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ORIGINAL ARTICLE

ZNF804A Variation May Affect Hippocampal-Prefrontal Resting-State Functional Connectivity in Schizophrenic and Healthy Individuals

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Abstract The *ZNF804A* variant rs1344706 has consistently been associated with schizophrenia and plays a role in hippocampal-prefrontal functional connectivity during working memory. Whether the effect exists in the resting state and in patients with schizophrenia remains unclear. In this study, we investigated the *ZNF804A* polymorphism at rs1344706 in 92 schizophrenic patients and 99 healthy controls of Han Chinese descent, and used resting-state functional magnetic resonance imaging to explore the functional connectivity in the participants. We found a significant main effect of genotype on the resting-state functional connectivity (RSFC) between the hippocampus and the dorsolateral prefrontal cortex (DLPFC) in both schizophrenic patients and healthy controls. The homozygous *ZNF804A* rs1344706 genotype (AA) conferred a high risk of schizophrenia, and also exhibited significantly decreased resting functional coupling between the left hippocampus and right DLPFC ($F(2,165) = 13.43$, $P < 0.001$). The RSFC strength was also correlated with cognitive performance and the severity of psychosis in schizophrenia. The current findings identified the neural impact of the *ZNF804A* rs1344706 on hippocampal-prefrontal RSFC associated with schizophrenia.

Keywords Schizophrenia · *ZNF804A* · Imaging genetics · Hippocampus · Dorsolateral prefrontal cortex

Introduction

Schizophrenia is a severe neuropsychiatric disorder with a complex etiology, which exhibits a considerable level of heritability [1, 2]. Previous studies have shown that abnormal brain structure and function are important intermediate phenotypes of schizophrenia [3, 4]. Imaging genetics and genomics research has further linked genetic variations to brain structure and function, indicating that genetic risk factors impact cognition, emotion, and behavior in both healthy persons and patients with diseases [5, 6].

The altered hippocampal–prefrontal connectivity along with related cognitive impairments in patients with schizophrenia might be an important aspect of the pathophysiology [7, 8]. Meyer-Lindenberg and colleagues found that, during a working memory task with a low load, both controls and patients with schizophrenia showed a negative correlation between activity in the hippocampal formation and that in the contralateral dorsolateral prefrontal cortex (DLPFC). In contrast, when switched to a high working memory load, the correlation remained in patients but diminished in controls, suggesting a region-specific alteration of hippocampal-DLPFC functional connectivity in

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schizophrenia [9]. The hippocampal–DLPFC coupling during working memory activation has also been reported in healthy relatives of patients [10] and healthy carriers of risk genotypes for schizophrenia [11, 12].

Zinc finger protein 804A (*ZNF804A*) is one of the candidate genes for schizophrenia recognized by genome-wide association studies of European samples (rs1344706, $P = 1.61 \times 10^{-7}$) [13]. In Asian populations, a few association studies have been conducted for *ZNF804A* rs1344706 and the results were inconsistent [14–17]. Overexpression of *Znf804a* mRNA in rat neural progenitor cells has been reported to significantly change the expression of schizophrenia-associated genes [18]. A landmark study of neuronal function with suppressed expression of *ZNF804A* in both human and rat cells has revealed its function in neurite formation, the maintenance of dendritic spine morphology, and responses to activity-dependent stimuli [19]. All these results suggest that *ZNF804A* is one of the most intriguing and promising risk genes for schizophrenia and a psychotic phenotype [20].

Rs1344706, a single nucleotide polymorphism (SNP) located at intron 2 of *ZNF804A*, has been reported to confer a risk of schizophrenia across several populations, A being the risk allele [21]. Neuroimaging studies have indicated its potential effect on brain structure [22, 23]. Efforts have also been made to understand its effect on multiple brain functions. Reduced inter-hemispheric DLPFC connectivity with a higher rs1344706 risk status has been reported during working memory, and this persists in both resting and cognitive states. However, the increased connectivity between the left hippocampus and right DLPFC has been exclusively reported in the n-back working memory task [11, 24]. Paulus and colleagues also reported positive functional connectivity between the left hippocampus and right DLPFC for the AA genotype and negative functional connectivity for the CC and CA genotypes [25].

Previous functional magnetic resonance imaging (fMRI) studies mainly concentrated on the association between the *ZNF804A* polymorphism and functional coupling of the left hippocampus and right DLPFC during working memory tasks. However, the effect of the *ZNF804A* polymorphism on the resting-state functional connectivity (RSFC) in patients with schizophrenia remains elusive. In the current study, we investigated the effects of *ZNF804A* rs1344706 on the RSFC of the hippocampus voxel-wise in the whole brain in patients with schizophrenia and healthy controls. Then, together with cognitive performance and assessment of psychosis severity, we tested whether their effects on RSFC are associated with behavior.

Materials and Methods

Participants

A total of 92 patients with schizophrenia and 99 healthy controls, who are all Han Chinese from northern China, were recruited in the Peking University Sixth Hospital. In the patients, two experienced psychiatrists made a diagnosis according to the *Structured Clinical Interview for Diagnostic and the Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR) Axis I Disorders (SCID, patient edition)*. Patients with any other neurological disorder, a history of severe medical illness, substance dependence, pregnancy, or treatment with electroconvulsive therapy within the past 6 months, and those with a diagnosis of any other Axis I disorder, were excluded. The healthy controls were screened using SCID (non-patient edition) and recruited if they had no history of mental and/or neurological disorder, drug or alcohol abuse, traumatic brain injury, or visible brain lesions on conventional MRI. Written informed consent was given by all patients and their legal guardians (i.e., parents) and by all healthy controls. This study was approved by the Medical Research Ethics Committees of Mental Health of Peking University Sixth Hospital.

Assessment of Symptomatology and Cognitive Performance

The severity of symptoms in the schizophrenic patients was evaluated by trained and experienced psychiatrists within one week of MRI scanning, using the Positive and Negative Syndrome Scale (PANSS). Category Fluency Test-animal naming (CFT) [26], the Digit Symbol Substitution Task (DSST) from the Wechsler Adult Intelligence Scale-III (WAIS-III) [27], and the Wechsler Memory Scale-Revised (WMS-R) [28] were also assessed to measure cognitive performance in the speed of processing and memory domains that are commonly impaired in schizophrenia [29–31].

Genotyping

Peripheral blood samples were collected from all participants and genomic DNA was extracted using a Qiagen QIAamp DNA Mini Kit (Germany). The SNP *ZNF804A* rs1344706 was selected from dbSNP (http://www.ncbi.nlm.nih.gov/SNP/) and genotyped using the TaqMan SNP genotyping assay on an ABI PRISM 7900 Sequence Detection System (Applied Biosystems, Foster City, CA), as described previously [32]. Investigators were blind to the case or control status during the genotyping process.

We repeated the genotyping assay in 1% of the samples and the results were 100% concordant. The DNA extraction and genotyping were centrally processed at the Key Laboratory of Mental Health in Beijing, China.

Imaging Data Acquisition

MRI scans were performed on a Siemens 3.0 Tesla Trio magnetic resonance scanner (Siemens Medical Systems, Erlangen, Germany) in Peking University Third Hospital. The resting-state functional imaging data were acquired with the following parameters: repetition time = 2000 ms, echo time = 30 ms, field of view = 220 × 220 mm^2, matrix = 64 × 64, flip angle = 90°, voxel size = 3.4 × 3.4 × 4.0 mm^3, 33 slices and 240 volumes. Before scanning, all participants were instructed to move as little as possible, keep their eyes closed, think of nothing in particular, and avoid falling asleep. After scanning, they were asked whether they fell asleep to reconfirm.

Resting-State fMRI Pre-processing

Data preprocessing of resting-state fMRI was completed using DPARSF (Data Processing Assistant for Resting State fMRI Advanced Edition, http://rfmri.org/DPARSF). The following steps were performed: (1) discarding the first 10 volumes from each participant; (2) slice timing correction; (3) realigning the volumes to the middle volume; (4) regressing out nuisance covariate signals including white matter, cerebrospinal fluid, and global signals; (5) spatial normalization by DARTEL (Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra); (6) smoothing with a 4-mm Gaussian kernel after resampling to 3-mm isotropic voxels; (7) linear regression to remove the effects of linear trends; and (8) temporal bandpass filtering (0.01–0.1 Hz). Specifically, healthy controls and schizophrenic patients exhibiting a maximum displacement of >3 mm in any of the cardinal directions (x, y, z) or a maximum rotation (x, y, z) of >3° were excluded. Finally, a total of 94 healthy controls and 79 schizophrenic patients were included in the functional connectivity analyses.

Statistical Analyses

The effect of gender was analyzed using Pearson's χ^2 test, and differences in continuous demographic variables, cognitive performance, and PANSS scores were analyzed by one-way ANOVA. Hardy-Weinberg equilibrium between expected and observed genotype distributions was tested using the χ^2 test. Correlations between cognitive performance, symptom severity, and RSFC strength were computed using two-tailed Pearson correlations. These analyses were performed using the Statistical Package for the Social Sciences for Windows, version 13.0 (SPSS, Chicago, IL). $P < 0.05$ was considered statistically significant.

The functional imaging analysis was performed using SPM8 (http://www.fil.ion.ucl.ac.uk/spm). We calculated individual voxel-wise hippocampal RSFC maps with a mask of the whole brain by taking the left and right hippocampus as regions-of-interest (ROIs). Hippocampal masks were constructed using the automated anatomical labeling atlas implemented in Wake Forest University Pickatlas [33]. Individual RSFC maps were generated by calculating the Pearson correlation coefficient between the average blood oxygen level-dependent time series in the ROIs and those of each voxel in the whole brain. Then, the correlation coefficients were converted to z values by Fisher's z transformation to improve normality. The individual z maps were then entered into a two-way ANOVA with diagnosis and genotype as between-subject factors and age and gender as covariates. A voxel-level threshold of $P < 0.001$ with a cluster extent $k > 10$ was considered significant. For each diagnostic group, a partial correlation analysis with age and gender as covariates in order to exclude their potential effect [34, 35] was further performed by genotype subgroup to explore the associations between the RSFC strength and cognitive performance as well as those between the RSFC strength and PANSS scores in patients.

Results

Cognitive Performance

There were no significant differences in gender distribution, age, education, and genotype between schizophrenic patients and healthy controls. Both case and control samples in each diagnostic group were in Hardy-Weinberg equilibrium ($P > 0.05$). Patients had significantly poorer cognitive performance in WMS-R ($P = 1 \times 10^{-6}$), DSST ($P < 1 \times 10^{-6}$), and CFT ($P = 0.001$) (Table 1). A significant difference of gender distribution was only found among the genotype subgroups of *ZNF804A* rs1344706 in schizophrenia due to fewer females in the AA subgroup ($P = 0.011$). No significant differences in WMS-R, DSST, and CFT were found between the genotype subgroups of rs1344706 in either diagnostic group (Table 2).

Effect of Diagnosis on Hippocampal-Thalamic RSFC

Two-way ANOVA showed a significant main effect of diagnosis on RSFC. We found increased RSFCs between

Table 1 Demographic and clinical characteristics of healthy controls and schizophrenic patients.

Variables	Schizophrenic patients	Healthy controls	F or χ^2	P value
Gender (male/female)	49/30	49/45	1.71	0.19
Age (years)	27.2 ± 6.8	25.8 ± 5.4	2.30	0.25
Education (years)	13.9 ± 2.7	13.7 ± 3.5	0.19	0.64
Genotype of rs1344706 (CC/CA/AA)	18/41/20	24/45/25	0.30	0.86
WMS-R score[a]	93.75 ± 21.07	109.31 ± 17.00	25.19	<0.001
DSST score[b]	52.26 ± 13.33	67.67 ± 13.79	52.99	<0.001
CFT score[b]	18.68 ± 5.35	21.73 ± 6.48	10.66	0.001
PANSS total score	76.85 ± 12.94	n.a.	n.a.	n.a.
PANSS positive score	23.63 ± 4.33	n.a.	n.a.	n.a.
PANSS negative score	18.51 ± 5.70	n.a.	n.a.	n.a.
PANSS general score	35.68 ± 5.43	n.a.	n.a.	n.a.

Data are given as mean ± SD; P-values refer to one-way ANOVA (parametric data) and the χ^2 test (categorical data). WMS-R, Wechsler Memory Scale-Revised; DSST, Digit Symbol Substitution Test; CFT, Category Fluency Test-animal naming; PANSS, Positive and Negative Syndrome Scale; n.a., not applicable. [a]Data were from 59 patients and 94 controls; [b]Data were from 74 patients and 93 controls.

the bilateral hippocampus and the right thalamus in schizophrenic patients, compared with the healthy controls (Table S1, Fig. S1). We maintained a cluster-level false discovery rate (FDR) at $P < 0.05$ using a voxel-level threshold of $P < 0.001$ with a cluster extent k > 10.

Effect of *ZNF804A* rs1344706 on Hippocampal-Frontal RSFC

Two-way ANOVA showed a significant main effect of genotype on the RSFC between the bilateral hippocampus and the right DLPFC. No significant interactive effect between genotype and diagnosis was found. As the hippocampus on each side had similar results, we only present results for the left hippocampus here. Data for the right hippocampus are available on request.

When the left hippocampus was treated as the seed region, genotype had a significant effect on the RSFC between the left hippocampus and the right dorsolateral superior and middle frontal gyri in the right DLPFC (Table S2). Results in the right dorsolateral superior frontal gyrus are shown in Fig. 1A (details of the right dorsolateral middle frontal gyrus are shown in Table S3 and Fig. S2) and those in the left middle frontal gyrus are shown in Table S4 and Fig. S3.

Furthermore, we extracted the mean functional connectivity strength of the right DLPFC with the peak MNI coordinates (x = 21, y = 42, z = 33) as the center and 6 mm as the radius (the sphere regions were similar to the frontal regions as shown in Fig. S4 and the results were almost the same). The results also showed a significant genotype effect on the mean RSFC strength of the right DLPFC ($P = 4.70 \times 10^{-4}$). The *post hoc* analysis showed that the RSFC strength was higher in CA heterozygotes than in CC ($P = 0.019$) and AA ($P < 0.001$) homozygotes (Fig. 1B). This significant difference still existed for each diagnosis. In the control group, CA heterozygotes showed higher RSFC strength than CC ($P = 0.037$) and AA ($P = 0.004$) homozygotes. In the schizophrenic group, CA heterozygotes only showed higher RSFC strength than AA ($P = 0.012$) homozygotes, with no significant difference from CC homozygotes ($P = 0.202$). When CC and CA were grouped as C carriers, as has been done in many previous studies exploring the effects of *ZNF804A* variation [23, 36–38], the mean RSFC strength in the CC/CA group was higher than that in AA homozygotes in both the control ($P = 0.023$) and schizophrenia ($P = 0.025$) groups (Fig. 1C).

Correlations Between RSFC and Behavioral Measures

With age and gender as confounding factors, partial correlation analysis showed that, in CA heterozygotes, the strength of RSFC was significantly negatively correlated with the DSST score, and positively correlated with scores of total and general psychopathological syndrome in the schizophrenic group. For AA (risk) homozygotes, the RSFC strength was significantly negatively correlated with the WMS-R score in the control group while positively correlated with the WMS-R score, and negatively correlated with the PANSS total score in the schizophrenic group (Table 3).

Taken together, for CA heterozygotes, higher hippocampal-prefrontal functional connectivity may predict a lower speed of information processing in healthy controls and schizophrenic patients, and higher PANSS scores for total and general symptoms indicating the severity of psychosis

Table 2 Demographic and clinical characteristics of healthy controls and schizophrenic patients with different genotypes of rs1344706.

	rs1344706									
	SZ					HC				
	CC	CA	AA	F or χ^2	P value	CC	CA	AA	F or χ^2	P value
Gender (male/female)	10/8	21/20	18/2	9.00	0.01	12/12	23/22	14/11	0.21	0.90
Age (years)	27.8 ± 6.7	27.5 ± 6.9	26.2 ± 7.0	0.36	0.70	24.5 ± 5.2	26.0 ± 5.4	26.8 ± 5.7	1.24	0.30
Education (years)	14.1 ± 2.3	14.3 ± 2.5	12.8 ± 3.3	2.08	0.13	14.4 ± 2.8	13.8 ± 3.0	12.9 ± 4.3	1.25	0.29
WMS-R score[a]	91.47 ± 18.30	99.57 ± 21.12	86.39 ± 20.39	2.36	0.10	115.46 ± 12.76	108.07 ± 15.26	105.64 ± 21.96	2.34	0.10
DSST score[b]	56.18 ± 9.79	53.46 ± 14.27	46.70 ± 12.92	2.75	0.07	68.48 ± 13.39	68.07 ± 14.50	66.20 ± 13.29	0.20	0.82
CFT score[b]	19.59 ± 4.95	19.24 ± 5.70	16.85 ± 4.78	1.65	0.20	20.83 ± 5.18	22.05 ± 7.13	22.04 ± 6.57	0.31	0.74
PANSS total score	82.67 ± 10.19	73.15 ± 14.89	79.20 ± 7.79	4.13	0.02	n.a.	n.a.	n.a.	n.a.	n.a.
PANSS positive score	24.50 ± 3.81	23.32 ± 4.85	23.50 ± 3.68	0.47	0.62	n.a.	n.a.	n.a.	n.a.	n.a.
PANSS negative score	19.78 ± 5.96	17.03 ± 5.63	20.35 ± 5.03	2.99	0.06	n.a.	n.a.	n.a.	n.a.	n.a.
PANSS general score	38.39 ± 6.56	34.66 ± 5.09	35.35 ± 4.32	3.17	0.05	n.a.	n.a.	n.a.	n.a.	n.a.
Chlorpromazine equivalent dose (mg/day)	436.11 ± 219.50	438.75 ± 205.53	482.50 ± 163.25	0.86	0.65	n.a.	n.a.	n.a.	n.a.	n.a.

Data are given as mean ± SD; P-values refer to one-way ANOVA (parametric data) and the χ^2 test (categorical data). SZ, schizophrenic patients; HC, healthy controls; WMS-R, Wechsler Memory Scale-Revised; DSST, Digit Symbol Substitution Test; CFT, Category Fluency Test-animal naming; PANSS, Positive and Negative Syndrome Scale; n.a., not applicable. [a]Data were from 59 patients and 94 controls; [b]Data were from 74 patients and 93 controls.

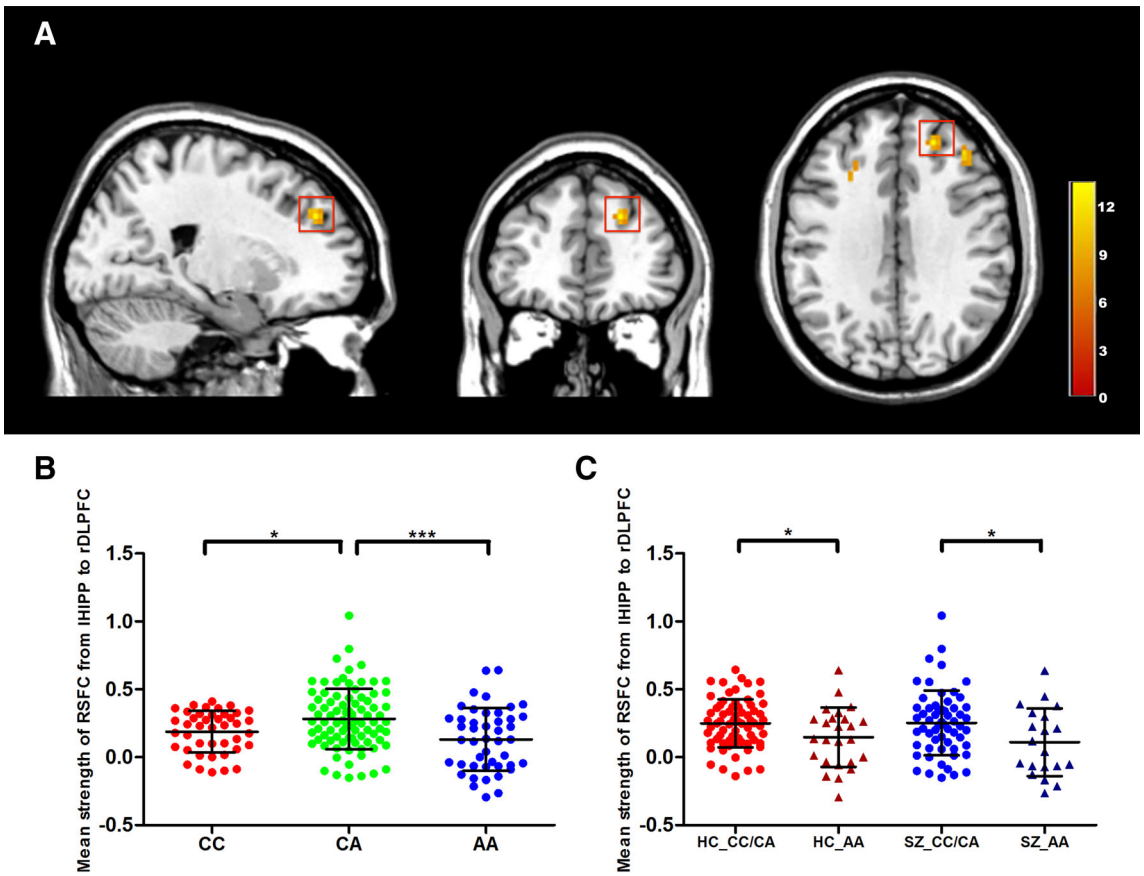

Fig. 1 Effects of *ZNF804A* rs1344704 on left hippocampal-frontal functional connectivity. **A** Frontal region (red box) with a significant genotype main effect for functional connectivity from the left hippocampus to the right dorsolateral prefrontal cortex (DLPFC) (peak MNI coordinates: x = 21, y = 42, z = 33, peak *F*-score = 13.43, $P < 0.001$, cluster size = 19). The color bar indicates the F-score. **B** Scatterplot of the mean strength of resting-state functional connectivity (RSFC) (mean ± SD) between the left hippocampus and the frontal region in the genotype groups. The Y-axis indicates the Z-score. **C** Scatterplot of the mean strength of RSFC (mean ± SD) from the left hippocampus to the right DLPFC in the genotype groups (CC/CA and AA genotype groups) in different diagnostic groups. The Y-axis indicates the Z-score. SZ, schizophrenic patients; HC, healthy controls; lHIPP, left hippocampus; rDLPFC, right dorsolateral prefrontal cortex; $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

Table 3 Significant correlations of the left hippocampus-right DLPFC resting-state functional connectivity with behavior.

	Cognitive Performance		PANSS score	
	SZ	HC	SZ	HC
CA genotype	DSST ($r = -0.341$, $P = 0.045$)	DSST ($r = -0.305$, $P = 0.047$)	Total ($r = 0.363$, $P = 0.025$)	–
	–	–	General ($r = 0.389$, $P = 0.014$)	–
AA genotype	WMS-R ($r = 0.610$, $P = 0.012$)	WMS-R ($r = -0.551$, $P = 0.006$)	Total ($r = -0.486$, $P = 0.041$)	–

The mean strength of resting-state functional connectivity (RSFC) between the left hippocampus and the right dorsolateral frontal region was significantly correlated with different measures of cognitive performance and symptom severity in patients. SZ, schizophrenic patients; HC, healthy controls; DSST, Digit Symbol Substitution Test; WMS-R, Wechsler Memory Scale-Revised; PANSS, Positive and Negative Syndrome Scale; Total, total PANSS score; General, score for general psychopathological syndrome in PANSS.s

in schizophrenia. For AA (risk) homozygotes, schizophrenic patients showed a lower hippocampal-prefrontal functional connectivity, which may predict worse memory functions, a slower speed of word processing, and more severe symptoms.

Discussion

In this study, we used an imaging genetic approach to investigate the effects of *ZNF804A* genetic variation on the resting-state fMRI in a sample of schizophrenic patients

and healthy individuals. We found that schizophrenic patients who were homozygous for the rs1344706 risk allele (AA) exhibited a lower strength of RSFC between the bilateral hippocampus and the right DLPFC. The mean RSFC strength between these two regions in patients was positively correlated with the WMS-R and CFT scores, and negatively correlated with the PANSS score. This means that a lower RSFC strength indicates a worse cognitive performance and a more severe psychiatric syndrome in patients. The current findings confirmed the likelihood that this SNP confers susceptibility to schizophrenia and identified potential neural mechanisms linking rs1344706 with schizophrenia.

Schizophrenia is regarded as a disorder of connectivity between components of large-scale brain networks [39–42]. The thalamus and hippocampus have been reported to exhibit greater fMRI activity during a sensory gating task, which is a common deficit in schizophrenia [43]. Both of these regions also show increased whole-brain functional connectivity strength with all other voxels in the brain compared with healthy controls [44]. Duan and colleagues found that the frequency of delta bursts in particular thalamic nuclei has a causal role in producing the working memory deficits in schizophrenia [45]. Furthermore, a higher functional connectivity with the hippocampal complex as the seed, between the hippocampal complex and the thalamus, has been reported in patients with auditory hallucinations alone, when compared with patients who had audio-visual hallucinations in the resting state [46], which suggested the specific functional involvement of the hippocampus and thalamus in schizophrenia. Our results revealed the functional coupling between the hippocampus and thalamus in schizophrenia; this is consistent with previous findings and might provide a potential pathogenesis of schizophrenia.

Previous studies reported a dysregulation of functional coupling between the left hippocampus and the right DLPFC during working memory in patients with schizophrenia and high-risk individuals [11, 24], and reduced hippocampal-prefrontal coupling during the resting state in chronic schizophrenia [47, 48]. Rasetti *et al.* reported the effect of the *ZNF804A* rs1344706 polymorphism on left hippocampus-right DLPFC coupling during the n-back working memory task in patients with schizophrenia, their healthy siblings, and healthy controls [10]. Recently, a study using both fMRI and diffusion tensor imaging data from healthy individuals has replicated the association between rs1344706 and the left hippocampus-right DLPFC coupling during the n-back working memory task, and also found impaired integrity of the white matter connection from the left hippocampus to the posterior cingulate cortex, one of the sub-connections between the left hippocampus and the right DLPFC, in healthy controls homozygous for the risk allele [38]. Therefore, our findings extend previous studies by reporting a consistent association between rs1344706 and left hippocampus-right DLPFC coupling during the resting state in schizophrenic patients.

The SNP rs1344706 has been associated with episodic and working memory in patients from different ethnic groups [49, 50] and with functional coupling of the right DLPFC and the anterior cingulate cortex during a cognitive control task [51]. The DLPFC is known to be a site for sustained attention and working memory [52, 53] and the hippocampus is believed to have close relationship with many areas of the cerebral cortex and constitutes a memory network to modulate and facilitate communication [54]. The correlation of hippocampus-DLPFC resting-state functional connectivity and cognitive performance demonstrated in our study provides further evidence that *ZNF804A* variation modulates the cortical network connectivity involved in memory and executive control. The rs1344706 allele has also been associated with the core symptoms of schizophrenia and the response to antipsychotic treatment. Larger white matter volumes and more severe symptoms have been reported in risk allele carriers with schizophrenia spectrum disorders [22]. When treated with antipsychotics, patients homozygous for the risk allele show poorer improvement of positive symptoms [55] and first-episode patients who are risk allele carriers exhibit significantly less improvement in the total PANSS score and positive sub-score [56]. Our results showing a correlation between RSFC strength and symptom severity in schizophrenia adds evidence for rs1344706 playing a role in the development of schizophrenia and being a potential target for future intervention.

Interestingly, in our study, effects of the *ZNF804A* polymorphism on the functional connectivity between the left hippocampus and the right DLPFC showed an "inverted U-shaped" form. We speculate that the highest RSFC strength in CA heterozygotes might result from cis-effects on ZNF804A expression in the brain. Ilaria Guella and colleagues found that total RNA of postmortem DLPFC samples from individuals with rs1344706 heterozygous for CA exhibited higher *ZNF804A* allelic expression than those homozygous for AA [57]. Another prior analysis revealed that the risk allele (A) was significantly associated with higher ZNF804A expression in postmortem prefrontal brain tissue from Irish control samples (n = 30, A: 32/60, C: 28/60) [58] whereas the opposite effect was found later in Caucasian patients with schizophrenia, using the same methods [59]. Various studies have demonstrated a cis-effect of rs1344706 on ZNF804A expression with controversial direction. Our results were partly consistent with the transcript expression model in which ZNF804A expression in CA heterozygotes

is similar to CC homozygotes but significantly higher than in AA homozygotes [60]. Further study is needed to explore whether the distinguishing functional connectivity indeed has a relationship with the expression of ZNF804A.

Finally, several limitations of our study need to be clarified. First, we specifically focused on functional connectivity from the hippocampus based on previous findings; further studies focused on the functional connectivity of other brain regions and more functional measures are needed. Second, although we replicated significant differences of hippocampal-prefrontal connectivity among individuals of the three genotypes, the differences were not significant in multiple testing corrections. It would be necessary to expand the sample to verify the stability and reliability of the results. In general, the study sheds light on the effect of a risk variant on functional connectivity during resting-state that needs to be verified in an independent sample.

In conclusion, our findings add to the evidence supporting *ZNF804A* as a promising risk gene for schizophrenia, and suggest that *ZNF804A* variation may be involved in the development and progression of schizophrenia by affecting resting-state brain function.

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Compliance with Ethical Standards

Conflict of interest All authors claim that there are no conflicts of interest.

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