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Differential clinical, structural and P300 parameters in schizophrenia patients resistant to conventional neuroleptics

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Abstract

Schizophrenia is a heterogeneous clinical condition that may reflect a variety of biological processes. In particular, treatment-resistant (TR) schizophrenia may have a distinct neurobiological substrate. Within the context of clinical data, a simultaneous study with different imaging techniques could help to elucidate differences in cerebral substrates among schizophrenia patients with different responses to treatment.

In the present work we used a set of biological data (basal and longitudinal volumetry, and P300 event-related potential measurements) to compare TR and treatment-responsive chronic schizophrenia patients with healthy controls. The TR patients showed higher baseline clinical scores, a more severe basal profile of brain alterations, as well as a different outcome as regards to volume deficits. These data support the notion that biological substrates vary among groups of different psychotic patients, even when they have the same diagnosis, and that those substrates may be related to the response to treatment.

Keywords: Brain lobes; Morphometry; MRI; P300; Schizophrenia; Treatment-resistance

1. Introduction

Schizophrenia is a heterogeneous clinical condition that may include a variety of biological processes. Many structural brain abnormalities described in this syndrome vary in degree and localization, and they do not occur in all patients (Shenton et al., 2001), which suggests the possibility of identifying subgroups with relevant clinical correlates within the diagnosis of schizophrenia. This possibility is supported by the findings of combined frontal/temporal deficits and greater cortical change associated with a poorer longitudinal course in the "kaepelinian" form of schizophrenia, a proposed poor-outcome variety of the condition (Keefe et al., 1996, 1988). More severe imaging abnormalities, such as ventricular enlargement (Davis et al., 1998) have been associated with this form of illness. Along the same line, the rate of response to anti-psychotics could be related to a distinct biological substrate in schizophrenia, since differences in brain structure have been described in patients with favourable and unfavourable outcomes (Staal et al., 2001).

From this perspective, we decided to use different clinical and biological data to attempt to characterize subgroups within the diagnosis of schizophrenia. The general goal of the study was to

Abbreviations: GM, grey matter; WM, white matter; CSF, cerebrospinal fluid; MRI, magnetic resonance imaging; ERP, event-related potential; TR, treatment-resistant; PANSS, positive and negative symptoms scale; SCID, Structured Clinical Interview for DSM-IV; DSM, Diagnostic and Statistical Manual; FOV, field of view; ROI, region of interest; ICV, intracranial volume; ROC, receiver operating characteristic.

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elucidate brain differences between groups of schizophrenia patients respectively responsive and resistant to classical anti-psychotics. By using different imaging data, we expected to gather information that would allow a better understanding of the substrate of the brain differences between these groups.

Within that framework, our first specific hypothesis was that there would be significant basal differences in brain structures associated with both types of resistance to treatment. If there are distinguishable anatomical characteristics between treatment-resistant (TR) and treatment-responsive schizophrenia patients, we would also expect that the former would be differentiated from non-resistant patients by other features related to structural alterations of functional relevance, such as P300 parameters. Third, we also surmised that TR patients would show a progressive reduction in GM cortical volume after a follow-up period, using longitudinal magnetic resonance imaging (MRI) assessments. The available literature indeed reports changes in these variables in some groups of patients with schizophrenia but not in others (Ford, 1999; Gur et al., 1998; Ho et al., 2003; Keshavan et al., 2000), supporting its potential relevance for our purposes.

2. Patients and methods

We used the MRI data from 93 subjects, 49 with schizophrenia (33 males; 37 of the paranoid and 12 of the undifferentiated subtypes), and 44 healthy controls (20 males). In order to optimize the discriminative capacity of the analyses, we included all the available clinical, structural and electrophysiological data from samples previously studied by our group for other purposes (Molina et al., 2005a,b,d, 2004a, 2003).

The diagnoses were based on DSM-IV criteria and were confirmed by a SCID (patient version). The symptoms in the patients were initially evaluated with the PANSS (Kay et al., 1987) and were re-evaluated with that same scale 6 months later in 39 out of the initial 49 patients (Table 1). Illness duration was defined as the time elapsed since the first time the patient met the criteria for a diagnosis of schizophrenia or other related diagnoses that later evolved into the illness such as a schizophreniform disorder). One of two experienced investigators (JS or VM) assessed duration, but reliability in their estimations about this parameter was not measured.

All patients were included during an episode of exacerbation of their psychotic symptoms while they were spending a brief stay at a psychiatric unit. They then returned to the community after a short in-patient treatment. They were divided into two groups:

2.1. TR schizophrenia

This group included of 30 patients (21 males) resistant to conventional treatment according to the usual criteria, similar to those of (Kane et al., 1988). Treatment resistance was defined as the persistence of clinically relevant positive symptoms (at least one positive symptom in the PANSS scoring 5 or more) and a CGI score equal to or higher than 4, despite the use at adequate doses of two different classical anti-psychotics for a period longer than 2 months each. A diagnosis of treatment resistance was made by gathering data from past clinical records (from the hospital and community mental health services); information from their treating psychiatrists and families, and clinical examination of the patients. These patients were living in the community, in spite of their persisting symptoms, but an increase in the severity of these and/or significant behavioural disturbances had made their admission to the psychiatric unit of a hospital necessary.

Until enrolment, all these patients had received only conventional neuroleptics. In all cases, treatment consisted solely of haloperidol during the month prior to the first MRI study (at a fixed dose of 10 mg/d except in the case of intolerance, for 4.5±3.2 weeks, with biperiden as needed if extrapyramidal symptoms appeared. Haloperidol was administered in order to confirm resistance to treatment before initiating treatment with clozapine.

2.2. Non-TR schizophrenia

The non-TR patients ($n=19$, 12 males) had a similar disease duration to the TR group. They were enrolled during a period of clinical psychotic exacerbation, but they did not meet the resistance criteria because all of them had previously responded to haloperidol during the preceding year. These criteria were used to rule out the possibility that biological differences between TR and non-TR patients might merely be due to differences in the severity of the psychotic symptoms or in illness duration. Regarding the former group, these patients had received only conventional neuroleptics prior to enrolment. In 12 of these cases, the relapse was probably related to discontinuation.

Our sample included a higher than usual proportion of TR patients, perhaps due to their inclusion in the study during their stay at a psychiatric unit, in turn probably reflecting greater severity than community samples.

After enrolment, the patients were treated with atypical neuroleptics throughout the study (clozapine in resistant patients (initial doses after escalation: 410±339 mg/day; final doses 260.9±211.2 mg/day), and olanzapine in non-resistant patients (initial

Table 1
Clinical and demographic values in the three subgroups shown as mean (sd)

	Resistant ($n=30$)	Non-resistant ($n=19$)	Controls ($n=44$)
Age	35.8 (11.7)	37.8 (12.5)	29.4 (9.7)
Sex (m:f ratio)	21:9	12:7	20:24
Parental SES	2.0 (0.9)	2.1 (1.0)	2.3 (0.9)
Education (yr)	10.9 (8.7)	12.0 (9.5)	12.9 (7.4)
Duration	9.92 (7.4)	11.9 (8.5)	
Total PANSS	102.6 (16.9)*	87.6 (21.8)	
Positive PANSS	24.45 (6.0)*	20.66 (6.0)	
Negative PANSS	26.12 (7.2)	27.9 (9.6)	
General PANSS	50.66 (10.7)	44.01 (13.9)	
Total PANSS improvement	25.3 (15.7)	19.5 (13.4)	
Positive PANSS improvement	30.3 (21.0)	27.5 (20.8)	
Negative PANSS improvement	13.8 (15.5)	14.01 (10.0)	
General PANSS improvement	27.3 (20.5)	18.2 (15.2)	

*$p<.05$.

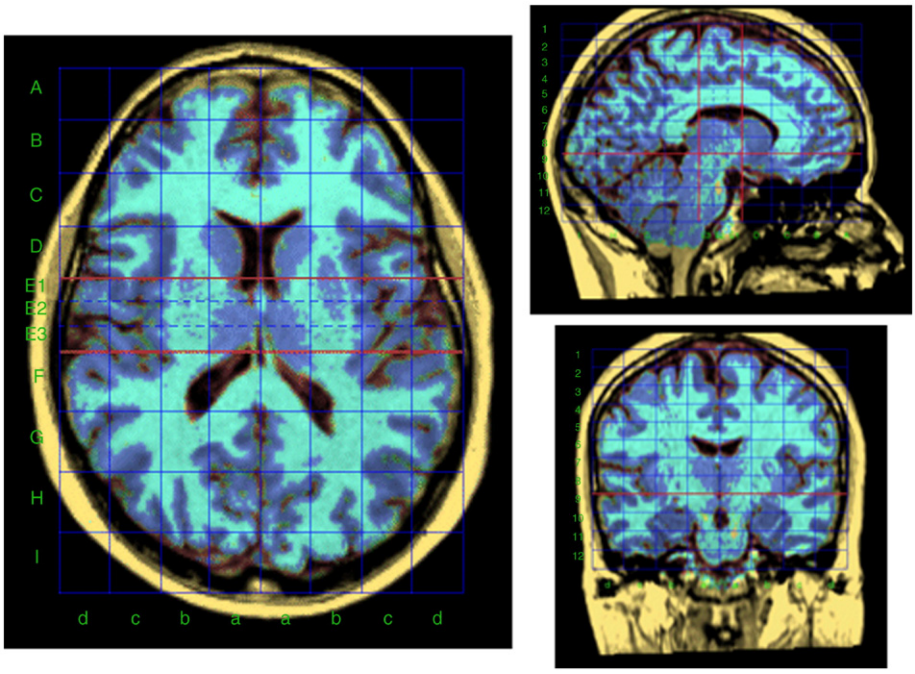

Fig. 1. Example of a Talairach proportional grid system built upon a particular brain. Cerebral tissue is segmented into grey and white matter and cerebrospinal fluid. This segmentation and the Talairach grid allow volume measurements for each ROI, described as a series of grid cells (see Methods).

dose 20 mg/d, mean final dose 15.4 (SD 9.5 mg/d)), with no other drugs except occasional benzodiazepines in the event of insomnia. We did not record additional medication along the follow-up period, but the prescription of medications with known effects on brain morphology, such as lithium or anti-depressants, were exclusion criteria for longitudinal analyses.

Of the 19 patients in the non-TR group, only 11 (5 women/6 men) could be evaluated with MRI longitudinally. In six of the remaining eight patients, therapy with olanzapine was switched due to an exacerbation of their symptoms or an insufficient response before the end of follow-up, and the other two patients were unavailable for follow-up. These patients were monitored after discharge in the same setting by one of two investigators (VM or JS). In the TR group, follow-up MRI scans were available in 13 patients (9 males). Of the other 17 TR cases, 6 were lost to follow-up, 5 refused a new MRI exam, and 6 had their treatment switched to another atypical due to intolerance to clozapine or incompliance.

There were no significant differences between the groups in age or parental socioeconomic status (Hollingshead and Frederick, 1953). Controls were matched with the patients in their levels of education.

The exclusion criteria for both groups of patients and controls were as follows: neurological illness; MRI findings judged clinically relevant from a neurological perspective by a radiologist blind to the diagnosis; a history of cranial trauma with loss of consciousness; substance dependence over the previous 3 years (except for caffeine or nicotine); substance abuse over the previous 6 months (a urine analysis at intake was used to rule out current consumption of cannabis, cocaine, amphetamines and opiates.), and antecedents of any other axis I psychiatric processes or treatment (or any current treatment having a known CNS action in addition to neuroleptics and benzodiazepines for insomnia). Alcohol abuse was discarded though clinical examination and biochemical parameters (gamma glutamyl transferase and corpuscular volume).

After receiving full information, the patients and their relatives and the controls signed a consent form. The study was approved by an independent ethics committee.

3. Imaging methods

The procedures used to determine the study variables have been published in detail in previous studies (Martin-Loeches et al., 2001; Molina et al., 2005c, 2003). The following is a summary of the key methodological aspects:

3.1. MRI method

3.1.1. MRI acquisition

Baseline MRI data were acquired in all patients and controls in the study. Magnetic resonance imaging studies were acquired on a Philips Gyroscan 1.5 T scanner using a T1-weighted 3D gradient echo sequence with the following parameters: matrix size 256×256, pixel size 0.9×0.9 mm (FOV 256 mm), flip angle 30°, echo time 4.6 ms, slice thickness of 1.5 mm. T2-weighted sequences were also acquired for verification of CSF segmentations and for other clinical purposes (Turbo-Spin Echo, turbo factor 15, echo time 120 ms, matrix size 256×256, slice thickness 3.5 mm).

3.1.2. Segmentation and ROI definition

To obtain volume measurements of the main brain lobes, we used a method for semi-automated segmentation of the brain

based on the Talairach reference system, similar to the one described in (Andreasen et al., 1996; Kates et al., 1999). Basically, we used a two-step procedure. The first involved editing the MRI to remove skull and extracranial tissue, and an initial segmentation of cerebral tissues into grey matter (GM), white matter (WM), and cerebrospinal fluid (CSF) (Ashburner and Friston, 1997). In a second stage, we applied the Talairach reference system (Talairach and Tournoux, 1988) to define regions of interest (ROIs) and to obtain volume data. MR images were processed using locally developed software that incorporates a variety of image processing and quantification tools (Desco et al., 2001).

The initial segmentation of cerebral tissues was performed using an automated method, currently included as a standard algorithm in the SPM2 (Statistical Parametric Mapping) program (Ashburner and Friston, 1997). The method performs a cluster analysis with a modified mixture model and *a priori* information about the likelihoods of each MRI voxel being one of 4 tissue types: GM, WM, CSF, and "other tissues." The *a priori* information consisted of anatomical templates that represented an 'average' brain and provided data about the spatial distribution of the different brain tissues. The algorithm also removes the effect of radiofrequency field inhomogeneities (Ashburner and Friston, 2000). This segmentation was checked for inconsistencies and corrected manually whenever necessary by an experienced radiologist blind to the diagnosis.

In the second stage, the edited MRI (without extracranial tissue) was used to build the Talairach grid, and ROIs were obtained by superimposing the 3D tissue masks corresponding to WM, GM, and CSF onto each subject's Talairach reference grid (Fig. 1), where the regions of interest were defined as sets of cells. On this MRI with the Talairach grid, the volumes for each tissue type were measured by totalling the data from the grid cells associated with each ROI (Desco et al., 2001) (Fig. 1).

The validity of the Talairach-based procedure as a suitable automated segmentation tool in schizophrenia research has been demonstrated previously (Andreasen et al., 1996; Kates et al., 1999). In our implementation, all manual procedures involved were performed by a single operator, thus avoiding any potential inter-rater variability. The repeatability of the SPM tissue segmentation algorithm ranged from 95 to 99% for total volumes of GM and WM, and from 89% to 99% in CSF (Chard et al., 2002; Gispert et al., 2004). The reliability of the method was assessed by repeating the whole segmentation procedure in a sample of 5 randomly selected cases. The ICC values ranged from 0.96 to 0.99 for regional GM measurements, and from 0.89 to 0.99 for the CSF data.

The ROIs included in the analysis were the frontal, parietal, temporal and occipital lobes, defined using the boundaries described previously for the Talairach method (Andreasen et al., 1996; Kates et al., 1999). ROIs were measured bilaterally, adding the left and right sides together. Intracranial volume (ICV) was measured by adding the total GM, WM and CSF volumes, including the cerebellum.

3.2. Longitudinal anatomical changes

The long-term progression of morphologic changes with atypical treatment was determined using data from 22 patients and 11 healthy controls. The longitudinal change in volume was measured as the difference between the initial and final volume of each ROI. To increase precision in the control of possible calibration drifts in the MRI equipment between the two scans, we calculated a correction factor as the quotient between the initial (baseline) and final intracranial volume (ICV) (E_ICV = ICV1 / ICV2), assuming that the total ICV should be equal in both scans. Thus, for each ROI the magnitude of the relative change in volume between the baseline (Vol1) and final (Vol2) MRI was calculated as follows:

$$\text{Longitudinal change} = \left[\left(\left(\text{Vol2} \times \text{E\_ICV}\right) - \text{Vol1}\right)/\text{Vol1}\right] \times 100$$

This value therefore expresses the percentage of change in each tissue and structure with respect to baseline values.

3.3. P300

In addition to the MRI findings, data on P300 ERP amplitude were obtained at baseline in 24 patients and 24 controls. To elicit the P300 component, the standard odd-ball paradigm was used. Accordingly, subjects heard binaural tone-bursts (duration 50 ms, rise and fall time 10 ms, and intensity 90 dB). A total of 200 tones (80% at 1000 Hz and 20% at 2000 Hz) were presented and the subjects were instructed to mentally count the

Table 2
Raw volumes and residuals (deficit or excess as compared to normal after age and ICV normalisation, see Methods) in the three groups

	Resistant (n=30)		Non-resistant (n=19)		Controls (n=44)	
	Raw	Residuals	Raw	Residuals	Raw	Residuals
ICV	1392.75 (135.1)		1450 (1410)		1397.29 (157.4)	
WM, frontal	109.83 (15.0)	9.77 (11.7)*	102.09 (13.0)	−2.81 (10.6)	103.96 (16.2)	0.12 (7.9)
GM, frontal	121.20 (19.6)	−20.08 (14.2)**	136.74 (19.2)	−8.70 (11.8)	145.06 (19.4)	−0.91 (10.4)
WM, parietal	116.73 (15.9)	13.50 (11.6)**	107.39 (13.2)	−.76 (9.5)	102.88 (15.1)	−0.91 (9.0)
GM, parietal	101.74 (13.1)	−8.77 (15.5)*	109.28 (14.2)	−14.51 (10.0)	116.08 (15.0)	1.36 (8.2)
WM, occipital	50.6 (57.0)	8.56 (5.6)**	45.08 (6.6)	.95 (5.0)	42.47 (6.6)	0.14 (3.7)
GM, occipital	56.33 (7.7)	−8.59 (7.7)**	64.15 (8.9)	−2.76 (6.6)	67.61 (9.3)	0.52 (5.6)
WM, temporal	65.56 (8.7)	3.10 (6.4)	66.24 (6.8)	1.28 (5.0)	62.88 (8.8)	1.13 (6.4)
GM, temporal	131.9 (12.4)	−6.67 (9.9)*	140.64 (13.7)	−2.63 (6.4)	140.09 (16.5)	−0.50 (6.8)

Asterisks show the comparison with controls (ANCOVA, with Sidak *post-hoc* test) *p<.05; **p<.001.
ICV: intracranial volume. No statistically significant difference was found between non-TR and controls.

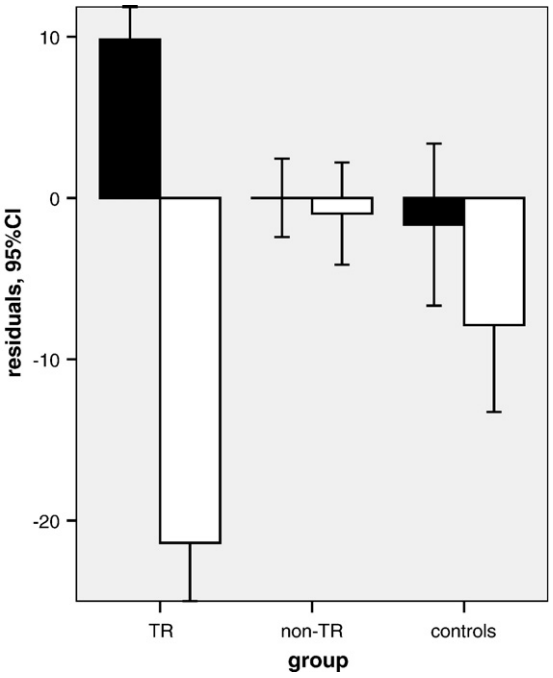

Fig. 2. Confidence intervals of baseline GM (white bars) and WM (black bars) frontal residuals by groups. TR: treatment-resistant ($n=30$); non-TR: non-treatment-resistant ($n=19$) and controls ($n=44$).

number of appearances of 2000-Hz tones, which were considered as the targets. Electroencephalograms were recorded from the mid-line parietal (Pz) site. The P300 component was defined as the most positive peak at each electrode between 250 and 500 ms after target stimulus onset. The method has been described in detail in other publications (Martin-Loeches et al., 2001; Molina et al., 2004a).

4. Statistical methods

Baseline PANSS scores (total and positive, negative and general sub-scales) were compared between the groups of patients with schizophrenia using Student's *t* tests. Clinical data about the treatment response rate (defined as the percentage change in clinical ratings from the baseline value) between the groups with schizophrenia was compared using Wilcoxon tests because the distribution of the data was not normal.

For the analysis of volume data, since age and total cranial size are factors known to affect regional cerebral volumes, their effect was removed by using the residuals from the regression models obtained from a group of 57 healthy individuals, (Molina et al., 2003), following the procedure of (Pfefferbaum et al., 1992). After this correction, volume variables were expressed as deviations from the expected volumes of healthy individuals of the same age and brain size as the patients (Molina et al., 2003). Thus, negative or positive residuals respectively represent a quantitative measurement of atrophy or hypertrophy in a given region for each subject. These data were used to test for differences in brain structure between resistant and non-resistant patients, using an ANCOVA model with sex as a covariate and *post-hoc* tests (Sidak).

To test for longitudinal changes in brain anatomy, owing to the smaller sample sizes we used non-parametric Mann–Witney *U* tests between controls and respectively TR and non-TR patients. We also compared the rates of change in both patient groups directly, using the same test. Weight changes from the baseline were compared between groups with Mann–Whitney *U* test, owing to their possible influence in cerebral measurements.

To compare P300 amplitudes among groups, we also used Kruskal–Wallis and Mann–Whitney *U* tests between each group of patients and controls, with adjustment for multiple comparisons.

Since electrophysiological and follow-up longitudinal data were not available in all patients, we repeated the comparisons of baseline MRI and P300 data only in the subgroups with all the three sets of data available (i.e, those with baseline, electrophysiological and follow-up MRI data available). These comparisons were performed using non-parametric Kruskall–Wallis and Mann–Whitney *U* tests.

In order to assess the sensitivity and specificity of the results, we performed a discriminant analysis using variables with significant between-group differences (i.e, between TR and non-TR cases) as predictive variables, with cross-validation. A receiver-operating characterisitc (ROC) curve analysis was performed using the variables previously selected by the discriminant analysis as the most discriminant between groups.

5. Results

5.1. Clinical data

There were no statistically significant differences in sex distribution between the TR and non-TR groups of the enrolled patients ($\chi^2=5.48$, df=2, $p=.14$). The control group included

Table 3
Comparison of P300 ERP amplitude (in μV) and structural longitudinal changes by group (expressed as percentage of volume changes with respect to the corresponding volume at intake, corrected for the error ratio for intracranial volume) see Methods

	Resistant	Non-resistant	Controls
P300 amplitude (μV) (*N*)	5.46 (5.8) (*n*=11)**	8.37 (5.2) (*n*=13)*	11.7 (2.7) (*n*=24)
Age	34.3 (8.7)	35.4 (10.3)	30.4 (8.5)
F:M ratio	2:9	3:10	10:14
Duration	11.9 (10.3)	9.8 (8.71)	
Frontal GM change (*N*)	7.8 (7.9) (*n*=11)**	−5.3 (5.3) *n*=11	.05 (4.0) *n*=11
Age	31.5 (5.8)	41.0 (11.3)	28.4 (6.1)
F:M ratio	4:7	5:6	5:6
Duration	10.7 (7.9)	12.3 (7.9)	
Frontal WM change	−7.58 (6.8)**	0.9 (7.6)	1.75 (6.9)
Parietal WM change	−7.57 (7.9)**	3.8 (5.7)	4.06 (7.3)
Parietal GM change	9.24 (10.1)***	−7.0 (4.1)	−3.49 (3.6)
Occipital WM change	−9.62 (7.4)**	−1.0 (6.7)	2.95 (8.0)
Occipital GM change	16.11 (10.9)***	−0.1 (6.5)	−1.40 (6.0)
Temporal WM change	−6.74 (10.3)	−1.0 (6.7)	−.20 (6.5)
Temporal GM change	2.82 (7.6)	2.6 (5.3)	−1.19 (3.3)
Months between scans	28.7 (11.8)	25.6 (9.9)	27.5 (14.0)

Age values, sex distibution and duration shown for the patients with frontal changes assessed also apply to the other strucutral assessments in this table. Age, disease duration, and time period between scans are also shown. Asterisks show statistical differences with controls *$p<.05$; **$p<.01$; ***$p\leq.001$).

Table 4
Raw volumes and residuals and P300 amplitudes in the subjects within the three subgroups with all data available

	Resistant (n=13)		Non-resistant (n=11)		Controls (n=11)	
	Raw	Residuals	Raw	Residuals	Raw	Residuals
ICV	1452.96 (132.19)		1494.20 (186.02)		1469.96 (131.10)	
WM, frontal	112.08 (16.82)	8.66 (10.59)	107.35 (12.90)	−1.08 (9.96)	103.15 (13.27)	−1.22 (7.60)
GM, frontal	129.26 (13.74)	−20.34 (10.38)**	14.82 (16.53)	−4.28 (6.15)	154.85 (17.69)	2.28 (11.36)
WM, parietal	119.27 (18.03)	12.48 (10.25)	112.07 (15.10)	0.36 (10.25)	104.76 (18.46)	−3.09 (12.72)
GM, parietal	106.37 (11.36)	−11.79 (11.15)	113.99 (9.61)	−0.99 (8.21)	117.66 (17.01)	−2.40 (7.04)
WM, occipital	51.93 (8.33)	7.98 (5.12)**	47.59 (7.09)	2.09 (5.55)	43.19 (7.83)	−1.35 (5.49)
GM, occipital	59.25 (7.06)	−9.23 (7.11)	68.51 (9.32)	0.77 (6.85)	68.32 (9.50)	−1.79 (6.28)
WM, temporal	66.16 (8.66)	2.50 (5.43)	66.18 (6.65)	−0.72 (4.96)	59.96 (6.52)	−3.99 (5.23)
GM, temporal	136.49 (10.28)	−7.90 (10.03)	142.16 (15.97)	−3.87 (5.92)	148.27 (8.39)	1.92 (6.38)
P300 amplitude (μV)	3.73 (3.33)**		9.21 (5.23)		12.54 (2.62)	

Comparison of the residuals was made using Kruskall–Wallis with Mann–Whitney *post-hoc* tests (**p<.001, significant after Bonferroni adjustment).

significantly more females and sex was therefore included as a cofactor in the analyses. There were no significant age differences between the groups of patients with schizophrenia or between those groups and the healthy subjects (t<1.3, p=ns in all cases).

Disease duration was not significantly different among the groups of patients (F=.28, df=2,47; p=.75).

There was a significant difference in clinical scores between the groups of patients with schizophrenia upon inclusion in the study, the TR patients showing the greatest severity on the total (t=2.25, df=48, p=.03) and the positive (t=2.08, df=42, p=.04) sub-scales of the PANSS (Table 1).

We found no significant differences in symptoms change rates after six months between TR (treated with clozapine) and non-TR patients (treated with olanzapine) with schizophrenia (Table 1). In both groups, there was a significant decrease in positive, general, and total scores (p<.05).

5.2. Basal anatomical data

A significant group effect was detected for GM residuals in the frontal (F=20.0, df=2, p<.001), parietal (F=4.25, df=2, 90 p=.013), temporal (F=4.56, df=2, p=.013), and occipital (F=14.08, df=2, p<.001) lobes. Moreover, significant group effects were detected for WM residuals in the frontal (F=6.67, df=2, p=.002), parietal (F=16.96, df=2, p<.001), and occipital (F=27.91, df=2, p<.001) lobes. There was no significant interaction between sex and group effects in any of the variables studied.

The *post-hoc* comparisons revealed that the TR patients had significantly less GM in the frontal and occipital regions and significantly more WM in the frontal, parietal and occipital regions as compared to the controls. These differences survived a Bonferroni adjustment for the number of comparisons (p<.005 was considered significant). These *post-hoc* comparisons also revealed the absence significant differences between the non-TR patients and controls (Table 2, Fig. 2).

5.3. Longitudinal anatomical changes

Longitudinal changes in controls were not significant (t test for one sample, with the null hypothesis of no change). With respect to longitudinal changes in GM and WM, the TR patients showed significant increases in GM from baseline to follow-up studies as compared with normal subjects in the following regions: frontal z=2.52, p=.01; parietal z=3.31, p=.001; occipital z=3.51, p=.001. Moreover, the TR patients showed a more marked decrease in WM than normal subjects in the frontal (z=2.71, p=.005), parietal (z=2.92, p=.003) and occipital (z=2.98, p=.002) regions. These differences were significant after Bonferroni adjustment.

As compared to the healthy controls, non-TR patients showed a slightly greater parietal GM decrease (z=2.13, p=.03), which was not significant after adjustment for multiple comparisons (Table 3). In the direct comparison between both patient groups, significant differences were found in the outcome of frontal (z=2.98, U=20, p=.002), parietal (z=2.75, U=24, p=.005) and occipital (z=2.69, U=25, p=.006) regions.

The patients weight increased in both TR (mean 3.2 kg, SD 4.5) and non-TR groups (mean=4.9 kg, SD=6.2). The difference in weight gain between groups was not statistically significant.

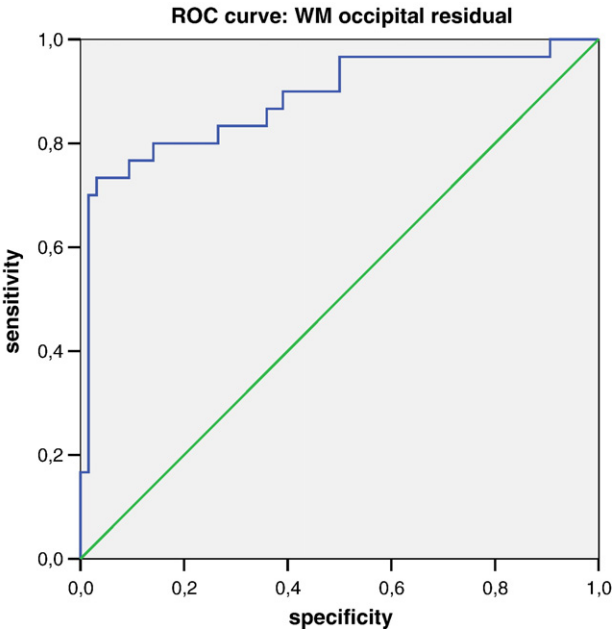

Fig. 3. ROC curve showing the capacity of the WM occipital residual to predict the classification of TR, non-TR patients and controls.

5.4. P300 potential

We observed that there were no significant differences in sex distribution, age, or in disease duration in the groups with P300 data available.

There was a significant effect of group on P300 amplitude ($\chi^2=15.50$, df=2, $p<.001$). The *post-hoc* tests revealed less amplitude in the TR cases than in the controls ($z=-3.62$, $U=30$; $p<.001$), significant after Bonferroni adjustment. The non-TR group also showed a lower amplitude of P300 ERP than the controls ($z=-2.67$, $U=95$, $p=.007$) (Table 3).

5.5. Comparison of patients with all data available

This group was made up of the patients with follow-up MRI studies, all of them also having baseline MRI and P300 data available. In this sub-sample, essentially the same results as those obtained in the group including all the patients were replicated, with significant differences between groups in frontal GM ($\chi^2=20.35$, df=2, $p<.001$), and occipital WM ($\chi^2=13.42$, df=2, $p<.001$). These differences were due to a lower frontal GM residual ($U=8$, $z=-3.67$, $p<.001$) and a higher WM occipital residual ($U=30$, $z=2.40$, $p=.01$) in TR patients as compared to controls (Table 4). A significant group effect was also detected in P300 amplitude ($\chi^2=12.82$, df=2, $p=.001$), also due to a lower amplitude in the TR patients. The other parameters with significant baseline differences between the complete TR and non-TR groups showed the same trend (less GM and more WM in TR patients).

5.6. Sensitivity and specificity

The stepwise discriminant analysis selected the occipital WM residuals as having the strongest predictive value (Wilk's $\lambda=0{,}641$, $\chi^2=21.12$, $p<.0001$). In this analysis, 76.7% of the TR patients and 85.0% of the non-TR patients were classified correctly. When the controls were also included, the stepwise procedure also included occipital WM and temporal WM residuals as predictors (76.3% of the TR patients, 79.5% of the non-TR patients and 70.0% of controls were correctly classified). In the first step, occipital WM was also selected (Wilk's $\lambda=0{,}564$, $F=35.20$, df=2,91, $p<.0001$).

The ROC curve obtained using the occipital WM residual as the classifying variable revealed an area under the curve of 0.848 (standard error 0.057, confidence interval 0.736 to 0.961, $p<.001$), as shown in Fig. 3.

6. Discussion

Consistent with our major hypothesis, we found significant clinical and biological differences between the TR and non-TR schizophrenia patients. These differences included greater clinical severity in the TR sample at baseline and in the different baseline cerebral anatomical and electrophysiological parameters. Moreover, these groups showed different changes in cerebral volumes after treatment with atypicals. The structural differences had a significant degree of sensitivity and specificity, which supports the existence of a distinct group with marked frontal deficits and a poor response to treatment within the diagnosis of schizophrenia.

In this study, the chronic schizophrenia patients with a history of resistance to conventional treatment showed an improvement with clozapine that was similar to the improvement with olanzapine observed in other schizophrenia patients with no definite history of resistance, age and duration being similar. From another perspective, the response to clozapine in our patients, with severe morphologic changes, was similar to the response to olanzapine in cases with less severe brain changes. This finding is in keeping with studies that have revealed a particular benefit of clozapine for patients with extensive volumetric changes (Lauriello et al., 1998; Molina et al., 2003, 2004b).

The combination of results obtained in this study supports the idea that within our sample of patients it is possible to differentiate at least two biologically different subtypes in chronic schizophrenia. The TR group appeared to be characterized by extensive cortical atrophy, an expansion of WM, and a decrease in P300 amplitude. The non-TR group had milder changes. It seems reasonable to speculate that both types of schizophrenia would reflect a different biological substrate. Overall, the results are consistent with those of a previous study comparing patients with TR and non-TR schizophrenia, greater volume deficits being seen in the former (Lawrie et al., 1995). From a quantitative point of view, the magnitude of the GM deficit in our patients taken together as a group, is very similar to that found in other studies in chronic patients (Premkumar et al., 2006). The greater severity of the cortical deficits in our TR patients is also consistent with a study that found that only patients with unfavourable progression had a significant deficit in the prefrontal cortex and an enlargement of the ventricles (Staal et al., 2001).

All our patients were studied years after the onset of their illness. Accordingly, we cannot know whether the basal structural changes in the TR cases were a cause or a consequence of the poor response to treatment. Despite this, to answer this issue indirectly, findings in first-episode (FE) patients could be considered. There are consistent data showing no significant differences in cortical volumes between FE patients and healthy controls (Cahn et al., 2002; Nopoulos et al., 1995) and the absence of structural differences between FE that evolved or not into schizophrenia (Molina et al., 2006), although such data may also point to more subtle anatomical alterations in other regions, such as the hippocampus (Laakso et al., 2001; Velakoulis et al., 1999) or thalamus (Gilbert et al., 2001). From this standpoint, a more marked longitudinal increase in anatomical changes has been described in the initial years of the disease in patients with unfavourable progression as compared to patients with a better prognosis (Ho et al., 2003). This finding suggests that our TR patients may have developed at least part of their morphologic changes after the onset of psychosis. If the absence of a severe cortical GM decrease in FE schizophrenia is assumed, the TR patients could have undergone a more pronounced cortical GM loss after onset.

The electrophysiological data and the longitudinal changes may help to characterize the substrate of the GM deficit in

treatment-resistant patients. The changes in P300 amplitude are consistent with the GM deficit in this group, since cortical generators have been described for that potential (Pae et al., 2003; Soltani et al., 2000). The decrease in P300 amplitude appears to support the functional importance of the GM deficit. This seems consistent with the relationship described between hypofrontality and decreased P300 amplitude in first-episode schizophrenia (Molina et al., 2005e). On the whole, patients with TR schizophrenia may show a cortical GM deficit with repercussions on information processing.

Some of the data reported here are uncommon in the published literature, such as the higher volume of WM in the TR group. Nevertheless, it has been described that there may be an excess of WM in schizophrenia, at least in one subgroup of patients (Lawrie and Abukmeil, 1998). Furthermore, patients with the deficit syndrome, characterized by poor treatment response (Buchanan et al., 1998), may also have excess parietal WM (Buchanan et al., 1993). This may be related to the excess of WM in our TR patients. The difficulty inherent to recruiting TR patients for imaging studies may have contributed to the rarity of this finding.

Occipital and temporal WM residuals were selected as classification predictors. That outcome might be due to a hypothetically greater extension of brain abnormalities in TR patients. In other words, anterior areas would be affected in both subtypes of patients, which would decrease its predictive power. That would also be coherent with the deficit in frontal GM in both types of patient in our sample. The reasons underlying the greater expansion of WM in TR patients cannot be studied with our methods and may deserve further analyses with adequate tools. It could be speculated that myelin synthesis could be stimulated by a factor with greater intensity in the TR sample; that factor could be related to a chronic glutamatergic hyperactivation state since hyperactivity is related to increased myelination in other disease states (Adamsbaum et al., 1996; Krishnan et al., 1994). Such a hyperactivity state may be indeed present in schizophrenia (Molina et al., 2005f; Volk and Lewis, 2002), and may be more decreased by clozapine, according to PET results (Cohen et al., 1997; Molina et al., 2005b).

Another unusual finding in our study is the severity of the parieto-occipital changes in the TR group. However, it has previously been shown that schizophrenia patients with "unfavourable progression" may have a greater volume deficit in posterior regions as compared to patients with a better prognosis (Mitelman et al., 2003), in agreement with our data.

Perhaps the most unexpected finding is that in the resistant group the baseline structural changes were partially reversed by clozapine. Although the impact of clozapine on brain volumes has not been studied by other groups, in recent years several similar findings have been reported in independent samples with other atypicals in mixed treatments. It has been found that atypical anti-psychotic treatment can reduce the rate of volume loss in schizophrenia (Dazzan et al., 2005; James et al., 2004; Lieberman et al., 2005), at least compared with what seems to occur with conventional treatments, or even that it is able to increase GM cortical volume (Garver et al., 2005; Molina et al., 2005d). Other recent studies in which some patients were treated with atypical and others with typical neuroleptics have reported no decreases in GM (DeLisi et al., 2004; Ho et al., 2003), with the possible exception of juvenile onset cases (Gogtay et al., 2004).

According to our results and others from basic research, the effect of clozapine in increasing GM may not be the same with olanzapine. In a recent study, the authors treated macaque monkeys with haloperidol or olanzapine for 17 to 27 months (Dorph-Petersen et al., 2005). They found both treatments to produce a diffuse and significant decrease in frontal and parietal gray and white matter volumes.

If confirmed, this special outcome might be taken into account to understand the basis of the structural deficits in schizophrenia. Concerning its substrate, a huge increase of connections would be required to explain a GM volume increase susceptible to being detected with MRI, and cortical neurogenesis in the adult brain seems unlikely. Another potential explanation refers to cortical glia. A proliferation of glial cells together with cortical hypertrophy have been observed in the prefrontal cortex of primates after treatment with typical and atypical neuroleptics (Selemon et al., 1999). A glial deficit is supported in schizophrenia by specific neuropathological evidence (Katsel et al., 2005; Rajkowska et al., 2002; Stark et al., 2004; Uranova et al., 2004). Olanzapine can increase the number of dividing glial cells in the frontal cortex of the adult rat (Wang et al., 2004), but according to our data clozapine might induce a greater glial proliferation, which can be investigated with the appropriate tools.

Nevertheless, clinical improvement was not associated with a GM increase in our olanzapine group, which should be taken into account on interpreting the possible relevance of GM changes. This implies that brain volume changes may not be associated with the degree of treatment response, Recent data further complicate the interpretation, since temporal GM expansion after a short discontinuation of treatment may be related to a worsening of negative symptoms (McClure et al., 2006).

Among the limitations of our study, in some cases no data concerning the patients' lifetime doses of anti-psychotics, P300, and longitudinal MRI changes were available, although we did observe that the sub-samples in which each of the biological variables was found were representative of the corresponding total samples. Moreover, the results from the patients with all data available replicated the same pattern. Another limitation is the different sex distribution between the controls and patients, although this was corrected for statistically in the comparisons. We did not assess the reliability of the measurements of illness duration between observers, but this probably did not affect the main differences in our study. In addition, intracranial size, the primary morphological brain difference between men and women, was corrected for when calculating the residuals used in the study. Moreover, other clinical dimensions, such as deficit schizophrenia (Carpenter et al., 1988) that might be related to resistance were not assessed in our sample. Finally, among the limitations to our study no multivariate analysis including the three types of data was performed owing to the reduced number of cases with no missing data for each of the three data sources.

Although a combined analysis is warranted in a multi-dimensional design, our choice was to provide the most robust evidence for each data set (clinical, MRI, P300).

In conclusion, the data presented here are consistent with the possibility that schizophrenia patients who are resistant and non-resistant to conventional treatment have different clinical and biological characteristics. TR patients are characterized by a more marked clinical severity as well as a GM deficit, increased WM, a decreased P300 amplitude, and a partial reversibility of volume deficits with clozapine. A genetic study of these two types of patients would therefore be of enormous interest.

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