidazoleacetic acid, thromboxane B2 (TXB2), and arachidonic acid (AA) showed a regular variation trend after MDD patients' treatment. A new metabolite-pathway network containing 4 SMs and 8 pathways was accessed after further integration analysis. The sample size calculation showed that a verification set of 84-135 subjects (containing healthy controls and MDD paients) was desired to confirm the results of this study.

Conclusion The results indicate that the transition in metabolic pathways during the occurrence and treatment process of MDD is mainly dominated by transformations in lipid metabolism and its relevant signaling pathway system. Additionally, histidine metabolism is also engaged. Subsequent large-scale validation study is acquired to evaluate whether the selected metabolites have the potential to diagnose and access the therapeutic effect of MDD, and to explore the probable mechanism of MDD in combination with other technologies.

Background

Major depression disorder (MDD) is a mental disease that not only seriously damages health and curtails the quality of human life, but also causes countless tragedies. Although a slight difference was between high-income countries and middle-income countries, the overall 12-month prevalence of MDD has reached 6%(1). In a systematic analysis of global burden of disease in 2015, depression ranked as one of the top 10 causes of disability in humans, especially among adolescents and middle-aged adults (2).

Metabolomics can provide complementary information on genomic and proteomic strategies, reflecting the results of complex biochemical networks, and thus generating basic information about the underlying

biological state of the system(3). It is increasingly used in the analysis of depression. Previous studies have shown that depression is noticeably related to transformations in metabolism of the body, including lipid metabolism, tryptophan–kynurenine pathway, fatty acid metabolism and phospholipid metabolism(4–6). And some metabolites such as oxalic acid and stearic acid which have the potential to be biomarkers may provide reference for the diagnosis and prediction(7).

In present study, Ultra-Performance Liquid Chromatography - Mass Spectrometry (UPLC-MS) was used to conduct extensive targeted metabolomics analysis based on a self-built database to scan differential metabolites in plasma, and to integrate and analyze the pathways correlated with MDD. In addition, MDD patients were followed during hospitalization, and more information on development in plasma metabolites was gleaned by self-control study before and after treatment. We aimed to explore the metabolic transformations in MDD patients under the real treatment state without intervention, and preliminarily search the potential mechanism of MDD changes in metabolism. This pilot study also laid a groundwork for further research.

Materials And Methods

Study design

12 controls and 12 MDD patients were enrolled in this study. Patients were followed up for two months with routine treatment in the hospital. The details were represented in flow diagram (Figure 1).

A total of 47 plasma samples were used for metabolomic analysis. During hospitalization, 12 MDD patients were followed up and sampled once a month. The samples were divided into three groups according to treatment time: 0m (untreated after admission, n=12), 1m (treat for one month, n=11), 2m (treat for two months, n=12). Only one blood sample was collected from each healthy control (n=12).

Inclusion and exclusion criteria of subjects

This study was approved by the Ethics Committee of Huazhong University of Science and Technology ([2013] IEC(S007)). Informed consent of the participants was obtained after the nature of the procedures had been fully explained. The study was carried out in accordance with the principles of the Helsinki Declaration as revised 1989.

The criteria for selection for patients in the MDD group were:1) patients who meet the diagnostic criteria for depression in the third edition of the Chinese Classification and Diagnostic Standard for Mental Disorders (CCMD-3) and obtain their informed consent ; 2) Hamilton Depression Rating Scale-17(HDRS-17) was higher than 25; 3Patients aged between 18 and 60.

The exclusion criteria were:1) Suffering from any other disease, especially neurological and psychiatric diseases, hepatobiliary diseases; 2) Upon review of pre-study laboratory data and thorough physical

examination, clinically significant abnormalities were found; 3) Had received any investigational drug within the past 3 months (prior to the first dosing in this study); 4) Consuming more than 28 units of alcohol per week; 5) Smoking more than 5 cigarettes (or equivalent amount of tobacco) per day; 6) HBsAg positive; 7) Other conditions considered ineligible by the researcher (e.g., infirm, etc.).

The inclusion and exclusion criteria of control group were the same as for the MDD group, except the HDRS-17 being ≤ 7.

Plasma sampling

Residual blood analysis was performed on a routine monthly sample of MDD patients with normal medical behavior during a two-month hospital stay. The collected blood samples were immediately centrifuged at 3000r/min for 10min, and the plasma was separated and stored at -80℃ for analysis.

Sample was thawed on ice, vortex for 10 seconds, and 300 μl of pure methanol was added into 50 μl of plasma, whirl the mixture for 3 min and centrifuge it with 12,000 rpm at 4 ℃ for 10 min. Then collect the supernatant and centrifuge it at 12,000 rpm at 4 ℃ for 5 min. Leave in a refrigerator at -20 ℃ for 30 min, centrifuge at 12000 r/min at 4 ℃ for 3 min, and take 150 μl of supernatant in the liner of the corresponding injection bottle for on-board analysis.

Plasma metabolome analysis

The sample extracts were analyzed using an UPLC-MS system (UPLC, ExionLC AD，https://sciex.com.cn/; MS, QTRAP® System, https://sciex.com/). The column was an ACQUITY UPLC HSS T3 C18 (1.8 μm, 2.1 mm*100 mm, Waters, Milford, MA, USA), column temperature was 40 ℃. Mobile phase A was water (containing 0.1% formic acid) and mobile phase B was acetonitrile (containing 0.1% formic acid). The gradient elution procedure is set as follows: 95:5 V/V at 0 min, 10:90 V/V at 10.0 min, 10:90 V/V at 11.0 min, 95:5 V/V at 11.1 min, 95:5 V/V at 14.0 min.

For MS, Linear Ion trap (LIT) and triple quadrupole (QQQ) scans were acquired on a triple quadrupole-linear ion trap mass spectrometer (QTRAP). The QTRAP® UPLC-MS system equipped with an electrospray ionization (ESI) Turbo Ion-Spray interface, operating in positive and negative ion mode. The ESI source operation parameters were as follows: source temperature 500 ℃; ion spray (IS) voltage 5500 V (positive), -4500 V (negative); ion source gas I, gas II, curtain gas (CUR) were set at 55, 60, and 25.0 psi, respectively; the collision gas (CAD) was high. Instrument tuning and mass calibration were performed with 10 and 100 μmol/L polypropylene glycol solutions in QQQ and LIT modes, respectively. A specific set of multiple reaction monitoring (MRM) transitions were monitored for each period according to the metabolites eluted within this period.

In addition, quality control (QC) was prepared by equal mixing of extracts from each sample. During the process of instrumental analysis, one quality control sample is inserted every 10 samples to be tested,

and the total ion chromatogram (TIC) of the essential spectrum detection and analysis of different quality control QC samples was analyzed by overlapping analysis. If the curves of total ion flow detected by metabolites have a high overlap, indicated that retention time (RT) and peak intensity are consistent, then the stability of the instrument can be considered acceptable and the repeatability and reliability of the data can be guaranteed.

Data analysis

Based on the self-established local database, the extracted ion chromatogram (XIC) of each sample was preliminarily processed by Analyst 1.6.3 (AB SCIEX LLC, Framingham, MA, USA) to obtain the basic information of detectable substances in the sample, including RT and characteristic ion signal strength. Multiquant software was used for the integration and correction of chromatographic peaks, and the peak area represented the relative content of the corresponding substance.

Differential metabolite analysis

Principal component analysis (PCA) and orthogonal partial least squares-discriminant analysis (OPLS-DA) were used for preliminary analysis of the original data. PCA is a multi-dimensional statistical analysis method for unsupervised pattern recognition, which is helpful to preliminarily understand the total metabolic differences and the variation degree between samples within the group. OPLS-DA is a supervised analytical method combined with partial least squares-discriminant analysis (PLS-DA) and orthogonal signal correction (OSC), which can remove irrelevant differences to better screen differential variables. The variable-importance in projection (VIP) of the OPLS-DA model is analyzed and combined with P-value or fold change of univariate analysis, differential metabolites between two groups were selected. Next, Kyoto Encyclopedia of Genes and Genomes (KEGG, https://www.kegg.jp/) database was used to annotate the differential metabolites to analyze the potential metabolite-related pathways.

Moreover, the relative contents of SDMs were standardized and normalized, then K-means clustering was used to analyze the variation trend of the relative contents of differential metabolites in plasma of each group, and to find out the variation pattern of differential metabolites that may be related to the occurrence and development of MDD. Finally, spearman correlation analysis was conducted to explore the relationship between these significantly different metabolites and MDD severity, and the related metabolites and KEGG pathways were integrated into a new MDD-related metabolic mechanism network.

Statistical analysis

R software (version 3.5.0, www.r-project.org/) was used for PCA analysis. ComplexHeatmap (R) (version 2.2.0) was used for heatmap producing. MetaboAnalystR (R) (version 1.0.1) was used for OPLS-DA analysis.

The statistical analyzes were performed using SPSS (version 5.0, IBM, Armonk, NY, USA). The characteristic parameters of subjects were expressed as mean ±S.D.. P values < 0.05 were considered statistically significant.

Graphpad (version 8.3.0.) was used for histogram drawing. And Cytoscape (version 3.7.2) was used for relational network diagrams.

Multiple regression analysis: f^2(Anticipated effect size) =0.15, α（two-tailed）=0.05, power level=0.8, at least 4 or 14 metabolites with significantly different characteristics were predicted (https://www.danielsoper.com/statcalc/calculator.aspx?id=1).

Results

Participants' characteristic

A total of 12 MDD patients and 12 healthy subjects were accepted as the main cohort (Table.1).

Table.1

Participant characteristics

Group	Controls	MDD
Sex(male/female)	0/12	6/6
Age	24.92±1.44	39.33±18.13
BMI	20.22±1.30	22.16±2.95
HDRS-17: Hamilton Depression Rating Scale-17; BMI: Body Mass Index. The age and BMI of subjects are represented as mean±S.D.		

Patients received conventional treatment without design or intervention to preserve the real condition of hospitalization. The drug regimens included antidepressants such as selective serotonin reuptake inhibitors (SSRIs), selective serotonin and norepinephrine reuptake inhibitors (SSNRIs), and traditional Chinese medicine. In addition, some adjuvant medications were included in the treatment regimen for sedation, hypnosis, mood stabilization, relief of delusions or hallucinations, and protection of the liver from damage caused by antidepressants.

This is a pilot study for preliminary exploration, the gender and age of subjects are not included in the influencing factors. There was significant difference in HDRS-17 scores between control group and 0m group(p＜0.0001). After treatment, HDRS-17 scores of 1m group and 2m group were significantly decrease compared with 0m group(p＜0.0001)(Figure 2).

Plasma metabolic profile of UPLC-MS

607 metabolites could be detected in 47 plasma samples and attributed to the local database. The chromatograms of the mixed QC samples are shown in Figure 3. In addition, the TIC curves of different QC samples in the overlap analysis showed high overlap, indicating the stability of the instrument signal, the analysis of the data is repeatable and high accuracy (Figure 3).

Principal component analysis (PCA)

The difference of plasma metabolites between each group could be preliminarily obtained by PCA. In the 3D-PCA score plot, the trend of separation between data points can preliminarily represent the difference of plasma metabolites in each group. There was an obvious partition trend among the four groups, indicating the disparities of metabolite composition. The QC samples are closely aggregated, and obviously separated from other samples, indicating the stability of the detection method (Figure 4a).

Specifically, the separation between the control group and the 0m group indicated MDD causes differences in plasma metabolic profile. The separation between the 0 m, 1 m, and 2 m groups indicated that routine treatment can affect the metabolic transformations caused by MDD

Orthogonal partial least-squares discriminant analysis (OPLS-DA)

OPLS-DA combines the OSC and PLS-DA analysis method, which can decompose the X matrix information into Y-related and Y-unrelated categories, and remove the unrelated differences to screen the difference variables. Through pair comparison of plasma of each group, the difference of comparison groups can be obtained. There were significant metabolic differences between control group and 0m group (Figure 4b-g). In addition, the comparison of 0m with 1m and 2m showed that the metabolic difference increased with the advance of hospitalization time.

Screening of differential metabolites in plasma

Differences in metabolites between comparison groups were further screened by multivariate statistical analysis (OPLS-DA VIP value) and univariate statistical analysis (P value or fold change (FC)). In this study, the threshold of significant difference was VIP$\geq$ 1, and $|Log_2FC| \geq 1$.

The Volcano Plot can visually show the difference in the metabolites detected in the samples between the two groups and the statistical significance of the difference (Figure 5a-c). The number of SDMs were shown in Table 2. It was clear that the quantity of SDMs between control group and 0m group was the

highest, reaching 79. However, compared with the 0m group, the amount of SDMs of the 1m group and the 2m group was significantly reduced.

Table 2

The number of significantly different metabolites (SDMs) in each pairwise comparison group

Group	Total SDMs	Down-regulated	Up-regulated
Control vs 0m	79	37	42
0m vs 1m	39	24	15
0m vs 2m	29	12	17

SDMs' relative contents were normalized to obtain the clustering heatmap, from which the change rule of metabolites between groups could be observed intuitively (Figure 6). Clearly, the clustering of these metabolites separated the plasma of healthy controls (control group) from that of MDD patients (0m, 1m□2m). However, the clustering of SDMs could not effectively isolate the samples of MDD patients treated for different time, suggest that hospitalization for a limited period can't significantly improve the plasma metabolic spectrum disorders that MDD induces.

K-means analysis of Significant different metabolites

In addition, 74 specific metabolites (SMs) showed an ideal variation trend in their relative contents, which could be divided into 4 kinds of variation laws was filtered by K-means clustering analysis□Figure 5d□. Interestingly, the relative levels of SMs in class 1 and class 2 tended to decline in 0m group compared to controls, and then increased after treatment. On the contrary, the relative content of SMs in class 3 and class 4 tend to increase in 0m groups compared to controls and then decrease after treatment. Therefore, the changes in the relative content of these SMs may reflect the changes in the metabolic level between healthy controls and MDD patients from the side, as well as the differences in the metabolic level of MDD patients before and after treatment. The detail of SMs was shown in additional file 1.

KEGG enrichment analysis

KEGG database is used to annotate the information of SDMs and enrich them into metabolic pathways, which is helpful to analyze the changes of plasma metabolic network. The KEGG enrichment plot (Figure 5e-g) shows the concentration of SDMs in KEGG pathways in the comparison group.

The enrichment pathway between controls and 0m group were mainly lipid and fatty acid metabolism, including bile acid metabolism, cholesterol metabolism, linoleic acid metabolism, and arachidonic acid metabolism, etc. And the enrichment degree of amino acid metabolism pathways is also significant. What's more, lots of SDMs were enriched in metabolic pathways related to the nervous system and

inflammation. The top 10 KEGG pathways and 14 related SDMs in additional file 2 were selected for further metabolite-pathway integration analysis (additional file 2) □exogenous metabolic processes have been excluded□. These SDMs and their involved pathways may play a crucial role in the metabolic abnormalities caused by MDD.

Plasma metabolic profiles were also different between 0m and 1m, 2m groups, but the degree of enrichment of pathways and the number of metabolites belonging to each pathway was small. SDMs between 0m and 1m group were enriched significantly in fructose and mannose metabolism, fatty acid biosynthesis, phenylalanine metabolism, pyrimidine metabolism, biosynthesis of unsaturated fatty acids, porphyrin and chlorophyll metabolism. And the SDMs of 0m group and the 2m group were enriched in fructose and mannose metabolism, pyrimidine metabolism, and ABC transporters.

MDD associated metabolite-pathway network integration

SMs selected by K-means clustering analysis was integrated with the top 10 pathways and related 14 SDMs between controls and 0m group to further explore the underlying mechanism of metabolic transformations in the occurrence and treatment of MDD. Finally, we obtained a metabolite-pathway network consisting of 4 SMs and 8 pathways (Figure 7a).

Furthermore, we conducted correlation analysis between the relative content of the 4 SMs and HDRS-17 score of all subjects, thus the relationship between these SMs and the severity of depression can be tentatively explored. The absolute value of Spearman correlation coefficient (|Cij|) □0.2 with P □0.05 was considered to be significantly correlated. Interestingly, TXB_2, AA were positively correlated (p□0.01□with HDRS-17 scores, while 9-HPODE, imidazoleacetic acid were negative correlated (p□0.01) with HDRS-17 scores(Figure 7b). It suggests that these SMs are closely related to the severity of depression.

Sample size estimation of the validation set

To further investigate whether 4 SMs can be used as a biomarker for the diagnosis and prognosis of MDD□referring to the method of Mocking et al. (8), we used multiple regression analysis to calculate the sample size of the validation set for future studies to verify the results of this study. Taking the threshold of at least 4 SMs, Cohen's f^2=0.15□the minimum sample size was calculated as 84□In addition, the other 10 SDMs listed in Additional file 2 belong to the top 10 enrichment pathways that are closely related to the occurrence of MDD, and their role in the potential mechanism of MDD changes in metabolism cannot be ignored. Therefore, taking the threshold of at least 14 SDMs□Cohen's f^2=0.15□the minimum sample size was calculated as 135. The results showed that a validation set of between 84 and 135 subjects (including healthy controls and MDD patients) was needed to confirm the results of the study.

Discussion

In the present study, we used UPLC-MS to detect different metabolites in the plasma of controls and MDD patients. And we followed MDD patients' hospitalization for two months. Samples of this metabolomics study were collected under the non-interventional medical condition, the self-control of patients avoid the influence of confounding factors, which could reflect the metabolic changes of patients under the real treatment condition.

Finally, we constructed a new network consisting of 4 SMs and 8 enrichment pathways. The relative contents of these SMs showed a regular trend of change in the plasma samples of each group, and significantly correlated with the scores of HDRS-17 scale. These results suggest that they are correlated with the severity of MDD and may have the potential to be biomarkers for the diagnosis and treatment of MDD. 8 enrichment pathways associated with the 4 SMs are involved in the possible mechanisms of MDD changes in metabolic processes, which are explained in detail as follows.

Lipid is important for maintaining normal brain function and activity, abnormal lipid metabolism is thought to be linked to many cerebral disorders, including depression(9). Among the 4 SMs selected, AA, TXB_2 and 9-HPODE are important participants in lipid metabolism. In present study, it was found that the lipid metabolism processes of MDD patients, including arachidonic acid metabolism, linoleic acid metabolism, primary bile acid biosynthesis, cholesterol metabolism, etc. were significantly altered compared with healthy controls.

Linoleic acid (LA) is one of the essential fatty acids in human body, which is important for regulating homeostasis(10, 11). 9-HPODE is one of the products of linoleic acid peroxidation, which has been shown to be involved in the oxidative stress of brain tissue caused by Alzheimer's disease(12). The results of our study showed that the plasma content of 9-HPODE in MDD patients was significantly decreased compared with the healthy controls, and its content was increased after treatment. Moreover, the relative content of 9-HPODE was also negatively correlated with the subjects' HDRS-17 scores. However, the relationship between linoleate peroxide and depression has not been clarified in previous studies. Therefore, the role of linoleate metabolism in the process of depression still needs further studies to confirm.

Arachidonic acid (AA) is an important product of linoleic acid metabolism. Cyclooxygenase (COX), lipoxygenase (LOX), Cytochrome P450 (CYP450) and other enzymes catalyze the formation of eicosanoid family of mediators, including thromboxanes (TXs), prostaglandins (PGs), leukotrienes (LTs), hydroxy eicosatetraenoic acid (HETE), which are closely related to the regulation of the inflammatory process(13, 14). Once an imbalance of pro-inflammatory and anti-inflammatory shows in the central nervous system, diseases including depression may appear(15, 16). In our study, plasma contents of AA and its downstream stable pro-inflammatory product, TXB_2 in MDD patients increased significantly compared with healthy controls, and then showed a downward trend after treatment, and their relative levels were positively correlated with the subjects' HDRS-17 score. In addition, although the relative contents of Leukotriene B4 (LTB_4, the pro-inflammatory metabolite of AA) and the unstable metabolite (±) 15-HETE did not change regularly among the groups, their contents in 0m group were significantly

increased compared with controls. The results indicated that the metabolic process of AA was significantly activated in MDD patients, suggesting that inflammatory events may occur in MDD patients.

AA is closely related to retrograde endocannabinoid signaling. Endocannabinoid system (ECS) consists type 1 and type 2 cannabinoid receptors (CBR1 and CBR2), endogenous ligands such as N-arachidonoylethanolamide (AEA) and 2-arachidonoylglycerol(2-AG), and a series of related enzymes and transporters. ECS plays an important role in the regulation of central nervous system functions such as energy metabolism, inflammation, behavioral selection and synaptic plasticity(17). AEA and 2-AG which are derivatives of AA generally play a role in the signal transmission of different brain regions and synapses through a retrograde mechanism, namely(18). The metabolic glutamate receptors (mGRAs), serotonergic receptors 5-HT2a and 5-HT2c, can regulate synaptic transmission by triggering the endocannabinoid retrograde signaling(19). ECS is involved in both short-term and long-term depression, and the loss of retrograde signaling may be one of the causes of depression(20). In our study the contents of AA and L-glutamate were significantly increased in plasma of 0m group compared with control group. Arachidonic acid is one of the hydrolytic product of two endocannabinoids(21), and glutamate is thought to be one of the neurotransmitters that activate the ECS(22). However, since the changes in key intermediate links such as receptors and enzymes related to the endocannabinoid retroactive signaling pathway are still unknown, further studies are needed to confirm the potential connection between the ECS and MDD.

Moreover, AEA can also produce activity through the activation of transient receptor potential V1(TRPV1) channel in addition to the retrograde signaling pathway. The TRP channel family is increasingly considered as a sensor of nociceptive stimulation and can also be indirectly regulated by a variety of inflammatory mediators(23). Arachidonic acid metabolism can activate the TRP channels, especially the TRPV4, TRPV1 and TRPM8 channels (24, 25). We found that compared with healthy controls, plasma contents of arachidonic acid and its metabolites LTB_4 and (±)15-HETE were increased in MDD patients, both of which are activated molecules of the TRP channels. Although the link between depression and the TRP channels has not been clearly understood (26), the possibility of the regulation of TRP channels as the potential mechanism of MDD cannot be ruled out, but the confirmation of the pathway needs more exploration.

What is known to all that the decrease of 5-hydroxytryptamine (5-HT) level in the brain is one of the main causes of depression. 5-HT can stimulate the release of AA by inducing phospholipaseA2 (PLA2) signaling through the 5-HT2 receptor. Then AA and its metabolites can in turn inhibit the release of neurotransmitters including 5-HT(27-29). Moreover, the KEGG pathway network constructed in present study showed that long-term depression was also associated with the above processes. Our study found that arachidonic acid metabolism was activated in MDD patients, suggesting that there may be crossover between arachidonic acid metabolism and 5-HT system, but there are still many complex processes involved between them, further studies on the receptors and enzymes involved in the intermediate processes are needed.

Bile acid not only has the characteristics of local cleaning agent, but also is an important signal molecule in the human body. They may lead to MDD by altering intestinal epithelial cell permeability, and may also regulate neuroinflammation through the signaling pathway mediated by takeda G protein-coupled receptor5 (TGR5), thus influencing the depressive phenotype of animals(30). In addition, the changes of expression of farnesoid X receptor (FXR) which can be activated by bile acid is one of the reasons induced depression animal behavior and neuroinflammation(31). And the activation of FXR can alleviate liver inflammation by inhibiting arachidonic acid metabolism and reducing the level of the pro-inflammatory metabolite LTB_4, which in turn inhibits the nuclear factor κ light-chain enhancer of activated B cells (NF-κB)(32). We found that compared with control group, the plasma contents of cholesterol, TXB_2 and LTB_4 in 0m group were significantly increased, while glycochenodeoxycholic acid and glycocholic acid were significantly decreased, indicating abnormal bile acid metabolism process in MDD patients. However, the change trend of related indicators is not completely consistent with previous studies(33). Whether the expression of FXR changes in MDD patients, and whether the disturbance of bile acid system causes the activation of neuroinflammation, these questions require further exploration before they can be answered.

In our study, we also found changes in histidine metabolism in MDD patients. Compared with control group, the contents of L-glutamic acid and N-acetylhistamine in 0m group were increased, and the contents of Imidazoleacetic acid and 3-N-methyl-L-histidine were decreased. Moreover, the relative content of Imidazoleacetic acid showed an upward trend after treatment. L-histidine can produce L-glutamic acid through a series of complex metabolic pathways. And glutamate system plays an important role in the pathophysiological processes of depression(34). Unfortunately, the relationship between histidine metabolism and depression has not been directly reported and the relevant mechanisms need to be explored in the future.

Changes in plasma metabolic profile

The result shows that HDRS - 17 scores of 0 m, 1 m and 2 m group were significantly increased compared with controls, and the HDRS-17 score of MDD patients gradually decreased with the prolongation of treatment time, indicated that patient's depressive symptoms were gradually relieved during the treatment. Moreover, there were significant differences in plasma metabolic profiles between MDD patients and controls, but metabolic transformations before and after treatment were not significant. These results suggest that the effect of two months of conventional treatment on the change of metabolic abnormalities in MDD patients is limited. Depression is a heterogeneous disease with a complex mechanism, and the classification of the subtypes of depression is not perfect at present, which leads to great differences in the internal metabolic changes of patients with similar depressive symptoms. Our study suggests that short-term conventional drug therapy can effectively improve patients' mental symptoms, while it cannot accurately act on the abnormal related metabolic pathways in patients, resulting in the inconsistency between the external behavioral symptoms and the changes in the internal biochemical indicators. Fortunately, we also found some changes of plasma metabolic profiles

and pathway disturbances in MDD patients during treatment, which may be a potential target for antidepressants and provide a new idea for individualized and precise treatment of MDD.

Study limitations

There are limitations to our study. As a pilot study at the exploratory stage, this study only included 12 control group and 12 MDD patients, and did not strictly control the subjects' gender and age. Although we followed up the patient for two months during his hospitalization, we failed to to obtain the patient's prognosis information after discharge. MDD is a multi-target chronic disease with complex mechanism, which not only involves changes in the metabolic process, but is also closely related to hormone levels protein and gene levels related to each pathway. The network of possible mechanisms for MDD that we got from the metabonomic alone is deficient in some intermediate details.

Conclusion

The results of present study indicate that the occurrence of MDD can significantly affect the metabolic level of the body, and conventional drug intervention can improve the metabolic abnormalities of MDD patients to a certain extent. The integrated metabolite-pathway network suggested that the metabolic abnormalities in MDD patients were mainly related to lipid metabolism and histidine metabolism as well as the changes of their downstream pathways. It is planned to establish a large-scale validation which specifies gender and age to confirm the predictive ability of related metabolites as potential biomarkers for MDD diagnosis and prognosis evaluation, and further conduct in-depth studies on the potential molecular mechanisms of related pathways such as proteins and genes to confirm the results of our study.

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Huazhong University of Science and Technology ([2013] IEC(S007)). Informed consent of the participants was obtained after the nature of the procedures had been fully explained. The study was carried out in accordance with the principles of the Helsinki Declaration as revised 1989.

Consent for publication

Not applicable

Availability of data and materials

The datasets generated and analysed during the current study are not publicly available to protect participants' privacy, but are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

XL wrote the original draft, did the formal analysis of data, organize the figures. SZ provided the clinical samples and information of patients. JT did the UPLC-MS analysis. Ying Zhu organized the data. XZ made the original figures; YL reviewed and edited the manuscript, did the project administration and Funding acquisition. All authors reviewed the manuscript.

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Figures

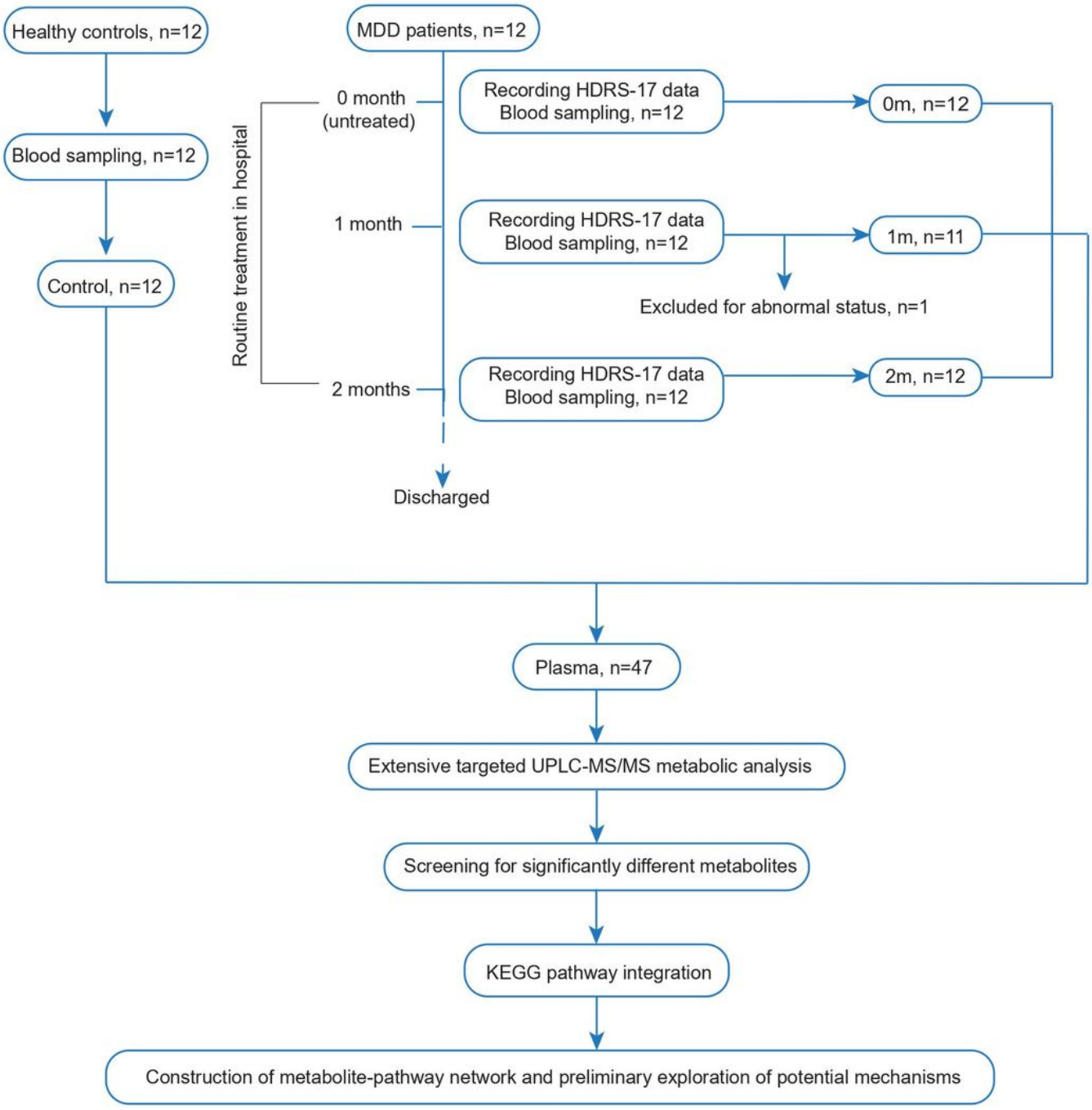

Figure 1

Flowchart of the study A total of 47 plasma samples were used for metabolomic analysis. During hospitalization, 12 MDD patients were followed up and sampled once a month. The samples were divided into three groups according to treatment time: 0m (untreated after admission, n=12), 1m (treat for one month, n=11), 2m (treat for two months, n=12). Only one blood sample was collected from each healthy control (n=12).

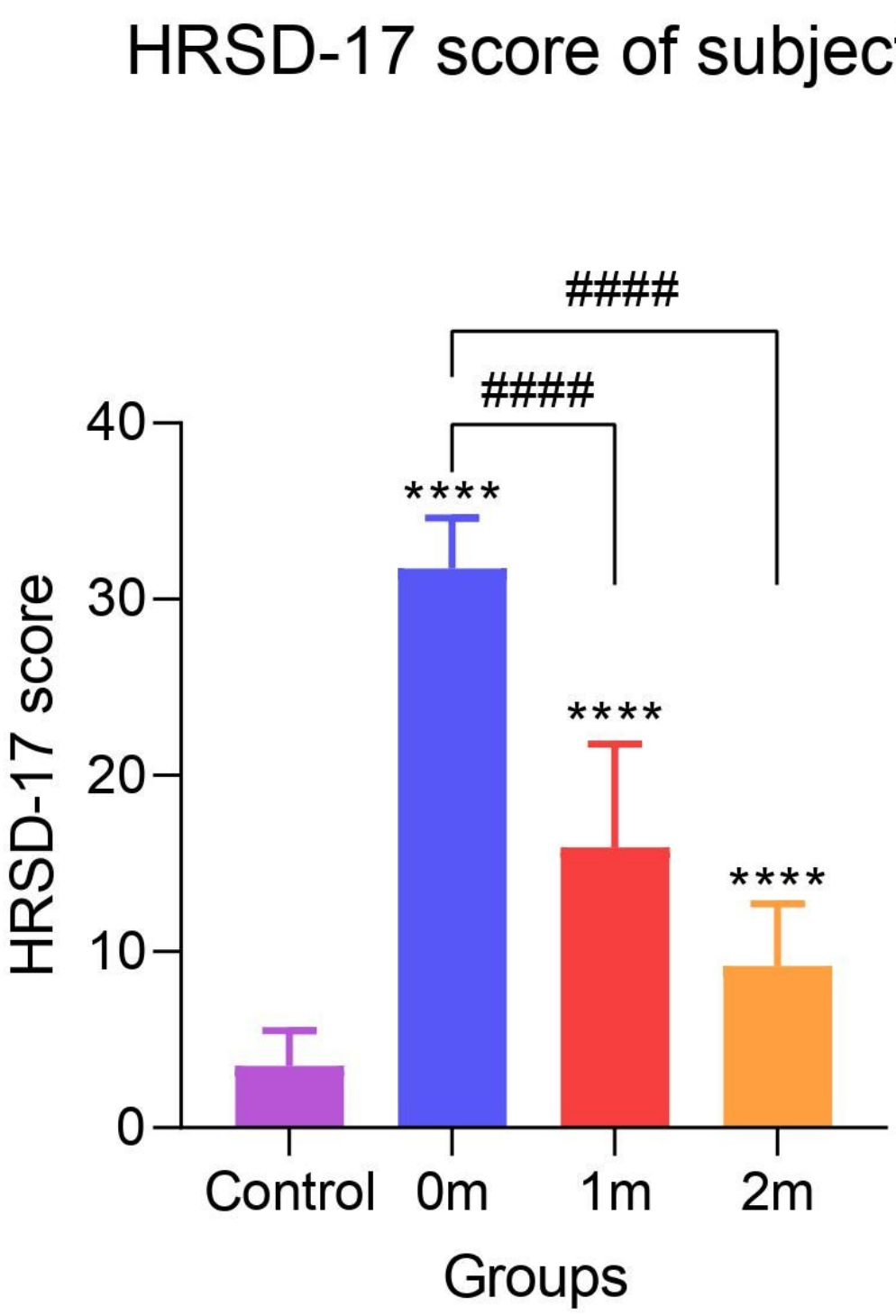

Figure 2

HDRS-17 score of subjects Control(n=12), 0m(n=12) , 1m(n=11), 2m(n=12).Data were means±S.D., unpaired t test****0.0001, compared with control group; ####0.0001, compared with 0m group.

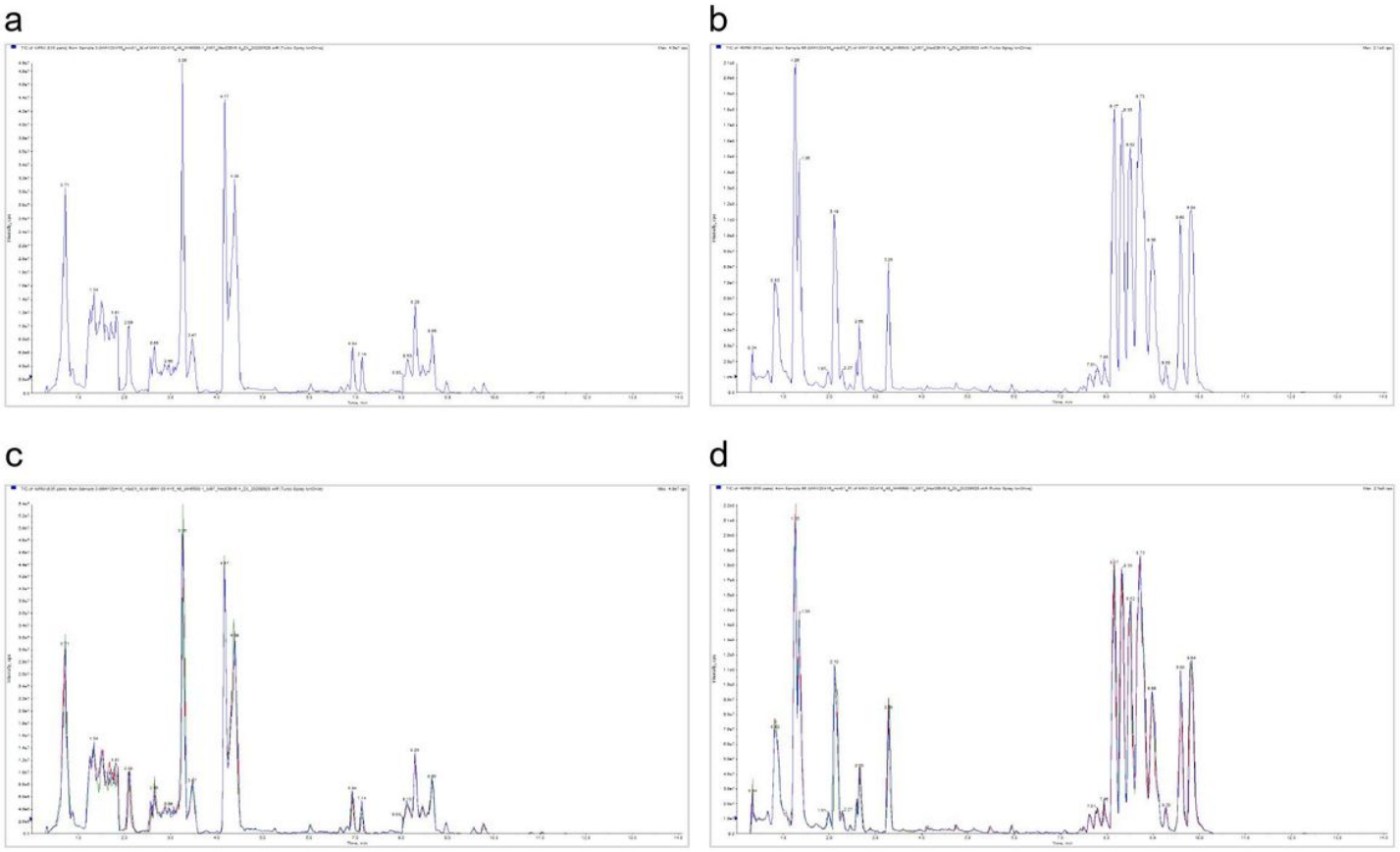

Figure 3

Chromatograms of QC samples in different modes ab.TIC of mixed plasma extract sample: a. negative ion mode. b. positive ion mode. cd. TIC overlap diagram of different QC samples: c. negative ion mode; d. positive ion mode.

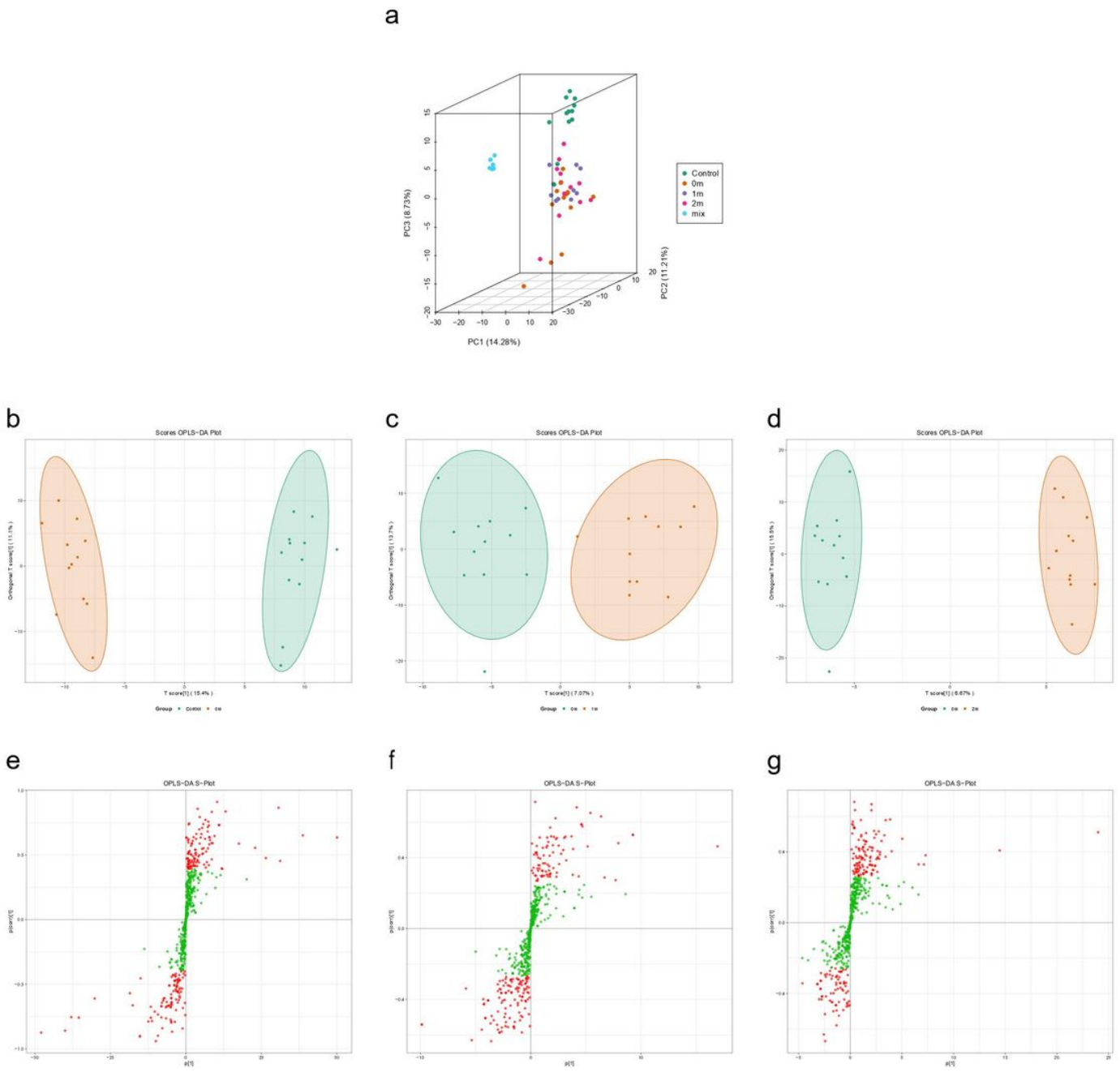

Figure 4

Differential model analysis of plasma metabolites a. 3D-score plot of PCA was derived based on UPLC-MS information of metabolites detected in healthy controls (Control, green), untreated depressed patients (0m, orange), depressed patients treated for one month (1m, purple) and depressed patients treated for two months (2m, pink), as well as the quality control (QC, blue). Each point represents an individual sample. bcd. Score plot of OPLS-DA of plasma from each comparison group (pairwise comparison). b.

Control vs 0m; c. 0m vs 1m; d. 0m vs 2m. efg. S-plot of OPLS-DA of plasma from each comparison group (pairwise comparison). e. Control vs 0m; f. 0m vs 1m; g. 0m vs 2m.

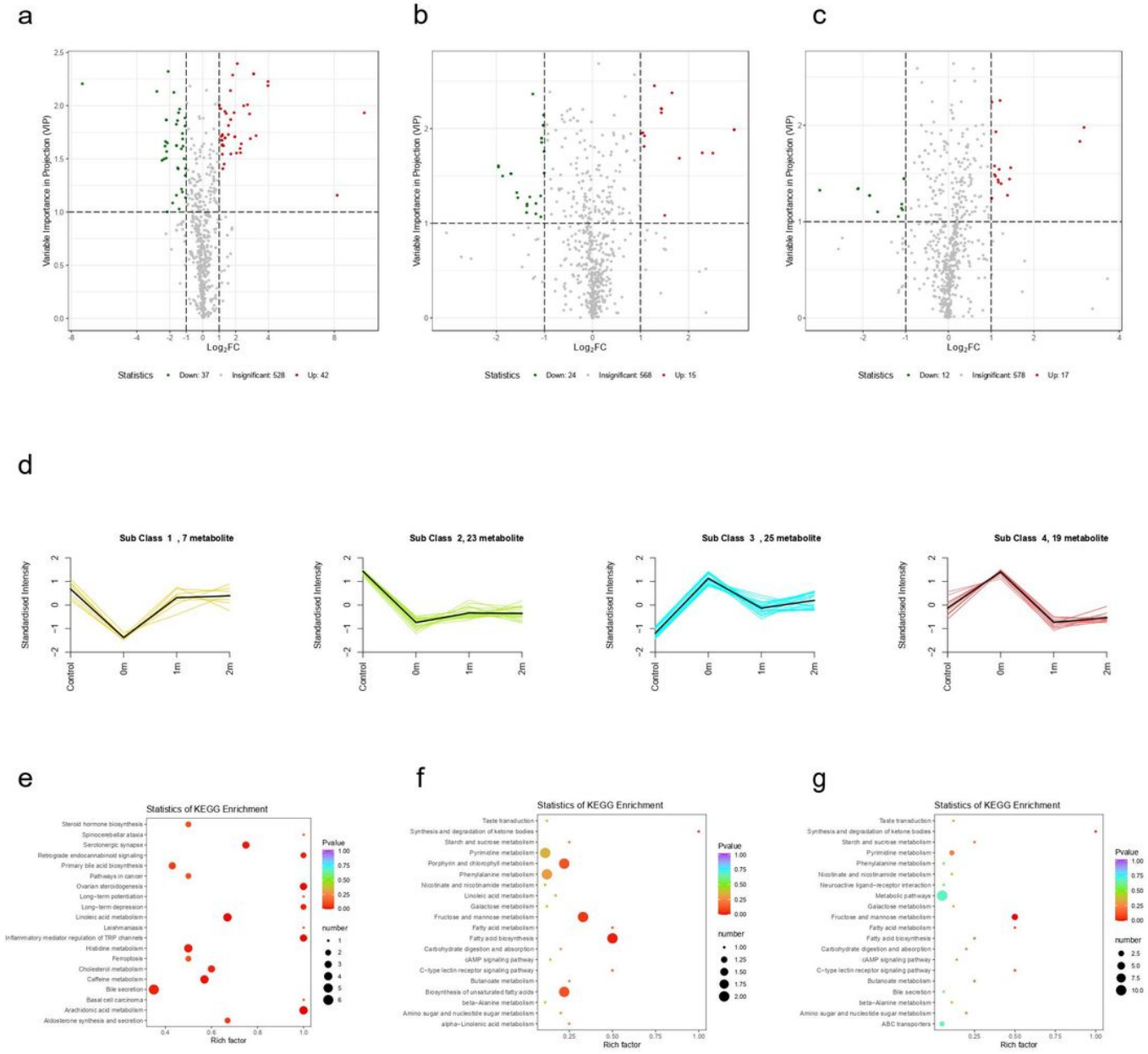

Figure 5

Significantly different metabolites (SDMs): Metabolite filtrating and pathway attribution abc. Volcano Plot of plasma metabolites in comparison groups. a. Control vs 0m; b. 0m vs 1m; c. 0m vs 2m. Each dot represents a metabolite. The vertical axis is VIP value, and the abscissa is Log2FC. Each dot represents a metabolite, the colored dots with VIP≥1 and absolute value of Log2FC ≥1 are considered to be

significantly different. The red dots represent significantly up-regulated metabolites, while the green dots represent significantly down-regulated metabolites. d. K-means plot of change trends in relative content of specific metabolites (SMs). The change trend of the 4 classifications in the figure is consistent with the change rule of the relative content of metabolites with the development of the disease. The abscissa represents the sample grouping and the ordinate represents the standardized relative metabolite content. efg. KEGG enrichment plot. e. Control vs 0m; f. 0m vs 1m; g. 0m vs 2m. The rich factor represents the ratio of the number of SDMs enriched in this pathway to the number of all metabolites detected and assigned to this pathway, the size of the point represents the amount of SDMs enriched, P-value represents the significance of enrichment in the pathway (indicated by the color of the dot, warm colors represent high significance and cool colors represent low significance).

Figure 6

Clustering heatmap of SDMs in comparison groups Horizontal represents sample information and vertical represents differential metabolite information. The color of block represents the scale of SDMs which was obtained by standardizing the relative metabolite content. Warm colors represent high scale and cool colors represent low scale.

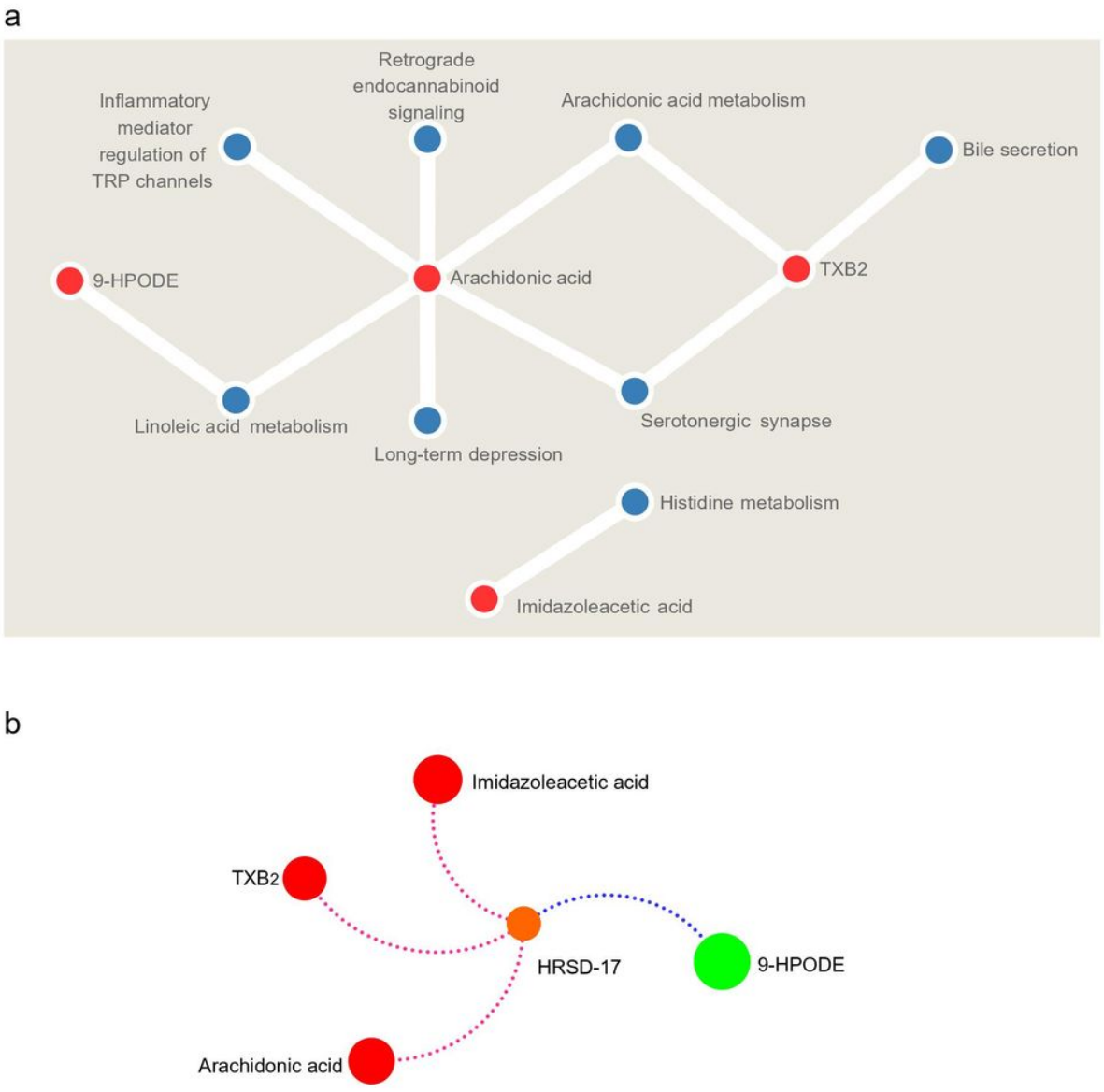

Figure 7

Metabolites-pathway integration a. Metabolite-pathway network closely related to the occurrence and development of MDD. Nodes representing SMs are labeled red, and nodes representing related pathways are labeled blue. b. Correlation between SMs and HDRS-17 scores. |Cij|>0.2, $p < 0.05$. The node size represents |Cij|. Red nodes, positively correlated with HDRS-17 scores; green nodes, negatively correlated with HDRS-17 scores.

Supplementary Files

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

- Additionalfile1.docx
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