N-acetylaspartate normalization in bipolar depression after lamotrigine treatment

Paul E Croarkin[a], **M Albert Thomas**[b], **John D Port**[c], **Joshua M Baruth**[d], **Doo-Sup Choi**[e], **Osama A Abulseoud**[a], and **Mark A Frye**[a]

[a]Department of Psychiatry and Psychology, Mayo Clinic, Rochester, MN

[b]Department of Radiology, Psychiatry, and Biomedical Engineering, Geffen School of Medicine, University of California at Los Angeles, Los Angeles, CA

[c]Department of Radiology, Mayo Clinic, Rochester, MN

[d]University of Louisville School of Medicine, Louisville, KY

[e]Department of Molecular Pharmacology and Experimental Therapeutics, Mayo Clinic, Rochester, MN, USA

Abstract

Objectives—To examine *N*-acetylaspartate (NAA), a general marker of neuronal viability, and total NAA (tNAA), the combined signal of NAA and *N*-acetylaspartylglutamate, in bipolar depression before and after lamotrigine treatment. Given that NAA is synthesized through direct acetylation of aspartate by acetyl-coenzyme A-*L*-aspartate-*N*-acetyltransferase, we hypothesized that treatment with lamotrigine would be associated with an increase in NAA level.

Methods—Patients with bipolar depression underwent two-dimensional proton magnetic resonance spectroscopy of the anterior cingulate at baseline (n = 15) and after 12 weeks of lamotrigine treatment (n = 10). A group of age-matched healthy controls (n = 9) underwent scanning at baseline for comparison.

Results—At baseline, patients with bipolar depression had significantly lower NAA [mean standard deviation (SD) = 1.13 (0.21); p = 0.02] than controls [mean (SD) = 1.37 (0.27)]. Significant increases in NAA [mean (SD) = 1.39 (0.21); p = 0.01] and tNAA [mean (SD) = 1.61 (0.25); p = 0.02] levels were found after 12 weeks of lamotrigine treatment.

Conclusions—These data suggest an NAA deficit in bipolar depression that is normalized after lamotrigine treatment. Future research is warranted to evaluate whether baseline NAA level is a potential biomarker for identifying lamotrigine response patterns and whether this functional brain change has an associated clinical response.

Corresponding author: Mark A. Frye, MD, Department of Psychiatry and Psychology, Mayo Clinic, 200 First Street SW, Rochester, MN 55905, Fax:, mfrye@mayo.edu.

Keywords

bipolar depression; bipolar disorder; lamotrigine; *N*-acetylaspartate; neuroprotection; two-dimensional proton magnetic resonance spectroscopy

Lamotrigine is an anticonvulsant approved by the US Food and Drug Administration for maintenance mood stabilization of bipolar I disorder (1). Its acute antidepressant properties are recognized increasingly, both through meta-analysis (2, 3) and placebo-controlled evaluation (4–6). The anticonvulsant activity of lamotrigine is thought to be mediated by voltage-dependent sodium channel blockade, with subsequent presynaptic inhibition of aspartate (Asp) and glutamate (Glu) resulting in an overall reduction in neuronal excitability (7–10). Similar to lithium, lamotrigine also has been shown to be neuroprotective against Glu excitotoxicity in a number of cellular and animal models (9, 11, 12). Recent work has highlighted evidence of its neuroprotective properties, mediated through histone deacetylase inhibition and chromatin remodeling, and both dose- and time-dependent increases in antiapoptotic Bcl-2 mRNA and protein levels (9). How these mechanisms confer antidepressant effects in bipolar depression is unclear.

N-Acetylaspartate (NAA) is an abundant neuronal metabolite broadly, conceptualized as a marker of mitochondrial activity and neuronal integrity (13). Putative functions include: maintenance of brain fluid balance as an osmolyte, providing a source of acetate for myelin synthesis (14); serving as a precursor for synthesis of *N*-acetylaspartylglutamate (NAAG); and regulating Glu metabolism (15, 16). NAA is synthesized in neuronal mitochondria from *L*-aspartate and acetyl coenzyme A through Asp *N*-acetyltransferase (17). An oligodendrocytic enzyme, aspartoacylase hydrolyzes NAA and thereby provides acetate for myelin synthesis (14). Glu and NAA metabolism are linked through the Glu-glutamine (Gln) and tricarboxylic acid cycles (18, 19) because such NAA may also serve as a Glu pool and buffer of glutamatergic excitoxcity (16).

Proton magnetic resonance spectroscopy (MRS) is a noninvasive functional imaging technique that can quantify NAA cortical deficits and measure dynamic changes (20, 21). This type of MRS resonance spectra of NAA is a dominant singlet peak at 2.02 ppm. Typically, the NAA signal is total NAA (tNAA), which is comprised of NAA and NAAG (22, 23). However, conventional one-dimensional MRS is limited given the resonance signal overlap. Two-dimensional (2D) localized correlated spectroscopy (L-COSY) facilitates better separation of overlapping cerebral metabolites and allows a more accurate quantification of NAA and tNAA through a novel prior-knowledge fitting algorithm (ProFit; QuantumSoft). Early work in 2D MRS has focused on metabolite discrimination and reproducibility in healthy controls (24, 25), cross-sectional evaluation of frontal white and gray matter in chronic hepatitis C infection (26), and different mood states in bipolar disorder (27).

The aim of this exploratory study was to use 2D MRS and L-COSY to quantify anterior cingulate NAA and tNAA in patients with bipolar depression and evaluate change in NAA and tNAA after 12 weeks of treatment with lamotrigine. We hypothesized that patients with

bipolar depression have decreased NAA levels in the anterior cingulate cortex and that lamotrigine therapy corrects this deficit.

Methods

The study protocol was approved by the University of California at Los Angeles (UCLA), Harbor–UCLA Medical Center (Los Angeles, CA, USA), and Mayo Clinic (Rochester, MN, USA) institutional review boards. Every study participant gave written informed consent, with diagnosis confirmed with the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorder*s* (Fourth Edition, Text Revision) (28). Study inclusion criteria involved a diagnosis of bipolar I or bipolar II disorder and a current major depressive episode of at least moderate symptom severity, defined as a score of   16 on the Montgomery–Åsberg Depression Rating Scale (29). Ratings were conducted by the principal investigator (MAF). Secondary severity measures included the Inventory of Depressive Symptoms (30). Further inclusion and exclusion criteria are reviewed elsewhere (31, 32). Baseline MRS scans were collected in 15 patients with bipolar depression and nine healthy controls. A second scan was obtained in 10 patients after 12 weeks of open-label treatment with lamotrigine. Five patients dropped out of the study before the second scan because of: hospitalization for depression (n = 1), rash [full resolution with drug discontinuation (n = 2)], administrative discharge (n = 1), and declined second scan (n = 1) (31, 32).

Magnetic resonance imaging/MRS acquisition and processing

Scans were performed with a 1.5-T MRS scanner (GE Medical Systems, Inc.) with echo-speed gradients using a head transmit and receive coil. Anatomical landmarks based on a human brain reference atlas were used in a systematic manner for voxel positioning. Acquisitions included a sagittal whole-brain image for orientation and positioning, a T1-weighted axial image [repetition time (TR)/echo time (TE), 800 sec/8 msec; 35 slices with 4-mm thickness] for voxel placement, and a water-suppressed, single-voxel scan (TR/TE, 3 sec/30 msec; 256 averages with $3 \times 3 \times 3$-cm^3 voxel size) in the anterior cingulate cortex. Thereafter, for 2D MRS, an L-COSY sequence of 3 slice-selective radio frequency pulses was employed. This technique resembles one-dimensional point-resolved spectroscopy sequence, but a 90° pulse replaces the final 180° pulse for volume localization and coherence transfer. Two-dimensional spectral coding was inserted between the second and third pulses. Spectral recording involved the following parameters: TE at 30 msec; TR at 2,000 msec; total number of scans at 800 and including 8 averages for each T1 increment of 1.6 msec; and $3 \times 3 \times 3$-cm^3 voxel size. Total duration was approximately 26 minutes.

Prior knowledge of the following metabolites was included in the ProFit algorithm (33) for quantification: creatine, NAA, glycerylphosphorylcholine, phosphorylcholine, free choline (Ch), alanine, Asp, γ-aminobutyric acid (GABA), glucose, glutamine (Gln), Glu, glycine, glutathione, lactate, myoinositol (mI), NAAG, phosphoethanolamine, taurine, scyllo-inositol, and ascorbate. The 2D L-COSY spectra from the 1.5-T GE data were then processed with a modified UCLA version of ProFit code (26) because ProFit was originally developed for processing Philips data (Fig. 1). The one-dimensional spectra from the same

participant are provided as a reference (Fig. 2). Measurement accuracy was characterized with use of Cramér-Rao lower bound. Further details regarding the methodology for quantification of the L-COSY spectrum can be found in our prior publications (26, 33).

Demographic characteristics were examined with a χ^2 test. Spectroscopic differences between healthy controls, patients with bipolar depression at baseline, and patients with bipolar depression after 12 weeks of lamotrigine treatment were examined with analysis of variance models and the posthoc least significant difference pairwise multiple comparison test. Alpha was set at 0.05 for all statistical tests. Analyses were computed with SPSS Statistics 21.0 (IBM). Remission at posttreatment scan was defined with a Montgomery–Åsberg Depression Rating Scale score of < 8. Spectroscopic metabolite findings among patients with remission and patients without remission were then examined with analysis of variance.

Results

Patients (n = 15) and controls (n = 9) had no significant differences in sex ($\chi^2 = 0.49$, p = 0.48) or age ($\chi^2 = 17.12$, p = 0.31). Patients with bipolar depression had a mean standard deviation (SD) Montgomery–Åsberg Depression Rating Scale score of 26.27 (5.64) and an Inventory of Depressive Symptomatology mean (SD) score of 31.00 (7.38). Among the three groups, there were significant differences in mean NAA [$F(2,31) = 5.15$, p = 0.01] and mean tNAA [$F(2,31) = 3.49$, p = 0.04] levels (Table 1 and Fig. 3). Patients with bipolar depression had significantly lower levels of NAA (p = 0.02) and tNAA (p = 0.06) than healthy controls. After 12 weeks of lamotrigine treatment, patients with bipolar depression had significant increases in NAA (p = 0.01) and tNAA (p = 0.02) compared with patients at baseline after 12 weeks of lamotrigine treatment. No significant differences in glutamate and glutamine (Glx) and Glu levels were found among the healthy controls and participants with bipolar depression at baseline. At posttreatment, Glx and Glu levels were elevated in participants with bipolar depression (Table 1).

There were significant differences in the mean tNAA to Glx ratio among these groups [$F(2,31) = 4.25$, p = 0.02]. Patients with bipolar depression at baseline had a lower tNAA/Glx than healthy controls [mean (SD) = 1.11 (0.10) versus 1.44 (0.33); p = 0.01]. There were no significant differences in NAA to Glu and tNAA to Glu ratios among the three groups. Spearman rho correlation coefficients between changes in tNAA and Glx, in NAA and Glu, and in tNAA and Glu did not show any statistically significant correlation. Of the 10 patients with bipolar depression who had a second MRS scan, five achieved remission with lamotrigine treatment. No statistically significant differences were found among patients whose bipolar depression remitted and those who did not have remission in baseline or post-treatment neurometabolites.

Discussion

The present study is the first, to our knowledge, to show an NAA deficit in bipolar depression that normalized after treatment with lamotrigine. In this relatively small sample, there was no relation between clinical remission with lamotrigine and NAA levels because

NAA increased regardless. Hence, this brain change is unrelated to mood changes. Although this is an exploratory study, its strengths include a hypothesis-driven analysis of drug mechanism of action (i.e., increased intraneuronal Asp and acetyl coenzyme A levels). The baseline deficit in NAA corrected with lamotrigine treatment is consistent with preclinical and animal models of lamotrigine-associated neuroprotection (9, 12, 34) and NAA deficit normalization with lithium (20, 35, 36). For example, the NAA deficit, as a state marker, contributes to excessive glutamatergic tone (37, 38) and thereby leads to cell death and resultant decreases in NAA. The normalization of an NAA deficit could be mediated through activating Asp *N*-acetyltransferase, which promotes NAA synthesis. Also, lamotrigine may inhibit aspartoacylase, a catalytic enzyme in oligodendrocytes, or NAAG synthase in astrocytes. This increase in NAA levels may be associated with a reduction in Glu levels (39, 40).

In prior work examining NAA as a biomarker, decrements were generally thought to be related to neuronal loss. This may be an overly simplistic approach because other pathophysiologic changes could deplete NAA in a manner that is responsive to pharmacologic treatments (41). For example, postmortem brain studies consistently show reduced glial cell density in bipolar disorder (42–44). Rodent models of depression also show that compromised glial cell function induces depressive-like behaviors (45). Although NAA is synthesized in neurons and sensitive to mitochondrial integrity where it is synthesized, it is taken up and metabolized in glial cells (14). Therefore, reduced NAA levels in bipolar depression could relate in part to dysregulations in glial cell functioning or integrity, and this could be amenable to pharmacologic treatments. However, making definitive conclusions is difficult regarding the temporal relation of NAA reductions with compromised glial cell function based on a cross-sectional study.

Previously, investigators have recognized that dynamic change in NAA may occur in relation to other therapeutic drug interventions (46, 47). In a landmark study by Moore et al. (20), 12 adult patients with bipolar disorder showed an increase in NAA after a four-week course of lithium. Other studies (35, 36), but not all studies (48), have reported a lithium-associated increase in NAA. In at least one study, the NAA increase directly correlated with brain lithium levels (49). The lithium-associated increase in NAA appears to be primarily in studies where an initial deficit was reported (40, 50). However, this NAA normalization or deficit amelioration has not been observed with divalproex (49, 51). Early work on the Glu reuptake inhibitor riluzole in bipolar depression reported a drug-associated increase in NAA, which also had a positive association with symptomatic improvement (47).

The present study's smaller sample size and different MRS methodology did not replicate previous findings of Glx and Glu increases (32, 37, 52). However, at baseline, patients with bipolar depression had lower tNAA to Glx ratios than healthy controls. Prior work shows correlations between NAA and Glu changes (19), indicates that the metabolism of these neurochemicals are coupled (16–18), and contends that comparing their ratios may have merit in quantifying disease burden in psychiatric disorders (13, 19). This result suggests that patients with bipolar depression have a lower NAA level or a higher Glx/Glu level, or both. The present data suggest that further study of NAA and glutamatergic ratios in bipolar

depression is warranted because it may provide a sensitive measure to monitor pharmacologic treatments.

Limitations of the present study include its small sample size. This study was able to detect only a brain effect and likely was underpowered to detect any relation with NAA change and clinical effects of lamotrigine therapy. This characteristic does raise questions about the clinical significance of the present findings. Larger studies are needed to examine potential neurochemical and clinical correlations. Further, the size of the voxel extends the region of interest beyond one anatomic region and included bilateral pregenual anterior cingulate, anterior midcingulate cortex, and medial prefrontal cortex (superior frontal gyrus). In addition, cerebrospinal fluid corrections were performed, but tissue segmentation of spectroscopic data was not performed. Fortunately, the midline anterior cingulate placement of the MRS voxel does ensure a low contribution of white matter (10%). Finally, prior knowledge-based peak fitting programs, such as ProFit and LCModel (LCMODEL, Inc.), do not fit NAAG, Gln, and other weaker metabolites with acceptable Cramér-Rao lower bounds. Hence, a common practice is to report both NAA and tNAA. Even though spectral dispersion is better and metabolite peaks can be resolved less ambiguously with 2D L-COSY than with one-dimensional point-resolved spectroscopy (PRESS) at 1.5 T, peak separation between NAA and NAAG is not sufficient to resolve NAAG. A 2D MRS resolves sensitive metabolites such as NAA, Glu, Ch, and mI more effectively than weaker metabolites (such as NAAG and Gln). More metabolites can be better resolved at 3 T and, more recently with our group's work, at 7 T (53).

Limitations of the current findings need to be considered in the context of understanding the molecules that contribute to resonance peaks of an MRS spectrum at a given field strength, typical concentrations, correlation times in the brain, and metabolic inter-conversions. For example, the NAA resonance peak may have contributions from *N*-acetyl sugars, which are at a high level in the gangliosides found in synaptic membranes, and uridine diphosphate-*N*-acetyl sugars, in the synthetic pathways of gangliosides. Even with L-COSY, Glu, Gln, and GABA cannot be quantified reliably at 1.5 T. The majority of the Glu resonance is from the metabolic pool rather than the neurotransmitter pool. Within the neurotransmitter pool, it is also not possible to differentiate neuronal, synaptic, and glial Glu metabolites. Findings with NAA/Glx ratios must be viewed with caution. These were included as exploratory measures, with the prospect of selecting a more sensitive measure in the future (54).

In conclusion, after 12 weeks of lamotrigine treatment, NAA and tNAA levels normalized in patients with bipolar depression. This lamotrigine-associated brain change (i.e., increase in neuronal viability) may be related to increasing intraneuronal storage of Asp and, subsequently, NAA. Future research is encouraged to evaluate whether baseline NAA could be a potential biomarker for identifying lamotrigine response patterns and whether this functional brain change has an associated clinical response.

Acknowledgements

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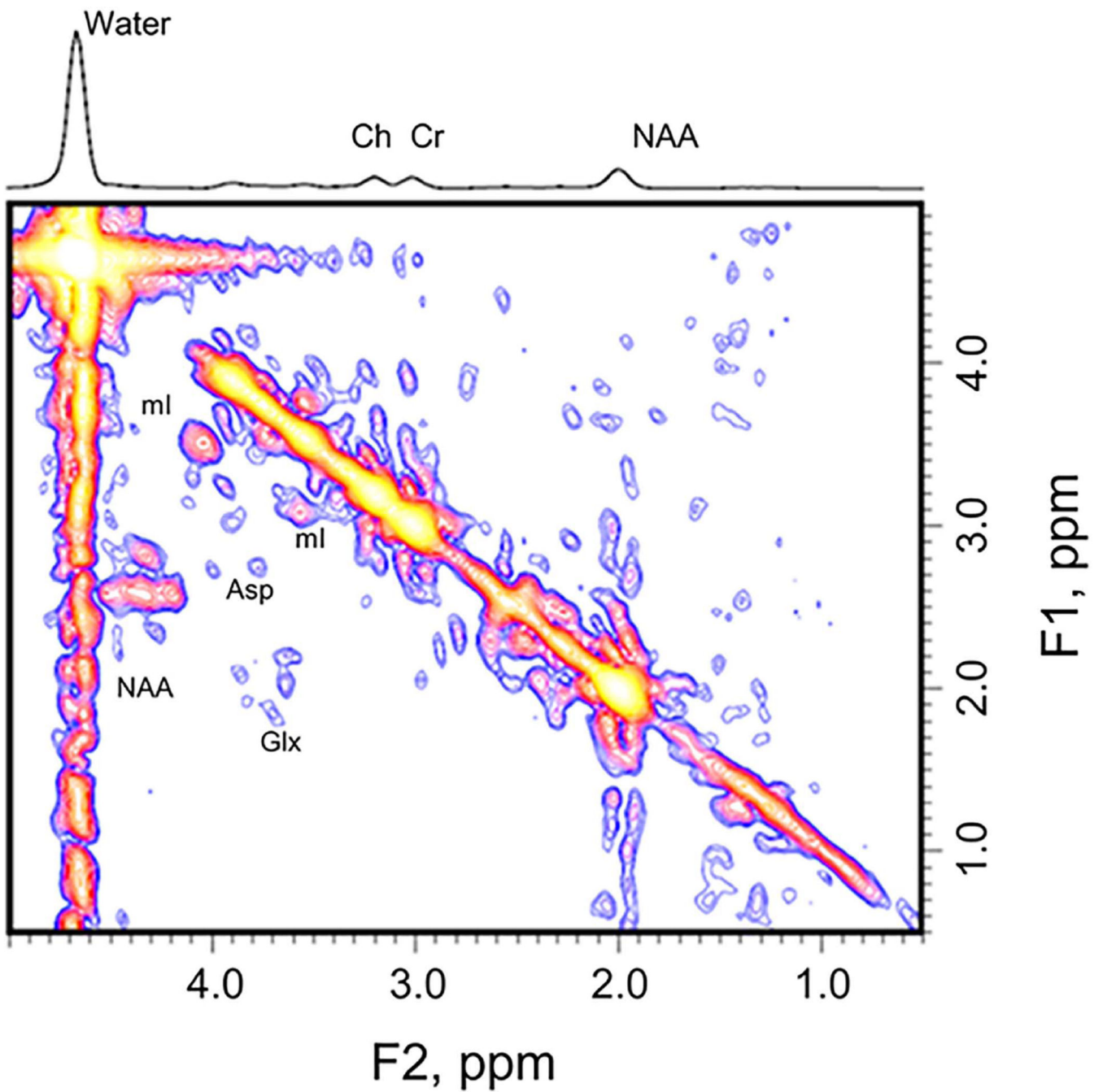
Fig. 1.
A two-dimensional localized correlated spectroscopy spectrum of a patient with bipolar depression. Asp = aspartate; Ch = free choline; Cr = creatine; Glx = the combined signal from glutamate and glutamine; mI = myoinositol; NAA = *N*-acetylaspartate.
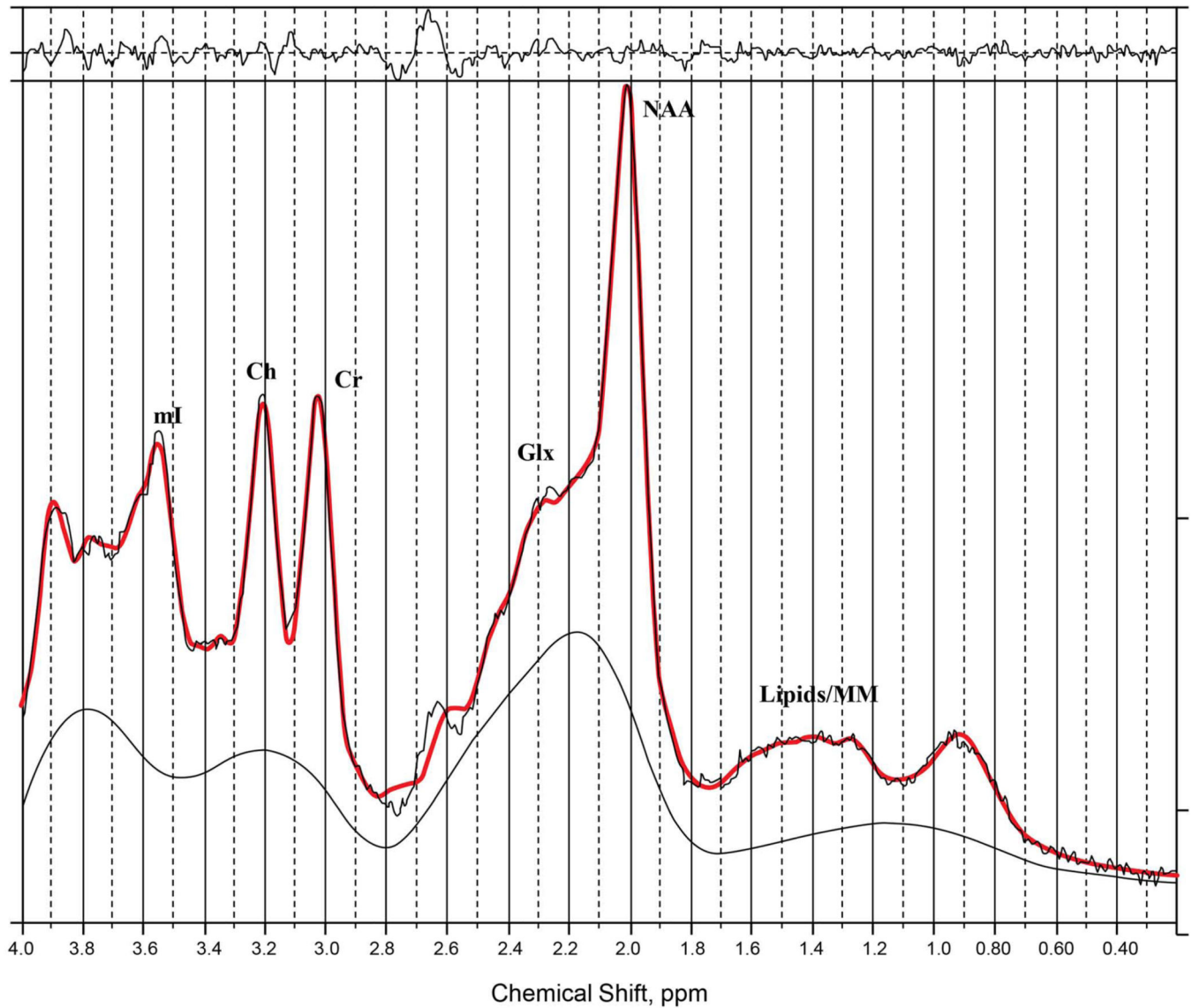
Fig. 2.
A one-dimensional localized correlated spectroscopy spectrum from the same patient with bipolar depression. Image supplied for reference. Ch = free choline; Cr = creatine; Glx = the combined signal from glutamate and glutamine; mI = myoinositol; NAA = *N*-acetylaspartate.
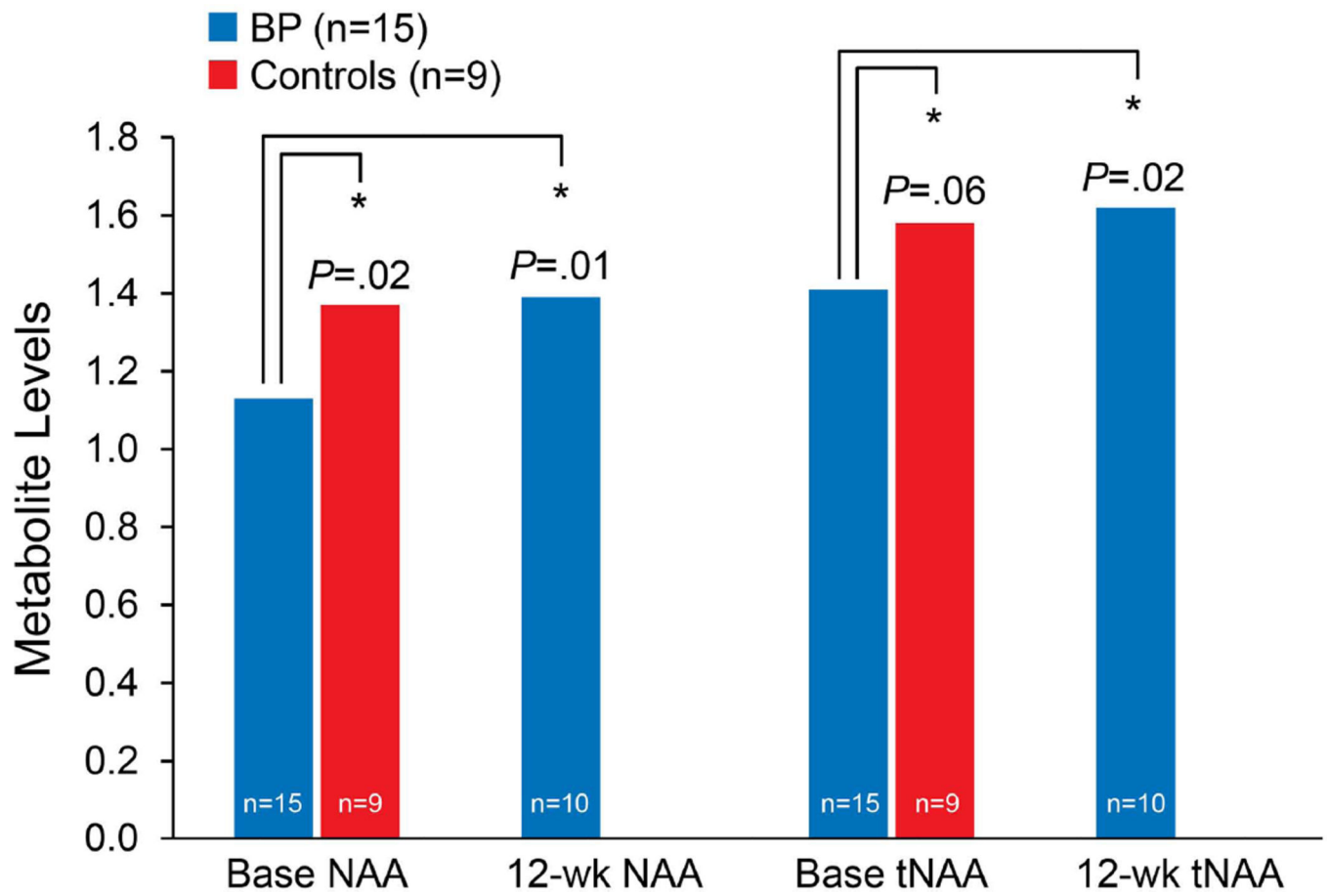
Fig. 3.
N-acetylaspartate (NAA) and total *N*-NAA (tNAA) levels at baseline and after 12 weeks of lamotrigine treatment. Base = baseline; BP = bipolar depression.

Table 1

Baseline and post-treatment neurometabolites

Neurometabolite[a]	Controls (n = 9)	BP Dep Baseline (n = 15)	Controls versus BP Dep Baseline LSD p-value (Cohen's d)[b]	BP Dep Post-treatment (n = 10)	Controls versus BP Dep Post-treatment LSD p-value (Cohen's d)[b]	BP Dep Baseline versus BP Dep Post-treatment LSD p-value (Cohen's d)[b]
NAA	1.37 (0.27)	1.13 (0.21)	0.02 (0.99)	1.39 (0.21)	0.84	0.01 (1.24)
tNAA	1.58 (0.26)	1.41 (0.14)	0.06	1.61 (0.25)	0.70	0.02 (0.99)
Glx	1.13 (0.20)	1.27 (0.12)	0.59	1.83 (1.18)	0.03 (−0.83)	0.04 (−0.67)
Glu	1.04 (0.16)	1.13 (0.11)	0.69	1.62 (1.01)	0.03 (−0.80)	0.04 (−0.68)
tNAA/Glx	1.44 (0.33)	1.11 (0.10)	0.01 (1.35)	1.10 (0.42)	0.02 (−0.90)	0.98
tNAA/Glu	1.55 (0.34)	1.26 (0.16)	0.04 (1.09)	1.24 (0.48)	0.05	0.92
NAA/Glu	1.37 (0.44)	1.00 (0.16)	0.03 (1.12)	1.10 (0.52)	0.14	0.50

BP Dep = bipolar depression; Glu = glutamate; Glx = glutamine and glutamate; LSD = least significant difference; NAA = *N*-acetylaspartate; tNAA = total NAA.

[a] Values are presented as mean (standard deviation) unless specified otherwise.

[b] Cohen's *d* provided for statistically significant findings.