



## <sup>1</sup>H Magnetic resonance spectroscopy study of dorsolateral prefrontal cortex in unipolar mood disorder patients

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### Abstract

Neuroimaging and postmortem studies have suggested the involvement of the dorsolateral prefrontal cortex (DLPFC) in the pathophysiology of unipolar disorder. We examined with in vivo <sup>1</sup>H magnetic resonance spectroscopy (MRS) the levels of specific metabolites in the DLPFC of adult unipolar patients and the role of illness chronicity on DLPFC abnormalities. Nineteen unmedicated unipolar mood disorder patients and 19 age- and gender-matched healthy controls underwent a short echo-time <sup>1</sup>H MRS examination localized to an 8-cm<sup>3</sup> single voxel placed in the left DLPFC. There were no significant differences in metabolite levels, including *N*-acetylaspartate (NAA), phosphocreatine plus creatine (PCr+Cr) and choline-containing-compounds (GPC+PC), between the two groups. However, NAA/PCr+Cr ratios were significantly lower in the chronic than in the less chronically ill patients and healthy controls. The low levels of NAA/PCr+Cr ratios in the left DLPFC of unipolar patients who had been more chronically ill suggest a potential role for illness chronicity in neuronal abnormalities in the DLPFC in unipolar disorder. This could possibly be accounted for by neurodegenerative processes arising with the

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progression of the illness. Future  $^1\text{H}$  MRS investigations should longitudinally examine the role of illness chronicity on DLPFC abnormalities and their relationship with the symptoms of unipolar disorder.

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## 1. Introduction

In vivo proton magnetic resonance spectroscopy ( $^1\text{H}$  MRS) is a non-invasive approach capable of measuring levels of important metabolites in specific brain regions that are involved in key physiological brain processes and possibly implicated in the pathophysiology of unipolar disorder (Stanley et al., 2000; Stanley, 2002). They include *N*-acetylaspartate (NAA), a measure reflecting viability of neurons; phosphocreatine plus creatine (PCr+Cr), which are high-energy phosphate metabolites; glycerophosphocholine plus phosphorylcholine (GPC+PC, or choline-containing-compounds) where PC is a precursor of membrane phospholipids and GPC is a breakdown product of membrane phospholipids; and myo-inositol (INO), a second messenger metabolite (Brambilla et al., 2002; Stanley et al., 2000; Tsai and Coyle, 1995).

The dorsolateral prefrontal cortex (DLPFC, Brodmann's areas 9 and 46) is an important area of the prefrontal cortex receiving projections from higher order association regions (Nauta, 1971) and playing a key role in cognitive and executive brain functions (Quintana and Fuster, 1999). In recent years, abnormally decreased blood flow, metabolism, and volumes in the DLPFC in unipolar disorder have been reported in controlled structural (Coffey et al., 1993; Kumar et al., 1997) and functional brain-imaging studies (Dolan et al., 1993; Ebert et al., 1993; Galynker et al., 1998), which could worsen with a longer length of illness (Axelson et al., 1993; Beats et al., 1991; Lacerda et al., 2002). Additionally, post-mortem studies reported reductions in neuronal and glial density in the DLPFC of subjects with major depression (Rajkowska et al., 1999). Furthermore, DLPFC stimulation with transcranial magnetic stimulation (TMS) appeared to be effective in treating unipolar patients with refractory depression (George

et al., 2000; Pascual-Leone et al., 1996). Therefore, the DLPFC, especially on the left side, may be a critical prefrontal sub-region that could be implicated in the neuronal circuitry underlying the pathophysiology of unipolar mood disorder (Bench et al., 1995; Rajkowska et al., 1999; Soares and Mann, 1997; Soares et al., 1996).

In vivo controlled  $^1\text{H}$  spectroscopy studies have shown alterations of specific metabolites in certain anatomical regions in unipolar patients, such as increased levels of choline in the basal ganglia (Charles et al., 1994; Hamakawa et al., 1998; Vythilingam et al., 2003) and the prefrontal cortex (Farchione et al., 2002; Frey et al., 1998; Kumar et al., 2002; Steingard et al., 2000), and decreased concentrations of glutamatergic metabolites in the anterior cingulate (Auer et al., 2000; Mirza et al., 2004; Pfleiderer et al., 2003). Specifically, three  $^1\text{H}$  MRS studies investigated the left DLPFC in major depressive disorder, showing abnormally increased choline levels in treatment-naïve pediatric patients (Farchione et al., 2002), and increased choline and INO concentrations (Kumar et al., 2002) and reduced glutamatergic metabolite levels in elderly subjects (Michael et al., 2003). Therefore, it is unclear what alterations are present in the left DLPFC of adult unipolar patients. Furthermore, Michael et al. (2003) reported an inverse correlation of NAA with duration of illness in the left DLPFC in unipolar patients. Thus, the role of illness chronicity on possible DLPFC abnormalities is an intriguing issue that needs to be further explored, particularly in view of prior findings of more severe structural brain changes with the progression of the illness (Axelson et al., 1993; Greenwald et al., 1996; Roy-Byrne et al., 1998).

In the present study, we examined with  $^1\text{H}$  MRS the left DLPFC of adult unipolar patients and also explored the role of illness chronicity on DLPFC abnormalities.

## 2. Methods

### 2.1. Subjects

Nineteen unmedicated unipolar patients (mean age  $\pm$  S.D. =  $37.4 \pm 12.0$  years; 13 females, 6 males; 13 depressed, 6 euthymic) as diagnosed by the Structured Clinical Interview for DSM-IV (SCID) (Spitzer et al., 1994) were recruited. They had been off all psychotropic drugs for at least 2 weeks prior to the MR examination, and had no axis I comorbid psychiatric disorders, current medical problems, or alcohol/substance abuse within the 6 months preceding the study. The Hamilton Depression Rating Scale (HDRS) (Hamilton, 1960) was utilized to rate the severity of clinical symptoms. All available clinical information was retrieved from patients' interviews and medical charts. Length of illness was defined as the current age minus the age at onset of the first

depressive episode, defined according to DSM-IV. The SCID interviews, from which we collected most of the clinical information, including the age at onset, were completed at the Depression and Manic Depression Prevention Program (directed by Dr. Ellen Frank), by trained social workers and registered nurses who have extensive experience in doing SCID interviews. Nineteen age- and gender-matched healthy controls (mean age  $\pm$  S.D. =  $37.0 \pm 11.5$  years; 13 females, 6 males) with no DSM-IV axis I disorders, as determined by the SCID-IV, non-patient version, and without any current medical problems, current or prior history of substance abuse/dependence or any psychiatric disorders in self or in first-degree relatives were studied. All subjects signed informed consent forms after having understood all issues involved in participation in the study protocol. This research study was approved by the University of Pittsburgh biomedical IRB.

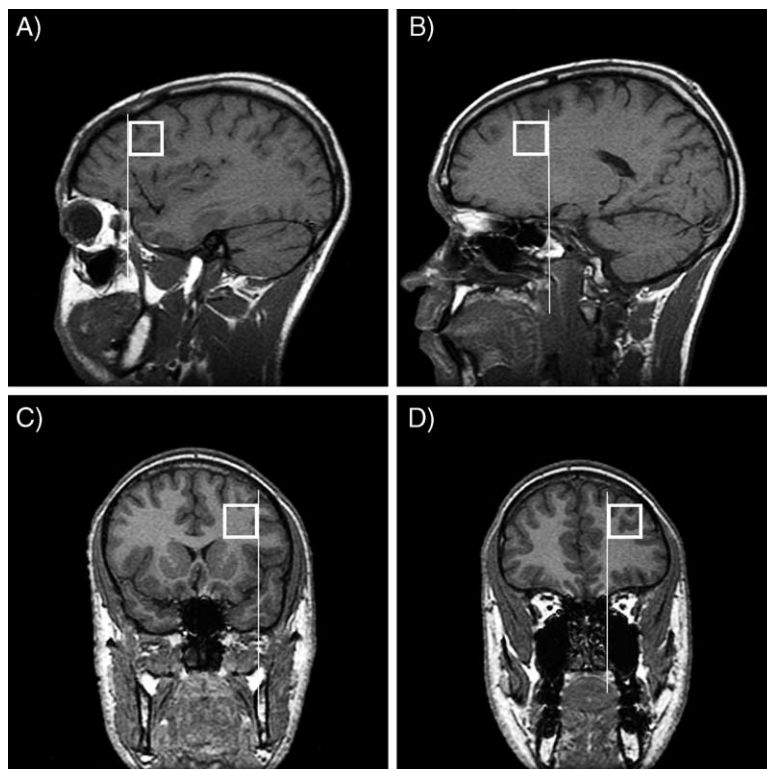


Fig. 1.  $^1\text{H}$  MRS volume of interest in left dorso-lateral prefrontal cortex. The white box represents the location of the volume of interest ( $2 \times 2 \times 2 \text{ cm}^3$ ) in the left dorsolateral prefrontal cortex (DLPFC). Sagittal (A and B) and coronal (C and D)  $^1\text{H}$  MR images are presented.

## 2.2. $^1\text{H}$ MRI/MRS procedure

In vivo  $^1\text{H}$  MRS was conducted on a 1.5 T GE Signa Imaging System (General Electric Medical Systems, Milwaukee, WI). A set of sagittal and coronal scout images was first obtained to verify patient position and image quality, to locate a midline sagittal image, and to position the MRS voxel. Sagittal slices covering the entire brain were obtained using a fast spin echo (FSE) sequence (TR=25 ms, TE=17 ms, flip angle=40°, FOV=24 cm, slice thickness=3 mm, NEX=1, matrix size=256×192) for tissue segmentation of the  $^1\text{H}$  MRS spectroscopy voxels. The single-voxel short-TE MRS data were collected using the Simulated Echo acquisition mode (STEAM) sequence (TE=20 ms, TM=13.6 ms, TR=6 s, bandwidth=2 kHz, 2048 complex data points, 96 acquisitions, voxel dimension 2.0×2.0×2.0 cm<sup>3</sup>). This 8-cm<sup>3</sup> voxel was placed in the left DLPFC, which was identified on the set of sagittal and coronal  $^1\text{H}$  MR images (Fig. 1). The position of the voxel was visually inspected and adjusted based on identifiable anatomical landmarks in reference to standard brain atlases (Jackson and Duncan, 1996; Yuh et al., 1994). The

superior frontal sulcus, the lateral fissure, and the genu of the corpus callosum were used as anatomical boundaries for the voxel placement. Water unsuppressed spectra with a TR of 10 s were also collected for absolute quantification (2 acquisitions).

The post-processing and quantification steps for the short-TE STEAM MRS data were 100% automated. The unsuppressed water spectrum was used to correct for any eddy current effects. No apodization was applied, and any residual water signal was removed by using the operator-independent SVD-based method (de Beer et al., 1992). Five Gaussian damped sinusoids were used to model the five predominant singlets (NAA at 2.01 ppm, PCr+Cr at 3.02, 3.93 ppm, GPC+PC at 3.21 ppm and INO at 3.54 ppm) in the time domain using the Marquardt-Levenberg algorithm. To ensure that the signals of overlapping and of lesser amplitudes (i.e., metabolites with multiplet structures and macromolecules) would have a negligible influence on the fitting of the singlets, the first 37 ms of the free-induction decay (FID) signal were omitted in the fitting. This has been shown to reliably and accurately quantify NAA, PCr+Cr and GPC+PC (Stanley et al., 2002) (Fig. 2). The unsuppressed water

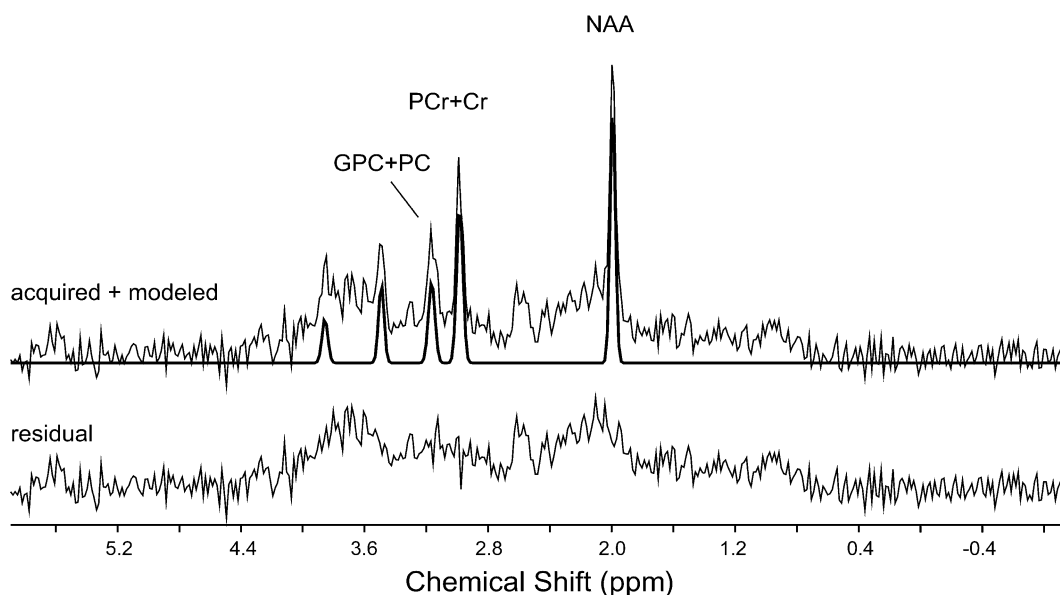


Fig. 2. Representative proton magnetic resonance spectroscopy spectrum of the left dorsolateral prefrontal cortex. A representative quantified in vivo short-TE spectrum of the dorsolateral prefrontal cortex (DLPFC) showing the Fourier transform of the acquired signal (thin line and no apodization) and the modeled signal (thick line) with the residual below. The three main quantified singlets, NAA, PCr+Cr and GPC+PC, are labeled accordingly.

signal, along with the appropriate correction factors, were applied to obtain absolute quantification values with units of mmol/kg wet weight.

### 2.3. Statistical analyses

All analyses were performed using SPSS for Windows software, version 11.0 (SPSS Inc., Chicago), and two-tailed significance level was set at  $P < 0.05$ . Student's  $t$ -test was performed to compare  $^1\text{H}$  metabolite levels and voxel composition between healthy individuals and unipolar patients. The effects of chronicity were investigated with ANCOVA with age as covariate. Pearson's correlation coefficient and Spearman's rho correlation coefficient were used to correlate age and clinical variables to the measured metabolite levels.

### 3. Results

The measured  $^1\text{H}$  MRS metabolites as well as the CSF, gray matter, and white matter voxel contents did not differ significantly in the left DLPFC between unipolar disorder patients and healthy controls (Student's  $t$ -test,  $P > 0.05$ ) (Table 1).

GPC+PC directly correlated with age in healthy controls ( $r = 0.40$ ,  $P = 0.01$ ), but not in unipolar patients ( $r = -0.34$ ,  $P = 0.11$ ). No other significant correlations were found between age and any

Table 1  
 $^1\text{H}$  MRS metabolite measures and voxel composition for left dorsolateral prefrontal cortex in unipolar patients and matched healthy subjects

	Unipolar patients ( $N=19$ )	Healthy controls ( $N=19$ )	Student's $t$ -test	
			$t$	$P$
NAA	$8.22 \pm 0.86$	$7.97 \pm 0.70$	-0.95	0.35
PCr+Cr	$6.46 \pm 1.09$	$6.09 \pm 0.77$	-1.14	0.26
GPC+PC	$1.15 \pm 0.41$	$1.14 \pm 0.34$	-0.08	0.94
NAA/PCr+Cr	$1.40 \pm 0.43$	$1.38 \pm 0.16$	-0.12	0.90
NAA/GPC+PC	$2.38 \pm 0.53$	$2.65 \pm 0.88$	1.14	0.26
GPC+PC/PCr+Cr	$0.54 \pm 0.17$	$0.57 \pm 0.18$	1.15	0.26
Gray matter (ml)	$3.36 \pm 0.84$	$2.98 \pm 0.58$	-1.64	0.11
White matter (ml)	$4.14 \pm 0.95$	$4.54 \pm 0.62$	1.54	0.13
CSF (ml)	$0.17 \pm 0.18$	$0.16 \pm 0.15$	-0.07	0.94

NAA= $N$ -acetyl-aspartate, PCr+Cr=phosphocreatine plus creatine, GPC+PC=choline-containing molecules. Absolute values are expressed as mmol/kg wet weight.

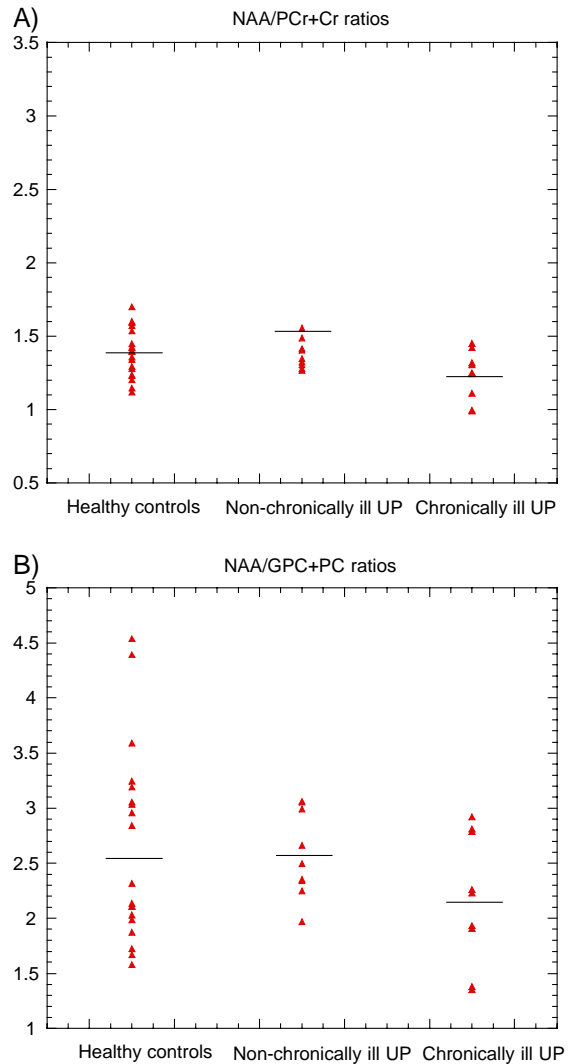


Fig. 3.  $N$ -acetyl-aspartate ratios in the left dorsolateral prefrontal cortex of healthy subjects and non-chronically and chronically ill unipolar patients. Chronically ill unipolar (UP) patients ( $N=9$ ) had significantly lower levels of NAA/PCr+Cr than non-chronically ill UP patients ( $N=10$ ) and healthy controls ( $N=19$ ) ( $F=8.62$ ,  $P=0.01$ ;  $F=4.80$ ,  $P=0.038$ , respectively) (A), and of NAA/GPC+PC than non-chronically ill UP subjects ( $F=5.24$ ,  $P=0.037$ ) (B).

measured metabolite levels in either group (Pearson's correlation coefficients,  $P > 0.05$ ). Length of illness (mean  $\pm$  S.D. =  $13.3 \pm 11.3$  years; median = 11.5), age at onset (mean  $\pm$  S.D. =  $26.1 \pm 11.3$  years; median = 24.5), number of prior depressive episodes (mean  $\pm$  S.D. =  $13.5 \pm 27.1$ ; median = 4.0), and HDRS scores (mean  $\pm$  S.D. =  $17.3 \pm 10.6$ ; median = 16.5) did

not significantly correlate with any measured metabolites (Spearman correlation coefficients,  $P > 0.05$ ).

The patient group was divided into chronically and non-chronically ill subgroups according to the median length of illness. Patients with chronic illness ( $N=9$ ) had significantly decreased levels of NAA/PCr+Cr and NAA/GPC+PC compared with non-chronically ill patients ( $N=10$ ) (mean  $\pm$  S.D. =  $1.23 \pm 0.17$  and  $1.54 \pm 0.55$ ;  $F=8.62$ ,  $P=0.01$ ; mean  $\pm$  S.D. =  $2.17 \pm 0.59 \pm 0.59$  and  $2.57 \pm 0.39$ ;  $F=5.24$ ,  $P=0.037$ , respectively) and of NAA/PCr+Cr versus healthy controls (mean  $\pm$  S.D. =  $1.23 \pm 0.17$  and  $1.38 \pm 0.16$ ;  $F=4.80$ ,  $P=0.038$ ). No significant differences were found for any measured metabolite levels between non-chronically ill unipolar patients and healthy controls (ANCOVA, age as covariate,  $P > 0.05$ ) (Fig. 3).

#### 4. Discussion

No significant differences in the left DLPFC  $^1\text{H}$  MRS metabolite levels (i.e. NAA, PCr+Cr, GPC+PC) were found between unmedicated unipolar disorder patients and age- and gender-matched healthy individuals. Two prior  $^1\text{H}$  MRS studies reported abnormally increased GPC+PC, but not NAA or PCr+Cr levels in the left DLPFC of depressed patients (Kumar et al., 2002; Farchione et al., 2002). The discrepancies between these studies and ours in regard to the GPC+PC resonance may be due to differences in the patient samples involved. Kumar et al. (2002) studied 20 patients with late-life major depression (mean age  $\pm$  S.D. =  $69.9 \pm 82$  years), whereas Farchione et al. (2002) investigated 11 depressed pediatric patients (mean age  $\pm$  S.D. =  $14.3 \pm 1.9$  years), of which 7 had another psychiatric disorder. On the contrary, our sample was composed of relatively young euthymic or mild-to-moderately depressed patients with illness onset in adulthood. Therefore, these three investigations are not comparable in terms of patient population. Furthermore, no differences for GPC+PC levels were reported in another  $^1\text{H}$  MRS report exploring the left DLPFC in resistant unipolar depression (Michael et al., 2003). It should be noted that Kumar et al. (2002) and Michael et al. (2003) also showed in the left DLPFC of elderly depressed patients, respectively, abnormally increased INO levels and decreased glutamate+glutamine (glx) metabolite concentrations. In our study, we

did not include values for these smaller metabolites as they were not reliably and accurately quantified by our post-processing procedure, and therefore we are not able to add any further information on those.

Interestingly, we here reported significantly reduced NAA/PCr-Cr ratios in the DLPFC of more chronically ill unipolar patients compared with healthy individuals and less chronically ill patients, suggesting neuronal impairment in DLPFC with the progression of the illness. Consistently, a recent  $^1\text{H}$  MRS study showed that NAA levels in the left DLPFC is inversely correlated with duration of depressive illness in unipolar disorder patients (Michael et al., 2003). This is supported by reports showing decreased blood flow, metabolism, and neuronal size in DLPFC in unipolar disorder (Coffey et al., 1993; Dolan et al., 1993; Ebert et al., 1993; Kumar et al., 1997; Galyner et al., 1998; Rajkowska et al., 1999), which could potentially be worse with the longer length of the illness.

NAA is the second most abundant amino acid after glutamate in the human brain and is the second most prominent peak in the proton spectrum after water. It accounts for approximately 85% of the proton signal of the *N*-acetyl group, whereas NAAG accounts for the remaining 15% (Pouwels and Frahm, 1998). NAA is a neuronal compound exclusively found only in mature neurons and neuronal processes and therefore is thought to be a marker of neuronal integrity, viability, and activity (Simmons et al., 1991; Urenjak et al., 1993). Although its specific neuronal function is still unclear, there are several putative roles for NAA, including involvement in de novo synthesis of fatty acids, initiation of protein synthesis, NAAG metabolism, and aspartate storage (Tsai and Coyle, 1995).

Neurodegenerative processes evolving as a consequence of the course of unipolar illness could possibly explain our findings. Abnormally low NAA concentrations have been reported in various neurodegenerative disorders by several in vivo  $^1\text{H}$  MRS studies (i.e. Alzheimer's Disease, lateral amyotrophic sclerosis, multiple sclerosis) (Davie et al., 1994; Kwo-On-Yuen et al., 1994; Kalra et al., 1998), and also in bipolar disorder (Winsberg et al., 2000) and schizophrenia (Bertolino et al., 1998). Thus, decreased levels of NAA are non-specific changes shared by several chronic neuropsychiatric diseases, which could reflect brain degenerative processes.

The strengths of the study include the selection of homogeneous unmedicated unipolar disorder patients, the careful matching of unipolar and healthy subjects, and the use of a reliable method that accurately resolves NAA, PCr+Cr, and GPC+PC. We have also addressed the potential tissue heterogeneity within the voxel by examining proportions of these tissue compartments within the voxels. However, the findings of the present study should be seen cautiously due to a few potential limitations. The relatively small sample size may have reduced the statistical power of our analyses, and small abnormalities in the metabolite concentrations in unipolar patients may not have been detected. However, we selected carefully diagnosed and unmedicated unipolar patients and well-matched healthy controls, which is a strength of our analyses, as it eliminates potential confounding factors from the current use of psychotropic medications, age, and gender. Also, the single-voxel approach does not allow us to generalize our findings to other brain regions. Last, our methods did not allow us to resolve the smallest  $^1\text{H}$  MRS peaks, such as GABA and aspartate.

In conclusion, this study reported unaltered  $^1\text{H}$  MRS metabolite concentrations in the left DLPFC of unmedicated unipolar patients compared with age- and gender-matched healthy individuals. Interestingly, abnormally reduced levels of NAA in DLPFC were reported in more chronically ill unipolar patients, suggesting that chronicity may play a major role in neuronal impairment in DLPFC in unipolar disorder. This could potentially be accounted for by neurodegenerative processes with the progression of unipolar illness. Thus, illness chronicity is an important factor to be taken into account in MRS studies of brain metabolites in unipolar patients. Longitudinal  $^1\text{H}$  MRS investigations in never-treated patients will be instrumental to further examine the role of illness chronicity on NAA levels and characterize the role of neurodegenerative mechanisms in the pathophysiology of unipolar disorder.

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