A Metabolomics–based Approach for Ranking the Depressive Level in Chronic Unpredictable Mild Stress Rat Model

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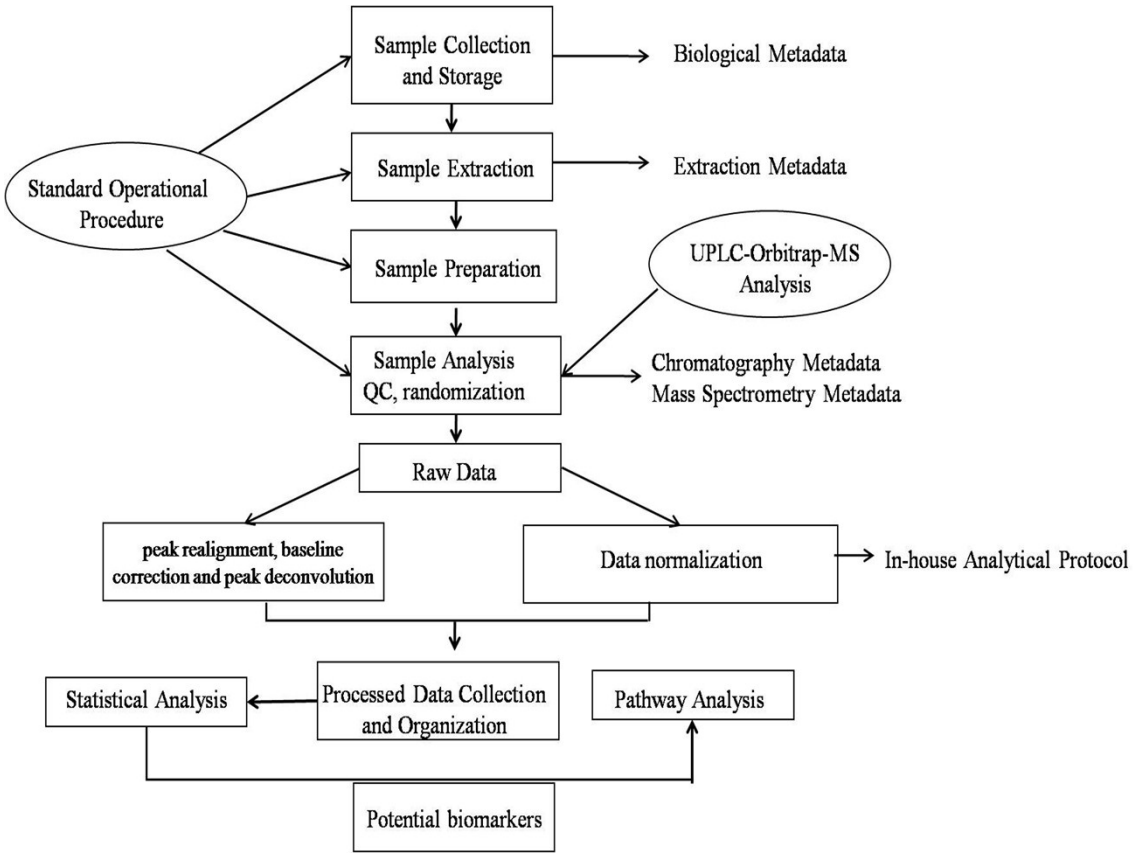
The experimental procedure of entire work



Biochemical interpretation for the remaining metabolites in plasma, hippocampus and PFC samples

1. Amino acids metabolism

The significant alteration of several branched chain amino acids (BCAAs) were also noticed in model group. In this study, the level of BCAAs including isoleucine (Ile) and valine (Val) was down-regulated in hippocampus after CUMS procedure compared to that of control group. BCAAs had been proved critical to human life and they were particularly involved in stress, energy and muscle metabolism[1, 2]. Recent studies showed that Ile help to form glutamine astrocytes to maintain the nitrogen balance in brain[3, 4]. It could be transported through the blood–brain barrier and participated in the synthesis of glutamate by providing amino group. When combined with glutamine, the glutaminyl-isoleucine complex proved to be involved in the control of locomotivity. The locomotivity of rats which received glutaminyl-isoleucine complex injection in ventral tegmental area had been greatly improved which was partly due to the alteration of transmit afferent glutamatergic projections to PFC induced by glutaminyl-isoleucine[5]. Based on the behavioral results especially for OPT, a decrease of locomotivity was observed due to the metabolic disturbance of Ile. Moreover, BCAAs also participated in the anabolism, energy metabolism and the regulation of blood glucose[3]. BCAAs also shared the same carrier-mediated transfer system with serotonin so the abnormal level of BCAAs could had an impact on the concentration of serotonin which was closely related to MDD. Ile was capable to participate in the biosynthesis of both carbohydrates and fats which suggested it was also involved in the perturbance of energy metabolism. Generally, these results indicated that MDD was a complicated psychiatric disorder in which lots of metabolic pathways were involved and metabolomics study could provide new thoughts of MDD from an overall perspective so as to deepen the understanding for it.

Glycine (Gly) was a simple, nonessential amino acid which acted as a coagonist with aspartic acid (Asp) in stimulation of NMDA receptors. Contrary to Asp which functioned as an excitatory neurotransmitter, Gly was another kind of inhibitory neurotransmitter and the regulation for the dynamic balance between the level of Asp and Gly was vital for NMDA receptors to perform normal function[6]. The decreased level of Gly along with the evaluation level of Asp might due to the parafunction of NMDA receptor-mediated neurotransmission. Besides, as a non-essential amino acid, the change of digestive and absorptive capacity along with the loss of appetite might be respond for the significant reduction of Gly which was observed in our study.

Beta-alanine was formed in vivo by the degradation of dihydrouracil and carnosine. It was a component of the naturally occurring peptides carnosine, anserine and pantothenic acid which was related to the biosynthesis of coenzyme A (CoA)[7]. As was mentioned above, NAA was synthesized from aspartic acid and acetyl-coA, and there was a metabolic disturbance of NAA in model rats which was also related to the changing level of beta-alanine.

Moreover, intracerebral ventricle administration of beta-alanine could induce a significantly inhibited exploratory behavior in animal model which was consistent with our results in OPT[8]. Therefore, these results indicated that the dysregulation of beta-alanine was also a factor which would induce depressive-like behaviors in CUMS model.

Lysine (Lys) was an essential amino acid and the deficiency of Lys was responsible for immunodeficiency and the requirement for it was increased when experiencing stress. The significant reduction of Lys level had been found in patients suffering Parkinson's disease and MDD[9, 10]. Besides, when received lysine therapy, it could normalize the level and had been associated with improvement of some patients with these conditions. Some researchers believed that the deficiency of Lys had an impact on the release of serotonin in amygdala and norepinephrine in hypothalamus. However, the exact mediating mechanism remained unclear, so it was necessary to further look into the function of Lys in MDD on this basis which might provide a new research focus for MDD in the future.

2. Energy metabolism

Blood derived glucose is the major source for energy metabolism in the brain, and the metabolic disturbance of glucose in blood has been proved to be related with some central nervous system (CNS) disease including schizophrenia and bipolar disorder[11, 12]. It had been observed that the level of glucose and sorbitol which was synthetically produced from glucose was decreased, suggesting there was a perturbance in the glucose metabolism through sorbitol pathway[13]. Besides, the concentration of citric acid and succinic acid were significantly decreased, and there were another two key metabolites involved in the citric acid cycle which was closely associated in the energy metabolism. Previous research had found a deficiency for peripheral concentration of glucose in MDD patients[14], worse still, and both the turbulence of glucose metabolism could greatly affect the energy metabolism and the energy deficiency would lead to symptoms like exhaustion and fatigue which were common in depressive patients.

Glycerol was an important component of triglycerides (i.e. fats and oils) and of phospholipids. It was a three-carbon substance that formed the backbone of fatty acids in fats. When the body used stored fat as a source of energy, glycerol and fatty acids were released into the bloodstream. The glycerol component was capable to be converted to glucose by the liver and provides energy for cellular metabolism. In conjunction with the observed lipids metabolic disturbance in this study, we could infer that the abnormal level of glycerol was involved in the perturbation for both energy metabolism and lipids metabolism.

3. Lipid metabolism

The remarkably changed level of several lipid species including PCs, LPCs, LPEs, linolenic acid and

dihydroxyacetone phosphate was observed in CUMS model rats which suggested that the pathogenesis of MDD may be associated with the abnormalities in lipid metabolism. Linolenic acid was a kind of polyunsaturated fatty acid which functioned as the precursor of the principal omega-6 polyunsaturated fatty acid arachidonic acid. Arachidonic acid could participate in the regulation of neuronal signal transduction as a second messenger. The lower level of linolenic acid indicated an association of MDD with abnormality in fatty acid metabolism. DHAP was an important intermediate in lipid biosynthesis and ATP synthesis, therefore, the decreased level of DHAP might indicate a decreased generation of ATP in model rats. Some studies had pointed out the disturbances of lipids metabolism was closely associated with the CNS diseases such as MDD or Alzheimer's disease [15, 16] . By the hydrolysis action of phospholipase A2, the lysophospholipids could be biosynthesized from phosphoglycerides. Researchers had explored the relationship between phospholipase A2 gene and the pathogenesis of bipolar disorder and found the level of activity of phospholipase was increased in the serum of depression subjects[17]. The evaluated concentration of LPCs was probably related to the oxidative stress via the 5-lipoxygenase pathway, and the enhanced oxidative stress induced by LPCs could act on endothelial cells and lead to some diseases including metabolic syndrome, cardiovascular and cerebrovascular diseases[18, 19]. Moreover, a lipidomics indicated a high correlation between the concentration of PC-O in plasma and depressive symptoms. Plasmalogen which functioned as an antioxidant to defend oxidative stress could be synthesized in the desaturation of alkyl-acyl PCs and PEs, and therefore, the results indicated the enhanced oxidative stress and reduced antioxidation ability were involved in the pathogenesis of MDD. Additionally, the disturbed metabolism of some unsaturated fatty acids such as docosahexaenoic acid (DHA) and 3-hydroxy-hexadecanoic acid (HHA) which acted as the important constituent of cytomembranes also had been observed in CUMS model. For DHA, it was one of the most common omega-3 essential fatty acids in brain phospholipids and it had been reported that the reduction level of DHA could finally result in a decrease for serotonin which was closely related to MDD[20]. DHA was a major component of neuron membranes in the brain which played a key role in the development of brain cell especially for the formation of synapse. It was able to keep the normal nerve function and metabolism activity and was also involved in the formation of memory. Study suggested that DHA could protect neuron from damage and down-regulate the expression of caspase-3 protein which proved to be a hallmark of apoptosis[21]. In this study, the decreased level of DHA in model rats had been observed which was correspond with previous study which indicated a metabolic disorder for DHA after CUMS procedure. Besides, HHA was an intermediate in fatty acid biosynthesis which was converted from 3-oxo-tetradecanoic acid via fatty-acid synthase and 3-oxoacyl- acyl-carrier-protein reductase. The reduction of both DHA and HHA indicated that the CUMS procedure could further affect the fatty acid

metabolism.

4. Antioxidant metabolism

The level of azelaic acid which could inhibit the generation of reactive oxygen species (ROS) via the inhibition of enzyme activity for NADPH oxidase was significantly lower in the plasma of CUMS model rats. Studies have shown that MDD had a close relationship with the increased oxidative stress[22, 23], and this may partly due to the down-regulation of azelaic acid. The reduction of azelaic acid in plasma was likely to indicate a decline in the function of antioxidant defense system which could finally cause the increased oxidative stress.

Myo-inositol was a cyclic polyalcohol which functioned as a second messenger in the metabolism of phosphatidylinositol and involved in the synthesis of membrane inositol phosphates. It was also mainly in the astrocyte and played an important role in the cellular signal transduction in phosphatidylinositol cycle astrocyte. Besides, it was one of the most important osmoregulators and had a crucial role in trophic function of cells and antioxidation. Abnormal level of myo-inositol and the metabolism of phosphatidylinositol could probably be associated with the pathophysiology of psychiatric disorders. An evaluation of plasma myo-inositol was observed in model group, and it was regulated by the antidepressive treatment which suggested abnormal level of myo-inositol and the metabolism of phosphatidylinositol could probably be associated with the pathophysiology of MDD.

Supplementary Information Table.1

S-Table.1 Stressor weekly schedule

Time	Stressor	Lasting period
Monday	Cage cleaning, changing the soiled padding and weighing the rats	8:00
	Food and water deprivation	13:00
Tuesday	Sucrose preference test	9:00-11:00
	Paired housing	15:00-8:00
Wednesday	Single housing	8:00
	Soiled cage	15:00-8:00
	White noise	20:00-23:00
Thursday	Cage cleaning and changing the solid padding	8:00
	Food deprivation	8:00-20:00
	Cage tilting	20:00-8:00
Friday	Water deprivation	8:00-20:00
	Stroboscopic illumination	20:00-23:00
	Cage tilting	23:00-11:00
Saturday	Paired housing	8:00-20:00
	Overnight illumination	20:00-8:00
Sunday	Soiled cage	15:00-8:00
	White noise	12:00-15:00

Supplementary Information Table.2

S-Table.2 Food consumption and body weight of rats during the period of chronic stress

Week	Item (g)	Control	Model	Treat
5	Food Consumption	215.0±11.6	210.1±8.2	220.5±13.4
	Body weight	291.7±17.8	308.2±26.7	302.3±22.7
8	Food Consumption	231.6±15.2	199.6±17.2*	204..9±9.5*
	Body weight	435.8±24.7	361±28.8*	374.9±36.3*
12	Food Consumption	243.6±10.6	210.5±7.9*	231.7±13.7
	Body weight	498.2±35.7	417.5±29.5**	450.1±41.3

Supplementary Information Table.3

S-Table.3 Retention time shift of identified metabolites in plasma

No	Metabolite	t_R (min)	Shift (min)
1	L-Phenylalanine[a]	2.53	0.06
2	Gamma-Aminobutyric acid[a]	7.04	0.11
3	L-Tryptophan[a]	4.54	0.14
4	Glycine[a]	0.91	0.02
5	Dopamine[a]	1.08	0.04
6	Beta-Alanine[a]	6.92	0.09
7	Azelaic acid[a]	1.51	0.03
8	Myoinositol[a]	0.67	0.02
9	Glucose[a]	0.65	0.01
10	L-Kynurenine[a]	5.85	0.05
11	Hypoxanthine[a]	0.65	0.02
12	Glycerol[a]	0.94	0.01
13	Quinolinic acid[a]	0.94	0.02
14	Kynurenic acid[a]	0.69	0.02
15	N-Acetyl-L-aspartic acid[a]	4.28	0.05
16	Citric acid[a]	1.08	0.02
17	Sorbitol[a]	5.33	0.10
18	Corticosterone[a]	6.85	0.15

198 Supplementary Information Table.4

199 **S-Table.4** Retention time shift of identified metabolites in hippocampus

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No	Metabolite	t_R (min)	Shift (min)
1	L-Tryptophan[a]	4.46	0.06
2	L-Tyrosine[a]	1.04	0.02
3	Citric acid[a]	1.02	0.04
4	L-Valine[a]	5.22	0.07
5	N-Acetyl-L-aspartic acid[a]	4.43	0.10
6	L-Kynurenine[a]	5.96	0.06
7	Quinolinic acid[a]	0.90	0.02
8	Inosine[a]	1.87	0.05
9	Glutathione[a]	0.98	0.02
10	3-Hydroxy-hexadecanoic acid[a]	5.07	0.09
11	L-Isoleucine[a]	1.23	0.03
12	L-Phenylalanine[a]	2.50	0.03
13	Myoinositol[a]	0.62	0.02
14	Gamma-Aminobutyric acid[a]	6.90	0.03
15	Glycerol[a]	0.92	0.01
16	L-Serine[a]	1.72	0.04
17	Homocysteine[a]	1.98	0.05
18	Succinic acid[a]	1.42	0.04
19	Beta-Alanine[a]	7.01	0.18

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228 Supplementary Information Table.5

229 **S-Table.5** Retention time shift of identified metabolites in PFC

No	Metabolite	t_R (min)	Shift (min)
1	Kynurenic acid[a]	0.73	0.02
2	L-Tryptophan[a]	4.46	0.06
3	Dopamine[a]	0.96	0.08
4	Quinolinic acid[a]	0.99	0.07
6	Inosine[a]	1.90	0.06
7	Linolenic acid[a]	8.99	0.23
8	L-Dopa[a]	0.75	0.02
9	5-Hydroxyindoleacetic acid[a]	0.81	0.02
10	N-Acetyl-L-aspartic acid[a]	4.45	0.12
11	Docosahexaenoic acid	5.78	0.17
12	L-Isoleucine[a]	1.24	0.04
13	Gamma-Aminobutyric acid[a]	7.09	0.16
14	Homovanillic acid[a]	0.68	0.02
15	L-Kynurenine[a]	5.98	0.08
16	L-Phenylalanine[a]	2.52	0.05
17	L-Lysine[a]	5.27	0.13
18	Tyramine[a]	9.65	0.20
19	L-Tyrosine[a]	1.05	0.03
20	L-Aspartic acid[a]	1.14	0.04

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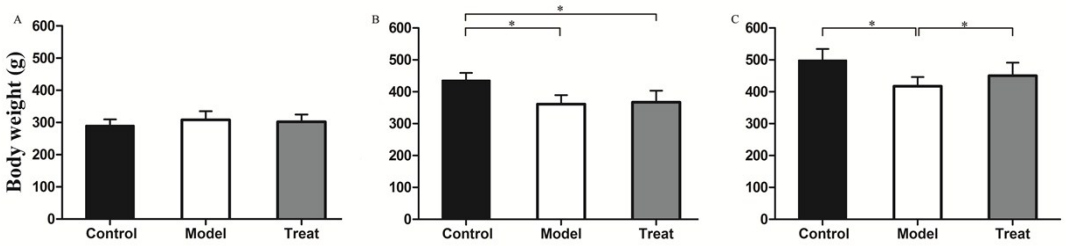
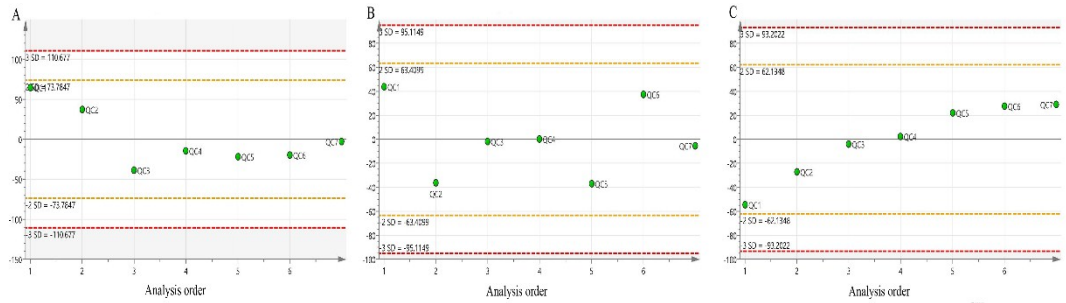
Supplementary Information Fig.1



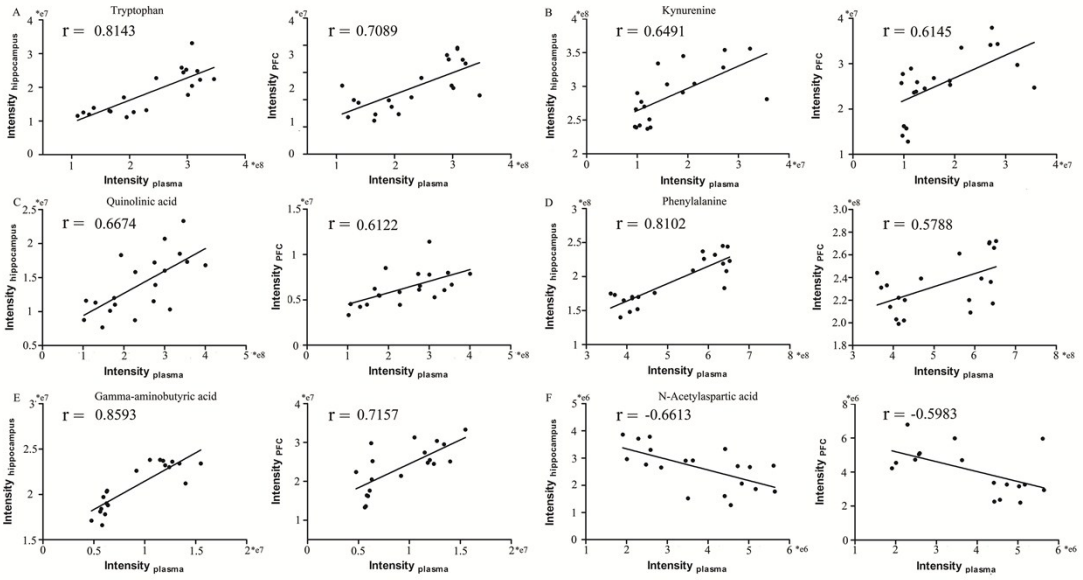
S-Fig.1 The body weight of baseline (a), week3 (b) and week7 (c) for control, model and treated group.Data are represented as mean±SD. * means a statistically significant difference at $p<0.05$.

Supplementary Information Fig.2



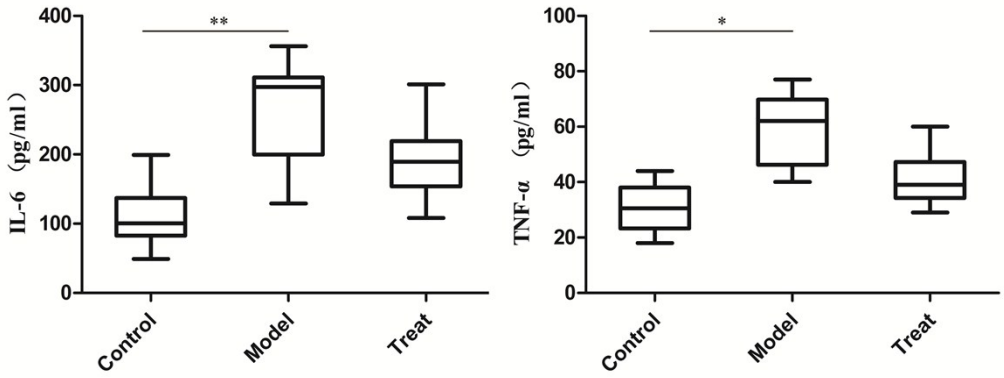
S-Fig.2 The PCA score plot of each QC sample in plasma, hippocampus and PFC.

Supplementary Information Fig.3



S-Fig.3 The binary logistic regression plot for the simplified panel between plasma, hippocampus and PFC

Supplementary Information Fig.4



S-Fig.4 The box plot of IL-6 and TNF-α in the plasma of rats.

** means a statistically significant difference at $p<0.01$, * means a statistically significant difference at $p<0.05$.

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