Decrease of Gray Matter Volume in Treatment-refractory Schizophrenia Patients

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ABSTRACT

Objective: To investigate the gray matter alterations in treatment-refractory schizophrenia (TRS) patients and to explore the correlations between gray matter alterations and clinical characteristics in these patients.

Methods: Voxel based morphometry (VBM) in conjunction with statistical parametric mapping on the structural magnetic resonance images of TRS patients (n=24) and healthy controls (n=21) was performed to assess differences between the two groups in gray matter volume(GMV).

Results: We found GMV decreased in the left olfactory, bilateral insula and right anterior cingulate of TRS patients compared to healthy controls ($p<0.05$, FWE corrected). However, we did not find any correlation between gray matter reduction and clinical characteristics in these patients.

Conclusion: TRS patients have GMV decreased in the left olfactory, bilateral insula and right anterior cingulate.

Key words:treatment-refractory schizophrenia; gray matter volume; voxel based morphometry (VBM)

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INTRODUCTION

Schizophrenia, characterized by delusions, hallucinations, formal thought disorder, personality disturbance, and cognitive dysfunction, is a complex neuropsychiatric disorder. The pathophysiology underlying this disease is remain unknown. Schizophrenia has a lifetime risk of about 1% with frequently chronic and socially disabling. A body of cohort studies indicate that 20-30% of patients with schizophrenia meet criteria for treatment-refractory schizophrenia (TRS)[1]. TRS was defined by the following two criteria: (1) at least three periods of treatment in the preceding 5 years with antipsychotic drugs (from at least two different chemical classes) at dosages equivalent to or greater than 600 mg/day of chlorpromazine for a period of 4 weeks, each without significant symptomatic relief, or patients having not tolerated the side effects of antipsychotics and; (2) no period of good functioning within the preceding 5 years [2, 3]. TRS has more

outstanding impact on rehabilitation of social function and quality of life. It has been the focus and difficulty of psychiatrists.

A number of neuroimaging studies on schizophrenia have provided overwhelming evidences that the disease is a disorder involving widespread abnormalities of brain structure[4]. The structural abnormalities include enlargement of the lateral and third ventricles, and reduced lateral temporal cortical, medial temporal, and prefrontal lobe volumes in the schizophrenia patients than the healthy control[5]. The neurobiological processes underlying these structural abnormalities are believed to be central to the pathophysiology of schizophrenia[6]. However, few studies have been performed on the brain structures of TRS patients by neuroimaging [7]. In this study, armed with voxel based morphometry (VBM), we investigated the changes of gray matter volume(GMV) in TRS patients aimed to explore the brain structural alterations in the TRS patients.

OBJECTS AND METHODS

Objects

Patients group: The TRS patients were recruited from the Department of Psychiatry, Affiliated Nanjing Brain Hospital of Nanjing Medical University between March 2013 and December 2014. Inclusion criteria: (1) being in line with the TRS diagnosis standard (Kane Standard); a patient who had no response to the treatment of two or three kinds of atypical antipsychotics at least for 4 to 6 weeks; (2) aged 18 to 50 years old; (3) subjects were fully informed about the measurement and MRI scanning in the study. Written informed consent forms were obtained from all subjects or their legal guardians. The protocols used in this study was approved by the Ethics Committee (Nanjing Brain Hospital of Nanjing Medical University Review Board, No. KY44, 2011). Criteria of exclusion are: (1) with organic brain diseases, infectious diseases, other chronic somatic diseases, history of psychoactive substance abuse; (2) constrained dications of magnetic resonance imaging examination. The TRS patients were diagnosed by two professional psychiatrists.

Control group: Healthy subjects with matched age, gender, and average education were recruited as a control group. The criteria of healthy subjects include: (1) no mental disorder, and the current state of mind was good; (2) without a family history of psychosis in the three generations. (3) aged 18 to 50 years old. Criteria of exclusion are: (1) with organic brain disease, infectious diseases or other chronic somatic diseases; (2) contraindications of magnetic resonance imaging examination.

Methods

High-resolution T1-weighted Imaging Acquisition

Magnetic resonance imaging (MRI) was performed on a 3.0T Siemens MRI scanner (Verio, Siemens Medical System) at Nanjing Brain Hospital of Nanjing Medical University. A standard birdcage head coil was used, along with foam pads for limiting head motion. High-resolution whole brain volume T1-weighted images were acquired sagittally with a 3D spoiled gradient echo pulse sequence. The scanning parameters are: repetition time (TR) = 2000 msec; echo time (TE) = 30 ms; flip angle = 8º; slice thickness = 1 mm; Gap = 0mm; 1 NEX; FOV=256×256mm; matrix size=256×256 mm; voxel size=1×1×1mm3; 176 slices.

MRI Data Analysis

All structural data were processed with the Statistical Parametric Mapping SPM8 software package (http://www.fil.ion.ucl.ac.uk/spm), with voxel-based morphometry toolbox (VBM8) (http://dbm.neuro.uni-jena.de/vbm). The VBM8 toolbox combines tissue segmentation, bias correction, and spatial normalization into a unified model[8] . A Hidden Markov Random Field (HMRF) model was used to introduce spatial constraints into the segmentation process to improve accuracy of tissue segmentation[9] . In the spatial normalization step, the high-dimensional Dartel normalization approach (VBM-Dartel) was chosen. Images were multiplied (modulated) by the Jacobian determinants from the normalization step to preserve volume information. Modulated gray matter images were smoothed with an 8 mm FWHM Gaussian kernel for statistical analyses.

Statistical Analyses

To examine differences in regional GMV between groups, a voxel-wise two-sample t-test analysis was carried out in SPM8. The total gray matter volume, age, gender, and years of education were set as confounding covariates. An absolute threshold mask of 0.1 was used to avoid possible edge effects around the border between gray matter and white matter. Clusters of 100 voxels (a cluster size equal to 1×1×1 mm3) or greater, surviving a family-wise error (FWE) corrected threshold of $p<0.05$ were considered significant.

Statistical analyses of demographic data were conducted with SPSS 15.0 software (SPSS, Chicago, Illinois). Independent-sample t test and $\chi 2$ test were used to compare demographic data between two

groups. In addition, we extracted the mean volumes of the clusters that had shown differences between groups in VBM analysis. Correlations between mean volumes of the clusters and clinical variables including PANSS scores (positive symptoms, negative symptoms, general psychopathology, total score), duration of illness, and chlorpromazine (CPZ) equivalent value were calculated by partial correlation analysis controlling for ROI GMV ($p<0.05$).

Results

Characteristics of Research Samples

After quality control, we eliminated poor quality MRI data samples. The final samples contained 24 TRS patients and 21 healthy controls. Demographics of TRS patients and control subjects, and course of disease, PANSS score, CPZ equivalent dose, etc. of TRS patients were shown in Table 1.

Table 1. Demographic and clinical characteristics of TRS patients and control subjects

	TRS patients (n=24)	control subjects (n=21)	P value
Years of Age (mean ± SD)	31.9 ± 9.3	34.1 ± 7.6	0.38
Sex(Male/Female)	14/10	15/6	0.36
Chinese Han/others	24/0	21/0	
Right/Left -handed	24/0	21/0	1
Education(years)(mean±SD)	12.0 ± 2.9	13.6±3.1	0.09
Course of disease (years)	13.9 ± 9.3		
PANSS-total score	98.2 ± 11.0		
PANSS-positive symptoms	25.9 ± 7.3		
PANSS-negative symptoms	24.0 ± 5.3		
PANSS-general psychopathology	48.2 ± 5.7		
CPZ-equivalent (mg)	697.3 ± 245.9		

PANSS:Positive and Negative Syndrome Scale

Brian maps of representative axial slices showing the differences of GMV between TRS patients group and control group.

Results from VBM analysis revealed that the GMV of left olfactory, bilateral anteriorinsular and right cingulate was reduced in TRS group (see Table 2 and Fig.1).

Table 2. Regions of reduced GMV of left olfactory, bilateral insula and right nterior cingulate in TRS group in comparison with the control group

Brodmann area	Anatomical Region	Cluster Size (No.Voxel)	Voxel level (FEW corrected)	MNI (mm) X	Y	Z
25	left olfactory	1384	2.84E-06	-1.5	9	-12
13	left insula	789	2.40E-05	-42	1.5	3
13	right insula	2665	3.24E-05	37.5	9	4.5
10	right anterior cingulate	559	1.05E-03	1.5	48	10.5

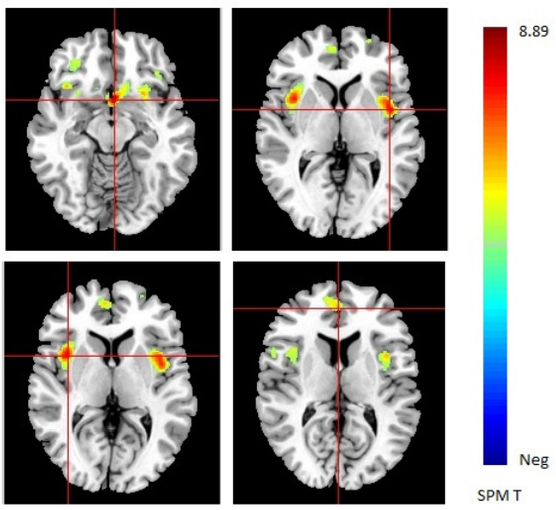

Fig.1 Brian maps showing the differences of GMV between TRS patients group and control group.

Significance level: The FWE corrected P <0.05, voxel>100

Region of Interest (ROI) Analysis

We found GMV decreased in the left olfactory, bilateral insula and right anterior cingulate of TRS patients compared to healthy controls ($p<0.05$, FWE corrected). No correlation was found between the mean ROI area of GMV in TRS patients with illness duration, education, CPZ equivalent dose, PANSS scores (positive symptoms, negative symptoms, general psychopathology, total score) ($P> 0.05$).

DISCUSSION

With the application of the second generation of antipsychotic drugs, considerable progress has been made in the treatment of schizophrenia. However, it will continue to be a challenge for clinicians to treat the TRS. TRS has special characteristics of psychopathology and clinical symptom. The neurobiologic mechanism underlying TRS is largely unknown.

In this study, we measured changes of the gray matter in the brains of TRS patients by performing high resolution T1-weighted imaging. The results showed the TRS patients had general deficits of the gray matter in their brains including bilateral insula, left olfactory and right anterior cingulate.

Insula plays important roles in emotional processing, sensory stimulation, and perception of the body's inner state[10]. A lot of studies had reported that the gray matter was reduced in the unilateral or bilateral insular in the patients with chronic schizophrenia. The results suggest that the GMV alteration of insular may be degenerative[11,12]. In this study, we found that the volume of the gray matter in the bilateral insula of TRS patients' brains was significantly reduced. The results support the conclusion that the insula should be involved in pathophysiology of schizophrenia.

Anterior cingulate is the main component of the limbic system in the central nervous system. It mainly involves emotion, learning and memory processing. However, its role in schizophrenia remains largely unknown. Sheng et al. reported that the first onset patients of schizophrenia displayed the defects of gray matter in their anterior cingulate[13]. Velakoulis et al. found that the volume of gray matter was decreased in the anterior cingulate of the patients suffering from chronic schizophrenia and it became smaller during the course of the disease[14]. Liu et al. demonstrated that abnormalities in the anterior cingulate as manifested by reduced surface area may contribute to cognitive dysfunction in schizophrenia[15]. In this study, we found that the TRS patients also accompanied with GMV reduction in their right anterior cingulate. Taken together, all the observations suggest that continuously reducing in the volume of gray matter in anterior cingulate may be responsible for poor responses of schizophrenia patients to drug.

Olfactory bulb and olfactory tract lay bellow the basal forebrain, modeling the olfactory sulcus. Olfactory bulb mitral cells synapses with neuroepithelium, retrieving information throughout the olfactory tract to primary olfactory areas and other regions that play crucial roles in emotion regulation and executive planning. Olfactory functional impairment may be a biological marker of schizophrenia susceptibility[16]. Performing a meta-analysis on olfactory dysfunction in schizophrenia patients, high risk groups and first degree relatives,

Moberg PJ et al. revealed that both schizophrenia patients and high risk individuals had moderate degree of olfactory dysfunction and their first degree relatives had mild or moderate olfactory dysfunction [17]. Being consistent with the olfactory functional impairment in schizophrenia, peripheral olfactory structures including neuroepithelial dysfunctional processes were found to be abnormal [18] and significant decrease in olfactory bulb volume and shallower olfactory sulcus were found in schizophrenia patients. These structural changes are stable over time and are correlated with worst olfactory performances [19]. Interestingly, results from other observations showed that the schizophrenia patients had normal orbital sulci [20]. Shallower olfactory sulcus, a structure occurring at early embryogenesis, is often disturbed during early development. However, the orbital sulcus, which does not develop until the third trimester, is relatively immune to early insults. The developmental differences of the two structures support the hypothesis that the defined temporal window – the first half of gestation – for neurodevelopment (at least for olfactory structures) is crucial for patients suffering schizophrenia. In this study, we found that the volume of gray matter in the left olfactory cortex was decreased in the TRS patients. The result provided one more piece of evidence about olfactory dysfunction in schizophrenia. Because there are rare reports of abnormal olfactory cortex in the study of common schizophrenia, the findings may suggest TRS is a more severe schizophrenia subgroup[21]. Our results implied that the damage of the olfactory cortical gray matter may reflect the specific lesion in the brain of TRS patients.

Schizophrenia is characterized by progressive GMV decreases and lateral ventricular volume increases. Some of these neuroanatomical alterations may be associated with antipsychotic treatment. But, the putative mechanism of action of antipsychotics on GMV is unknown and can only be inferred in vivo from animal studies. It is important to note that association is not causation and thus GMV decreases being correlated with cumulative exposure to antipsychotic treatments should be interpreted cautiously. Antipsychotic treatment may not be the only factor associated to longitudinal GMV decreases in schizophrenia[22]. This is supported by progressive brain changes are already present before the onset and may in particular occur during transition of psychosis in antipsychotic-naïve subjects.

In summary, we showed in this study that TRS patients have extensive decrease in gray matter and the defective areas include left olfactory cortex, bilateral insula and right anterior cingulate. However, whether the defects are responsible for the poor response of the TRS patients to the drug needs further investigation.

DISCLOSURE STATEMENT

The authors do not have any actual or potential conflicts of interest.

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