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Effect of clozapine on white matter integrity in patients with schizophrenia: A diffusion tensor imaging study

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ABSTRACT

Several diffusion tensor imaging (DTI) studies have reported disturbed white matter integrity in various brain regions in patients with schizophrenia, whereas only a few studied the effect of antipsychotics on DTI measures. The aim of this study was to investigate the effect of 12 weeks of clozapine treatment on DTI findings in patients with schizophrenia, and to compare the findings with those in unaffected controls. The study included 16 patients with schizophrenia who were assessed with the Positive and Negative Syndrome Scale, a neurocognitive test battery, and DTI at baseline and 12 weeks after the initiation of clozapine treatment. Eight unaffected controls were assessed once with the neurocognitive test battery and DTI. Voxel-wise analysis of DTI data was performed via tract-based spatial statistics (TBSS). Compared with the control group, the patient group exhibited lower fractional anisotropy (FA) in 16 brain regions, including the bilateral superior longitudinal fasciculi, inferior fronto-occipital fasciculi, superior and inferior parietal lobules, cingulate bundles, cerebellum, middle cerebellar peduncles, and left inferior longitudinal fasciculus, whereas the patients had higher FA in six regions, including the right parahippocampus, left anterior thalamic radiation, and right posterior limb of the internal capsule before clozapine treatment. After 12 weeks of treatment with clozapine, white matter FA was increased in widespread brain regions. In two of the regions where FA had initially been lower in patients compared with controls (left inferior fronto-occipital fasciculus and superior parietal lobule), clozapine appeared to increase FA. An improvement in semantic fluency was correlated with the increase in FA value in the left inferior fronto-occipital fasciculus. An increase in FA following 12 weeks of treatment with clozapine suggests that this treatment alters white matter microstructural integrity in patients with schizophrenia previously treated with typical and/or atypical antipsychotics and, in some locations, reverses a previous deficit.

1. Introduction

Neuroimaging and neurophysiological studies suggest that a disturbance in connectivity between different brain regions is responsible for the clinical symptoms of schizophrenia (Bramon et al., 2004; Meyer-Lindenberg et al., 2005). Dysconnectivity is thought to result from aberrant wiring of connections during development, and from aberrant synaptic plasticity. Findings of dysconnectivity (Meyer-Lindenberg et al., 2005) and white matter abnormalities (Dracheva et al., 2006) in the brains of patients with schizophrenia have led to an increase in the use of diffusion tensor imaging (DTI), a magnetic resonance imaging (MRI) technique used to evaluate structural connectivity in the human brain in specific white matter bundles. Fractional anisotropy (FA) is a DTI parameter widely used to indicate the motional anisotropy of water molecules; a reduction in FA may be indicative of white matter impairment (Beaulieu, 2002; Assaf and Pasternak, 2008).

Numerous DTI studies have compared findings in patients with schizophrenia with findings in normal controls, and most have observed lower FA in diverse white matter regions in patients than in controls (Kubicki et al., 2005; Kanaan et al., 2005; Buchsbaum et al., 2006a; Mitelman et al., 2006; Ellison-Wright and Bullmore, 2009). The corpus callosum, cingulum, uncinate fasciculus, superior and inferior longitudinal fasciculus, fornix, and anterior limb of the internal capsule are some of the brain regions that have frequently been reported to have lower FA, based on region of interest (ROI) studies (Foong et al., 2000; Sun et al., 2003; Buchsbaum et al., 2006b; Ashtari et al., 2007; Mori et al., 2007;

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Price et al., 2008; Karlsgodt et al., 2008; Fitzsimmons et al., 2009). Voxel-based studies that have analyzed white matter throughout the brain, rather than preselecting limited regions of the brain for analysis, report even more brain regions with lower FA (Ellison-Wright and Bullmore, 2009; Melonakos et al., 2011; Sugranyes et al., 2012; Roalf et al., 2013). In a meta-analysis of 15 voxel-based DTI studies, 407 patients with schizophrenia were compared with 383 controls, and 112 coordinates with lower FA (number of coordinates in the studies ranging from 2 to 19) were detected in the patients (Ellison-Wright and Bullmore, 2009). A more recent meta-analysis of 23 studies reported inconsistent findings, with coordinates representing low FA scattered across the brain in patients with schizophrenia; the genu and splenium of the corpus callosum, the right anterior corona radiata, and the posterior thalamic radiation bilaterally were the most frequently reported regions (Melonakos et al., 2011).

Differences in reports of low FA in patients with schizophrenia have been associated with methodological and clinical differences, and the possible effects of antipsychotics (Buchsbaum et al., 2006b; Kubicki et al., 2007; Konrad and Winterer, 2008; Melonakos et al., 2011; Henze et al., 2012). Although the effect of antipsychotics on DTI measures is an important parameter that should be controlled for when interpreting relevant findings, our knowledge of the effect is quite limited. Some cross-sectional studies on the relationship between antipsychotic dose and FA values found that there was not an association (Foong et al., 2000), whereas others reported a negative association (Kuroki et al., 2006) or a positive association (Minami et al., 2003; Okugawa et al., 2004). The literature includes only two longitudinal follow-up studies on the effects of antipsychotics on DTI. Garver et al. (2008) assessed whole-brain mean diffusivity (Dm) in 13 acutely psychotic, drug-free patients with schizophrenia before and after 28 days of antipsychotic drug treatment (risperidone, ziprasidone, or haloperidol), and reported that there was a significant decrease in Dm in the right pyramidal tract, left temporal lobe, and cingulate gyrus in the treatment responders. In the second study, first episode drug-naïve patients with schizophrenia were reported to have a significant decrease in FA of white matter around the bilateral anterior cingulate gyrus and the right corona radiata of the frontal lobe compared with controls, following 6 weeks of antipsychotic treatment that included several typical and atypical antipsychotics (Wang et al., 2013).

Antipsychotic drugs induce anatomical and molecular changes in the brain, and their effect on neuroplasticity via modification of synaptic connections is thought to be important to their mechanism of action (Konradi and Heckers, 2001). Clozapine is a prototypical atypical antipsychotic that has been shown to be effective in the treatment of refractory schizophrenia (Kane et al., 1988; McEvoy et al., 2006). Preclinical studies have found that clozapine has a positive effect on neuroplasticity (Critchlow et al., 2006) and myelin integrity (Xu et al., 2009; Xu et al., 2010). Structural imaging studies showed that clozapine could reverse the increase in basal ganglia volume induced by typical antipsychotics (Chakos et al., 1995) and that the reduction in the severity of negative symptoms in response to clozapine was associated with an increase in right prefrontal gray matter volume (Arango et al., 2003). A magnetic resonance spectroscopy (MRS) study reported that 8 weeks of clozapine treatment resulted in an increase in the *N*-acetyl aspartate/creatine ratio in the left dorsolateral prefrontal cortex, which was considered evidence of the positive effects of clozapine on neuronal functioning and integrity (Ertugrul et al., 2009). In that study the left dorsolateral prefrontal cortex was the only region studied with MRS and no data regarding possible alterations in other brain regions were obtained. Clozapine was also shown to affect brain metabolism and function in multiple brain regions. An animal study found that clozapine caused metabolic changes in 32 brain regions acutely and 19 regions chronically (Wotanis et al., 2003). Human neuroimaging studies showed that clozapine had effects in various brain regions, including the limbic cortex (Cohen et al., 1997), hippocampus (Lahti et al., 2003), anterior cingulate cortex (Lahti et al., 2004), and components of the fronto-striato-thalamic pathway, especially the prefrontal cortex (Molina Rodríguez et al., 1996; Lahti et al., 2003; Ertugrul et al., 2009).

To the best of our knowledge, no study has investigated the effect of clozapine treatment on white matter integrity based on DTI measures. In view of the results of preclinical and clinical studies, it is plausible to expect that clozapine will alter connectivity by changing white matter integrity. As such, the aim of the present study was to investigate the effect of 12 weeks of clozapine treatment on white matter in patients with schizophrenia based on DTI measures, and to compare the findings with those in unaffected controls. A whole-brain approach was chosen for the following reasons: (1) Patients with schizophrenia have been reported to have lower FA in multiple brain regions compared with controls (Kubicki et al., 2005; Kanaan et al., 2005; Buchsbaum et al., 2006a; Mitelman et al., 2006; Ellison-Wright and Bullmore, 2009). (2) The results of earlier neuroimaging studies on clozapine have indicated effects in multiple brain regions, not in any specific anatomical region (Molina Rodríguez et al., 1996; Cohen et al., 1997; Lahti et al., 2003; Lahti et al., 2004; Ertugrul et al., 2009). (3) As the first DTI study on the effect of clozapine in patients with schizophrenia, the present exploratory study aimed to detect all probable changes anywhere in brain via voxel-based analysis. For voxel-based analysis, the present study used tract-based spatial statistics (TBSS), which is suggested to improve the sensitivity, objectivity, and interpretability of the analysis of multi-subject diffusion imaging (Smith et al., 2006), and is increasingly being used in the study of white matter organization in patients with schizophrenia (Chen et al., 2013; Lee et al., 2013; Roalf et al., 2013).

2. Methods

2.1. Participants

This study was conducted at the Department of Psychiatry in the Hacettepe University School of Medicine. The study included 16 consecutive outpatients diagnosed with schizophrenia based on DSM-IV criteria that were treated with clozapine due to treatment refractoriness or intolerance to previous typical and/or atypical antipsychotics. In addition, eight unaffected controls matched to the patients on age, gender, and level of education were recruited for the study. Exclusion criteria were alcohol or drug abuse/dependency, any major medical or neurological disorder, and history of head trauma that would affect neuroradiological assessment. The study protocol was approved by the Hacettepe University School of Medicine Ethics Committee (LUT 10/02), and all participants gave informed consent. The Structured Clinical Interview for DSM Disorders (First, 1997; Corapcioglu et al., 1999) was used to confirm the patients' diagnoses and to exclude any Axis-I disorder in the controls. Table 1 presents the demographic and clinical characteristics of the two groups.

2.2. Procedure

Psychopathology, neurocognitive functioning, and DTI in the patient group were assessed at baseline while the patients were using their current antipsychotic medication, and again 12 weeks after the initiation of clozapine treatment. Psychopathology was assessed using the Positive and Negative Syndrome Scale (PANSS) (Kay et al. 1987; Kostakoglu et al., 1999) and the Clinical Global Impression Scale (CGIS). The neuropsychological test battery included the following tests: Digit Span Test (Wechsler, 1987); Auditory Consonant Trigram Test (Anil et al., 2003); Word and Category Fluency Test (Benton and Hamsher, 1978); Rey Auditory-Verbal Learning Test (RAVLT) (Lezak, 1995); Wechsler Visual Memory Scale-Visual Reproduction Scale (Wechsler, 1987); and Trail Making A and B (Spreen and Strauss, 1998).

Clozapine treatment was initiated following baseline assessment, and the current antipsychotic medication was discontinued following gradual tapering.

Table 1
Demographic and clinical variables of the study groups.

	Patients ($n=16$) Mean (S.D.)	Controls ($n=8$) Mean (S.D.)	Analysis p
Age	34.3 (11.25)	33.88 (11.70)	1.00
Gender			1.00
Male	10	5	
Female	6	3	
Years of education	10.94 (3.66)	11.38 (3.20)	0.88
Age of onset	22.25 (7.46)		
Duration of illness[a]	11.62 (9.17)		
Hospitalization number	1.81 (1.60)		
Medication dose, mg/day[b]	572.54 (268.81)		
Medication type			
Typical	1		
Atypical	10		
Both types	3		
Unmedicated	2		
Clozapine dose, mg/day	317.19 (98.62)		

[a] Years.
[b] Chlorpromazine-equivalent dosage at baseline.

The clozapine dose was titrated according to each patient's clinical status. The mean clozapine dose at week 12 of treatment was 317.19 ± 98.62 mg/day. All patients completed the assessment after 12 weeks of treatment. The patients' white blood cell counts were assessed weekly. No serious adverse effects were observed. Controls were assessed once with DTI and the neuropsychological test battery.

2.3. Diffusion tensor imaging

2.3.1. Image acquisition

All participants that met the inclusion criteria underwent structural MRI with DTI of the brain using a 1.5-Tesla scanner equipped with a 30 mT/m gradient system and an eight-channel phase-array head coil (Symphony, Tim, Siemens, Erlangen, Germany). Sagittal and axial T1-weighted images (repetition time (TR)/echo time (TE: 500/50 ms; matrix: 192×256; slice thickness: 5 mm; interslice gap: 10%) and axial T2-weighted images (TR/TE: 4500/100 ms; matrix: 192×256; slice thickness: 3 mm; interslice gap: 0) were obtained to exclude the presence of lesions and to obtain anatomical data for DTI planning.

DTI was performed using a single-shot echo-planar imaging sequence in the axial plane, parallel to the anterior–posterior commissures covering the entire brain (TR/TE: 5814 /98 ms; maximum b factor: 1000 s/mm^2; 64 independent directions; field of view: 23 cm; matrix: 128×128; slice thickness: 3 mm; interslice gap: 0).

2.3.2. Processing and analysis of DTI data

TBSS (Smith et al., 2006)—included in the FSL v.4.0 software package (Centre for Functional MR Imaging of the Brain, Oxford University, Oxford, UK, http://www.fmrib.ox.ac.uk/fsl)—was used for whole-brain voxel-wise statistical analysis. Preprocessing of the diffusion-weighted data included correction of head motion and Eddy current, diffusion tensor fitting (FSL DTIFit), and calculation of FA maps. FA maps were registered and aligned to the average space as input for TBSS, and the mean FA skeleton was computed. A permutation-based inference with 500 permutations was performed for voxel-wise statistics on FA. Then, threshold-free cluster enhancement output was obtained and corrected for multiple comparisons. Family-wise error (FWE)-corrected maps were obtained with P values < 0.05. Then, cluster-based thresholding was performed, which included Gaussian smoothing, application of a threshold (t: 1.5), and forming clusters from 26 neighboring suprathreshold voxels. TBSS maps were obtained for the following two comparisons: (1) Patients vs. controls at baseline; (2) Patients at baseline vs. patients following clozapine treatment.

White matter coordinates of clusters with significant FA change on the corrected threshold-cluster extent voxel maps were extracted as regions of interest (ROIs), and were registered to and overlaid onto an anatomical Montreal Neurology Institute (MNI) template (www.fmrib.ox.ac.uk/fsl/data/FMRIB58). The ROIs were noted according to the Johns Hopkins University WM tractography atlas and the International Consortium for Brain Mapping DTI-81 WM atlas, which are available in FSL. Next, mean FA of the ROIs was calculated for each participant to be used in correlational analysis.

2.4. Statistical analysis

Statistical analysis was performed using SPSS v.15.0 for Windows. Comparisons of baseline FA values in the patient and control groups, and of the FA values in the patients before clozapine and after clozapine treatment, were performed via the TBSS method (family-wise error (FWE)-corrected, $P < 0.05$). Patients and controls were compared using the Mann–Whitney U-test for numerical variables and the chi-square test for nominal variables. The normality of PANSS, CGIS, and neurocognitive test scores in the patients was assessed using the Shapiro–Wilk test, and in cases of normal variance the paired sample t-test was used; otherwise, the Wilcoxon-signed rank test was used to compare PANSS, CGIS, and neurocognitive scores in the patients before and after clozapine treatment. The relationships between changes in PANSS, CGIS, and neurocognitive test scores, and changes in FA scores after 12 weeks of clozapine treatment, were evaluated using Spearman's correlation analysis. Correction for multiple comparisons was done for correlational analysis by the Machado method (Machado, 2007). The adjusted significance threshold, α, was 0.004 for correlational analysis.

3. Results

3.1. Demographic and clinical characteristics of the study groups

There were not any significant differences in mean age, level of education, or gender ratio between the schizophrenia and control groups (Table 1). The comparison of baseline and week-12 psychopathology assessments in the schizophrenia group showed that the PANSS total score, as well as the PANSS positive, negative, and general psychopathology subscale scores, improved significantly ($P < 0.001$), as did the CGIS score ($P=0.001$) (Table 2). In terms of neurocognitive test performance, the patients had significantly lower word fluency ($P=0.038$), category fluency (animal ($P=0.027$), name ($P=0.000$), and alternation ($P=0.000$)) scores, auditory consonant trigrams (ACT) score ($P=0.016$), RAVLT immediate memory ($P=0.045$), RAVLT learning ($P=0.023$), and RAVLT cumulative learning ($P=0.011$) scores, Trail Making-A score ($P=0.006$), and visual reproduction-delayed recall score ($P=0.027$) than the controls at baseline. Significant improvement in category fluency name ($P=0.045$) and alternation scores ($P=0.045$), RAVLT immediate memory ($P=0.019$) and RAVLT learning scores ($P=0.004$), and visual reproduction-delayed recall score ($P=0.001$) was observed in the patient group after 12 weeks of clozapine treatment (Table 2).

3.2. Comparison of baseline FA measures in the schizophrenia and control groups

TBSS showed that FA was significantly lower at baseline in 16 regions (including the bilateral superior longitudinal fasciculi, inferior fronto-occipital fasciculi, cingulate bundles, inferior parietal lobules, superior parietal lobules, cerebellum, middle cerebellar peduncles, left inferior longitudinal fasciculus, and sagittal stratum) in the patient group than in the control group. On the other hand, TBSS showed that FA was significantly higher at baseline in six regions (including the right parahippocampus, posterior limb of the internal capsule, sagittal stratum, left anterior thalamic radiation, and bilateral optic radiation) in the patient group than in the control group. Fig. 1 shows the regions that differed significantly ($P < 0.05$) between the two groups according to TBSS. The coordinates and the mean FA values for these regions are shown in Table 3.

3.3. Comparison of FA measures at baseline and after 12 weeks of clozapine treatment in the patients with schizophrenia

As compared with baseline values, TBSS showed that FA in the patient group increased significantly after 12 weeks of clozapine treatment in 31 brain regions, including the corpus callosum, bilateral inferior longitudinal fasciculi, uncinate fasciculi, thalamus, left parahippocampus, fornix, cingulate bundle, inferior fronto-occipital fasciculus, superior parietal lobule, and cerebellum. There was no brain region in which FA decreased after

Table 2
The clinical and neuropsychological results of the study groups.

	Controls $n=8$	Patients (visit 1) $n=16$	Patients (visit 2) $n=16$	Statistics	
	Mean (S.D.)	Mean (S.D.)	Mean (S.D.)	Control/visit 1[a]	Visit 1/Visit 2[b,c]
PANSS total		83.25 (12.57)	56.31 (12.19)		$t=7.087$[b] **$p=0.000$**
PANSS positive		22.44 (4.03)	12.44 (3.58)		$t=7.402$[b] **$p=0.000$**
PANSS negative		21.06 (4.15)	16.00 (3.86)		$t=5.589$[b] **$p=0.000$**
PANSS general psychopathology		39.75 (6.94)	28.19 (6.40)		$t=6.829$[b] **$p=0.000$**
CGI-S		4.62 (0.81)	2.81 (0.91)		$z=-3.449$[c] **$p=0.001$**
Word fluency	28.62 (9.41)	18.44 (10.87)	21.62 (12.74)	$z=-2.085$ **$p=0.038$**	$z=-1.877$[c] $p=0.061$
Category fluency-animal	19.88 (4.29)	14.81 (6.53)	14.31 (7.31)	$z=-2.179$ **$p=0.027$**	$z=-0.126$[c] $p=0.900$
Category fluency-name	24.25 (3.01)	15.38 (6.08)	17.19 (5.89)	$z=-3.283$ **$p=0.000$**	$z=-2.000$[c] **$p=0.045$**
Category fluency-alternation	9.12 (1.80)	4.69 (2.60)	6.06 (2.72)	$z=-3.289$ **$p=0.000$**	$z=-2.611$[c] **$p=0.009$**
Digit span-forward	6.00 (1.77)	5.44 (2.10)	6.00 (2.42)	$z=-0.475$ $p=0.653$	$z=-1.557$[c] $p=0.120$
Digit span-backward	5.88 (2.90)	4.31 (1.99)	4.62 (1.96)	$z=-1.238$ $p=0.238$	$z=-0.914$[c] $p=0.361$
Digit span total	11.88 (4.36)	9.75 (3.47)	10.62 (4.10)	$z=-0.954$ $p=0.350$	$z=-1.878$[c] $p=0.060$
ACT	47.50 (8.32)	36.25 (10.45)	38.81 (8.40)	$z=-2.361$ **$p=0.016$**	$z=-1.427$[c] $p=0.154$
RAVLT-immediate recall	6.50 (1.77)	4.75 (1.81)	6.12 (2.03)	$z=-2.018$ **$p=0.045$**	$t=-2.627$[b] **$p=0.019$**
RAVLT-5 learning	11.00 (2.07)	8.25 (2.89)	8.81 (2.93)	$z=-2.285$ **$p=0.023$**	$t=1.013$[b] $p=0.327$
RAVLT 1-5 cumulative learning	47.50 (10.10)	33.75 (11.91)	39.2 (13.07)	$z=-2.483$ **$p=0.011$**	$t=-3.40$[b] **$p=0.004$**
RAVLT- delayed recall	9.12 (3.27)	7.19 (2.74)	6.4 (2.94)	$z=-1.392$ $p=0.172$	$t=-1.441$[b] $p=0.170$
WMS-visual reproduction-immediate	31.38 (4.14)	25.88 (9.08)	27.25 (8.97)	$z=-1.472$ $p=0.153$	$z=-0.804$[c] $p=0.422$
WMS-visual reproduction-delayed	28.50 (6.52)	18.94 (10.39)	26.25 (10.12)	$z=-2.213$ **$p=0.027$**	$z=-3.184$[c] **$p=0.001$**
Trail making A- time	31.00 (7.78)	69.50 (65.55)	63.12 (66.78)	$z=-2.696$ **$p=0.006$**	$z=-1.657$ $p=0.098$
Trail making B-time	100.00 (65.06)	135.54 (78.78)	103.00 (57.62)	$z=-0.991$ $p=0.351$	$z=-1.836$ $p=0.066$

[a] Mann–Whitney *U*-test.
[b] Paired samples *t*-test.
[c] Wilcoxon signed-rank test. ACT: Auditory Consonant Trigram. RAVLT: Rey's Auditory–Verbal Learning Test, WMS: Wechsler Memory Scale

clozapine treatment. Fig. 2 shows the regions with a significant increase in FA ($P<0.05$) between baseline and 12 weeks of clozapine treatment according to TBSS; the coordinates and the mean FA values for these regions are presented in Table 4.

The effect of clozapine treatment on the regions that were found to be significantly different between patients at baseline and controls by TBSS (the regions presented in Table 3) was examined in an additional analysis. The mean FA values of the same cluster of voxels were calculated for post-clozapine data in patients with schizophrenia. The baseline and week-12 FA values of these regions were compared by paired samples *t*-test, and significant increases in the FA in the left superior parietal lobule ($t=-2.37$, $P<0.05$) and the left inferior fronto-occipital fasciculus ($t=-6.12$, $P<0.001$) were detected.

3.4. Relationship between change in FA values and change in psychopathology and neurocognitive scores

The correlations between FA values and psychopathology and neurocognitive test scores that significantly changed after clozapine treatment were computed. Improvement in category fluency was significantly correlated with the observed increase in FA in the left inferior fronto-occipital fasciculus after correction for multiple comparisons ($r=0.738$, $P=0.001$) (Fig. 3). There were no other significant correlations between the change in FA values and psychopathology and neurocognitive test scores after correction for multiple comparisons.

4. Discussion

The aim of the present study was to investigate the effect of clozapine on white matter integrity in patients with schizophrenia based on measurement of FA, and to compare FA values in patients with schizophrenia with values in controls. The present findings show that the patients with schizophrenia had lower baseline FA values than the controls in 16 regions, including the left inferior longitudinal fasciculus, bilateral superior longitudinal fasciculi, inferior fronto-occipital fasciculi, cingulate bundles, superior and inferior parietal lobules, cerebellum, and middle cerebellar

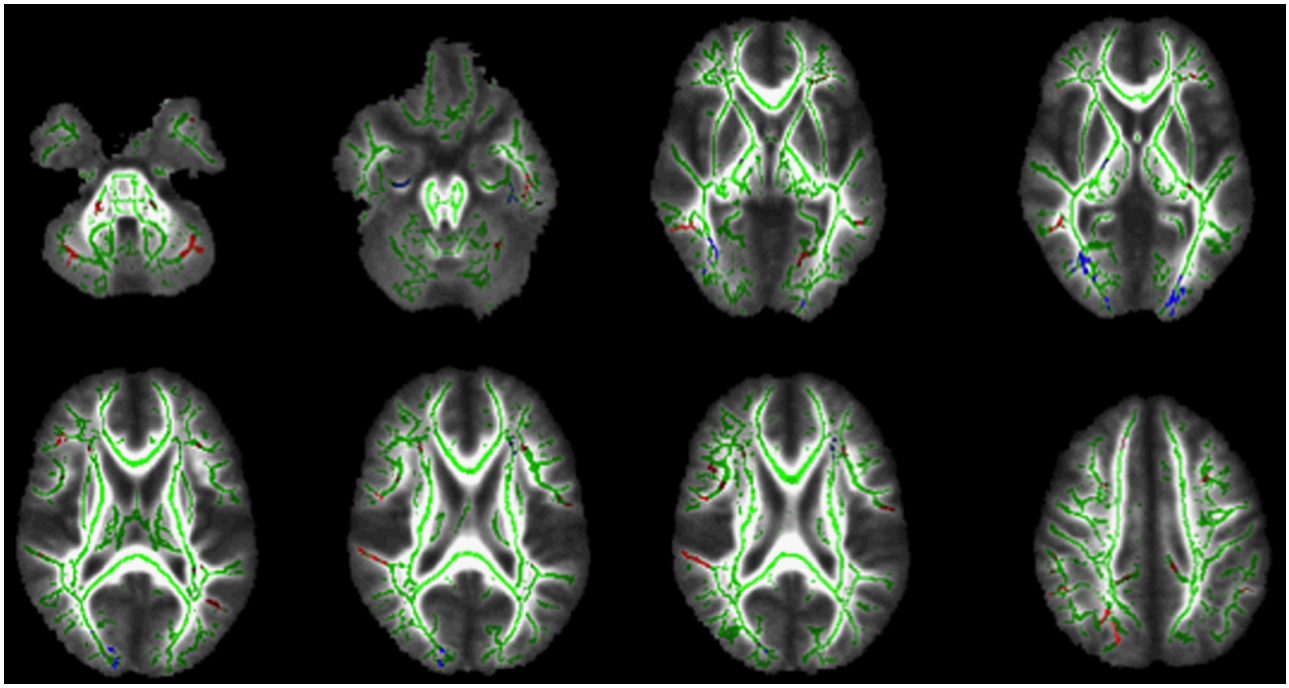

Fig. 1. TBSS (FWE-corrected threshold-cluster extend voxel *P* maps) show regions of significantly lower (in red) and higher (in blue) FA in patients with schizophrenia, as compared to gender- and age-matched controls ($P < 0.05$). An FA skeleton projected onto a mean FA map is shown in green. (For interpretation of the references to color in this figure legend,the reader is referred to the web version of this article)

Table 3
The mean FA values and MNI coordinates of the brain regions which are significantly different in patients at baseline compared with controls according to TBSS (FWE corrected, $P < 0.05$).

Regions of brain	MNI coordinates			Patients (visit 1) ($n=16$)	Controls ($n=8$)
	x	*y*	*z*	Mean (S.D.)	Mean (S.D.)
Schizophrenia FA < control FA					
Superior longitudinal fasciculus (R)	44	78	73	0.422 (0.127)	0.443 (0.105)
Superior longitudinal fasciculus (L)	143	80	70	0.302 (0.149)	0.381 (0.100)
Inferior longitudinal fasciculus(L)	137	108	53	0.336 (0.010)	0.362 (0.087)
Inferior frontooccipital fasciculus (R)	49	156	66	0.235 (0.086)	0.269 (0.076)
Inferior frontooccipital fasciculus (L)	112	152	68	0.279 (0.067)	0.308 (0.055)
Sagittal stratum (L)	124	90	80	0.540 (0.099)	0.576 (0.051)
Cingulum (R)	82	94	109	0.287 (0.067)	0.318 (0.089)
Cingulum (L)	99	96	109	0.306 (0.099)	0.357 (0.143)
Inferior parietal lobule (R)	36	92	89	0.255 (0.108)	0.366 (0.129)
Inferior parietal lobule (L)	135	64	86	0.240 (0.108)	0.276 (0.124)
Superior parietal lobule (R)	77	81	131	0.233 (0.099)	0.280 (0.121)
Superior parietal lobule (L)	104	88	112	0.184 (0.082)	0.292 (0.092)
Cerebellum (R)	58	61	38	0.260 (0.061)	0.303 (0.083)
Cerebellum(L)	126	65	38	0.225 (0.056)	0.256 (0.079)
Middle cerebellar peduncle (R)	74	93	41	0.555 (0.101)	0.572 (0.071)
Middle cerebellar peduncle (L)	101	90	41	0.466 (0.083)	0.479 (0.078)
Schizophrenia FA > control FA					
Parahippocampus(R)	63	105	49	0.257 (0.030)	0.245 (0.023)
Optic radiation (R)	72	31	80	0.252 (0.050)	0.239 (0.091)
Optic radiation (L)	109	35	77	0.238 (0.071)	0.192 (0.059)
Posterior limb of internal capsule (R)	71	114	76	0.673 (0.068)	0.667 (0.035)
Anterior thalamic radiation (L)	114	153	89	0.328 (0.043)	0.319 (0.064)
Sagittal stratum (R)	58	57	75	0.403 (0.068)	0.358 (0.071)

L: left. R: right

peduncles, and that after 12 weeks of clozapine treatment, FA values in the patients with schizophrenia increased in 31 regions, including the splenium, genu and the body of the corpus callosum, bilateral inferior longitudinal fasciculi, uncinate fasciculi, thalamus, anterior thalamic radiation, middle cerebellar peduncles, left superior longitudinal fasciculus, inferior fronto-occipital fasciculus, parahippocampus, fornix, cingulate bundle, superior parietal lobule and cerebellum.

The observation that at baseline, FA was lower in multiple brain regions in patients with schizophrenia in this study is in accordance with earlier DTI studies that have consistently reported lower FA in various white matter regions in patients with

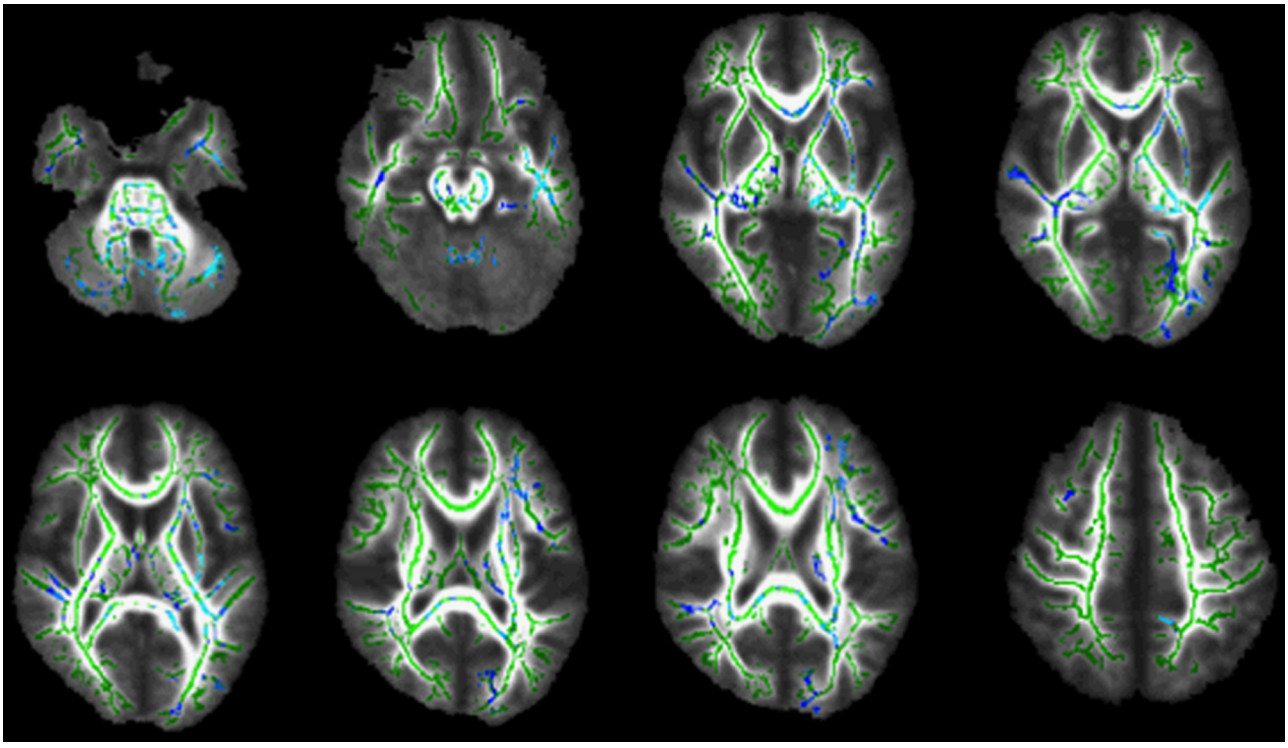

Fig. 2. TBSS (FWE-corrected threshold-cluster extend voxel *P* maps) shows regions with significantly higher FA (in blue) in patients with schizophrenia following 12 weeks of clozapine treatment, as compared to baseline ($P < 0.05$). An FA skeleton projected onto a mean FA map is shown in green. (For interpretation of the references to color in this figure legend,the reader is referred to the web version of this article)

Table 4
The mean FA values and MNI coordinates of the brain regions which are significantly different in patients at week 12 of clozapine treatment compared with baseline according to TBSS (FWE corrected, $P < 0.05$).

Regions of brain	MNI coordinates			Before clozapine (visit 1) ($n=16$)	After clozapine (visit 2) ($n=16$)
After clozapine FA > Before clozapine FA	*x*	*y*	*z*	Mean (S.D.)	Mean (S.D.)
Parahippocampus (L)	119	102	50	0.212 (0.043)	0.227 (0.036)
Fornix (L)	93	113	88	0.268 (0.090)	0.277 (0.063)
Cingulum (L)	101	96	109	0.231 (0.110)	0.286 (0.105)
Uncinate fasciculus (R)	70	147	62	0.305 (0.057)	0.431 (0.086)
Uncinate fasciculus (L)	109	142	59	0.274 (0.137)	0.309 (0.123)
Superior longitudinal fasciculus (L)	133	77	74	0.396 (0.145)	0.408 (0.146)
Inferior longitudinal fasciculus (R)	46	111	57	0.410 (0.070)	0.429 (0.069)
Inferior longitudinal fasciculus (L)	133	114	52	0.385 (0.119)	0.426 (0.084)
Inferior frontooccipital fasciculus (L)	119	179	67	0.218 (0.088)	0.253 (0.087)
Sagittal stratum (L)	126	86	80	0.545 (0.081)	0.570 (0.054)
Optic radiation (L)	111	34	77	0.213 (0.063)	0.220 (0.056)
Splenium of corpus callosum	85	94	88	0.799 (0.166)	0.801 (0.201)
Genu of corpus callosum	91	150	74	0.693 (0.179)	0.730 (0.160)
Body of corpus callosum	84	129	96	0.486 (0.155)	0.495 (0.161)
Anterior limb of internal capsule (L)	110	135	83	0.368 (0.101)	0.394 (0.117)
Posterior limb of internal capsule (R)	65	108	78	0.627 (0.085)	0.645 (0.102)
Posterior limb of internal capsule (L)	112	108	78	0.741 (0.053)	0.765 (0.053)
Thalamus (R)	81	117	84	0.370 (0.081)	0.374 (0.050)
Thalamus(L)	99	113	84	0.330 (0.044)	0.332 (0.061)
Anterior thalamic radiation (R)	82	116	71	0.451 (0.092)	0.486 (0.127)
Anterior thalamic radiation (L)	100	112	87	0.315 (0.057)	0.331 (0.057)
External capsule (L)	123	115	78	0.296 (0.050)	0.300 (0.065)
Superior parietal lobule (L)	100	82	122	0.208 (0.122)	0.228 (0.088)
Inferior parietal lobule (R)	40	84	91	0.333 (0.159)	0.343 (0.171)
Acustic radiation (R)	44	104	80	0.294 (0.123)	0.308 (0.127)
Acustic radiation (L)	128	95	78	0.462 (0.071)	0.472 (0.099)
Cerebellum (L)	128	61	41	0.210 (0.045)	0.238 (0.048)
Middle cerebellar peduncle (R)	74	94	35	0.248 (0.143)	0.277 (0.168)
Middle cerebellar peduncle (L)	102	93	35	0.277 (0.158)	0.315 (0.157)
Corticospinal tract (R)	79	109	51	0.5453 (0.126)	0.564 (0.093)
Corticospinal tract (L)	100	104	51	0.600 (0.139)	0.611 (0.168)

L: left. **R**: right.

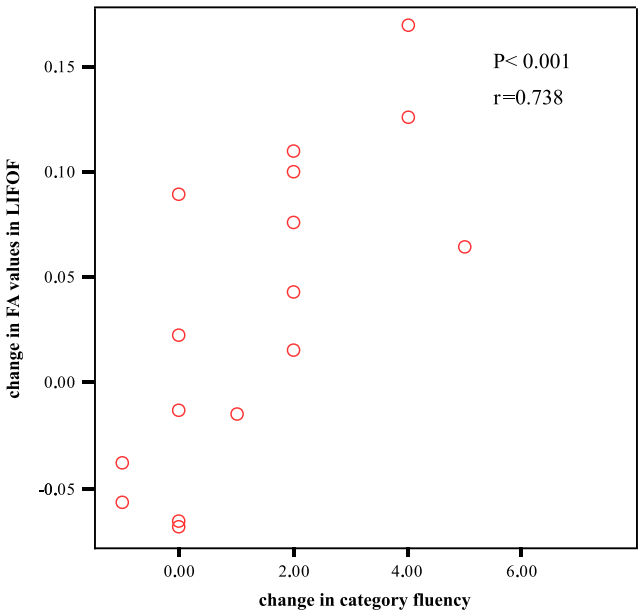

Fig. 3. Correlation between the change in FA values in left inferior fronto-occipital fasciculus (LIFOF) and change in category fluency scores after 12 weeks of clozapine treatment in patients with schizophrenia.

schizophrenia than in controls (Kakeda and Korogi, 2010; Melonakos et al., 2011). White matter changes in patients with schizophrenia have been explained by numerous theories (Ellison-Wright and Bullmore 2009). According to one theory, disturbance in white matter integrity is uniform in the brain, possibly due to genetic abnormalities in the proteins that control myelination (Konrad and Winterer, 2008). Several studies found abnormal expression of myelin/oligodendrocyte-related genes in patients with schizophrenia, suggesting a disruption in oligodendrocyte function (Hakak et al., 2001; Tkachev et al., 2003; Sugai et al., 2004; Dracheva et al., 2006). Another theory proposes that rather than uniform white matter reduction, specific white matter tracts are affected, either as a cause of or a consequence of a disorder in the gray matter regions that they connect (Konrad and Winterer, 2008; Ellison-Wright and Bullmore, 2009). The results of the present study show that FA was lower in the brain regions that are components of specific white matter tracts important to the pathophysiology of schizophrenia. The present findings confirmed earlier reports of low FA in the superior longitudinal fasciculus (Shergill et al., 2007; Seal et al., 2008), inferior longitudinal fasciculus (Ashtari et al., 2007), inferior fronto-occipital fasciculus (Seal et al., 2008), cingulate bundles (Takei et al., 2009), parietal lobes (Ardekani et al., 2003; Chen et al. 2013), cerebellum and middle cerebellar peduncles (Magnotta et al., 2008; Okugawa et al., 2004) in patients with schizophrenia; on the other hand, contrary to some earlier reports, FA of the corpus callosum was not significantly different compared with FA in the same region in controls (Ardekani et al., 2003; Hubl et al., 2004; Roalf et al., 2013). There are some previous studies in which no difference was found in FA values of the corpus callosum, similar to our results (Buchsbaum et al., 1998; Foong et al., 2002; Mandl et al., 2010). Inconsistency is also present regarding laterality. There is still not enough information to reach a conclusion about why some findings are bilateral while others are unilateral. For any region where there is a positive result, a negative result is also reported (Kanaan et al., 2005; Ellison-Wright and Bullmore, 2009; Melonakos et al., 2011). The heterogeneity of the disorder, clinical variables such as severity and duration of illness, dose and duration of previous antipsychotic use, and methodological differences such as ROI versus voxel-based approach have been suggested to be responsible for the variance in reported results (Kanaan et al., 2005). The most common interpretation of lower FA is that it reflects lower white matter integrity (Kanaan et al., 2005); however, our findings regarding low FA in multiple regions do not specify the nature of the change. The density, diameter, or directionality of the fibers, and the thickness of the myelin sheaths all affect the diffusion of water molecules and, therefore, may be responsible for the low FA observed in patients with schizophrenia (Beaulieu, 2002).

An interesting result of the present study is that FA in the patients with schizophrenia was higher in six regions, including the right parahippocampus, posterior limb of the internal capsule, sagittal stratum, left anterior thalamic radiation, and bilateral optic radiation than in the controls at baseline. Although some studies reported low FA in the parahippocampus (Ardekani et al., 2003), bilateral posterior limb of the internal capsule (Skelly et al., 2008), and left anterior thalamic radiation (Skelly et al., 2008), no study has reported higher FA in these regions in schizophrenia patients than in controls. On the other hand, higher FA was reported in such regions as the arcuate fasciculus, superior longitudinal fasciculus, and genu of the corpus callosum in patients with schizophrenia with auditory hallucinations than in those without auditory hallucinations and healthy controls (Hubl et al., 2004; Shergill et al., 2007; Rotarska-Jagiela et al., 2009). High FA was considered to be evidence of an increase in connectivity in these regions and was suggested to be responsible for the occurrence of hallucinations (Rotarska-Jagiela et al., 2009). Schizophrenia is a heterogeneous disorder with a variable clinical picture and, as such, differences in tissue abnormalities are not unexpected. Clozapine treatment was indicated in the patients with schizophrenia in the present study because they were refractory to other previously used medications, and the higher FA in various brain regions may have been related to the pathophysiology of refractoriness, or the influence of the previously used antipsychotic medications. Persistent psychotic symptoms may be the cause of or the result of inappropriate or maladaptive wiring (Meyer-Lindenberg, 2011). Considering the lower FA in multiple white matter tracts observed in the present study, we think that higher FA in other regions might be the result of compensatory mechanisms that evolved during the course of illness.

The most striking result of the present study is the significant increase in FA values in multiple brain regions important to interhemispheric and intrahemispheric connectivity after 12 weeks of clozapine treatment in the patients with schizophrenia. Disturbances in the corpus callosum and the frontothalamic, frontotemporal, cortical-thalamic-cerebellar-cortical pathways have been reported in numerous studies. Those findings support the dysconnection hypothesis of schizophrenia (Okugawa et al., 2005; Okugawa et al., 2006; Ellison-Wright and Bullmore, 2009); therefore, the FA increase in major white matter tracts following 12 weeks of clozapine treatment observed in the present study suggests that clozapine might change white matter integrity and, subsequently, connectivity in the regions associated with schizophrenia. An interesting finding is that among the regions that were significantly different between the patients and controls at baseline, the left inferior fronto-occipital fasciculus and the superior parietal lobule were the ones which had significantly increased FA values after clozapine treatment. The lower FA at baseline seems to be reversed in these regions, while FA values also increased in several other regions which do not exactly overlap with those showing group differences at baseline. Complicated neurodevelopmental and neurodegenerative processes are thought to be involved in the pathophysiology of schizophrenia and, as such, it may not be reasonable to expect the structural and functional disturbances to be fully reversible or treatable. On the other hand, considering the plasticity of the brain and the possible

neuroplastic effects of antipsychotics, reducing the severity of symptoms via correction of dysconnectivity seems to be a possibility (Konradi and Heckers, 2001). Our results suggest that the alteration of connectivity may not only involve a reversal of the disturbance in some regions, but also an adaptative or maladaptive compensatory change in others. This may be a reason why, after clozapine treatment, the FA values increased in several regions other than the ones that differed from values in controls in the baseline comparison.

The mechanism by which white matter microstructure changed in response to 12 weeks of clozapine treatment in the present study remains unknown. A follow-up study that investigated the effect of antipsychotics on DTI measures reported that there was a significant decrease in mean diffusivity (Do) in the right pyramidal tract, left temporal lobe, and cingulate gyrus in treatment responders after 28 days of antipsychotic (risperidone, ziprasidone, or haloperidol) treatment, and an antipsychotic-induced cascade was suggested to partially restore myelin integrity and functional connectivity concomitant with the antipsychotic effect (Garver et al., 2008). On the other hand in a recent study, first episode, drug-naïve patients with schizophrenia were reported to have a significant decrease in FA of white matter around the bilateral anterior cingulate gyrus and the right corona radiata of the frontal lobe, compared with healthy controls, following 6 weeks of treatment with several typical and atypical antipsychotics (risperidone, olanzapine, quetiapine, aripiprazole, sulpiride, and haloperidol) (Wang et al., 2013). The authors concluded that the acute reduction in white matter FA might be due to the effects of antipsychotic medication during the early phase of treatment, and discussed the potential toxic effects of antipsychotic medication such as oxidative stress and excitatory neurotoxicity, and also pointed out that it was not possible to entirely exclude the effects of underlying progression of illness. Our study is different from the previous longitudinal studies as it includes a longer follow-up period, and specifically assesses the effect of clozapine, which has not been studied previously. In addition, the patients included in the present study were chronic patients who had been treated previously, and had their baseline assessment while they were on their current antipsychotic. Therefore, the observed FA differences from controls at baseline could have been exacerbated by the previous exposure to antipsychotics, and the increase in FA values in multiple white matter tracts after 12 weeks of clozapine treatment could have resulted from the switching of the patients' current antipsychotic medication.

Although the present study is the first to investigate the effect of clozapine on white matter based on DTI measures, several neuroimaging studies on the effect of clozapine on brain structures and functions have been published (Molina Rodríguez et al., 1996; Arango et al., 2003; Lahti et al., 2003; Ertugrul et al., 2009). Clozapine was reported to increase the *N*-acetyl aspartate/creatine ratio in the left dorsolateral prefrontal cortex after 8 weeks of treatment, which was interpreted as evidence of the positive effect of clozapine on neuronal function and integrity (Ertugrul et al., 2009). Preclinical studies also suggest that there is a possible neuroplastic effect of clozapine (Bai et al., 2003; Lu and Dwyer, 2005; MacDonald et al., 2005; Critchlow et al., 2006; Ozdemir Rezaki et al., 2012), in addition to a protective role, on myelination (Xu et al., 2009, 2010); as such, the change in axonal parameters, in addition to increased myelination, might have played a role in the observed increases in FA in the present study. The specific effect of clozapine on FA measures could be better studied in first episode drug naïve patients, but this is not possible in many countries considering the current practice regarding initiation of clozapine treatment only in patients with intolerance or resistance to other antipsychotics.

The clinical importance of the increase in FA values in multiple regions observed during clozapine treatment needs cautious interpretation. Data on the relationship between DTI measures and clinical variables are inconsistent (Peters et al., 2010). There are several studies in which an inverse correlation was shown between psychopathology and FA values (Skelly et al., 2008; Nakamura et al., 2012), while others showed a positive correlation (Karlsgodt et al., 2008; Cheung et al., 2011) or no correlation (Liu et al., 2013). Wang et al. (2013) reported an improvement in positive symptoms while FA decreased after 6 weeks of antipsychotic treatment, but the correlation was not significant. They suggested that in spite of the improvement in symptoms, antipsychotics could not arrest or reverse a deteriorating process occurring in the brains of patients with schizophrenia. Contrary to their results, in the present study, PANSS scores improved while the FA increased in several regions after 12 weeks of clozapine treatment, although the correlations were not statistically significant. Besides, the increase in FA values in the left inferior fronto-occipital fasciculus was significantly correlated with the improvement in semantic fluency. Since correlations cannot be assumed to reflect a causal relationship, the improvement in semantic fluency after clozapine treatment may not necessarily be a consequence of the increased FA in this region. However, the inferior fronto-occipital fasciculus, which connects occipital cortex to orbitofrontal and temporal-basal regions, has been reported to have a crucial role in the semantic system (Duffau et al., 2005; Martino et al., 2010), and the correlation between low FA values in inferior fronto-occipital fasciculus and the cognitive deficits has been shown in previous studies (Lee et al., 2013; Liu et al., 2013). The observed relationship between the change in FA values in the left inferior fronto-occipital fasciculus and semantic fluency after clozapine treatment needs to be confirmed in future studies. Although we do not know what the increase in FA values actually mean at a microstructural level in this study, considering that FA is accepted as a measure of white matter integrity, and that it is consistently shown to be lower in both first episode and chronic patients with schizophrenia compared with controls (Ellison-Wright and Bullmore, 2009; Nakamura et al., 2012; Lee et al., 2013), these results led us to the conclusion that the increase in FA values, at least in some of the regions such as the left inferior fronto-occipital fasciculus, after 12 weeks of clozapine treatment can be regarded as an 'improvement'.

A strength of the present study was the use of TBSS to evaluate whole-brain white matter, which is more informative than ROI approaches which only analyze predetermined areas; however, isotropic DTI with higher resolution would be obtained on a 3-Tesla or higher system and that would likely yield more extensive abnormalities. In the present study, FA was measured in an effort to compare the present findings to those of previous studies on FA. Investigation of other diffusion indices, such as axial and radial diffusivity, would definitely yield more data about alterations in brain tissue.

A shortcoming of the present study is the absence of a rescan of the control group. A second DTI assessment of the controls at week 12 would have permitted a comparison of FA between patients on clozapine and controls at week 12, and would enabled us to ensure that the reported differences between the two measurements for the patients with schizophrenia were due to the change in the type of antipsychotic, rather than other factors, such as an alteration in FA which might also be present in controls, or variations in technical parameters. The results of previous longitudinal studies do not support the probability of an increase in FA values of normal controls in 12 weeks (Mitelman et al., 2009a, 2009b). Besides, we do not expect a change in FA values due to technical factors since all DTI scans were performed using the same scanner, which was not upgraded during the study time period, with the same parameters,

carried out by the same technician, and were assessed by the same radiologist. The study is also limited by the small sample size and relatively low duration of follow-up. In fact, previous longitudinal studies showed a change in FA values in patients with schizophrenia even after 6–8 weeks of antipsychotic treatment (Garver et al., 2008; Wang et al., 2013); therefore, a period of 12 weeks seems to be reasonable to assess the effect of clozapine on FA values. However, considering the long duration of illness, and the possible long-term beneficial effects of clozapine treatment (Rosenheck et al., 1999), a longer follow-up would ensure a more accurate assessment of the effect of clozapine.

The present study is the first longitudinal study to investigate the effect of clozapine on brain tissue via DTI and the first to assess the relationship of post-treatment changes in FA with symptoms and cognitive functioning in patients with schizophrenia. The present findings support the current information on the role of dysconnectivity in the pathophysiology of schizophrenia, and suggest that clozapine changed white matter microstructure in the corpus callosum and other brain regions involved in fronto-thalamic, fronto-temporal, and cortical-thalamic-cerebellar-cortical connectivity. These findings shed light on the unique effect of clozapine in the treatment of schizophrenia, but they must be confirmed by other large-scale, longer-term studies.

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