Healthy siblings of schizophrenia patients have an almost 9-fold higher risk for developing the illness than the general population. Disruption of white matter (WM) integrity as indicated by reduced fractional anisotropy (FA) derived from diffusion tensor imaging (DTI), is believed to be the key substrate of schizophrenia. However, it remains unclear whether schizophrenia patients and their healthy siblings share a specific pattern of disruption of WM integrity that may be related to the disease risk. The objective of this study is to determine whether a specific brain regional pattern of disruption of WM integrity is shared by schizophrenia patients and their healthy siblings. We investigated brain white matter abnormalities by voxel-based analysis of white matter FA data acquired from diffusion tensor imaging in 34 pairs of schizophrenia patients and their healthy siblings, as well as in 32 healthy controls. Both schizophrenia patients and their healthy siblings showed reduced white matter FA in the left prefrontal cortex and the hippocampus in comparison to healthy controls, without significant difference between patients and siblings. In marked contrast, only schizophrenia patients exhibited reduced white matter FA in the left anterior cingulate cortex in comparison to both siblings and controls, without significant difference between siblings and controls. Thus, schizophrenia patients and their healthy siblings share disruption of WM integrity in the left prefrontal cortex and the hippocampus that may be related to higher risk of healthy siblings to develop schizophrenia, which may be eventually attributed to additional disruption of WM integrity in the left anterior cingulate cortex.

1. Introduction

Schizophrenia is a highly heritable psychiatric disorder characterized by highly heterogeneous symptoms including positive, negative symptoms and cognitive deficits (Mueser and McGurk, 2004). In addition, schizophrenia patients and their healthy siblings share similar genetic backgrounds and early-life environments. These similarities are believed to underlie the almost 9-fold higher risk for siblings to develop...
schizophrenia compared with the general population (Sadock Bj, 2007). Comparison between healthy siblings and healthy controls is important because both of them are free of antipsychotic medications, which may cause brain changes in schizophrenia patients and thus may confound imaging results if patients were compared with healthy controls alone. Furthermore, it is well-known that healthy siblings have a similar pattern of cognitive deficits, although milder, as compared to schizophrenia patients (Snitz et al., 2006). Thus, comparison amongst schizophrenia patients, healthy siblings and healthy controls likely provides additional insights.

Cognitive functions include executive control, decision making as well as learning and memory. Ample evidence shows that the prefrontal cortex (PFC), the hippocampus and the anterior cingulate cortex (ACC) play distinct roles in cognitive functions, and dysfunctions of these brain regions are associated with schizophrenia (Barch, 2005). Functional magnetic resonance imaging (fMRI) studies have documented that cognitive tasks induce different brain activation patterns in schizophrenia patients and healthy controls while structural MRI studies have demonstrated reduced white matter (WM) volume in the PFC of schizophrenia patients (Glahn et al., 2005; Ho et al., 2003; Walterfang et al., 2006). The hippocampus is critical for the formation of certain types of memory (Squire et al., 2004), and it is also implicated in many psychiatric disorders including schizophrenia. Neuro-pathological, structural and functional MRI studies have demonstrated abnormal neural circuitry and decreased volume of the hippocampus in schizophrenia patients (Heckers, 2001; Heckers et al., 1998). Furthermore, nonpsychotic first-degree relatives of schizophrenia patients also exhibit reduced volume of the hippocampus (Boos et al., 2007). Finally, the ACC plays crucial roles in monitoring and detecting conflict in ongoing information processing (Botvinick et al., 2001) that may relate to cognitive functions such as decision making, deficits of which are the cardinal feature of schizophrenia. Decreased volume, neuronal density, and activation of the ACC are consistently observed in schizophrenia (Yucel et al., 2002; Baiano et al., 2007; Benes, 1998). Thus, cognitive deficits associated with dysfunction of the PFC, the hippocampus and the ACC are amongst the most consistent findings in schizophrenia (Barch, 2005).

WM contains neural fibers that connect neurons within a given brain region or neurons from different brain regions. WM deficiency has long been assumed to be a key neural abnormality in schizophrenia and increasing evidence supports this theory (Walterfang et al., 2006). Fractional anisotropy (FA) derived from diffusion tensor imaging (DTI) is the most commonly used measure to represent WM integrity. Substantial evidence suggests that schizophrenia patients show abnormal WM FA in multiple brain regions compared to healthy controls (Kryiakopoulos et al., 2008). For example, many studies find that schizophrenia patients exhibit decreased WM FA in the frontal and temporal lobes, genu and splenium of the corpus callosum, internal capsule, cingulum bundle, uncinate and arcuate fasciculus (Konrad and Winterer, 2008), with decreased WM FA in the frontal and temporal lobes as the most consistent finding (Hao et al., 2006; Lim et al., 1999; Szczesko et al., 2008). Other studies, however, report decreased WM FA in the parietal association cortex, cerebellum peduncle, and anterior thalamic radiation (Kanaan et al., 2005; Kubicki et al., 2007). Furthermore, evidence shows that schizophrenia patients not only show overall reduced FA but also increased WM FA in specific brain regions. Increased WM FA in the left arcuate fasciculus was found in patients with auditory hallucinations compared with those without auditory hallucination or healthy controls (Hubl et al., 2004). Moreover, some studies demonstrate that schizophrenia patients show neither reduced nor increased WM FA relative to healthy controls (Foong et al., 2002; Price et al., 2005; Wang et al., 2003). These inconsistent findings may relate to differences in genetic background, life environment or medications. Finally, WM FA reductions have been demonstrated in schizophrenia and in individual at high genetic risk for schizophrenia (DeLisi et al., 2006; Hoptman et al., 2008; Munoz et al., 2008). Therefore, examining the WM integrity in schizophrenia patients, their healthy siblings and healthy controls may improve our understanding of the pathophysiology for schizophrenia.

We used voxel-based analysis (VBA) to identify brain regions with abnormal WM FA in schizophrenia patients and their healthy siblings as compared with healthy controls. We hypothesized that WM FA reductions in the PFC and hippocampus would be shared by schizophrenia patients and their healthy siblings, representing increased genetic liability, while WM FA reduction in the ACC would be identified only in schizophrenia patients, representing a marker of disease progression.

2. Methods

2.1. Participants

Using the Structured Clinical Interview patient version for DSM-IV (SCID-P) (First MB, 1998), thirty-four schizophrenia patients were recruited from inpatient and outpatient units of the Department of Psychiatry, the Second Xiangya Hospital of Central South University, Changsha, Hunan province, PR China. The inclusion criteria were: a) age between 17 and 45; b) met DSM-IV criteria for schizophrenia; c) nine years of education or above; d) having at least one healthy sibling who was willing to participate in the study; e) the total scores of Positive and Negative Syndrome Scale (Kay et al., 1987) for psychopathology >60; f) right-handed by a determination of hand preference (Annett, 1970). The exclusion criteria were: a) any contraindications to MRI scanning; b) alcohol or substance abuse history; c) history of receiving electroconvulsive therapy; d) chronic neurological disorders.

Thirty-four healthy siblings of schizophrenia patients, one sibling for each of the identified patients, were recruited. The inclusion and exclusion criteria were the same as those of the schizophrenia group except that the healthy siblings did not meet the DSM-IV diagnostic criteria of any psychiatric disorders by SCID non-patient version. Each schizophrenia patients and his/her corresponding sibling had the same biological parents. Thirty-two healthy controls were recruited from a community sample. The inclusion and exclusion criteria for these subjects were the same as those of the healthy siblings group with the exception that their first-degree relatives had no history of any psychiatric disorders.

All subjects gave informed consent for participation in the study after the risks and benefits of their participation were
explained in detail. The study was approved by the ethics committee of the Second Xiangya Hospital of Central South University.

2.2. Image acquisition and analysis

MRI was performed on a 1.5-T GE Signa Twinspeed MR scanner (General Electric Medical System, Milwaukee, USA). A standard head coil was used for radio frequency (RF) transmission and reception of the nuclear magnetic resonance (NMR) signal. Head motion was minimized with restraining foam pads provided by the manufacturer.

Diffusion weighted images were acquired with a single-shot echo planar imaging (EPI) sequence aligned to the straight axial plane. The diffusion sensitizing gradients were applied along thirteen non-collinear directions \( b = 1000 \, \text{s/mm}^2 \), together with an acquisition without diffusion weighting \( b = 0, b_0 \) images. Thirty contiguous axial slices were acquired with slice thickness of 4 mm and no gap. The acquisition parameters were as follow: TR = 12,000 ms; TE = 107 ms; Matrix = 128 × 128; FOV = 240 mm × 240 mm; NEX = 5.

The methods of imaging processing are similar to those described previously (Hao et al., 2006). Parametric images of b0 and FA were generated using DTI-Studio software and transformed from DICOM format to ANALYZE format for further processing using Statistical Parametric Mapping (SPM2, Wellcome Department of Cognitive Neurology, London, UK). For each subject, the b0 image was first normalized to the standard Montreal Neurological Institute (MNI) space using SPM2 implementing on MATLAB 6.5, and the transformation matrix was applied to the FA maps in order to normalize the FA maps to the standard MNI space. All images were re-sampled with a final voxel size of 2 × 2 × 2 mm\(^3\). Each normalized FA map was spatially smoothed with an 8-mm Full Width at Half Maximum (FWHM) Gaussian kernel in order to decrease spatial noise and compensate for the inexact nature of normalization.

The normalized and spatially smoothed FA maps were further analyzed using SPM2. A one-way analysis of variance (ANOVA) was performed voxel-by-voxel for FA maps with the single factor “group” (schizophrenia patients, healthy siblings and healthy controls). To identify the white matter regions most consistently involved and to reduce the problem of multiple testing, clusters containing more than 100 neighboring voxels (800 mm\(^3\)) with difference of \( p < 0.005 \) (uncorrected) were identified across three groups. For visualization of the regions showing significantly different FA values across three groups, the significant clusters were superimposed onto T1-weighted brain template of SPM2.

The average regional FA values corresponding to these identified clusters in FA analyses were calculated using the individual FA. The average FA values were compared by one-way ANOVA followed by least significant difference (LSD) (SPSS 12.0) across schizophrenia patients, healthy siblings and healthy controls. Meanwhile, one-way ANOVA was also used to compare age and completed years of education and Chi-square test was used to compare gender composition across the three groups.

3. Results

3.1. Demographic and clinical characteristics

Demographic and clinical characteristics of schizophrenia patients, healthy siblings and healthy controls were summarized in Table 1. There was no significant difference in age \( (F_{2, 97} = 0.295, \, p = 0.745) \), completed years of education \( (F_{2, 97} = 2.01, \, p = 0.140) \) or gender composition \( (\chi^2 = 0.003, \, p = 0.999) \) across the schizophrenia patients, healthy siblings and healthy controls.

3.2. Comparison of FA maps by groups

Using DTI and VBA, we identified clusters showing significant difference across schizophrenia patients, healthy siblings and healthy controls. These clusters were in the PFC (upper row in Fig. 1), the hippocampus (middle row in Fig. 1) and the ACC (lower row in Fig. 1) of the left cerebral hemisphere. The details of these identified clusters were summarized in Table 2.

3.3. Comparison of FA values by groups

We retrieved WM FA values from these identified clusters. In the left PFC, one-way ANOVA revealed a significant group main effect \( (F_{2, 97} = 7.689, \, p = 0.001) \). Post hoc tests indicated

<table>
<thead>
<tr>
<th>Table 1</th>
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<tr>
<td><strong>Variable</strong></td>
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<tr>
<td><strong>Mean</strong></td>
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<tr>
<td><strong>Age (years)</strong></td>
</tr>
<tr>
<td><strong>Years of education</strong></td>
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<tr>
<td><strong>Gender</strong></td>
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<tr>
<td>Men</td>
</tr>
<tr>
<td>Women</td>
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<tr>
<td><strong>Handedness</strong></td>
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<tr>
<td>Left</td>
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<tr>
<td><strong>Chlorpromazine equivalent (mg)</strong></td>
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<tr>
<td><strong>Age of onset (years)</strong></td>
</tr>
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<td><strong>Duration of illness (months)</strong></td>
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*One-way analysis of variance.*
that FA values in both schizophrenia patients and healthy siblings were lower than those in healthy controls ($p < 0.001$ for patients and $p = 0.007$ for siblings vs. controls; left panel in Fig. 2A), while there was no significant difference between patients and siblings ($p = 0.293$). Cumulative probability plot confirmed that WM FA values in the order of low to high were from schizophrenia patients, healthy siblings and healthy controls (right panel in Fig. 2A).

In the left hippocampus, one-way ANOVA revealed a significant group main effect ($F_{2, 97} = 7.933, p = 0.001$). Post hoc tests indicated that FA values in both schizophrenia patients and healthy siblings were lower than those in healthy controls ($p < 0.001$ for patients and $p = 0.04$ for siblings vs. controls; left panel in Fig. 2B), without significant difference between patients and siblings ($p = 0.056$). Cumulative probability plot illustrated that WM FA values in the order of low to high were from schizophrenia patients, healthy siblings and healthy controls (right panel in Fig. 2B).

In the left ACC, one-way ANOVA revealed a significant group main effect ($F_{2, 97} = 6.527, p = 0.002$). Post hoc tests indicated that FA values in schizophrenia patients were lower than those in both healthy siblings and healthy controls ($p = 0.001$ for siblings and $p = 0.022$ for controls vs. patients; Fig. 2C), without significant difference between siblings and controls ($p = 0.243$). WM FA values in the order of low to high were surprisingly from schizophrenia patients, healthy controls and healthy siblings.

### Table 2
Brain regions showing significant difference by comparison of FA maps across schizophrenia patients, healthy siblings and healthy controls.

<table>
<thead>
<tr>
<th>Brain regions (white matter)</th>
<th>Hemisphere</th>
<th>Voxels</th>
<th>MNI coordinates at center of cluster</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prefrontal cortex</td>
<td>Left</td>
<td>170</td>
<td>−24 −28 16</td>
<td>0.001</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>Left</td>
<td>137</td>
<td>−26 −24 −14</td>
<td>0.001</td>
</tr>
<tr>
<td>Anterior cingulate cortex</td>
<td>Left</td>
<td>119</td>
<td>−14 4 44</td>
<td>0.002</td>
</tr>
</tbody>
</table>

### 4. Discussions

Using a VBA in DTI studies, we found that schizophrenia patients and their healthy siblings shared disruption of WM integrity in the left PFC and hippocampus while only schizophrenia patients exhibited disruption of WM integrity in the left ACC. Since the PFC, hippocampus and ACC are critical in different aspects of cognitive functions, these findings would suggest that disruption of WM integrity may also underlie some cognitive deficits in schizophrenia.

Extensive evidence implicates that WM abnormality may occur before the onset of schizophrenia (Marenco and Weinberger, 2000; Delisi, 2008). In the present study, we
extracted WM FA values from the identified WM clusters in the left PFC and hippocampus of each subject and found that WM FA values consistently in an order of low to high were from the left PFC and hippocampus of schizophrenia patients, healthy siblings and healthy controls (right panel in Fig. 2A–B). Remarkably, schizophrenia patients and their healthy siblings shared reduced WM FA in the left PFC and the hippocampus as compared with healthy controls (left panel in Fig. 2A–B). This pattern of disruption of WM integrity may provide an explanation why healthy siblings have an about 9-fold higher risk to develop schizophrenia and share some similar cognitive deficits with patients (Snitz et al., 2006). However, we also found that only schizophrenia patients showed disruption of WM integrity in the left ACC as compared with either their healthy siblings or healthy controls. Interestingly, healthy siblings but not healthy controls showed the highest WM FA values in the left ACC (Fig. 2C). Explanation for this finding could be more complex since schizophrenia patients are medicated and their WM FA values may be affected by the illness, while their healthy siblings may be benefited from compensation of higher WM FA values as compared with either schizophrenia patients or healthy controls. Nevertheless, since ACC is critical for cognitive functions such as thinking or decision making, neural disconnectivity in the left ACC could be associated with the transition from disease risk to schizophrenia.

It is assumed that even relatively simple information is processed by distributed neural networks (Pastor et al., 2000). Abnormalities of neural networks can be revealed by brain imaging studies. Evidence shows that defects in white
matter can result in alterations in dopaminergic function and behavior related to psychiatric disorders such as schizophrenia (Roy et al., 2007). WM FA is associated with cognitive functions such as executive control (Nestor et al., 2004), verbal learning (Szeszko et al., 2008), and IQ (Schmithorst et al., 2005). Lower WM FA positively correlates with poor performance on various cognitive tasks and more severe psychotic symptoms (Szeszko et al., 2008). WM FA reduction in the hippocampus may also occur in the early stage of schizophrenia and is associated with the cognitive deficits of schizophrenia (White et al., 2007). Our present findings that WM FA is reduced in the left PFC and hippocampus of schizophrenia patients are consistent with previous reports (Buchsbaum et al., 2006, 1998; Kalus et al., 2004). Furthermore, evidence demonstrates that gray matter volumetric reduction is found in the PFC and hippocampus (Gur et al., 2007) of schizophrenia patients. WM volume is characterized by progressive reduction in these brain regions despite receiving antipsychotic drug treatment (Ho et al., 2003). Moreover, relatives also exhibit PFC and hippocampus volume reduction, although these changes are not as marked as those in schizophrenia patients (Lawrie et al., 2008). Abnormal PFC and hippocampus activation, as revealed by functional MRI studies, has also been identified in schizophrenia patients and their healthy relatives (Macdonald et al., 2008; Lawrie et al., 2008; Becker et al., 2008; Fussar-Poli et al., 2007; Callicott et al., 2003). Taken together, these studies strongly suggest that abnormalities of the PFC and the hippocampus are a consistent feature in both schizophrenia patients and healthy first-degree relatives or healthy siblings. The present findings are consistent with previous reports (Meyer-Lindenberg et al., 2005; Weinberger et al., 1992a,b) and further strengthen the neural disconnectivity hypothesis of schizophrenia.

Ample evidence also implicates that abnormal ACC may be the key feature of schizophrenia (Fornito et al., 2008a,b). Our present findings are consistent with previous reports that only schizophrenia patients exhibited neural disconnectivity in the left ACC (Becker et al., 2008). Evidence demonstrates that the ACC volume is decreased (Baiano et al., 2007; Koo et al., 2008), which is associated with impaired executive control in schizophrenia patients (Szeszko et al., 2000). Abnormal ACC activities during interference and attention tasks have been reported in schizophrenia (Yucel et al., 2007). Previous DTI study indicates that WM FA is reduced in the ACC of schizophrenia patients (Konrad and Winterer, 2008). An interesting notion is that the ACC monitors response conflict that recruits PFC to reduce conflict (Gehring and Knight, 2000). Other aspects of cognitive functions such as working memory and speech are also engaged in the ACC (Barch, 2005). Remarkably, co-activation of the ACC and PFC is observed in various cognitive tasks, indicating the importance of neural connectivity between these regions (Paus, 2001). Thus, disruption of WM integrity in the ACC, the PFC and the hippocampus may not only associate with cognitive deficits in both schizophrenia patients and healthy siblings, but also may relate to the neuropathology of schizophrenia.

Assessment of the risk to develop schizophrenia in an individual is difficult in a clinical setting. However, in the present study, the average FA values (see Fig. 2A–B) of the left PFC and hippocampus in healthy siblings may serve as the criteria of the risk for schizophrenia, by which an individual may be of higher disease risk if the FA values of the left PFC and hippocampus are lower than the criteria. In contrast, neural disconnectivity in the left ACC is not present in healthy first-degree relatives of schizophrenia patients as compared with healthy controls (Becker et al., 2008; MacDonald et al., 2006). Thus, reduced WM FA in the ACC may be used as an indicator for an individual with cognitive deficits or with other brain diseases likely to convert to schizophrenia.

Some evidence shows that reduced WM FA may not be associated with age, but another report shows a negative correlation between WM FA and age in schizophrenia (Kyrkakopoulos et al., 2008). Our present study did not find a correlation between WM FA values and ages (see Fig. 2A–C). Furthermore, since both healthy siblings and healthy controls are free of medications, and thus the present findings that schizophrenia patients and healthy siblings shared reduced WM FA in the left PFC and hippocampus could exclude the role of medication as consistent with previous reports (Mori et al., 2007). This view is further supported by evidence that medication-naïve patients with schizophrenia also show WM FA reduction in the brain regions (Cheung et al., 2007).

In summary, reduced WM FA in the left PFC and the hippocampus may underlie disease risk of schizophrenia, whereas reduced WM FA in the left ACC may reflect the transition from the risk to schizophrenia. Future research may provide further understanding of the relationship between the WM FA reductions and the cognitive impairments in schizophrenia patients and their healthy siblings. Furthermore, a longitudinal study is necessary to confirm whether some of the younger siblings will develop schizophrenia, as well as the extent to which this finding may help to predict risk of developing schizophrenia amongst high risk subjects.

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Contributors
All authors were involved in the design and implementation of the study and the writing of the manuscript. Authors Yihui Hao, Qiang Yan, Baoci Shan and Zhenbing Liu devised the concept. Authors Yihui Hao and Qiang Yan carried out the analysis. Authors Lin Xu, Zhimin Xue, Tianzi Jiang, Baoci Shan and Zhenbing Liu supervised the study. Authors Haihong Liu, Xueqin Song and Yoshi Kanoke were involved in collecting the imaging data or clinical information.

Conflict of interest
All authors report no conflict of interests.

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