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The Disrupted-in-Schizophrenia-1 Ser704Cys polymorphism and brain neurodevelopmental markers in schizophrenia and healthy subjects

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ABSTRACT

Increasing evidence has implicated the role of Disrupted-in-Schizophrenia-1 (*DISC1*), a potential susceptibility gene for schizophrenia, in early neurodevelopmental processes. However, the effect of its genotype variation on brain morphologic changes related to neurodevelopmental abnormalities in schizophrenia remains largely unknown. This magnetic resonance imaging study examined the association between *DISC1* Ser704Cys polymorphism and a range of brain neurodevelopmental markers [cavum septi pellucidi (CSP), adhesio interthalamica (AI), olfactory sulcus depth, and sulcogyral pattern (Types I, II, III, and IV) in the orbitofrontal cortex (OFC)] in an all Japanese sample of 75 schizophrenia patients and 87 healthy controls. The Cys carriers had significantly larger CSP than the Ser homozygotes for both schizophrenia patients and healthy controls. The Cys carriers also exhibited a reduction in the Type I pattern of the right OFC in the healthy controls, but not in the schizophrenia patients. The *DISC1* Ser704Cys polymorphism did not affect the AI and olfactory sulcus depth in either group. These results suggested a possible role of the *DISC1* genotype in the early neurodevelopment of human brains, but failed to show its specific role in the neurodevelopmental pathology of schizophrenia.

1. Introduction

The Disrupted-in-Schizophrenia-1 (*DISC1*) gene, one of the candidates for a schizophrenia-susceptibility gene (Millar et al., 2000; St Clair et al., 1990), is thought to be involved in neurodevelopment and synaptic plasticity within various brain areas (Austin et al., 2003; Meyer and Morris, 2008; Schurov et al., 2004). In addition to the possible genotype effect of a functional single nucleotide polymorphism (SNP) on exon 11 (*rs821616*, a serine to cysteine substitution at codon 704) on brain morphology and function in healthy subjects (Callicott et al., 2005; Hashimoto et al., 2006; Li et al., 2013; Thomson et al., 2005), our preliminary study suggested that it might differentially affect the gray matter volume of the neocortical and limbic regions in schizophrenia patients and healthy controls (Takahashi et al., 2009). Although recent whole-brain gray matter analysis using voxel-based morphometry (VBM) failed to replicate our earlier findings (Kido et al., in press), the possibility still exists that its genotype variation is specifically related to brain morphologic changes that are closely related to abnormal early neurodevelopment in schizophrenia.

Several magnetic resonance imaging (MRI) studies of potential 'brain neurodevelopmental markers' have implicated the role of aberrant neurodevelopmental processes in the pathophysiology of schizophrenia (Pantelis et al., 2005). For example, a large cavum septi pellucidi (CSP), which is formed by the incomplete fusion of the septum pellucidum (Rakic and Yakovlev, 1968), may be related to fetal neurodevelopmental abnormalities of the corpus callosum and limbic structures in schizophrenia (Trzesniak et al., 2011b). While our previous MRI study showed no difference in the size and prevalence of CSP in a large sample of schizophrenia patients compared with controls (Takahashi et al., 2007), a recent meta-analysis suggested that a large CSP was more common in schizophrenia (Trzesniak et al., 2011b). The adhesio interthalamica (AI), a narrow bridge connecting the medial surfaces of the thalami, is variable in size among individuals and missing in about 20% of human brains (Rosales et al., 1968). Previous neuroimaging studies have demonstrated that schizophrenia patients are more likely to have a smaller AI (Takahashi et al., 2008; Trzesniak et al., 2011a), possibly reflecting early developmental abnormalities. In

Abbreviations: AI, adhesio interthalamica; ANCOVA, analysis of covariance; ANOVA, analysis of variance; CASH, Comprehensive Assessment of Symptoms and History; CSP, cavum septi pellucidi; *DISC1*, Disrupted-in-Schizophrenia-1; HWE, Hardy–Weinberg equilibrium; LOS, lateral orbital sulcus; MOS, medial orbital sulcus; MRI, magnetic resonance imaging; OFC, orbitofrontal cortex; SANS, Scale for the Assessment of Negative Symptoms; SAPS, Scale for the Assessment of Positive Symptoms; SNP, single-nucleotide polymorphism; TOS, transverse orbital sulcus; VBM, voxel-based morphometry.

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addition to these neurodevelopmental markers located in the midline brain regions, gross morphologic changes of the orbitofrontal cortex (OFC) in schizophrenia (Nakamura et al., 2007; Takahashi et al., 2013a; Takayanagi et al., 2010) are likely to reflect abnormal neurodevelopment during the gestational period.

Altered OFC patterns (Chakirova et al., 2010) and abnormal CSP (Choi et al., 2008) in subjects at high genetic risk of schizophrenia may support a genetic influence on such gross morphologic changes in schizophrenia. Furthermore, since recent animal data (Osbun et al., 2011; Shen et al., 2008) as well as genetic analyses in patients with callosal agenesis (Osbun et al., 2011) suggest a crucial role for *DISC1* in callosal development, it is possible that its genotype variation may influence the size of CSP. However, VBM approach which we used to explore the genotype effect of *DISC1* on brain morphology (Kido et al., in press) cannot examine the gross brain characteristics. It thus remains largely unknown as to whether *DISC1* genotype could influence the CSP and other neurodevelopmental markers in patients with schizophrenia as well as in healthy subjects.

In this MRI study, we aimed to investigate the effects of *DISC1* Ser704Cys SNP on a range of neurodevelopmental markers in schizophrenia patients and matched healthy controls. On the basis of previous MRI observations in schizophrenia, we selected the size and prevalence of CSP and AI (Trzesniak et al., 2011a,b), depth of the olfactory sulcus (Takahashi et al., 2013a), and the OFC sulcogyral pattern (Nakamura et al., 2007) as possible neurodevelopmental markers. Despite evidence implicating the role of *DISC1* in early neurodevelopmental processes of human brains, there have also been questions about *DISC1* as a genetic risk factor of schizophrenia (Sullivan, 2013). We therefore predicted that variation in the *DISC1* genotype could be related to the morphology of these structures regardless of diagnosis, but we also explored its specific role in the gross brain abnormalities of schizophrenia.

2. Methods

2.1. Subjects

Seventy-five patients with schizophrenia (41 males and 34 females; mean age = 27.4 years, SD = 6.1) who met the ICD-10 research criteria (World health organization, 1993) were recruited from the inpatient and outpatient clinics of the Department of Neuropsychiatry of Toyama University Hospital. The patients were diagnosed following a structured clinical interview by psychiatrists using the Comprehensive Assessment of Symptoms and History (CASH; Andreasen et al., 1992). Clinical symptoms were rated at the time of scanning using the Scale for the Assessment of Negative Symptoms (SANS) and the Scale for the Assessment of Positive Symptoms (SAPS) (Andreasen, 1984). Seventy patients were right-handed and five patients were mixed-handed. Sixty-nine patients were receiving antipsychotic medication at the time of scanning; 26 patients were being treated with typical antipsychotics and 43 patients were receiving atypical antipsychotics.

The control subjects consisted of 87 right-handed healthy volunteers (45 males and 42 females; mean age = 26.4 years, SD = 6.6) recruited from members of the local community, hospital staff, and university students. They were asked to complete a questionnaire consisting of 15 items concerning their personal (13 items; including a history of obstetric complications, substantial head injury, seizures, neurological or psychiatric disease, impaired thyroid function, hypertension, diabetes, and substance abuse) and family (2 items) histories of illness. Subjects with any personal or family history of psychiatric illness among their first-degree relatives were excluded.

Demographic and clinical data of the subjects in this study are presented in Table 1. All subjects were Japanese and physically healthy at the time of the study. None had a lifetime history of serious head trauma, neurological illness, serious medical or surgical illness, or substance abuse. All participants were also screened for gross brain abnormalities (except a large CSP) by neuroradiologists. This cohort was the same as that of our recent VBM study that examined the genotype effects of *DISC1* and related molecule (*YWHAE*) on whole-brain gray matter (Kido et al., in press). They were also partly included in our previous MRI studies, in which we investigated the CSP (49/75 patients and 46/87 controls; Takahashi et al., 2007), AI (31/75 patients and 29/87 controls; Takahashi et al., 2008), and OFC (45/75 patients and 38/87 controls; Nishikawa et al., in submission). The Committees on Medical Ethics of Toyama University and Nagoya University Graduate School of Medicine approved this study. Written informed consent was obtained from all subjects.

2.2. SNP genotyping

Genomic DNA was extracted from EDTA-containing venous blood samples according to standard procedures. The genotyping of the Ser704Cys SNP (*rs821616*) of the *DISC1* gene was performed using TaqMan assays (Applied Biosystems, Foster City, CA). TaqMan® SNP Genotyping Assay and Universal PCR Master Mix were obtained from Applied Biosystems. Allelic-specific fluorescence was measured using the ABI PRISM 7900 Sequence Detector System (Applied Biosystems).

2.3. MRI procedures

MR images were obtained using a 1.5T Magnetom Vision (Siemens Medical System, Inc., Erlangen, Germany) with a three-dimensional gradient-echo sequence FLASH (fast low-angle shots) yielding 160–180 contiguous T1-weighted slices of 1.0 mm thickness in the sagittal plane, according to the participants' head size. The entire scan was obtained in approximately 14 min. The imaging parameters were as follows: repetition time = 24 ms; echo time = 5 ms; flip angle = 40°; field of view = 256 mm; and matrix size = 256 × 256 pixels. The voxel size was 1.0 × 1.0 × 1.0 mm. A total intracranial volume was estimated by calculating the sum of gray matter, white matter, and cerebrospinal fluid whole brain volumes using the VBM8 toolbox (http://dbm.neuro.uni-jena.de/vbm/) for Statistical Parametric Mapping 8 (SPM8, Wellcome Institute of Neurology, University College London, UK, http://www.fil.icon.ucl.ac.uk/spm).

2.4. Assessment of the neurodevelopmental markers

The images were processed on a Linux PC (Fujitsu Limited, Tokyo, Japan) using Dr. View software (AJS, Tokyo, Japan). The brain images were realigned in three dimensions and then reconstructed into entire contiguous coronal images with a 1-mm thickness, perpendicular to the anterior commissure–posterior commissure line. Assessment of the neurodevelopmental markers was performed by one rater (TT), who was blind to the subjects' identity. High intra- and inter-rater reliabilities (>0.8) have been established for all of the following structures (AI, CSP, olfactory sulcus depth, and OFC pattern) on this sample (Nishikawa et al., in submission; Takahashi et al., 2007, 2008, 2014).

2.4.1. Midline brain structures

As described in detail elsewhere (Takahashi et al., 2007, 2008), the rater counted the number of 1-mm coronal slices where each midline structure (AI and CSP) was clearly seen (Fig. 1). The length of the AI and CSP (in mm) was equal to the number of these slices. We considered the AI as present when it could be identified on three or more slices on both coronal and axial views (Takahashi et al., 2008). A CSP equal to or greater than 6 mm was defined as large on the basis of previous reports (e.g., Kasai et al., 2004; Nopoulos et al., 1997).

2.4.2. Olfactory sulcus depth

On each coronal slice, the olfactory sulcus was traced beginning with the deepest point of the sulcus and ending inferiorly with a tangent line connecting the top surfaces of the gyrus rectus and medial orbital gyrus (Rombaux et al., 2009; Takahashi et al., 2013a) (Fig. 1). The average