ORIGINAL ARTICLE

Decreased N-Acetylaspartate in Children with Familial Bipolar Disorder

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Background: *Relatively low levels of brain N-acetylaspartate, as measured by magnetic resonance spectroscopy, may indicate decreased neuronal density or viability. Dorsolateral prefrontal levels of N-acetylaspartate have been reported to be decreased in adults with bipolar disorder. We used proton magnetic resonance spectroscopy to investigate dorsolateral prefrontal N-acetylaspartate levels in children with familial bipolar disorder.*

Methods: *Subjects were 15 children and adolescents with bipolar disorder, who each had at least one parent with bipolar disorder, and 11 healthy controls. Mean age was 12.6 years for subjects and controls. Subjects were allowed to continue current medications. Proton magnetic resonance spectroscopy at 3-Tesla was used to study 8 cm^3 voxels placed in left and right dorsolateral prefrontal cortex.*

Results: *Bipolar subjects had lower N-acetylaspartate/Creatine ratios only in the right dorsolateral prefrontal cortex ($p < .02$). No differences in myoinositol or choline levels were found.*

Conclusions: *Children and adolescents with bipolar disorder may have decreased dorsolateral prefrontal N-acetylaspartate, similar to adults with BD, indicating a common neuropathophysiology. Longitudinal studies of at-risk children before the onset and during the early course of bipolar disorder are needed to determine the role of prefrontal N-acetylaspartate as a possible risk marker and/or indication of early bipolar illness progression.* Biol Psychiatry 2003;53:1059–1065 © *2003 Society of Biological Psychiatry*

Key Words: MRS, NAA, bipolar disorder, children, adolescents, offspring

Introduction

Proton magnetic resonance spectroscopy (^{1}H-MRS) is a noninvasive procedure using magnetic resonance technology, which provides data regarding levels of neuronal substrates, including N-acetylaspartate (NAA), choline (Cho), myoinositol (mI), and creatine/phosphocreatine (Cr) (Miller et al 1991). NAA is an amino acid found in high concentrations within neurons, and not within glial cells, and therefore may serve as a marker of neuronal density or integrity (Urenjak et al 1993). Cr is distributed within gray and white matter and is commonly used as a reference for the amount of brain tissue contained within the analyzed voxel (Ross and Michaelis 1994). Relatively low NAA/Cr ratios may indicate decreased neuronal density or viability.

Dorsolateral prefrontal (DLPF) levels of NAA have been found to be decreased in adults with bipolar disorder (BD) compared to healthy controls (Winsberg et al 2000). This finding, implying decreased neuronal density in DLPF in BD, was supported by a histopathological study of postmortem brains reporting decreased neuronal and glial density in the dorsolateral prefrontal cortex (DLPFC) of BD patients (Rajkowska et al 2001). Decreased density may furthermore represent decreased functioning, as a functional magnetic resonance imaging (fMRI) study found decreased activation of right DLPFC in adults with BD viewing fearful faces as compared to controls (Yurgelun-Todd et al 2000).

Other studies have implicated prefrontal regions in the pathophysiology of BD. Decreased subgenual prefrontal gray matter, glia, and blood flow have been reported in familial BD patients (Drevets et al 1997; Ongur et al 1998). Positron emission tomography (PET) studies have supported decreased prefrontal metabolism and blood flow in bipolar depressed states (Baxter et al 1989; Ketter et al 2001; Martinot et al 1990; Reischies et al 1989).

Early neuroimaging studies of pediatric mood disorders also implicate prefrontal abnormalities. In a magnetic resonance imaging (MRI) study, children with nonfamilial major depressive disorder had larger left prefrontal volumes than controls (Nolan et al 2002). Another study reported decreased left subgenual prefrontal cortex volume in adolescent females with MDD compared to controls (Botteron et al 2002). These studies support children and adolescents with mood disorders having prefrontal abnormalities similar to adults with mood disorders.

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In pediatric BD, there have been two published ^{1}H-MRS studies. The first study found no differences between subjects and controls in levels of NAA in frontal and temporal cortex (Castillo et al 2000). This negative finding involved primarily nonlimbic areas such as temporal cortex; hence, lower neuronal density in BD could be specific to limbic and paralimbic areas such as DLPF. The second study was of acutely manic children, reporting decreased mI concentrations in anterior cingulate cortex (ACC) after lithium treatment (Davanzo et al 2001). No differences in NAA/Cr levels were found in ACC of bipolar subjects compared to controls either before or after lithium treatment. There have been no prefrontal ^{1}H-MRS studies of euthymic bipolar children, but children with MDD have been reported to have elevated Cho levels in left anterior medial cortex (Steingard et al 2000) and left DLPFC (Farchione et al 2002).

Therefore, we wished to use ^{1}H-MRS to study DLPF NAA/Cr ratios in children and adolescents with BD. We studied subjects with BD who had at least one biological parent with BD, a more homogenous cohort that may represent a highly heritable form of BD and therefore perhaps a form with distinct neurobiological findings. We hypothesized that DLPF NAA/Cr ratios would be lower in bipolar offspring with BD than in healthy controls.

Methods and Materials

This protocol was approved by the Stanford University Panel of Medical Research in Human Subjects. Fifteen patients and 11 healthy volunteers were recruited from an ongoing study of bipolar offspring and from the community. After obtaining oral and written informed consent from parents and oral and written assent from their offspring, semistructured interviews were conducted. Patients had at least one parent with bipolar I or II disorder as diagnosed by the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I) (First et al 1995), administered by a trained masters-level clinician (KD) and/or board certified psychiatrist (KC). All subjects, patients, and healthy volunteers were evaluated by the affective disorders module of the Washington University in St. Louis Kiddie Schedule for Affective Disorders and Schizophrenia (WASH-U-KSADS) (Geller et al 1996, 2001), and the Schedule for Affective Disorders and Schizophrenia for School-age Children, Present and Lifetime (K-SADS-PL) (Kaufman et al 1997). Subjects were evaluated either by a child psychiatrist (KC) or a trained masters-level research assistant (KD), who were both aware of parental diagnosis. Interrater reliability was established at the outset by rating videotaped interviews, observing trained rater interviews, and performing interviews with observation by a trained rater, as described by Geller et al (1998) (four consecutive patients with 100% agreement on diagnoses). Diagnostic decisions were ultimately made by a child psychiatrist (KC), based on personal interview, discussion with the research assistant, and written notes of parental and subject responses to individual WASH-U-KSADS questions. Current and lifetime diagnoses were established according to DSM-IV criteria. Parents were euthymic at the time of their own and their child's interview. Patients in this study all received a diagnosis of bipolar I disorder. Age of onset of BD was determined as the earliest period to the closest month that patients met criteria for a manic or depressive episode, as defined by the DSM-IV.

Healthy volunteers did not have a DSM-IV psychiatric diagnosis, had both parents without any diagnosis by SCID-I, and did not have a first or second degree relative with BD, as determined by the Family History Research Diagnostic Criteria (Andreasen et al 1977).

Subjects were all outpatients at the time of scanning. Patients with BD were administered the clinician-rated Young Mania Rating Scale (YMRS) (Fristad et al 1995; Young et al 1978) and completed the Childhood Depression Inventory (CDI) (Kovacs 1985), with the help of a parent if they were less than 12 years old. Patients with BD had psychostimulants discontinued for 24 hours before the magnetic resonance spectroscopy (MRS) scan, primarily due to a concurrent, separate fMRI study of attention. The effects of psychostimulants on neurometabolites measured by ^{1}H-MRS are unknown; regardless, as the half-life of the majority of psychostimulants available at the time of this study was less than 8 hours, 24 hours was considered adequate time for at least cessation of behavioral effects. They were allowed to continue any other current medications, such as mood stabilizers or antidepressants, due to the risk of mood destabilization. Subjects were scanned on a 3T GE Signa scanner with Echospeed gradients, using a custom-built head coil with a 50% advantage in signal-to-noise ratio than the standard GE coil. Eighteen axial slices (4-mm thick, .5-mm skip) parallel to the anterior-posterior commissure plane covering the whole brain were obtained with a temporal resolution of 3 seconds using a T2-weighted gradient echo spiral pulse sequence (repeat time [TR] = 3000 milliseconds, echo time [TE] = 30 milliseconds, flip angle = 89° and 1 interleave). The field of view was 200 mm and the in-plane spatial resolution was 3.125 mm. To aid in voxel segmentation, high-resolution T1-weighted spoiled grass gradient recalled (SPGR) three-dimensional MRI sequences with the following parameters used: TR = 35 milliseconds; TE = 6 milliseconds; flip angle = 45°; 24-cm field of view; 124 slices in coronal plane; 256 × 192 matrix; acquired resolution = 1.5 × .9 × 1.2 mm^3. The images were reconstructed as a 124 × 256 × 256 matrix with a 1.5 × .9 × .9 mm^3 spatial resolution.

For ^{1}H-MRS, a 2 × 2 × 2 cm voxel was prescribed in the right and then left DLPF from the first axial slice above the lateral ventricles (Figure 1). As slices were 5-mm thick, the voxel was placed anywhere from 0 mm to 5 mm above the lateral ventricles, immediately anterior to a line drawn between the anterior aspects of the lateral ventricles and as far lateral as possible, while remaining in the cerebrum and visually maintaining approximately equal parts gray and white matter. An investigator blind to diagnosis (NA) visually inspected each voxel placement to ensure proper placement fully within the brain and that spectra contained no sizable lipid peaks or rolling baselines. The MRS data were acquired using a spin-echo series of TR/TE 2000/35 milliseconds with a preselected region of interest for point-resolved spectroscopy (PRESS). The MRS scan, including the

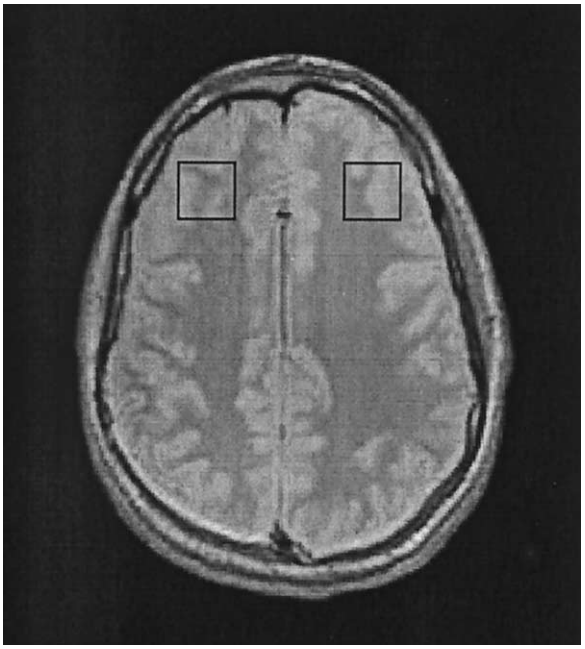

Figure 1. Placement of magnetic resonance spectroscopy voxels in bilateral dorsolateral prefrontal cortex.

number of excitations (NEX), was 1 minute, 44 seconds in length. The fully automated PROBE/SV quantification tool (General Electric Medical System, Milwaukee, WI) was used to process MRS data. Each of the five spectral peaks associated with NAA, Cr, Cho, mI, and water (H_2O) was quantitated by Marquardt Leavenworth curve fitting over that line region. Before curve fitting, line widths were normalized and a Lorentzian-to-Gaussian transformation was performed.

The percentage of each type of tissue (cerebrospinal fluid [CSF] and gray and white matter) within each spectroscopic voxel was calculated by placing a $2 \times 2 \times 2$ cm voxel on the SPGR slice, anatomically and spatially corresponding to the slice used to place the MRS voxel on the T2 image. Tissue within that voxel was segmented into gray, white, and CSF, as described by Otsu (1979). This algorithm approximates a normal distribution for each of gray, white, and CSF intensities. For each histogram bin, the algorithm calculates the probability of belonging to each tissue type. The result is a set of threshold values to approximate tissue classifications. The voxels within each tissue compartment were then counted and recorded.

Statistical Analysis

NAA/Cr ratios were considered primary outcome measures. Other metabolite ratios were considered secondary and exploratory. For primary outcome measures, a one-tailed test was used secondary to our a priori hypothesis of decreased NAA/Cr in bipolar subjects. A significance threshold was set at $p < .025$, as a correction for multiple comparisons (right and left hemispheres). As MRS data were not normally distributed, with three statistical outliers (greater than two standard deviations from the mean) in the bipolar group, nonparametric tests were used. Mann–Whitney *U* was used to compare NAA/Cr ratios between patients and healthy volunteers. For secondary outcome measures, a two-tailed test was used, with a significance threshold of $p < .05$ (no correction for multiple comparisons). Analyses of variance (ANOVAs) were performed for secondary measures, which were normally distributed. Spearman rank correlations were used to test correlations of variables with metabolite ratios. Demographic information between groups was analyzed using unpaired *t* tests and χ^2 analyses.

Results

Cohort

Mean age was 12.6 ± 2.9 years for both patients and controls; age range was 9.1 years to 18.2 years for both groups. Groups did not differ significantly by age. The bipolar group had more males numerically than the control group (87% vs. 55%), but this was not a significant difference ($\chi^2 = 3.3$, $df = 1$, $p = .09$, Fisher exact). Furthermore, there was no significant interaction of gender with NAA/Cr ratios ($F = 3.2$, $df = 1$, $p = .09$).

Mean YMRS score for the bipolar group was 11.1 ± 9.1 and mean CDI score was 12.6 ± 7.6. Subjects were considered clinically euthymic. Three subjects were considered to have substantial mood elevation symptoms, with YMRS scores greater than 19. One of these subjects had substantial mixed symptoms, with a CDI score of 30. Although symptomatic, these subjects did not meet DSM-IV criteria for a syndromal mood episode. The 12 other subjects in the bipolar group had YMRS and CDI scores less than 20. There were no significant correlations of YMRS or CDI scores with NAA/Cr ratios in right or left hemispheres.

All subjects in the bipolar group except one were taking psychotropic medications. Seventy-nine percent of these subjects had significant past exposure (more than 2 months) to stimulants: 43% to tricyclic antidepressants, 71% to serotonin reuptake inhibitors, 50% to antipsychotics, and 86% to mood stabilizers, including 36% with exposure to lithium, 64% with exposure to valproate, and 14% with exposure to carbamazepine (Table 1). No subjects in the control group had previous exposure to psychotropic medications. Ninety-two percent of subjects in the bipolar group had comorbid psychiatric diagnoses, with attention-deficit/hyperactivity disorder (ADHD) the highest at 87% (Table 1). No subjects in the bipolar group had a present or past substance-use disorder.

NAA/Cr

Mean right DLPF NAA/Cr ratios were significantly lower in the bipolar group compared to the control group (1.61 ± .12 vs. 1.68 ± .08, $p < .02$; Mann–Whitney *U*) (Figure 2). Mean left DLPF NAA/Cr ratios were not significantly

Table 1. Demographics and Voxel Compositions of Subjects

	Bipolar	Control
n	15	11
Mean Age, Years (SD)	12.6 (2.9)	12.6 (2.9)
Gender, % Male	87	55
SES (SD)	3.9 (.9)	4.0 (.6)
Race		
African American	1 (7)	1 (9)
Hispanic	1 (7)	0 (0)
Asian	0 (0)	1 (9)
Caucasian	13 (87)	9 (82)
IQ (SD)	111 (9.5)	114 (6.5)
Handedness, % Right	93	100
Comorbid Diagnoses (%)		
ADHD	13 (87)	0 (0)
Anxiety D/O	4 (27)	0 (0)
Oppositional defiant D/O	8 (53)	0 (0)
Past Psychotropic Medication Exposure, %		
Stimulants	80	0
TCAs	47	0
SSRIs	73	0
Atypical ADs	47	0
Lithium	40	0
Valproate	67	0
Carbamazepine	13	0
Antipsychotics	47	0
Any mood stabilizer	87	0
First degree relatives with mood disorder, %	54	0
Right DLPF Gray, % (SD)	41.3 (8.6)	42.9 (5.5)
Right DLPF White, % (SD)	56.5 (8.6)	56.1 (7.8)
Left DLPF Gray, % (SD)	41.8 (6.5)	44.0 (5.9)
Left DLPF White, % (SD)	56.0 (6.6)	54.5 (6.7)

ADHD, attention-deficit/hyperactivity disorder; ADs, antidepressants; DLPF, dorsolateral prefrontal cortex; D/O, disorder; IQ, intelligence quotient; SD, standard deviation; SES, socioeconomic status; SSRIs, selective serotonin reuptake inhibitors; TCAs, tricyclic antidepressants.

different between groups (Table 2). No differences in mI/Cr or Cho/Cr levels were found (Table 2).

As we did not collect absolute levels of metabolites, we performed analyses referencing to Cho and Cr. NAA/Cho and NAA/Cho+Cr tended to yield the findings seen with NAA/Cr (Table 2).

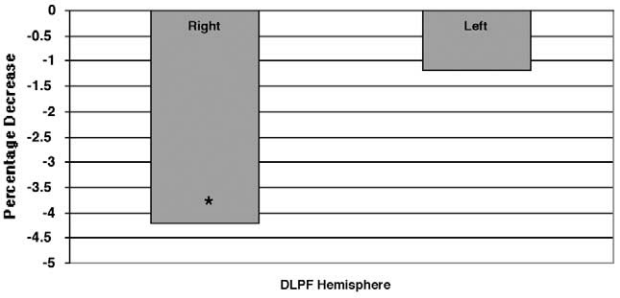

Figure 2. Percentage decrease in DLPF NAA/Cr in bipolar subjects compared to controls. DLPF, dorsolateral prefrontal cortex; NAA, N-acetylaspartate; Cr; creatine-phosphocreatine; *$p = .02$.

Table 2. Neurometabolite Levels in DLPF

Metabolite Ratio	Control Group ($n = 11$)	Bipolar Group ($n = 15$)	p
Right NAA/Cr	1.68 ± .08	1.61 ± .12	.02
Left NAA/Cr	1.61 ± .07	1.59 ± .13	.16
Right NAA/Cho	2.20 ± .17	2.09 ± .17	.10
Left NAA/Cho	2.17 ± .16	2.03 ± .18	.06
Right NAA/Cho+Cr	.95 ± .05	.91 ± .06	.03
Left NAA/Cho+Cr	.92 ± .04	.89 ± .06	.16
Right Cho/Cr	.77 ± .06	.77 ± .08	.84
Left Cho/Cr	.75 ± .07	.78 ± .07	.16
Right mI/Cr	.46 ± .03	.47 ± .05	.47
Left mI/Cr	.49 ± .07	.46 ± .05	.55

Cho, choline; Cr, creatine-phosphocreatine; DLPF, dorsolateral prefrontal cortex; mI, myoinositol; NAA, N-acetylaspartate.

NAA/Cr ratios did not correlate significantly with age in right ($r = -.1$, $p = .63$) or left DLPF ($r = .18$, $p = .38$). Duration of bipolar illness tended to correlate inversely with NAA/Cr ratios in right DLPF (Spearman rank rho = $-.419$, $p = .065$) (see Figure 3).

Voxel Composition

Voxel segmentation revealed no significant differences between the bipolar or control groups in percent voxel composition of gray/white matter or CSF (white: $t = .74$, $p = .45$; gray: $t = -.26$, $p = .79$; CSF: $t = -.17$, $p = .86$). Voxel compositions are presented in Table 1.

Discussion

We found decreased NAA/Cr ratios in right prefrontal cortex in children with BD who had at least one parent

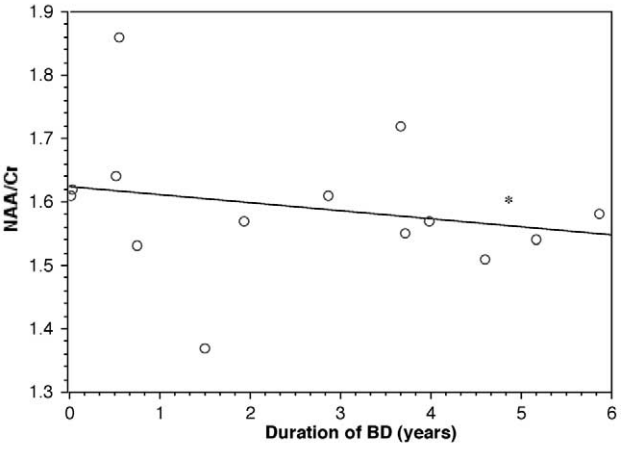

Figure 3. Duration of bipolar illness versus NAA/Cr in right DLPF of patients with bipolar disorder. BD, bipolar disorder; NAA, N-acetylaspartate; Cr, creatine-phosphocreatine; DLPF, dorsolateral prefrontal cortex; *$p = .065$.

with BD compared to healthy volunteers. This finding is similar to results obtained from ^{1}H-MRS studies of adults with BD (Winsberg et al 2000) and schizophrenia (Bertolino et al 1998). An overall regional decrease in NAA/Cr may indicate lower neuronal density or viability and therefore dysfunction in the DLPF. The DLPF has been suggested to have a role in mediation of affect and has been implicated to have a role in the pathophysiology of mood disorders in general and BD specifically. Previous studies have found decreased DLPF metabolism and blood flow in patients with primary (unipolar and bipolar) and secondary (Parkinson and Huntington disease and stroke) depression (for review, see Ketter et al 2001). Using fMRI, researchers reported decreased right DLPF activity in adults with BD viewing fearful faces (Yurgelun-Todd et al 2000). Winsberg et al (2000) found decreased NAA/Cr levels in bilateral DLPF of adults with BD. Finally, a histopathological study of postmortem brains reported decreased neuronal and glial density in DLPF of BD patients (Rajkowska et al 2001). These studies together support a possible pathophysiological role of the DLPF in BD, with putative decreased neuronal density in DLPF leading to abnormal function in patients with BD.

The DLPF also has been hypothesized to play a role in higher attention processing. As 87% of our bipolar subjects had a comorbid diagnosis of ADHD, it is possible that the decrease in NAA signal is related to the ADHD condition as well as to BD. Reduced left DLPF NAA levels have been reported in adults with ADHD (Hesslinger et al 2001), and bilateral globus pallidus NAA decreases were noted in children with ADHD (Jin et al 2001). Determining the individual contributions of these two disorders would be challenging, as ADHD is highly comorbid with pediatric BD (Faraone et al 1997) and may be an early phenotypic feature of the disorder itself. As only two bipolar subjects in this study did not have comorbid ADHD, we could not meaningfully separate groups based on presence of ADHD for further analysis.

Furthermore, as we did not assess other brain regions, we do not know if our findings represent a decrease in NAA/Cr specifically in right DLPF or reflect a global decrease in NAA; however, other researchers have found no decreases in NAA levels in frontal lobes of children with BD (Castillo et al 2000) or in temporal lobes of both adults (Stoll et al 1995; Yurgelun-Todd et al 1993) and children (Castillo et al 2000) with BD. Hence, it is possible that decreased neuronal density in BD could be specific to limbic and paralimbic areas such as DLPF.

Another potential confound of this study is that the majority of bipolar subjects were taking psychotropic medications at the time of scanning. Lithium may increase brain NAA concentrations in adults (Moore et al 2000), but it is not known what the effect combinations of mood stabilizers and other medications may have on relative neuronal density in the prefrontal cortex.

We did not find increased mI/Cr ratios, as had been previously reported in children with BD (Davanzo et al 2001); however, in that study, all children were acutely manic and had mI levels measured in ACC. Thus, it may be that higher mI concentrations may occur in only acutely manic children and/or only in ACC. Furthermore, in our study, metabolites other than NAA were considered secondary outcome measures. Therefore, negative findings regarding these metabolites should be approached with caution, as we did not correct for multiple comparisons and as our sample size was only able to detect large effects.

Decreased NAA/Cr ratios were only found in right, not left, DLPF. Some previous MRS studies of children with mood disorders have supported left-sided prefrontal differences. Steingard et al (2000) found increased Cho in left orbitofrontal cortex in depressed children, but the right side was not assessed. Farchione et al (2002) reported increased Cho in left but not right DLPF in children with major depression; however, Castillo et al (2000) found both frontal lobes to have increased glutamate in children with BD (no NAA differences were noted). Some studies of bipolar depression also have supported abnormalities in left greater than right prefrontal cortex. For example, PET and single photon emission computer tomography (SPECT) studies of bipolar depression have found more marked hypofrontality in the left hemisphere (Baxter et al 1985; Bonne et al 1996; Delvenne et al 1990); however, another SPECT study failed to detect such lateralized changes in the depressed phase of BD (Tutus et al 1998). Therefore, while a clear laterality has not been established in mood disorders, many studies point to left-sided greater than right-sided abnormalities. We found the reverse to be true in this study, which might represent a separate pathophysiology for early-onset BD or perhaps an early manifestation of right-sided dysfunction, which eventually leads to a compensatory left-sided dysfunction later in the progression of the disorder.

It is unclear if relatively low concentrations of NAA in DLPF occur as a trait marker (present since birth), occur shortly before the onset of fully developed BD, or only occur after the onset of BD as a type of degenerative process. In this study, right DLPF NAA/Cr tended to decrease as duration of bipolar illness increased, supporting a neurodegenerative hypothesis; however, this effect may have been due to duration of exposure to psychotropic medications, which likely increased in conjunction with increasing duration of illness. A study of adults with BD found decreased NAA/Cr in both right and left DLPF (Winsberg et al 2000), with greater decreased NAA/Cr in left (8.9%) than right (6.3%) DLPF. Adults with bipolar I

disorder had a mean right DLPF NAA/Cr ratio of 1.56 ± .09, lower than that in our children with BD, again supporting a gradual neurodegenerative process in patients with BD as they age; however, in that study, controls had a mean NAA/Cr ratio of 1.74 ± .18 in the right DLPF, slightly higher than that in our younger controls. Since NAA/Cr ratios are thought to gradually decrease throughout the brain with age due to increasing Cr concentrations (Pfefferbaum et al 1999), our findings may not be comparable to this previous study. Measurement of absolute NAA concentrations would help control for these age-related differences.

Limitations

Our study was limited by the relatively small sample size of patients and controls. All bipolar subjects were euthymic, but inclusion of three patients with significant mood symptoms may prevent limiting specificity of results to only euthymic patients. Furthermore, patients continued psychotropic medications, most of which have not been studied in regard to their effects on neurometabolites, and stimulants were discontinued for only 24 hours. For example, antipsychotics have been reported to increase NAA/Cr ratios in DLPF in patients with schizophrenia (Bertolino et al 2001). A normalizing phantom was not used in this study to control for scanner drift, which may cause variable detection of neurometabolite concentrations over time. Similarly, test/retest checks of voxel placement were not done to prevent slight variations in voxel placement across subjects. Cr was used as a reference, as absolute concentrations of metabolites were not obtained. Cr has historically been used as a stable reference for gray matter in the brain, but more recently, the stability of levels of this metabolite has been questioned. Therefore, we performed analyses referencing to other metabolites, which tended to yield results similar to using Cr as a reference. A small fraction of the NAA signal is thought to be due to the presence of unidentified macromolecules with similar resonance; however, the effect of these macromolecules on NAA/Cr ratios in our research subjects could not be determined at this time. We also cannot generalize the finding of decreased NAA to other portions of the brain. Finally, all patients were bipolar offspring and mostly Caucasian; therefore, these results may not be generalizable to children with BD without a bipolar parent or non-Caucasian children with BD.

Nevertheless, this study has demonstrated intriguing abnormalities of neurometabolite concentrations in children and adolescents with BD. Decreased prefrontal NAA/Cr ratios may indicate decreased neuronal density, leading to functional abnormalities in prefrontal cortex. A longitudinal study of greater numbers of bipolar offspring before and after the onset of fully developed BD is required to further investigate the role of NAA in the pathogenesis of BD.

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