Neuropsychiatric Disease and Treatment

Dovepress

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ORIGINAL RESEARCH

Plasma disturbance of phospholipid metabolism in major depressive disorder by integration of proteomics and metabolomics

This article was published in the following Dove Press journal:
Neuropsychiatric Disease and Treatment

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Introduction: Major depressive disorder (MDD) is a highly prevalent mental disorder affecting millions of people worldwide. However, a clear causative etiology of MDD remains unknown. In this study, we aimed to identify critical protein alterations in plasma from patients with MDD and integrate our proteomics and previous metabolomics data to reveal significantly perturbed pathways in MDD. An isobaric tag for relative and absolute quantification (iTRAQ)-based quantitative proteomics approach was conducted to compare plasma protein expression between patients with depression and healthy controls (CON).

Methods: For integrative analysis, Ingenuity Pathway Analysis software was used to analyze proteomics and metabolomics data and identify potential relationships among the differential proteins and metabolites.

Results: A total of 74 proteins were significantly changed in patients with depression compared with those in healthy CON. Bioinformatics analysis of differential proteins revealed significant alterations in lipid transport and metabolic function, including apolipoproteins (APOE, APOC4 and APOA5), and the serine protease inhibitor. According to canonical pathway analysis, the top five statistically significant pathways were related to lipid transport, inflammation and immunity.

Conclusion: Causal network analysis by integrating differential proteins and metabolites suggested that the disturbance of phospholipid metabolism might promote the inflammation in the central nervous system.

Keywords: major depressive disorder, plasma proteomics, iTRAQ, metabolomics, integrative analysis

Introduction

Major depressive disorder (MDD) is a severe and highly prevalent psychiatric disorder affecting 3% of the global population.[1] It is a complex disease characterized by pervasive and persistent low mood and loss of interest or pleasure.[2] A survey on the global burden of 220 diseases using disability-adjusted life years suggested that MDD was a key contributor to disability among the nonfatal consequences of disease and injury.[3] Furthermore, consequences of depressive episodes include serious impairments in social functioning[4] and even suicidal ideation and attempts.[5] Indeed, approximately 2%–7% of MDD patients commit suicide.[6] The pathophysiology of depression is not yet understood, but current theories center around monoaminergic systems,[7] immunological dysfunction[8] and hypothalamic–pituitary–adrenocortical (HPA) axis dysfunction.[9] However, a clear causative etiology of MDD remains largely unknown. Therefore, improved understanding of the molecular mechanisms

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http://dx.doi.org/10.2147/NDT.S164134

of disease progression and discovery of novel therapeutic targets are urgently needed for patients with MDD.

The central nervous system (CNS) and peripheral tissues have interactions. In recent years, multi-omics techniques including genomics,[10] proteomics[11] and metabolomics[12] have been used to discover peripheral biomarkers of depression, such as neurotrophic factors, neurotransmitters, amino acids, lipids and carbohydrates. In contrast to the single-omics approach, researchers have used multiple combinatorial approaches to explore the disease state.[13] Combined proteomics and metabolomics analysis is particularly attractive. Metabolomics data enable functional interpretation of proteomics data and aid in understanding their regulatory relationships. Accordingly, combined proteomics and metabolomics analysis has been used to determine the molecular mechanisms of various diseases such as cancer,[14] cardiovascular disease[15] and mental disorder.[16] Moreover, in our previous study, we performed combined analysis of proteomics and metabolomics data from cerebellar tissue of chronic mild stress (CMS)-treated depressed rats and CON.[17] We found abnormal cerebellum energy metabolism in depression, including disturbance of amino acid, glycolytic and tricarboxylic acid (TCA) cycle enzymes and mitochondrial respiratory chain dysfunction. Although this provides clues to the pathophysiology of depression, rat models do not adequately explain changes in the human body during disease conditions. Consequently, combined proteomics and metabolomics analysis in human plasma samples is needed to further explore molecular changes in depressed patients and how peripheral tissues affect CNS changes.

Here, we aimed to identify critical protein alterations in the plasma of patients with MDD. Furthermore, we integrated our proteomics and previous metabolomics data of depressed patients[18] to explore significantly perturbed pathways in MDD.

Participants and methods

Ethics approval and informed consent

The ethics committee of Chongqing Medical University reviewed and approved the protocol of this study and the procedures used for sample collection and analysis. All subjects provided written informed consent after detailed introduction of the study. All procedures were performed according to the Declaration of Helsinki.

Participants

Patients with MDD were recruited from the Psychiatric Department of the First Affiliated Hospital of Chongqing Medical University, Chongqing, China. The inclusion criteria were diagnosis of MDD according to *Diagnostic and Statistical Manual of Mental Disorders*, 4th Edition (DSM-IV) criteria for MDD,[19] first-episode and treatment-naïve patients, patients aged 18–60 years, patients with Hamilton depression score (on the 17-item scale) >18 and patients with no comorbidity. Healthy CON matched for age, sex and body mass index (BMI) were enrolled from the Medical Examination Center at Chongqing Medical University. In total, 20 first-episode, drug-naïve, depressed patients and 20 demographically matched CON were included.

Samples for proteomics and metabolomics analyses were all screened from our clinical sample database (contains ~2,000 samples) with the same inclusion criteria. Table S1 gives information regarding patients used for metabolomics analysis.

Sample collection and preparation

Peripheral blood samples (5 mL) were collected in EDTA tubes (BD Vacutainer catalog no 367863; BD, Franklin Lakes, NJ, USA) by venipuncture between 8:00 and 10:00 am, and then immediately placed on ice and centrifuged at 3,000 rpm at 4°C for 15 min. The aliquoted plasma samples were stored at −80°C within 1 h of collection. Pooled plasma samples were generated by combining equal volumes of all 20 individual plasma samples from both groups. The protein concentration of each pooled sample was determined by using a commercial Bradford Protein Assay Kit (Beyotime, Shanghai, China). Protein normalization was confirmed by 12% sodium docecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

Isobaric tags for relative and absolute quantification (iTRAQ) labeling and strong cation exchanger (SCX)-based fractionation

Before labeling, protein digestion for resultant peptide mixtures was performed according to the pooled sample preparation procedure described previously.[20] Briefly, the proteins (100 μg) in each sample were reduced, blocked on cysteine, alkylated and subsequently digested with trypsin overnight at 37°C. Prior to iTRAQ labeling, digested samples were desalted with a C18 cartridge (Sigma-Aldrich Co., St Louis, MO, USA; EMD Millipore, Billerica, MA, USA) to remove urea, salt and other reagents. Then, the purified sample was evaporated to dryness with a SpeedVac (RVT4104; Thermo Fisher Scientific, Waltham, MA, USA). After resuspension with 30 μL 0.5 M triethylammonium bicarbonate (pH 8.5), samples were labeled with iTRAQ reagents following the protocol provided by the manufacturer. Proteins

obtained from control samples were labeled with iTRAQ Reagent-8plex Multiplex Kit (AB Sciex, Framingham, MA, USA), which contained 113, 114 and 115 reporter tags. Proteins obtained from patients with MDD were labeled with 116, 117 and 118 reporter tags. iTRAQ-labeled peptides were mixed and fractionated by SCX chromatography using the AKTA Purifier 100 system (GE Healthcare UK Ltd, Little Chalfont, UK). For each test, 33 SCX fractions were collected followed by a C18 cartridge (66872-U; Sigma-Aldrich Co.; EMD Millipore) for desalination.[21]

Liquid chromatography–mass spectrometry (LC–MS)/MS-based quantitative protein analysis

The labeled peptides were analyzed on a Q Exactive mass spectrometer (Thermo Fisher Scientific) coupled with an EASY-nanoLC system. A nanoViper C18 trap column (100 µm × 2 cm, 5 µm particle size; Thermo Fisher Scientific) and a C18 analytical column (10 cm × 75 µm, 3 µm particle size; Thermo Fisher Scientific) were used. The peptide mixtures were first injected into buffer A (0.1% formic acid) at a flow rate of 300 nL/min. The separation gradient was 0–55 min, buffer B (0.1% formic acid and 84% acetonitrile) from 0% to 55%; 55–57 min, buffer B from 55% to 100% and 57–60 min, buffer B remained 100%. Then, the eluted peptides were analyzed by a Q Exactive mass spectrometer. The mass range of positive ion mode was from 300 to 1,800 m/z, the number of scan ranges was 20, the primary mass spectrometry resolution was 70,000 (m/z 200), the resolution for high-energy collisional dissociation (HCD) spectra was 17,500 (m/z 200) and the maximum inject times were set at 10 and 60 ms. Dynamic exclusion time was 40.0 s. The top 10 abundant precursor ions were used for MS/MS analysis with normalized collision energy at 30 eV and an underfill ratio of 0.1%.

Data analysis

Obtained MS/MS spectra were processed with Proteome Discoverer 1.4 (Thermo Fisher Scientific) and subsequently searched against the UniProt Human database, which contained 156,639 sequences, downloaded on January 5, 2017 (http://www.uniprot.org), using Mascot engine (version 2.2; Matrix Science, London, UK). Search parameters were as follows: trypsin as digestion enzyme, allowance of two missed cleavages, oxidized methionine and iTRAQ 8-plex (Y) as variable modifications with carbamidomethyl (C), iTRAQ modification at the peptide N-terminus and iTRAQ 8-plex (K) as fixed modifications. The peptide mass tolerance and fragment mass tolerance were set to 20 ppm and 0.1 Da, respectively. Furthermore, all peptide ratios were normalized by median protein ratio, which should be 1. A decoy database search strategy was adopted to estimate the false discovery rate (FDR) for peptide identification. For this study, a high peptide confidence (1% FDR) was selected based on the assumption that expression of most proteins does not change. This bias correction mitigates the systematic errors arising from samples in each experimental condition that were not combined in exactly equal amounts. The software identified a median average protein ratio, which was corrected to unity, and then, this factor was applied to all quantification results.[22]

Protein identifications were grouped depending on peptide matches from all samples. Protein ratios were calculated based on the median of unique peptides. Proteins with p-values ≤ 0.05 and fold changes ≥ 1.2 were considered as significantly regulated.[23] Differential plasma proteins were profiled by functional classification through PANTHER (http://www.pantherdb.org/).[42]

Demographic data and clinical characteristics were analyzed using two-tailed Student's t-test and Fisher's exact test by SPSS 21.0 (IBM Corporation, Armonk, NY, USA).

Ingenuity Pathway Analysis (IPA)

IPA software (Qiagen NV, Venlo, the Netherlands) was used to integrative proteomics and metabolomics analyses (including biological function annotation, canonical pathways and relevant networks). The association between uploaded data and canonical pathways was measured by two means: 1) statistical significance – Fisher's exact test was used to calculate p-values to determine the probability that the association between the uploaded molecules and the canonical pathways is explained by chance and 2) the ratio of the number of uploaded molecules that map to the given pathway divided by the total number of molecules in the canonical pathway.

Results

Demographic and clinical characteristics of participants

In total, 20 patients with MDD and 20 healthy CON were included. There were no significant differences in the age, sex or BMI. Demographic and clinical characteristics of the participants are summarized in Table 1.

Quantitative protein analysis

After SCX fractionation and subsequent LC–MS/MS analysis, 370,351 MS/MS spectra were obtained, of which 21,213 peptide spectrum matches were assigned to 3,829 peptides (2,816 unique peptides) with an FDR of 1%. Moreover, these identified peptides corresponded to a set of 669 proteins.

Table 1 Demographic and clinical features of recruited subjects[a]

	CON (n = 20)	MDD (n = 20)	*p*-value
Age (years)[b]	34.8 ± 10.1	37 ± 9.0	0.57
Sex (M/F)	8/12	5/15	0.50
BMI[b]	22.5 ± 2.4	22 ± 3.1	0.96
HAMD[b]	–	27.65	–

Notes: [a]CON. [b]Values are expressed as mean ± SD (range).
Abbreviations: CON, controls; MDD, major depressive disorder; M, male; F, female; BMI, body mass index; HAMD, Hamilton depression scale.

Ultimately, 74 differentially expressed proteins were identified ($p < 0.05$, unique peptide >1, 1.2-fold change; Figure 1A). Among these 74 significantly differentially expressed proteins, 11 showed upregulation and 63 downregulation (Table S2). The differential proteins in both sample groups were evaluated by hierarchical clustering analysis (Figure 1B) and classified by gene ontology (GO) categories of biological processes (Figure 1C). Functions of the differential proteins were mainly concentrated in three areas: metabolic process (GO: 0008152), cellular process (GO: 0009987) and biological regulation (GO: 0065007). Hence, significant changes in these biological functions may relate to disease development.

Bioinformatics analysis

To further investigate the function of these 74 differentially expressed proteins, IPA software was used. According to canonical pathway analysis, the top five statistically significant pathways were related to lipid transport, inflammation and immunity (Figure 2A). As the top-ranking canonical pathway, the liver X receptor (LXR)/retinoid X receptor (RXR) activation pathway is involved in the regulation of lipid metabolism, inflammation and cholesterol catabolism. This finding is also supported by our previous study.[24]

Thus, to explore the regulatory relationship between differential proteins and lipids, we uploaded significant lipid metabolites from LC–MS/MS analysis to the IPA database

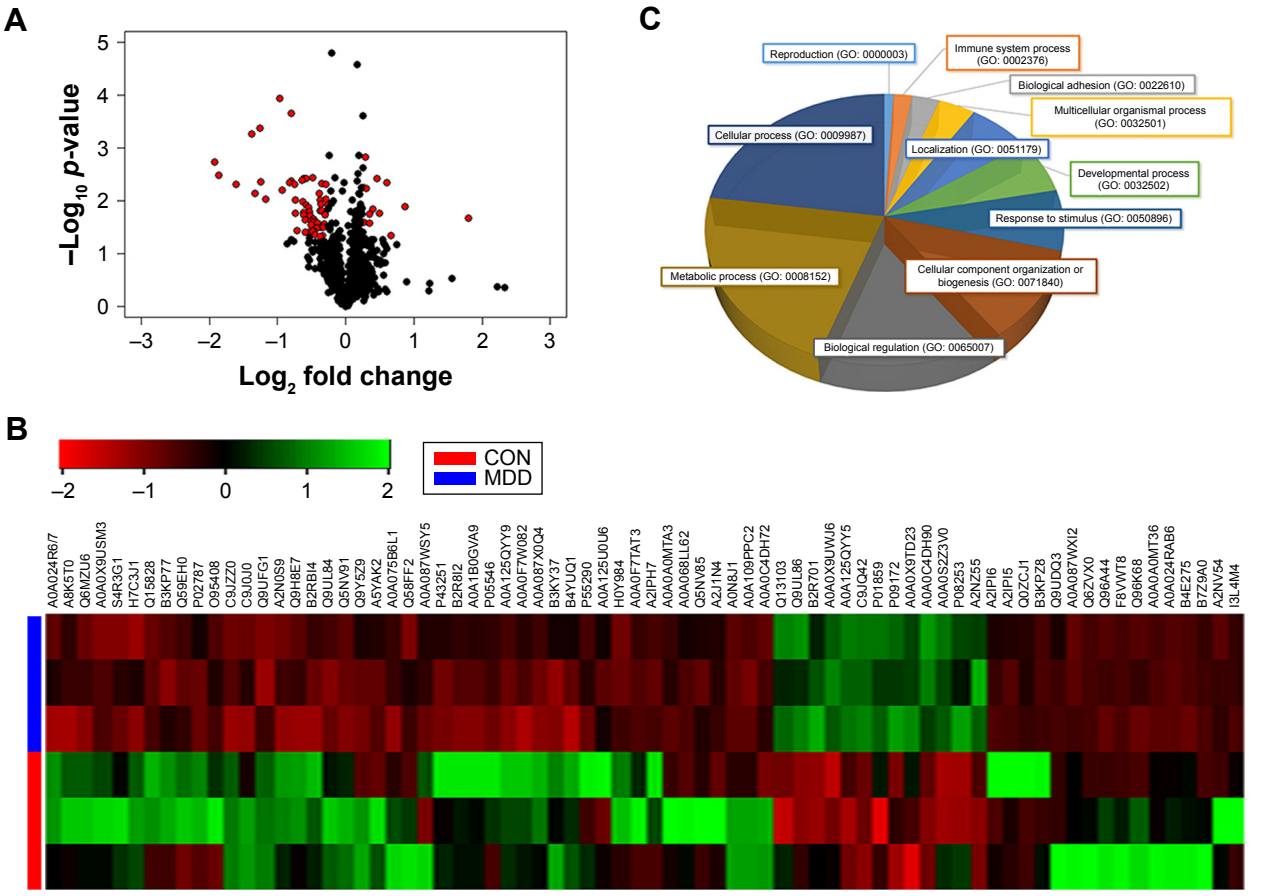

Figure 1 Differential proteins in patients with depression.
Notes: (**A**) The volcano plot of differential proteins. The red dots represent 74 differential proteins selected from 669 identified plasma proteins (*p*-value <0.05 and fold change >1.2). (**B**) Hierarchical cluster analysis of differential plasma proteins associated with MDD. Up- and downregulated proteins are indicated by green and red hues, respectively. The color intensity indicates the protein expressional level as displayed. Red and blue represent samples from healthy CON and depressed patients, respectively. (**C**) Functional protein classification using PANTHER (http://www.pantherdb.org)[42] with GO annotation. Proteins are classified under the ontology of biological process.
Abbreviations: MDD, major depressive disorder; CON, controls; GO, gene ontology.

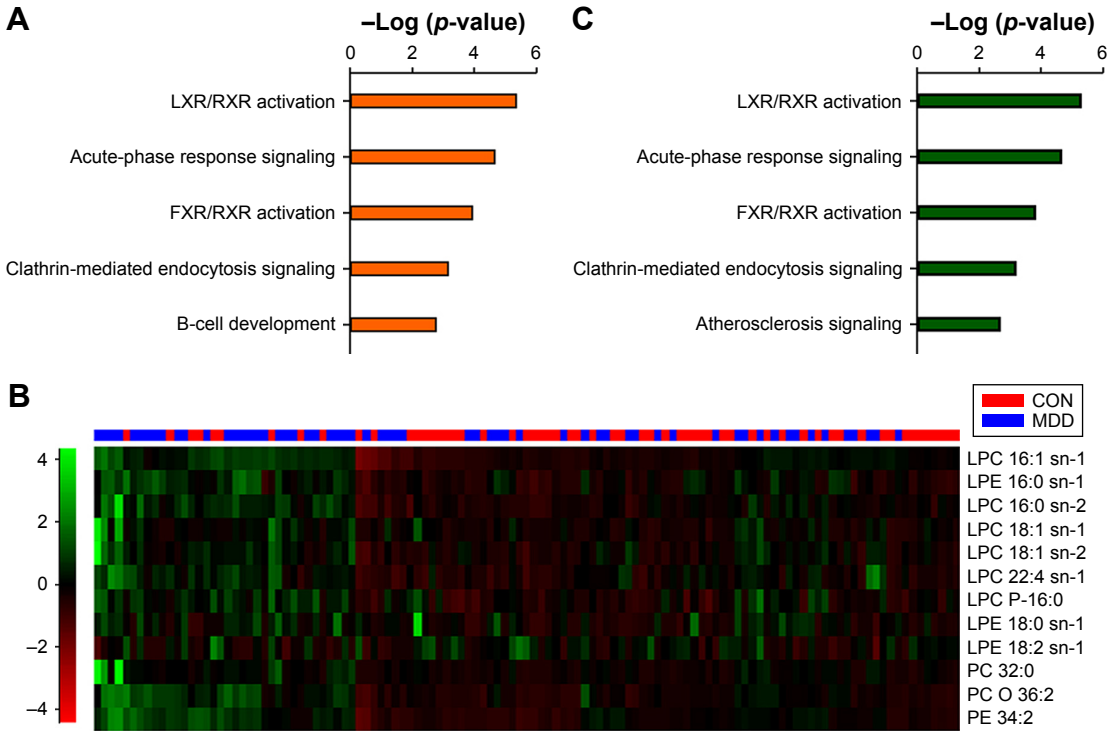

Figure 2 Integration analysis of proteomics and metabolites.
Notes: (**A**) Top five canonical pathways of differential proteins. (**B**) Heat plot of the differential lipids. Up- and downregulated proteins are indicated by green and red hues, respectively. (**C**) Top five pathways of combining differential proteins and metabolites. The significantly enriched pathways are almost identical.
Abbreviations: LXR, liver X receptor; RXR, retinoid X receptor; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; FXR, farnesoid X receptor; PC, phosphatidylcholine; PE, phosphatidylethanolamine.

and performed an integrative analysis based on a causal network.[18] The metabolites were classified into four categories: fatty acyls, amino acids, phospholipids and bile acids, alcohols and derivatives based on their chemical structure and properties (Table S3). Of these metabolites, phospholipids showed a general increase (Figure 2B).

By combining differential proteins and lipids, the top five significant pathways were highly similar to the pathways of differential proteins (Figure 2C). Network analysis identified a causal network of lipid metabolism that showed the interaction between proteins and lipids (Figure 3). In this network, apolipoprotein E (APOE) was the central node and showed downregulation (Figure 3). Furthermore, hemoglobin subunit beta (HBB), haptoglobin (HP), serotransferrin (TF), apolipoprotein A-V (APOA5), complement factor H (CFH), and immunoglobulin gamma (IgG) formed a downregulated co-expression pattern with APOE.

Discussion

This study is the first to combine the proteomics and lipid metabolomics data to investigate the pathophysiological mechanisms underlying MDD in human plasma. Our IPA analysis results show that LXR/RXR activation is the top-ranking pathway for combined differentially expressed proteins and lipids. Previously, we obtained a similar result in proteomics study of the chronic unpredictable mild stress (CUMS) mouse model of depression,[25] also finding a significant change in the LXR/RXR activation pathway. The RXR is a nuclear receptor that mediates the biological effects of retinoid. Moreover, RXRα is a dimeric partner of the type II nuclear receptor that contains LXR. Furthermore, LXR can be activated by oxysterol ligands and forms a heterodimer with RXR. Together, LXR and RXR are involved in lipid metabolism.[26] Notably, depression is highly related to disturbance of lipid metabolism, as found in chronic restraint stress (CRS)-treated rats[27] and also previously by us in blood from depressed patients.[21] Furthermore, our previous research in CUMS-treated mice also showed that proteins involved in lipid metabolism may simultaneously participate in the immune regulation process.[28] This suggests that disrupted lipid metabolism and neuroinflammation may coordinate together, leading to depression.

Additionally, due to their structural function, lipids may contribute to neuronal processes by influencing various signaling pathways directing neuronal survival or death.[29] Lipids play an important role in CNS as modulators of the redox state and inflammation. Certain lipids are downregulated in an experimental model of Parkinson's disease (PD) and,

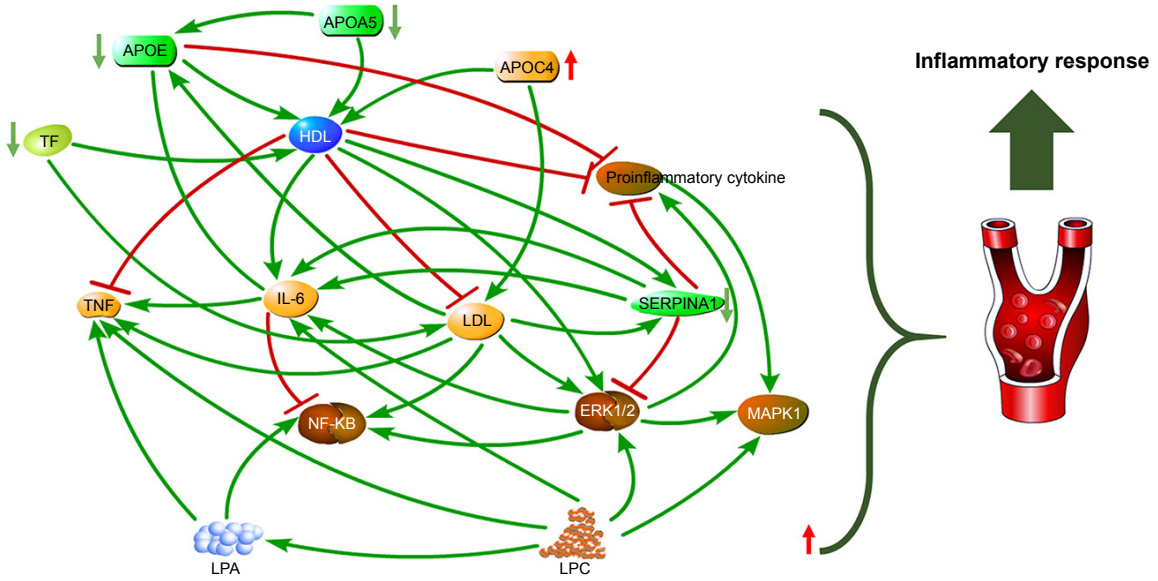

Figure 3 Plasma phospholipid metabolism disturbance in patients with depression.
Note: Downregulation of apolipoproteins leads to elevated LDL and decreased HDL, which in turn leads to increased lysophospholipid expression and increases in proinflammatory cytokines including IL-6 and TNF, resulting in inflammatory response.
Abbreviations: LDL, low-density lipoprotein; HDL, high-density lipoprotein; IL, interleukin; LPA, lysophosphatidic acid; LPC, lysophosphatidylcholine; SERPINA1, serine protease inhibitor; TF, serotransferrin; APOE, apolipoprotein E; APOA5, apolipoprotein A-V.

in particular, lysophosphatidylcholine (LPC; 16:0) and LPC (18:1), which are both important to neuroinflammatory signaling, appear to be upregulated.[30]

APOE is a key regulator of lipid metabolism. As the ligand for low-density lipoprotein (LDL) receptors, APOE is closely related to lipoprotein metabolism and immunomodulation. A growing body of research indicates that APOE is involved in many immunological processes, including inhibition of T-cell proliferation, regulation of macrophage function, promotion of lipid antigen presentation (via CD1) to natural killer T cells as well as modulation of inflammation and oxidation.[31,32] APOE is produced by macrophages, and its secretion has been shown to be confined to typical monocytes in peripheral blood mononuclear cells (PBMCs). Secretion of APOE by monocytes is downregulated by inflammatory cytokines and upregulated by transforming growth factor (TGF)-beta.[33] In the nervous system, non-neuronal cell types, most notably astroglia and microglia, are the primary producers of APOE, while neurons preferentially express the receptors for APOE.[34] Here, APOE was found to be downregulated in an integrative network (Figure 3). We could hypothesize that this is the result of astroglia and microglia dysfunction. As a part of the blood–brain barrier (BBB), astroglia dysfunction may lead to enhanced permeability of BBB. Consequently, more peripheral inflammatory factors may enter the brain causing neuroinflammation. On the other hand, the reduction of apolipoprotein-affected lipid transport in plasma may result in decreased high-density lipoprotein (HDL) and increased LDL.[35] HDL helps inhibit oxidation[36] and inflammation,[37] and promotes the platelet aggregation.[38] Therefore, the activation of oxidation and inflammation in MDD may be caused by the downregulation of HDL. Our results suggest that upregulation of the pro-inflammatory cytokine interleukin (IL)-6 may be caused by a general increase in lysophospholipids released by LDL. Lysophosphatides result from hydrolysis of phospholipids by enzymatic action of phospholipase A2. Indeed, LPC, lysophosphatidylethanolamine (LPE) and other hemolytic molecules can produce lysophosphatidic acid (LPA). The role of LPA in astrocytes suggests that LPA may alter the permeability of the BBB under physiological or pathological conditions.[39,40] This may involve increased mRNA levels in the entire proinflammatory network, as found in autopsied brain from patients with depression.[41] Additionally, LPA increases neurotoxicity of excitatory amino acids and inhibits glutamate uptake by glial cells. The downregulation of apolipoproteins leads to decreased HDL, which can accept and promote excretion of lysophospholipids. This process may promote these neurotoxic substances, which are more detrimental to the CNS.

There are several limitations to our study. First, the cohorts used in the proteomics and metabolomics analyses are different. Consequently, we used a method based on biological function of pathways for integra-

tion analysis instead of investigating the relationship between differential proteins and metabolites at the mathematical level. Second, iTRAQ and label free, as two very popular quantitative proteomic analysis methods, each has its own advantages and can complement each other. We only used an iTRAQ approach here, and the combination of these two methods should be considered in future studies. Third, our proteomics findings were not validated by a secondary method such as Western blot. It is worth noting that this study primarily combined proteomics and metabolomics analyses of plasma samples from patients with depression and healthy CON. Following bioinformatics analysis, phospholipid metabolism dysregulation was identified as significantly altered in patients with depression. Thus, the results showed links between differential proteins and metabolite biomarkers, and may provide novel insight into further research on antidepressants, such as potential treatment targets. Further study in this filed should include more investigation to validate regulations among molecules.

Conclusion

In this study, we used an iTRAQ-based quantitative proteomics approach to compare plasma protein expression between patients with depression and healthy CON. Altogether, we identified 74 differentially expressed proteins, mainly associated with dysregulation of lipid metabolism pathway. In particular, aberrant expression of apolipoproteins may play a role in the pathogenesis of depression. Moreover, combined analysis of differential proteins and metabolites suggests that disturbance of phospholipid metabolism coupled with abnormal expression of apolipoproteins may promote the inflammation in the CNS and ultimately lead to depression.

Acknowledgments

This work was supported by Major Scientific Instrument and Equipment Development Project of China (Grant No 2012YQ120044) and National Key R&D Program of China (Grant No 2017YFA0505700).

Disclosure

The authors report no conflicts of interest in this work.

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Supplementary materials

Table S1 Demographic and clinical features of recruited subjects of metabolomics analysis

Variable	CON (n = 59)	MDD (n = 60)	*p*-value
Age (years)[a]	38.28 ± 1.24	37.87 ± 1.02	0.458
Sex (M/F)	30/29	30/30	0.926
BMI[a]	22.25 ± 2.11	22.04 ± 3.00	0.691
HAMD[a]	–	25.03	–

Note: [a]Values are expressed as mean ± SD (range).
Abbreviations: CON, controls; MDD, major depressive disorder; M, male; F, female; BMI, body mass index; HAMD, Hamilton depression scale.

Table S2 Significantly differential proteins in depressed patients

Accession	Gene name	Ratio[a]	Two-tailed Student's *t*-test *p*-value[b]
B2RBI4	CTNNAL1	3.469	2.11E – 02
Q9Y5Z9	UBIAD1	1.818	1.28E – 02
Q5NV91	IGLV3-27	1.523	4.48E – 03
A0A075B6L1	IGLC7	1.412	1.69E – 02
B2R8I2	HRG	1.373	3.76E – 03
A5YAK2	APOC4	1.312	1.45E – 02
A0A1B0GVA9	RYR3	1.278	2.63E – 02
P05546	SERPIND1	1.268	1.76E – 02
Q58FF2	GRP94c	1.234	5.78E – 03
P43251	BTD	1.224	1.50E – 03
A0A087WSY5	CPB2	1.205	5.67E – 03
Q9UDQ3	CACNA2D1	0.828	1.21E – 02
Q96A44	SPSB4	0.817	9.24E – 03
A0A087WXI2	FCGBP	0.814	4.82E – 03
F8VWT8	BRF1	0.804	2.74E – 02
A0A0C4DH90	IGKV3OR2-268	0.798	3.16E – 02
C9JQ42	GYG1	0.791	4.51E – 02
P01859	IGHG2	0.781	1.55E – 02
H0Y984	BST1	0.772	1.53E – 02
P55290	CDH13	0.745	3.29E – 02
I3L4M4	GEMIN4	0.726	3.76E – 02
A0A024RAB6	HSPG2	0.718	2.20E – 02
A0A0S2Z3V0	APOE	0.714	3.63E – 03
A0A0A0MT36	IGKV6D-21	0.713	3.80E – 02
Q5NV85	IGLV3-12	0.705	2.45E – 02
C9JZZ0	APOA5	0.698	1.90E – 02
P09172	DBH	0.693	3.78E – 02
P08253	MMP2	0.691	1.61E – 02
Q13103	SPP2	0.668	1.22E – 02
B4YUQ1	TGM4	0.668	3.79E – 03
A0A0C4DH72	IGKV1-6	0.663	3.97E – 02
A0A0F7W082	HLA-A	0.597	9.76E – 03
O95408	HBB	0.593	4.87E – 03
A0A0F7TAT3	IGHV4-39	0.574	4.22E – 03
P02787	TF	0.574	2.25E – 04
A0A087X0Q4	IGKV2-40	0.567	4.60E – 03
A0A0A0MTA3	IGKJ5	0.524	6.24E – 03
Q15828	CST6	0.443	9.47E – 03
Q6MZU6	IGH	0.423	4.40E – 03
B3KP77	HP	0.418	4.20E – 04
A2N0S9	VH6DJ	0.650	1.64E – 02
Q9UFG1	DKFZp434I2115	0.640	4.02E – 03
A8K5T0	CFH	0.397	7.33E – 03

(*Continued*)

Table S2 (*Continued*)

Accession	Gene name	Ratio[a]	*t*-test *p*-value[b]
C9J0J0	CCDC126	0.384	5.42E – 04
A0A024R6I7	SERPINA1	0.274	3.26E – 03
A0A125QYY9		1.202	2.52E – 02
Q9UL84		1.578	4.51E – 02
B3KY37	DSE	0.511	1.15E – 04
H7C3J1	TSSK4	0.263	1.84E – 03
A0A0X9USM3		0.275	3.30E – 03
A2IPH7		0.653	3.75E – 03
A2NZ55		0.783	4.74E – 03
Q59EH0		0.327	4.96E – 03
Q96K68	SNC73	0.763	7.33E – 03
S4R3G1	MNX1	0.197	4.81E – 06
B4E275	PIGA	0.767	9.71E – 03
A0A109PPC2		0.645	1.05E – 02
A2IPI6		0.779	1.16E – 02
Q9UL86		0.684	1.32E – 02
A0A0X9TD23		0.831	1.44E – 02
B7Z9A0	GSN	0.599	1.70E – 02
A2IPI5		0.807	1.80E – 02
Q0ZCJ1		0.808	1.85E – 02
Q9H8E7		0.650	1.87E – 02
A0A0X9UWJ6		0.661	2.33E – 02
A0A125QYY5		0.734	2.50E – 02
A0A068LL62		0.722	2.61E – 02
A0A125U0U6		0.753	2.68E – 02
B2R701	PI16	0.705	2.87E – 02
B3KPZ8	TKT	0.830	3.05E – 02
Q6ZVX0		0.776	3.16E – 02
A2J1N4		0.609	3.73E – 02
A2NV54		0.732	4.39E – 02
A0N8J1		0.767	4.76E – 02

Note: [a]Values >1 indicate higher levels in depressed patients relative to healthy CON, and values <1 indicate lower levels in depressed patients relative to healthy CON. [b]The *p*-values were obtained by two-tailed Student's *t*-test.

Abbreviations: APOE, apolipoprotein E; APOA5, apolipoprotein A-V; HBB, hemoglobin subunit beta; TF, serotransferrin; HP, haptoglobin; CFH, complement factor H; SERPINA1, serine protease inhibitor; CON, controls.

Table S3 Significantly differential metabolites in depressed patients

ID	Name	Class	*p*-value[a]	VIP	Ratio[b]
HMDB02014	Carnitine C14:2	Fatty acyls	3.01E – 04	1.19	0.735
HMDB13331	Carnitine C14:3	Fatty acyls	2.83E – 04	1.12	0.799
HMDB00756	Carnitine C6:0	Fatty acyls	1.75E – 04	1.52	0.732
HMDB00791	Carnitine C8:0	Fatty acyls	2.75E – 05	1.7	0.667
HMDB13324	Carnitine C8:1	Fatty acyls	1.98E – 03	1.53	0.743
HMDB00651	Carnitine C10:0	Fatty acyls	8.39E – 05	1.8	0.702
HMDB00824	Carnitine C3:0	Fatty acyls	1.06E – 02	1.4	0.851
HMDB00696	L-methionine	Amino acid	1.34E – 05	1.91	0.876
HMDB00929	L-tryptophan	Amino acid	1.79E – 05	2.06	0.828
HMDB10383	LPC 16:1 sn-1	Phospholipids	1.75E – 04	1.8	1.248
HMDB11503	LPE 16:0 sn-1	Phospholipids	1.07E – 04	2.05	1.277
HMDB10382	LPC 16:0 sn-2	Phospholipids	4.61E – 03	1.36	1.066
HMDB10385	LPC 18:1 sn-1	Phospholipids	1.71E – 03	1.46	1.102
HMDB10408	LPC 18:1 sn-2	Phospholipids	1.19E – 03	1.57	1.17
HMDB10401	LPC 22:4 sn-1	Phospholipids	5.62E – 03	1.44	1.212
HMDB10407	LPC P-16:0	Phospholipids	7.97E – 04	1.88	1.233
HMDB11130	LPE 18:0 sn-1	Phospholipids	5.27E – 03	1.65	1.187
HMDB11507	LPE 18:2 sn-1	Phospholipids	2.41E – 03	1.67	1.231
HMDB00564	PC 32:0	Phospholipids	2.49E – 03	1.63	1.149
HMDB08001	PC 32:1	Phospholipids	2.07E – 04	1.57	1.334
HMDB13418	PC O 36:2	Phospholipids	2.50E – 05	2.02	0.828
HMDB09056	PE 34:2	Phospholipids	1.95E – 04	1.46	1.262
HMDB00626	Deoxycholate	Bile acids, alcohols and derivatives	6.91E – 06	1.29	0.551
HMDB00631	Glycodeoxycholic acid	Bile acids, alcohols and derivatives	2.21E – 04	1.66	1.693
HMDB00708	Glycoursodeoxycholic acid	Bile acids, alcohols and derivatives	4.85E – 03	1.37	1.379
HMDB00951	Taurochenodeoxycholate	Bile acids, alcohols and derivatives	1.73E – 02	1.12	1.255
HMDB00761	Lithocholic acid	Bile acids, alcohols and derivatives	1.17E – 04	1.46	0.559

Note: [a]The *p*-values were obtained by two-tailed Student's *t*-test. [b]Values >1 indicate higher levels in depressed patients relative to healthy CON, and values <1 indicate lower levels in depressed patients relative to healthy CON.

Abbreviations: LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; CON, controls; VIP, variable importance plot; PC, phosphatidylcholine; PE, phosphatidylethanolamine.