Journal of Affective Disorders 208 (2017) 139–144

Contents lists available at ScienceDirect

Journal of Affective Disorders

journal homepage: www.elsevier.com/locate/jad

1H-magnetic resonance spectroscopy study of glutamate-related abnormality in bipolar disorder

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ARTICLE INFO

Keywords:
Bipolar disorder
Glutamate
Glutamine
Lithium
Sodium valproate
Magnetic resonance spectroscopy

ABSTRACT

Background: Previous studies of patients with bipolar disorder (BD) using magnetic resonance spectroscopy (MRS) have shown neurophysiological abnormalities related to the glutamate (Glu)-glutamine (Gln) cycle, membrane turnover, and neuronal integrity, although the results were neither consistent nor conclusive. Recently it has been reported the Gln/Glu ratio is the most useful index, quantifying neuronal-glial interactions and the balance of glutamatergic metabolites In this MRS study, we elucidated the abnormalities of metabolites in a larger sample of patients with BD with a high-field MRI system.

Methods: Sixty-two subjects (31 patients with BD and 31 healthy controls [HC]) underwent 3T proton MRS (1H-MRS) of the anterior cingulate cortex (ACC) and left basal ganglia (ltBG) using a stimulated echo acquisition mode (STEAM) sequence.

Results: After verifying the data quality, 20 patients with BD and 23 age- and gender-matched HCs were compared using repeated-measures analysis of covariance (ANCOVA). Compared to the HC group, the BD group showed increased levels of Gln, creatine (Cr), N-acetyl aspartate (NAA), choline (Cho), and an increased ratio of Gln to Glu in the ACC, and increased Gln and Cho in the ltBG. These findings remained after the participants with BD were limited to only euthymic patients. After removing the influence of lithium (Li) and sodium valproate (VPA), we observed activated glutamatergic neurotransmission in the ACC but not in the ltBG.

Limitations: The present findings are cross-sectional and metabolites were measured in only two regions.

Conclusions: Our results support a wide range of metabolite changes in patients with BD involved in glutamatergic neurotransmission, membrane turnover, and neuronal integrity. Moreover, the elevation of Gln/Glu ratio suggested that hyperactivity of glutamatergic neurotransmission in the ACC is a disease marker for BD.

1. Introduction

Bipolar disorder (BD) is characterized by recurrent episodes of depression and mania. It is a main cause of disability among young people, leading to cognitive and functional impairment and raised mortality, particularly death by suicide. Many studies have gradually elucidated the pathogenesis and pathophysiology of BD. Recently, glutamatergic neurotransmission in BD has been revealed by application of proton magnetic resonance spectroscopy (1H-MRS), which can noninvasively measure in vivo metabolite levels, including N-acetyl aspartate (NAA), choline (Cho), creatine (Cr), glutamine (Gln), and glutamate (Glu), in specific brain regions. Glutamatergic neurotransmission can be assessed by measuring Gln, Glu, and Glx, which comprises Gln and Glu. Elevated Glx is one of the most replicated findings in BD (Taylor, 2014; Yüksel and Öngür, 2010). Gigante et al. (2012) conducted a systematic review of 9 articles including 162 BD subjects and reported that Glx levels were significantly elevated in several regions of the brain, even if the assessed area was limited to the

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http://dx.doi.org/10.1016/j.jad.2016.08.046
Received 1 June 2016; Received in revised form 11 July 2016; Accepted 23 August 2016
Available online 08 October 2016
0165-0327/

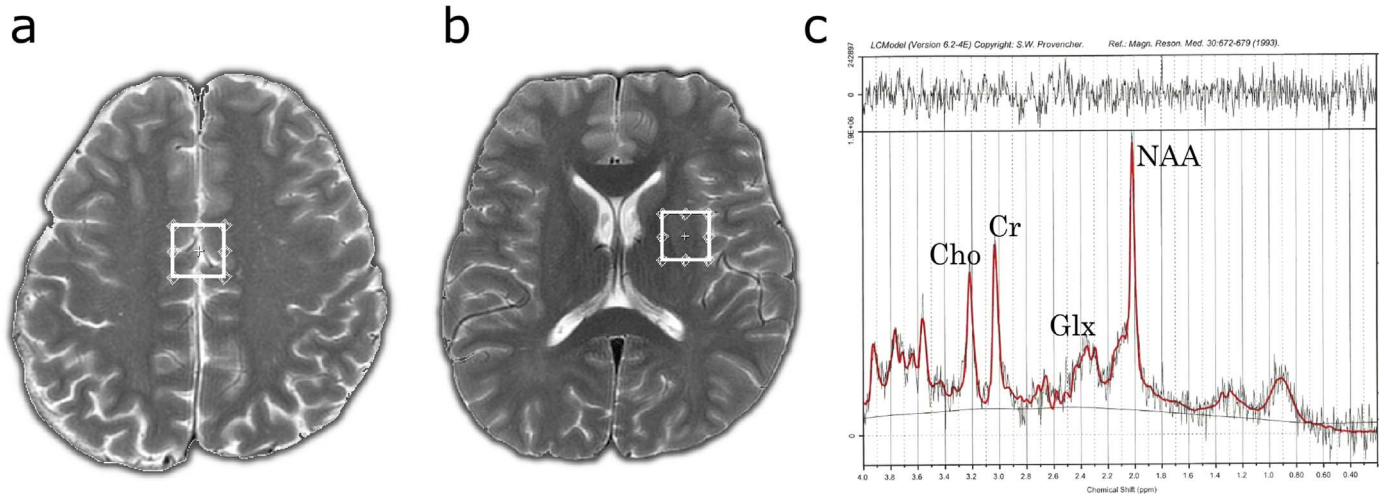

Fig. 1. Region of interest in (ROI) (a) the anterior cingulate cortex (ACC) and (b) the left basal ganglia (ltBG); (c) Sample magnetic resonance spectrum in LC Model. Abbreviations: Cho=Choline; Cr=creatine; Glx=Glutamate+Glutamine; NAA=N-acetyl aspartate.

frontal brain.

Most earlier 1H-MRS studies with 1.5 T scanners reported Glx (an overlapping resonance of Glu and its precursor Gln), whereas more recent studies, using 3 T scanners, can measure Glu and Gln separately. Gln is synthesized from Glu in astrocytes in a reaction catalyzed by Gln synthetase (GS) (Martinez-Hernandez et al., 1977). Synthesized Gln is transferred to presynaptic neurons and is the precursor for synaptic Glu; these process constitute the glutamine-glutamate cycle (Albrecht et al., 2010). The MRS signal of Glu and Gln represents more than just Glu and Gln at the synaptic cleft, however, because they play roles in intermediate metabolism. The Glu signal is dominated by the intracellular transmitter pool, and several measures indicate the use of Glu as a neurotransmitter (Erecińska and Silver, 1990). The Gln/Glu ratio is the most useful index, quantifying neuronal-glial interactions and the balance of glutamatergic metabolites. The Gln/Glu ratio increases and reductions may reflect increased and decreased glutamatergic neurotransmission (Hall et al., 2015). Several 1H-MRS studies reported abnormalities of Glu and Gln in patients with BD (reviewed by Yüksel and Öngür, 2010), whereas only five studies have reported Glu and Gln separately (Frye et al., 2007b; Kaufman et al., 2009; Moore et al., 2007; Ongür et al., 2008; Soeiro-de-Souza et al., 2015). Furthermore, only four reports have been published regarding the Gln/Glu ratio in patients with BD, three of which suggested elevated Gln/Glu in the anterior cingulate cortex (ACC) of patients with BD (Brennan et al., 2010; Ongür et al., 2008) and reduced Glu/Gln (Soeiro-de-Souza et al., 2015). Significant differences in glutamate concentration were reported between patients in depressive or manic episodes (Xu et al., 2013), however, whether glutamatergic neurotransmission is changed by mood-state shifts in BD is still unclear (Brady et al., 2014). The influence of medication on metabolite levels is also unclear (Brambilla et al., 2004; Friedman et al., 2004; Grošić et al., 2014; Machado-Vieira et al., 2015; Moore et al., 2000; O'Donnell et al., 2003; Shibuya-Tayoshi et al., 2008; Strawn et al., 2012; Szulc et al., 2013, 2011). It is thus difficult to interpret metabolite levels in MRS owing to the heterogeneity created by varying medication regimes.

In addition to the ACC, the basal ganglia (BG) are also highly involved in the pathophysiology of BD (Abler et al., 2008; Delvecchio et al., 2012; Hulvershorn et al., 2012; Hummer et al., 2013). However, published MRS studies of BD have reported varying results in the BG, indicating no significant change in Glx (Dager et al., 2004; Frye et al., 2007a; Kaufman et al., 2009), decreased Glx (Port et al., 2008), or elevated Glx (Castillo et al., 2000) with respect to levels in healthy controls.

In the present study, we investigated the hypothesis that metabolite abnormalities in the brain, particularly in the ACC and BG, would be present in patients with BD when compared to healthy controls.

2. Methods

2.1. Subjects

Thirty-one subjects with bipolar disorder and 31 control subjects participated in this study. The subjects were recruited from Tokushima University Hospital, Japan and signed written informed consent forms in accordance with the guidelines of the ethical committee at Tokushima University. The ethical committee approved this protocol. Subjects with BD were assessed using the DSM-IV TR (American Psychiatric Association, 2000). All patients were assessed using the Structured Interview Guide for the Hamilton Depression Rating Scale (SIGH-D) and the Young Mania Rating Scale (YMRS; Young et al., 1978) on the scan day. Control subjects were assessed using the Zung Self-Rating Depression Scale (SDS) and had no history of an Axis I psychiatric illness as determined by the DSM-IV TR. All subjects were Japanese and none had a serious medical illness, neurodevelopmental disorder, or history of head injury, drug or alcohol abuse, or of taking hormonal drugs. All subjects were right-handed.

2.2. 1H-MRS procedure

1H-MRS was performed employing a 3 T magnetic resonance imaging (MRI) instrument (DISCOVERY MR750, GE, Milwaukee, USA) and a STEAM sequence with water suppression by CHESS pulses (TE=18 ms, TR=5000 ms, acquisition=64 times). Neurochemical compounds that can be identified in short-echo 1H-MRS include Cr, Glu, Gln, NAA, and Cho. The area under each of the magnetic resonances is proportional to the concentration of the particular compound. Metabolite levels were estimated using linear combination model (LCModel) software (Provencher, 1993). Our basis-set was constructed from original in vivo data for each metabolite. On the basis of previous reports of functional anomalies, the regions of interest (ROIs) for 1H-MRS were set as the anterior cingulate cortex and left basal ganglia, ROI size=6.0 ml, using three oriented images. For a reference slice of the ROI of the ACC, an axial cut was chosen ~1 cm above the upper end of the body of the lateral ventricles. The ROI was centered on the frontal interhemispheric fissure, 3 cm in front of the central fissure and 2 cm above the corpus callosum (Fig. 1a). A reference slice of the ltBG was placed between the Sylvian fissure and the lateral ventricles to encompass the lenticular nucleus (Fig. 1b). Representative 1H-MRS spectra of each compound in the ACC from one subject are shown in Fig. 1c.

We measured gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF) voxel contents in order to correct the partial volume effect for each metabolite level. T1-weighted images (3D-SPGR) were acquired using the following parameters: TE=4.2 ms, TR=10 ms, slice thickness=0.8 mm, matrix=512×512, FOV=24 cm×24 cm, and flip angle=15°, and segmented into three tissues based on the intensity of each voxel using SPM8 (http://www.fil.ion.ucl.ac.uk/spm/software/spm8/). In the ROIs used in the MRS scan, the voxels of each tissue were counted and the volume of each tissue were calculated. These processes were performed using SPM8 functions, the MarsBaR software package (http://sourceforge.net/projects/marsbar/files/marsbar/), and a Matlab-based script developed in-house. To obtain the tissue-composition-corrected metabolite intensities, each raw metabolite level was calculated using the following formula: [met corrected]=[raw met]/[1−CSF ratio].

2.3. Statistical procedures

Statistical tests were performed using PASW Statistics version 18 (SPSS Inc.). We used Student's *t*-test for comparisons of age, educational duration, height, body weight and percent voxel composition of GM, WM and CSF, and chi-square tests for comparisons of gender and smoking status between the BD and control groups. Significance levels were set at $p \leq 0.05$. Data are given as means ± standard deviations.

Pearson's correlation analyses were used to determine the association between medication (lithium (Li) concentration, sodium valproate (VPA) concentration, and chlorpromazine (CP) equivalent) and metabolite levels. The significance level was set at $p \leq 0.05$. For the group comparisons of metabolite levels, a repeated-measures ANCOVA using metabolite concentrations as the dependent variables with a between-subject factor (patients vs. controls), a within-subjects factor (metabolites: Gln/Glu, Gln, Glu, Cr, NAA, and Cho), and gender and age as covariates. If Mauchly's sphericity test indicated a violation of the homogeneity assumption ($p < 0.05$), the F value was corrected by adjusting the degrees of freedom by Greenhouse–Geisser epsilon (ε).

If a significant interaction between between-subject factors and any other factor (metabolite levels) was found, post hoc multiple comparisons were performed using the Bonferroni method. We regarded metabolite levels as a within-subjects factor because previous studies reported that metabolites levels were mutually interrelated.

3. Results

3.1. Subject demographics and clinical characteristics

Of 31 patients with BD and 31 control subjects, 11 patients with BD and 8 control subjects whose Cramer-Rao lower bounds of metabolite values (Cr, Gln, Glu, NAA, or Cho) were larger than 20% were excluded from analysis. Twenty patients with BD (7 female, 13 male; mean age 45.0 ± 12.4 years) and 23 healthy control participants (8 female, 15 male; mean age 46.4 ± 11.3 years) were included in the analysis. In patients with BD, the mean duration of illness was 12.6 ± 7.4 years, and the mean age at onset was 32.4 ± 10.4 years. In the BD group, 1 patient was hypomanic (YMRS > 8), 5 were depressed (17-item SIGH-D > 8), and 14 were euthymic (YMRS≤8, 17-item SIGH-D≤8) at the time of the scan. Eight patients had a diagnosis of bipolar disorder type I, and 12 patients had a diagnosis of type II.

There were no significant differences between patients and controls with respect to gender, age, or smoking status. There was significant difference between patients and controls with respect to years of education (14.2 ± 2.9 and 16.4 ± 2.6 years, respectively) (Table 1).

Patients with BD had received various medications (Table 2). Five patients were taking both Li and VPA, and three patients were taking both Li and lamotrigine (Supplementary Table 1).

Table 1
Demographic and clinical data of participants.

Variable	Bipolar group		Healthy controls		Statistics		
	n=20		n=23				
	Mean	SD	Mean	SD	χ^2/t	df	p
Age (years)	45.0(24–64)	12.4	46.4(25–67)	11.3	−.385[a]	41	.702
Gender (female/male)	7/13		8/15		.000[b]	1	.988
Smoker/non-smoker	5/15		7/16		.157[b]	1	.692
Education (years)	14.2	3.0	16.4	2.6	−2.571[a]	41	.014*
Duration of illness (years)	12.6(1–26)	7.41					
Age of onset (years)	32.4(15–53)	10.39					
17-item HDRS score	6.45(1–20)	6.07					
21-item HDRS score	7.45(1–23)	6.97					
YMRS	1.75(0–20)	4.54					
SDS			29.7(20–44)	7.1			
ACC GM, %	57.3	11.4	55.0	6.5	0.809[a]	41	0.423
WM, %	26.3	14.8	22.5	5.0	1.179[a]	41	0.245
CSF, %	16.4	7.8	22.5	8.2	−2.492[a]	41	0.017*
ltBG GM, %	21.2	6.7	19.9	4.1	0.769[a]	41	0.446
WM, %	77.8	7.6	79.0	4.9	−0.640[a]	41	0.526
CSF, %	1.0	1.2	1.0	1.4	0.129[a]	41	0.898

HDRS, Hamilton depression rating scale; YMRS, Young mania rating scale; SDS, Zung self-rating depression scale.
[a] Student's *t*-test.
[b] χ^2 tests.
* $p < 0.05$ significant.

Table 2
Dose of medication.

	n	Dose	Serum concentration
Lithium	16	837.5 ± 247.3(400–1200)	0.76 ± 0.21(0.43–1.09)
Sodium valproate	7	914.3 ± 323.7(400–1200)	55.7 ± 27.9(7.46–83.1)
Lamotrigine	4	262.5 ± 170.1(50–400)	
Antipsychotics	7	418.6 ± 243.5(75.8–625)	
Benzodiazepine	14	13.4 ± 8.06(5–27)	

Antipsychotic dose is shown as chlorpromazine equivalent. Benzodiazepine dose is shown as diazepam equivalent.

3.2. Average percent voxel composition of GM, WM and CSF in subjects by group

No significant difference was found in GM, WM and CSF composition between BD group and HC group, except for the CSF composition in the ACC (Table 1). Therefore, all metabolite concentrations in the ROI were corrected for CSF by dividing the percentage of brain tissues in each ROI, under the assumption that the metabolite concentrations in CSF were equal to zero (Bustillo et al., 2001).

3.3. MRS results

3.3.1. Metabolite changes in all patients

Repeated-measures ANCOVAs showed a significant interaction between subjects (BD or control) and metabolite concentration ($F_{[4.527,\ 176.550]}$=4.318, p=0.001). A post hoc MANCOVA with subjects as a main factor, metabolite concentrations as the dependent variables, and gender and age as covariates revealed a significant effect of subjects for Gln/Glu ($F_{[1,39]}$=6.02, p=0.019), Cr ($F_{[1,39]}$=21.99, $p < 0.001$), Gln ($F_{[1,39]}$=13.18, p=0.001), NAA ($F_{[1,39]}$=6.61, p=0.01), and Cho

Table 3
Comparison of metabolite levels between bipolar patients and controls.

	Bipolar group (n=20)		Control group (n=23)		ANCOVA1: Covariates (age, gender)			ANCOVA2: Covariates (age, gender, Li, VPA)		
	Mean	SD	Mean	SD	F	df	p	F	df	p
ACC										
Gln/Glu	0.66	0.24	0.54	0.09	6.02	39	0.019*	8.87	37	0.005*
Cr	7.29	0.98	6.27	0.59	21.99	39	0.000*	8.90	37	0.005*
Gln	5.22	1.69	3.97	0.76	13.18	39	0.001*	10.38	37	0.003*
Glu	7.95	1.18	7.39	0.80	3.84	39	0.057	0.26	37	0.610
NAA	7.79	1.46	6.99	0.71	6.61	39	0.014*	8.89	37	0.005*
Cho	2.14	0.36	1.68	0.18	41.28	39	0.000*	8.31	37	0.007*
ltBG										
Gln/Glu	0.84	0.26	0.75	0.14	2.78	39	0.103	0.25	37	0.624
Cr	6.45	0.91	6.16	0.91	1.28	39	0.266	0.07	37	0.786
Gln	4.77	1.29	4.13	0.90	4.79	39	0.035*	0.27	37	0.606
Glu	5.83	1.00	5.55	0.71	1.08	39	0.305	1.87	37	0.180
NAA	6.22	0.90	6.52	0.90	1.54	39	0.222	0.10	37	0.755
Cho	1.94	0.27	1.77	0.32	4.77	39	0.035*	0.44	37	0.513

ACC: anterior cingulate cortex; ltBG: left basal ganglia.
Gln: glutamine; Glu: glutamate; Cr: creatine; NAA: N-acetylaspartate; Cho: choline.
* Significant main effect of illness in repeated-measured ANCOVA ($p < 0.05$).

($F_{[1,39]}$=41.28, $p < 0.001$) in the ACC, and Gln ($F_{[1,39]}$=4.79, $p < 0.05$) and Cho ($F_{[1,39]}$=4.77, $p < 0.05$) in the ltBG after using the Bonferroni correction (Table 3). The levels of these metabolites were higher in subjects with BD than in the healthy controls. There were no significant effects for other metabolites (Table 3).

In the BD group, there were significant positive correlations between Li concentration and Gln/Glu and Cho in the ltBG (Gln/Glu: r=0.547, p=0.043; Cho: r=0.604, p=0.049) and between VPA concentration and Glu in the ACC (r=0.929, p=0.003). There was no significant correlation between CP equivalent and metabolites.

We additionally performed a repeated-measures ANCOVA using age, gender, Li concentration, and VPA concentration as covariates. Repeated-measures ANCOVAs showed a significant interaction between subjects (BD or control) and metabolite concentration ($F_{[4.378, 161.990]}$=3.509, p=0.007). Post hoc MANCOVAs with subjects as a main factor, metabolite concentrations as dependent variables, and gender, age, Li concentration, and VPA concentration as covariates revealed a significant effect of subjects for Gln/Glu ($F_{[1,37]}$=8.872, p=0.005), Cr ($F_{[1,37]}$=8.898, p=0.005), Gln ($F_{[1,37]}$=10.380, p=0.003), NAA ($F_{[1,37]}$=8.886, p=0.005), and Cho ($F_{[1,37]}$=8.310, p=0.007) in the ACC after the Bonferroni correction (Table 3). The levels of these metabolites were higher in subjects with BD than in controls. There were significant correlations in 9 of the 10 possible combinations of 5 metabolites in the left basal ganglia, and in all pairs in the ACC ($p < 0.05$). There were no significant effects in other metabolites (Table 3).

3.3.2. Metabolite changes in euthymic patients

Repeated-measures ANCOVAs showed a significant interaction between subjects (euthymic or control) and metabolite concentration ($F_{[4.453, 146.961]}$=3.424, p=0.008). Post hoc MANCOVAs with subjects as a main factor, metabolite concentrations as dependent variables, and gender and age as covariates revealed a significant effect of subjects for Gln/Glu ($F_{[1,33]}$=4.971, p=0.033), Cr ($F_{[1,33]}$=15.251, $p < 0.001$), Gln ($F_{[1,33]}$=10.247, p=0.003), NAA ($F_{[1,33]}$=5.245, p=0.029), and Cho ($F_{[1,33]}$=27.033, $p < 0.001$) in the ACC and Gln ($F_{[1,33]}$=4.214, p=0.048) and Cho ($F_{[1,33]}$=4.149, p=0.050) in the ltBG after Bonferroni correction (Table 4). The levels of these metabolites were higher in subjects with BD than in controls. There were no significant effects in other metabolites (Table 4).

We additionally performed a repeated-measures ANCOVA using age, gender, Li concentration, and VPA concentration as covariates. The repeated-measures ANCOVA showed a significant interaction between subjects (euthymic or control) and metabolite concentration ($F_{[4.294, 133.110]}$=2.642, p=0.033). A post hoc MANCOVA with subjects as a main factor, metabolite concentrations as dependent variables, and gender, age, Li concentration, and VPA concentration as covariates revealed a significant effect of subjects for Gln/Glu ($F_{[1,31]}$=9.401, p=0.004), Cr ($F_{[1,31]}$=4.459, p=0.043), Gln ($F_{[1,31]}$=8.234, p=0.007), and NAA ($F_{[1,31]}$=6.499, p=0.016) in the ACC after Bonferroni correction (Table 4). The levels of these metabolites were higher in subjects with BD than in controls. There were no significant effects in other metabolites (Table 4).

4. Discussion

Subjects with BD had significantly increased Gln/Glu ratios and levels of Gln in the ACC and levels of Gln in the ltBG. The findings of activated glutamatergic neurotransmission, such as increased Gln/Glu and Gln levels, are consistent with previous MRS studies (Dager et al., 2004; Frye et al., 2007b; Michael et al., 2003; Soeiro-de-Souza et al., 2015). These findings remained after limiting participants with BD to only those who were currently euthymic. The Gln/Glu ratio is the most useful index, quantifying neuronal-glial interactions and the balance of glutamatergic metabolites (Brennan et al., 2010). There were three reports about Gln/Glu of patients of BD. Dager et al. reported elevated Gln/Glu in acute mania compared to controls and schizophrenia patients (Ongür et al., 2008). Brennan et al. (2010) reported that riluzole increased Gln/Glu in BD, though they have not compared Gln/Glu between BD and HC. Soeiro-de-Souza et al. (2015) reported the reduced Glu/Gln, which is equivalent to increased Gln/Glu, in ACC of euthymic patients compared to HC. Our results are consistent with the report by Soeiro-de-Souza et al., although we could not find increased Gln/Glu in ltBG. We reconfirmed that hyperactivity of glutamatergic neurotransmission in the ACC is a disease marker for BD. The role of glutamatergic neurotransmission in the BG of patients with BD has been inconsistently reported thus far. As the basal ganglia have been variably linked to mood and affective regulation (Alexander et al., 1986; Soares and Mallinger, 1997; Strakowski et al., 2005), these results indicate that abnormality of Gln level in the ltBG is related to the pathophysiology of BD.

In the exploratory analysis, we found significant positive correlations between Li concentration and Gln/Glu and Cho in the ltBG and between VPA concentration and Glu in the ACC. No significant correlation was found between CP equivalent and metabolites. Our findings are similar to those of studies reporting that lithium treatment increased Glu/Cr and Glx/Cr in the ACC of patients with bipolar depression (Machado-Vieira et al., 2015) and increased Glu in the hippocampus of patients with BD (Colla et al., 2009; Zanetti et al.,

Table 4
Comparison of metabolite levels between euthymia and controls.

	Euthymia group (n=14)		Control group (n=23)		ANCOVA3: Covariate (age, gender)			ANCOVA4: Covariate (age, gender, Li, VPA)		
	Mean	SD	Mean	SD	F	df	p	F	df	p
ACC										
Gln/Glu	0.67	0.28	0.54	0.09	4.97	33	0.033*	9.40	31	0.004*
Cr	7.12	1.01	6.27	0.59	15.25	33	0.000*	4.46	31	0.043*
Gln	5.18	1.84	3.97	0.76	10.25	33	0.003*	8.23	31	0.007*
Glu	7.93	1.36	7.39	0.80	2.82	33	0.102	0.03	31	0.873
NAA	7.61	1.38	6.99	0.71	5.25	33	0.029*	6.50	31	0.016*
Cho	2.08	0.40	1.68	0.18	27.03	33	0.000*	3.11	31	0.088
ltBG										
Gln/Glu	0.82	0.27	0.75	0.14	1.99	33	0.168	0.04	31	0.842
Cr	6.40	0.94	6.16	0.91	1.01	33	0.322	0.07	31	0.800
Gln	4.72	1.41	4.13	0.90	4.21	33	0.048*	0.36	31	0.552
Glu	5.88	1.04	5.55	0.71	1.48	33	0.233	0.99	31	0.326
NAA	6.12	0.89	6.52	0.90	2.09	33	0.157	0.16	31	0.692
Cho	1.94	0.31	1.77	0.32	4.15	33	0.050*	0.28	31	0.599

ACC: anterior cingulate cortex; ltBG: left basal ganglia.
Gln: glutamine; Glu: glutamate; Cr: creatine; NAA: N-acetylaspartate; Cho: choline.
* Significant main effect of illness in repeated-measured ANCOVA ($p < 0.05$).

2015). Treatment with VPA also increased Glu in the ACC of patients with BD (Strawn et al., 2012). Conversely, earlier studies reported that lithium treatment decreased Glx levels (Friedman et al., 2004; O'Donnell et al., 2003). The effect of medication on MRS measurements is not fully clear. Further replication studies are needed to confirm this confounding effect. Additionally, after removing the medication effects of Li and VPA, activated glutamatergic neurotransmission was still observed in the ACC, but not in the ltBG. Similar results were observed for euthymic patients. Our findings indicate that glutamatergic neurotransmission may contribute to the pathogenesis of BD and may be modulated by mood stabilizers.

Subjects with BD also showed significantly increased NAA in the ACC. A recent systematic review of MRS studies reported that patients with BD had significantly lower NAA levels in the basal ganglia and a tendency to higher NAA in the dorsolateral prefrontal cortex than was observed in healthy controls (Kraguljac et al., 2012b). This report is similar to our findings regarding the NAA levels in the ACC. NAA is used in MRS studies as a marker of neuronal integrity to reflect neuronal viability (Demougeot et al., 2004), and a significantly positive correlation between NAA/Cr and Glx/Cr has been demonstrated (Kraguljac et al., 2012a; Waddell et al., 2011; Wang et al., 2015). The increased NAA in our study should be interpreted as increased glutamatergic neurotransmission rather than increased neuronal viability. Next, we found that patients with BD had significantly higher Cho levels in the ACC and ltBG and higher Cr levels in the ACC. These findings suggested that patients with BD have higher membrane turnover (Yildiz-Yesiloglu and Ankerst, 2006). Given the above findings regarding the change in other metabolites, the elevated Cho in patients with BD might be related to neurotoxicity due to increased glutamatergic neurotransmission. Most MRS studies reported that Cr, a measure of energy utilization, tends to be stable, and it has often been used as an internal standard comparison (Malhi et al., 2002). However, a previous study also reported elevated Cr levels in patients with BD (Frye et al., 2007b). It is thus possible that Cr level is a meaningful measurement for BD pathophysiology, and therefore, Cr should not be used as an internal standard, especially in studies of BD. In order to avoid the confounding effects of Cr levels, we used tissue-composition-corrected metabolite levels in this study.

5. Limitations

There are several methodological considerations and limitations in our study. First, we measured the metabolite levels in only two regions, the ACC and ltBG. The pathology of BD is related to other regions of the brain as well. Second, because MRS cannot selectively measure synaptic glutamatergic metabolite levels, we must carefully interpret the results regarding this. Third, we must consider the influence of medication. We attempted to use statistical analyses to remove the influence of Li and VPA on metabolism as much as possible in this study. Fourth, the diagnosis of our participants include bipolar type I and type II. Different diagnostic subtype could effect on the metabolite levels, while our small sample size may not have enough statistical power to elucidate it. Further study focusing on the difference of diagnostic subtype is needed.

In conclusion, we found that subjects with BD showed a significantly increased Gln/Glu ratio, increased Gln, Cr, NAA, and Cho in the ACC, and increased Gln and Cho in the ltBG. These results suggested that a wide range of metabolic processes, including glutamatergic neurotransmission, is associated with the pathophysiology of BD.

Contributors

HK and MN designed the study and conducted the analysis. HK, MN, SS, JI, SN, NK, SW, HU, MK, MI, MT, MO, and TO recruited participants and discussed the results. HK wrote the first draft. MN and TO revised the manuscript. CNY, YF, and MH performed 1H-MRS and LCModel analysis. All authors contributed to and approved the final manuscript.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.jad.2016.08.046.

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