Published in final edited form as:

Bipolar Disord. 2012 September ; 14(6): 607–617. doi:10.1111/j.1399-5618.2012.01040.x.

Frontal lobe bioenergetic metabolism in depressed adolescents with bipolar disorder: a phosphorus-31 magnetic resonance spectroscopy study

Xian-Feng Shi^{a,b}, Douglas G Kondo^{a,b,c}, Young-Hoon Sung^{a,b}, Tracy L Hellem^a, Kristen K Fiedler^a, Eun-Kee Jeong^d, Rebekah S Huber^a, and Perry F Renshaw^{a,b,c}

^aThe Brain Institute, University of Utah School of Medicine, Salt Lake City, UT, USA

^bDepartment of Psychiatry, University of Utah School of Medicine, Salt Lake City, UT, USA

^cVISN 19 Mental Illness Research, Education and Clinical Center (MIRECC), Department of Veterans Affairs Medical Center, Salt Lake City, UT, USA

^dDepartment of Radiology, University of Utah School of Medicine, Salt Lake City, UT, USA

Abstract

Objectives—To compare the concentrations of high-energy phosphorus metabolites associated with mitochondrial function in the frontal lobe of depressed adolescents with bipolar disorder (BD) and healthy controls (HC).

Methods—We used *in vivo* phosphorus-31 magnetic resonance spectroscopy (³¹P-MRS) at 3 Tesla to measure phosphocreatine (PCr), beta-nucleoside triphosphate (β-NTP), inorganic phosphate (Pi), and other neurometabolites in the frontal lobe of eight unmedicated and six medicated adolescents with bipolar depression and 24 adolescent HCs.

Results—Analysis of covariance, including age as a covariate, revealed differences in PCr ($p = 0.037$), Pi ($p = 0.017$), and PCr/Pi ($p = 0.002$) between participant groups. Percentage neurochemical differences were calculated with respect to mean metabolite concentrations in the HC group. Post-hoc Tukey–Kramer analysis showed that unmedicated BD participants had decreased Pi compared with both HC (17%; $p = 0.038$) and medicated BD (24%; $p = 0.022$). The unmedicated BD group had increased PCr compared with medicated BD (11%; $p = 0.032$). The PCr/Pi ratio was increased in unmedicated BD compared with HC (24%; $p = 0.013$) and with medicated BD (39%; $p = 0.002$). No differences in β-NTP or pH were observed.

Conclusions—Our results support the view that frontal lobe mitochondrial function is altered in adolescent BD and may have implications for the use of Pi as a biomarker. These findings join volumetric studies of the amygdala, and proton MRS studies of n-acetyl aspartate in pointing to potential differences in neurobiology between pediatric and adult BD.

Corresponding author: Xian-Feng Shi, Ph.D., The Brain Institute, University of Utah, 383 Colorow Drive, Salt Lake City, UT 84108, USA, Fax: 801-585-0625, xianfeng.shi@hsc.utah.edu.

Disclosures

DGK has received research support from Ridge Diagnostics, Eli Lilly & Co., and GlaxoSmithKline. PFR has received research support from GlaxoSmithKline and Roche; and serves as a consultant to Kyowa Hakko, Novartis, and Roche. X-FS, Y-HS, TLH, KKF, E-KJ, and RSH declare that they have no commercial associations or conflicts of interest to disclose.

Keywords

adolescent; bipolar disorder; depression; ^{31}P -MRS

Among adults with bipolar disorder (BD), up to 65% report the age of onset in childhood or adolescence (1–3). In persons 10–24 years of age, who make up 27% of the world's population, BD is the fourth leading cause of disability (4). Patients with BD experience long delays to diagnosis and appropriate treatment (2, 3). In fact, patients with childhood-onset BD experience a lag between symptom onset and treatment averaging 16.8 years (5). In spite of BD's prevalence and severity, and the health care system's inability to identify cases in a timely fashion, its neurochemical basis remains obscure (6, 7). Improvement in diagnosing pediatric BD is a critical barrier to progress in the field (8). One approach advocated by both the National Institute of Mental Health (NIMH) Strategic Plan (9) and the Research Agenda for DSM-V (10), is to identify neuroimaging biomarkers that reflect BD's pathophysiology (11).

Converging lines of evidence implicate mitochondrial dysfunction in the pathophysiology of BD (12), including molecular (13–15), family-based (16–19), expression analysis (20–22), proteomic (23), *in vitro* (24–26), cerebrospinal fluid (CSF) (27, 28), post-mortem (29–31), and animal studies (32–35). As reviewed by Stork and Renshaw (36), studies of BD utilizing the neuroimaging method magnetic resonance spectroscopy (MRS) provide an internally consistent body of evidence for mitochondrial involvement in BD.

MRS is a method that allows *in vivo* analysis of the neurochemical alterations associated with psychiatric disorders (37). Phosphorus-31 MRS (^{31}P -MRS) is the only method capable of non-invasive measurement of the phosphorus metabolites reflecting mitochondrial function in the living human brain (38). These include phosphocreatine (PCr), inorganic phosphate (Pi), and beta-nucleoside triphosphate (β -NTP) (largely adenosine triphosphate, or ATP).

Psychotropic medication affects the brain regions of interest (ROI) to neuroimaging researchers (11). The effects of medication class, duration of drug exposure, and polypharmacy have yet to be elucidated (39, 40). Moreover, neurochemical alterations in psychiatric disorders could result from medication effects, disease processes (41), or both. In pediatric populations, the interaction between the brain and medication is further complicated by neurodevelopment (42). These confounds make it difficult to interpret cross-sectional neuroimaging data acquired from medicated BD patients.

By contrast, neuroimaging of medication-free BD patients provides investigators with the opportunity to identify biomarkers of both bipolar illness and treatment response (43), without confounding by current treatment (39). In fact, inclusion of medicated participants in cross-sectional BD neuroimaging studies is considered a major methodological weakness (44). Notwithstanding this, expert recommendations for cross-sectional studies of BD include comparison of neuroimaging findings in medicated versus unmedicated individuals (39).

Frontal lobe regions such as the dorsolateral and medial prefrontal cortex (PFC) and anterior cingulate are known to be involved in mood regulation (45). Correspondingly, PFC and cingulate functions including emotion processing, executive functioning, and decision-making are abnormal in juvenile BD (46). Frontal lobe abnormalities have also been reported in functional magnetic resonance imaging (MRI) studies (47), morphology studies (48), and studies of treatment response in pediatric BD (49). Published studies of pediatric BD using proton MRS (^1H -MRS) have also largely focused on the frontal lobe for neurochemical analysis (45, 50–56). To investigate whether bioenergetic metabolism is altered in the same region as the convergent chemical and neurobiological findings previously reported in adolescent BD, we selected a frontal lobe ROI for interrogation with ^{31}P -MRS.

The mitochondrial hypothesis of BD(12) suggests the possibility that ^{31}P -MRS has the potential to identify biomarkers of BD. Studies of adults with BD have used ^{31}P -MRS to compare high-energy phosphate concentrations in BD patients and healthy controls (HC) (57–61), but to our knowledge this method has not been applied to study adolescent BD. We hypothesized that medication-free adolescents with bipolar depression would demonstrate lower concentrations of cerebral PCr compared with HC, in parallel with findings in adults (58, 60).

Patients and methods

Patients

The University of Utah Institutional Review Board (IRB) approved the study. We obtained written assent from adolescents, and written consent from parents or guardians prior to study procedures. The BD participants were recruited to participate in a clinical trial (62), through clinician referrals and IRB-approved advertising. Consecutive adolescents who met inclusion criteria were enrolled. Inclusion criteria for BD participants were: outpatient status; male or female 13–18 years of age; a primary diagnosis of BD I, BD II, or not otherwise specified (NOS); and a current major depressive episode of > 2 weeks' duration with a Children's Depression Rating Scale-Revised (CDRS-R) (63) raw score > 40. Participants could be medication free; for those taking medication, we required their regimen to be stable for 2 weeks. Exclusion criteria for participants with BD were: clinically significant abnormality on complete blood count, comprehensive metabolic profile, thyroid-stimulating hormone or urinalysis; psychotic symptoms; high risk for suicidal behavior; primary Axis I diagnosis other than BD; Young Mania Rating Scale (YMRS) score > 10 (64); known or suspected mental retardation per parent/guardian report; contraindication to MRI (e.g. ferromagnetic implant or claustrophobic anxiety); and positive urine drug screen. Inclusion criteria for HC were: male or female 13–18 years of age; and absence of an Axis I psychiatric or substance use disorder. Exclusion criteria for HCs were: clinically significant neurological or medical condition; contraindication to MRI; and positive urine drug screen. Pregnant females and nursing mothers were excluded from participation in either the BD or HC group. Diagnoses were established with the Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version (K-SADS-PL) (65), administered by a board-certified child-and-adolescent

psychiatrist (DGK). Participants with BD and HC underwent identical psychiatric evaluations prior to ^{31}P -MRS neuroimaging.

Methods

Phosphorus spectroscopic neuroimaging—Participants were instructed that they could discontinue scanning at any time, if they experienced discomfort. Neuroimaging data were acquired using a Siemens Trio 3 Tesla whole-body MRI system (Siemens Medical Solutions, Erlangen, Germany) with Avanto gradients (45 mT/m strength and 150 T/m/s slew rate) and a $^{31}\text{P}/^1\text{H}$ double-tuned volume head coil (Clinical MR Solutions, LLC, Brookfield, WI, USA). ^{31}P spectroscopic imaging data were acquired using a two-dimensional MRS imaging pulse sequence (2D MRSI) with the following parameters: field of view (FOV) $20 \times 20 \times 2.5 \text{ cm}^3$; receiver bandwidth = 2.5 kHz; repetition time (TR)/echo time (TE) = 3000/2.3 msec; flip angle = 90° ; average number = 24; vector size = 1024; matrix size = 8×8 ; and voxel dimension = $2.5 \times 2.5 \times 2.5 \text{ cm}^3$. To facilitate voxel placement, high-resolution T_1 -weighted images were acquired using a three-dimensional magnetization-prepared rapid gradient echo acquisition (MPRAGE) pulse sequence with the following parameters: TR/TE/inversion times (TI) = 2000/3.37/1100 msec; flip angle = 8° ; FOV = $256 \times 192 \times 144 \text{ mm}^3$; matrix size = $256 \times 192 \times 144$; $1 \times 1 \times 1 \text{ mm}^3$ spatial resolution; bandwidth = 300 Hz/pixel. To effectively saturate unnecessary fat signal from scalp, six outer volume saturation bands were placed in the scalp and skull regions. Figure 1 depicts the ROI and displays a representative ^{31}P -MRS spectrum. The anterior commissure–posterior commissure (AC-PC) line was identified on the midsagittal images acquired by MPRAGE pulse sequence. A two-dimensional MRSI grid was placed immediately above the plane formed by the AC-PC line and covers the anterior cingulate cortex, corpus callosum and occipital lobe. Board-certified radiologists reviewed the anatomic images to rule out the presence of intracranial abnormalities.

Phosphorus metabolite data analysis—All ^{31}P spectra were preprocessed using a MATLAB-based application (The MathWorks, Inc., Natick, MA, USA). Each spectrum was apodized with 10 Hz Lorentzian line broadening before zero-filling and Fast Fourier Transform. The zero- and first-order phase corrections were performed in all spectra. The signal intensity of each metabolite was obtained using the Advanced Method for Accurate, Robust and Efficient Spectral Fitting of MRS data with use of prior knowledge (AMARES) (66) fitting algorithm within the software application jMRUI 4.0 (67). Metabolite concentrations were calculated as a percentage of the total phosphorus (TP) signal acquired from the ROI. Intracellular pH was calculated according to the method of Petroff et al. (68).

Statistical analysis

Statistical analyses were performed using JMP 9.0.2 (SAS Institute Inc., Cary, NC, USA). Levene's test was used to confirm the homogeneity of variances between the groups. The goodness of fit of the metabolite distributions was assessed using the Shapiro–Wilk test. With participant age included as a covariate, we tested our *a priori* hypotheses using analysis of covariance (ANCOVA). Due to the limited sample size, gender effects were not considered. Group assignment (medicated BD, unmedicated BD, or HC) was the independent variable, with metabolite concentrations in the frontal lobe ROI serving as

dependent variables and participant age included as a covariate. Post-hoc analyses were conducted using the Tukey–Kramer Honestly Significant Difference (HSD) test (69, 70), which simultaneously compares all possible pairwise differences between group means.

Results

³¹P-MRS scans of 14 depressed adolescents with BD and 24 HC were acquired. No intracranial abnormalities were detected on participants' anatomical MRIs. Demographic data for unmedicated BD, medicated BD, and HC participants are presented in Table 1. There were no significant between-group differences between BD and HC in age, educational level, or handedness. Participants' history of exposure to psychotropic medication is presented in Table 2. In the unmedicated BD group, four participants were medication naïve. The four remaining unmedicated BD participants had a mean medication-free period prior to entering the study of 70 ± 47.5 weeks (range: 28–116 weeks). Table 3 displays ANCOVA results comparing ³¹P-MRS results across the three participant groups. Significant group differences were found in PCr [$F(2,34) = 3.637$; $p = 0.037$], Pi [$F(2,34) = 4.609$; $p = 0.017$], and PCr/Pi [$F(2,34) = 7.343$; $p = 0.002$]. There were no significant differences in frontal lobe β -NTP concentration [$F(2,34) = 0.825$; $p = 0.447$], or pH [$F(2,34) = 0.250$; $p = 0.780$].

Post-hoc Tukey–Kramer HSD pairwise comparisons are shown in Figure 2. We found increased PCr in unmedicated BD participants compared with medicated BD ($p = 0.032$). Mean PCr in HC was not significantly different from medicated BD ($p = 0.063$). The unmedicated BD participants demonstrated decreased Pi compared with both HC ($p = 0.038$) and medicated BD ($p = 0.022$). The PCr/Pi ratio was increased in unmedicated BD compared with HC ($p = 0.013$).

To evaluate the influence of brain tissue components within the ROI, the MPRAGE images were segmented into gray matter, white matter, and CSF using FSL [Functional Magnetic Resonance Imaging of the Brain (FMRIB) Software Library, Release 4.1, Oxford, UK]. The resulting volumes are shown in Figure 3. There was no statistically significant difference in tissue component volume among the medicated BD, unmedicated BD, and HC groups.

Discussion

We report what, to our knowledge, is the first ³¹P-MRS study of pediatric BD. Our *a priori* hypothesis that frontal lobe PCr would be reduced in depressed adolescents with BD was not confirmed. This result could be due to a lack of statistical power associated with our sample size, a limitation recognized as pervasive in neuroimaging studies of BD (71–75). In the search for neuroimaging biomarkers of BD, it is necessary to compare HC with medication-free participants because of the established effects of medication on brain chemistry (76–81) and brain structure (44, 82, 83). Another potential explanation for our results is that adolescent BD and adult BD display dissimilar neurochemical characteristics. For example, lithium administration *decreases* prefrontal N-acetylaspartate (NAA) levels in adolescent BD (84), but *increases* NAA concentrations in the prefrontal cortex of lithium-treated BD adults (85). Longitudinal MRS studies will be required to determine whether brain

metabolism in BD changes with age, or whether the discrepancies are due to ascertainment bias (42). Adding to the support for neurobiological differences between pediatric and adult BD are the studies documenting decreased amygdala volume in pediatric BD, and contrapositive results in adults with BD (11).

We found decreased PCr concentration in medicated BD adolescents compared with the unmedicated BD group. The medicated BD cohort also demonstrated a trend toward decreased PCr ($p = 0.063$) compared with HC, suggesting that psychotropic medication may play a role in reducing PCr levels in the frontal lobe. This result is consistent with previous findings in adults with BD, which show decreased PCr in comparison with HC (58) regardless of mood state, and a negative correlation between Hamilton Depression Rating Scale score and PCr (60). The frontal cortex is known to undergo maturation through the age of 24 years (86).

Changes in β -NTP (58–60, 87) and pH (87) have not been found in ^{31}P -MRS studies of adults with bipolar depression. We therefore did not anticipate alterations in β -NTP or pH in our adolescent participants, and none were demonstrated in this study.

An unexpected finding was reduced Pi in our medication-free participants, in whom Pi was decreased compared with HC (17%; $p = 0.038$) and with medicated BD (24%; $p = 0.022$). If the ‘mitochondrial hypothesis’ of BD were true, how might a finding of decreased Pi be related? A partial answer may lie in the fact that at least three independent research groups have concluded that oxidative phosphorylation, the ubiquitous metabolic pathway for ATP production, is principally controlled by Pi (88–90). Furthermore, it is thought that the only Pi that is detectable by nuclear magnetic resonance is involved in oxidative phosphorylation (88, 91). Mammalian cells in which oxidative phosphorylation is impaired can reduce the concentration of free Pi, via compartmentation to the inner mitochondrial membrane, which immobilizes the phosphorus ions and renders them *invisible* to MRS (92–95). In addition, Pi has a direct effect *in vitro* on glucose utilization in cortical neurons (96). It has been posited that decreased ATP consumption leads to a fall in cytosolic Pi, to a level that balances ATP synthesis and utilization (97, 98), thus stabilizing the cell’s phosphorylation potential (92–94).

Additional support for the relevance of Pi to the pathophysiology of BD is provided by the enduring observation that the activity of sodium–potassium adenosine triphosphatase (Na^+/K^+ -ATPase), an enzyme partially regulated by Pi (99–103), is altered in patients with BD (104, 105). In addition, there is a genetic association between BD and mutations in Na^+/K^+ -ATPase (106, 107). The normal response of lymphocyte Na^+/K^+ -ATPase to incubation with lithium is absent in the lymphocytes of BD patients, regardless of their medication status (108). In animal models, decreased neuronal Na^+/K^+ -ATPase activity increases susceptibility to chronic variable stress depression paradigms (109).

Na^+/K^+ -ATPase is the receptor for digitalis-like compounds (DLC), which are implicated in mood regulation (110, 111). Furthermore, binding for DLC in BD is separable from that observed in both major depressive disorder and schizophrenia (112). Neutralization of DLC alters catecholamine metabolism in Sprague–Dawley rats: ventral tegmental area dopamine

levels rise by 55%, and the hippocampal norepinephrine/dopamine ratio increases more than 15-fold (113). Finally, blocking DLC elicits an antidepressant-like response in the Flinders Sensitive Line of genetically depressed rats (113). Further study is needed to determine if, and how, Na⁺/K⁺-ATPase activity and cerebral Pi levels are relevant to the pathophysiology of BD.

This study has a number of limitations, in addition to the afore-mentioned sample size. Our results may be limited by the study's cross-sectional design, diagnostic heterogeneity, and the fact that participants with comorbid diagnoses were not excluded. By parsing the adolescents with BD into medication-free and medicated subgroups, we avoided the array of confounders introduced by the uncertain effects of medication, medication class, duration of exposure to medication, and polypharmacy (39).

If replicated by other investigators, the finding of reduced frontal lobe Pi concentration in medication-free adolescents with bipolar depression could serve as an initial step toward establishing a biomarker for the illness. Additional ³¹P-MRS studies of pediatric BD are indicated, including larger-scale studies aimed at biomarker identification and longitudinal investigations designed to elucidate the natural history of BD and the effects of treatment.

Acknowledgments

This work was supported by National Institute of Mental Health grant MH058681 (PFR); a NARSAD Young Investigator Award from the Brain and Behavior Research Foundation (DGK); and by the Utah Science Technology and Research Initiative at the University of Utah (USTAR). The jMRUI software package was kindly provided by the participants of the EU Network programs: Human Capital and Mobility, CHRX-CT94-0432 and Training and Mobility of Researchers, ERB-FMRX-CT970160.

References

1. Perlis RH, Miyahara S, Marangell LB, et al. Long-term implications of early onset in bipolar disorder: data from the first 1000 participants in the systematic treatment enhancement program for bipolar disorder (STEP-BD). *Biol Psychiatry*. 2004; 55:875–881. [PubMed: 15110730]
2. Lish JD, Dime-Meenan S, Whybrow PC, Price RA, Hirschfeld RM. The National Depressive and Manic-depressive Association (DMDA) survey of bipolar members. *J Affect Disord*. 1994; 31:281–294. [PubMed: 7989643]
3. Leverich GS, Post RM, Keck PE Jr, et al. The poor prognosis of childhood-onset bipolar disorder. *J Pediatr*. 2007; 150:485–490. [PubMed: 17452221]
4. Gore FM, Bloem PJ, Patton GC, et al. Global burden of disease in young people aged 10–24 years: a systematic analysis. *Lancet*. 2011; 377:2093–2102. [PubMed: 21652063]
5. Post RM, Kowatch RA. The health care crisis of childhood-onset bipolar illness: some recommendations for its amelioration. *J Clin Psychiatry*. 2006; 67:115–125. [PubMed: 16426098]
6. Goodwin, FK.; Jamison, KR. *Manic-Depressive Illness: Bipolar Disorder and Recurrent Depression*. 2. New York: Oxford University Press; 2007.
7. Belmaker RH, Agam G. Bipolar disorder: neurochemistry and drug mechanisms. *Discov Med*. 2005; 5:191–198. [PubMed: 20704909]
8. Carlson GA, Findling RL, Post RM, et al. AACAP 2006 Research forum—advancing research in early-onset bipolar disorder: barriers and suggestions. *J Child Adolesc Psychopharmacol*. 2009; 19:3–12. [PubMed: 19232018]
9. Insel TR. Translating scientific opportunity into public health impact: a strategic plan for research on mental illness. *Arch Gen Psychiatry*. 2009; 66:128–133. [PubMed: 19188534]

10. Kupfer, DJ.; First, MB.; Regier, DA. A Research Agenda for DSM-V. Arlington: American Psychiatric Publishing; 2002.
11. Phillips ML, Vieta E. Identifying functional neuroimaging biomarkers of bipolar disorder: toward DSM-V. *Schizophr Bull.* 2007; 33:893–904. [PubMed: 17562698]
12. Kato T, Kato N. Mitochondrial dysfunction in bipolar disorder. *Bipolar Disord.* 2000; 3:180–190. [PubMed: 11256685]
13. Kato T, Kunugi H, Nanko S, Kato N. Association of bipolar disorder with the 5178 polymorphism in mitochondrial DNA. *Am J Med Genet.* 2000; 96:182–186. [PubMed: 10893494]
14. Kato T. Role of mitochondrial DNA in calcium signaling abnormality in bipolar disorder. *Cell Calcium.* 2008; 44:92–102. [PubMed: 18177933]
15. Washizuka S, Kakiuchi C, Mori K, et al. Association of mitochondrial complex I subunit gene NDUFB2 at 18p11 with bipolar disorder. *Am J Med Genet B Neuropsychiatr Genet.* 2003; 120B: 72–78. [PubMed: 12815743]
16. Siciliano G, Tessa A, Petrini S, et al. Autosomal dominant external ophthalmoplegia and bipolar affective disorder associated with a mutation in the ANTF1 gene. *Neuromuscul Disord.* 2003; 13:162–165. [PubMed: 12565915]
17. Mancuso M, Filosto M, Bellan M, et al. POLG mutations causing ophthalmoplegia, sensorimotor polyneuropathy, ataxia, and deafness. *Neurology.* 2004; 62:316–318. [PubMed: 14745080]
18. McMahon FJ, Stine OC, Meyers DA, Simpson SG, DePaulo JR. Patterns of maternal transmission in bipolar affective disorder. *Am J Hum Genet.* 1995; 56:1277–1286. [PubMed: 7762550]
19. Munakata K, Fujii K, Nanko S, Kunugi H, Kato T. Sequence and functional analyses of mtDNA in a maternally inherited family with bipolar disorder and depression. *Mutat Res.* 2007; 617:119–124. [PubMed: 17320116]
20. Konradi C, Eaton M, MacDonald ML, Walsh J, Benes FM, Heckers S. Molecular evidence for mitochondrial dysfunction in bipolar disorder. *Arch Gen Psychiatry.* 2004; 61:300–308. [PubMed: 14993118]
21. Kvjao M, Dhilla A, Swor DE, Karayiorgou M, Gogos JA. Evidence implicating the candidate schizophrenia/bipolar disorder susceptibility gene G72 in mitochondrial function. *Mol Psychiatry.* 2008; 13:685–696. [PubMed: 17684499]
22. Konradi C, Sullivan SE, Clay HB. Mitochondria, oligodendrocytes and inflammation in bipolar disorder: evidence from transcriptome studies points to intriguing parallels with multiple sclerosis. *Neurobiol Dis.* 2012; 45:37–47. [PubMed: 21310238]
23. Pennington K, Beasley CL, Dicker P, et al. Prominent synaptic and metabolic abnormalities revealed by proteomic analysis of the dorsolateral prefrontal cortex in schizophrenia and bipolar disorder. *Mol Psychiatry.* 2008; 13:1102–1117. [PubMed: 17938637]
24. Modica-Napolitano JS, Renshaw PF. Ethanolamine and phosphoethanolamine inhibit mitochondrial function in vitro: implications for mitochondrial dysfunction hypothesis in depression and bipolar disorder. *Biol Psychiatry.* 2004; 55:273–277. [PubMed: 14744468]
25. Cui J, Shao L, Young LT, Wang JF. Role of glutathione in neuroprotective effects of mood stabilizing drugs lithium and valproate. *Neuroscience.* 2007; 144:1447–1453. [PubMed: 17184924]
26. Bachmann RF, Wang Y, Yuan P, et al. Common effects of lithium and valproate on mitochondrial functions: protection against methamphetamine-induced mitochondrial damage. *Int J Neuropsychopharmacol.* 2009; 12:805–822. [PubMed: 19149911]
27. Regenold WT, Phatak P, Marano CM, Sassan A, Conley RR, Kling MA. Elevated cerebrospinal fluid lactate concentrations in patients with bipolar disorder and schizophrenia: implications for the mitochondrial dysfunction hypothesis. *Biol Psychiatry.* 2009; 65:489–494. [PubMed: 19103439]
28. Båve U, Nybom R, Landén M, Wetterberg L. Micrometer-sized thread-like and/or spherical particles in the first fraction of cerebrospinal fluid in patients with bipolar disorder. *Bipolar Disord.* 2010; 12:298–305. [PubMed: 20565436]
29. Sun X, Wang JF, Tseng M, Young LT. Downregulation in components of the mitochondrial electron transport chain in the postmortem frontal cortex of subjects with bipolar disorder. *J Psychiatry Neurosci.* 2006; 31:189–196. [PubMed: 16699605]

30. Andreazza AC, Shao L, Wang JF, Young LT. Mitochondrial complex I activity and oxidative damage to mitochondrial proteins in the prefrontal cortex of patients with bipolar disorder. *Arch Gen Psychiatry*. 2010; 67:360–368. [PubMed: 20368511]
31. Iwamoto K, Bundo M, Kato T. Altered expression of mitochondria-related genes in postmortem brains of patients with bipolar disorder or schizophrenia, as revealed by large-scale DNA microarray analysis. *Hum Mol Genet*. 2005; 14:241–253. [PubMed: 15563509]
32. Kubota M, Kasahara T, Nakamura T, Ishiwata M, Miyauchi T, Kato T. Abnormal Ca²⁺ dynamics in transgenic mice with neuron-specific mitochondrial DNA defects. *J Neurosci*. 2006; 26:12314–12324. [PubMed: 17122057]
33. Kasahara T, Kubota M, Miyauchi T, et al. Mice with neuron-specific accumulation of mitochondrial DNA mutations show mood disorder-like phenotypes. *Mol Psychiatry*. 2006; 11:577–593. [PubMed: 16619054]
34. Valvassori SS, Rezin GT, Ferreira CL, et al. Effects of mood stabilizers on mitochondrial respiratory chain activity in brain of rats treated with d-amphetamine. *J Psychiatr Res*. 2010; 44:903–909. [PubMed: 20334877]
35. Riegel RE, Valvassori SS, Elias G, et al. Animal model of mania induced by ouabain: evidence of oxidative stress in submitochondrial particles of the rat brain. *Neurochem Int*. 2009; 55:491–495. [PubMed: 19447152]
36. Stork C, Renshaw PF. Mitochondrial dysfunction in bipolar disorder: evidence from magnetic resonance spectroscopy research. *Mol Psychiatry*. 2005; 10:900–919. [PubMed: 16027739]
37. Dager SR, Corrigan NM, Richards TL, Posse S. Research applications of magnetic resonance spectroscopy to investigate psychiatric disorders. *Top Magn Reson Imaging*. 2008; 19:81–96. [PubMed: 19363431]
38. Zhu XH, Du F, Zhang N, et al. Advanced in vivo heteronuclear MRS approaches for studying brain bioenergetics driven by mitochondria. *Methods Mol Biol*. 2009; 489:317–357. [PubMed: 18839099]
39. Phillips ML, Travis MJ, Fagiolini A, Kupfer DJ. Medication effects in neuroimaging studies of bipolar disorder. *Am J Psychiatry*. 2008; 165:313–320. [PubMed: 18245175]
40. Pan L, Keener MT, Hassel S, Phillips ML. Functional neuroimaging studies of bipolar disorder: examining the wide clinical spectrum in the search for disease endophenotypes. *Int Rev Psychiatry*. 2009; 21:368–379. [PubMed: 20374150]
41. Yurgelun-Todd DA, Ross AJ. Functional magnetic resonance imaging studies in bipolar disorder. *CNS Spectr*. 2006; 11:287–297. [PubMed: 16641834]
42. Marsh R, Gerber AJ, Peterson BS. Neuroimaging studies of normal brain development and their relevance for understanding childhood neuropsychiatric disorders. *J Am Acad Child Adolesc Psychiatry*. 2008; 47:1233–1251. [PubMed: 18833009]
43. Delbello MP, Strakowski SM. Neurochemical predictors of response to pharmacologic treatments for bipolar disorder. *Curr Psychiatry Rep*. 2004; 6:466–472. [PubMed: 15538996]
44. Savitz J, Nugent AC, Bogers W, et al. Amygdala volume in depressed patients with bipolar disorder assessed using high resolution 3T MRI: the impact of medication. *Neuroimage*. 2010; 49:2966–2976. [PubMed: 19931399]
45. Caetano SC, Olvera RL, Hatch JP, et al. Lower N-acetyl-aspartate levels in prefrontal cortices in pediatric bipolar disorder: a (1)H magnetic resonance spectroscopy study. *J Am Acad Child Adolesc Psychiatry*. 2011; 50:85–94. [PubMed: 21156273]
46. Bearden CE, Glahn DC, Caetano S, et al. Evidence for disruption in prefrontal cortical functions in juvenile bipolar disorder. *Bipolar Disord*. 2007; 9(Suppl 1):145–159. [PubMed: 17543033]
47. Dickstein DP, Finger EC, Skup M, Pine DS, Blair JR, Leibenluft E. Altered neural function in pediatric bipolar disorder during reversal learning. *Bipolar Disord*. 2010; 12:707–719. [PubMed: 21040288]
48. Baloch HA, Hatch JP, Olvera RL, et al. Morphology of the subgenual prefrontal cortex in pediatric bipolar disorder. *J Psychiatr Res*. 2010; 44:1106–1110. [PubMed: 20488457]
49. Pavuluri MN, Passarotti AM, Harral EM, Sweeney JA. Enhanced prefrontal function with pharmacotherapy on a response inhibition task in adolescent bipolar disorder. *J Clin Psychiatry*. 2010; 71:1526–1534. [PubMed: 20816040]

50. Chang K, Adleman N, Dienes K, Barnea-Goraly N, Reiss A, Ketter T. Decreased N-acetylaspartate in children with familial bipolar disorder. *Biol Psychiatry*. 2003; 53:1059–1065. [PubMed: 12788251]
51. Patel NC, Cecil KM, Strakowski SM, Adler CM, DelBello MP. Neurochemical alterations in adolescent bipolar depression: a proton magnetic resonance spectroscopy pilot study of the prefrontal cortex. *J Child Adolesc Psychopharmacol*. 2008; 18:623–627. [PubMed: 19108667]
52. Cecil KM, DelBello MP, Morey R, Strakowski SM. Frontal lobe differences in bipolar disorder as determined by proton MR spectroscopy. *Bipolar Disord*. 2002; 4:357–365. [PubMed: 12519095]
53. Olvera RL, Caetano SC, Fonseca M, et al. Low levels of N-acetyl aspartate in the left dorsolateral prefrontal cortex of pediatric bipolar patients. *J Child Adolesc Psychopharmacol*. 2007; 17:461–473. [PubMed: 17822341]
54. Patel NC, DelBello MP, Cecil KM, et al. Lithium treatment effects on Myo-inositol in adolescents with bipolar depression. *Biol Psychiatry*. 2006; 60:998–1004. [PubMed: 17056394]
55. Patel NC, DelBello MP, Bryan HS, et al. Open-label lithium for the treatment of adolescents with bipolar depression. *J Am Acad Child Adolesc Psychiatry*. 2006; 45:289–297. [PubMed: 16540813]
56. Sassi RB, Stanley JA, Axelson D, et al. Reduced NAA levels in the dorsolateral prefrontal cortex of young bipolar patients. *Am J Psychiatry*. 2005; 162:2109–2115. [PubMed: 16263851]
57. Kato T, Takahashi S, Shioiri T, Inubushi T. Alterations in brain phosphorous metabolism in bipolar disorder detected by in vivo 31P and 7Li magnetic resonance spectroscopy. *J Affect Disord*. 1993; 27:53–59. [PubMed: 8432961]
58. Kato T, Takahashi S, Shioiri T, Murashita J, Hamakawa H, Inubushi T. Reduction of brain phosphocreatine in bipolar II disorder detected by phosphorus-31 magnetic resonance spectroscopy. *J Affect Disord*. 1994; 31:125–133. [PubMed: 8071475]
59. Deicken RF, Fein G, Weiner MW. Abnormal frontal lobe phosphorous metabolism in bipolar disorder. *Am J Psychiatry*. 1995; 152:915–918. [PubMed: 7755123]
60. Kato T, Shioiri T, Murashita J, et al. Lateralized abnormality of high energy phosphate metabolism in the frontal lobes of patients with bipolar disorder detected by phase-encoded 31P-MRS. *Psychol Med*. 1995; 25:557–566. [PubMed: 7480436]
61. Hamakawa H, Murashita J, Yamada N, Inubushi T, Kato N, Kato T. Reduced intracellular pH in the basal ganglia and whole brain measured by 31P-MRS in bipolar disorder. *Psychiatry Clin Neurosci*. 2004; 58:82–88. [PubMed: 14678462]
62. Kondo DG, Sung YH, Hellem TL, et al. Open-label uridine for treatment of depressed adolescents with bipolar disorder. *J Child Adolesc Psychopharmacol*. 2011; 21:171–175. [PubMed: 21486171]
63. Poznanski, EO.; Mokros, HB. Children's Depression Rating Scale. Los Angeles: Western Psychological Services; 1996. Revised (CDRS-R) Manual
64. Young RC, Biggs JT, Ziegler VE, Meyer DA. A rating scale for mania: reliability, validity and sensitivity. *Br J Psychiatry*. 1978; 133:429–435. [PubMed: 728692]
65. Kaufman J, Birmaher B, Brent D, et al. Schedule for affective disorders and schizophrenia for school-age children-present and lifetime version (K-SADS-PL): initial reliability and validity data. *J Am Acad Child Adolesc Psychiatry*. 1997; 36:980–988. [PubMed: 9204677]
66. Vanhamme L, van den Boogaart A, Van Huffel S. Improved method for accurate and efficient quantification of MRS data with use of prior knowledge. *J Magn Reson*. 1997; 129:35–43. [PubMed: 9405214]
67. Naressi A, Couturier C, Devos JM, et al. Java-based graphical user interface for the MRUI quantitation package. *MAGMA*. 2001; 12:141–152. [PubMed: 11390270]
68. Petroff OA, Prichard JW, Behar KL, Alger JR, den Hollander JA, Shulman RG. Cerebral intracellular pH by 31P nuclear magnetic resonance spectroscopy. *Neurology*. 1985; 35:781–788. [PubMed: 4000479]
69. Kramer CY. Extension of multiple range tests to group means with unequal numbers of replications. *Biometrics*. 1956; 12:307–310.
70. Tukey JW. The philosophy of multiple comparisons. *Stat Sci*. 1991; 6:100–116.

71. Yildiz A, Sachs GS, Dorer DJ, Renshaw PF. 31P Nuclear magnetic resonance spectroscopy findings in bipolar illness: a meta-analysis. *Psychiatry Res.* 2001; 106:181–191. [PubMed: 11382540]
72. Cerullo MA, Adler CM, Delbello MP, Strakowski SM. The functional neuroanatomy of bipolar disorder. *Int Rev Psychiatry.* 2009; 21:314–322. [PubMed: 20374146]
73. Kempton MJ, Geddes JR, Ettinger U, Williams SC, Grasby PM. Meta-analysis, database, and meta-regression of 98 structural imaging studies in bipolar disorder. *Arch Gen Psychiatry.* 2008; 65:1017–1032. [PubMed: 18762588]
74. Hallahan B, Newell J, Soares JC, et al. Structural magnetic resonance imaging in bipolar disorder: an international collaborative mega-analysis of individual adult patient data. *Biol Psychiatry.* 2011; 69:326–335. [PubMed: 21030008]
75. Chen CH, Suckling J, Lennox BR, Ooi C, Bullmore ET. A quantitative meta-analysis of fMRI studies in bipolar disorder. *Bipolar Disord.* 2011; 13:1–15. [PubMed: 21320248]
76. Brennan BP, Hudson JI, Jensen JE, et al. Rapid enhancement of glutamatergic neurotransmission in bipolar depression following treatment with riluzole. *Neuropsychopharmacology.* 2010; 35:834–846. [PubMed: 19956089]
77. Yildiz A, Moore CM, Sachs GS, et al. Lithium-induced alterations in nucleoside triphosphate levels in human brain: a proton-decoupled 31P magnetic resonance spectroscopy study. *Psychiatry Res.* 2005; 138:51–59. [PubMed: 15708301]
78. Yoon SJ, Lyoo IK, Kim HJ, et al. Neurochemical alterations in methamphetamine-dependent patients treated with cytidine-5'-diphosphate choline: a longitudinal proton magnetic resonance spectroscopy study. *Neuropsychopharmacology.* 2010; 35:1165–1173. [PubMed: 20043005]
79. Forester BP, Finn CT, Berlow YA, Wardrop M, Renshaw PF, Moore CM. Brain lithium, N-acetyl aspartate and myo-inositol levels in older adults with bipolar disorder treated with lithium: a lithium-7 and proton magnetic resonance spectroscopy study. *Bipolar Disord.* 2008; 10:691–700. [PubMed: 18837863]
80. Jensen JE, Daniels M, Haws C, et al. Triacetyluridine (TAU) decreases depressive symptoms and increases brain pH in bipolar patients. *Exp Clin Psychopharmacol.* 2008; 16:199–206. [PubMed: 18540779]
81. Moore CM, Biederman J, Wozniak J, et al. Mania, glutamate/glutamine and risperidone in pediatric bipolar disorder: a proton magnetic resonance spectroscopy study of the anterior cingulate cortex. *J Affect Disord.* 2007; 99:19–25. [PubMed: 17005256]
82. Lyoo IK, Dager SR, Kim JE, et al. Lithium-induced gray matter volume increase as a neural correlate of treatment response in bipolar disorder: a longitudinal brain imaging study. *Neuropsychopharmacology.* 2010; 35:1743–1750. [PubMed: 20357761]
83. Germana C, Kempton MJ, Sarnicola A, et al. The effects of lithium and anticonvulsants on brain structure in bipolar disorder. *Acta Psychiatr Scand.* 2010; 122:481–487. [PubMed: 20560901]
84. Patel NC, DelBello MP, Cecil KM, Stanford KE, Adler CM, Strakowski SM. Temporal change in N-acetyl-aspartate concentrations in adolescents with bipolar depression treated with lithium. *J Child Adolesc Psychopharmacol.* 2008; 18:132–139. [PubMed: 18439111]
85. Brambilla P, Stanley JA, Nicoletti MA, et al. 1H magnetic resonance spectroscopy investigation of the dorsolateral prefrontal cortex in bipolar disorder patients. *J Affect Disord.* 2005; 86:61–67. [PubMed: 15820271]
86. Giedd JN. Linking adolescent sleep, brain maturation, and behavior. *J Adolesc Health.* 2009; 45:319–320. [PubMed: 19766933]
87. Kato T, Takahashi S, Shioiri T, Inubushi T. Brain phosphorous metabolism in depressive disorders detected by phosphorus-31 magnetic resonance spectroscopy. *J Affect Disord.* 1992; 26:223–230. [PubMed: 1479134]
88. Brazy PC, Mandel LJ. Does availability of inorganic phosphate regulate cellular oxidative metabolism? *News Physiol Sci.* 1986; 1:100–103.
89. Chance B, Leigh JS Jr, Kent J, et al. Multiple controls of oxidative metabolism in living tissues as studied by phosphorus magnetic resonance. *Proc Natl Acad Sci U S A.* 1986; 83:9458–9462. [PubMed: 3467315]

90. Bose S, French S, Evans FJ, Joubert F, Balaban RS. Metabolic network control of oxidative phosphorylation: multiple roles of inorganic phosphate. *J Biol Chem.* 2003; 278:39155–39165. [PubMed: 12871940]
91. Freeman D, Bartlett S, Radda G, Ross B. Energetics of sodium transport in the kidney. Saturation transfer 31P-NMR. *Biochim Biophys Acta.* 1983; 762:325–336. [PubMed: 6830878]
92. Iles RA, Stevens AN, Griffiths JR, Morris PG. Phosphorylation status of liver by 31P-n.m.r. spectroscopy, and its implications for metabolic control. A comparison of 31P-n.m.r. spectroscopy (in vivo and in vitro) with chemical and enzymic determinations of ATP, ADP and Pi. *Biochem J.* 1985; 229:141–151. [PubMed: 4038253]
93. Balaban RS. Regulation of oxidative phosphorylation in the mammalian cell. *Am J Physiol.* 1990; 258:C377–C389. [PubMed: 2138418]
94. Brown GC. Control of respiration and ATP synthesis in mammalian mitochondria and cells. *Biochem J.* 1992; 284:1–13. [PubMed: 1599389]
95. Iotti S, Lodi R, Gottardi G, Zaniol P, Barbiroli B. Inorganic phosphate is transported into mitochondria in the absence of ATP biosynthesis: an in vivo 31P NMR study in the human skeletal muscle. *Biochem Biophys Res Commun.* 1996; 225:191–194. [PubMed: 8769116]
96. Glinn M, Ni B, Paul SM. Inorganic phosphate enhances phosphonucleotide concentrations in cultured fetal rat cortical neurons. *Brain Res.* 1997; 757:85–92. [PubMed: 9200502]
97. From AH, Zimmer SD, Michurski SP, et al. Regulation of the oxidative phosphorylation rate in the intact cell. *Biochemistry.* 1990; 29:3731–3743. [PubMed: 2340268]
98. Zhang J, Shorr L, Yoshiyama M, et al. Hyperperfusion and cardioplegia effects on myocardial high-energy phosphate distribution and energy expenditure. *Am J Physiol.* 1994; 267:H894–H904. [PubMed: 8092293]
99. Apell HJ, Roudna M, Corrie JE, Trentham DR. Kinetics of the phosphorylation of Na,K-ATPase by inorganic phosphate detected by a fluorescence method. *Biochemistry.* 1996; 35:10922–10930. [PubMed: 8718885]
100. Gatto C, Helms JB, Prasse MC, Arnett KL, Milanick MA. Kinetic characterization of tetrapropylammonium inhibition reveals how ATP and Pi alter access to the Na⁺-K⁺-ATPase transport site. *Am J Physiol Cell Physiol.* 2005; 289:C302–C311. [PubMed: 15788490]
101. Pedemonte CH, Beauge L. Inhibition of (Na⁺,K⁺)-ATPase by magnesium ions and inorganic phosphate and release of these ligands in the cycles of ATP hydrolysis. *Biochim Biophys Acta.* 1983; 748:245–253. [PubMed: 6313060]
102. Stein WD. Energetics and the design principles of the Na/K-ATPase. *J Theor Biol.* 1990; 147:145–159. [PubMed: 2177514]
103. Beauge L. Breakdown of Na⁺/K⁺-exchanging ATPase phosphoenzymes formed from ATP and from inorganic phosphate during Na⁺-ATPase activity. *Eur J Biochem.* 2001; 268:5627–5632. [PubMed: 11683886]
104. Looney SW, el-Mallakh RS. Meta-analysis of erythrocyte Na,K-ATPase activity in bipolar illness. *Depress Anxiety.* 1997; 5:53–65. [PubMed: 9262935]
105. Coppen A, Shaw DM, Malleson A, Costain R. Mineral metabolism in mania. *Br Med J.* 1966; 1:71–75. [PubMed: 5902530]
106. Goldstein I, Lerer E, Laiba E, et al. Association between sodium- and potassium-activated adenosine triphosphatase alpha isoforms and bipolar disorders. *Biol Psychiatry.* 2009; 65:985–991. [PubMed: 19058785]
107. Mynett-Johnson L, Murphy V, McCormack J, et al. Evidence for an allelic association between bipolar disorder and a Na⁺, K⁺ adenosine triphosphatase alpha subunit gene (ATP1A3). *Biol Psychiatry.* 1998; 44:47–51. [PubMed: 9646882]
108. Wood AJ, Smith CE, Clarke EE, Cowen PJ, Aronson JK, Grahame-Smith DG. Altered in vitro adaptive responses of lymphocyte Na⁺,K⁺-ATPase in patients with manic depressive psychosis. *J Affect Disord.* 1991; 21:199–206. [PubMed: 1648581]
109. Kirshenbaum GS, Saltzman K, Rose B, Petersen J, Vilsen B, Roder JC. Decreased neuronal Na⁺,K⁺-ATPase activity in Atp1a3 heterozygous mice increases susceptibility to depression-like endophenotypes by chronic variable stress. *Genes Brain Behav.* 2011; 10:542–550. [PubMed: 21418141]

110. Traub N, Lichtstein D. The mood cycle hypothesis: possible involvement of steroid hormones in mood regulation by means of Na⁺, K⁺-ATPase inhibition. *J Basic Clin Physiol Pharmacol*. 2000; 11:375–394. [PubMed: 11248948]
111. Bagrov YY, Manusova NB, Egorova IA, Fedorova OV, Bagrov AY. Endogenous digitalis-like ligands and Na/KATPase inhibition in experimental diabetes mellitus. *Front Biosci*. 2005; 10:2257–2262. [PubMed: 15970492]
112. Goldstein I, Levy T, Galili D, et al. Involvement of Na(+), K(+)-ATPase and endogenous digitalis-like compounds in depressive disorders. *Biol Psychiatry*. 2006; 60:491–499. [PubMed: 16712803]
113. Goldstein I, Lax E, Gispan-Herman I, et al. Neutralization of endogenous digitalis-like compounds alters catecholamines metabolism in the brain and elicits anti-depressive behavior. *Eur Neuropsychopharmacol*. 2012; 22:72–79. [PubMed: 21700431]

Fig. 1.

Voxel placement (left), axial slice location (top, right), and a representative phosphorus-31 magnetic resonance spectroscopy (^{31}P -MRS) spectrum (bottom, right). PME = phosphomonoester; Pi = inorganic phosphate; PDE = phosphodiester; PCr = phosphocreatine; NTP = nucleoside triphosphate.

Fig. 2.

Bar graphs depicting frontal lobe phosphorus metabolite levels and post-hoc Tukey–Kramer pairwise comparisons: **(A)** PCr/TP; **(B)** Pi/TP; **(C)** PCr/Pi; and **(D)** beta-NTP/TP. Error bars represent 95% confidence intervals. HC = healthy controls; BD-MED = medicated bipolar disorder; BD-UNMED = unmedicated bipolar disorder; PCr = phosphocreatine; TP = total phosphate; Pi = inorganic phosphate; NTP = nucleoside triphosphate. The values in parentheses refer to the percentage difference of metabolites with respect to mean metabolite concentrations in the HC group.

Fig. 3.

The volume percentage of gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF) in the region of interest. Error bars represent 95% confidence intervals. HC = healthy controls; BD-MED = medicated bipolar disorder; BD-UNMED = unmedicated bipolar disorder.

Table 1

Characteristics of adolescents with bipolar disorder (BD) and healthy controls

	Unmedicated BD (n = 8)	Medicated BD (n = 6)	Healthy controls (n = 24)	p-value ^a
Age (years) mean $\pm$ SD	15.6 $\pm$ 1.0	15.5 $\pm$ 0.8	15.7 $\pm$ 1.7	0.99 ^a
Gender (female), n	5	2	11	–
CDRS-R raw score, mean $\pm$ SD	60.4 $\pm$ 11.0	55.7 $\pm$ 13.9	20.8 $\pm$ 3.9	< 0.01 ^a
Medication naïve, n (%)	4 (50)	0 (0)	24 (100)	–
Duration unmedicated (weeks), mean $\pm$ SD ^b	70.0 $\pm$ 47.5	N/A	N/A	–

CDRS-R = Children's Depression; SD = standard deviation.

Rating Scale-Revised.

^aOne-way analysis of variance.^bFour of eight unmedicated BD participants had prior exposure to psychotropic medication.

Table 2

Diagnoses, medication status, and family history of depressed adolescents with bipolar disorder (BD)

Age/sex	Diagnoses	Current medication(s)	Medication(s) exposure history	Family history
15 F	BD-II ADHD combined ODD	None (unmedicated for 28 weeks prior to study)	Age 5–12: methylphenidate immediate release Age 12–13: aripiprazole 10 mg, lamotrigine 100 mg Age 14: quetiapine 50 mg AM and 200 mg HS	Siblings: two brothers with BD Maternal: cousin with BD Paternal: none known
17 F	BD-NOS ADHD inattentive	None (medication naïve)	Medication naïve	Unknown: patient was adopted
15 F	BD-I ADHD combined GAD Eating disorder NOS	None (unmedicated for 106 weeks prior to study)	Age 12–13: citalopram 20 mg, trazodone 50 mg	Siblings: brother with ASD, sister with ADHD and anxiety disorder Maternal: mother with BD, grandfather with BD, cousin with BD Paternal: father with cocaine, opioid, and alcohol dependence
16 M	BD-II ADHD combined Social phobia Impulse control disorder NOS	None (medication naïve)	Medication naïve	Siblings: sister with BD Maternal: mother with BD, grandmother and grandfather had mood disorder, great uncle completed suicide Paternal: none known
17 F	BD-NOS Alcohol dependence (sustained full remission)	None (unmedicated for 30 weeks prior to study)	Age 16: citalopram 40 mg (induced mania) Age 17: divalproex sodium 1500 mg	Siblings: none known Maternal: grandmother with schizoaffective disorder, bipolar type, great grandfather with BD Paternal: none known
13 M	BD-NOS ADHD combined Social phobia Encopresis	None (medication naïve)	Medication naïve	Siblings: none known Maternal: mother with BD, cocaine dependence, antisocial personality disorder Paternal: none known
17 M	BD-I ADHD combined Cannabis dependence (early full remission)	Aripiprazole 10 mg Trazodone 100 mg (duration of this regimen = 68 weeks)	Age 7–13: methylphenidate extended release 54 mg Age 14: lisdexamfetamine 70 mg, fluoxetine 20 mg Age 15: venlafaxine extended-release 225 mg Age 16: risperidone 2 mg	Siblings: sister with BD Maternal: mother with BD Paternal: father with MDD
16 M	BD-II ADHD combined	Methylphenidate extended-release 36 mg Melatonin 3 mg (duration prior to study = 92 weeks)	Age 12–13: atomoxetine 60 mg Age 14: risperidone 1 mg	Siblings: none known Maternal: none known Paternal: uncle with BD, uncle with recurrent MDD, grandmother with MDD and anxiety disorder
15 F	BD-II GAD Cannabis abuse	None (unmedicated for 116 weeks prior to study)	Age 12: citalopram 20 mg Age 13: escitalopram 10 mg	Siblings: sister with BD and cannabis abuse Maternal: none known Paternal: father with BD, ADHD, alcohol dependence, great uncle completed suicide

Age/sex	Diagnoses	Current medication(s)	Medication(s) exposure history	Family history
17 M	BD-I Social phobia GAD	Lamotrigine 200 mg Citalopram 10 mg (duration prior to study = 150 weeks)	Age 12–14: risperidone 2.5 mg	Siblings: none known Maternal: mother with MDD and anxiety disorder Paternal: father with BD, grandmother with BD, cousin completed suicide
18 F	BD-I GAD	Bupropion extended-release 150 mg (duration prior to study = 8 weeks)	Age 14: venlafaxine extended-release 150 mg Age 15: escitalopram 20 mg	Siblings: brother with BD Maternal: mother with MDD, great uncle with BD, great grandmother with BD, great grandfather with alcohol dependence Paternal: father with MDD, grandfather with MDD, cousin with BD, great grandfather with alcohol dependence
17 M	BD-I GAD	SAM-e 200 mg (duration prior to study = 2 weeks)	N/A (SAM-e was the first psychotropic exposure)	Siblings: none known Maternal: none known Paternal: none known
17 F	BD-II ADHD combined	Escitalopram 40 mg Dexamethylphenidate extended-release 20 mg Topiramate 50 mg (duration prior to study = 130 weeks)	Age 7: fluoxetine 20 mg Age 8: sertraline 200 mg Age 9–13: citalopram 20 mg, clonazepam 1 mg, trazodone 12.5 mg	Siblings: brother with ADHD, sister with ADHD Maternal: mother with MDD, aunt with MDD requiring ECT, uncle with MDD, uncle with ADHD Paternal: none known
17 M	BD-NOS ADHD combined	None (medication naïve)	Medication naïve	Unknown: the patient was adopted

ADHD = attention-deficit hyperactivity disorder; AM = morning; ASD = autism spectrum disorder; BD-I = bipolar I disorder; BD-II = bipolar II disorder; ECT = electroconvulsive therapy; F = female; GAD = generalized anxiety disorder; HS = bedtime; NOS = not otherwise specified; M = male; MDD = major depressive disorder; ODD = oppositional defiant disorder; SAM-e = S-adenosylmethionine.

Frontal lobe phosphorus-31 magnetic resonance spectroscopy (^3P -MRS) metabolite concentrations in adolescents with unmedicated bipolar disorder [(BD-UNMED) group A], medicated bipolar disorder [(BD-MED) group B], and healthy controls [(HC) group C]

Table 3

Metabolite	BD-UNMED (n = 8)	BD-MED (n = 6)	HC (n = 24)	ANCOVA		Pairwise group p-value ^a		
				F(2,34)	p-value	(A, B)	(B, C)	(A, C)
PCr/TP	0.145 (0.013)	0.129 (0.003)	0.140 (0.012)	3.637	0.037	0.032	0.063	0.649
Pi/TP	0.045 (0.007)	0.058 (0.009)	0.054 (0.008)	4.609	0.017	0.022	0.574	0.038
PCr/Pi	3.321 (0.772)	2.281 (0.395)	2.668 (0.455)	7.343	0.002	0.002	0.233	0.013
β -NTP/TP	0.127 (0.013)	0.135 (0.013)	0.127 (0.015)	0.825	0.447	0.556	0.377	0.990
PCr/ β -NTP	1.153 (0.198)	0.961 (0.098)	1.127 (0.188)	2.518	0.096	0.108	0.943	0.091
pH	7.062 (0.042)	7.050 (0.022)	7.053 (0.034)	0.250	0.780	0.781	0.961	0.826
PME/TP	0.139 (0.028)	0.134 (0.016)	0.135 (0.015)	0.152	0.859	0.880	0.992	0.871
PDE/TP	0.183 (0.019)	0.194 (0.015)	0.183 (0.021)	0.820	0.449	0.539	0.445	0.999
Mg ²⁺ ($\mu\text{mol/L}$)	0.209 (0.083)	0.165 (0.021)	0.248 (0.200)	0.687	0.510	0.852	0.460	0.804

Values are indicated as mean [standard deviation (SD)]. The total phosphorus (TP) concentration was used for standardization.

ANCOVA = analysis of covariance; PCr = phosphocreatine; PDE = phosphodiester; Pi = inorganic phosphate; PME = phosphomonoester; Mg = magnesium; β -NTP = beta-nucleoside triphosphate;

^aTukey Honestly Significant Difference correction was applied following ANCOVA for multiple comparison, and age in years was included as a covariate.