Lower *N*-Acetyl-Aspartate Levels in Prefrontal Cortices in Pediatric Bipolar Disorder: A ^{1}H Magnetic Resonance Spectroscopy Study

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Objective: The few studies applying single-voxel ^{1}H spectroscopy in children and adolescents with bipolar disorder (BD) have reported low *N*-acetyl-aspartate (NAA) levels in the dorsolateral prefrontal cortex (DLPFC), and high myo-inositol / phosphocreatine plus creatine (PCr+Cr) ratios in the anterior cingulate. The aim of this study was to evaluate NAA, glycerophosphocholine plus phosphocholine (GPC+PC) and PCr+Cr in various frontal cortical areas in children and adolescents with BD. We hypothesized that NAA levels within the prefrontal cortex are lower in BD patients than in healthy controls, indicating neurodevelopmental alterations in the former. **Method:** We studied 43 pediatric patients with *DSM-IV* BD (19 female, mean age 13.2 ± 2.9 years) and 38 healthy controls (19 female, mean age 13.9 ± 2.7 years). We conducted multivoxel in vivo ^{1}H spectroscopy measurements at 1.5 Tesla using a long echo time of 272 ms to obtain bilateral metabolite levels from the medial prefrontal cortex (MPFC), DLPFC (white and gray matter), cingulate (anterior and posterior), and occipital lobes. We used the nonparametric Mann–Whitney *U* test to compare neurochemical levels between groups. **Results:** In pediatric BD patients, NAA and GPC+PC levels in the bilateral MPFC, and PCr+Cr levels in the left MPFC were lower than those seen in the controls. In the left DLPFC white matter, levels of NAA and PCr+Cr were also lower in BD patients than in controls. **Conclusions:** Lower NAA and PCr+Cr levels in the PFC of children and adolescents with BD may be indicative of abnormal dendritic arborization and neuropil, suggesting neurodevelopmental abnormalities. J. Am. Acad. Child Adolesc. Psychiatry, 2011;50(1): 85–94. **Key words:** magnetic resonance spectroscopy, bipolar disorder, prefrontal cortex, *N*-acetyl aspartate

Proton magnetic resonance spectroscopy (^{1}H MRS) is a noninvasive neuroimaging technique that measures neurochemicals such as *N*-acetyl aspartate (NAA), as well as the choline-containing compounds glycerophosphocholine plus phosphocholine (GPC+PC) and the compounds phosphocreatine plus creatine (PCr+Cr).

NAA is related to myelin formation and participates in the energy metabolism of neuronal mitochondria.[1,2] During early development, NAA levels increase in parallel with dendritic arborization and the formation of synaptic connections, and NAA is therefore considered a neuroaxonal marker of tissue function.[3,4] Low NAA levels can occur as a consequence of neuropil reduction and axonal metabolic dysfunction.[5]

The peaks in GPC+PC levels include very small amounts of glycerophosphocholine (a metabolite in the catabolic pathway of membrane phospholipids) and phosphocholine (a precursor of membrane phospholipids), and membrane phospholipids in the bilayer structure function as a barrier between cellular components such as neuronal dendrites and synaptic connections. The GPC+PC compound consists of membrane phospholipid metabolites involved in membrane synthesis and breakdown, and products of recep-

tor-mediated lecithin hydrolysis also serve as important second messengers in signal cascades that control cell growth. This compound is also a precursor of the synthesis of acetylcholine, the neurotransmitter involved in memory and cognition.[6]

The PCr+Cr peaks contain mostly phosphocreatine and creatine. The reaction between phosphocreatine and creatine serves as energy storage of phosphates and the adenosine 5′-triphosphate/adenosine diphosphate ratio. Therefore, PCr+Cr is essential for the regeneration of adenosine 5′-triphosphate consumed by the cell, and decreased PCr+Cr concentrations suggest decreased energy metabolism.[7,8]

In adults with bipolar disorder (BD), phosphorus MRS (^{31}P-MRS) that measures high-energy phosphate metabolism, and ^{1}H MRS suggest a mechanism of mitochondrial dysfunction that involves a decrease in total cellular energy production and altered phospholipid metabolism.[1] However, ^{1}H MRS has been underused as a tool in the study of pediatric BD and the underlying neurodevelopmental processes.[9-11]

The few studies applying single-voxel ^{1}H MRS in children and adolescents with BD, compared to healthy controls, have reported lower NAA levels and NAA/PCr+Cr ratios in the dorsolateral prefrontal cortex (DLPFC),[12,13] lower levels of NAA and GP+PC in the orbitofrontal cortex,[14] higher myo-inositol/PCr+Cr ratios in the anterior cingulate,[15,16] and lower glutamate plus glutamine levels and higher glutamate plus glutamine/PCr+Cr ratios in the frontal lobes and basal ganglia.[17] However, other studies demonstrate that NAA levels in the anterior cingulate and DLPFC of pediatric BD patients are comparable to those observed in healthy controls,[16,18] Table 1[12-25] summarizes current available evidence on ^{1}H MRS findings among children and adolescents with BD.

Currently, in a single MRI scan session, multiple in vivo ^{1}H MRS spectra can be acquired to simultaneously measure metabolite levels in multiple brain areas.[7,26] This approach would broaden the neurochemical investigation of specific brain areas that are interconnected and functionally related to the symptomatology of mood disorders, mainly the fronto-limbic areas, is the model proposed by various researchers.[10,11] Specifically in pediatric BD, a comprehensive neurochemical examination of these frontal areas would shed light on how this disorder affects the developing brain.

Previously, we reported lower NAA levels within the left DLPFC of children and adolescents with type I or type II BD compared to healthy controls in a single-voxel H MRS approach.[20] Now, in our current study we enlarged our sample to include those with BD not otherwise specified (NOS) and also adopted a multivoxel ^{1}H MRS approach so as to simultaneously evaluate the following a priori regions of interest (ROIs): the medial prefrontal cortex (MPFC), the DLPFC (white and gray matter), and the cingulate (anterior and posterior), to obtain a neurochemical profile of these various frontal areas, all of which are involved in mood regulation. Indeed, PFC and cingulate functions, such as decision making, executive functions, and emotion integration, have been reported as abnormal in BD youth.[27] We hypothesized that NAA levels in the DLPFC, MPFC and anterior cingulate are lower in children and adolescents with BD compared with healthy controls, which could be indicative of neurodevelopmental abnormalities. As a control region, we included the occipital cortex in our study, as it is not believed involved in any neural pathway that regulates mood and therefore was expected to present normal levels of the neurochemicals evaluated.

METHOD

Subjects

Children and adolescents to be included in the control group were recruited from newspaper and television advertisements and flyers posted in the community. Children and adolescents with BD were referred by local psychiatrists. In both cases, only those individuals between 8 and 17 years of age were included. Individuals with serious medical problems or contraindications to magnetic resonance imaging (MRI) were excluded. Patients were included if they had been diagnosed with BD according to *DSM-IV* criteria (1994). Patients with BD presenting with substance abuse or dependence in the 6 months preceding study enrollment were excluded, as were those with a history of schizophrenia, developmental disorders, eating disorders, Tourette syndrome, or mental retardation. For healthy controls, exclusion criteria were any lifetime Axis I *DSM-IV* psychiatric disorder and history of any Axis I psychiatric disorder in first-degree relatives.

A total of 43 pediatric patients with *DSM-IV*–diagnosed BD (27 with BD type I, 10 with BD type II, and 6 with BD NOS) were compared with 38 healthy controls. The two groups were comparable in terms of

TABLE 1 MRS Findings Among Children and Adolescents with Bipolar Disorder

Study	Sample	Age, y (mean ±SD)	Main Findings in BD patients compared with HC
Castillo et al. (2000)[17]	10 BD	8	↑ Glutamate/glutamine in basal ganglia and frontal lobes
	10 HC	Non-age matched	NAA: no differences between BD and HC in frontal and temporal cortices
Davanzo et al. (2001)[15]	11 manic BD	11.4	Trend toward ↑ mI/PCr+Cr in ACC.
	11 HC	age matched	↓ mI/PCr+Cr ratio in BD was associated with lithium treatment
Cecil et al. (2002)[14]	17 (8 mixed + 9 manic) BD	22.3 ± 7.3	↓ NAA and choline in medial orbital frontal cortex gray matter
	21 HC	21.7 ± 5.2	
Chang et al. (2003)[13]	15 euthymic BD	12.6 ± 2.9	↓ NAA in right DLFPC
	11 HC	12.6 ± 2.9	
Cecil et al. (2003)[19]	9 euthymic Mood disorder (7 BD, 2 MDD)	9.8 ± 1.4	↑ mI in frontal cortex A trend towards ↓ NAA and PCr+Cr in cerebellar vermis
	10 HC	10.8 ± 1.8	
Davanzo et al. (2003)[16]	10 manic BD	9.8 ± 2.0	↑ mI and mI/ PCr+Cr in ACC in BD versus IED and HC
	10 IED	9.6 ± 3.0	No differences in occipital cortex
	13 HC	11.7 ± 3.6	
Sassi et al. (2005)[12]	14 BD	15.5 ± 3.0	↓ NAA in left DLPFC
	18 HC	17.3 ± 3.7	
Gallelli et al. (2005)[21]	60 offspring of BD I or II (32 with BD and 28 with BD subsyndromal symptoms)	14.1 ± 3.0 12.2 ± 2.6	No differences in NAA/Cr ratios in left or right DLPFC
	26 HC	14.2 ± 2.8	
DelBello et al. (2006)[22]	20 manic or mixed BD pre- and post- 28 day olanzapine administration (11 remitters and 8 nonremitters)	14 ± 2 15 ± 2	↑ NAA in medial VPFC in BD olanzapine remitters (N = 11) compared to BD nonremitters (N = 8) Over time, ↓ NAA in BD nonremitters and ↑ NAA in BD remitters ↑ Choline from baseline to day 7 in medial and right VLPFC in BD
	10 HC	15 ± 2	
Patel et al. (2006)[23]	28 depressed BD type I pre and 42 day post-lithium	15.5 ± 1.5	↑ mI over time in medial and right lateral PFC ↑ mI on day 42 of lithium compared to those on day 7
Olvera et al. (2007)[20]	35 BD	13.2 ± 2.9	↓ NAA in left DLPFC
	36 HC	13.7 ± 2.6	
Patel et al. (2008)[18]	28 depressed BD	15.5 ± 1.5	↑ NAA in ACC
	10 HC	14.6 ± 1.8	↑ NAA, choline, PCr+Cr in left VLPFC ↑ NAA, PCr+Cr, mI in right VLPFC
Patel et al. (2008)[24]	28 depressed BD pre- and post-28 day lithium administration	15.5 ± 1.5	↓ NAA in medial VLPFC over time after lithium administration No difference in NAA in left or right VLPFC
Chang et al. (2009)[25]	10 mood dysregulation but not full BD, all BD offspring pre and post 12 week divalproex	11.3 ± 3.6	No change in NAA/PCr+Cr, mI/PCr-Cr, Cho/PCr+Cr in left and right DLPFC

Note: ↑ *increased or higher;* ↓ *decreased or lower; ACC= anterior cingulate cortex; BD= bipolar disorder patients; DLPFC= dorsolateral prefrontal cortex; HC= healthy controls; IED= intermitent explosive disorder; mI = myo-inositol; NAA = N-acetyl aspartate; PCr+Cr = phosphocreatine-creatine; VLPFC = ventral lateral prefrontal; WMH = white matter hyperintensities.*

TABLE 2 Sociodemographic Characteristics of Pediatric Bipolar Disorder Patients and Healthy Controls

Characteristic	Bipolar Patients (*n* = 43)	Healthy Controls (*n* = 38)	Analysis
Mean age, y (range)	13.2 ± 2.9 (8–17)	13.9 ± 2.7 (8–17)	$t = 1.13$, df = 79, $p = .26$
Gender, male/female (%/%)	24/19 (55.8/44.2)	19/19 (50.0/50.0)	Fisher's exact test: $p = .66$
Race/ethnicity, *n* (%)			
White	27 (62.7)	6 (15.8)	$\chi^2 = 19.0$ $p = .01$
Hispanic	12 (27.9)	26 (68.4)	
African American	02 (4.7)	4 (10.5)	
Asian	02 (4.7)	2 (5.3)	
Education (y)	7.2 ± 2.9	7.9 ± 2.7	$U = 694.0$ $p = .24$
Socioeconomic status[a]	43.2 ± 13.9	45.2 ± 14.6	$U = 463.5$ $p = .52$
Puberty degree, *n* (%)			
Prepubertal	11 (25.5)	4 (10.5)	
Onset	10 (23.3)	9 (23.7)	$\chi^2 = 4.2$ $p = .39$
Middle	5 (11.6)	6 (15.8)	
Advanced	14 (32.6)	16 (42.1)	
Postpubertal	3 (7.0)	3 (7.9)	

Note: [a]*Socioeconomic status expressed as the Hollingshead Two-Factor Index of Social Status.*

mean age, gender, education, socioeconomic status, and pubertal development. There were more Hispanic patients in the control group and more Caucasian subjects in the BD group. Sociodemographic variables are displayed in Table 2. The clinical characteristics of the pediatric BD patients are shown in Table 3.

Subject Assessment

Subjects and parents or legal guardians were interviewed separately using the Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime version (K-SADS-PL).[28] All interviews were reviewed by a board-certified child and adolescent psychiatrist. In five consecutive cases, the interrater agreement between interviewers and the child and adolescent psychiatrist was 100% for the following: BD type I, type II, and NOS; schizoaffective disorder; major depression; attention-deficit/hyperactivity disorder (ADHD); and oppositional defiant disorder. We rated the severity of mania and depression using the Young Mania Rating Scale (YMRS)[29] and the Children's Depression Rating Scale, Revised (CDRS),[30] respectively. Parental socioeconomic status was measured using the Hollingshead Two-Factor Index of Social Status.[31] Pubertal development was scored according to the Pubertal Development Scale.[32] Laterality was measured using the Edinburgh handedness inventory.[33]

The study design was approved by institutional review board of the University of Texas Health Science

TABLE 3 Clinical Characteristics of the Pediatric Bipolar Patients Sample

Clinical Characteristic	Bipolar Patients (*n* = 43)
YMRS (mean ± SD)	11.0 ± 9.03
CDRS (mean ± SD)	35.0 ± 14.6
Age of BD onset (mean ± SD), y	8.7 ± 3.0
Length of illness (mean ± SD), mo	47.7 ± 30.4
Last reported mood episode (%)	
Euthymic	8 (18.6)
Depressed	11 (25.6)
Hypomanic/manic	12 (27.9)
Mixed	12 (27.9)
Off medication, *n* (%)	12 (27.9)
Current comorbidities, *n* (%)	
ADHD	28 (65.1)
Oppositional defiant disorder	15 (34.9)
Conduct disorder	5 (11.6)
Panic disorder	2 (4.7)
Separation anxiety	10 (23.3)
Social phobia	5 (11.6)
Simple phobia	6 (14.0)
Generalized anxiety disorder	17 (39.5)
Obsessive compulsive disorder	3 (7.0)

Note: ADHD = attention deficit hyperactive disorder; BD = bipolar disorder; CDRS = Children's Depression Rating Scale, Revised; YMRS = Young Mania rating Scale.

Center at San Antonio. All subjects or their parents/legal guardians gave written informed consent.

Multivoxel Proton Magnetic Resonance Spectroscopy

Our multivoxel or chemical shift imaging (CSI) ^{1}H MRS study was performed on a 1.5-T Philips Intera scanner (8.1.1; Philips Medical Systems, Best, the Netherlands) in the Audie Murphy Division of the South Texas Veterans Health Care System. We used the point-resolved spectroscopy sequence (PRESS) to localize a region of interest, which resulted in the inferior slice being positioned just above the corpus callosum, including only the superior border of the latter. Above the first slice, four additional slices were acquired with 5-mm gaps, using 16 × 16 phase-encoding steps. We adjusted the slice angle to be parallel with the anterior commissure–posterior commissure line to exclude the sinus. Acquisition parameters were as follows: repetition time/echo time = 1.5 s/ 272 ms; field of view = 24 cm; spectral bandwidth = 2,000 Hz; complex data points = 1,024; CSI matrix size 16 × 16; nominal voxel size 1.5 × 1.5 cm; dimension of the region of interest localized by the PRESS sequence within the CSI= 119.14 ± 12.89 × 146.61 ± 16.12 × 2cm^3; number of measurements = 2; and acquisition time = 16 min. We acquired five T_1-weighted scout images to achieve appropriate placement of the multivoxel axial slice. The PRESS defined the anterior/posterior and right/left dimension of the region of interest defined in the axial slice. To reduce lipid contamination from the anterior, posterior, and lateral scalp, we positioned four outer volume saturation bands. To obtain absolute quantification, we also collected data related to water unsuppressed spectra using 8 × 8 phase-encoding steps and pulses for chemical shift selective imaging.[34]

Postacquisition Data Processing

The unsuppressed data were zero filled before the Fourier transform to match the 16 × 16 matrix before the spectral data were transferred to the workstation. The SpecTool version 3.2 software (Greg Metzger, 2001, Philips Medical Systems, Bothell, WA), which runs under the Philips Research Imaging Development Environment (PRIDE), was used to shift a particular voxel (within the 16 × 16 voxel-matrix) superimposed on T1weighted axial images over 12 predefined (left and right) brain areas (Figure 1): the MPFC, anterior cingulate, posterior cingulate, DLPFC white matter, DLPFC gray matter, and occipital lobe. This process was applied to the data related to water-suppressed spectra, as well as to those related to water unsuppressed spectra. We used the PRIDE to extract the complex time–domain signal of the shifted voxels, which was then quantified as a separate spectrum. Before the spectral fitting, we removed any residual water or lipid signals using Hankel-Lanczos singular value decomposition.

We obtained the absolute metabolite levels (mmol/kg wet weight) using the quantified water peak from the data related to water unsuppressed spectra.[34] We modeled the NAA, PCr+Cr and GPC+PC peaks in the time domain using Gaussian-modulated sinusoidal functions and the nonlinear Levenberg-Marquardt algorithm.[35] Spectra were systematically rejected if the chemical shift was not within the ±2 ppm window of the NAA, GPC+PC, and PCr+Cr peaks (2.01 ppm, 3.02 ppm and 3.20 ppm, respectively), as was any spectral peak with a linewidth ≥50 Hz (18% of voxels were rejected).

The T_1-weighted images were co-registered to the axial scout images, corrected for any B_1 field bias, the brain was extracted and the images segmented into partial volume maps of gray and white matter tissue, and CSF/extra-cortical space using FreeSurfer and FSL tools, in a fully automated procedure. We estimated the tissue fractions by extracting the region of interest matching the coordinates and size of the ^{1}H spectroscopy voxel from the segmented images using FSL tools. We estimated the proportion of gray and white matter, and CSF/extracortical space for each extracted ^{1}H spectroscopy voxel. The tissue fractions were used in the calculation of metabolite concentration levels, and there were no significant differences in tissue fractions between groups.

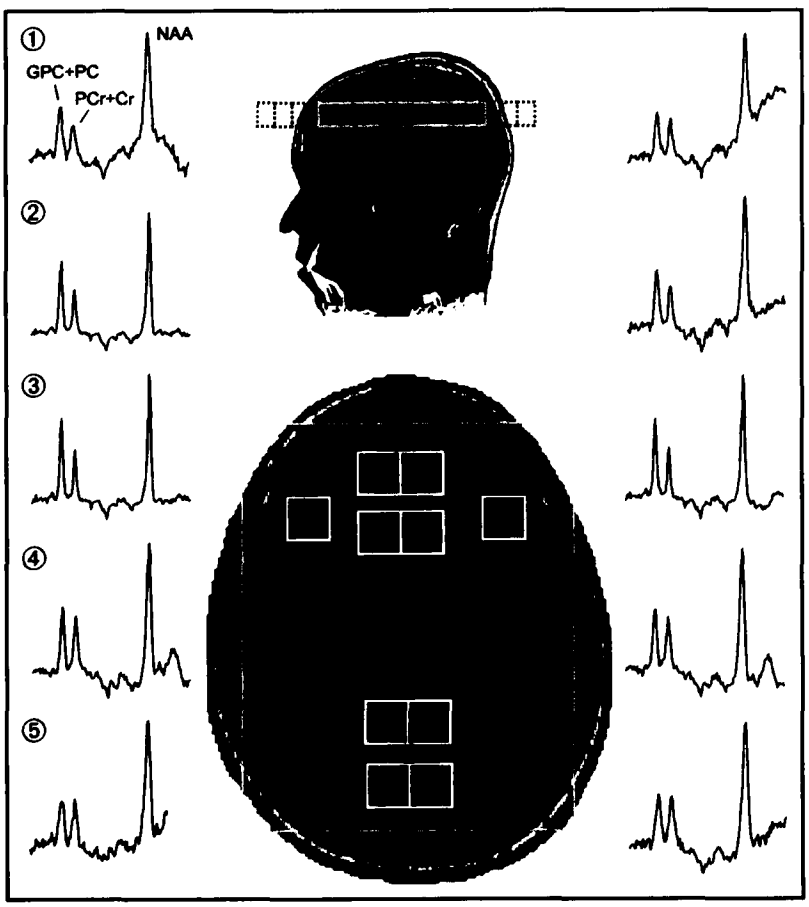
Statistical Analysis

We conducted the statistical analyses using the program Statistical Package for the Social Science, version 14 for Windows (SPSS Inc., Chicago, IL). We tested the data distribution using the Shapiro–Wilk test. To compare the spectroscopy values that did not follow normal distribution, we used the Mann–Whitney *U* test. To gain further insight into possible clinical contributions to our findings, we performed secondary analyses of clinical subtypes for the anatomical regions that presented significant differences between BD patients and controls. Because we had an independent hypothesis for each region, we applied a Bonferroni-corrected threshold controlling for the number of metabolites examined at each region. Therefore we adopted a significance level of $p < .017$ (two-tailed).

RESULTS

In the MPFC, and left DLPFC white matter, NAA levels were lower in the BD patients than in the controls. In addition, GPC+PC levels in the MPFC were lower in the BD patients than in the controls, as were PCr+Cr levels in the right MPFC and left DLPFC white matter. Means and statistical significance are shown in Table 4.

FIGURE 1 Position of the chemical shift imaging (CSI) voxels and spectra. Note: Circled numbers in figure are as follows: 1, right medial prefrontal cortex (MPC); 2, right dorsolateral prefrontal cortex (DLPFC) gray matter; 3, right anterior cingulate; 4, right posterior cingulate; and 5, right occipital lobe.



Secondary Analyses

To support our primary analyses, we conducted secondary analyses by determining the influence of clinical variables. In terms of the levels of the neurochemicals evaluated, we found no differences between the following subgroups: BD patients with ADHD or anxiety disorders versus BD patients without such disorders; BD type I patients versus BD type II or BD NOS patients; and BD patients who were medication naive or had been off medication for at least 2 weeks (n = 12) versus healthy controls.

DISCUSSION

Our principal finding was that NAA and GPC+PC levels within the bilateral MPFC, and PCr+Cr levels in the right MPFC of pediatric BD patients were lower than in those of healthy controls. In the left DLPFC white matter, the BD patients displayed lower NAA and PCr+Cr levels. The use of the multivoxel ^{1}H MRS allowed the generation of a neurochemical profile by assessing multiple brain areas simultaneously. Overall, children and adolescents with BD presented lower neurochemical levels in the areas that are involved in the pathophysiology of pediatric BD.[9-11] Because pediatric BD is a neurodevelopmental disorder that manifests itself according to the central nervous system maturation,[36] our results suggest that these frontal areas may be underdeveloped. A possible explanation is that the cytoarchitecture of the brain might be altered by the organization of the neuropil (dendrites, dendritic spines, and axon terminals), as well as by the influence of epigenetic factors.[37] Following this line, we can surmise that the lower ^{1}H MRS-determined levels of neurochemicals in BD patients could be indicative of lower synaptic density in the neuropil.

TABLE 4 Comparison of Pediatric Bipolar Patients and Healthy Controls

	Bipolar Patients		Healthy Controls		Statistics	
	Mean ± SD (mmol/kg)		Mean ± SD (mmol/kg)		*p*	
Region	**Left**	**Right**	**Left**	**Right**	**Left**	**Right**
Medial prefrontal						
NAA	12.88 ± 4.53	12.13 ± 3.27	15.53 ± 4.14	14.50 ± 3.93	.002[a]	.004[a]
PCr+Cr	10.47 ± 4.88	10.50 ± 5.11	11.86 ± 5.21	12.38 ± 4.55	.06	.005[a]
GPC+PC	2.11 ± 0.90	2.12 ± 0.92	2.64 ± 1.21	2.51 ± 0.73	.006[a]	.002[a]
DLPFC white matter						
NAA	11.75 ± 3.29	11.75 ± 3.51	12.83 ± 1.97	12.92 ± 1.90	.013[a]	.03
PCr+Cr	9.56 ± 3.87	9.18 ± 3.69	10.94 ± 2.22	10.21 ± 2.67	.011[a]	.02
GPC+PC	2.41 ± 0.98	2.42 ± 1.19	2.46 ± 0.47	2.59 ± 0.73	.04	.07
DLPFC gray matter						
NAA	15.23 ± 3.87	16.95 ± 5.26	17.70 ± 4.03	18.78 ± 3.85	.06	.09
PCr+Cr	11.66 ± 4.17	12.66 ± 4.52	13.73 ± 5.07	14.94 ± 4.93	.08	.05
GPC+PC	2.51 ± 1.18	2.54 ± 1.21	2.65 ± 0.82	2.60 ± 0.91	.07	.30
Anterior cingulate						
NAA	13.99 ± 3.29	14.23 ± 4.37	14.84 ± 2.80	14.62 ± 2.86	.04	.27
PCr+Cr	10.91 ± 3.85	10.73 ± 5.51	11.65 ± 2.57	11.24 ± 3.82	.09	.05
GPC+PC	2.77 ± 0.74	2.71 ± 0.77	2.90 ± 0.63	2.91 ± 0.70	.15	.13
Posterior cingulate						
NAA	13.76 ± 3.25	13.92 ± 3.69	13.96 ± 3.52	14.38 ± 3.13	.54	.39
PCr+Cr	10.0 ± 3.26	9.96 ± 3.10	9.72 ± 3.27	11.34 ± 4.05	.74	.08
GPC+PC	2.18 ± 0.84	2.22 ± 0.67	2.15 ± 0.86	1.98 ± 0.70	.88	.24
Occipital lobe						
NAA	15.09 ± 5.16	15.13 ± 4.56	16.21 ± 4.66	15.92 ± 3.80	.42	.27
PCr+Cr	11.25 ± 4.68	11.85 ± 4.73	12.16 ± 6.15	12.24 ± 6.42	.50	.83
GPC+PC	2.26 ± 1.19	2.15 ± 0.97	2.32 ± 1.61	2.26 ± 1.32	.51	.95

Note: DLPFC = dorsolateral prefrontal cortex; GPC+PC = glycerophosphocholine phosphocholine; NAA = N-acetyl-aspartate; PCr+Cr = phosphocreatine-creatine;

[a] *Significant findings at the Bonferroni-corrected threshold of .017.*

Prefrontal Cortex

Our principal finding related to the prefrontal cortex was that NAA levels within the MPFC and left DLPFC white matter were lower in pediatric BD patients than in healthy controls. This is in agreement with our previous reports and others of lower NAA levels[12,20] and NAA/PCr+Cr ratios in the DLPFC[13] of youths with BD. Lower NAA levels in prefrontal areas could be interpreted as a sign of neuronal dysfunction, neurodevelopmental delay, and moderately delayed myelination. However, our findings differ from those two studies presenting contrasting results.[17,21] Castillo et al (2000) used varying voxel sizes ($2\times2\times2$ cm^3 in some patients and $3\times3\times3$ cm^3 in others), thereby covering variable prefrontal cortex areas.[17] Gallelli et al (2005) used a mixed sample of BD offspring with BD or subsyndromal symptoms.[21]

Our pediatric BD sample presented lower GPC+PC in the MPFC, as well as lower PCr+Cr in the right MPFC and bilateral DLPFC white matter. Cecil et al (2002) also reported that GPC+PC levels are lower in the orbitofrontal cortex of adults with BD during manic episodes.[14] However, few studies have demonstrated abnormalities in GPC+PC levels in adults with BD.[38-40] In pediatric BD patients, lower GPC+PC levels might represent lower cell membrane content, indicating diminished maturation of the MPFC. In medication-free adults with BD, Frey et al (2007) also reported lower PCr+Cr in the left DLPFC,[38] suggesting mitochondrial dysfunction. In our study, we performed absolute quantification of metabolite concentrations (mmol/kg) rather than using the relative method, which expresses metabolite concentrations as a ratio to PCr+Cr. One previous MRS study demonstrated that different results can be obtained depending on whether absolute or relative levels of neurochemi-

cals are used.[41] It has recently been suggested that quantification of absolute levels is the preferred method for producing accurate and reproducible results.[42]

Most structural MRI studies have reported decreased volume in the prefrontal brain areas of BD subjects in comparison with those of controls.[43-45] Functional MRI findings have also suggested abnormalities in prefrontal areas.[46-48] Taken together, these results suggest impairment in the prefrontal cortical structure and function in pediatric BD.

Although the prefrontal cortex has extensive connections with specific regions involved in mood regulation, including the striatum, globus pallidus, substantia nigra and thalamus, it is also responsible for emotional expression and for the organization of cognitive functions.[49] The prefrontal cortex has been traditionally compartmentalized into two systems: the orbitomedial, which is involved in the modulation of and the automatic response to emotional stimuli; and the dorsal, which is involved in planning and the self-regulation of emotions.[50] Emotional regulation occurs by mechanisms such as reappraisal and attentional control that require the activation of lateral, medial and orbital frontal regions.[51] In fact, the DLPFC is one of the last regions to mature in the brain, probably due to its role in the integration of cognitive functions.[52] Our findings may indicate disruption of these circuits, possibly related to the brain maturation process, with the subsequent manifestation of poor emotional regulation.

Cingulate Cortex

We did not find any significant differences regarding neurochemical levels between pediatric BD patients and healthy controls. In previous studies of the anterior cingulate, no differences were found in terms of these metabolites,[15,16,53] one such study actually demonstrating higher NAA levels.[18] The anterior cingulate (Brodmann's areas 24, 25 and 33) is responsible for emotion integration, motor control and the arousal/motivation state.[54] Some of the well-known symptoms of BD, including cognitive deficits,[55] could be related to cingulate dysfunction or to its neurocircuitry.

Occipital Lobes

We also examined the occipital cortex as a control region, since it is not believed to be involved in any neural pathway that regulates mood. As expected, no differences were found between pediatric BD patients and healthy controls in terms of the levels of any of the neurochemicals examined in the occipital cortex.

Secondary Analyses

We did not find any effects of medication use in our sample. Studies that examine the direct effects of medication are conflicting. Moore et al found that treatment with risperidone resulted in an overall increase in the glutamate-glutamine/creatine ratio in the anterior cingulate.[53] Davanzo et al reported a decrease in anterior cingulate myo-inositol ratios in adolescents who responded to lithium, although there were no significant changes in choline/creatine, glutamate/creatine, or NAA/creatine ratios.[15] DelBello et al found differential neurochemical chemical changes in the ventral MPFC levels of NAA in adolescents treated with olanzapine, with remitters showing an increase and nonremitters a decrease. The investigators also found an overall increase in MPFC and right lateral PFC choline levels after 7 days of olanzapine administration, although they reported no changes in other metabolites.[22] Nevertheless, the various findings reported for medications in previous studies call for caution in interpreting the negative results of the present study.

Our sample also presented high levels of comorbidity with ADHD and anxiety disorders. We found no differences in terms of comorbid conditions in the secondary analyses. Individual studies investigating these effects, with adequate power to detect differences, are needed.

One major limitation of our study is that our BD patients presented with various mood states at the time of the scan. Another important limitation is that we chose to apply less conservative statistics because of the novelty of presenting the first multivoxel ^{1}H MRS data on pediatric BD patients.

In summary, we found that NAA and GPC+PC levels in the MPFC and PCr+Cr levels in the right MPFC, as well as NAA and PCr+Cr levels in the left DLPFC white matter, were lower in pediatric BD patients than in healthy controls. These results might indicate a reduced neuropil in pediatric BD, which would suggest neurodevelopmental abnormalities. Follow-up studies are needed to confirm our findings and to elucidate whether lower neurochemical levels are related to neurodevelopmen-

tal disorder, as well as to determine the relevance of these abnormalities for prognosis and for predicting treatment responses.

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