**Quantitative proteomic study of the plasma reveals acute phase response and LXR/RXR and FXR/RXR activation in the chronic unpredictable mild stress mouse model of depression**

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**Abstract.** Major depressive disorder is a severe neuropsychiatric disease that negatively impacts the quality of life of a large portion of the population. However, the molecular mechanisms underlying depression are still unclear. The pathogenesis of depression involves several brain regions. However, most previous studies have focused only on one specific brain region. Plasma and brain tissues exchange numerous components through the blood-brain barrier. Therefore, in the present study, plasma samples from control (CON) mice and mice subjected to chronic unpredictable mild stress (CUMS) were used to investigate the molecular pathogenesis of depression, and the association between the peripheral circulation and the central nervous system. A total of 47 significant differentially expressed proteins were identified between the CUMS and CON group by an isobaric tag for relative and absolute quantitation (iTRAQ) coupled with tandem mass spectrometry approach. These 47 differentially expressed proteins were analyzed with ingenuity pathway analysis (IPA) software. This revealed that the acute phase response, LXR/RXR and FXR/RXR activation, the complement system and the intrinsic prothrombin activation pathway were significantly changed. Four of the significant differentially expressed proteins (lipopolysaccharide binding protein, fibrinogen β chain, α-1 antitrypsin, and complement factor H) were validated by western blotting. The present findings provide a novel insight into the molecular pathogenesis of depression.

**Introduction**

Major depressive disorder (MDD), a severely debilitating mental disease, negatively affects the quality of life of a substantial percentage of the world population, and is associated with a lifetime morbidity of 4.4-20% (1). It is predicted that MDD will be the second most common illness in 2020, according to the 1990 Global Burden of Illness list. It is estimated that the economic burden of depression was $52.9 billion in 1990 and $83.1 billion in 2000 in the United States (2,3). Therefore, much attention has been given to depression research worldwide. Over the last few decades, many pathogenetic mechanisms have been put forward for MDD (4). However, the molecular mechanisms of MDD remain largely unknown. It is therefore urgent to elucidate the underlying mechanisms of MDD.

To gain insight into the pathogenesis of depression, our team previously used a proteomics approach on human plasma. However, the differential proteins in Xu et al (5) were different from those in Yang et al (6) and Song et al (7). A possible reason for the lack of concordance is variability in the factors that cause depression. The pathogenesis of depression may therefore differ among patients, accounting for the varying proteomic changes. Furthermore, some patients might have taken antidepressants that affect the metabolism of proteins in plasma (8). Therefore, we established a mouse model of chronic unpredictable mild stress (CUMS) to acquire more robust data (9). In this model, mice in the CUMS group are exposed to the same stressors at the same time. Both physical and mental stressors are given to imitate the risk factors that depressive patients encounter in daily life. Hence, the CUMS
model might well mimic human depression, and plasma from CUMS mice might help clarify the molecular changes in the disease.

MDD is a mental illness, and therefore, cerebrospinal fluid (CSF) and brain tissue samples might be better for proteomic studies investigating the molecular mechanisms of MDD; however, such samples are not practically accessible for living human subjects. In contrast, plasma samples can be obtained easily. Moreover, the pathogenesis of depression involves several brain regions, while most published papers only examine one region at a time (10-12). CSF, plasma and brain tissue exchange their molecular components through the blood-brain and CSF-brain barriers, which suggests that some proteins might be exchanged between the central nervous system and the peripheral circulation (13,14). Therefore, plasma samples might be better for examining the pathogenesis of depression (15).

Proteomics is a hypothesis-free approach, and it is a useful tool for discovering novel molecules involved in the pathogenesis of disease. It has been applied in a wide range of diseases, such as depression, schizophrenia and other psychiatric illnesses (16). In the present study, isobaric tags for relative and absolute quantitation (iTRAQ) was employed for identifying proteins differentially expressed between CON and CUMS mice. Furthermore, some of the proteins involved in the significantly changed pathways were validated by western blotting.

Materials and methods

Animals. A total of 40 adult male mice (8-10 weeks of age) were bought from Chongqing Medical University's animal facility. Unless indicated otherwise, the mice were maintained under standard conditions (12h-12 h light-dark cycle, lights on from 07:00 a.m. to 07:00 p.m.; temperature: 23±1°C; relative humidity of 40-60%; food and water available ad libitum). Chongqing Medical University's Ethics Committee approved all procedures, which were in accordance with the National Institutes of Health's Animal Research Guide. Efforts were made to reduce the number of deaths and to minimize suffering. (9).

CUMS protocol. The CUMS protocol was carried out according to our previously published papers, with some minor modifications. After adaptation and sucrose preference training, each lasting a week, mice were isolated into two groups: CUMS group (n=20) and CON group (n=20). The mice in the CUMS group were subjected to various repeated unpredictable mild stressors for 4 weeks, during which the sucrose preference test was performed. Minor stress sources included deprivation of water and food, paired housing, wet bedding, 45° cage tilt, night lighting, white noise, strobe, and odor exposure. All stressors were applied in a random order, and we did not repeat the same stressors over 2 consecutive days.

Behavioral test. The sucrose preference test (SPT) is one of the most important tests for evaluating depressive symptoms in mice. After 1 week of adaptation to the environment, each mouse was trained using two bottles of water for 1 week, one with 1% sucrose solution and the other with tap water. According to the baseline of sucrose preference, the mice were segregated into two groups with no significant difference. One group was exposed to CUMS stressors, while the other group was kept under standard conditions. Sucrose preference was measured starting at 8 am every Sunday morning for 24 h. During the test, the mice were placed in separate cages with equal access to the two bottles. The location of the two bottles was changed randomly to avoid position preference. The total weight consumed by the mouse was measured. Sucrose preference (SP) was calculated as follows: SP=(weight of 1% sucrose solution consumed/the weight of 1% sucrose solution consumed + the weight of water consumed) x 100%.

Open field testing was used to assess space exploration behavior. Before the test, the mice were placed in the experimental room for 30 min. The experiment was carried out in a soundproof room from 8 am to 1 pm. Only one mouse was placed in the open field test apparatus (44.5x44.5x45 cm) at a time, and allowed to freely explore the field for 6 min. The behavior was recorded and analyzed with an automatic video-tracking system (Smart, Panlab SL, Barcelona, Spain). The box was thoroughly cleaned with alcohol after each trial.

The forced swim test, also known as the behavioral despair test, which evaluates the rodent's response to the threat of drowning, was carried out in the experimental room from 8 am to 1 pm. In a separate test, the mouse was placed in the apparatus (height, 30 cm; diameter, 15 cm) for 5 min, and the pool was filled with tap water. The depth of the water was about 15 cm, and the water temperature was about 23±1°C. After each trial, the water was replaced with fresh water. Behavioral recording and analysis were performed using the Smart system mentioned above.

Immunodepletion of high abundance plasma proteins. Frozen plasma samples from the two groups (CON (n=20) and CUMS (n=20) were thawed, and equal-volume samples from six or seven mice (6,7) for each group were pooled to minimize the effect of individual variation (each group therefore generated three pooled samples). The pooled plasma samples were immunodepleted of high abundance plasma proteins using a Multiple Affinity Removal LC Column-Mouse 3 (Agilent Technologies, Inc., Santa Clara, CA, USA). The procedure was carried out according to the manufacturer's instructions. To evaluate the removal of high abundance proteins, samples of non-immunodepleted plasma and immunodepleted plasma were separated on 12.5% SDS-PAGE gels and then stained with Coomasie Blue (5,17).

iTRAQ labeling, and strong cation exchange (SCX) fractionation. After removal of high abundance proteins from plasma, the samples were quantified using the BCA assay. Then, 300-µg samples of total protein for each group were taken out for analysis. To these samples, 25 µl of SDT solution was added, and then DTT solution to a final concentration of 100 mM. These samples were then placed in a boiling water bath for 5 min and then cooled to room temperature. Thereafter, 200 µl of UA buffer (8 M urea, 150 mM Tris-HCl, pH 8.0) was added, and the samples were then centrifuged at 14,000 x g for 10 min. The supernatant was then added, and the samples were then centrifuged at 14,000 x g for 10 min. The supernatant was then added, and the samples were then centrifuged at 14,000 x g for 10 min. The supernatant was then added, and the samples were then centrifuged at 14,000 x g for 10 min.
30 min in a 30-kd ultrafiltration centrifuge tube. Subsequently, 200 µl of UA buffer was added to the samples and centrifuged at 14,000 x g for 30 min. After discarding the filtrate, 100 µl of IAA (50 mM IAA in UA) was added. The solution was shaken at 600 rpm for 1 min, followed by a 45 min incubation at room temperature. The samples were then centrifuged at 14,000 x g for 30 min. Thereafter, 100 µl of UA buffer was added to the concentrate and centrifuged at 14,000 x g, twice, 30 min each. After discarding the filtrate, 100 µl of 25 mM ABC was added, and the solution was centrifuged at 14,000 x g for 30 min. After discarding the filtrate, 40 µl of trypsin buffer (6 µg trypsin in 40 µl 100 mM ABC) was added. This solution was shaken at 600 rpm for 1 min followed by a 16-18-hour incubation at 37°C. Then, 40 µl of 25 mM ABC was added to the previous solution and centrifuged at 14,000 x g for 30 min. Subsequently, 0.1% TFA solution was added, and the OD280 was measured after desalting on a C18 cartridge (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany). Samples containing 100 µg of protein were labeled with the AB kit (iTRAQ Reagent-8plex Multiplex kit (AB Sciex, Foster City, CA, USA) according to the manufacturer's protocol (113, 114, 115 for the CON group; 116, 117, 118 for the CUMS group). The parameters used for SCX fractionation were as follows: AKTA Purifier 100 (GE Healthcare, Chicago, IL, USA), polysulfoethyl 4.6x100 mm column (5 µm, 200Å) (PolyLC Inc., Columbia, MD, USA), SCX Buffer A (10 mM KH2PO4 pH 3.0, 25% CAN), SCX Buffer B (10 mM KH2PO4 pH 3.0, 500 mM KCl, 25% CAN). The peptide fragments after iTRAQ labeling were mixed and subjected to SCX fractionation. For each test, 33 fractions were collected from each group and then combined into ten pools according to the SCX chromatogram, which were next desalted on a C18 cartridge (66872-U; Sigma-Aldrich; Merck KGaA) (9).

**Liquid chromatography-tandem mass spectrometry.** Each sample was separated using a nano-flow velocity HPLC liquid system, Easy nLC (Thermo Fisher Scientific, Inc., Waltham, MA, USA), and 0.1% formic acid aqueous solution (buffer A) and 0.1% formic acid acetonitrile aqueous solution (acetonitrile 84%) (buffer B) were used in the experiment. The column was equilibrated with 95% buffer A. Then, samples were loaded with the autosampler onto the Thermo Scientific EASY-column (2 cm x100 µm, 5 µm-C18) and separated on an analytical column (Thermo Scientific EASY-column, 75 µm x 100 mm, 3 µm-C18) at a flow rate of 300 nL/min. The liquid phase parameters were as follows: 0 to 55 min, buffer B from 0 to 50% with a linear gradient; 55 to 57 min, buffer B from 50 to 100% with a linear gradient; 57 to 60 min, buffer B maintained at 100%. After separation by capillary high performance liquid chromatography, each sample was analyzed by mass spectrometry using a Q-Exactive mass spectrometer (Thermo Finnigan, San Jose, CA, USA). The parameters were as follows: Analysis time, 60 min; detection method, positive ion; parent ion scanning range, 300-1,800 m/z; primary mass spectrometry resolution, 70,000 at m/z 200; AGC target, 3e6; primary maximum IT, 10 ms; number of scan ranges, 1; dynamic exclusion, 40.0 s. The mass/charge ratio of the fragments of the polypeptide and polypeptide were collected using the following parameters: MS2 activation type, HCD; isolation window, 2 m/z; secondary mass spectrometry resolution, 17,500 at m/z 200; microscans, 1; secondary maximum IT, 60 ms; normalized collision energy, 30 eV; underfill ratio, 0.1%. Subsequently, the original data was processed with Mascot 2.2 and Proteome Discoverer 1.4 software packages (Thermo Fisher Scientific, Inc.) for identification and quantitative analysis. The database was downloaded from Unipart (uniprot_mouse_78469_20150825.fasta, including 78649 series, downloaded on 2015-07-25). Peptide FDR was set at ≤0.01 (9).

**Ingenuity pathway analysis (IPA).** IPA software has been widely used in proteomics research (9,11). To determine the significant canonical pathways, networks of interacting proteins and models of functions and diseases, we uploaded the differentially expressed protein lists (with UniProt accession) and the directions of change of these proteins onto the IPA server (Qiagen, Inc., Valencia, CA, USA). All these analyses used Fisher’s exact test with P<0.5.

**Western blotting.** Frozen plasma samples were thawed and diluted 50 times using 1X PBS and 1X loading buffer. The volume of the loading buffer was one-fourth of the total volume. The proteins were denatured at 100°C for 10 min. Following SDS-PAGE, the proteins were transferred to PVDF membranes. After blocking in 5% non-fat milk power in TBST for 2 h at room temperature, the PVDF membrane was incubated for 10 h at 4°C with the following primary antibodies: Anti-hippocampal crossducible binding protein (LBP) antibody (1:500; Ruiying Biological, http://www.rlgene.com/), anti-fibrinogen β chain (FGB) antibody (1:1,000; Sangon Biotech Co., Ltd., Shanghai, China), anti-α-1 antitrypsin (SERPINA1) antibody (1:1,000; Abcam, Cambridge, UK), anti-complement factor H antibody (CFH) (1:500; Abcam). After three washes with THST, the membrane was incubated with anti-sheep or anti-rabbit secondary antibody (1:10,000) at room temperature for 2 h, and then the membrane was washed another three times with TBST (10 min each). The relative intensity of each protein was calculated with Quantity One software (version 4.6.7; Bio-Rad Laboratories, Inc., Hercules, CA, USA).

**Statistical analysis.** All of the data results were presented as mean ± standard deviation. Analysis of sucrose preference and body weight of mice was performed using ANOVA method. Behavioral data of the two groups of mice and the data of western blotting were analyzed by Student’s t-test. All data shown in this study were calculated by SPSS21.0 (IBM Corp., Armonk, NY, USA) used in our previous study. The threshold for statistical significance was set at P<0.05.

**Results**

**Assessment of the CUMS mouse model.** The CUMS mouse model was evaluated using the sucrose preference test (SPT), body weight measurement, the forced swim test (FST), and the open-field test (OFT). Given that the plasma samples used in this study were collected from the same batch of mice used in our previous publication (9), the results are only briefly described here. Body weight in the CUMS group was significantly lower than in the CON group after treatment (P<0.001).
Table I. Significant differentially expressed proteins identified by an iTRAQ coupled with LC-MS/MS method.

| UniProt accession | Gene symbol | Name | Unique peptides | CUMS/CON | t-test P-value |
|-------------------|-------------|------|----------------|---------|---------------|
| Q6LD55            | APOA2       | APOAII | 2              | 0.23    | 1.19E-03      |
| A2APX3            | CST3        | Cystatin-C (Fragment) | 2     | 0.44    | 3.28E-02      |
| Q9EQI5            | PPBP        | Chemokine (C-X-C motif) ligand 7, isoform CRA_b | 2    | 0.6     | 5.08E-03      |
| P98086            | C1QA        | Complement C1q subcomponent subunit A | 2 | 0.61    | 7.75E-04      |
| O55222            | ILK         | Integrin-linked protein kinase | 2     | 0.64    | 8.66E-03      |
| P23492            | PNP         | Purine nucleoside phosphorylase | 2     | 0.65    | 7.82E-03      |
| Q8CAG6            | PLEK        | Plekstrin | 2     | 0.67    | 9.63E-03      |
| Q5FW60            | MUP20       | Major urinary protein 20 | 2     | 0.66    | 7.91E-03      |
| Q8BP4F3           | N/A         | Putative uncharacterized protein | 3     | 0.67    | 1.69E-02      |
| A2AQ07            | TUBB1       | Tubulin β-1 chain | 2 | 0.67    | 6.90E-04      |
| P14106            | C1QB        | Complement C1q subcomponent subunit B | 5  | 0.69    | 3.03E-04      |
| P07310            | CKM         | Creatine kinase M-type | 4     | 0.7     | 1.86E-02      |
| Q02105            | C1QC        | Complement C1q subcomponent subunit C | 4  | 0.7     | 7.42E-03      |
| A7LNR1            | CD93        | CD93 antigen (Fragment) | 2     | 0.7     | 2.26E-02      |
| D3Z0Y2            | PRDX6       | Peroxiredoxin-6 | 3      | 0.71    | 8.35E-03      |
| A2AE89            | GSTM1       | Glutathione S-transferase Mu 1 (Fragment) | 2 | 0.71    | 8.21E-03      |
| Q8K0E8            | FGB         | Fibrinogen β chain | 32    | 0.71    | 2.34E-03      |
| P26039            | TLN1        | Talin-1 | 19     | 0.72    | 2.10E-03      |
| Q923D2            | BLVRB       | Flavin reductase (NADPH) | 6     | 0.72   | 4.08E-03      |
| P13634            | CA1         | Carbonic anhydrase 1 | 4     | 0.72    | 3.71E-03      |
| P16015            | CA3         | Carbonic anhydrase 3 | 3     | 0.74   | 3.81E-02      |
| B1AXY5            | B4GALT1     | β-1,4-galactosyltransferase 1 | 2 | 0.75    | 1.74E-02      |
| P61089            | UBE2N       | Ubiquitin-conjugating enzyme E2 N | 3  | 0.75    | 1.09E-02      |
| P32848            | PVALB       | Parvalbumin α | 3     | 0.75    | 2.21E-02      |
| P97336            | OBP1A       | Odorant binding protein 1a (Fragment) | 4  | 0.76    | 7.95E-04      |
| P06909            | CFH         | Complement factor H | 46    | 1.31    | 9.61E-03      |
| P31532            | SAA4        | Serum amyloid A-4 protein | 7  | 1.32  | 7.37E-03      |
| B2RXW7            | C4B         | Complement component 4B (Childo blood group) | 64 | 1.33   | 7.25E-03      |
| A1L3C5            | PRG4        | Prg4 protein | 4     | 1.34   | 4.24E-02      |
| P01027            | C3          | Complement C3 | 107   | 1.34   | 5.16E-03      |
| Q71KU9            | FGL1        | Fibrinogen-like protein 1 | 3     | 1.35   | 3.33E-03      |
| G3X8T9            | SERPINA3N   | Serine (Or cysteine) peptidase inhibitor, clade A, member 3N, isoform CRA_a | 18  | 1.37    | 2.41E-04      |
| Q03734            | SERPINA3M   | Serine protease inhibitor A3M | 7     | 1.39   | 2.97E-03      |
| Q8BJU6            | COL3A1      | Putative uncharacterized protein (Fragment) | 3  | 1.39    | 4.46E-02      |
| Q8VC7M3           | FGG         | Fibrinogen gamma chain | 31 | 1.41 | 5.87E-03      |
| Q9D8W4            | IGLV1       | β-2-microglobulin | 2     | 1.41   | 5.42E-03      |
| E9PV24            | FGA         | Fibrinogen α chain | 37    | 1.41   | 2.95E-03      |
| Q61805            | LBP         | Lipopolysaccharide-binding protein | 4  | 1.42    | 1.40E-02      |
| Q91X72            | HPX         | Hemopexin | 25    | 1.53   | 5.85E-04      |
The total distance traveled was not significantly different at baseline, but was significantly shortened after 28 days of CUMS (P<0.05). Compared to the CON group, immobility time was significantly increased (P<0.05) and sucrose preference was significantly lower in the CUMS group in the last week (P<0.05).

**Immunodepletion of high-abundance proteins.** To assess the efficacy of depletion, equal amounts of sample from each group were loaded onto a one-dimensional electrophoresis gel. After electrophoresis, the gel was stained with Coomassie Blue. More protein bands appeared after immunodepletion comparing with crude plasma samples (Fig. 1). This indicates that immunodepletion is an appropriate method for concentrating low abundance proteins.

**Plasma analysis by iTRAQ-based quantitative proteomics.** To identify differentially expressed proteins in CUMS mice compared with CON mice, 2D LC-MS/MS coupled with iTRAQ labeling was performed. As all samples from each group were mixed for experimentation, the main concern was systematic variation. It has been published that the iTRAQ approach for identifying and quantifying differentially expressed proteins on a large-scale entails at least a 30% technical variability (18). Therefore, a 1.3-fold change and unique peptides ≥2 threshold was used, as in our earlier study (5). Using this cut-off threshold, 47 proteins were found to be significantly differentially expressed between the CUMS group and the CON group. Among these 47 proteins, 22 were upregulated and the remainder were downregulated in the CUMS group (Table I).

**IPA analysis of the differential proteins.** To analyze the overall function of these 47 significantly expressed proteins, we uploaded them onto the IPA server. The top 5 canonical pathways were LXR/RXR activation, acute phase response signaling, FXR/RXR activation, complement system, and intrinsic prothrombin activation pathway (Table II). Four proteins related to these significantly changed canonical pathways were chosen for western blot validation. Additionally, IPA identified the following top five diseases and functions: Cell-to-cell signaling and interaction, developmental disorder, organismal injury and abnormalities, hereditary disorder, and immunological disease (Table III).

**Validation of differential proteins by western blotting.** Four significantly changed candidate proteins were chosen for validation using western blotting-lipopoly saccharide-binding protein, fibrinogen β chain, α-1 antitrypsin, and complement factor H. Compared with the CON group, α-1 antitrypsin and
lipopolysaccharide-binding protein were upregulated (P<0.05) (Fig. 2). In contrast, expression of fibrinogen β chain (P<0.05) was significantly reduced in the CUMS group (Fig. 2). These findings are concordant with our iTRAQ results. However, the expression levels of complement factor H were not significantly changed as assessed by western blotting (P>0.05) (Fig. 2), although they were significantly upregulated in CUMS mice by the iTRAQ method.

Discussion

The systemic response triggered by local inflammation, which can be seen in acute and chronic inflammation, is called the acute phase response. The function of acute phase proteins include opsonization, capturing microbes, complement activation, neutralizing enzymes and modulating immune responses. The acute phase proteins validated in this study included LBP, SERPINA1 and FGB, which are involved in the acute phase response pathway (Table II). LBP is used as a marker of a variety of inflammatory diseases and the development and prognosis of disease (19). Furthermore, depression and inflammation are closely related (20). LBP can also affect the innate immune function and Toll-like receptor 4 (21,22), which are involved in the pathogenesis of depression (23). Thus, LBP might be involved in the pathogenesis of depression. LBP is also associated with other
psychiatric disorders, such as Parkinson’s disease (24), and therefore, LBP may be closely related to central nervous system functions. The concentration of SERPINA1 in the plasma of depressed patients is increased (25), consistent with our results. SERPINA1 also has a relationship with the immune system, the apoptotic process and the inflammatory response (26). The concentration of FGB is reduced in the platelets of depressed patients (27), also consistent with our results. This suggests that FGB is closely related to the occurrence and development of depression. The plasma concentration of FGB is also abnormal in Rett syndrome (28), and Rett syndrome is a unusual genetic postnatal neurological disease negatively affecting the grey matter of the brain. Thus, FGB may be closely related to central nervous system functions.

Retinoid X receptors (RXRs) are nuclear receptors that mediate the biological effects of retinoids. RXRα is the dimerization partner for the type II nuclear receptors that include the liver X receptor (LXR). The LXR is activated by oxysterol ligands and forms a heterodimer with RXR. After the heterodimer is formed, LXR initiates the transcription of the target gene by binding to the LXR response element. LXR/RXR are involved in lipid metabolism, the inflammatory response and cholesterol and bile acid metabolism (29). Previous studies suggest that abnormalities in these processes lead to the development of depression (9,20). The farnesoid X receptor (FXR) is a member of the nuclear receptor family and is a key player in many metabolic pathways. FXR is activated by bile acids and their intermediates, which thus serves as a sensor of bile acid levels. Along with the retinoid X receptor (RXR), FXR plays a key role in linking bile acid regulation with lipoprotein and lipid and glucose metabolism (30). Energy metabolism is dysregulated in depressed patients and in the CUMS mouse model (5,10,11). Perturbations of the

Table III. Significant differentially changed diseases and functions with uploaded proteins (top 10).

| Category                              | P-value     | Molecules                                                                 |
|---------------------------------------|-------------|----------------------------------------------------------------------------|
| Developmental disorder                | 1.43x10^-3.95x03 | TUBB1, C3, ILK, C1QA, C1QC, HBA1/HBA2, C1QB, SERPINA7, FGG, C4A/C4B, CA3, HP, B4GALT1, CST3, APCS, PNP, SERPINA1, FGB, FGA, CA1, COL3A1 |
| Hereditary disorder                   | 1.43x10^-3.95x03 | APOA2, LRG1, C1QA, SERPINA3, C1QC, HBA1/HBA2, C1QB, SERPINA7, PRDX6, FGG, C4A/C4B, CA3, CST3, APCS, FGB, SERPINA1, CFH, CA1, TUBB1, PVALB, C3, HP, B4GALT1, PNP, FGA, COL3A1 |
| Immunological disease                 | 1.43x10^-3.95x03 | TUBB1, HPX, C3, C1QA, C1QC, HBA1/HBA2, C1QB, PRDX6, FGG, C4A/C4B, CA3, HP, B4GALT1, CST3, APCS, PNP, SERPINA1, FGB, PLEK, LBP, CFH, FGA, CA1, COL3A1 |
| Organismal injury and abnormalities   | 1.43x10^-3.95x03 | ITIH3, GSTM5, UBE2N, APOA2, LRG1, ILK, C1QC, TLN1, SERPINA3, C1QA, HBA1/HBA2, C1QB, SERPINA7, PRDX6, FGG, C4A/C4B, CA3, APCS, CST3, SERPINA1, FGB, LBP, CFH, CA1, PVALB, TUBB1, HPX, C3, KMK, CD93, BLVRB, HP, B4GALT1, PNP, FGL1, PLEK, FGA, COL3A1 |
| Cell-To-cell signaling and interaction| 6.08x10^-3.95x03 | C3, Ppbp, UBE2N, APOA2, CD93, ILK, C1QA, TLN1, FGG, C4A/C4B, B4GALT1, APCS, CST3, PNP, FGB, SERPINA1, PLEK, LBP, CFH, FGA, COL3A1 |
| Hematological system development and function | 6.08x10^-3.95x03 | UBE2N, APOA2, ILK, C1QC, TLN1, SERPINA3, C1QA, HBA1/HBA2, FGG, C4A/C4B, APCS, CST3, FGB, SERPINA1, CFH, LBP, HPX, C3, Ppbp, CD93, HP, B4GALT1, PNP, PLEK, FGA |
| Immune cell trafficking               | 6.08x10^-3.95x03 | C3, Ppbp, UBE2N, CD93, ILK, C1QA, TLN1, SERPINA3, FGG, C4A/C4B, HP, B4GALT1, CST3, APCS, FGB, SERPINA1, CFH, LBP, FGA |
| Inflammatory response                 | 7.5x10^-3.95x03 | TUBB1, HPX, C3, KMK, APOA2, UBE2N, CD93, ILK, SERPINA3, TLN1, C1QA, HBA1/HBA2, PRDX6, FGG, C4A/C4B, CA3, HP, B4GALT1, APCS, CST3, PNP, SERPINA1, FGB, PLEK, CFH, LBP, FGA, CA1, COL3A1 |
| Cellular movement                     | 6.12x10^-3.95x03 | C3, Ppbp, CD93, ILK, TLN1, SERPINA3, PRDX6, C4A/C4B, HP, B4GALT1, APCS, CST3, SERPINA1, FGB, CFH, LBP, FGA, COL3A1 |
| Hematological disease                 | 7.18x10^-3.95x03 | TUBB1, C3, KMK, APOA2, CD93, C1QA, HBA1/HBA2, FGG, C4A/C4B, HP, SERPINA1, FGB, FGL1, LBP, PLEK, CFH, FGA |

P-value: Fisher’s exact test; Molecules: The uploaded proteins mapped to relevant diseases and functions. The uploaded proteins involved in the Cell-To-Cell Signaling and Interaction are shown in Fig. 3 produced by IPA.
LXR/RXR and FXR/RXR pathways may be involved in the development of depression (31,32). In this study, according to the IPA, iTRAQ data and western blotting validation, LBP is involved in LXR/RXR activation (Table II), in accordance with a previous study (33). SERPINA1 is also related to the LXR/RXR and FXR/RXR pathways (Table II). Thus, LBP and SERPINA1 might be responsible for the occurrence and development of MDD via LXR/RXR and FXR/RXR pathways.

According to IPA analysis and previous studies, LBP is also involved in IL-10 signaling (34). FGB is related to the coagulation system, the extrinsic prothrombin activation pathway, and role of tissue factor in cancer (35). SERPINA1 participates in the coagulation system (36), atherosclerotic signaling (37), and IL-12 signaling and production in macrophages (Table II) (38). This suggests that these pathways likely contribute to the development of depression.

The various changes in proteins and pathways in the CUMS model might ultimately lead to a depressive state. In this study, the first five significantly changed functions and diseases identified by IPA were cell-to-cell signaling and interaction, developmental disorder, organismal injury and abnormalities, hereditary disorder, and immunological disease (Table III). These results suggest that depression is also related...
to structural abnormalities in the central nervous system (39) as well as intercellular interaction and signal transmission (Fig. 3). Therefore, synaptic neurotransmission might be perturbed in CUMS mice.

However, the expression levels of factor H were not significantly changed as assessed by western blotting, although they were significantly upregulated in CUMS mice by the iTRAQ method. A similar phenomenon has also been observed in other previous studies (5,6). The variability of dynamic range between iTRAQ and western blotting and the internal differences related to the steps of iTRAQ coupled with tandem mass spectrometry approach and western blotting analysis may lead to this discrepancy.

These results suggest that the CUMS mouse model is a suitable model of depression, and that peripheral plasma samples can, at least to an extent, provide some biomarkers of depression. The three significantly differentially expressed proteins (FGB, SERPINA1, LBP) are worthy of further studies on the molecular mechanisms of depression.

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