ZNF804A rs1344706 is Associated With Cortical Thickness, Surface Area, and Cortical Volume of the Unmedicated First Episode Schizophrenia and Healthy Controls

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The effects of ZNF804A rs1344706, a prominent susceptibility gene for schizophrenia, on gray matter (GM) structure in unmedicated schizophrenia (SZ) patients are still unknown, although several previous studies investigated the effects in medicated SZ patients and healthy controls (HC). Analyzing cortical thickness, surface area, and GM volume simultaneously may provide a more precise and complete picture of the effects. We genotyped 59 unmedicated first episode SZ patients and 60 healthy controls for the ZNF804A single nucleotide polymorphism (SNP) rs1344706, and examined between-group differences in cortical thickness, surface area, and cortical volume using a full-factorial 2×2 analysis of variance (ANOVA). We found the risk allele (T) in ZNF804A rs1344706, compared to the non-risk allele (G), was associated with thinner cortex in the bilateral precuneus, left precentral gyrus, and several other regions, associated with a smaller cortical surface area in the left superior parietal, precuneus cortex and left superior frontal, and associated with a lower cortical volume in the left superior frontal, left precentral, and right precuneus in SZ patients. In contrast, in the controls, the T allele was associated with the increased cortical measurements compared to the G allele in the same regions as those mentioned above. ZNF804A rs1344706 has significant, but different, effects on cortical thickness, surface area, and cortical volume in multiple regions of the brain cortex. Our findings suggest that ZNF804A rs1344706 may aggravate the risk for schizophrenia by exerting its effects on cortical thickness, surface area, and cortical volume in these brain regions.
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INTRODUCTION

Schizophrenia (SZ) has consistently showed high heritability, with heritability estimated at 73–90% [Sullivan et al., 2003]. A genome-wide association study found that a single nucleotide polymorphism (SNP) rs1344706 in ZNF804A is associated with schizophrenia [O'Donovan et al., 2008]. This association has been replicated in multiple independent samples, including the Han Chinese population [Donohoe et al., 2010], although differences were found in other studies [Li et al., 2012]. Actually, ZNF804A is expressed broadly throughout in the brain, especially during neural development, and is considered to be involved in the regulation of early neurodevelopment, adult neurogenesis, dendritic development, and neuronal maturation [Hill and Bray 2012].

The association between the variant in ZNF804A and the brain gray matter (GM) structure in vivo is a key factor to understand the neuropathological mechanism of the susceptibility gene in SZ. Previous studies have reported inconsistent results (Table I). Donohoe et al. [2011] detected that homozygous "AA" risk carriers with SZ had relatively larger gray matter volumes than heterozygous/homozygous non-carriers (AC/CC), particularly for hippocampal volumes. Schultz et al. [2014] found that AA carriers of SZ exhibited significantly thicker cortex in prefrontal and temporal regions and less disturbed superior temporal cortical folding. However, the other two studies in SZ reported no association between the GM and rs1344706 in ZNF804A [Wassink et al., 2012; Bergmann et al., 2013]. We noticed that the patients included in these studies were medicated and several studies suggested that the antipsychotics may influence brain GM [Ho et al., 2011; Fusar-Poli et al., 2013; Goghari et al., 2013]. The discrepancy of these results about the association between GM and ZNF804A in SZ may partly be contributed to the medication. A study in unmedicated SZ patients will avoid the influence of medication on the results.

Similarly, for healthy controls, most of studies on the association between rs1344706 and GM structure focused on one measure of GM morphometry, GM volume or cortical thickness (Table I) [Donohoe et al., 2011; Cousijn et al., 2012; Wassink et al., 2012; Bergmann et al., 2013]. GM volume was measured by summing the voxels of a given structure and is a product of cortical thickness and

cortical surface area substantially. The change in cortical volume can not reflect the precise changes of thickness and surface area [Fischl and Dale 2000]. From a biological perspective, the cortical surface is composed of the tops of ontogenetic columns that grow perpendicular to the cortical surface [Mountcastle 1997]. Thus, the cortical surface area is determined by the number of columns, and the cortical thickness is driven by the number of neurons within a column [Rakic 2007]. The two cortical representations, cortical surface area and thickness, show distinct features during different ontogenic stages of corticogenesis [Fischl and Dale 2000; Rakic 2007]. Recently, the cortical thickness and surface area have been used to investigate the pathology of SZ and other mental disorders [Palaniyappan and Liddle 2012]. Thus, analyzing the cortical thickness, surface area, and volume simultaneously will reveal more precise effects of ZNF804A rs1344706 on the cortical macrostructure.

The aim of this study was to examine the effects of ZNF804A rs1344706 on brain GM more precisely and completely. We analyzed three indices of GM (cortical thickness, surface area, and volume) separately in unmedicated first episode SZ patients and healthy controls. Because the genetic environment of the healthy controls is different from that of schizophrenic patients [Schultz et al., 2014], we analyzed the effects of the interaction between the ZNF804A rs1344706 genotype (non-risk allele carriers or G homozygous: GG; risk allele carriers or T carriers: GT/TT) and the diagnosis of schizophrenia (SZ patients and the healthy controls) on these three indices.

METHODS

Sample

We recruited 59 drug naïve first episode SZ patients from the Inpatient Unit of Department of Psychiatry, the Third Affiliated Hospital of Sun Yat-Sen University in Guangzhou, China. All patients were right-handed [Oldfield 1971] and met the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria for paranoid schizophrenia or schizophreniform disorder (after follow-up, a diagnosis of paranoid schizophrenia was

TABLE I. Summary of Previous Studies on Association of ZNF804A rs1344706 Genotype and Gray Matter Structure in Patients With Schizophrenia (SZ) and Healthy Controls (HC)

Reference	Year	Subjects	medicated	Indices	results
Schultz, et al	2014	55 SZ	Yes	Cortical thickness	Positive
		40 HC	No	Cortical folding	Positive
Bergmann, et al	2013	82 SZ	Yes	Cortical thickness	Negative
		152 HC	No		Negative
Wassink, et al	2012	335 SZ	Yes	Cortical volume	Negative
		198 HC	No		Negative
Cousijn, et al	2012	892 HC	No	Cortical volume	Negative
Donohoe, et al	2011	70SZ	Yes	Cortical volume	Positive
		38 HC	No		Negative
Voineskos	2011	62 HC	No	Cortical thickness	Positive
Lencz, et al	2010	39 HC	No	Cortical volume	Positive

Positive means there is association of ZNF804A rs1344706 genotype and gray matter structure, and negative means there is not.

established; n = 22). The DSM-IV diagnosis was performed by an experienced psychiatrist (QW, 14 years clinical experience) using the patient version of the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I/P). The severity of psychopathology at the time of MRI scanning was rated for each patient with SZ according to the Positive and Negative Syndrome Scale (PANSS) [Kay et al., 1987]. Family psychiatric history was obtained by interviewing the patients and their relatives when possible, who provided information on family history in details during the clinical interview. This study adopted the definition of family history as described by Xu et al. [2008]. Patients with positive family history (PFH) were defined as having at least one relative with schizophrenia in their first-degree or second-degree relatives; otherwise, they were defined as patients with negative family history (NFH). We also recruited 60 age- and gender-matched healthy subjects as the control group. They were screened for major medical, neurological, or psychiatric history. None of them had a current or history of mental illness or first-degree relatives with a psychotic disorder, according to the non-patient version of SCID. For all participants in this study, the exclusion criteria were as follows: (i) chronic neurological disorders; (ii) history of alcohol or substance abuse; (iii) history of electroconvulsive therapy; and (iv) contraindication to MRI scanning. This research was conducted in accordance with the Helsinki Declaration as revised 1989 and was approved by the Clinical Research Ethics Committee of the Third Affiliated Hospital of Sun Yat-Sen University. Informed consent was obtained from each subject.

Table II lists the demographic and clinical characteristics for the SZ patients and the healthy subjects. Analysis of variance tests (for numeric variables) and χ^2-test (for categorical variables) were conducted using the Statistical Package for Social Sciences (SPSS 17.0).

Genotyping

Genotyping was performed as described previously [Wei et al., 2012]. Briefly, genomic DNA from leukocytes in the blood was amplified by the polymerase chain reaction (PCR) to generate a 443 bp product spanning rs1344706. Primers were as follows: upper GAATCTAGA GTCATGCAGG, and lower CAAGTTATTC TCTAGAGTCC.

The healthy control group (GG = 10, GT = 12, TT = 38) and the patient group (GG = 17, GT = 16, TT = 26) were both in Hardy–Weinberg equilibrium as calculated with GENEPOP software package (http://genepop.curtin.edu.au/). In order to test the effect of the risk allele (T allele) on the brain cortical thickness, we divided both the SZ patients and the healthy controls into two subgroups, non-risk allele carriers (GG genotype) and risk allele carriers (GT/TT genotypes).

Structural Images Acquisition and Data Processing

MRI data were acquired on a 1.5-T GE Signa Twinspeed MRI scanner equipped with an eight-channel phased array head coil. High resolution T1-weighted brain structural MRI (sMRI) data were obtained using an inversion-recovery prepared 3D spoiled gradient echo (SPGR) pulse sequence (TR = 9.568 ms, TE = 4.132 ms, TI = 650 ms, flip angle = 15°, FOV = 240 × 240 mm², matrix = 256 × 256, slice thickness = 0.9 mm, and 192 sagittal slices).

We used the FreeSurfer software package (version 5.0.0, http://surfer.nmr.harvard.edu) to create a three-dimensional model of the cortical surface for estimating the cortical thickness and cortical surface area [Dale et al., 1999; Fischl et al., 1999]. The procedures were also described in our previous study [Li et al., 2014]. In brief, the implemented processing pipeline comprised removal of non-brain tissue, transformation to Talairach-like space, and segmentation of gray/white matter tissue. The white/gray matter boundary was tessellated and topological defects were corrected automatically. After intensity normalization and identifying transitions between white/gray matter, the boundary of the pial and gray matter was identified by detecting the greatest shifts in intensity through

TABLE II. Demographic and Clinical Characteristics of the Unmedicated Schizophrenia Patients and the Healthy Controls

| | Schizophrenia patients | | Healthy controls | | ANOVA/χ^2/t-test | |
	G homozygous (n = 17)	T carriers (n = 42)	G homozygous (n = 10)	T carriers (n = 50)	F/χ^2/t	P
Age (years)	27.1 (6.9)	26.0 (6.4)	27.6 (4.8)	25.2 (5.9)	F = 0.69	0.56
Gender (F/M)	8/9	20/22	4/6	23/27	χ^2 = 4.85	0.18
Years of education	12.4 (3.4)	11.9 (2.5)	13.8 (2.9)	13.2 (2.7)	F = 2.28	0.08
Positive scores of PANSS	22.4 (5.4)	25.0 (4.9)	NA	NA	t = -1.74	0.09
Negative scores of PANSS	16.9 (6.3)	14.5 (4.2)	NA	NA	t = 1.68	0.10
General scores of PANSS	35.3 (6.0)	36.7 (5.1)	NA	NA	t = -0.94	0.35
Total scores of PANSS	75.1 (14.1)	76.2 (8.3)	NA	NA	t = -0.37	0.72
Duration of psychosis (weeks)	119.3 (134.0)	101.9 (182.7)	NA	NA	t = 0.36	0.72
Age of onset (years)	24.4 (6.4)	24.2 (6.3)	NA	NA	t = 0.13	0.89
Subtypes: No. Para/Dis/Un	10/3/4	28/6/8	NA	NA		
Family psychotic history: No. PFH/NFH	3/14	7/35	NA	NA		

ANOVA, univariate analysis of variance; Dis, disorganized type; G homozygous, non-risk allele carriers (GG); NFH, negative family history; PANSS, positive and negative syndrome scale; Para, paranoid type; PFH, positive family history; T carriers, risk allele carriers (GT/TT); Un, undifferentiated type.

surface deformation. The entire cortex for each subject was then visually inspected and any inaccuracies in segmentation were manually edited. After creation of the cortical representations, we estimated cortical measures (cortical thickness, surface area, and volume) for each of these regions [Fischl et al., 2004]. All of the reconstructed cortical surfaces were mapped on a common spherical coordinate system using a spherical transformation. Surface maps were smoothed with a Gaussian kernel with a full-width-at-half-maximum (FWHM) of 10 mm. Cortical thickness was measured by calculating the shortest distance from the gray/white boundary to the gray/CSF boundary and vice versa at each vertex, and averaging these two values [Fischl and Dale 2000]. Estimates of the cortical surface area were obtained by computing the area surrounding each vertex on a triangular grid. The cortical volume was obtained by multiplying the surface area and thickness in each vertex across the cortical mantle.

Statistical Analysis

A general linear model (GLM) controlling for age, gender, and years of education was used to perform a 2×2 ANOVA with cortical thickness, area, and volume as dependent variables. The first factor was diagnosis (schizophrenia and healthy control), and the second was the presence/absence of a genotype of ZNF804A rs1344706 (non-risk and risk allele carriers). The right and left hemispheres were tested separately. A Monte Carlo simulation cluster analysis with 10,000 iterations and a cluster threshold of $P < 0.05$ was adopted to correct for multiple comparisons.

In order to further verify and analyze the influence of interactions between the genotype and a diagnosis of schizophrenia on brain morphometry, we extracted the mean cortical values of thickness, area, and volume at the significant clusters and quantified the differences (D) between the non-risk (G homozygous) and risk (T carriers) allele carriers in the patients with SZ and healthy controls, respectively, using the following equation:

$$D = (M_{\mathrm{G}} - M_{\mathrm{T}})/M_{\mathrm{G}} \qquad (1)$$

where M_{G} (M_{T}) represents the group averaged cortical thickness, or area, or volume in the non-risk allele carriers (risk allele carriers) for a given region.

RESULTS

Sample

Table II shows no significant difference ($P > 0.05$) in age, gender, and years of education between the two genotype subgroups, non-risk allele carriers (GG), and risk allele carriers (GT/TT), within the total sample and within each diagnostic group. None of the aforementioned demographic variables showed a significant interaction between genotype and diagnosis. Within the patient group, the two genotype subgroups showed no significant difference ($P > 0.05$) in the subtype, family history, total scores on PANSS (positive scores, negatives scores, and general scores), duration of psychosis, and age-at-onset of psychosis.

Effect of SNP Genotype on Brain Cortex

The brain morphological differences associated with a diagnosis of schizophrenia and the presence of the ZNF804A rs1344706 genotype were obtained by using a 2×2 ANOVA. Figure 1 shows sets of
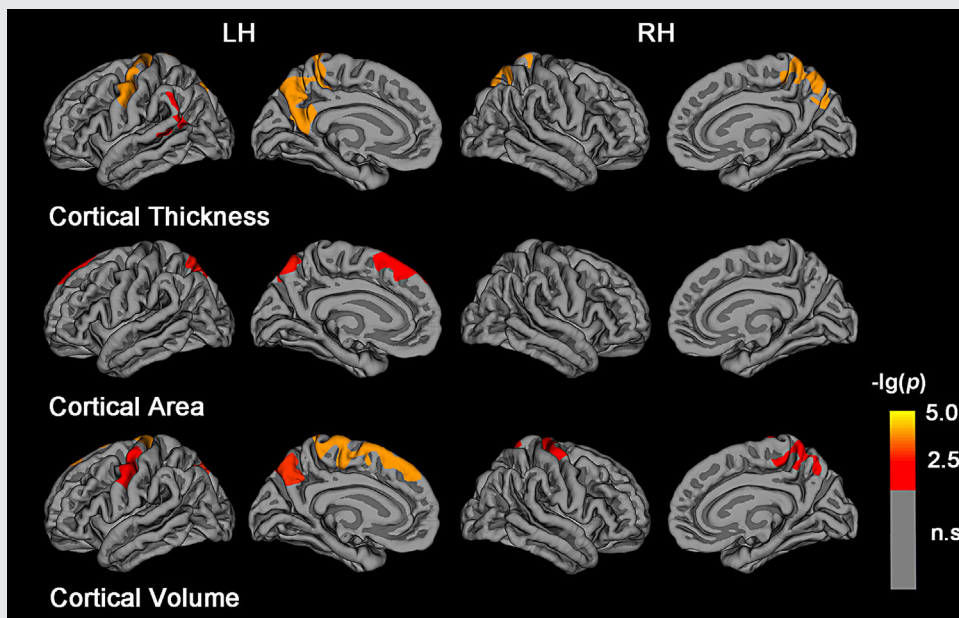

FIG. 1. Statistical maps displaying effects of the interaction between the ZNF804A rs1344706 genotype (non-risk allele carriers: GG; risk allele carriers: GT/TT) and diagnosis (schizophrenia patient vs. healthy control) on cortical thickness, surface area, and volume. LH (RH): left (right) hemisphere.

p-maps to indicate a significant interaction between diagnosis and genotype on the cortical thickness, surface area, and volume, separately.

Cortical thickness. We detected a significant effect of the interaction between the genotype and the diagnosis of schizophrenia on cortical thickness in the following four clusters with their corresponding cluster-wise probabilities (CWP) (Fig. 1 and Table III), the left precuneus, superior parietal (CWP = 0.0001); the left precentral cortex (CWP = 0.0001); the left superior temporal, inferior parietal cortex (CWP = 0.0040); and the right precuneus, superior parietal and paracentral gyrus (CWP = 0.0001).

Table IV lists the extracted mean cortical thickness in the four aforementioned clusters, in which the interaction between diagnosis and genotype was significant. The results showed that compared to non-risk allele carriers, the risk allele carriers had thinner cortical thickness in the bilateral precuneus (left: D = 2.8%; right: D = 2.2%) and left precentral gyrus (D = 1.1%) in the patients with SZ. But the healthy controls with risk allele carriers (GT/TT) showed a thicker cortical thickness in those clusters (left precuneus: D = −1.6%; right precuneus: D = −3.6%; left precentral: D = −7.9%) than did the healthy non-risk allele carriers (GG).

Cortical surface area. As for cortical surface areas, we detected significant interactions between genotype and a diagnosis of schizophrenia in these clusters, the left precuneus and superior parietal cortex (CWP = 0.0020), the left superior frontal cortex (CWP = 0.0124) (Fig. 1, Table III). From Table IV, we can see that in the SZ patients, the risk allele carriers had a decreased cortical surface area in these two clusters (left precuneus: D = 2.5%; left superior frontal: D = 1.0%), but in the healthy controls, the risk allele carriers had an increased cortical surface area, when compared to the non-risk allele carriers.

Cortical volume. Figure 1 also shows an interaction effect between genotype and a diagnosis of schizophrenia on the cortical volume in five clusters. They were located in the left superior frontal, paracentral, and precentral gyrus (CWP = 0.0001); the left precuneus and superior parietal gyrus (CWP = 0.0010); the left precentral gyrus (CWP = 0.0068); the right precuneus, superior parietal, and paracentral gyrus (CWP = 0.0208); and the right precuneus and superior parietal cortex (CWP = 0.0385). The details of these five clusters are listed in Table III.

An ROI-wise analysis was performed to determine the differences between the risk allele carriers and non-risk allele carriers for the five clusters (Table IV). In the patients, we found that the risk allele carriers had a smaller cortical volume in four clusters, the left superior frontal (D = 2.0%), left precuneus (D = 5.4%), left precentral (D = 0.5%), and right precuneus (D = 5.6%), compared to non-risk allele carriers. However, in the healthy controls, we detected an increased cortical volume for these four brain regions in the risk allele carriers compared to non-risk allele carriers (see Table IV for details).

DISCUSSION

The present study used sMRI to explore the potential association of the schizophrenia risk gene variant ZNF804A rs1344706 with the cortical thickness, surface area, and volume in unmedicated first episode SZ patients and a healthy population. We found that the risk allele (T) was associated with thinner cortical thickness, less cortical surface area, and a smaller cortical volume in multiple regions in the SZ patients, whereas the opposite effect was observed in the healthy controls, i.e., the risk allele (T) was associated with thicker cortical thickness, larger cortical surface area, and a bigger cortical volume in these regions.

It is important to note that antipsychotics may cause the increase or decrease of brain GM as several studies suggested antipsychotics is an important confounding factor in neuroimaging studies of SZ [Ho et al., 2011; Fusar-Poli et al., 2013; Goghari et al., 2013]. Different from previous studies, this case-controls study recruited

TABLE III. Clusters Showing Significant Interaction Between the ZNF804A rs1344706 Genotype (Non-Risk Allele Carriers and Risk Allele Carriers) and Diagnosis (Unmedicated Schizophrenia Patients and Healthy Controls) on Cortical Thickness, Surface Area, and Volume, Separately

Cortex area		Talairach coordinates (x, y, z)	Cluster size (mm^2)	CWP
Cortical thickness (mm)				
Precuneus, superior parietal	LH	(−17, −45, 55)	2742.3	0.0001
Precentral	LH	(−53, −7, 39)	1445.8	0.0001
Superior temporal, inferior parietal	LH	(−44, −54, 22)	1053.8	0.0040
Precuneus, superior parietal, paracentral	RH	(8, −60, 57)	1968.0	0.0001
Cortical surface area (mm^2)				
Precuneus, superior parietal	LH	(−16, −67, 59)	931.9	0.0020
Superior frontal	LH	(−7, 34, 50)	736.0	0.0124
Cortical volume (mm^3)				
Superior frontal, paracentral, precentral	LH	(−7, 34, 50)	2201.1	0.0001
Precuneus, superior parietal	LH	(−8, −73, 46)	1133.3	0.0010
Precentral	LH	(−53, −7, 39)	902.5	0.0068
Precuneus, paracentral, superior parietal	RH	(8, −59, 57)	784.6	0.0208
Precuneus, superior parietal	RH	(39, −10, 61)	699.2	0.0385

LH (RH), left (right) hemisphere; CWP, cluster-wise probability.

TABLE IV. Quantification of Brain Clusters Showing Significant Interactions Between ZNF804A rs1344706 Genotype [Non-Risk Allele Carriers: G Homozygous and Risk Allele Carriers: T Carriers] and Diagnosis [Unmedicated Schizophrenia Patients and Healthy Controls] on the Cortical Thickness, Surface Area, and Volume

| | | Mean cortical values: mean [SD] | | | | | |
| | | Schizophrenia patients | | | Healthy controls | | |
Cortex area		G homozygous (n = 17)	T carriers (n = 42)	Difference (D) in %	G homozygous (n = 10)	T carriers (n = 50)	Difference (D) in %
Cortical thickness [mm]							
precuneus, superior parietal	LH	2.47 [0.16]	2.40 [0.12]	2.8	2.43 [0.24]	2.47 [0.11]	−1.6
precentral	LH	2.73 [0.15]	2.70 [0.19]	1.1	2.53 [0.31]	2.73 [0.17]	−7.9
superior temporal, inferior parietal	LH	2.63 [0.16]	2.65 [0.14]	−0.8	2.56 [0.29]	2.71 [0.15]	−5.9
precuneus, superior parietal, paracentral	RH	2.27 [0.14]	2.22 [0.12]	2.2	2.20 [0.27]	2.28 [0.11]	−3.6
Cortical surface area (mm2)							
precuneus, superior parietal	LH	919.47 [131.76]	896.48 [102.56]	2.5	908.60 [80.28]	928.12 [103.75]	−2.1
superior frontal	LH	716.76 [71.09]	710.26 [79.31]	1.0	694.60 [61.36]	723.70 [66.24]	−4.2
Cortical volume [mm³]							
superior frontal, paracentral, precentral	LH	6778.06 [725.74]	6639.29 [849.17]	2.0	6771.70 [1486.40]	7189.78 [865.10]	−6.2
precuneus, superior parietal	LH	3423.59 [710.04]	3239.93 [540.52]	5.4	3590.80 [1096.23]	3626.76 [599.18]	−1.0
precentral	LH	2580.94 [337.99]	2594.26 [459.66]	0.5	2409.50 [578.10]	2710.78 [420.00]	−12.5
precuneus, paracentral, superior parietal	RH	2285.23 [276.06]	2158.05 [397.08]	5.6	2304.30 [747.25]	2383.32 [309.11]	−3.4
precentral	RH	1653.35 [227.66]	1662.26 [283.57]	−0.5	1598.10 [302.65]	1747.58 [277.95]	−9.4

The relative alteration of cortical thickness for a cluster was estimated by $D = (M_G - M_T)/M_G$, in which the term $M_G (M_T)$ represents the group averaged cortical thickness in the non-risk allele carriers (risk allele carriers) for the given ROI. LH (RH), left (right) hemisphere.

the SZ patients who were unmedicated and were in first episode stage. The variability of recruited patients in different studies may partly contributed to the inconsistence of our findings with those of several previous studies, which reported that ZNF804A rs1344706 was not associated with the GM structure [Wassink et al., 2012; Bergmann et al., 2013] or found the opposite effects of this variant compared with our results [Donohoe et al., 2011; Schultz et al., 2014]. This discrepancy may also be partly due to the different SZ subtypes [Yang et al., 2013], or different ethnicity [Li et al., 2011], or different processing methods [Schultz et al., 2014].

We found that the effects of ZNF804A rs1344706 on cortical thickness and on cortical surface area were not the same in the affected locations and on the size of the brain regions showing a significant interaction between diagnosis and genotype. Table III shows that the cortical thickness was affected in four clusters (the left precuneus, superior parietal; the left precentral; the left superior temporal, inferior parietal; and the right precuneus, superior parietal and paracentral gyrus), while the cortical surface area was affected in two cluster (the left precuneus, superior parietal; superior frontal cortex). According to the radial unit hypothesis [Pontious et al., 2008; Rakic et al., 2009], cortical thickness is influenced by the number of cells within a column but cortical surface area is determined by the number of cortical columns. These two factors are determined during different phases of neural cell proliferation. The length of the first phase may determine the number of radial units that participate in the overall expansion of the cortical surface, and the cell growth during the second phase may affect cortical thickness by regulating the number of neurons generated within each radial column [Rakic 2007; Rakic et al., 2009]. Thus, considering the differences between the influences on cortical thickness and surface area, and putting all the evidences we found together, we speculate that ZNF804A rs1344706 may be associated with both phases of neural cell proliferation in the left superior parietal and precuneus cortices and also with the second phase in additional brain regions. In addition, since the effects of ZNF804A rs1344706 on these cortical measures reflect different biological information [Rakic 2007; Pontious et al., 2008; Rakic et al., 2009], our findings provide further evidence that ZNF804A rs1344706 may be involved in the regulation of neurodevelopment [Chung et al., 2010; Hill and Bray 2012].

In this study, all three morphological measures (cortical thickness, surface area, and volume) showed that ZNF804A rs1344706 was associated with morphological changes in the GM in the left and/or right precuneus and other parts of parietal cortex. The precuneus is engaged in many high-order cognitive functions, including behavioral inhibition [Fuentes et al., 2012], problem solving [Jin et al., 2012], self-related processing and awareness [Lou et al., 2004; van der Meer et al., 2013], and episodic memory [Lepage et al., 2010]. Several studies found the precuneus is related to disorders of insight [Faget-Agius et al., 2012; Liemburg et al., 2012; van der Meer et al., 2013], conduct [Schiffer et al., 2013], and episodic memory [Lepage et al., 2010] in schizophrenia. With these evidences together, our findings in the present study suggest that the effect of ZNF804A rs1344706 on the precuneus and other parts of parietal cortex may be involved in the pathological mechanisms of conferring risk of schizophrenia.

We also found that the risk allele (T) in ZNF804A rs1344706, compared to the non-risk allele (G), was associated with a smaller cortical surface area in the left superior frontal, and with a lower cortical volume in the left superior frontal in schizophrenia. Previous studies also showed that ZNF804A s1344706 was associated with the prefrontal cortex [Esslinger et al., 2009; Nenadic et al., 2015; Schultz et al., 2014]. Many studies [Glahn et al., 2008] have demonstrated that the frontal cortex is associated with the neuropathological mechanism of schizophrenia. About the temporal cortex, we found that ZNF804A rs1344706 was associated with the cortical thickness in the left superior temporal in the SZ patients. This result is consistent with several previous studies, which showed that ZNF804A s1344706 was associated with the temporal cortex [Mohnke et al., 2014; Nenadic et al., 2015]. Temporal cortex also has been showed to be involved in the neuropathological mechanism of SZ [Sun et al., 2009]. Thus, the effect of ZNF804A rs1344706 on the prefrontal and temporal cortices may be also involved in the pathological mechanisms of conferring risk of schizophrenia.

In addition, we found that the effect of ZNF804A rs1344706 on the brain GM in the healthy controls was opposite to that in SZ patients. That is, the risk allele was associated with significantly decreased brain structural measures (thickness, surface area, and volume) in the SZ patients, but with the significantly increased brain structural measures in the healthy controls, in the precuneus, parietal, frontal and temporal cortices (Table III). This results are partly consistent with the previous finding about the differential effects of rs1344706 on gray matter [Schultz et al., 2014] or white matter [Wei et al., 2012] in healthy controls and SZ patients. This different effect of ZNF804A rs1344706 on the brain GM in the SZ and in the controls may be caused from variability of subjects or their different risk genetic environments [Prata et al., 2009; Nicodemus et al., 2010]. For example, an exonic SNP rs12476147, located in exon four of ZNF804A, was shown significantly association with SZ and allelic expression imbalance in the DLPFC, using a powerful within-subject design [Guella et al., 2014]. In the same study [Guella et al., 2014], the rs1344706 SZ risk allele was found the cis-regulatory variant directly responsible for this allelic expression imbalance in adult cortex. Taken together, the different effects of rs1344706 on the GM in SZ and control groups may be involved in neuropathological mechanisms of this risk variant rs1344706 conferring risk to schizophrenia.

There are several limitations to be considered in the present study. First, to avoid the confounding factors of antipsychotics, we included the unmedicated SZ patients only, and the sample size was relative smaller. Second, we analyzed rs1344706 only, while other SNPs or haplotypes in the same gene or in other genes interacting with ZNF804A may also be relevant. Third, the brain structural images were acquired on a 1.5 GE MRI scanner and the SNR was low which may affect the accuracy of our findings. To minimize the effect of low SNR on the calculations, we have screened the brain structural images, and selected these images had a high gray/white matter contrast into the data analysis. This may ensure the accuracy of segmentation of gray and white matter. No doubt, the future studies need to consider a larger sample size, to genotype other SNPs or haplotypes in the same gene or in other related genes, and to acquire relative higher SNR images by using a high- strength MRI scanner or by repeating MRI scans. Therefore, the future studies

need to consider a larger sample size, to genotype other SNPs or haplotypes in the same gene or in other related genes, and to acquire relative higher SNR images by using a high-strength MRI scanner or by repeating MRI scans.

In summary, for the first time, we investigated the effects of ZNF804A rs1344706 on the cortical morphometry in unmedicated SZ patients and healthy controls and found that the effects of the risk genetic variants (T) on the microstructure of the GM in precuneus, parietal, prefrontal, and temporal cortex in the schizophrenic patients were opposite to those found in the healthy controls. Our findings suggest that ZNF804A rs1344706 may aggravate the risk for schizophrenia by exerting its effects on cortical thickness, surface area, and cortical volume in these brain regions.

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