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doi:10.1017/S0033291706008968 Printed in the United Kingdom

# Hippocampal $^{1}$H MRS in patients with bipolar disorder taking valproate *versus* valproate plus quetiapine

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## ABSTRACT

**Background.** No study to date has examined the effects of mood stabilizer alone and the combination of mood stabilizer and atypical antipsychotic, quetiapine, on hippocampal neurochemical markers of bipolar disordered patients concurrently. We therefore undertook a proton magnetic resonance spectroscopy ($^{1}$H MRS) study of drug-free patients with bipolar disorder (drug-free group), patients undergoing valproate treatment (valproate group), patients administered valproate + quetiapine (valprote + quetiapine group) and healthy controls, focusing on the *in vivo* neuroanatomy of the hippocampus.

**Method.** Thirty patients from the Firat University School of Medicine Department of Psychiatry and 10 healthy controls gave written informed consent to participate in the study. The patients and controls underwent proton magnetic resonance spectroscopic imaging ($^{1}$H MRSI), and measures of *N*-acetylaspartate (NAA), choline-containing compounds (CHO), and creatine + phosphocreatine (CRE) in hippocampal regions were obtained.

**Results.** The drug-free patients had significantly lower NAA/CRE and NAA/CHO ratios compared with the valproate and valproate + quetiapine groups and the healthy controls. The lower NAA/CRE and NAA/CHO ratios remained statistically significant even after covarying for age or whole brain volume compared with the valproate and valproate + quetiapine groups and healthy controls. In *post hoc* comparisons, a significant difference was found between the valproate + quetiapine group and the valproate group only with regard to NAA/CHO.

**Conclusion.** Our findings suggest that valproate has a neuroprotective effect. In *post hoc* comparisons, a significant difference was found between the valproate + quetiapine and the valproate group with regard to NAA/CHO, indicating that the addition of quetiapine further increases the level of NAA and provides an additional neuroprotective effect.

## INTRODUCTION

Numerous investigations have performed structural and functional neuroimaging studies of mood disorders. The published studies of functional neuroimaging in bipolar disorder have revealed alterations in glucose metabolism, regional cerebral blood flow or high-energy phosphate metabolism in patients with bipolar disorder in the prefrontal and temporal cortex, basal ganglia and amygdala (Drevets *et al*. 1997; Blumberg *et al*. 1999). Functional neuroimaging studies lend further evidence to physiological abnormalities in cortical and subcortical fronto-limbic regions in bipolar disorder. Neuroimaging studies that examine neurochemistry in the living brain provide further support for the hypothesis that bipolar disorder is related to

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changes in neuronal viability and function. Proton magnetic resonance spectroscopy ($^1$H MRS), a recent development in MR technology, allows biochemical constituents to be assayed directly *in vivo*, such as choline-containing compounds (CHO), an index of membrane metabolism, creatine + phosphocreatine (CRE), involved in cell energetic metabolism, and *N*-acetyl-containing compounds (especially *N*-acetylaspartate, NAA). While CHO and CRE are present in neurons and in glial cells, NAA is found primarily in neurons (Urenjak *et al.* 1993) and in highest concentrations in pyramidal glutamatergic neurons (Moffett & Namboodiri, 1995). NAA was initially thought to represent a marker of neuronal structural integrity, but a number of more recent studies have demonstrated that NAA reductions are reversible, suggesting that NAA is sensitive to processes affecting the functioning of neurons (Richards, 1991; Cendes *et al.* 1997). NAA is also sensitive to mitochondrial oxidative phosphorylation and may correlate highly with tissue glutamate levels (Jenkins *et al.* 2000; Petroff *et al.* 2002). Low NAA is thought to represent loss of neurons and/or axons, reduction of interneuronal neuropil, and neuronal or axonal metabolic dysfunction or damage (Baxter *et al.* 1989; Drevets, 1999). $^1$H MRS studies concerning bipolar disorder focused on dorsolateral prefrontal cortex (DLPFC) and hippocampal regions. One $^1$H MRS study showed significant reductions in NAA peaks in the DLPFC of adult bipolar disorder subjects (Winsberg *et al.* 2000), whereas two other MRS studies (Hamakawa *et al.* 1999; Bertolino *et al.* 2003) did not find any differences in DLPFC or frontal lobes. Chang *et al.* (2003) reported reduced NAA levels in DLPFC in a sample of pediatric bipolar patients who had a parent with bipolar disorder. There is also extensive literature from functional imaging and postmortem studies in support of DLPFC dysfunction in bipolar disorder (Soares and Mann, 1997; Rajkowska *et al.* 2001). Studies using high-resolution MRS reveal that unmedicated patients with bipolar disorder have decreased levels bilaterally of NAA in the hippocampus (Bertolino *et al.* 2003), as compared with healthy control subjects. Therapeutic doses of lithium can reverse these decreased levels of NAA in the brain (Moore *et al.* 2000). Deicken *et al.* (2003) found low NAA bilaterally in the absence of smaller hippocampal volume as measured by magnetic resonance imaging (MRI), supporting the idea that NAA might be a more sensitive marker of neuronal damage or loss than quantitative MRI measurements of tissue loss.

Quetiapine is an atypical antipsychotic with established efficacy in the treatment of schizophrenia. It also shows efficacy in the treatment of acute mania and depression associated with bipolar disorder (Dando & Keating, 2005). Quetiapine, either as monotherapy or in combination with lithium or divalproex sodium, is generally well tolerated and effective in reducing manic symptoms in patients with acute bipolar mania and is approved for use in adults for this indication (Dando & Keating, 2005; Gao & Calabrese, 2005; McIntyre *et al.* 2005). As monotherapy, the drug is also effective in reducing depressive symptoms in patients with bipolar depression (Dando & Keating, 2005). It is associated with a low incidence of extrapyramidal symptom (EPS)-related adverse events and low EPS ratings in bipolar disorder. Quetiapine thus shows potential in the treatment of bipolar depression and represents a useful agent for the treatment of acute bipolar mania (Dando & Keating, 2005; Gao & Calabrese, 2005; McIntyre *et al.* 2005).

To the best of our knowledge, no study to date has examined the effects of mood stabilizer alone and the combination of mood stabilizer and an atypical antipsychotic, quetiapine, on hippocampal neurochemical markers of bipolar disordered patients. We have therefore performed a $^1$H MRS study in drug-free patients with bipolar disorder, those with bipolar disorder undergoing valproate treatment, those with valproate + quetiapine, and healthy controls, focusing on the *in vivo* neuroanatomy of the hippocampus.

## METHOD

### Subjects

Thirty patients (18 men and 12 women; mean age 29·8 years, S.D. 7·4) with bipolar disorder were selected from the Firat University School of Medicine Department of Psychiatry. No external funding source was used in this study. Of the patients, 10 were first applied patients who had never taken any drug for their condition AQ1

(drug-free group), 10 were undergoing valproate treatment (valproate group), and 10 were on valproate + quetiapine (valprote + quetiapine group). The valproate group subjects were selected from among bipolar disordered patients who had been treated with valproate alone ($\geqslant 75\,\mu$g/ml) for at least 6 months and demonstrated 50% or more reduction on the Young Mania Rating Scale (YMRS; Young *et al.* 1978) according to baseline. The severity of manic symptoms was assessed with the YMRS and that of depressive symptoms was evaluated by the Hamilton Depression Rating Scale (HDRS; Hamilton, 1960). Valprote + quetiapine group subjects had been on monotherapy with valproate ($\geqslant 75\,\mu$g/ml) for a minimum of 3 months prior to quetiapine initiation and demonstrated less than 30% reduction on the YMRS relative to baseline and were no better than 'minimally improved' on the Clinical Global Impression improvement item. A group of healthy controls (six men and four women; mean age 27·0 years, S.D. 7·9 years) were matched for age, sex, education and handedness. All patients fulfilled DSM-IV criteria for bipolar I disorder, based on a clinical interview conducted by the research psychiatrist and on the Structured Clinical Interview for DSM-IV (SCID). All subjects were right-handed. All subjects meeting the criteria were given details of the study and asked to sign a written informed consent form if they agreed to participate. The study was approved by the local ethics committee. The exclusion criteria included the presence of any co-morbid psychiatric disorder, current medical problems, or alcohol/substance abuse within the 6 months preceding the study. The healthy control subjects had no DSM-IV axis I disorders in self or in a first-degree relative, as determined by the SCID non-patient version, no current medical problems, neurological or psychiatric histories, and no use of psychoactive medication within 2 weeks of the study.

## Procedure

Multiple-slice proton magnetic resonance spectroscopic imaging ($^1$H MRSI) was performed on a conventional GE Signa Excite 1.5-Tesla MR imaging system (GE Medical Systems Milwaukee, WI, USA) equipped with self-shielded gradients, using the method of Duyn *et al.* (1993) as modified by our group; the procedure used in the present study was identical to that used in the report by Bertolino *et al.* (2003). The standard quadrature head coil was used in all cases. A set of T2-weighted, 5-mm-thick, oblique coronal images (FRFSE-XL, TR = 5000 ms, TE = 85 ms) was acquired in a plane perpendicular to the sylvian fissure, after a three-plane localizer. Phase encoding procedures were used to obtain a $32 \times 32$ array of spectra from volume elements in each selected slice. Each volume element (voxel) had nominal dimensions of $7{\cdot}5\text{ mm} \times 7{\cdot}5\text{ mm} \times 15\text{ mm}$ (0·84 ml). The actual volume was 1·4 mL, based on full width at half maximum (FWHM). The $^1$H MRSI sequence involves a Probe-P slice selection with TR of 1000 ms and TE of 144 ms for the acquisition. The pulse sequence also includes suppression of water and most of the signals arising from lipids in skull marrow and in surface tissues. To suppress lipid signals from the skull and scalp, the $^1$H MRSI sequence included an outer-volume saturation pulse. A rater blind to diagnosis manually drew the hippocampal region on the T1-weighted coaxial MR images. This was later transferred to the same location on the metabolite maps. The hippocampal region was drawn with reference to standard anatomic atlases (Duvernoy & Cabanis, 1991; Bertolino *et al.* 1996; Talairach & Tournoux, 1998). Voxels that were contained within the anatomic hippocampus but not observed in the metabolite maps were excluded to obtain exact calculations only on those containing $^1$H spectra.

After obtaining the second set of T1-weighted images, a three-dimensional MRI data set was acquired as 124 conventional sagittal slices [spoiled gradient echo pulse sequence (SPGR), TR = 24 ms, TE = 5 ms]. The locations of NAA, CHO and CRE peaks were determined automatically for all voxels. Cosine filters were used for apodization of the free induction decay to minimize bleed before Fourier transformation. Metabolite signals are reported as ratios of the area under each peak: NAA/CRE, NAA/CHO and CHO/CRE. The position of the hippocampal voxels and a sample MR spectrum are presented in Fig. 1.

## Statistical analysis

Analysis of covariance (ANCOVA), analysis of variance (ANOVA), $\chi^2$ and partial correlation

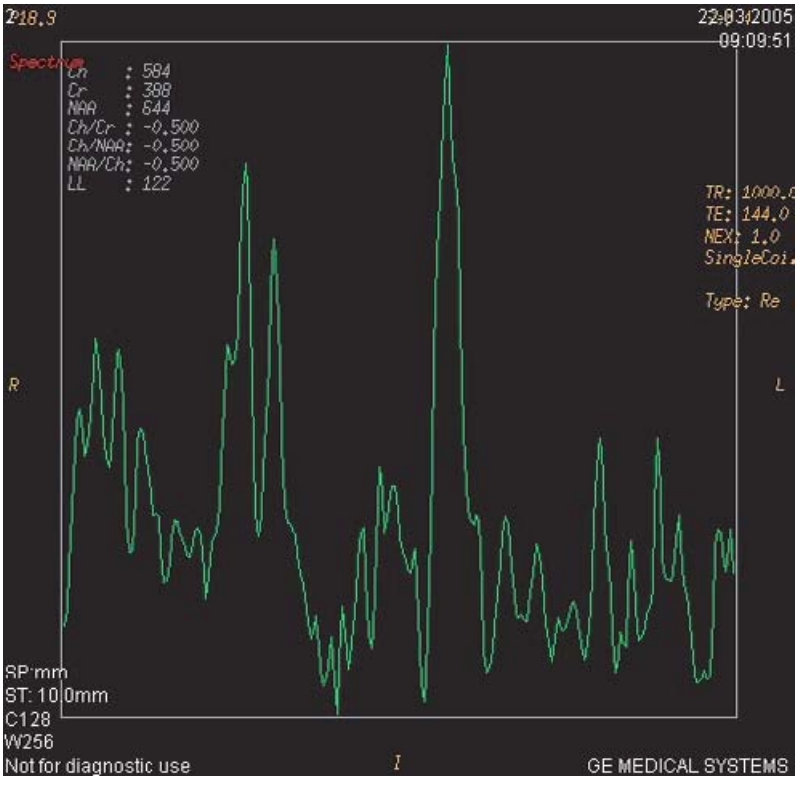


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FIG. 1. Position of hippocampal voxels and sample magnetic resonance spectrum.

analyses were conducted using SPSS for Windows, version 10.0 (SPSS Inc., Chicago, IL, USA).

## RESULTS

The drug-free group, the valproate group, the valproate + quetiapine group and the healthy control subjects did not differ with regard to age ($p > 0{\cdot}05$), gender ($p > 0{\cdot}05$), intracranial volume (ICV) ($1427{\cdot}2 \pm 141{\cdot}3$ cm$^3$ for the drug-free group, $1481{\cdot}6 \pm 157{\cdot}3$ cm$^3$ for the valproate group, $1455{\cdot}9 \pm 149{\cdot}7$ cm$^3$ for the valproate + quetiapine group, and $1439{\cdot}2 \pm 154{\cdot}4$ cm$^3$ for healthy controls; $p > 0{\cdot}05$), or handedness (all subjects were right-handed) ($p > 0{\cdot}05$). Clinical and sociodemographic characteristics of the healthy controls and patients with bipolar disorder are presented in Table 1. All inter- and intra-rater reliability scores were $\geqslant 0{\cdot}80$, demonstrating sufficient inter- and intra-reliability.

The unadjusted mean volumes of structures measured for all subjects are presented in Table 2. Whole brain volume, gray and white matter, and both sides of the hippocampus volumes did not differ among groups ($p > 0{\cdot}05$). The differences among groups with regard to whole brain volume, gray and white matter, and hippocampus volumes still remained statistically insignificant after covarying for age. Drug-free patients had significantly lower NAA/CRE ($F = 7{\cdot}41$, $p < 0{\cdot}01$) and NAA/CHO ($F = 4{\cdot}34$, $p < 0{\cdot}05$) ratios compared with valproate, valproate + quetiapine groups, or healthy controls. Lower NAA/CHO ($F = 6{\cdot}96$, $p < 0{\cdot}01$ age as covariate and $F = 7{\cdot}70$, $p < 0{\cdot}01$ whole brain volume as covariate) and NAA/CRE ($F = 3{\cdot}67$, $p < 0{\cdot}05$ age as covariate and $F = 3{\cdot}12$, $p < 0{\cdot}05$ whole brain volume as covariate) remained statistically significant even after covarying for age or whole brain volume compared with the valproate, valproate + quetiapine groups, or healthy controls. However, there was no

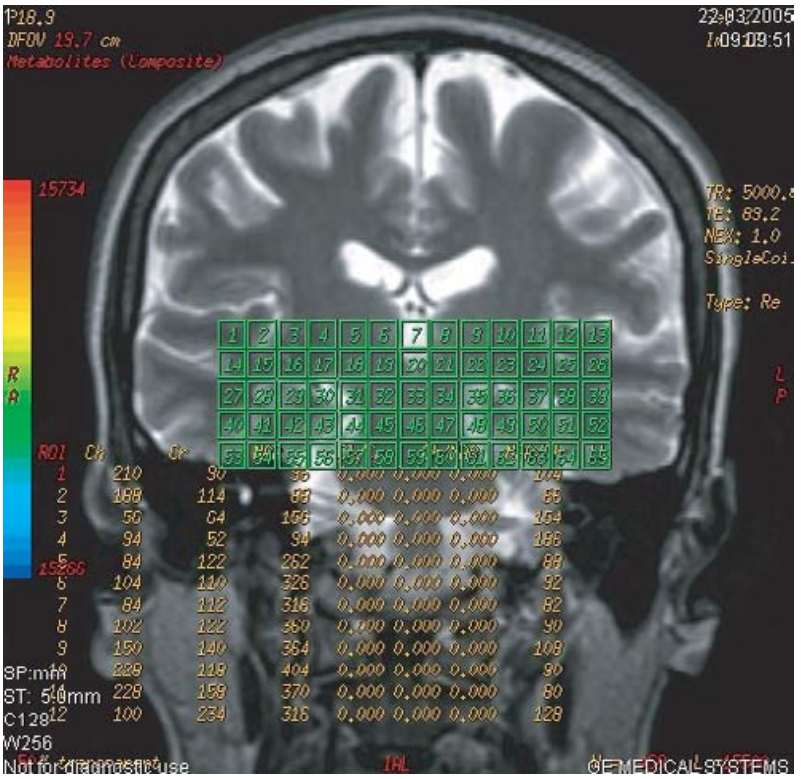


FIG. 1. (*cont.*)

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difference with regard to CHO/CRE among groups ($F=0{\cdot}35$, $p>0{\cdot}05$). In *post hoc* comparisons, a significant difference was found between the valproate + quetiapine group and the valproate group with regard to only NAA/CHO ($F=3{\cdot}79$, $p<0{\cdot}05$), but not between the valproate group and the healthy controls, nor between the valproate + quetiapine group and the healthy controls for NAA/CRE and CHO/CRE. No main effect of hemisphere or interaction of diagnosis by hemisphere was found for any metabolite ratio in any group. Further analyses with ANOVA on metabolite ratios in the hippocampus to check for gender effects did not reveal any significant effects ($p>0{\cdot}05$), suggesting that reductions in NAA ratios are not related to gender.

No significant correlation was found between hippocampus volume and ratio measures ($r=$ –0·12 and 0·18 for left and right hippocampus respectively in the total patient group, and $r=$ 0·09 and −0·16 respectively in healthy controls; $p>0{\cdot}05$). Correlation analyses revealed a significant negative correlation between NAA/CHO ratios and the YMRS for both sides of the hippocampus in the drug-free group ($r=$–0·50, $p<0{\cdot}05$ for left sides and $r=$–0·46, $p<0{\cdot}05$ for right sides).

## DISCUSSION

Neurochemical metabolite ratios in drug-free patients, in bipolar patients undergoing valproate treatment and in those on valproate + quetiapine have not been previously evaluated concurrently. As psychopharmacological interventions and psychotherapeutic approaches can affect the neurochemical metabolite ratios and volumetric measurements of the brain regions, we considered it important to evaluate how the mood stabilizer valproate and the combination of valproate + quetiapine affect the neurochemical metabolite ratios and brain volumes of interest in bipolar disordered patients. We

Table 1. *Clinical and demographic characteristics of normal control subjects and patients with bipolar disorder*

| | First-applying ($n=10$) | Valproate ($n=10$) | Valproate + quetiapine ($n=10$) | Controls ($n=10$) |
|---|---|---|---|---|
| Age (years) | 23·4 ± 5·6 | 25·8 ± 6·4 | 24·9 ± 5·1 | 24·3 ± 4·3 |
| Gender (F/M) | 6/4 | 5/5 | 6/4 | 5/5 |
| Age at onset (years) | 23·0 ± 5·2 | 22·3 ± 4·4 | 22·1 ± 4·0 | — |
| Education (high school) | 7 | 6 | 6 | 8 |
| Handedness (right) | 10 | 10 | 10 | 10 |
| Duration of iIlness (years) | — | 3·5 ± 2·2 | 2·8 ± 1·4 | — |
| Number of patients who were manic | 10 | 2 | 2 | — |
| Number of patients who were depressive | — | 3 | 2 | — |
| Number of patients who were mixed episode | — | 1 | 2 | — |
| Number of patients who were euthymic | — | 4 | 4 | — |
| YMRS score, patients with current manic or mixed episode | 17·9 ± 5·3 | 16·0 ± 2·1 | 15·5 ± 1·8 | — |
| HDRS score, patients with current depressive or mixed episode | — | 18·8 ± 0·9 | 17·8 ± 1·1 | — |
| Duration of treatment with valproate (months) | — | 8·3 ± 4·4 | 5·8 ± 3·1 | — |
| Duration of treatment with quetiapine (months) | — | — | 4·9 ± 2·8 | — |
| Dose of valproate (mg/day) | — | 1225·0 ± 226·8 | 950·0 ± 120·5 | — |
| Dose of quetiapine (mg/day) | — | — | 705·0 ± 95·2 | — |

F, Female; M, male; HDRS, Hamilton Depression Rating Scale; YMRS, Young Mania Rating Scale.
No significant differences exist between groups with regard to age, handedness and gender composition.

Table 2. *Volumetric measurements of the bipolar patients and healthy control subjects*

| | Drug-free group ($n=10$) | Valproate group ($n=10$) | Valproate + quetiapine group ($n=10$) | Healthy control group ($n=10$) | $p$ |
|---|---|---|---|---|---|
| ICV | 1427·2 ± 141·3 | 1481·6 ± 157·3 | 1455·9 ± 149·7 | 1439·2 ± 154·4 | > 0·05 |
| Whole brain volume | 1119·4 ± 111·7 | 1160·1 ± 144·5 | 1141·2 ± 133·6 | 1130·6 ± 133·2 | > 0·05 |
| Gray matter volume | 334·1 ± 30·9 | 353·8 ± 41·7 | 350·5 ± 37·8 | 335·2 ± 26·9 | > 0·05 |
| White matter volume | 785·3 ± 83·1 | 806·3 ± 94·1 | 790·7 ± 80·8 | 795·4 ± 90·3 | > 0·05 |
| Left hippocampus | 3·87 ± 0·53 | 3·88 ± 0·65 | 3·83 ± 0·55 | 3·91 ± 0·68 | > 0·05 |
| Right hippocampus | 3·73 ± 0·72 | 3·80 ± 0·60 | 3·77 ± 0·40 | 3·84 ± 0·48 | > 0·05 |
| NAA/CHO | 1·08 ± 0·12 | 1·26 ± 0·19 | 1·43 ± 0·29 | 1·34 ± 0·25 | < 0·01 |
| NAA/CRE | 1·40 ± 0·35 | 1·62 ± 0·41 | 1·67 ± 0·34 | 1·70 ± 0·42 | < 0·05 |
| CHO/CRE | 1·60 ± 0·21 | 1·59 ± 0·28 | 1·70 ± 0·31 | 1·62 ± 0·17 | > 0·05 |

ICV, Intracranial volume; NAA, *N*-acetylaspartate; CRE, creatinine; CHO, choline.

therefore performed a hippocampal $^1$H MRS study in patients with bipolar disorder who were drug free, patients who were taking valproate and those undergoing valproate + quetiapine treatment, focusing on the *in vivo* neurochemical metabolite ratios of hippocampus to further evaluate the hypothesis of neuronal functional pathology of the hippocampus in bipolar patients and their possible involvement in the pathophysiology of bipolar disorder, and to examine whether the changes in metabolite ratios would be state or trait marker of the disease.

This study revealed several important findings: (i) drug-free patients had significantly lower NAA/CHO and NAA/CRE ratios compared with valproate and valproate + quetiapine groups and healthy controls. Lower NAA/CHO and NAA/CRE remained statistically significant even after covarying for age or whole brain volume compared with valproate and valproate + quetiapine groups and healthy controls; (ii) in *post hoc* comparisons, a significant difference was found between the valproate + quetiapine group and the valproate group with regard to only NAA/CHO, but not between the valproate group and healthy controls, or the valproate + quetiapine group and healthy controls for NAA/CRE and CHO/CRE.

In patients with bipolar disorder, previous $^1$H MRS studies involving various anatomical sites have demonstrated conflicting results. Sharma *et al.* (1992) found increased NAA/CRE in the basal ganglia of bipolar patients. Stoll *et al.*

(1992) and Yurgelun-Todd *et al.* (1993) found no differences in NAA in the temporal lobes between bipolar and control subjects. Lower NAA measures in mood disorders have been reported. Thus, Renshaw *et al.* (1995), in a mixed group of patients with schizophreniform or bipolar disorder, found a reduction of NAA measures in the temporal lobes. Another $^{1}H$ MRS study showed significant reductions in NAA peaks in the DLPFC of adult bipolar disorder subjects (Winsberg *et al.* 2000), whereas other MRS studies did not find any differences in DLPFC (Bertolino *et al.* 2003) or frontal lobes (Hamakawa *et al.* 1999). Chang *et al.* (2003) reported reduced NAA levels in DLPFC in a sample of pediatric bipolar patients who had a parent with bipolar disorder. Bertolino *et al.* (2003) found lower NAA/CRE and NAA/CHO in the hippocampus of patients with bipolar disorder, as seen in our study. A reduction in NAA and CRE hippocampal concentrations has also been reported by Deicken *et al.* (2003). These results are consistent with the present data, and suggest that NAA/CHO and NAA/CRE reductions in our patients are due to lower levels of NAA.

The effects of mood stabilizers on neurochemical metabolite changes in brains of patients with bipolar disorder have been studied in a limited number of studies. In their study, Brambilla *et al.* (2005) found significantly higher NAA/CRE ratios in lithium-treated bipolar patients compared to unmedicated patients and healthy controls. It has been consistently shown that chronic treatment with lithium enhances NAA/CRE levels in the temporal lobes and basal ganglia of bipolar patients (Sharma *et al.* 1992; Silverstone *et al.* 2003). However, Moore *et al.* (2000) reported a small but significant increase in total brain NAA levels following 4 weeks of lithium treatment in bipolar patients and healthy persons. The results of increased NAA/CRE levels could be related to the neuroprotective properties of lithium (Manji *et al.* 2000). The effect of sodium valproate has been evaluated in only one study (Silverstone *et al.* 2003), which reported that bipolar patients chronically treated with lithium had a significant increase in NAA concentrations. However, Silverstone *et al.* (2003) found no significant increases in the sodium valproate-treated patients compared to controls and concluded that sodium valproate and lithium might not share a common mechanism of action in bipolar disorder involving neurotrophic or neuroprotective effects. Our findings suggest that valproate also has neuroprotective effects, in contrast to Silverstone *et al.* (2003). Furthermore, as determined by the finding that in *post hoc* comparisons, a significant difference was found in NAA/CHO between the valproate + quetiapine group and the valproate group, the addition of quetiapine further increased the level of NAA and indicated an additional neuroprotective effect. There is limited information in the literature on the neuroprotective role of quetiapine. Atypical antipsychotic drugs show neuroprotective effects that may be involved in the management of schizophrenia or mood disorders (Bai *et al.* 2002). It has been shown that atypical antipsychotic drugs can protect PC12 cells from death after serum withdrawal (Bai *et al.* 2002), from apoptosis induced by $MPP^{+}$ (Qing *et al.* 2003) and from oxidative stress induced by hydrogen peroxide (Wei *et al.* 2003). It has also been reported that chronic administration of quetiapine could be neuroprotective to hippocampal neurons in immobilization stress in rats (Xu *et al.* 2002). Our present findings support the neuroprotective effects of quetiapine. As NAA is thought to be a measure of neuronal integrity, our findings demonstrating decreased NAA/CRE suggest decreased hippocampus neuronal density or neuronal dysfunction in patients with bipolar I disorder. Because in this preliminary study no other brain regions have been investigated, it is possible that these findings are not exclusive to the hippocampal region and that other brain regions could show such changes as well.

Some particular limitations in our present findings should be considered. The number of subjects studied was small and the statistical thresholds applied were modest, thereby accentuating these risks and underscoring the importance of replication. In addition, only the hippocampus was investigated in this study; therefore our findings cannot be generalized to other brain regions that may be associated with the pathophysiology of bipolar disorder.

In summary, hippocampal neurochemical abnormalities seem to be present at the onset of bipolar I disorder, and to normalize by alleviating the disorder. Consequently, it is tempting to speculate that the decreased NAA is a trait

marker of bipolar disorder. Our findings suggest that valproate has a neuroprotective effect. Furthermore, as determined by the finding that in *post hoc* comparisons, a significant difference was found between the valproate + quetiapine group and the valproate group with regard to NAA/CHO, the addition of quetiapine further increases the level of NAA and provides an additional neuroprotective effect. Future longitudinal neuroimaging and neuropsychological studies with larger patient samples are warranted to confirm these preliminary findings and to better characterize the relevance of neurochemical abnormalities in the hippocampus in the pathophysiology of bipolar disorder.

## DECLARATION OF INTEREST

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