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No association of ZNF804A rs1344706 with white matter integrity in schizophrenia: A tract-based spatial statistics study

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HIGHLIGHTS

- Imaging genetics of ZNF804A (rs1344706) in schizophrenia.
- Whole-brain track-based DTI analysis of the effect of rs1344706.
- rs1344706 is not associated with brain fiber tract integrity in schizophrenia.

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ABSTRACT

Altered brain connectivity has been widely considered as a genetic risk mechanism for schizophrenia. Of the many susceptibility genes identified so far, ZNF804A (rs1344706) is the first common genetic variant associated with schizophrenia on a genome-wide level. Previous fMRI studies have found that carriers of rs1344706 exhibit altered functional connectivity. However, the relationship between ZNF804A and white matter structural connectivity in patients of schizophrenia remains unknown. In this study, 100 patients with schizophrenia and 69 healthy controls were genotyped at the single nucleotide polymorphism rs1344706. Diffusion tensor imaging (DTI) was conducted and analyzed with tract-based spatial statistics. Systematic statistical analysis was conducted on multiple diffusion indices, including fractional anisotropy, axial diffusivity, radial diffusivity, and mean diffusivity. Unpaired two-sample *t*-test revealed significant differences in fractional anisotropy and diffusivity between schizophrenia and control groups. A two-way ANOVA analysis was conducted to assess the main effects of and the interaction between schizophrenia and ZNF804A. Although significant main effects of the diagnosis of schizophrenia were found on radial diffusivity, no association between the ZNF804A (rs1344706) and white matter connectivity was found in the entire group of subjects or in a selected subgroup of age-matched subjects ($n = 72$).

1. Introduction

Schizophrenia (SZ) has consistently showed high heritability, with heritability estimated at 73–90% [24]. In a recent genome-wide association study, a single nucleotide polymorphism (SNP) rs1344706 in ZNF804A was identified to be associated with schizophrenia [16]. This association has been replicated in multiple independent samples [4], including the Han Chinese population [31]. ZNF804A is known to be expressed in the brain and is associated with white matter development. In particular, studies on zfp804a, the mouse homologue of ZNF804A, suggested that ZNF804A may be involved in the regulation of early neurodevelopment [2]. Furthermore, bioinformatic analyses of the conserved

Abbreviations: FA, fractional anisotropy; NFH, negative family history; PFH, positive family history; ROI, regions of interesting; SEL, selected group; SZ, schizophrenia; WM, white matter.

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mammalian sequence around rs1344706 suggested that the variant was associated with myelin transcription factor 1 and octamer-binding factor 6 [4]. Both transcription factors are involved in oligodendrocyte differentiation and proliferation [3,15], two critical processes contributing to abnormal white matter connectivity that has been widely implicated in schizophrenia. Despite this confirmed association between ZNF804A and schizophrenia, the relationship between rs1344706 and white matter connectivity remains to be elucidated [4].

Imaging genetics provides a unique tool for exploring and evaluating the functional impact of genetic polymorphisms [1]. Using functional MRI (fMRI), Esslinger et al. found that healthy carriers of rs1344706 exhibit no changes in regional brain activity but pronounced gene dosage-dependent reduced connectivity of dorsolateral prefrontal cortex across hemispheres and increased connectivity with hippocampus [5,6]. Using T1-weighted MRI, Wei et al. found that white matter (WM) intensity is increased in the left prefrontal lobe of patients carried with the risk-allele compared to non-risk allele carriers [28]. Although these findings are consistent with the "dysconnectivity" hypothesis of schizophrenia [19], neither fMRI nor T1-weighted MRI provides measure of white matter structural connectivity. In an attempt to address this issue, Voineskos et al. applied diffusion tensor imaging (DTI) to measure connectivity of major frontotemporal and interhemispheric white matter tracts in healthy carriers of the risk variant rs1344706 [26]. While cortical thickness was found to be reduced, no effect of the risk variant on microstructural integrity of white matter tracts was found. The effect of the risk variant on the white matter connectivity in patients with schizophrenia, however, remains to be unknown. Schizophrenia is a complex brain disorder involving multiple risk genes and environmental factors. It is possible that ZNF804A may interact with other risk factors in patients with schizophrenia. Such interaction may result in different neurobiological phenotypes that may be detectable by MRI [25,14].

The goal of the current study was to examine the effects of ZNF804A gene polymorphism rs1344706 on the WM connectivity in schizophrenia using the methods of DTI and tract-based spatial statistics (TBSS), an unbiased and hypothesis-free whole-brain approach. The effects of rs1344706 on five indices of diffusion were assessed using the general linear model.

2. Methods

2.1. Subjects

100 patients with schizophrenia ($n = 77$) or schizophreniform disorder (after follow-up, a diagnosis of schizophrenia was established; $n = 23$) were recruited in the Third Affiliated Hospital of Sun Yat-sen University. All participants were Han Chinese. The inclusion criteria were as follows: (a) age being between 18 and 45 years; (b) years of education being greater than 9 and (c) had to be right-handed (assessed by a 10-item questionnaire, the Edinburgh Handedness Inventory [17]). The exclusion criteria were: (a) presented with chronic neurological disorders; (b) had a history of alcohol or substance abuse; (c) had a history of electroconvulsive therapy. Diagnosis based on the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) was made by an experienced psychiatrist using patient version of the Structured Clinical Interview for DSM-IV (SCID) [7]. 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. [29]. 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). The Positive and Negative Syndrome Scale (PANSS) [11] was used to measure psychopathologic symptoms at the time of imaging.

69 healthy volunteers (all Han Chinese, unpaid) from the local community were recruited by advertisement. To be included in the study, these volunteers and their first-degree or second degree relatives [29] should not have history of mental disorder based on DSM-IV. Interview and assessment were conducted by the same experienced psychiatrist using SCID as for the patient group. Other inclusion and exclusion criteria were the same as those of the patients.

2.2. Genotyping

Genotyping was performed as described previously [28]. Briefly, genomic DNA from leukocytes in blood was amplified by polymerase chain reaction (PCR) to generate a 443 bp product spanning rs1344706. Primers were as follows: upper GAATCTAGA GTCATGCAGG, and lower CAAGTTATTC TCTAGAGTCC.

2.3. Image acquisition and analysis

Images were acquired on a 1.5-T GE Signa Twinspeed MRI scanner (General Electric Medical System, Milwaukee, WI, USA) equipped with a quadrature birdcage head coil. Diffusion-weighted images were acquired with a single-shot echo planar imaging (EPI) sequence. The diffusion gradients were applied along fifteen non-collinear directions ($b = 1000\ s/mm^2$), together with an acquisition of "$b0$ image" ($b = 0$). 35 contiguous axial slices were acquired with a slice thickness of 4 mm and no gap. The acquisition parameters were as follows: repetition time = 11,000 ms; echo time = 74.7 ms; matrix = 128×128; field of view = 240 mm $\times$ 240 mm.

Diffusion data were analyzed using the Diffusion Toolbox distributed with the FSL's software package (University of Oxford, UK) [22]. All diffusion-weighted images were first linearly registered to the corresponding non-diffusion weighted images. Diffusion tensors were obtained based on DTI and diagonalized to derive the fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (L1) and the radial diffusivity (L2 and L3).

Voxelwise statistical analysis of the FA, MD, L1, L2 and L3 values was carried out using TBSS [23], a program implemented in FSL [22]. The steps of this analysis are as follows. First, all subjects' FA images were skull-stripped and aligned into the MNI152 standard space using the nonlinear registration tool (FNIRT) within FSL. Next, the mean FA volume was created and thinned to create a mean FA skeleton, which represents the centers of all tracts common to the entire group of subjects. Each subject's aligned FA data were then projected onto this skeleton and the resulting data were fed into voxelwise cross-subject statistical testing.

An unpaired two-sample t-test was first performed to compare patients and controls without accounting for genetic differences. Then, a full-factorial two-way analysis of variance (ANOVA) was implemented within the framework of the general linear model using the FEAT algorithm in FSL [22]. Within this model, the first factor characterized if the subject had schizophrenia, and the second factor accounted for the presence of the risk-allele rs1344706 (T). Maps of t-statistics for main effects and interaction were generated by permutation testing ($n = 500$) using the randomize tool in FSL for each of the aforementioned tensor characteristics. To correct for multiple comparisons, statistical significance was inferred using the Threshold-Free Cluster Enhancement method [21]. After family-wise error correction, only those clusters with acceptable p-values were retained ($p < 0.05$).

To address potential confounds that may be caused by age-related white matter structural differences and unequal sample sizes, the analysis was carried out both in the entire group of