Glutamatergic Correlates of Bipolar Symptoms in Adolescents

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Abstract

Objectives: Prior studies demonstrate elevated cortical glutamate (Glu) in patients with bipolar disorder (BD). Studies assessing neurochemistry in early stages of bipolar illness before the emergence of manic symptoms are lacking. This study aimed to examine neurochemical correlates measured by proton magnetic resonance spectroscopy (¹H-MRS) and a dimensional measure of bipolarity in a sample of depressed adolescents.

Methods: Adolescent participants (aged 13–21 years) underwent a semistructured diagnostic interview and clinical assessment, which included the General Behavior Inventory Parent Version (P-GBI), a 73-item, parent-rated assessment of symptoms and behaviors. ¹H-MRS scans of a left dorsolateral prefrontal cortex (L-DLPFC) voxel (8 cm³) were collected using a two-dimensional *J*-averaged sequence to assess *N*-acetylaspartate (NAA), Glu, Glx (glutamate + glutamine), and NAA/Glx concentrations. We used generalized linear models to assess the relationships between P-GBI scores and metabolite levels in L-DLPFC.

Results: Thirty-six participants (17 healthy controls, 19 depressed) underwent ¹H-MRS scans and clinical evaluation with the P-GBI. There was a significant negative relationship between P-GBI score and L-DLPFC NAA/Glx in the whole sample. However, the magnitude of the effect was small and statistical significance was lost after correcting for multiple comparisons.

Conclusions: These preliminary results suggest that NAA/Glx may have utility as a marker of bipolar traits in healthy and depressed adolescents. If replicated, ¹H-MRS measures of glutamatergic metabolism anomalies might have a role in identifying depressed adolescents at risk for mixed symptom presentations or BD. Identifying bipolarity in the early stages of the disease would have a significant impact on treatment planning and prognosis. Further longitudinal studies should examine neurochemical correlates of mood state during the developmental emergence of BD.

Keywords: glutamate, NAA, ¹H-MRS, DLPFC, adolescent, bipolar disorder

Introduction

BIPOLAR DISORDER (BD) is a disabling disease characterized by recurrent episodes of depression and mania or hypomania. The underlying pathophysiology of BD is yet to be fully characterized. The onset of BD occurs in up to 65% of patients before the age of 19, yet the initial presentation of symptoms is often ambiguous (Lish et al. 1994; Kondo et al. 2014). Due, in part, to difficulties in making accurate diagnoses, childhood-onset BD is associated with a delay of first treatment of more than 16 years (Leverich et al. 2007).

In practice, many BD patients are initially diagnosed with and treated for unipolar depression (Smith et al. 2011), which involves medications that are ineffective for bipolar depression and may increase risk for additional manic or hypomanic episodes. The prevalence of treatment failures demands the need for better understanding the neurobiology of BD and improved methods for distinguishing unipolar and bipolar illness in adolescents (Judd et al. 2002).

Proton magnetic resonance spectroscopy (¹H-MRS) is a noninvasive method to study biochemical and neuronal changes underlying psychopathology. ¹H-MRS can detect *N*-acetylaspartate

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(NAA), glutamate (Glu), glutamine (Gln), the combined signal of glutamate and glutamine (Glx), creatine, choline-containing compounds, and several other metabolites in specific brain regions. To date, ^1H -MRS findings of BD have been mixed. However, evidence supports the view that dysregulation of the excitatory glutamatergic system is involved in the pathophysiology of BD (Yüksel and Öngür 2010; Zarate et al. 2010).

Specific ^1H -MRS findings in patients with BD include NAA reductions and Glu or Gln increases in frontal areas, such as the dorsolateral prefrontal cortex (DLPFC), anterior cingulate cortex (ACC), and hippocampus (Szulc et al. 2018). As a supplement to clinical assessment, ^1H -MRS may provide a biological measure for distinguishing bipolar and unipolar depression when symptoms are unclear or before the onset of a first manic or hypomanic episode, potentially guiding treatment and reducing exposure to less effective and higher risk interventions.

This study aimed to quantify neurometabolite concentrations by ^1H -MRS and to examine the link with dimensional measures of bipolar traits in a sample of healthy controls and depressed adolescents. While several frontal brain regions play a role in mood regulation, the left dorsolateral prefrontal cortex (L-DLPFC) is prominently active in behavioral adaptation and inhibition (Boschin et al. 2017). Our primary aim was to identify specific L-DLPFC spectroscopic measures associated with the severity of dimensions of behavioral and emotional dysregulation in both healthy and depressed youth.

We anticipated a negative relationship between the General Behavior Inventory Parent Version (P-GBI) (Youngstrom et al. 2001) scores and both NAA and NAA/Glx levels, and a positive relationship between P-GBI scores and Glx and Glu levels. We focused on Glx (rather than Gln and Glu) due to general concerns regarding the validity of Glu and Gln measures. Resolving Gln and Glu is challenging because of high coupling between protons that provide the signal in standard spectroscopy protocols (Govindaraju et al. 2000; Choi et al. 2006). Glx and NAA levels are easier to quantify compared with other spectroscopic measures. This approach anticipates that measures of Glx and NAA would have a rapid clinical translational potential compared with spectroscopic measures of Glu and Gln.

Methods

Overview

All study procedures were approved by the Mayo Clinic Institutional Review Board (Rochester, MN) before any recruitment or research activities. This was a prospective cross-sectional study of depressed adolescents and controls.

Participants and clinical assessments

Adolescents and young adults with depressive symptoms between ages 13 and 21 years were recruited from a psychopharmacology clinic. Healthy control (HC) participants were recruited from pediatric primary care clinics and through community advertising and had no history of psychiatric disorder or treatment. Written informed consent was obtained from the parents or guardians of participants younger than 18 years, and from participants 18 years and older. Written assent was obtained from participants younger than 18 years.

All participants underwent clinical assessment by a board-certified child and adolescent psychiatrist (P.E.C.), including a semistructured diagnostic interview [the Schedule for Affective Disorders and Schizophrenia for School Aged Children (K-SADS-

PL; Kaufman et al. 1997)] and a range of clinician- and participant- or parent-rated scales of symptomatology and psychosocial factors. Severity of depressive symptoms was rated using the Children's Depression Rating Scale, Revised [CDRS-R; Poznanski et al. 1984)], and the Quick Inventory of Depressive Symptomatology (17-item) Adolescent and Parent Self Report [QIDS-A17-SR; Bernstein et al. 2010)]. Healthy control participants had no current or past psychiatric diagnoses based on the interview with the K-SADS-PL.

The General Behavior Inventory (GBI) is a validated questionnaire that is designed to assess for symptoms of depression and mania/hypomania (Depue 1987; Youngstrom et al. 2001). It includes 73 Likert-type items and each item is rated on a four-point scale. Prior work suggests that the parent report version of the GBI (P-GBI) has clinical utility in establishing the presence of a bipolar spectrum disorder among adolescents (Depue 1987; Youngstrom et al. 2001, 2004; Findling et al. 2002). The primary caregiver rated each item with a four-point scale, where higher scores were indicative of greater degrees of dysregulation. Participants who were 18 and older filled out GBI forms themselves.

Eligibility criteria

Adolescents in the HC group had no current or historical psychiatric diagnosis, no current or previous psychopharmacologic or psychotherapeutic treatment, and had depression severity raw scores <30 on the CDRS-R. Participants in the depressed groups had current diagnoses of unipolar depressive disorders on the K-SADS-PL diagnostic interview and had CDRS-R raw scores of 35 or greater. Exclusion criteria for all participants consisted of lifetime history of mania or psychosis, the presence of an active substance use disorder (except nicotine), and any contraindication to magnetic resonance imaging (MRI), such as implanted ferromagnetic material or orthodontic hardware that would cause artifact in magnetic resonance images.

Proton magnetic resonance spectroscopy

Eligible participants (both healthy and depressed adolescents with no contraindication to MRI) underwent ^1H -MRS scans at 3 T to assess glutamatergic metabolite concentrations in brain regions of interest. An FAST 3D SPGR sequence was utilized to acquire volumetric data for voxel positioning and tissue segmentation. Spectroscopic data were acquired using a two-dimensional J-averaged PRESS (2DJ) sequence (repetition time [TR] = 2000 ms, echo time [TE] = 35–195 ms in 16 steps, TR = 2000 ms, eight averages, three-way phase cycling) designed specifically for improved Glu measurement (Hurd et al. 2004). Measurements were collected from an 8-cm³ voxel corresponding to the L-DLPFC positioned according to previously published methods (Port et al. 2008; Croarkin et al. 2015, 2016). Stimulants were held on the day of the scan.

Following the scan, quantitative metabolite concentrations were estimated using LCModel (Provencher 2001) software version 6.3-1K and a vendor-provided basis set. Scans were reviewed by a neuroradiologist (J.D.P.) to exclude scans with visible artifacts and verify integrity of spectra. Only metabolite measurements with a Cramér–Rao standard deviation <20% were included for analysis. Scans with signal-to-noise ratio <10 were excluded. Metabolites measured included Glu, NAA, and Glx. Metabolite concentration measurements were corrected to the cerebrospinal fluid fraction according to previously published methods (Port et al. 2008; Croarkin et al. 2016).

Statistical analysis

Statistical tests were performed using SPSS version 25 (IBM Corp., Armonk, NY) and JMP Pro 14.1.0 (SAS Institute, Inc., Cary, NC) software. The significance level was set at $\alpha=0.05$. The Benjamini–Hochberg false discovery rate (FDR) method was used to correct for multiple comparisons (Benjamini and Hochberg 1995).

Demographic and clinical characteristics of the overall sample and each group (healthy controls and depressed participants) were described with mean and standard deviation for continuous variables and counts and percentages for categorical variables. Group differences were assessed with independent samples *t*-tests or Mann–Whitney *U* tests for continuous variables and Pearson's chi-squared test or Fischer's exact test for categorical variables. Distributions of parameters were assessed with Shapiro–Wilk tests. Differences between participants who were on medication (Dep/Med+) and those who are not (Dep/Med–) were also included.

The primary outcome-dependent variable was the L-DLPFC metabolite measures (NAA, Glx, Glu, and NAA/Glx ratio). For the main aim, relationships between L-DLPFC NAA, Glu, Glx, and NAA/Glx and P-GBI total scores as a dimensional measure were examined using separate generalized linear models to test the hypothesis in the whole sample. Age, sex, depression severity, and medication status were assessed as potential covariates to assess robustness of results.

The secondary aim was to compare metabolite levels between the healthy group and depressed group. Student's *t* test and Mann–Whitney–*U* test were utilized for this aim.

Exploratory analyses included in-depth assessment of differences between HC and depressed as well as comparison between HC, Dep/Med+, and Dep/Med–. All metabolite levels across groups and associations between five different P-GBI scores (total, Depression subscale, Hypomania subscale, Biphasic subscale, and combination of Biphasic and Hypomania subscale) and four different metabolite levels (NAA, Glu, Glx, NAA/Glx) were studied (Pendergast et al. 2015).

Results

Thirty-six patients with both spectroscopy scans and P-GBI scores were included in analyses. The sample size for each model was also based on the quality of the scans and this varied for each metabolite. The quality filters applied were signal-to-noise ratio ≥ 10 and Cramér–Rao lower bounds $<20\%$. Quality was good for

NAA metabolite for all participants. All analyses with Glu excluded one healthy control. For Glx and NAA/Glx analyses, one healthy participant and one depressed participant were excluded.

Demographic characteristics of participants are reported in Table 1. There were no significant differences in age and sex between groups. P-GBI, CDRS-R, and QIDS-A17-SR scores were significantly higher in the depressed group ($p < 0.001$). A list of comorbidities and daily medications of all participants are reported in Supplementary Table S1. Eight patients were on psychotropic medications. Most common comorbidities were historical cannabis use disorder (6/36) and attention-deficit/hyperactivity disorder (ADHD) (4/36).

Primary aim: analyses for P-GBI score and ¹H-MRS-measured L-DLPFC metabolites in whole sample

In the generalized linear model analyses (Table 2), there were no significant relationships between P-GBI scores and L-DLPFC Glu [$\beta = 0.033$, $p = 0.372$, $p_{(FDR)} = 0.496$] and L-DLPFC NAA [$\beta = -0.006$, $p = 0.793$, $p_{(FDR)} = 0.793$]. There was a significant positive relationship between P-GBI score and L-DLPFC Glx [$\beta = 0.159$, $p = 0.042$, $p_{(FDR)} = 0.084$], and a negative relationship with L-DLPFC NAA/Glx ratio [$\beta = -0.002$, $p = 0.022$, $p_{(FDR)} = 0.084$] that became nonsignificant after correcting for multiple comparisons (corrected for 4 using FDR method, $q = 0.05$). Results of multivariate analyses assessing robustness of significant results are reported in Supplementary Table S2. In all significant models, the results remained robust after covarying for age, sex, and age and sex together. The most important cofounder appeared to be depression severity (CDRS-R score).

Secondary aim: ¹H-MRS-measured L-DLPFC metabolite comparisons between groups

Means and standard errors of NAA, Glu, Glx, and NAA/Glx are reported in Table 3. There were no significant differences in any of the metabolites between healthy and depressed participants.

Exploratory analyses: ¹H-MRS-measured L-DLPFC metabolites and subscales of P-GBI across HC, depressed, Dep/Med–, and Dep/Med+

Exploratory analyses are summarized in Supplementary Tables S2–S5. We examined the relationship between P-GBI

TABLE 1. DEMOGRAPHICS AND CLINICAL CHARACTERISTICS

Characteristic	Whole sample (n = 36)	Controls (n = 17)	Depressed (n = 19)	p-Value
Age, mean $\pm$ SD	16.19 $\pm$ 2.01	16.65 $\pm$ 2.21	15.79 $\pm$ 1.78	0.21
Female, n (%)	23 (63.9)	12 (70.6)	11 (57.9)	0.5
CDRS-R, mean $\pm$ SD	36.08 $\pm$ 19.15	19.12 $\pm$ 2.89	51.26 $\pm$ 13.81	<0.001
QIDS-A17-SR, mean $\pm$ SD	8.22 $\pm$ 6.87	2.71 $\pm$ 2.14	13.16 $\pm$ 5.77	<0.001
Current psychotropic medication, n (%)	8 (22.2)	0 (0)	8 (42.1)	0.003
P-GBI score, mean $\pm$ SD	34.64 $\pm$ 36.45	2.53 $\pm$ 6.14	63.37 $\pm$ 26.55	<0.001
P-GBI Depression subscale, mean $\pm$ SD	23.97 $\pm$ 26.56	1.35 $\pm$ 4.12	44.21 $\pm$ 21.05	<0.001
P-GBI Biphasic subscale, mean $\pm$ SD	3.44 $\pm$ 4.61	0.18 $\pm$ 0.53	6.37 $\pm$ 4.69	<0.001
P-GBI Hypomanic subscale, mean $\pm$ SD	5.97 $\pm$ 8.61	1 $\pm$ 2.26	10.42 $\pm$ 9.77	<0.001
P-GBI Biphasic + Hypomanic subscale, mean $\pm$ SD	9.69 $\pm$ 12.81	1.18 $\pm$ 2.63	17.32 $\pm$ 13.53	<0.001

p-Values are for assessing group differences. Sex was assessed via Pearson's chi square. Current psychotropic medication use was compared with Fischer's exact test due to expected cell values <5 . CDRS-R and all P-GBI scores were compared across groups with Mann–Whitney *U*. Age and QIDS-A17-SR are compared across groups with Student's *t* test.

CDRS-R, Children's Depression Rating Scale, Revised; QIDS-A17-SR, Quick Inventory of Depressive Symptomatology (17-item) Adolescent and Parent Self-Report; P-GBI, General Behavior Inventory Parent Version; SD, standard deviation.

TABLE 2. RELATIONSHIPS BETWEEN GENERAL BEHAVIOR INVENTORY PARENT VERSION AND PROTON MAGNETIC RESONANCE SPECTROSCOPY-MEASURED LEFT DORSOLATERAL PREFRONTAL CORTEX METABOLITES

Metabolite	N	β	SE	95% CI for $\hat{b}$	p-Value	P(FDR)
NAA	36	-0.006	0.024	-0.053 to 0.04	0.793	0.793
Glu	35	0.033	0.037	-0.039 to 0.105	0.372	0.496
Glx	34	0.159	0.079	0.005-0.313	0.042	0.084
NAA/Glx	34	-0.002	0.001	-0.004 to -0.0002	0.022	0.084

Separate generalized linear models assessing relationships of total P-GBI score with ^1H -MRS-measured L-DLPFC metabolites. Unstandardized parameter estimate (coefficient) for the variable (β), standard error of β , 95% CIs for β , p -values, and FDR-corrected p -values ($q=0.05$) for the relationship between the P-GBI and the L-DLPFC metabolites are reported. Quality filters for scans included signal-to-noise ratio ≥ 10 and Cramér-Rao lower bounds $< 20\%$. Some participants were excluded from analyses due to quality filters: Glu, one healthy control; Glx and NAA/Glx, one healthy participant and one depressed participant were excluded.

^1H -MRS, proton magnetic resonance spectroscopy; L-DLPFC, left dorsolateral prefrontal cortex; CI, confidence interval; FDR, false discovery rate; NAA, *N*-acetylaspartate; Glu, glutamate; Glx, glutamate + glutamine; SE, standard error; P-GBI, General Behavior Inventory Parent Version.

score and P-GBI subscales and L-DLPFC metabolites in the depressed group, healthy group, and together as a whole sample. Age, sex, and depression severity were added to significant generalized linear models to assess robustness of results (Supplementary Table S2). Only the relationship between the P-GBI Biphasic subscale and Glx in the depressed group suggested a trend after covarying for depression severity ($\beta = 1.394$, $p = 0.054$). This set of statistical analyses demonstrated no significant relationship between L-DLPFC 2DJ NAA levels and the P-GBI total score or any of the subscales across three models (healthy participants, depressed participants, and whole sample).

Similarly, Glu levels and none of the P-GBI scores were significantly associated in any of the models. However in the whole-sample analyses, there were significant positive associations between P-GBI total score ($p = 0.0042$), P-GBI Hypomania subscale ($p = 0.042$), P-GBI Biphasic subscale ($p = 0.018$), and sum of Hypomania and Biphasic subscales ($p = 0.038$), but not for the P-GBI Depression subscale ($p = 0.117$). There was no significant relationship with healthy participants. The depressed sample had similar results except the P-GBI Biphasic subscale, which had a significant relationship ($p = 0.032$). In whole sample, the NAA/Glx ratio had a significant relationship with P-GBI total score

($p = 0.022$) and P-GBI Biphasic subscale ($p = 0.017$), but there was no significant relationship in any other model.

Comparison of the relationship coefficients between the healthy group and depressed group can be approached in two ways: (i) four metabolite levels within the same P-GBI score or (ii) five different P-GBI scores within the same metabolite. We would like to emphasize that none of these models was significant except P-GBI Biphasic subscale and Glx association. These analyses are summarized in Supplementary Table S3.

The comparison of the relationship of the P-GBI score and L-DLPFC metabolites between the Dep/Med-, Dep/Med+, and HC is summarized in Supplementary Table S5. Two of these models had significant results. In Dep/Med- sample, there were significant relationships between Glx levels and P-GBI total score ($\beta = 0.458$, $p = 0.032$) and P-GBI Biphasic subscale score ($\beta = 2.161$, $p = 0.023$).

We also looked at the distribution of metabolites and P-GBI scores across HC, Dep/Med-, and Dep/Med+ (Supplementary Table S4). There was no significant difference between healthy controls and nonmedicated and medicated patients in any of the metabolites ($p > 0.05$). There were significant differences between groups in all of the P-GBI scores ($p < 0.001$). In all P-GBI score

TABLE 3. PROTON MAGNETIC RESONANCE SPECTROSCOPY-MEASURED LEFT DORSOLATERAL PREFRONTAL CORTEX METABOLITES BY GROUP

Metabolites	Scores (n)	P-GBI total		P-GBI depression		P-GBI hypomanic		P-GBI-biphasic		P-GBI hypomanic + biphasic	
		β	p	β	p	β	P	β	p	β	p
NAA	Healthy (17)	-0.003	0.985	0.0026	0.914	-0.188	0.668	1.453	0.433	-0.0081	0.831
	Depressed (19)	-0.006	0.906	-0.04	0.538	0.0057	0.688	0.024	0.937	0.043	0.675
Glu	Healthy (16)	-0.082	0.781	-0.291	0.502	0.287	0.719	1.430	0.675	0.272	0.693
	Depressed (19)	-0.085	0.239	0.097	0.288	0.009	0.964	0.261	0.534	0.012	0.933
Glx	Healthy (16)	0.805	0.309	1.134	0.336	1.304	0.552	15.305	0.077	1.591	0.394
	Depressed (18)	0.199	0.103	0.104	0.515	0.563	0.087	1.416	0.032	0.399	0.096
NAA/Glx	Healthy (16)	-0.01	0.22	-0.013	0.261	-0.019	0.398	-0.157	0.77	-0.02	0.282
	Depressed (18)	-0.002	0.198	-0.001	0.48	-0.004	0.302	-0.013	0.12	-0.003	0.347

Due to varying quality levels of scans for each metabolite, some participants were excluded from analyses: for Glu, one healthy control ($n = 35$); for Glx and NAA/Glx ($n = 34$), one healthy participant and one depressed participant were excluded. p -Values are for assessing group differences. Student's t -test was utilized for comparing all metabolite levels except NAA/Glx. Mann-Whitney U test was used to test hypothesis for NAA/Glx ratio.

Each β and p -value pair were generated via a separate generalized linear model. Scores were the only independent variables in these models. The results of models with added covariates such as age, sex, depression severity, current psychotropic medication use are available upon request. (i) P-GBI total score coefficients and P-GBI biphasic subscale coefficients had a consistent direction for both groups for each metabolite. In all models with P-GBI biphasic score, the healthy group had larger coefficients. P-GBI Depression subscale, Hypomania subscale, and combined Biphasic and Hypomania coefficients showed variation in directionality of the association of groups across metabolites. (ii) In NAA and Glu models, coefficients were varying in size and direction across the different score types for both healthy and depressed groups. In Glx models, all coefficients were positive and larger in the healthy group. In NAA/Glx models, all coefficients were negative across the different P-GBI scores.

NAA, *N*-acetylaspartate; Glu, glutamate; Glx, glutamate + glutamine; P-GBI, General Behavior Inventory Parent Version.

types, significant differences were between (i) Dep/Med– versus HC and (ii) Dep/Med+ versus HC. There was no significant difference between Dep/Med– versus Dep/Med+ (Supplementary Table S4).

Discussion

This is the first report to explore potential correlations between P-GBI measures of bipolar traits and L-DLPFC metabolite levels in an adolescent cohort of depressed and healthy individuals. Meta-analyses (Gigante et al. 2012; Taylor 2014) have suggested that BD is associated with increased brain Glx levels in various cortical regions, whereas studies on individuals with major depressive disorder (MDD) have shown lower Glx levels compared with healthy controls. ¹H-MRS is promising as a noninvasive means of distinguishing unipolar depression from bipolar depression (Gigante et al. 2012; Taylor 2014). The NAA/Glx ratio may offer a dynamic measure of Glu brain neurochemistry (Croarkin et al. 2015). Our results offer a first step into assessing NAA/Glx's potential role in assessing bipolarity in adolescents. The NAA/Glx ratio also has a pragmatic appeal, given the relative ease of acquisition for clinical implementation.

Primary results of this study suggest that if replicated with larger samples, L-DLPFC Glx levels and NAA/Glx ratio may be helpful in early suspicion and diagnosis of BD in adolescents. However, after adjusting for multiple comparisons, these results were not significant. This may be due to several reasons. First, the age range in our sample (13–21 years) is different than what the P-GBI was previously validated for (5–17 years). There were eight participants (six HC, two depressed) who were 18 years or older in this sample. This may have introduced heterogeneity and contributed to nonsignificant results. Second, medication use (8/36) among patients may have been a confounding factor and contributed to nonsignificant results. However, our exploratory analyses regarding the medication status of subjects suggested that this was not a significant factor.

Similarly, comorbidities are likely to increase heterogeneity. The small sample size limited power in all analyses. Future longitudinal studies will also be important. However, our results may serve as a reference point for future endeavors. Studies with larger sample sizes and enhanced methodologies, such as ultrahigh-field ¹H-MRS to provide greater resolution of Glu metabolites, are needed to better understand the role of ¹H-MRS in early diagnosis of BD.

Regarding the secondary aim and performance of each metabolite, in primary and exploratory analyses, Glx showed more promise compared with Glu. This may suggest that metabolites that are included in Glx other than Glu may also have relevance in the early identification of bipolar traits. In all models, Glx had a positive relationship with P-GBI scores. This is expected and in line with prior literature demonstrating associations with high glutamatergic state and bipolarity. NAA is conceptualized as a biomarker of neuronal integrity. In our sample, none of the models with NAA were significant and only the P-GBI total score had a consistent negative coefficient. This may be related to the small sample size or the subclinical nature of symptoms assessed by the P-GBI in this set of healthy and depressed adolescents.

In this set of exploratory analyses, the P-GBI Biphasic subscale had the strongest relationship among subscales with L-DLPFC metabolites and the coefficients were consistently larger. The Biphasic subscale was a narrower range subscale than P-GBI Hypomanic subscale (0–14 vs. 0–37). Also, the Biphasic subscale was the only subscale that had significant association in depressed participants: There was a significant association between Glx levels and P-GBI Biphasic score ($\beta = 1.416$, $p = 0.032$). The results also

showed a trend after covarying for depression severity, which was the most important cofounder in all other models ($\beta = 1.394$, $p = 0.054$).

One could speculate that features measured by the Biphasic subscale may appear earlier in the emergence of BD or that the Biphasic subscale may be a sensitive measure of mixed features in adolescents with major depressive disorder. Results of exploratory analyses are summarized in Supplementary Tables S2–S5. We did not correct for multiple comparisons since these were exploratory analyses. Results must be interpreted with caution.

There were consistencies and inconsistencies with respect to existing literature and our results. In our sample, there were no between-group differences in any metabolite level between depressed and healthy participants, which contrasts with prior findings in adult literature demonstrating decreased Glx in MDD (Yüksel and Öngür 2010). There are a few possible explanations. First, medication use in some of the depressed participants may have mitigated the decrease in Glx levels, but our exploratory analyses did not support this idea. Second, it may be due to differences in a developing brain and an adult brain.

Our sample was predominantly adolescent (24/36 aged <18) and Yüksel and Ongur (2010) reviewed the adult literature. Developmental MRI studies of healthy children and adolescents report an age-related increase in total cerebral volume, which is primarily due to increases in white matter, rather than changes in gray matter (Sowell et al. 2002). Beyond the natural developmental process, the same disorder may present with opposing findings in adolescents and adults. For example, MRI morphometric studies report that compared with healthy subjects, bipolar adults exhibit enlarged amygdala, striatal, and thalamic volumes (Strakowski et al. 2000). However, adolescents with BD exhibited smaller amygdala and enlarged putamen compared with healthy subjects (DeBello et al. 2004).

Third, the difference might be rooted in the comorbidities in our sample. Cannabis use disorder was comorbid in five of the depressed participants and one healthy control. Cannabis use was suggested to lower NAA in DLPFC in adolescents (Sneider et al. 2013). ADHD was comorbid in four of the depressed participants. Frontal/striatal glutamatergic resonances (Glx) were elevated in the children with ADHD compared with healthy control subjects, but no differences were noted in NAA, Cho, or Cr metabolite ratios (MacMaster et al. 2003). This also added heterogeneity in our sample and therefore may be contributing to these results.

On the contrary, our results were somewhat similar to another study. Wise et al. (2018) measured Glu levels in the dorsal ACC via ¹H-MRS in a group of medication-free individuals experiencing major depressive episodes with unipolar ($n = 20$) and bipolar diagnoses ($n = 9$), as well as healthy controls. Each depressed group, as well as their combination, had lower Glu levels than controls, however, only the unipolar group reached significance when compared with controls. When unipolar and bipolar groups were compared, no significant difference was found in Glu levels or Glx levels.

Two studies investigated spectroscopic markers in adolescents (Rosenberg et al. 2005; Patel et al. 2008). Rosenberg et al. (2005) found that Glu and Glx levels were significantly lower in depressed participants compared with healthy controls, contrasting our results. Patel et al. (2008) compared adolescents with BD who were in a depressed episode and healthy controls. Depressed BD adolescent patients had higher mean Glx levels in ACC, left ventrolateral prefrontal cortex, and right ventrolateral prefrontal cortex, although group differences did not reach significance thresholds, somewhat similar to our results.

A limitation of this study is that we did not examine individual items on the P-GBI that would measure traits more relevant to the DLPFC. Using a specific item (rather than the total summary score) might identify a more robust relationship between individual traits and spectroscopic metabolite levels. Our small sample size limited the power in all models, which might be a reason why none of the subgroup analyses showed significant results. Furthermore, this was a cross-sectional study, which makes it difficult to make inferences on causality and temporality of the findings.

The existing literature predominantly compares bipolar patients with euthymic or healthy controls, and unipolar depression patients with controls, without examining any dimensional clinical- or symptom-based measures in analyses. Considering the developmental trajectory of BD in adolescence and young adulthood, as well as potential metabolite differences in depressed versus euthymic versus manic/hypomanic mood states, longitudinal studies in this population will be essential in understanding the metabolic mechanisms of BD and brain changes that occur in response to BD treatments. This will facilitate the understanding of the role of NAA and Glx metabolism in the pathophysiology of BD. In addition, Glx, NAA, and NAA/Glx measured by spectroscopic imaging may ultimately be used in early differentiation of depressed adolescents, so that appropriate BD treatments can be implemented early in the course for those who will develop BD at a later age.

Conclusions

The present study suggested that P-GBI scores' L-DLPFC NAA/Glx had a negative relationship in a sample of depressed and healthy adolescents. The results of this study are limited because of the multiple comparisons performed on small sample sizes. If replicated, ¹H-MRS measures of glutamatergic metabolism anomalies might have a role in identifying depressed adolescents at risk for mixed symptom presentations or BD. Longitudinal studies will be required to determine the positive predictive value of NAA/Glx, with respect to the development of BD in children and adolescents with depressive symptoms. Identifying bipolarity in the early stages of the disease would have a significant impact on treatment planning and prognosis.

Clinical Significance

Glutamatergic metabolism has a complex role in adolescent mood disorders. This study suggests that ¹H-MRS measures of NAA/Glx may have utility as a marker or risk for bipolarity in adolescents. Spectroscopic measures of NAA/Glx also have a relatively high clinical translational potential in comparison with other methodologies and metabolite measures. Longitudinal studies of neurochemical correlates of mood and bipolarity in developing adolescents hold the prospect of refining the understanding of the underlying neurobiology and biomarker development efforts.

Disclaimer

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Authors' Contributions

A.I.S., C.P.L., J.D.P., A.C.-A., C.J.B., B.J.S., A.J.M., J.M.L., M.A.F., and P.E.C. contributed to the design of the study and interpretation of data. A.I.S., C.P.L., J.D.P., C.J.B., B.J.S., and P.E.C. acquired the data. A.I.S. and P.E.C. completed the statistical ana-

lyses. A.I.S., C.P.L., J.D.P., A.C.-A., C.J.B., B.J.S., A.J.M., J.M.L., M.A.F., and P.E.C. drafted, revised, critically reviewed, and approved the final submitted draft of the article. A.I.S. and P.E.C. had full access to all of the data in the study. P.E.C. takes responsibility for the integrity of the data and the data analysis.

Disclosures

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Supplementary Material

Supplementary Table S1
Supplementary Table S2
Supplementary Table S3
Supplementary Table S4
Supplementary Table S5

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