Metabolomic Biomarkers Related to Non-suicidal Self-injury in Patients with Bipolar Disorder

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Research Article

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Abstract

Background: Non-suicidal self-injury (NSSI) is an important symptom of bipolar disorder (BD) and other mental disorders and has attracted the attention of researchers lately. Metabolomics is a relatively new field that can provide complementary insights into data obtained from genomic, transcriptomic, and proteomic analyses of psychiatric disorders. The aim of this study was to identify the metabolic pathways associated with BD with NSSI and assess important diagnostic and predictive indices of NSSI in BD.

Method: Nuclear magnetic resonance spectrometry was performed to evaluate the serum metabolic profiles of patients with BD with NSSI (n = 31), patients with BD without NSSI (n = 46), and healthy controls (n = 10). Data were analyzed using an Orthogonal Partial Least Square Discriminant Analysis and a t-test. Differential metabolites were identified (VIP > 1 and $p < 0.05$), and further analyzed using Metabo Analyst 3.0 to identify associated metabolic pathways.

Results: Eight metabolites in the serum and two important metabolic pathways, the urea and glutamate metabolism cycles, were found to distinguish patients with BD with NSSI from healthy controls. Eight metabolites in the serum, glycine and serine metabolism pathway, and the glucose-alanine cycle were found to distinguish patients with BD without NSSI from healthy controls. Five metabolites in the serum and the purine metabolism pathway were found to distinguish patients with BD with NSSI from those with BD without NSSI.

Conclusions: Abnormalities in the urea cycle, glutamate metabolism, and purine metabolism played important roles in the pathogenesis of BD with NSSI.

Background

Bipolar disorder (BD) is characterized by biphasic moods that include depression and mania (in some cases, hypomania), which occur as recurrent episodes of changes in the behavior of individuals. Depressive episodes are more common in patients with BD. BD represents a chronic and recurrent disorder that affects approximately 1% of the global population(1, 2)**,** resulting in a number of disabilities in young people, such as cognitive and functional impairment, in addition to increased mortality, particularly from suicide and cardiovascular disease(3, 4)**.**

Non-suicidal self-injury (NSSI), defined as the direct and deliberate destruction of one's own bodily tissue (e.g., cutting, burning, and hitting oneself**)** in the absence of suicidal intent **(5)**, has recently seen a sharp rise among young people(6). As NSSI is the strongest predictor of future suicidal behavior (7, 8), it is important to probe its pathogenesis in patients with BD with NSSI. Following decades of progressive increase and growing scientific interest in the incidence of NSSI among adolescents and adults(6), NSSI is listed separately in the Diagnostic and Statistical Manual of Mental Disorders (fifth edition). However, there is little relevant research on the pathogenesis of NSSI. Studies suggested that the amygdala and nucleus accumbens may be potential treatment targets in persons who engage in NSSI (9, 10) .

Meanwhile, Zahid, et al. have reported an association between neural activity across the dorsolateral prefrontal cortex and suicidal ideation and self-injury risk (11). Related studies have also pointed to a relationship between abnormal gene methylation and NSSI (12, 13). Studies have shown that increased inflammation may change major neurotransmitter metabolism, thereby affecting frontal function and decreasing response inhibition, which is associated with increased behavioral impulsivity. This may explain the neurobiological basis of NSSI. However, little is known about the neurobiological mechanisms and biomarkers of NSSI.

Metabolomics is a promising approach for the identification of potential diagnostic and treatment response biomarkers of psychiatric disorders(14, 15). As blood samples are easier to obtain, it is possible to assess peripheral biomarkers. BD biomarkers in blood, serum, urine, and plasma have been probed using proton nuclear magnetic resonance (^{1}H NMR)(16-18), gas chromatography-mass spectrometry(19), and in vivo brain imaging experiments(20, 21). Significant metabolic markers, such as α-hydroxybutyrate, choline, and isobutyrate, among others, can differentiate patients with BD from healthy individuals (22-25) . However, little information on the biomarkers associated with NSSI is available. Therefore, a metabolomics study of blood samples may provide insightful information on the pathophysiology of BD. Thus, this study sought to assess the biomarkers of NSSI in patients with BD using metabolomics technologies.

Methods

Participants

This study was conducted at the Department of Psychiatry in Shanxi Bethune Hospital from January 2018 to August 2020. Inpatients and outpatients 15 to 45 years of age and diagnosed with BD during a depressive episode using the Diagnostic and Statistical Manual of Mental Disorders (fifth edition) were recruited.

Participants were classified into two groups based on their history of NSSI: BD with NSSI (N=31) and BD without NSSI (non-NSSI) (N=46). A history of NSSI was determined using information collected through direct interviews and medical record reviews. Participants were asked: “Have you experienced problems with self-inflicted injury or pain, not counting suicide attempts?”. A board-certified psychologist blinded to each patient’s clinical course double-checked all available information and confirmed a history of NSSI. Patients with suicidal intent or suicidal behavior were excluded from the study. Healthy individuals with no family history of psychiatric disorders were enrolled as the control group. None of the healthy subjects exhibited self-injurious behaviors.

The patients were diagnosed by at least two psychiatrists, and the diagnosis was confirmed by another experienced clinical psychiatrist. The exclusion criteria included a history of a neurological disorder, head injury resulting in loss of consciousness, and alcohol or substance abuse. Subjects who were diagnosed

with either borderline personality disorder or borderline personality traits were excluded. All participants were of Han Chinese origin.

Collection of clinical data and assessment of patients

Demographic and clinical information of the participants were collected using a self-designed case report form, which included information on age, sex, family history of psychiatric disorders, onset of disease, duration of disease, and presence of psychotic symptoms. Relevant information about life events, sleep time, work pressure and frequency, and method of self-injury were also collected. The HAMD-24 was selected to measure the level of depression and changes in its severity. Patients with BD were considered to have a depressive episode if their HAMD-24 score was >17. The demographic information of the participants is shown in Table 1.

Table 1

Demographic and clinical characteristics of all participants

Collection of NMR metabolomics data

Serum collection

Participants were requested to fast overnight. Their serum samples were collected between 8:00 am and 9:00 am. Blood was drawn into Vacutainer tubes, immediately placed on ice, allowed to clot for at least 30 min, and centrifuged at 1500 g for 15 min. The obtained serum was aliquoted, transferred into polypropylene tubes containing 0.01% (m/v) sodium azide, and stored at -80°C until assayed. The maximum duration of storage was two weeks.

For NMR spectroscopic analyses, serum samples were thawed and centrifuged at 13000r/min for 20 min at 4°C to separate any precipitate. Aliquots of 450 μL of the supernatants were diluted with 350 μL of D_2O and placed in 5.0 mm diameter NMR tubes.

^{1}H-NMR spectrum and data analysis

The proton spectra of the blood samples were collected using a Bruker Avance 600 spectrometer operating at a 600.13 MHz 1H frequency with a standard one-dimensional pulse sequence. Typically, 64 transients were collected into 16 K data points with a spectral width of 8000 Hz, an acquisition time of 0.945 s, and a relaxation delay of 2 s.

Variable		Group			Analysis	
		BD (NSSI) (N=31)	BD (Non-NSSI) (N=46)	HC (n=10)	F/c^2	p
Age (year)		25.48±7.78	26.78±9.94	26.9±8.14	0.139	0.870
Onset age (year)		19.52±5.56	22.09±5.37	-	0.153	0.046*
The total scores of HAMD-24		37.32±5.63	32.89±5.36	-	0.040	0.001*
Number（NSSI）		6.06±8.28	-	-		
Gender	male	11	21	3	1.288	0.525
	female	20	25	7		
marriage	1=unmarried	28	26	7		
	2=married	2	16	3		
	3=divorced	1	3	0	11.185	
	4=digamous	0	1	0	（0.083）	
	5=cohabit	0	0	0		
	6=widowed	0	0	0		
	7=separated	0	0	0		
family history	1=Yes	8	13	0	6.943	0.139
	2=No	22	33	9		
	9=unknown	1	0	1		
life events	1=No	15	30	10	8.830	0.012*
	2=Yes	16	16	0		
sleeptime	<5h	10	7	1	19.732	0.000*
	5-8h	13	31	1		
	>8h	8	8	8		
working pressure	1=No	10	27	7	10.877	0.028*
	2= Mild	8	11	3		
	3= severe	13	8	0		
Frequency （NSSI）	1= few	4	-	-		
	2= sometimes	8	-	-		
	3= frequently	11	-	-		

	4= persistently	8	-	-
way□NSSI□	1= cutting	17		
	2= scratching	8		
	3= hitting	3		
	4= biting	3		

Prior to Fourier transformation, the free induction decay was zero-filled and multiplied by an exponential function that corresponds to a line-broadening factor of 0.3 Hz in the frequency domain. Blood resonance assignments were performed according to the guidelines in previous literature and NMR databases.

^{1}H-NMR data were transported to a data matrix, and chemometrics analyses based on interval principal component analysis (iPCA) and partial least-squares discriminant analysis (PLS-DA) were performed using MestReNova (Mestrelab Research, Santiago de Compostella, Spain).

The Metaboanalyst 5.0 website (http://www.metaboanalyst.ca) was used for enrichment analysis of differential metabolites.

Data processing and statistical analyses

The processed ^{1}H NMR spectrum profile data were imported into the SIMCA-P 14.1 software (Umetric, Sweden) for orthogonal projections to latent structures discriminant analysis (OPLS-DA). The established score plot of the OPLS-DA model was used to assess the differences between group clusters. The differential metabolites were identified using a loading plot of the OPLS-DA model and a paired t-test performed using SPSS 21 software (VIP > 1 and $p < 0.05$). All continuous variables were analyzed using a two-tailed student's t-test or one-way analysis of variance, followed by the Bonferroni post-hoc test. Data were processed using SPSS 21.0. The t-test was used for the detection and identification of difference markers. Statistical significance was set at $p < 0.05$.

Results

Demographic data of the participants

Of the 77 participants included in the analysis, 31 were classified into the NSSI group.

There were no significant differences in age ($F = 0.139$, $p = 0.870$), sex ($\chi2 = 1.288$, $p = 0.525$), marital status ($\chi2 = 11.185$, $p = 0.083$), or family history ($\chi2 = 6.943$, $p = 0.139$) among the BD (NSSI), BD (non-NSSI), and HC (healthy control) groups. The BD (NSSI) group had a younger age at onset than the BD (non-NSSI) group ($F = 0.153$, $p = 0.046$). The BD (NSSI) group had higher total HAMD-24 scores than the BD (non-NSSI) group ($F = 0.040$, $p = 0.001$). There were significant differences in life events ($\chi2 = 8.830$, p

= 0.012), sleep time (χ2 = 19.389, p = 0.001), and work pressure (χ2 = 10.877, p = 0.028) between the BD (NSSI) and BD (non-NSSI) groups. (Table 1)

In the BD (NSSI) group, 61.3% of patients had frequent NSSI. The most common method of inflicting NSSI was cutting (54.8%), scratching (25.8%), hitting (9.7%), and biting (9.7%) (Table 1).

Analysis of ^{1}H NMR spectroscopy data

Figure 1 shows the typical ^{1}H NMR spectrum profiles of the BD and HC groups. Twenty-eight small molecule compounds were identified in the ^{1}H NMR spectrum profiles of participants in the two groups based on the findings of the assessment of the human metabolome database (http://www.hmdb.ca/) and related articles published previously (Table 2).

Table 2

Peak attribution in 1H-NMR spectra of differential metabolites among three group

	Metabolites	Chemical shift
1	HDL	0.874（m）
2	pantothenate	0.907（s）
3	Isoleucine	0.949（t）
4	Leucine	0.961（t）
5	3-Hydroxybutyric acid	1.21（d）
6	Lactate	1.33（d）
7	Acetic acid	1.927（s）
8	O-Acetyl glycoproteins	2.14（s）
9	Acetoacetate	2.28 (s), 3.44 (s)
10	Methionine	2.14（s）
11	Guanidinoacetate	3.80 (s)
12	Uracil	5.81 (d, 7.7 Hz), 7.55 (d, 7.7 Hz)
13	Histidine	7.04 (s), 7.84 (s)
14	Dimethylglycine	2.92 (s), 3.70 (s)
15	Creatine	3.04 (s), 3.93 (s)
16	Acetylcholine	3.23 (s)
17	Taurine	3.27 (t, J = 6.6 Hz), 3.42 (t, J = 6.6 Hz)
18	Scyllo-inositol	3.36 (s)
19	3-D-hydroxybutyrate	1.20(d)
20	Betaine	3.27(m)
21	Betaine	3.67(m),3.78(m)
22	Citrulline	3.73(s)
23	N-Acetyl glycoproteins	2.05 (s)
24	Glutamate	2.06 (m), 2.14 (m), 2.36 (m)
25	Glutamine	2.14 (m)
26	Acetone	2.23 (s)
27	Citric acid	2.53 (d, 16.1 Hz), 2.70 (d, 16.1 Hz)

28	Choline	3.20 (s), 4.06 (m)

Discriminative model construction

The score plot of the OPLS-DA model showed that the patients and healthy controls could be clearly separated with little overlap (Figure 2a). This indicates that the OPLS-DA model built with serum metabolites could be a potential tool for objectively diagnosing BD during a depressive episode. Moreover, the higher original Q2 and R2 values than their corresponding permutated values demonstrated that the OPLS-DA model was valid and not over-fitted (Figure 2b), which further confirmed the robustness of these results.

The serum macro profile analysis of each group showed that the HC group was significantly separated from the BD group, indicating that the endogenous metabolites in the BD group differed from those in the HC group. However, a further comparison of the BD (NSSI) and BD (non-NSSI) groups is required.

Analysis of plasma metabolites and metabolic pathways

Differences in the plasma metabolite and metabolic pathways associated with the BD (NSSI) and HC groups

To further identify differential metabolites responsible for samples separation, the loading plot was constructed based on the OPLS-DA model (Figure 3). A total of eight endogenous differential metabolites were detected (variable importance in projection [VIP] >1, P <0.05)—lipid, 3-hydroxybutyric acid, pyruvate, citline, and creatinine were significantly increased (*P <0.05, **P <0.01), and oxidized glutathione, glycerol, and β-glucose were significantly decreased (*P <0.05, **P <0.01) in the BD (NSSI) group compared with the HC group (Table 3).

Table 3

Peak area of metabolites in serum ^{1}H-NMR spectra of HC and BD (NSSI) groups

Metabolites	Peak area after normalization	
	HC	BD (NSSI)
HDL	0.763±0.102	1.170±0.153**
3-Hydroxybutyric acid	0.477±0.172	4.489±1.854**
pyruvic acid	0.181±0.025	0.556±0.160**
oxidized glutathione	3.109±0.434	1.694±0.489**
Glyceryl	0.880±0.087	0.711±0.054**
Citrulline	0.746±0.077	1.257±0.219*
Creatinine	0.591±0.116	1.213±0.612**
β-glucose	40.352±4.027	29.679±6.850**

Relative peak area of potential biomarkers found in serum Compared with control group□*P< 0.05□**P< 0.01

Differential metabolites were imported into the Metaboanalyst 5.0 website to further analyze the metabolic pathways associated with BD (NSSI). Multiple differential metabolic pathways were screened using an impact value above 0.05 (Figure 5). These metabolic pathways included the urea cycle, the glutamate metabolism pathway, and the pyruvaldehyde degradation pathway.

Differences in the plasma metabolite and metabolic pathways associated with the BD (non-NSSI) and healthy control groups

To further identify differential metabolites responsible for samples separation, the loading plot was constructed based on the OPLS-DA model (Figure 4). A total of eight endogenous differential metabolites were detected (VIP >1 and P <0.05)—lipids, pantothenate, alanine, glyceryl, dimethylglycine, and valine were significantly increased (*P <0.05, **P <0.01), and N-acetyl glycoproteins and ascorbate were significantly decreased (*P <0.05, **P <0.01) in the BD (non-NSSI) group compared with the HC group(Table 4).

Table 4

Peak area of metabolites in the serum ^{1}H-NMR spectra of the HC and BD (non-NSSI) groups

Metabolites	Peak area after normalization	
	HC	BD (Non-NSSI)
HDL	0.699±0.147	0.994±0.149**
Pantothenate	0.472±0.099	1.001±0.165**
Alanine	0.058±0.039	0.318±0.218**
N-Acetyl glycoproteins	0.874±0.162	0.641±0.110**
Glyceryl	0.490±0.078	1.956±0.196**
Dimethylglycine	0.706±0.123	1.349±0.259**
Ascorbate	1.496±0.263	0.680±0.248**
Valine	0.035±0.016	0.266±0.082**
Relative peak area of potential biomarkers found in serum Compared with control group□*P< 0.05□**P<0.01		

Differential metabolites were imported into Metaboanalyst 5.0 website to further assess the metabolic pathways associated with BD (non-NSSI). Multiple differential metabolic pathways were screened using an impact value above 0.05 (Figure 6.). These metabolic pathways consisted of the glycine and serine metabolism pathway and the glucose-alanine cycle.

Differences between the plasma metabolites and metabolic pathways in the BD (non-NSSI) and BD (NSSI) groups

A total of five endogenous differential metabolites were detected (VIP >1, P <0.05) —xanthine, niacinamide, adenosine, hypoxanthine, and histidine, which were significantly higher in the BD (NSSI) group than in the control group (P < 0.05, P < 0.01) (Table 5).

Table 5

Peak area of metabolites in the serum ^{1}H-NMR spectra of the BD (NSSI) and BD (non-NSSI) groups

Metabolites	Peak area after normalization	
	BD(NSSI)	BD(Non-NSSI)
Xanthine	0.122±0.322*	-0.008±0.061
Niacinamide	0.040±0.110*	-0.005±0.036
Adenosine	0.103±0.266*	0.001±0.016
Hypoxanthine	0.120±0.307*	0.002±0.017
Histidine	0.094±0.261*	-0.006±0.050

Relative peak area of potential biomarkers found in serum
Compared with control group: *P<0.05

Differential metabolites were imported into Metaboanalyst 5.0 website to further analyze the metabolic pathways associated with BD (NSSI). Multiple differential metabolic pathways were screened based on an impact value above 0.05 (Figure 7). These metabolic pathways consisted of the purine metabolism and methylhistidine metabolism pathways.

Discussion

This study identified the metabolic pathways associated with BD (NSSI) and assessed the important diagnostic and predictive indices of NSSI in BD. To the best of our knowledge, this is the first study to identify the differential metabolites of BD (NSSI) and BD (non-NSSI).

NSSI is a characteristic manifestation of BD. The highest rates of NSSI are observed during adolescence; due to its rapidly increasing prevalence, NSSI requires more clinical attention (26). In a previous study, 5.1% to 24% of people who inflict self-injury reported that they had initiated this behavior before age 11–13 years (27). In this study, we observed that the onset age of patients in the BD (NSSI) group was lower than that of those in the BD (non-NSSI) group, which is consistent with previous results. Cutting, scratching, burning, hitting, and biting are some of the most commonly used methods of inflicting NSSI. Most self-injurers cut themselves using a sharp object, such as a knife or blade, mainly on the forearms, legs, and/or abdomen(28), which was consistent with our research. In a previous study, a multivariate regression analysis revealed that young age, unemployment, a higher monthly family income, single status, impulsivity, long duration of illness, and life stressors were risk factors for NSSI in patients with depression and BD (29). In addition, adverse family life events may expose an individual to a greater degree of stress throughout their lifespan and may serve as triggers for NSSI. Studies confirmed that life stressors and adverse interpersonal experiences are associated with an increased risk of NSSI (30, 31) , which is consistent with the present result. In the present study, we also found that sleep problems were most common in the BD (NSSI) group than in the BD (non-NSSI) group or healthy group. Previous research suggests that multiple sleep variables, including poor sleep quality and frequent nightmares, are

associated with and are independent risk factors for NSSI(32, 33) . Therefore, we suspect that interventions that improve sleep quality and sleep duration or reduce life stress may concomitantly decrease the risk of NSSI.

A series of studies of BD demonstrated abnormalities of energy metabolism in patients with BD(34). Several studies have confirmed that lipid metabolic disorders or abnormalities is concerned with neuropsychiatric disorders, such as BD, schizophrenia, and major depressive disorder. Previous studies confirm that there is a high prevalence of elevated triglycerides, cholesterol, low-density lipoprotein, and glucose levels and low high-density lipoprotein (HDL) level in patients with BD(35). In this study, we found that HDL is a common differential metabolite in the BD (NSSI) and BD (non-NSSI) groups, which suggested the importance of lipid metabolism in BD, that is consistent with previous studies (35, 36). Results showed that sphingolipids and glycerolipids were increased, whereas glycerophospholipids were decreased, in serum samples from patients with BD. Moreover, studies also showed that elevated lipid level is associated with smaller brain structures in patients with BD(37). Future research is needed to verify the changes in the HDL levels of BD patients compared with those of healthy individuals and the specific mechanisms of lipid changes in the pathogenesis of BD.

The results of the present study indicate that 3-hydroxybutyric acid, pyruvic acid, oxidized glutathione, glyceryl, citrulline, creatinine, and β-glucose are characteristic markers of bipolar NSSI. Patients in the BD (NSSI) group showed higher lipid, 3-hydroxybutyric acid, pyruvic acid, citrulline, and creatinine levels and lower oxidized glutathione, glyceryl, and β-glucose levels than the healthy controls. Kamonwad et al. reported that patients with BD have increased salivary levels of glutathione and oxidized glutathione compared to controls (38). Rosa et al. (39) documented decreased levels of glutathione and increased levels of glutathione disulfide in the plasma of patients with BD. Previous studies have also revealed higher serum levels of pyruvate and N-acetyl glutamate in patients with BD than in healthy controls (34, 40), which is consistent with the findings of this study. However, previous studies have insufficiently focused on correlation between the abovementioned metabolites and NSSI in patients with BD.

This study showed that the urea cycle and the glutamate metabolism pathway are significant metabolic pathways in BD (NSSI). The urea cycle is a metabolic pathway for the disposal of excess nitrogen, which primarily starts from the removal of ammonia from the blood. For the urea cycle, also known as the ornithine cycle, when amino acids are metabolized in the body, ammonia is produced and subsequently synthesized into urea through. Studies have suggested that an abnormality in the urea cycle (or arginine metabolism) is associated with BD (41, 42). The results of this study indicated that an abnormal urea cycle is associated with BD (NSSI). Lan et al. (43) reported that glutamate levels are increased in the post-mortem brains of patients with BD. This is consistent with the results of the BD (NSSI) group in the present study. Glutamate is the most abundant excitatory neurotransmitter in the mammalian brain; glutamate metabolism is involved in the synthesis of gamma-aminobutyric acid (GABA), and abnormalities in the GABAergic system contribute to the pathophysiology of mood disorders(44). As such, changes in glutamate neurotransmission may be involved in the etiology of BD. Xu et al. reported that state-related abnormalities in oxidative and glutamate metabolism are associated with BD(45).

Increasing evidence suggests that changes in inflammatory mediators are involved in the pathogenesis of mood disorders(46, 47). Meanwhile, studies have demonstrated links between alterations in inflammation and glutamate metabolism in mood disorders(48, 49). This indicates that inflammatory mediators, glutamate metabolism and oxidative stress are closely related to the pathogenesis of BD(NSSI). Thus, therapeutic strategies targeting amino acid metabolism such as glutamate may be effective in patients with BD (NSSI), and increased inflammation as reflected in C-reactive protein levels may be helpful in the diagnosis of BD (NSSI).

In clinical practice, identifying specific diagnostic markers of NSSI in patients with BD will provide a strong basis for the recognition and treatment of NSSI. Thus, we also compared the metabolic differences between BD (NSSI) and BD (non-NSSI) groups. Five endogenous differential metabolites, including xanthine, niacinamide, adenosine, hypoxanthine, and histidine, were significantly higher in the BD (NSSI) group than in the BD (non-NSSI) group. Adenosine, a purine nucleoside, may contribute to the pathophysiology of mental disease by interacting with dopamine and glutamate receptors through A1 and A2A receptors; thus, modulating dopaminergic and glutamatergic neurotransmission (50, 51). Zhang et al. reported that levels of the purines guanine and guanosine are decreased in the brains of patients with BD (52). However, assessments of changes in purine and adenosine metabolism levels are lacking. This study shows that the important metabolic pathways associated with BD (NSSI) are the purine and methylhistidine metabolism pathways. The purinergic system is a critical neurotransmitter system with uric acid (UA) as its end-product. Recent studies have shown that the patients with BD have the highest UA levels among healthy controls and those with other mental disorders (53, 54) , which is involved in the occurrence and development of mental illnesses such as BD and MDD(55, 56) . A series of studies have demonstrated a direct association between UA levels and associated purinergic dysfunction. The purinergic system is involved in the neurodevelopment and pathophysiological processes of psychotic disorders, such as the genesis, differentiation of neurocytes and inflammation of neuroglial cells (57-59). Growing evidence suggests that oxidative stress and the purine/adenosine system play key roles in the development and progression of mental diseases, such as BD(60, 61). We suggest that NSSI in patients with BD is related to an increase in oxidative stress levels. Post-mortem and imaging studies showed an increasingly complex interaction between the mitochondrial, purinergic, and oxidative systems, which are associated with psychiatric disorders(62). These results suggest that an increase in purinergic-UA metabolism and oxidative stress levels may be a significant mechanism underpinning BD (NSSI), which may be related to mitochondrial dysfunction.

It has been hypothesized that gout and BD may share similar pathophysiological mechanisms, such as purinergic dysfunction. Previous research has shown that patients with BD have an increased risk of gout (63, 64). We observed increased purine synthesis in the BD (NSSI) group in this study, indicating that the incidence of gout was higher in patients with BD (NSSI). This suggests that purine-UA metabolism is a potential therapeutic target in the treatment of BD (NSSI). As xanthine and hypoxanthine levels are elevated in BD (NSSI), allopurinol, an inhibitor of xanthine oxidase, is used to treat and prevent gout. Allopurinol and febuxostat(65), two potent inhibitors of UA accumulation, have demonstrated antimanic and antidepressant effects in clinical and preclinical studies, and may be used as add-on therapy for BD

(NSSI) to reduce rates of self-injury. Tomoya et al. suggested that adenosine modulator adjuvant therapy is more beneficial than a placebo in treating manic episodes of BD (66). As adenosine levels were altered in this study, we suspect that adenosine modulator adjuvant therapy may be effective for BD (NSSI). These drugs can be used as potential therapeutic options for patients with BD (NSSI).

In this study, purine and amino acid metabolism were found to be altered in patients with BD (NSSI) compared to that in healthy controls, which is consistent with the findings of a previous study(67). As the final metabolite of purine, UA acts on neurons presynaptically and postsynaptically, and on specific receptors in the glial cell membrane that can affect activities of other neurotransmitters involved in the pathophysiological process of mood disorders, including dopamine, GABA, glutamate, and serotonin(68). It has been suggested that purinergic-UA metabolism is associated with glutamate metabolism, further affecting oxidative stress and is involved in the pathogenesis of NSSI, all of which are related to mitochondrial dysfunction(69-71). This provides important evidence for the diagnosis and treatment of BD (NSSI). These findings provide a basis for further research on the pathogenesis of NSSI, and are highly significant with regard to the diagnosis, recognition, and treatment of NSSI.

Limitations

This study has some limitations. First, we did not control for the effects of psychotropic medications, which may affect plasma metabolite profiling. Second, the sample size was limited. Adequately powered studies are warranted in the future to confirm our preliminary conclusions. Third, although we controlled for main clinical characteristics, blood metabolites may be influenced by additional factors that we did not consider, including cardiovascular health and dietary patterns.

Conclusion

This study demonstrated that purine and amino acid metabolism are greatly enhanced in BD with NSSI than that in BD without NSSI, providing evidence of the relationship between the purinergic system, glutamate metabolism, and the pathogenesis of NSSI in patients with BD. In addition, the results of this study indicate that xanthine, hypoxanthine, and adenosine may be potential biomarkers of NSSI in patients with BD. These findings suggest that abnormalities in the purinergic system, urea cycle, and glutamate metabolism play important roles in the pathogenesis of BD. Further investigations are needed to elucidate the relationship between purinergic-UA metabolism, amino acid cycling, and oxidative stress. In addition, future studies which are stringently designed with larger samples are required to validate our results and confirm our conclusions.

Abbreviations

BD, bipolar disorder; NSSI, non-suicidal self-injury; UA, uric acid; HAMD, Hamilton Depression Scale; ^{1}H NMR, proton nuclear magnetic resonance; PCA, Principal Component Analysis; iPCA, interval principal component analysis; PLS-DA, partial least-squares discriminant analysis; OPLS-DA, orthogonal projections to latent structures discriminant analysis; OSC, orthogonal signal correction; HDL, high-density lipoprotein; HC, healthy control

Declarations

• Ethics approval and consent to participate

The authors state that they have obtained approval from The Medical Research Ethics Committee of Shanxi Bethune Hospital (the Approval Notice Number: YXLL-2020-001) and have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

• Consent for publication

Not applicable.

• Availability of data and material

All data used during the study appear in the submitted article.

• Competing interests

The authors declare that they have no competing interests.

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• Authors’ contributions

Y Ren designed and performed the study and modified the manuscript. XJ Guo and J Jia collected and analyzed the data and wrote the manuscript. ZY Zhang, YT Miao, P Wu and YQ Bai performed or contributed to the experiments. All authors reviewed and approved the final manuscript.

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Figures

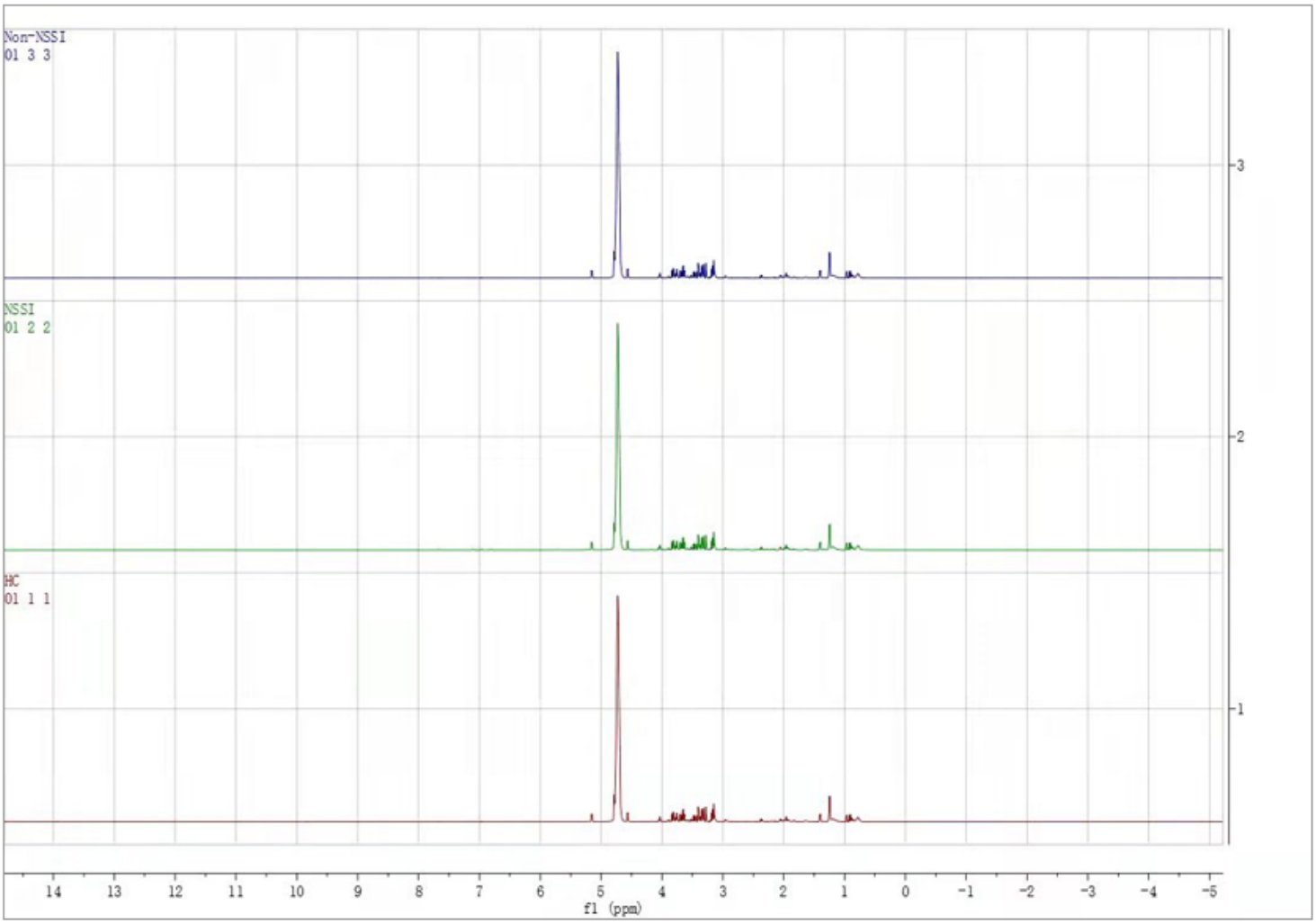
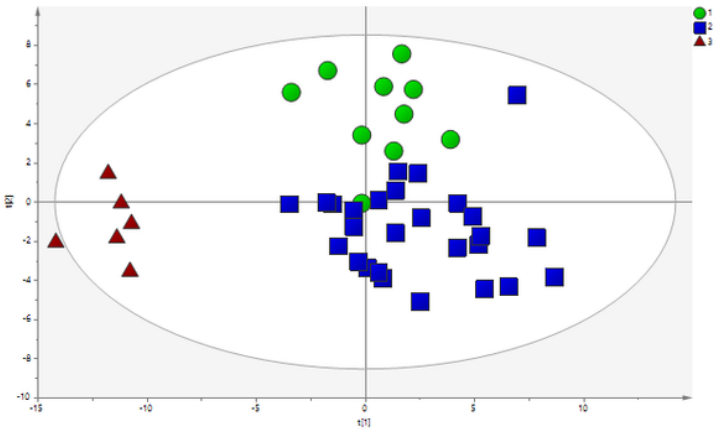
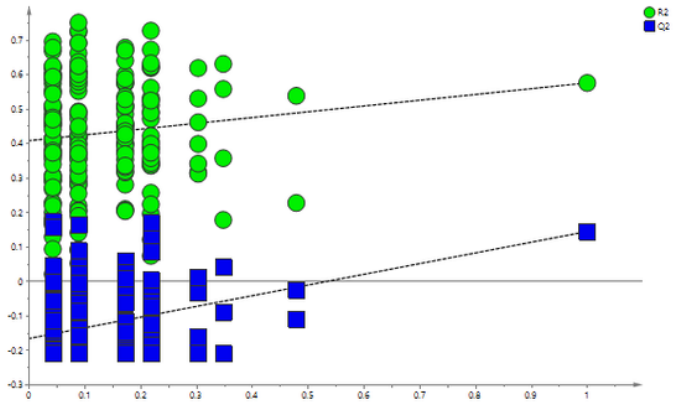


Figure 1

Proton nuclear magnetic resonance spectra of blood samples from patients with bipolar disorder and healthy controls



a



b

Figure 2

Proton nuclear magnetic resonance spectra of the partial least-squares discriminant analysis of the serum of patients in the three groups (circle: bipolar disorder with non-suicidal self-injury (NSSI) group; square: bipolar disorder without NSSI; triangle: healthy control group)

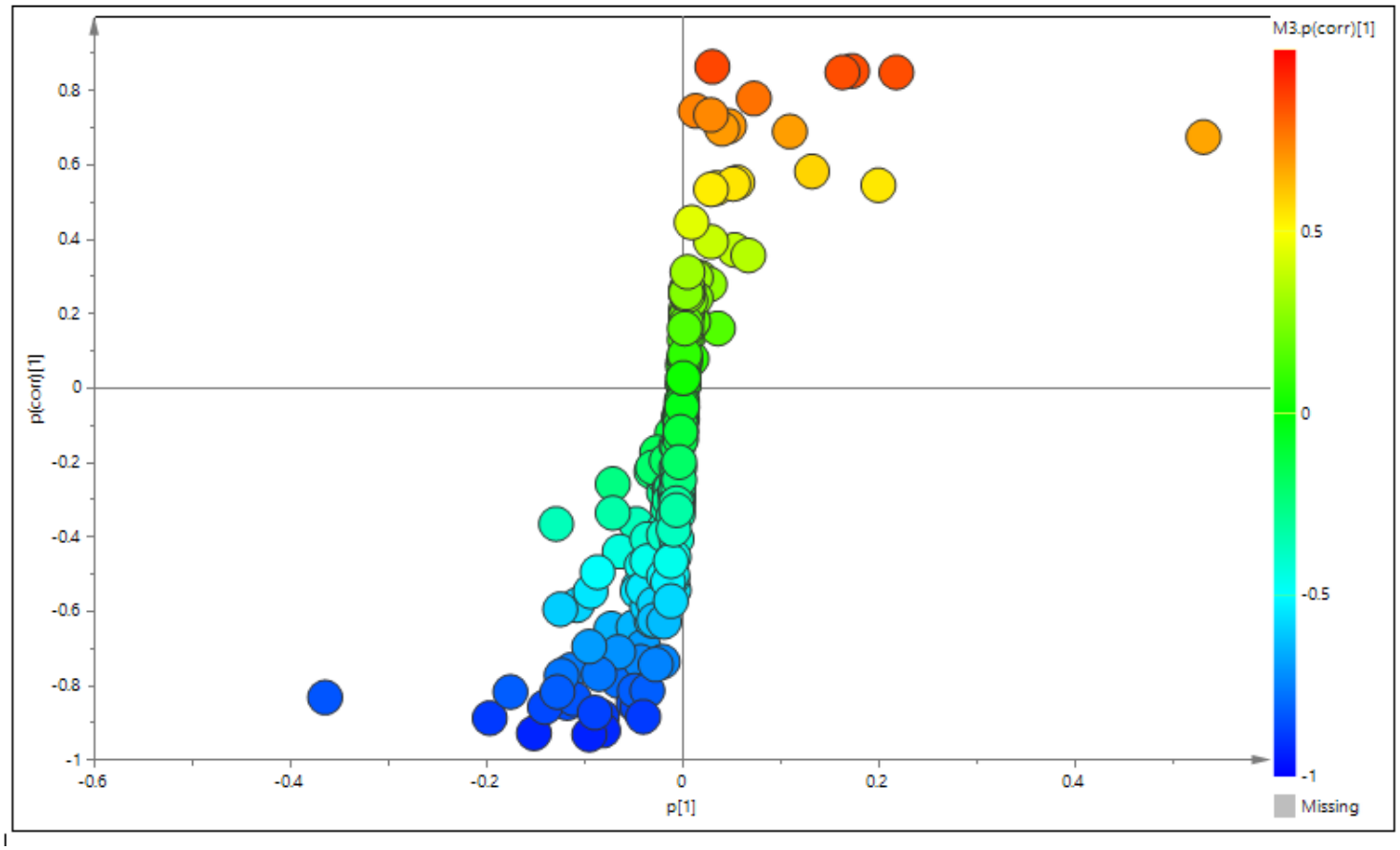


Figure 3

PCA plots of the OPLS-DA of the healthy control group and BD (NSSI) group, which were validated using S-plots.

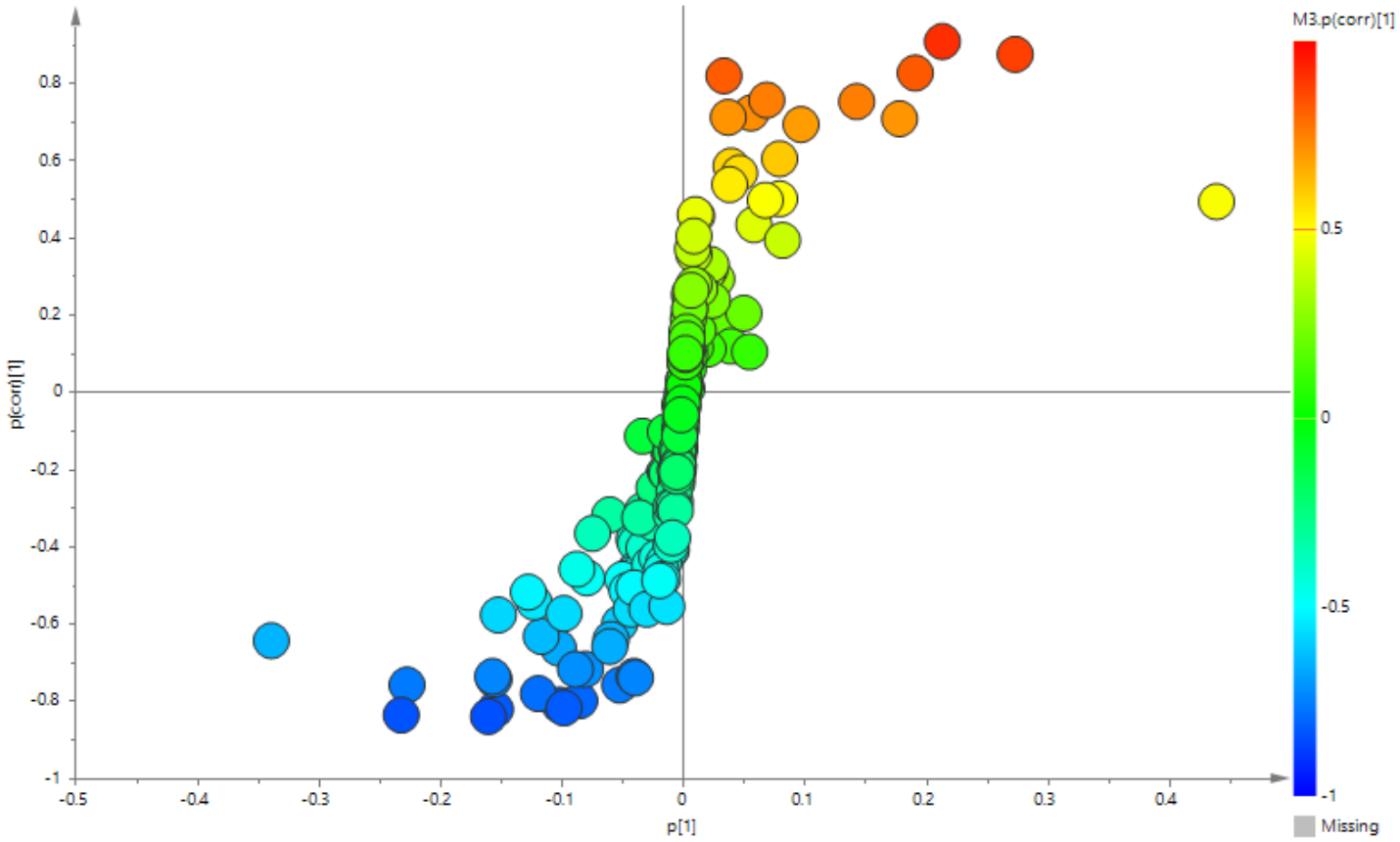


Figure 4

PCA plots of the OPLS-DA of the healthy control group and BD (non-NSSI) group, which were validated using S-plots.

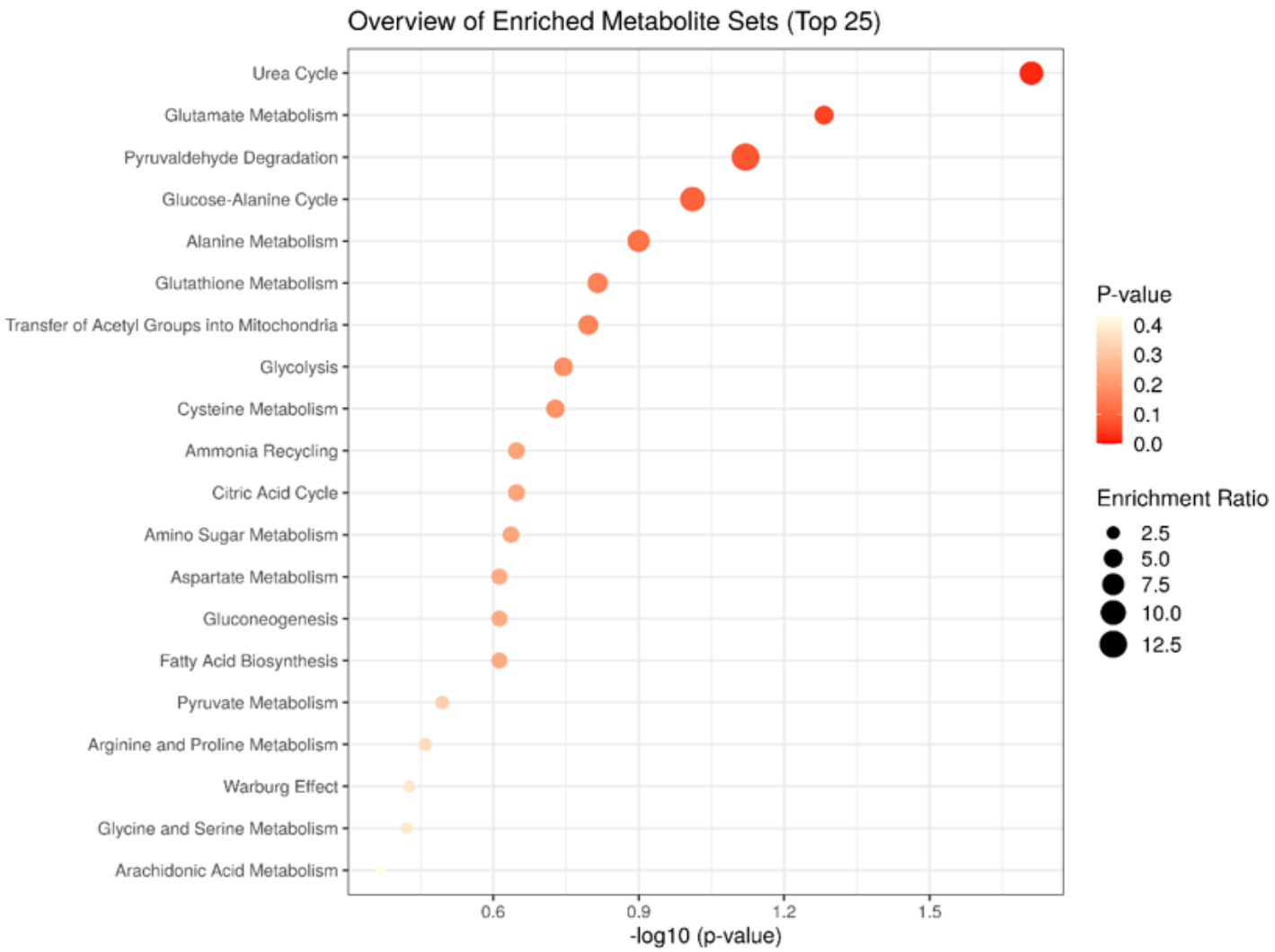


Figure 5

Metabolic pathways associated with bipolar disorder with non-suicidal self-injury

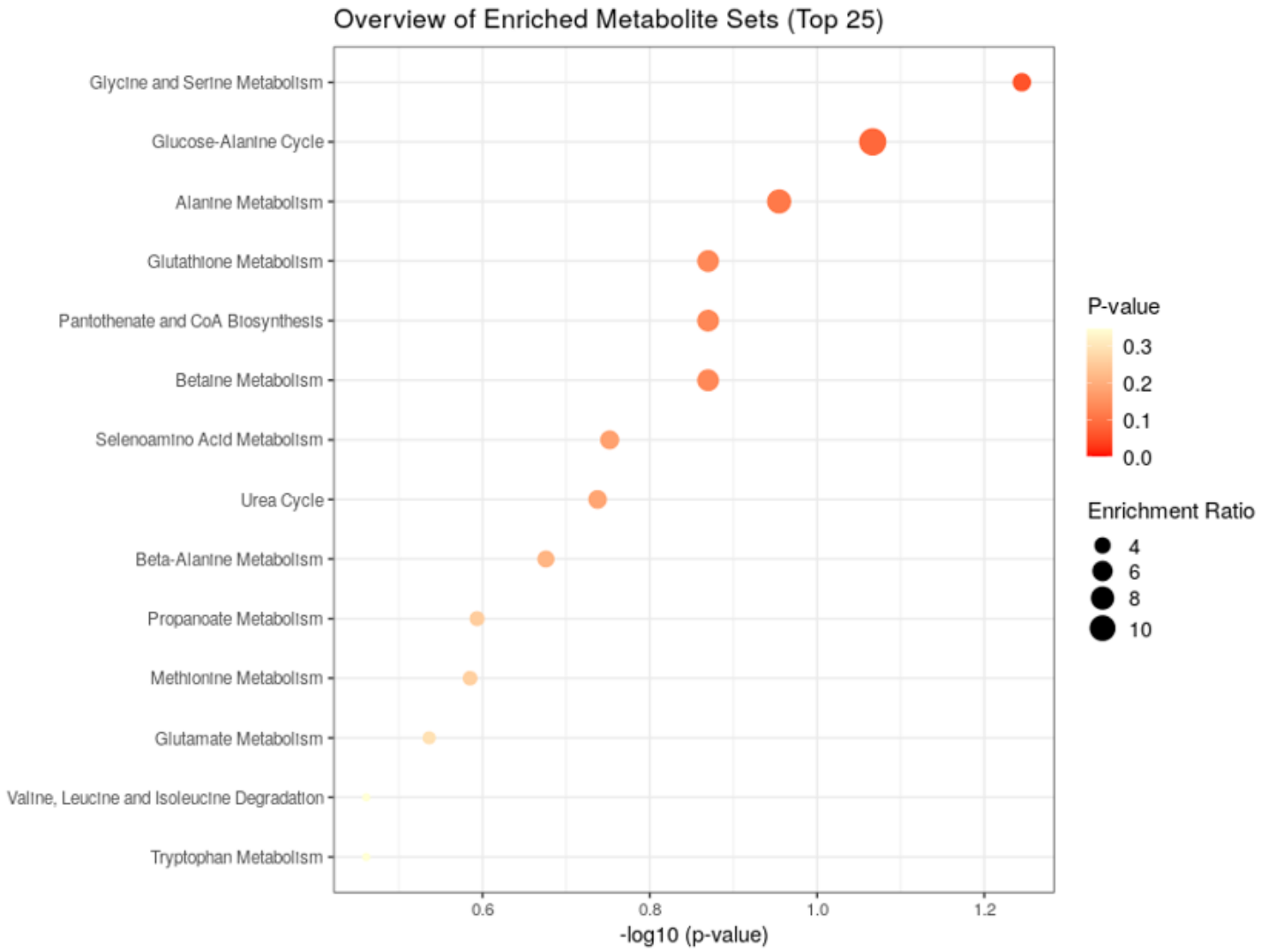


Figure 6

Metabolic pathways associated with bipolar disorder without non-suicidal self-injury

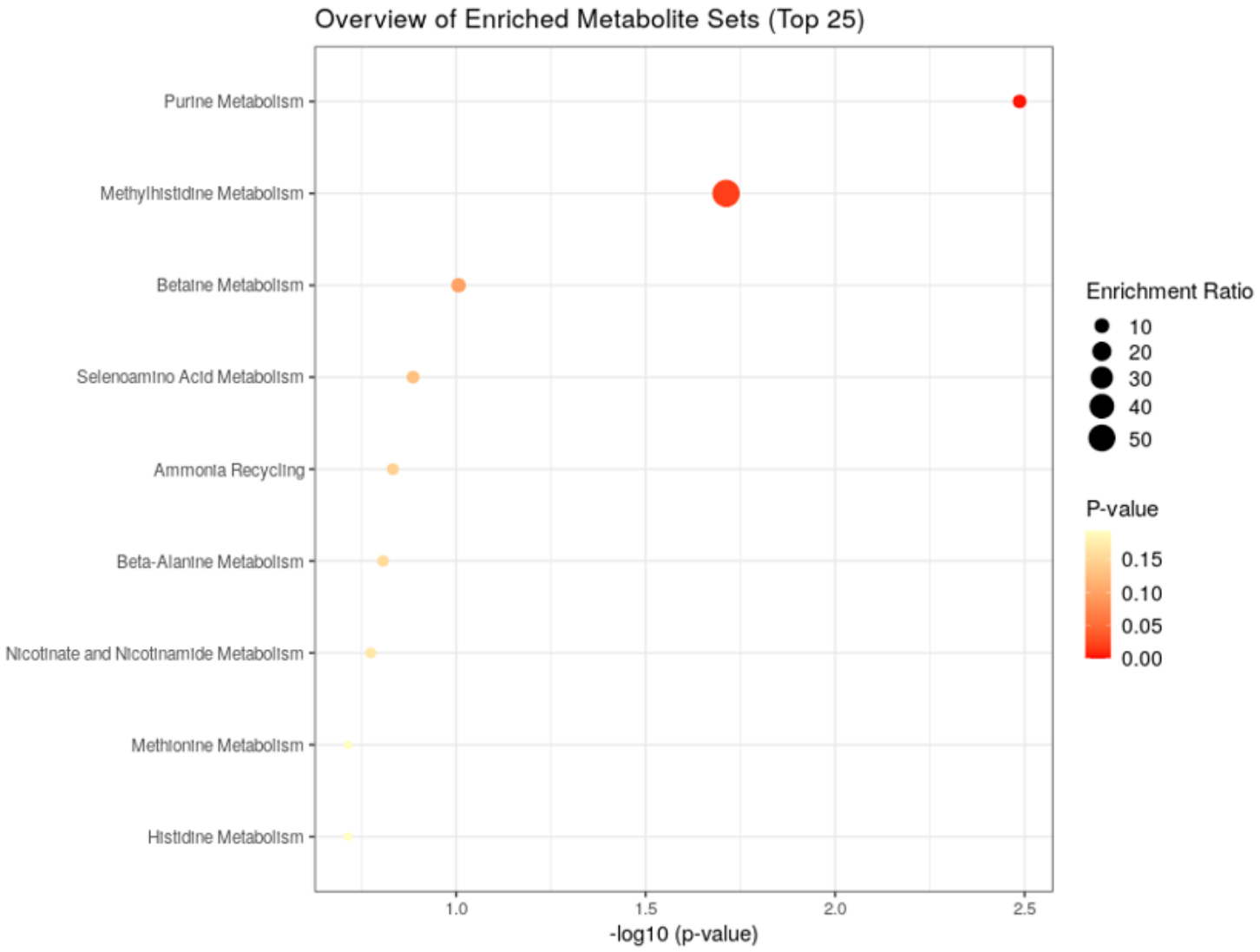


Figure 7

Comparison of metabolic pathways associated with bipolar disorder with and without non-suicidal self-injury