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ORIGINAL PAPER

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Biochemical changes in the cingulum in patients with schizophrenia and chronic bipolar disorder

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Abstract Biochemical changes have been reported in vivo in the brain in schizophrenia patients using 1H-magnetic resonance spectroscopy (MRS). The aim of this study was to assess the specificity of biochemical changes occurring in schizophrenia patients, in a direct comparison with bipolar disorder patients. Fourteen patients with chronic paranoid schizophrenia, 17 euthymic type I bipolar patients with no previous history of psychotic symptoms and 15 healthy controls were included, most of them were female. They underwent a study with MRS: proton spectra were acquired using a Signa 1.5 T CVI scanner, with a localised single voxel PRESS sequence. *N*-acetyl aspartate (NAA), Creatine (Cr), and Choline (Cho) metabolite resonance intensities were all quantified in the cingulum, a region of interest in schizophrenia and bipolar disorder. Schizophrenia patients showed a significantly higher Cho/Cre as well as lower NAA/Cho ratios as compared with controls and bipolar patients. No significant differences were found among the three groups as regards NAA/Cre levels. These data are consistent with an increase in the concentration of choline in the cingulum in chronic schizophrenia, at least in this predominantly female group. Such an increase seems to be more intense than in psychosis-free bipolar disorder patients.

Key words spectroscopy · *N*-acetyl-aspartate · choline · schizophrenia · bipolar disorder

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Introduction

Spectroscopic magnetic resonance of 1H enables in vivo studies of certain aspects of brain biochemistry and the levels of *N*-acetyl-aspartate (NAA) and Choline (Cho), amongst other substances, to be quantified. NAA is found almost exclusively in neurons and, as a result, is a possible marker for neuronal integrity, viability and function [42]. NAA is also found within oligodendrocyte-type 2 astrocyte (O-2A) progenitor cells [42], and mature oligodendrocytes may also express it [4]. However, when the spectral profiles derived from cultured glial and neuronal cells were analysed, a NAA peak was only detected in the neuronal line [18]. Thus, the NAA signal may be assumed to be mostly if not totally of neuronal origin. In this respect, the intensity of the choline signal in the magnetic resonance spectroscopy (MRS) mainly reflects the choline present in membrane phospholipids and, to a lesser extent, free choline, and may be a marker of cell membrane integrity which increases in the presence of high membrane turnover [38].

Schizophrenia and bipolar disorder share certain epidemiological, clinical and neuropharmacological features. Bipolar disorder patients frequently show psychotic and even first rank symptoms. Bipolar

and schizophrenia respond to dopamine blockade. Besides, many patients with schizophrenia also show symptoms of depression or mania. Finally, schizophrenia and mania share a number of characteristics such as the onset in young adults, earlier in males in both cases and the frequent occurrence of life events prior to the onset or relapse [27]. As a result, the comparison of cerebral biochemical features in both syndromes is of interest.

The prefrontal concentration of NAA may be decreased in chronic schizophrenia [36]. However, results are less consistent regarding metabolite concentrations in bipolar disorder. A decrease in NAA levels in the dorsolateral prefrontal region have been reported in long-term bipolar patients [44], as well as in the orbital, medial frontal and cingulum regions, expressed as absolute values in patients suffering manic or mixed episodes [9]. Using a multi-voxel technique, Bertolino et al. [3], described changes in NAA in the hippocampal region but neither in the dorsolateral prefrontal cortex nor in the cingulum. However, more recent studies found no changes in NAA levels in the frontal region in bipolar disorder patients [1, 12]. Finally, in a post-mortem study, there were no significant differences in NAA concentration in bipolar patients compared with healthy controls [29].

Magnetic resonance spectroscopy studies of the cingulum can be useful to understand the cerebral substrates of schizophrenia and bipolar disorder, as changes in this region may play a role in both processes [14, 32, 40]. This region is particularly appropriate for single voxel spectroscopy given its uniform topography. The anterior cingulum is divided into two regions in terms of its projections, function and cell architecture, namely the anterior or affective region including areas 25, 24, 32 and 33, and the dorsal or cognitive region which was described and classified as areas 24′and 32′ [8]. This cognitive subregion may play a relevant role in modulating attention, cognitive processing and response selection prior to the triggering of motor action [34].

In order to study the biochemical substrates involved in schizophrenia and bipolar disorder, in the present report NAA/Cre, Cho/Cre, NAA/Cho ratios were compared in the anterior cingulum in a group of patients with chronic schizophrenia and type I bipolar disorder with no previous history of psychosis.

Methods

Subjects

The study included 31 outpatients, 17 of them euthymic bipolar patients (type I) with no previous history of psychotic symptoms (14 women, mean age = 37, SD = 10) and 14 paranoid schizophrenic patients (11 women, mean age = 34, SD = 9), all according to the DSM-IV diagnostic criteria. A semi-structured clinical interview (SCID, patient version) was used to confirm the diagnosis with information provided by patients, their relatives and healthcare professionals.

In the bipolar group, 16 patients were receiving lithium at the time of the MRS studies, with a mean exposure time to therapeutic doses of 59 months. Moreover, within the same group 11 patients were exposed to atypical neuroleptics for a mean of 8 months at the time of the imaging test. In the schizophrenia group no patients were receiving lithium and 12 were receiving atypical neuroleptics at the time of the study with a mean exposure time of 55 months. None of the patients (schizophrenia or bipolar ones) had consumed alcohol in the last 3 years, which could be ensured on the basis of a long therapeutic relationship and information from relatives.

After providing patients with written information, we obtained written consent from both the patients and their relatives. The ethics committees of the participating centres endorsed the study.

We also included 15 healthy control subjects (11 women, mean age = 34 and SD = 8) with no significant differences in either gender or age distribution. The educational level was below graduate level with no significant differences between the three groups in the educational and socio-economic level of their parents. The Hollingshead Index was used to evaluate parental, as well as patients socioeconomic level [19].

Exclusion criteria for patients and controls included residual schizophrenia; neurological disease; MRI findings were deemed clinically relevant from a neurological perspective by a radiologist blind to diagnosis; history of head injury with loss of consciousness; history of substance abuse (other than caffeine or nicotine); history of axis I psychiatric disorders or treatment (other than schizophrenia or bipolar disorder), or current treatment with a known CNS depressant or stimulant (in controls).

Imaging methods

Spectroscopy

All the proton spectra were acquired using a Signa 1.5 T CVI scanner, from the Department of Radiology in the CERCO centre (Seville), with a localised single voxel point resolved spectroscopy (PRESS) [6], sequence (TE = 35 ms; TR = 1,500 ms; Acquisition Number of averages 8), average voxel volume = 30.5 cc (17.5–44.8). Being this voxel larger than the usual one in this kind of research, this allowed a good signal-to-noise ratio and thus a lower number of averages were needed. Sample volumes were placed encompassing the centre of both cingulates, planned in an axial 3D, T1 weighted sequence with spoiled gradients as defined below, with the anterior/posterior margins limited by the cingulate sulcus, the inferior margin being the slice just above the corpus callosum and the superior margin being the calculated slice from the thickness of corpus callosum as measured perpendicular to the axial acquisition plane. Lateral margins are planned symmetrically encompassing the grey matter of the cingulate gyrus (Fig. 1). The water signal was suppressed by selective chemical shift suppression [17].

The spectra were automatically processed with PROBE-p [23] and the metabolite resonance intensities were quantified for NAA, creatine (Cr), and choline (Cho) (Fig. 2). NAA values are expressed in relative units, as proportional to Cr and to Cho, and Cho as proportional to Cr.

Segmentation and VOI spectra definition

In order to control the partial volume effect of grey matter when defining the biochemical differences, the images were segmented into grey and white matter and were quantified within the voxel.

Structural magnetic resonance imaging studies were acquired using a T1-weighted 3D gradient echo sequence with spoiled gradients as follows: matrix size 256 × 256, pixel size 0.96 × 0.96 mm (FOV 256 mm), flip angle 30°, repetition time 40 ms, echo time 6 ms, slice thickness 1.3 mm.

For the purpose of segmentation we used a three-step procedure using automated programmes included in the Functional Magnetic

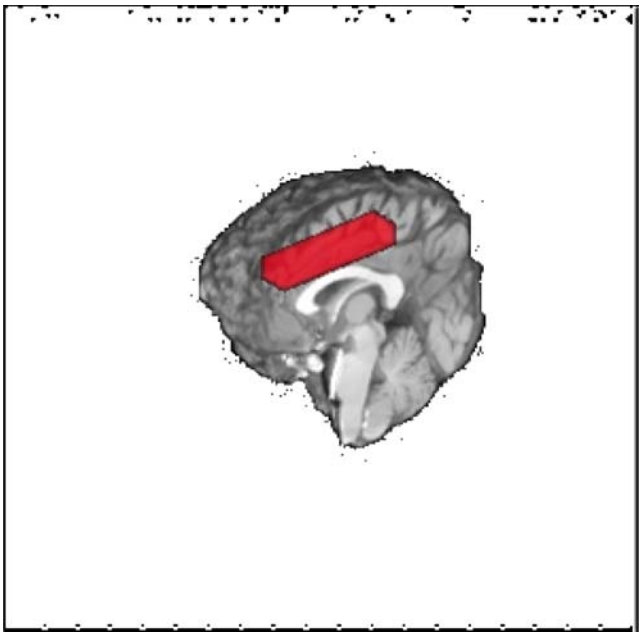

Fig. 1 Image of the spectroscopy voxel, placed to encompass the center of both cingula, with the anterior/posterior margins limited by cingulate sulcus, the inferior margin being the slice just above the corpus callosum and the superior margin being the calculated slice from the thickness of corpus callosum as measured perpendicular to acquisition axial plane

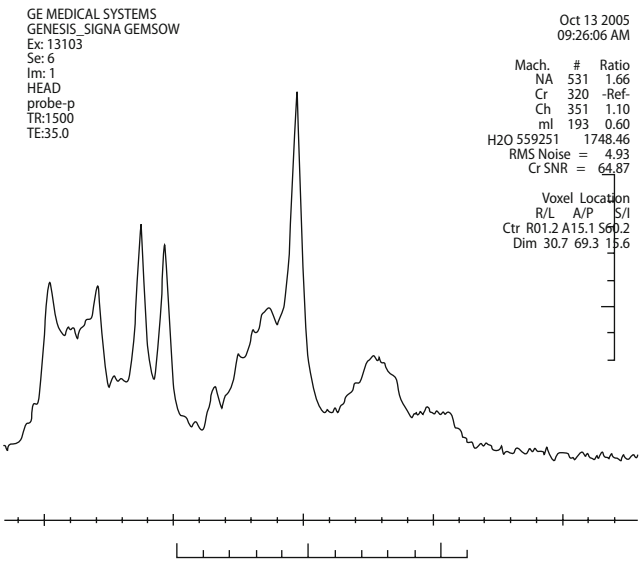

Fig. 2 Typical ^{1}H-MRS spectrum obtained form a patient in our group

Resonance Imaging of the Brain software library (FMRIB). The first step involved editing the MRI to remove skull and extracranial tissue using the automated programme Brain Extraction Tool (BET) [35]. In the second step, a 3D noise reduction filter was applied using Smallest Univalue Segment Assimilating Nucleus (SUSAN) [46]. In the third step, brain tissues were automatically segmented into grey matter (GM), white matter (WM), and cerebrospinal fluid (CSF) using FMRIB's Automated Segmentation Tool (FAST) [46], whilst also correcting for spatial intensity variations (also known as bias field or RF non-homogeneities). The underlying method is based on a hidden Markov random field model and an associated Expectation–Maximisation algorithm. This segmentation was checked for inconsistencies and manually corrected whenever necessary by an experienced radiologist blind to the diagnosis.

Then, we used Analysis of Functional Neuroimages (AFNI) [10] to automatically define the voxel volume of the spectra, using the box coordinates from the DICOM header information of the Spectroscopy. Ultimately the total volume of the spectra box and the volumes of GM, WM and CFS contained in the spectra box were quantified.

Data analysis

Age was compared between groups with ANOVA, and sex distribution with a χ^2 test. Illness duration was compared between bipolar and schizophrenia groups with a t test (Table 1).

The significance of the differences across groups in metabolite concentration was assessed using a General Linear Model (GLM), with diagnosis as the main factor (controls, bipolar, schizophrenia), and three covariates (gender, age and GM in each voxel) For each outcome (NAA/Cre, Cho/Cre, NAA/Cho ratios), the overall null hypothesis was tested (H0: control = bipolar = schizophrenia) (Table 2). The model was progressively refined by eliminating covariates that did not explain variance of the outcomes, until a final model was reached where only diagnosis and GM in voxel remained (Table 3). In this final model, apart from overall null hypothesis also pair-wise tests of the differences between groups (bipolars vs. control, schizophrenia vs. control and schizophrenia vs. bipolar) were conducted using Student's t test with Sidak's correction (Table 3). The same procedure was applied to compare biochemical values only in female subjects.

Finally, we also assessed the correlation coefficients between the different outcomes (NAA/Cre, Cho/Cre, NAA/Cho ratios) and clinical scores, duration of the disease in years, and duration of the treatment with lithium and atypical neuroleptics.

Results

No significant differences were found between groups in terms of age ($F_{2,43} = 0.50$; $P = 0.61$) or gender ($\chi^2_2 = 0.38$; $P = 0.83$) There was no difference in disease duration between the schizophrenia and the bipolar disorder groups ($t_{29} = 0.35$; $P = 0.73$).

Differences in outcomes were not explained significantly by sex and age, nor did gender modify significantly the effect of the group. Only diagnosis and GM showed a significant relationship with the outcome (Table 2). In the final models the comparison of biochemical variables revealed no significant differences in the NAA/Cre levels between groups ($F_{2,40} = 0.20$; $P = 0.815$). However, statistically significant differences were detected in the NAA/Cho levels between groups ($F_{2;40} = 3.46$; $P = 0.04$) due to a lower NAA/Cho level in the schizophrenia patients compared with controls ($T_{40} = -2.48$; $P = 0.05$) (Table 3).

There were also significant between-groups differences in Cho/Cre levels ($F_{2,24} = 5.95$, $P = 0.005$). Schizophrenia patients showed significantly higher Cho/Cre ratios as compared with healthy controls ($T_{40} = 3.23$; $P = 0.006$) and bipolar patients ($T_{40} = 2.69$; $P = 0.039$) (Table 3).

A similar pattern of differences was evident for Cho/Cre ratios when only females were compared between groups (between groups difference for Cho/Cre; $F_{2,40} = 3.37$; $P = 0.047$). The difference of NAA/

Table 1 Socio-demographic, clinical, treatment and biochemical data for patients and controls

	Controls		Bipolar		Schizophrenia	
	15		17		14	
Age (mean ± td)	34	±8	37	±10	34	±9
Parental socioeconomic status	2.1		2.1		2.0	
Patients socioeconomic status	2.0		3.0		3.2	
School years	12.7	±3	9.1	±3	7.6	±2
Illness duration (years)			14	±8	13	±7
Female subjects	11	73.3%	14	82.4%	11	78.6%
Positive PANSS					14.40	±7.5
Negative PANSS					22.27	±13.3
Total PANSS					74.93	±27.6
Lithium treatment (*N*)	0		16	94.1%	0	
Lithium duration, months (mean, SD)			59	±47		
Atypical antipsychotics (*N*)	0		11	64.7%	12	85.7%
Atypical antipsychotics duration (months) (mean ± SD)			18	± 21	55	± 35
% Grey matter voxels (mean ± td)	50.89	8.33	48.76	6.60	44.72	4.00
NAA/Cre (mean ± SD)	1.62	±0.11	1.58	±0.19	1.65	±0.11
NAA/Cho (mean ± SD)	1.81	±0.25	1.73	±0.34	1.63	±0.23
Cho/Cre (mean ± SD)	0.91	±0.11	0.94	±0.11	1.02	±0.11

Table 2 General linear model (GLM) for each outcome, with group as main factor and controlling for sex, age, grey matter and the interaction between group and sex

ANOVAs	NAA/Cre		NAA/Cho		Cho/Cre	
	$F_{2,40}$	*P*	$F_{2,40}$	*P*	$F_{2,40}$	*P*
Group	0.32	0.729	4.39	**0.019**	6.14	**0.005**
Sex	0.64	0.428	0.87	0.358	1.78	0.191
Age	0.09	0.762	2.03	0.162	2.33	0.135
Grey matter	9.43	**0.004**	12.49	**0.001**	3.42	**0.072**
Group × sex	1.09	0.347	1.07	0.353	0.54	0.585

Bold values indicate a significance level $P < 0.05$

Table 3 Comparison of biochemical variables between the three groups, using a GLM (controlling only for grey matter)

ANOVAs	NAA/Cre		NAA/Cho		Cho/Cre	
	$F_{2,40}$	*P*	$F_{2,40}$	*P*	$F_{2,40}$	*P*
Group	0.20	0.815	3.46	**0.041**	5.95	**0.005**
Grey matter	13.61	**0.001**	17.42	**0.000**	5.17	**0.028**
Differences between groups	Differ.	P-Sidak	Differ.	P-Sidak	Differ.	P-Sidak
Bipolar-control	−0.01	0.97	−0.04	0.960	0.02	0.893
Schizo-control	0.01	0.99	−0.22	**0.050**	0.13	**0.006**
Schizo-bipolar	0.03	0.89	−0.19	0.125	0.11	**0.039**

Bold values indicate a significance level $P < 0.05$

Cho ratios among female subgroups was not statistically significant ($F_{2,40} = 1.49$; $P = 0.24$).

There was no significant association between any ratio and positive PANSS ($r = -0.18$, $P = 0.55$; $r = 0.29$, $P = 0.34$), negative PANSS ($r = 0.14$, $P = 0.65$; $r = -0.07$, $P = 0.81$) or total PANNS ($r = -0.24$, $P = 0.94$; $r = 0.06$, $P = 0.83$) scores. No significant correlation was found between duration of treatment with lithium or atypical neuroleptics, or the duration of the disease, and NAA/Cre, NAA/Cho or Cho/Cre ratios (Fig. 3–5).

Discussion

The results suggest an increase in Cho concentration in the cingulum of patients with chronic schizophrenia compared with healthy controls and bipolar patients, although probably not a lesser concentration of NAA, in a predominantly female sample. These comparisons have been controlled for the possible confounding effects of gender, age and partial volume effect and may support some differences in the cere-

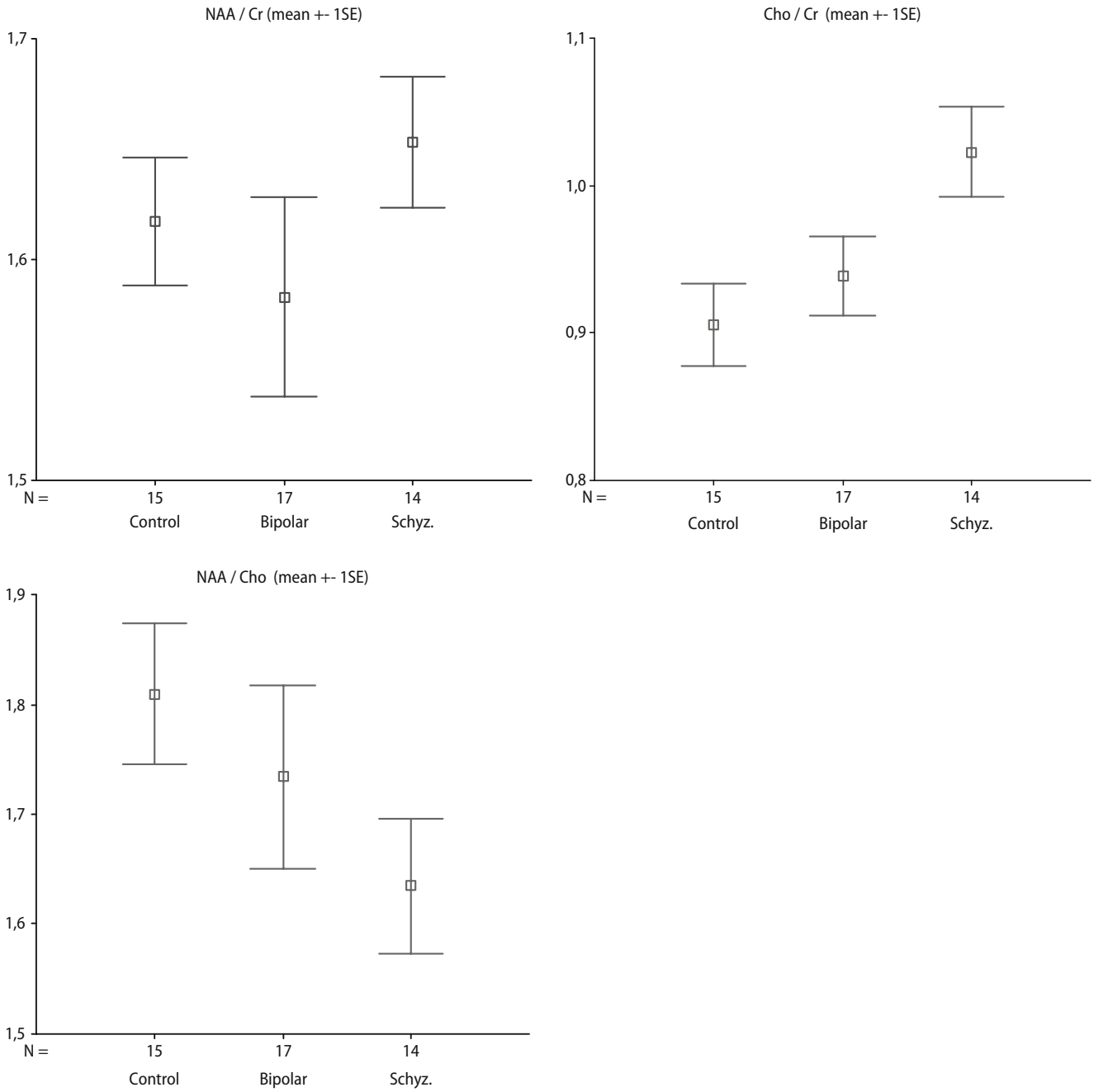

Fig. 3–5 For each outcome assessed: NAA/Cr, NAA/Cho and Cho/Cr graphs with 1 mean ± 1 SE were devised for the three groups of diagnoses

bral substrate of schizophrenia and non-psychotic bipolar disorder.

The lower NAA/Cho and higher Cho/Cr ratios in the schizophrenia patients would be consistent with the results reported by Yamasue et al. [45], who described increased levels of Cho and no significant change in NAA in schizophrenia in the cingulate anterior to the genus of the corpus callosum in patients treated with typical neuroleptics. Recently, Jessen et al. [21] have described an increase in Cho levels in the anterior cingulum in at-risk subjects who later developed schizophrenia compared with those at-risk subjects who did not progress to that diagnosis. Other authors also report increased levels of Cho in the frontal lobe of schizophrenia patients [5, 7]. However, agreement is not complete on this point, as Deicken et al. [13] and Ende et al. [15] found no changes in Cho levels in the anterior cingulate region in schizophrenia patients treated with typical neuroleptics.

The absence of any significant differences in NAA levels in the schizophrenia group is inconsistent with

a significant part of current literature. This absence may be due to changes restricted to one or some regions, such as the prefrontal and hippocampal ones, where such a decrease is a frequent finding [36]. The second explanation may be an increase in NAA levels due to chronic treatment with atypical neuroleptics as described by Bertolino et al. [2, 3]. Moreover, and not incompatible with the preceding possibilities, it can also relate to the high proportion of females in our schizophrenia group, as available data support a higher structural damage of the brain on male patients [28].

The absence of NAA changes in bipolar patients is consistent with previous results reported for the anterior cingulate region [1, 3] and the dorsolateral prefrontal cortex [3]. However, it disagrees with other studies. For instance, a decrease in NAA was reported in the prefrontal region in a long-term bipolar sample [44]. Also, Cecil et al. [9] when studying a single voxel encompassing the orbital gyrus and cingulate gyrus, reported a decrease in NAA expressed relatively to the standard water measurement; this sample comprised patients during manic or mixed episodes with a history of psychotic symptoms. These results, together with the intermediate metabolite values obtained in the present study in the bipolar patients (i.e., between controls and schizophrenia patients), may suggest a biological heterogeneity in the brains of patients with bipolar disorder.

The absence of differences in Choline levels in the bipolar group as compared to controls is in agreement with similar results in that illness [37]. For instance, Bertolino et al. [3] who included the anterior and posterior cingulum, prefrontal dorsolateral cortex and the inferior frontal gyrus amongst other regions, did not find significant changes in Cho/Cre in any of these areas. Also Ohara et al. [30] found no differences in the basal ganglia in euthymic bipolar patients. Nevertheless, other results did not agree, as an increase in choline expressed as Cho/NAA, in the anterior right cingulum [25], and an increase in choline in the striatum [37] have been reported in bipolar patients.

In the present sample, most of the bipolar patients were chronically treated with lithium. Previous studies have suggested that chronic lithium may increase NAA concentration [26, 33], but as far as we know lithium has no significant effects on choline levels. Thus, the lack of significant differences in the Cho/Cr and NAA/Cho ratios between bipolar and control subjects might not be an effect of lithium.

The likely higher Cho levels in schizophrenia patients can be interpreted in different ways. First, it may point to an increase in phospholipid turnover with membrane destruction which, if neuronal, would be consistent with certain converging lines of research in schizophrenia that suggest a disruption of cortical synaptic circuits [24]. The reported association between the duration of untreated psychosis (DUP) and choline level [39] would specially agree with that possibility. However, if the Cho increase originated from neuronal membrane destruction, it should be accompanied by NAA decrease, which was not found in our group. If not neuronal, a glial origin for Cho increase seems conceivable. In other words, the possibility of changes in glial cells would account for increases in choline, which would also be compatible with the absence of decreased NAA in the same region. Such a glial involvement is also supported by a recent result showing a decrease in mio-inositol (a glial marker) levels in elderly schizophrenia patients along with an increase in frontal Cho signal [11].

Speculatively those glial changes may be due to glial proliferation or destruction that would increase the choline signal. In fact, glial proliferation has been described in monkeys under chronic treatment with atypical anti-psychotic drugs [31], which could suggest one possible mechanism for the observed increase: a glial proliferation induced by treatment. Nevertheless, no correlation was found between Cho levels and duration of treatment with atypical neuroleptics in the schizophrenia group, as would be expected in that case. Even so, the absence of such a correlation may be due to a limited effect over time. Alternatively, the increase in Cho in the schizophrenia group may be related to the decrease in glial cells found in schizophrenia patients at the prefrontal and cingulum regions [41, 43], as well as to the alterations of glial-related gene expression at that level reported in schizophrenia [22].

Alcohol consumption may also be a factor related to increased Cho levels in schizophrenia, since a positive correlation between alcohol consumption and frontal Cho signals can be found [16]. However, the patient's alcohol consumption in the present sample has been carefully discarded, and thus a different amount of alcohol intake is unlikely to explain the differences here found.

The main limitations to the study are the small sample sizes and the effect that chronic treatment might have upon metabolites. Also, despite being the most prevalent method adopted in current literature, the method of relative quantification used can have technical limitations relating to the possibility of variations in the ratios due to changes in the numerator, denominator or both [20]. Regardless of these limitations, it seems reasonable to conclude that the data supports changes in cerebral biochemistry in chronic schizophrenia, in a predominantly female sample, in the cingulate region, which is more intense than those seen in psychosis-free bipolar disease and different from those described in the prefrontal region.

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