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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

Table 1
Clinical description of schizophrenia patients and healthy controls with each *DISC1* genotype.

	Schizophrenia patients		Controls		Diagnosis effect	Genotype effect
	Ser/Ser	Cys carriers	Ser/Ser	Cys carriers		
	($N = 56$)	($N = 19$)	($N = 65$)	($N = 22$)		
Male/female	30/26	11/8	33/32	12/10	$\chi^2 = 0.14, p = 0.708$	$\chi^2 = 0.20, p = 0.655$
Age (years)	27.6 ± 6.2	27.0 ± 5.7	26.6 ± 6.8	25.6 ± 5.8	$F(1,158) = 0.98, p = 0.324$	$F(1,158) = 0.45, p = 0.502$
Height (cm)	163.9 ± 9.2	167.1 ± 6.1	164.9 ± 7.7	166.5 ± 10.0	$F(1,158) = 0.02, p = 0.878$	$F(1,158) = 2.43, p = 0.121$
Education (years)[a]	13.7 ± 1.9	13.8 ± 2.1	16.0 ± 2.2	15.7 ± 2.4	$F(1,157) = 30.11, p < 0.001$; Con > Sz	$F(1,157) = 0.12, p = 0.725$
Parental education (years)[a]	12.9 ± 2.3	12.4 ± 2.2	13.1 ± 2.5	13.5 ± 2.3	$F(1,157) = 2.24, p = 0.136$	$F(1,157) = 0.05, p = 0.829$
Age of onset (years)	22.8 ± 5.1	21.7 ± 4.2	–	–	–	$F(1,73) = 0.65, p = 0.424$
Duration of illness (years)	4.5 ± 4.9	5.3 ± 5.6	–	–	–	$F(1,73) = 0.28, p = 0.598$
Duration of medication (years)	3.0 ± 3.8	3.2 ± 3.6	–	–	–	$F(1,73) = 0.04, p = 0.839$
Drug dose (haloperidol equivalent, mg/day)[b]	8.5 ± 7.2	9.4 ± 9.1	–	–	–	$F(1,73) = 0.19, p = 0.662$
Total SAPS score[c]	30.3 ± 27.5	29.4 ± 21.8	–	–	–	$F(1,71) = 0.02, p = 0.897$
Total SANS score[c]	51.2 ± 21.5	58.7 ± 23.6	–	–	–	$F(1,71) = 1.66, p = 0.202$

Values represent means ± SDs, except where noted. Con, controls; SANS, Scale for the Assessment of Negative Symptoms; SAPS, Scale for the Assessment of Positive Symptoms; Sz, schizophrenia.
[a] Data missing for one patient.
[b] The different typical and atypical antipsychotic dosages were converted into haloperidol equivalence in accordance with the guideline by Toru (2008).
[c] Data missing for two patients.

depth of the sulcus was calculated as follows: sum of the depth in all slices containing the sulcus/slice number.

2.4.3. OFC sulcogyral pattern classification

The medial orbital sulcus (MOS), lateral orbital sulcus (LOS), and transverse orbital sulcus (TOS) were highlighted on consecutive 1-mm coronal slices, and then viewed in the axial plane for OFC pattern classification based on the definition by Chiavaras and Petrides (2000). The OFC sulcogyral patterns were classified according to the continuity of the 'H-shaped' sulcus consisting of the MOS, TOS, and LOS; for Type I the MOS is disconnected while the LOS is intact, for Type II both the MOS and LOS are continuous, and for Type III both the MOS and LOS are disconnected (Bartholomeusz et al., 2013) (Fig. 2). In rare instances where the MOS was continuous, but the LOS was disconnected, this pattern was classified as Type IV (Chakirova et al., 2010).

2.5. Statistical analysis

The demographic and clinical differences between the groups were examined using an analysis of variance (ANOVA) or χ^2 test. The deviation from Hardy–Weinberg equilibrium (HWE) was tested using the χ^2 goodness-of-fit test. Group differences (schizophrenia *vs* controls, Ser homozygotes *vs* Cys carriers) in nominal measures such as the prevalence of CSP (CSP ≥ 1 slice), large CSP (CSP ≥ 6 slices), and AI, as well as the OFC sulcogyral pattern distribution, were evaluated using the χ^2 test. The CSP length was log-transformed for the following analyses because of their skewed distribution. The lengths of the CSP (log) and AI were analyzed using an analysis of covariance (ANCOVA) with age and intracranial volume as covariates, with diagnosis and genotype as between-subject factors. The olfactory sulcus depth was analyzed by a similar ANCOVA model, but the hemisphere was used as a within-subject variable. Post-hoc Scheffe's tests were used to follow-up any significant main effects or interactions. The relation between these potential neurodevelopmental markers and clinical variables, as well as the relation between these anatomical measures, in schizophrenia in each genotype was examined using Spearmen's rank correlations for continuous measures [CSP length (log), AI length, and olfactory sulcus depth] with Bonferroni correction or ANCOVA with each brain measure as a between-subject factor for nominal measures. As we have previously demonstrated the genotype effect of *YWHAE* (*rs28365859*), a gene encoding *DISC1*-interacting molecule, on the OFC pattern especially in healthy subjects (Takahashi et al., 2014), we also examined the gene–gene interaction between the *YWHAE* (protective C allele carriers *vs* G allele homozygotes) and *DISC1* Ser704Cys SNPs on potential neurodevelopmental markers using the χ^2 test (for nominal anatomical measures) or ANCOVA with the genotype of each SNP as independent variables (for continuous anatomical measures) in 72 patients and 86 controls. Statistical significance was defined as $p < 0.05$.

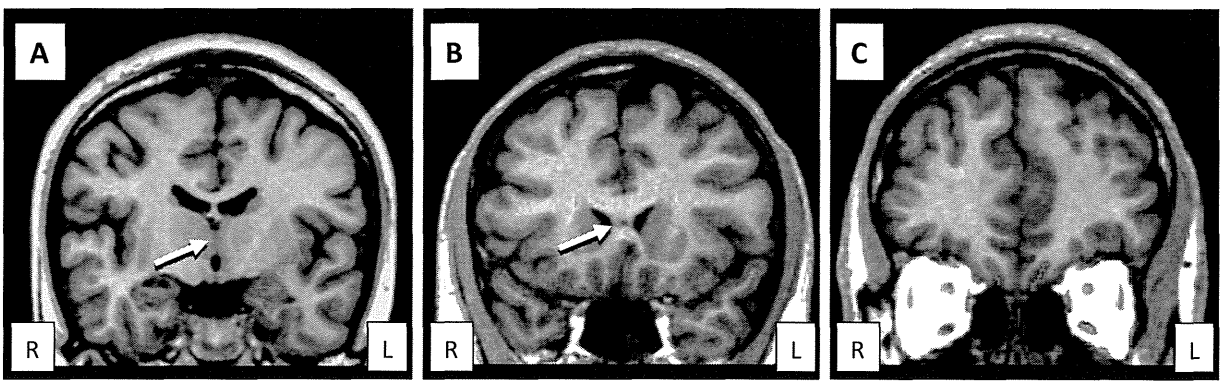

Fig. 1. Sample coronal slices showing the adhesio interthalamica (A, arrow), cavum septum pellucidum (B, arrow), and olfactory sulcus (C, colored in red). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

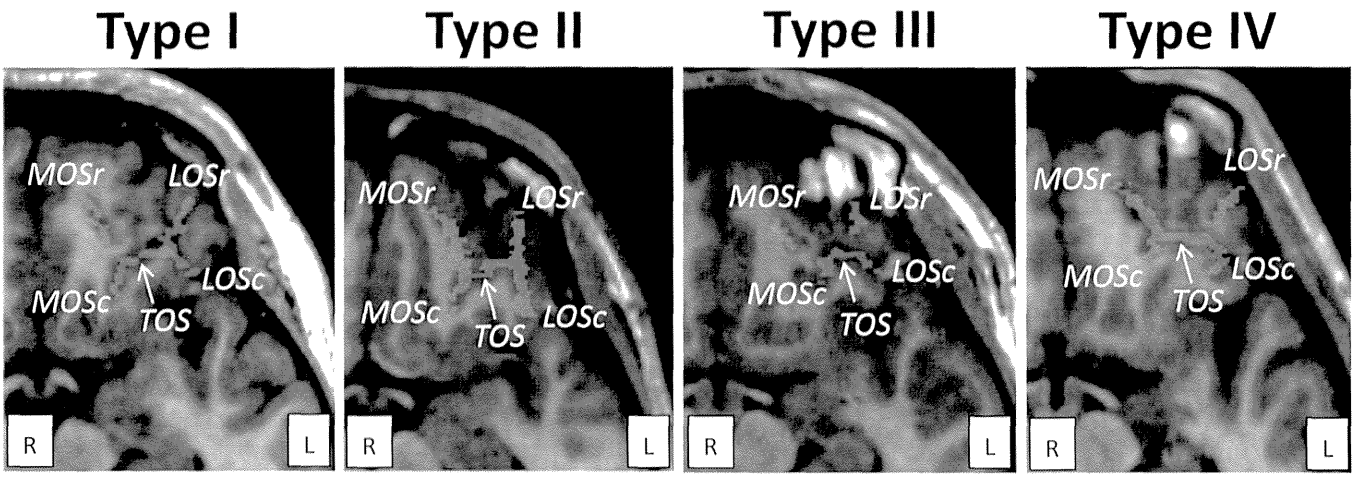

Fig. 2. Classification of the orbitofrontal sulcogyral pattern on an axial view. Note that these sulci were identified using orthogonal views in three directions and colored on consecutive coronal slices. c, caudal portion; *LOS*, lateral orbital sulcus; *MOS*, medial orbital sulcus; r, rostral portion; *TOS*, transverse orbital sulcus. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3. Results

3.1. Sample characteristics and genotyping results

The two groups were matched for age, height, sex, and parental education, but the controls had attained a higher level of education than the schizophrenia patients. Clinical or demographic data did not differ between the Ser homozygotes and Cys carriers in the schizophrenia and control groups (Table 1). There were two Cys homozygotes in the current sample (one male patient and one male control). The observed genotype frequency was within the distribution expected according to the HWE. The patients with schizophrenia and the healthy comparisons did not differ significantly in genotype distributions ($\chi^2 < 0.01$, $p = 0.995$).

3.2. Diagnosis effect on the neurodevelopmental markers

The AI was smaller and more often absent in schizophrenia compared with the healthy subjects, but there was no significant group difference in the size and prevalence of the CSP (Table 2). The patients were also characterized by a shallower olfactory sulcus bilaterally, as well as increased Type III and decreased Type I expression in the right OFC, as compared with controls (Table 2).

3.3. Genotype effect and diagnosis-by-genotype interaction on the neurodevelopmental markers

The Cys carriers (risk allele group) had significantly larger CSP (post-hoc test, $p = 0.009$) and higher prevalence of CSP (≥1 slice)

Table 2
Brain measures of schizophrenia patients and healthy controls with each *DISC1* genotype.

	Schizophrenia patients		Controls		Diagnosis effect	Genotype effect
	Ser/Ser	Cys carriers	Ser/Ser	Cys carriers		
	($N = 56$)	($N = 19$)	($N = 65$)	($N = 22$)		
Intracranial volume (ml)	1422 ± 142	1485 ± 182	1405 ± 115	1411 ± 139	$F(1, 157) = 3.23$, $p = 0.074$	$F(1, 157) = 1.94$, $p = 0.165$
CSP (≥1 slice) present; *N* (%)	43 (76.8)	18 (94.7)	52 (80)	22 (100)	$\chi^2 = 0.40$, $p = 0.526$	$\chi^2 = 8.00$, $p = 0.005$
Large CSP (≥6 slices) present; *N* (%)	5 (8.9)	1 (5.3)	4 (6.2)	3 (13.6)	$\chi^2 < 0.01$, $p = 0.991$	$\chi^2 = 0.22$, $p = 0.637$
CSP length (log)	0.23 ± 0.40	0.30 ± 0.25	0.21 ± 0.37	0.50 ± 0.41	$F(1, 156) = 3.15$, $p = 0.078$	$F(1, 156) = 6.63$, $p = 0.011$
AI absent; *N* (%)	13 (23.2)	6 (31.6)	6 (9.2)	1 (4.5)	$\chi^2 = 8.93$, $p = 0.003$; Sz > Con	$\chi^2 = 0.04$, $p = 0.836$
AI length (mm)	7.1 ± 3.3	5.7 ± 4.0	9.7 ± 3.4	9.0 ± 3.2	$F(1, 156) = 18.15$, $p < 0.001$; Sz < Con	$F(1, 156) = 2.01$, $p = 0.158$
Olfactory sulcus depth (mm)					$F(1, 156) = 77.82$, $p < 0.001$; Sz < Con	$F(1, 156) = 0.02$, $p = 0.894$
Left	11.0 ± 1.5	11.4 ± 1.5	13.0 ± 1.2	12.9 ± 1.1		
Right	11.6 ± 1.6	11.8 ± 1.4	13.6 ± 1.4	13.6 ± 1.4		
Left OFC pattern; *N* (%)					$\chi^2 = 5.28$, $p = 0.153$	$\chi^2 = 0.85$, $p = 0.839$
Type I	25 (44.6)	7 (36.8)	37 (56.9)	13 (59.1)		
Type II	7 (12.5)	4 (21.1)	11 (16.9)	1 (4.5)		
Type III	24 (42.9)	8 (42.1)	16 (24.6)	8 (36.4)		
Type IV	0 (0.0)	0 (0.0)	1 (1.5)	0 (0.0)		
Right OFC pattern; *N* (%)					$\chi^2 = 9.93$, $p = 0.019$[a]	$\chi^2 = 0.92$, $p = 0.821$
Type I	27 (48.2)	14 (73.7)	52 (80.0)	12 (54.5)		
Type II	6 (10.7)	0 (0.0)	6 (9.2)	3 (13.6)		
Type III	22 (39.3)	5 (26.3)	7 (10.8)	7 (31.8)		
Type IV	1 (1.8)	0 (0.0)	0 (0.0)	0 (0.0)		

Values represent means ± SDs, except where noted. AI, adhesio interthalamica; Con, controls; CSP, cavum septum pellucidum; OFC, orbitofrontal cortex; SANS, Scale for the Assessment of Negative Symptoms; SAPS, Scale for the Assessment of Positive Symptoms; Sz, schizophrenia.

[a] Patients had a decrease in Type I ($\chi^2 = 6.31$, $p = 0.012$) and an increase in Type III ($\chi^2 = 8.44$, $p = 0.004$) expression compared to controls.

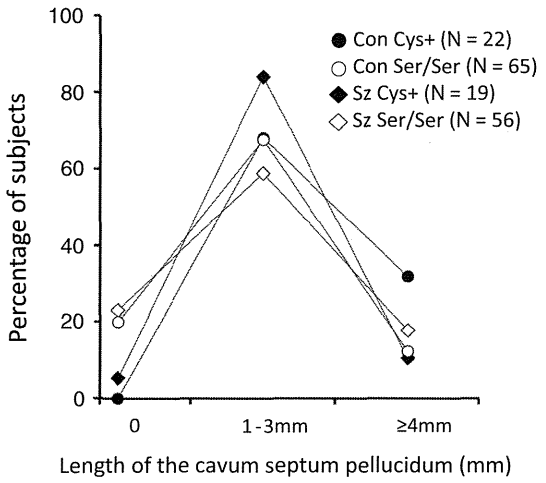

Fig. 3. Length of the cavum septum pellucidum (CSP) in the study participants. Con, controls; Cys+, Cys carriers; Ser/Ser, Ser homozygotes; Sz, schizophrenia.

compared to the Ser homozygotes for both schizophrenia patients and healthy comparisons (Table 2, Fig. 3), but no diagnosis-by-genotype interaction was found. There was no significant main effect of genotype or diagnosis-by-genotype interaction on the AI and olfactory sulcus depth. The Cys carriers exhibited a decrease in Type I ($\chi^2 = 5.48$, $p = 0.019$) and increase in Type III ($\chi^2 = 5.39$, $p = 0.020$) pattern on the right OFC in the healthy controls, but not in the schizophrenia patients (Fig. 4). Thus, diagnosis-by-genotype interaction was observed only for the OFC pattern among possible neurodevelopmental markers investigated in this study.

3.4. Neurodevelopmental markers and clinical variables in schizophrenia

The patients with a left OFC Type I pattern had a significantly higher total SAPS score compared to those with a Type III pattern (post-hoc test, $p = 0.033$) in the Ser homozygote group [$F(2, 50) = 4.75$, $p = 0.013$], but not in the Cys carrying group [$F(2, 15) = 0.44$, $p = 0.651$]. There was no significant relation between other neurodevelopmental markers (CSP, AI, and olfactory sulcus) and clinical variables (onset age, illness duration, dose/duration of medication, and symptom severity) in either genotype group.

3.5. Possible relation between neurodevelopmental markers in schizophrenia

There was no significant relation between the neurodevelopmental markers (CSP, AI, olfactory sulcus depth, and OFC pattern) in either genotype group, suggesting that different neurodevelopmental marker abnormalities might occur in different subsets of patients.

3.6. Possible interaction between DISC1 and YWHAE on neurodevelopmental markers

There were no significant gene–gene interaction effects on the CSP length, AI length, and olfactory sulcus depth in whole subjects or in either diagnostic group. χ^2 tests showed a significant difference between the 4 genotype groups only in the prevalence of CSP (≥1 slice) ($\chi^2 = 8.48$, $p = 0.037$), but this could be explained by the effect of *DISC1* SNP alone ($\chi^2 = 8.00$, $p = 0.005$). The *YWHAE* genotype alone did not affect the morphology of these neurodevelopmental markers except the left OFC patterns as reported elsewhere (Takahashi et al., 2014).

4. Discussion

To our knowledge, this is the first MRI study to report the genotype effect of *DISC1* Ser704Cys SNP on several brain morphologic characteristics closely related to early neurodevelopment in both schizophrenia and healthy comparisons. The subjects carrying the Cys allele, which may increase the susceptibility to schizophrenia (Qu et al., 2007), had a significantly larger size and higher prevalence of CSP (≥1 slice) than the Ser homozygotes regardless of diagnosis. The Cys carriers also exhibited a decrease in Type I and increase in Type III pattern on the right OFC in the healthy controls, but not in the schizophrenia patients. For the AI and olfactory sulcus depth, no significant genotype effect was found in either group. These findings suggest that the genotype variation of *DISC1* is related to normal brain development, but that its effect alone cannot explain the changes in neurodevelopmental markers of schizophrenia investigated in this study.

Consistent with several studies in healthy subjects suggesting that Cys carriers have structural and functional abnormalities in limbic and other brain regions (Brauns et al., 2011; Di Giorgio et al., 2008; Hashimoto et al., 2006; Trost et al., 2013), the Cys carriers in this study had a significantly larger CSP than the Ser homozygotes. In normal brain development, the CSP (i.e., an incomplete fusion of the septum pellucidum) is consistently present in prematures, but begins to close just before term probably due to the rapid growth of the corpus callosum and limbic system structures (Sarwar, 1989; Shaw and Alvord, 1969). The CSP itself is thought to be a normal anatomical variant, but it is possible that the genotype variation of *DISC1* could influence the neurodevelopmental processes in the limbic and callosal regions (Austin et al., 2003; Meyer and Morris, 2008; Schurov et al., 2004) and that an incomplete fusion of the septum pellucidum due to such a neurodevelopmental alteration might also be associated with subsequent attenuated cortical maturation (Raznahan et al., 2011) and reduced brain connectivity (Li et al., 2013; Liu et al., in press) reported in healthy subjects carrying the Cys allele.

On the other hand, we found no relation between the genotype variation of *DISC1* and an unusually large CSP (≥6 mm in length), which has been implicated in midline neurodevelopmental abnormality

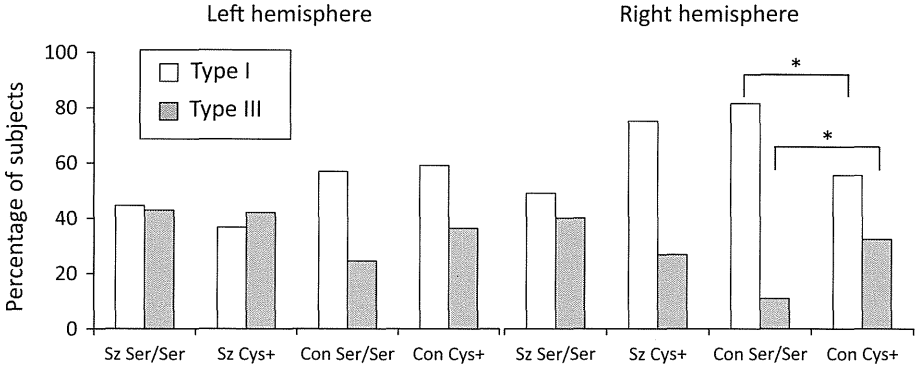

Fig. 4. Distribution of the orbitofrontal sulcogyral pattern (Type I *vs* Type III) in each diagnostic group. Con, controls; Cys+, Cys carriers; Ser/Ser, Ser homozygotes; Sz, schizophrenia. *$p < 0.05$.

(Bodensteiner and Schaefer, 1990). Several MRI studies have reported an increased prevalence of a large CSP in schizophrenia (e.g., Kasai et al., 2004; Nopoulos et al., 1997) or genetic high-risk individuals (Choi et al., 2008), but such findings have not been consistently replicated. In addition to the lack of *DISC1* genotype effect on the length or prevalence of the CSP (≥1 slice) specific to schizophrenia, the present findings suggest that the effect of *DISC1* Ser704Cys SNP alone cannot explain the CSP abnormalities, if present, in schizophrenia. However, patients with a large CSP may exhibit smaller volumes of limbic structures (Kasai et al., 2004; Takahashi et al., 2007), more severe symptoms (Flashman et al., 2007; Kasai et al., 2004), and greater cognitive deficits (Flashman et al., 2007; Nopoulos et al., 2000) compared to those without it (reviewed by Trzesniak et al., 2011b), implicating a significant role of abnormally large CSP in the pathophysiology of schizophrenia. Since the current sample included only 13 subjects with a large CSP (6 patients and 7 controls), our findings warrant further studies with a larger sample size to confirm the association of *DISC1* with a large CSP.

The important finding of this study was the significant effect of the *DISC1* genotype on the right OFC sulcogyral pattern, which develops predominantly during the gestational period from 28 to 44 weeks (Chi et al., 1977; Kringelbach and Rolls, 2004), in healthy subjects. The exact mechanism of the development of the OFC sulcogyral pattern remains unclear, but the cortical folding in human brains is strongly regulated by genetic factors (Bartley et al., 1997; Gregorio et al., 2009) and likely reflects critical neurodevelopmental events, such as neuronal migration, local neuronal connection, and synaptic development (Armstrong et al., 1995; Rakic, 1988). The present findings are likely to support recent animal data, which suggest the significant role of a *DISC1*-related protein in axon elongation of the OFC (Sekiguchi et al., 2011), as well as MRI findings demonstrating the impact of *DISC1* variations in OFC gray matter reduction in healthy subjects (Carless et al., 2011; Wei et al., 2012). Given the time periods at which the OFC H-shaped sulcus and CSP develop as well as the lack of genotypic effects on the AI and olfactory sulcus, both of which are considered to reflect neurodevelopment during early gestation (Chi et al., 1977; Rosales et al., 1968), our data may also suggest that the genotype variation of *DISC1* is related to normal brain development after the mid-late gestational period. Furthermore, in combination with functional MRI data that healthy subjects carrying the Cys allele have less efficient prefrontal function (Prata et al., 2008), our results of decreased Type I pattern of the right OFC in Cys carriers may support the hypothesis that the Type I OFC pattern is associated with more efficient neural organization and better axonal connectivity with other brain regions (Bartholomeusz et al., 2013).

Despite a recent MRI finding that individuals at increased genetic risk of schizophrenia partly share an altered OFC pattern with patients with schizophrenia (Chakirova et al., 2010), we did not find any significant genotype effect of *DISC1* on the OFC pattern in schizophrenia. However, a significant relation between the left Type I pattern and positive symptoms only in the Ser homozygote patients suggests that the *DISC1* genotype variation may have some relevance to the symptomatology of schizophrenia. Given that schizophrenia is a heterogeneous disorder with a multifactorial etiology (Harrison and Weinberger, 2005; Sawa and Snyder, 2002), further analyses of *DISC1*-related and other susceptibility genes, as well as their interactions with environmental factors, will be required to clarify the molecular basis related to the neurodevelopmental pathology of schizophrenia.

A few possible confounding factors in this study should be taken into account. First, we examined only a single SNP of the *DISC1* gene and its interaction with *YWHAE*, a gene encoding one of the *DISC1*-interacting molecules (Ikeda et al., 2008), in a relatively small sample. For the analysis of sulcal patterns, for example, some of the four subgroups (types II and IV) were very small. The potential role of genetic variation in *DISC1*, as well as its interaction with other genetic/non-genetic factors, should be further tested in larger cohorts. Second, we examined schizophrenia patients with an illness duration of approximately 5 years, while illness chronicity (Haijma et al., 2013) and medication (Andreasen et al., 2013; Lieberman et al., 2005) can significantly affect brain morphology. Although there was no difference in these variables between the patients with and without the Cys allele (Table 1) and gross cortical folding patterns remain rather stable throughout life in healthy subjects (Armstrong et al., 1995; Magnotta et al., 1999), a recent longitudinal MRI study demonstrated the possibility that the size of the CSP could change during the course of schizophrenia (Trzesniak et al., 2012). On the other hand, our longitudinal studies showed that the CSP length and olfactory sulcus depth remained stable over time in schizophrenia but that the AI might exhibit age-related atrophy in both schizophrenia and controls (Takahashi et al., 2013a, 2013b). It is thus possible that the anatomical measures investigated in this study may be affected by disease course. The present findings should be thus replicated using patients at earlier illness stages. Finally, because aberrations of *DISC1* are likely to be a generalized risk factor in major psychiatric disorders including bipolar disorder and autism (Crepel et al., 2010; Duff et al., 2013), the possible effect of variation in the *DISC1* genotype on brain morphology should be examined in various psychiatric disorders in future studies.

5. Conclusion

This preliminary study suggested that genotype variation in *DISC1* may be related to the normal development of the midline brain region and cortical folding in the OFC. However, we did not observe a genotype effect of *DISC1* on possible neurodevelopmental markers specific to schizophrenia, suggesting the role of other genetic and/or environmental factors in the development of gross morphologic abnormalities in schizophrenia.

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PLOS ONE

The Polymorphism of *YWHAE*, a Gene Encoding 14-3-3Epsilon, and Brain Morphology in Schizophrenia: A Voxel-Based Morphometric Study

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Abstract

Background: *YWHAE* is a possible susceptibility gene for schizophrenia that encodes 14-3-3epsilon, a Disrupted-in-Schizophrenia 1 (*DISC1*)-interacting molecule, but the effect of variation in its genotype on brain morphology remains largely unknown.

Methods: In this voxel-based morphometric magnetic resonance imaging study, we conducted whole-brain analyses regarding the effects of *YWHAE* single-nucleotide polymorphisms (SNPs) (*rs28365859, rs11655548, and rs9393*) and *DISC1* SNP (*rs821616*) on gray matter volume in a Japanese sample of 72 schizophrenia patients and 86 healthy controls. On the basis of a previous animal study, we also examined the effect of *rs28365859* genotype specifically on hippocampal volume.

Results: Whole-brain analyses showed no significant genotype effect of these SNPs on gray matter volume in all subjects, but we found significant genotype-by-diagnosis interaction for *rs28365859* in the left insula and right putamen. The protective C allele carriers of *rs28365859* had a significantly larger left insula than the G homozygotes only for schizophrenia patients, while the controls with G allele homozygosity had a significantly larger right putamen than the C allele carriers. The C allele carriers had a larger right hippocampus than the G allele homozygotes in schizophrenia patients, but not in healthy controls. No significant interaction was found between *rs28365859* and *DISC1* SNP on gray matter volume.

Conclusions: These different effects of the *YWHAE* (*rs28365859*) genotype on brain morphology in schizophrenia and healthy controls suggest that variation in its genotype might be, at least partly, related to the abnormal neurodevelopment, including in the limbic regions, reported in schizophrenia. Our results also suggest its specific role among *YWHAE* SNPs in the pathophysiology of schizophrenia.

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Introduction

Schizophrenia is a heterogeneous psychiatric disorder with a multifactorial etiology in which multiple susceptibility genes interact with environmental factors [1,2]. Convergent evidence from neuroimaging studies in schizophrenia suggests subtle but widespread gray matter (GM) reductions predominantly in the frontal and temporo–limbic regions (e.g., hippocampus), at least partly as a consequence of early neurodevelopmental insult [3,4]. These brain morphologic changes in schizophrenia could be useful endophenotypes for unraveling the molecular etiopathology of this complex psychiatric disorder [5,6].

The Disrupted-in-Schizophrenia 1 (*DISC1*) gene [7,8], which is thought to be involved in mechanisms of neurodevelopment and synaptic plasticity in cortical and limbic regions [9–13], has been one of the candidate genes for schizophrenia [14,15]. In addition to the possible effect of *DISC1* genotype variation on brain function and structure in the hippocampus [16] and cingulate cortex [17] in healthy subjects, our preliminary magnetic resonance imaging (MRI) study suggested that it might differentially affect GM volume of the neocortical and limbic regions in schizophrenia patients and healthy controls [18]. Several other MRI studies of *DISC1* in schizophrenia have yielded inconsistent results [reviewed by Duff et al. [19] and there have also been questions about *DISC1* as a genetic risk factor of schizophrenia [20]. However, *DISC1* interacts with a complex formed by related molecules [13] and the genetic variation in such *DISC1*-interacting molecules might have a significant role in the pathophysiology of schizophrenia.

YWHAE is a gene encoding 14-3-3epsilon, one of the *DISC1*-interacting molecules that is thought to play a crucial role in neuronal development via transport of the NudE-like (*NUDEL*)/lissencephaly-1 (*LIS1*) complex [13,21], and is a possible susceptibility gene for schizophrenia as identified in a Japanese population [22]. Genetic and expression evidence indicated that a functional single-nucleotide polymorphism (SNP) in the 5′ flanking region (*rs28365859*) was associated with schizophrenia, with subjects with the C allele having a reduced risk of the illness [22]. In addition, animal studies using genetically modified 14-3-3epsilon-deficient mice showed developmental defects of hippocampal neurons [21] as well as working memory deficits [22], which is one of the prominent features of schizophrenia [23]. Despite these observations supporting the significant role of *YWHAE* in the neurobiology of schizophrenia, the possible association between variation in its genotype and brain morphology in schizophrenia remains largely unknown.

In this MRI study, we used voxel-based morphometry (VBM), which allows automated whole-brain analysis, to explore the effects of a *YWHAE* SNP (*rs28365859*) on regional GM volume in a Japanese sample of schizophrenia patients and matched healthy controls. On the basis of the potential role of *YWHAE* in neuronal development as well as previous MRI findings in schizophrenia [3,4], we predicted significant diagnosis-by-genotype interaction predominantly in frontal and temporo–limbic regions, with patients with the protective C allele having a larger GM volume. As previous animal studies suggested the impact of *YWHAE* on the hippocampus [21], we also examined the effect of its genotype specifically on hippocampal volume using small volume correction (SVC) of VBM analyses, with the hypothesis that subjects with the C allele would have a larger hippocampal volume, especially in schizophrenia patients.

To investigate the specificity of the effect of *rs28365859* on brain morphology, we also examined two putative non-risk SNPs in *YWHAE* (*rs11655548* that was associated with schizophrenia but located in the intron region and *rs9393*, a functional SNP with no difference in genotype distribution between schizophrenia and controls) [22]. Possible interaction effect between *rs28365859* and *DISC1* Ser704Cys SNP (*rs821616*) on brain morphology was also examined.

Methods

Ethics statement

This protocol was approved by Committee on Medical Ethics of Toyama University and Nagoya University Graduate School of Medicine. After a complete and detail description of the study was given, subjects provided written informed consent. Clinical staff explained the nature of the study to the subjects, the risks and benefits, and the option not to participate in this research. If the mental status of a subject was impaired to the point where s/he could not understand these issues, the subject was not asked to participate in this research. If there was a possibility that the capacity of a participant to consent was compromised, an additional consent form was obtained from the next of kin, care takers, or guardians of such subjects.

Subjects

Seventy-two patients with schizophrenia (39 males and 33 females; mean age = 27.5 years, SD = 6.0) who met the ICD-10 research criteria [24] were recruited from 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) [25]. Clinical symptoms were rated at the time of scanning using the Scale for the Assessment of Negative Symptoms (SANS) [26] and the Scale for the Assessment of Positive Symptoms (SAPS) [27]. Sixty-eight patients were right-handed and four patients were mixed-handed.

The control subjects consisted of 86 right-handed healthy volunteers (45 males and 41 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.

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 by neuroradiologists. The subject overlap with our previous publication included 30/72 schizophrenia patients and 28/86 controls, where we reported the effect of *DISC1* Ser704Cys polymorphism (*rs821616*) on brain morphology [18].

SNP genotyping

Genomic DNA was extracted from EDTA-containing venous blood samples according to standard procedures. The genotyping of SNPs in *YWHAE* (*rs28365859*, *rs11655548*, and *rs9393*) and *DISC1* (*rs821616*) was performed by 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).

MRI procedures

MR images were obtained using 1.5 T 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. 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. The scanner was

Table 1. Clinical and *YWHAE* genotypic description of schizophrenia patients and healthy controls.

	Schizophrenia patients		Controls		Group comparisons
	C allele carriers	G homozygotes	C allele carriers	G homozygotes	
	(*n*=34)	(*n*=38)	(*n*=32)	(*n*=54)	
Male/female	14/20	25/13	19/13	26/28	Chi-square = 3.95, $p=0.27$
Age (years)	27.2±5.9	27.9±6.2	25.5±6.6	27.0±6.6	F (3,154) = 0.85, $p=0.47$
Height (cm)	162.3±8.7	166.4±8.1	166.9±9.6	164.5±7.4	F (3,154) = 2.22, $p=0.09$
Body weight (kg)	56.3±9.5	62.1±11.6	57.9±9.9	57.1±9.7	F (3,154) = 2.48, $p=0.06$
Education (years)	13.9±1.7	13.6±2.1	16.0±2.2	15.9±2.3	F (3,153) = 13.79, $p<0.01$; Con>Sz
Parental education (years)	13.0±1.8	12.4±2.5	13.2±2.5	13.3±2.4	F (3,153) = 1.22, $p=0.30$
Age of onset (years)	21.7±4.1	23.3±5.1	-	-	F (1,70) = 2.21, $p=0.14$
Duration of illness (years)	5.4±5.8	4.4±4.6	-	-	F (1,70) = 0.64, $p=0.43$
Duration of medication (years)	2.9±3.9	3.2±3.7	-	-	F (1,70) = 0.11, $p=0.75$
Drug dose (haloperidol equivalent, mg/day)	8.2±7.2	9.3±8.3	-	-	F (1,70) = 0.37, $p=0.55$
Total SAPS score[a)]	32.3±26.3	28.3±26.6	-	-	F (1,69) = 0.40, $p=0.53$
Total SANS score[a)]	53.1±24.1	52.2±20.6	-	-	F (1,69) = 0.03, $p=0.87$
Total gray matter volume (mm^3)	631.3±46.6	658.0±64.4	655.6±52.3	654.5±57.2	F (3,154) = 1.74, $p=0.16$

Values represent means ± SDs. Con, controls; SANS, Scale for the Assessment of Negative Sympoms; SAPS, Scale for the Assessment of Positive Symptoms; Sz, schizophrenia.
[a)]Data missing for one patient.
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calibrated weekly with the same phantom to ensure measurement stability.

T1-weighted MR images were processed using Statistical Parametric Mapping 8 (SPM8, Wellcome Institute of Neurology, University College London, UK, http://www.fil.icon.ucl.ac.uk/spm) running under MATLAB R2012b (The MathWorks Inc., USA). The images were preprocessed using the VBM8 toolbox (http://dbm.neuro.uni-jena.de/vbm/), which is an extension of the unified segmentation model consisting of spatial normalization, bias field correction, and tissue segmentation [28]. Registration to the stereotactic space of the Montreal Neurological Institute (MNI) consisted of linear affine transformation and nonlinear deformation using high-dimensional Diffeomorphic Anatomical Registration through Exponential Lie Algebra (DARTEL) normalization [29]. Estimation options were set as follows: extremely light bias regulation; bias cut-off full width at half maximum (FWHM) = 30 mm; affine regulation = International Consortium for Brain Mapping (ICBM) space template of East Asian brains; and the others were defaults. The normalized and segmented images were modulated by applying a nonlinear deformation, which allows comparison of absolute amounts of tissue corrected for individual differences in brain size. The bias-corrected, modulated, and warped tissue maps were then written with an isotopic voxel resolution of 1.5×1.5×1.5 mm and smoothed with an 8-mm FWHM Gaussian kernel [30,31].

Exploratory whole-brain analysis of regional GM volume

First, we performed whole-brain analyses using SPM8 to explore the effects of genotype and genotype-by-diagnosis interaction for each of *YWHAE* (*rs28365859*, *rs11655548*, and *rs9393*) and *DISC1* (*rs821616*) SNPs on GM volume in all subjects. These effects were statistically assessed using a full factorial model for a 2×2 ANOVA, with diagnosis and genotype status as independent variables, and age and sex as covariates of no interest in SPM8. In order to avoid type I error, the significance level was set at $p<0.0001$ (uncorrected for multiple comparison), and the extent threshold of cluster size was set at $k>50$. We also explored the gene-gene interaction between *rs28365859* and *rs821616* on brain morphology using a full factorial model for a 2×2 ANOVA, with genotype status of each SNP as independent variables.

Using the Wake Forest University (WFU) PickAtlas [32], we then performed small volume corrections (SVCs) for each brain region including the clusters with a significant genotype effect or interaction. Each region was defined using the Automated Anatomical Labeling (AAL) atlas [33]. For the regions of interest (ROIs) with significant genotype-by-diagnosis interaction, the genotype effect was examined separately in the patients and controls, with age and sex as covariates of no interest. For these SVC analyses, a family-wise error-corrected (FWE) voxel level threshold of $p<0.05$ was applied to account for multiple comparisons of the results. Voxel coordinates were given as an indication of location in a standardized brain. Voxels were localized in MNI space and transformed into Talairach and Tournoux coordinates [34] using the WFU PickAtlas [35,36].

Hypothesis-driven ROI analysis for hippocampus

On the basis of a previous postmortem rat experiment [21], we also examined the effect of *rs28365859* on bilateral hippocampi defined by the AAL atlas (FWE, $p<0.05$). For this hypothesis-driven ROI analysis, we examined the effect of genotype in all subjects as well as in each diagnostic group. Age and sex were used as covariates of no interest in these analyses.

Statistical analysis

Demographic and clinical differences between groups were examined by using chi-square test or one-way analysis of variance (ANOVA) with post hoc Scheffé's test. Genotypes were tested for

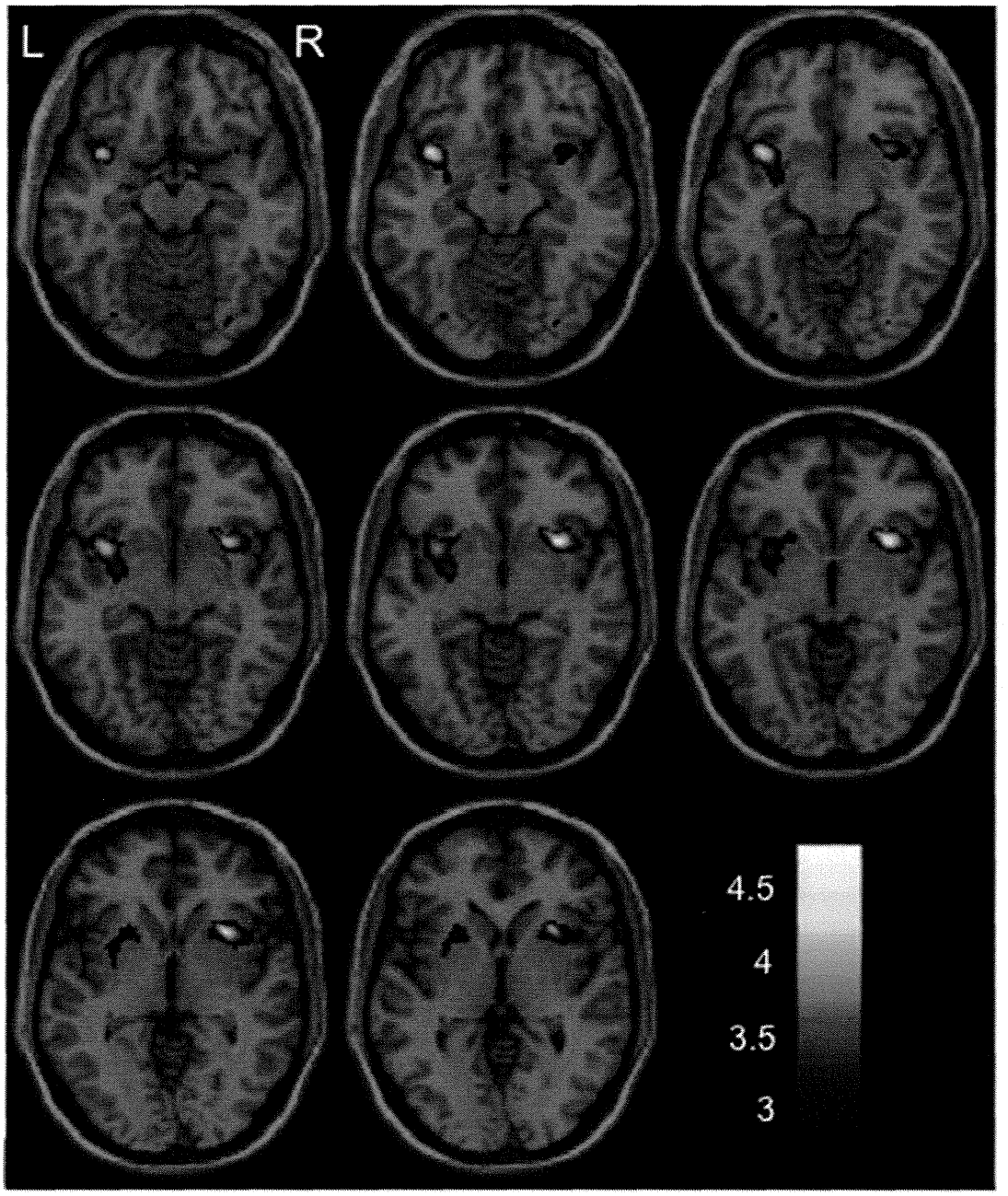

Figure 1. The *YWHAE* (*rs28365859*) genotype-by-diagnosis interaction on gray matter volume. The regions showing interaction in all subjects are displayed by a hot colormap. The color bar shows t values corresponding to the color in the figure.
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Hardy–Weinberg equilibrium (HWE) using the chi-square goodness-of-fit test. Since the number of subjects with C allele homozygosity of *rs28365859* was quite small (3 schizophrenia patients and 4 control subjects), and on the basis of a previous report on lymphocytes of healthy control subjects [22], the study participants were categorized into C allele carriers (protective allele group) or G allele homozygotes. For other *YWHAE* and *DISC1* SNPs, on the basis of minor allele frequency [22] and previous report [18], the subjects were divided into G allele carriers *vs* A allele homozygotes (*rs11655548* and *rs9393*) and T allele homozygotes *vs* A allele carriers (*rs821616*), respectively. Statistical significance was defined as $p<0.05$.

Results

Sample characteristics and genotyping results

Groups were matched for age, sex, height, body weight, and total GM volume, but the controls had attained a higher level of education than the schizophrenia patients (Table 1). In Table1, the different typical and atypical antipsychotic dosages were converted into haloperidol equivalent according to the guidelines by Toru [37]. There was no significant difference in clinical and demographic data between *YWHAE (rs28365859)* C allele carriers and G allele homozygotes in both schizophrenia and control groups. The genotype frequencies of the SNPs investigated in this study were within the distribution expected according to the

Table 2. Effect of *rs28365859* genotype and genotype-by-diagnosis interaction on gray matter volume.

	Brain region	Contrast	Covariates	Talairach coordinate			Cluster size	*p*
				x	y	z		
Interaction on whole brain								
	Rt putamen		age, sex	32	13	−5	125	<0.0001 (uncorrected)
	Lt insula		age, sex	−39	10	−11	108	<0.0001 (uncorrected)
Interaction on SVC								
	Rt putamen		age, sex	32	13	−5	168	0.001 (FWE-corrected)
	Lt insula		age, sex	−39	10	−11	232	0.004 (FWE-corrected)
Genotype effect on SVC[a]								
	Rt putamen	ConC−>ConC+	age, sex	30	16	−1	60	0.023 (FWE-corrected)
	Lt insula	SzC+>SzC−	age, sex	−36	8	−11	52	0.047 (FWE-corrected)
		SzC+>SzC−	age, sex, doi, med	−36	8	−11	68	0.037 (FWE-corrected)

ConC+, controls with C allele; ConC−, controls without C allele; doi, duration of illness; FWE, family-wise error; Lt, left; med, daily medication dose; Rt, right; SVC, small volume correction; SzC+, schizophrenia patients with C allele; SzC−, schizophrenia without C allele.

[a]There were no suprathreshold clusters for other contrasts.

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HWE. As shown in Table 1, patients with schizophrenia and healthy comparisons did not differ significantly in genotype distributions (chi-square = 1.62, $p = 0.204$) and allele frequencies (chi-square = 1.00, $p = 0.317$) of *rs28365859*.

For the other SNPs, *rs11655548* (3 patients and 3 controls), *rs9393* (3 patients and 1 control), and *rs821616* (3 patients) were not detected for some participants. There was a group difference in the genotype distribution only for *rs9393* (chi-square = 5.65, $p = 0.018$; less G allele carriers in the patients), but such a difference was not found in a larger sample including the current sample ($n = 332$) or in a large independent Japanese sample ($n = 3157$) [22].

Exploratory whole-brain analysis of regional GM volume

There was no significant genotype effect of *YWHAE* SNPs or *rs821616* on GM volume in all subjects. However, we found significant genotype-by-diagnosis interactions for *rs28365859* in the left insula and right putamen GM volume (uncorrected $p < 0.0001$, extent threshold k>50; Table 2 and Fig. 1), which were confirmed by subsequent FWE-corrected SVC analyses (left insula, $p = 0.004$; right putamen, $p = 0.001$) (Table 2). Other SNPs (*rs11655548, rs9393,* and *rs821616*) had no genotype-by-diagnosis interaction. There was no significant gene-gene interaction on GM volume between *rs28365859* and *rs821616*.

On the basis of significant genotype-by-diagnosis interactions of *rs28365859*, we then separately investigated its genotype effect on GM volume in schizophrenia and control groups. The protective C allele carriers had a significantly larger left insula than G homozygotes only for the schizophrenia patients (FWE-corrected $p = 0.047$, Fig. 2), while the controls with G allele homozygosity had a significantly larger right putamen than the C allele carriers (FWE-corrected $p = 0.023$, Fig. 3) (Table 2). The C allele was also related to smaller left insula in controls (FWE-corrected $p = 0.144$) and larger right putamen in schizophrenia patients (FWE-corrected $p = 0.078$), although these effects were not statistically significant. The findings reported herein did not change even when we added the illness duration and medication dose as covariates for the SVC analyses for the schizophrenia patients (Table 2).

Hypothesis-driven ROI analysis for hippocampus

The protective C allele carriers of *rs28365859* had a significantly larger right, but not left, hippocampal volume than the G allele homozygotes (FWE-corrected $p = 0.009$, Table 3). For the analyses in each diagnostic group, such an effect of *YWHAE* genotype was significant only in schizophrenia patients (FWE-corrected $p = 0.009$, Table 3 and Fig. 4). That result in schizophrenia remained the same even when we added illness duration and medication as covariates (Table 3).

Discussion

This is the first structural MRI study to report the relationship between the functional polymorphism of *YWHAE*, a gene encoding 14-3-3epsilon, and brain morphology in patients with schizophrenia and healthy controls. While no significant difference was found in clinical and demographic data between the *YWHAE (rs28365859)* C allele carriers (protective allele group) and G allele homozygotes in both schizophrenia and control groups, the exploratory whole-brain analysis of regional GM volume demonstrated significant genotype-by-diagnosis interaction of *rs28365859* on the left insula and right putamen. Subsequent SVC analyses showed that the protective C allele carriers had a significantly larger left insula than G homozygotes only for the

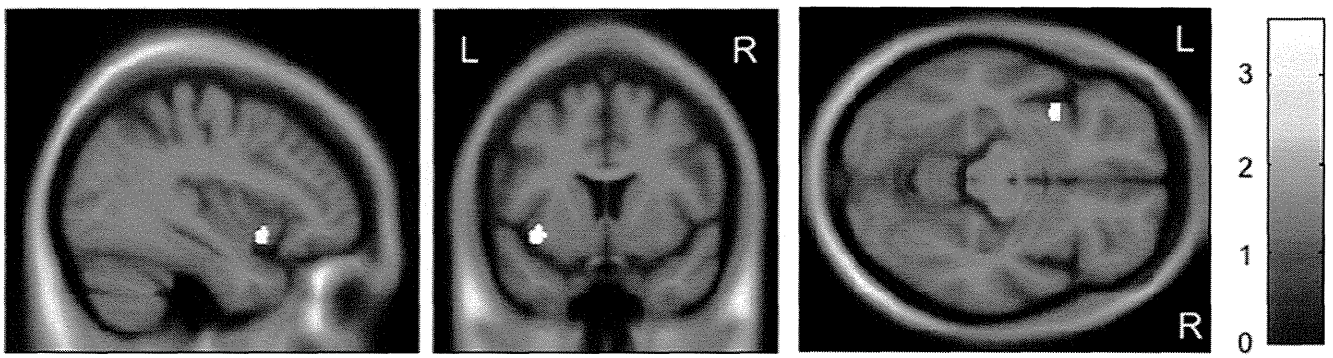

Figure 2. Impact of the *rs28365859* genotype on gray matter volume of left insula in schizophrenia. Age, sex, illness duration, and medication dose were used as covariates. The protective C allele carriers had a significantly larger left insula than the G homozygotes. Anatomical localizations are displayed on the normal template MR images in three directions. The color bar shows t values corresponding to the color in the figure.
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schizophrenia patients, while the controls with G allele homozygosity had a significantly larger right putamen than the C allele carriers. Furthermore, the hypothesis-driven ROI analysis revealed that the subjects with the C allele had a larger hippocampal volume, especially for schizophrenia patients. Our report using a Japanese cohort thus suggests that the genotype variation of 14-3-3epsilon, a *DISC1*-interacting molecule associated with neuronal development [13,21], may be at least partly related to the abnormalities in brain morphology reported in schizophrenia. Importantly, we found no significant genotype effect of non-risk *YWHAE* SNPs (*rs11655548* and *rs9393*) on GM volume, supporting the specific role of *rs28365859* in the pathophysiology of schizophrenia [22].

Our finding of preserved insula GM volume in schizophrenia patients with protective C allele of *rs28365859* is consistent with the literature suggesting a significant role of insula pathology in schizophrenia [38]. GM reduction of the insula, which plays crucial roles in emotional and various cognitive functions as a component of the limbic integration cortex [39], has been repeatedly described in schizophrenia [40,41]. GM reduction or dysfunction of the insula has also been implicated in the manifestation of psychotic symptoms and cognitive impairments [38]. The exact neurobiological basis for these GM changes of the insula in schizophrenia remains unknown, but the defects in gyrification [42], cytoarchitectural abnormalities [43,44], and significant volume reduction prior to the illness onset [45,46] imply early neurodevelopmental abnormalities in this region. A lack of insular GM abnormalities in non-psychotic co-twins within monozygotic twins discordant for schizophrenia [47] suggests that the insular findings in schizophrenia are also attributable to non-genetic factors. In this study, healthy controls with C allele had a non-significantly smaller left insula compared to G homozygotes. The reason for this opposite direction of volume changes related to the same allele between schizophrenia patients and controls is unclear, but our earlier MRI study demonstrated that the *DISC1 (rs821616)* genotype variation could also differently affect the insula GM volume in schizophrenia patients and healthy comparisons [18]. The current evidence for *DISC1* alone as a genetic risk factor of schizophrenia is not strong [20]. Indeed, the present study did not support its effect on brain morphology in schizophrenia. However, considering that *DISC1* interacts with a complex formed by related molecules (including 14-3-3epsilon) during processes involved in neuronal development, such as axonal elongation [13], the present results raise the possibility that the genetic variation of *DISC1*-interacting molecules might have an additive or independent role in alterations of the neural development in schizophrenia, especially regarding the insula pathology [38]. The potential role of genetic variation in *DISC1*-interacting molecules and its interaction with other genetic/non-genetic factors in the pathophysiology of schizophrenia should be further tested through *in vitro* and *in vivo* studies.

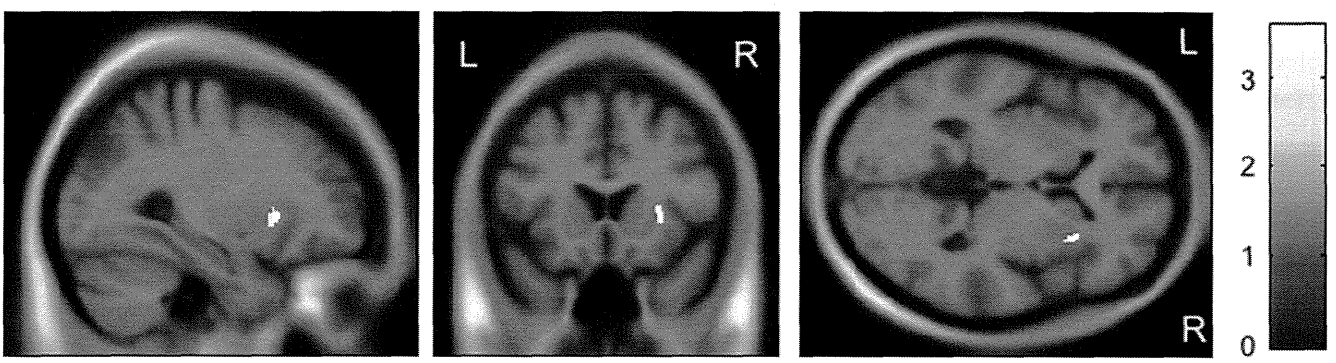

Figure 3. Impact of the *rs28365859* genotype on gray matter volume of the right putamen in healthy controls. The G allele homozygotes had a significantly larger right putamen than the C allele carriers. Anatomical localizations are displayed on the normal template MR images in three directions. The color bar shows t values corresponding to the color in the figure.
doi:10.1371/journal.pone.0103571.g003

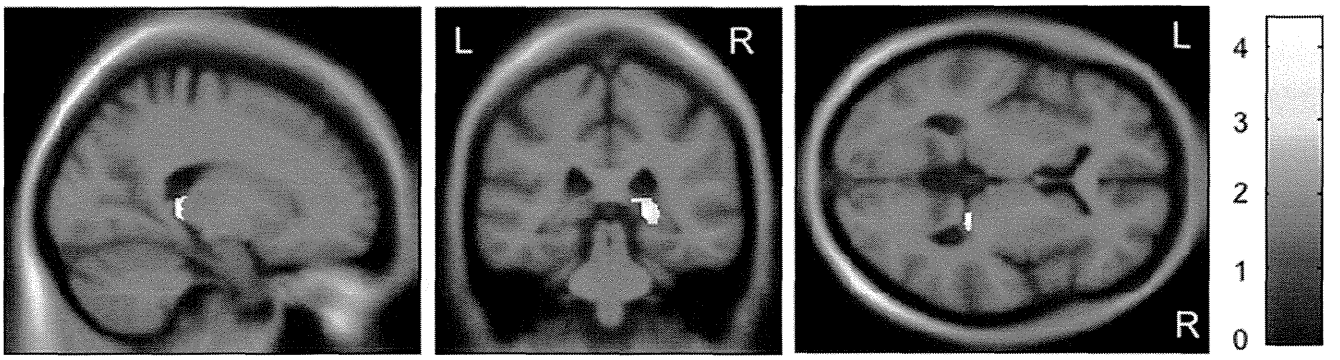

Figure 4. Impact of the *rs28365859* genotype on gray matter volume of the right hippocampus in schizophrenia. Age, sex, illness duration, and medication dose were used as covariates. The protective C allele carriers had a significantly larger right hippocampus than the G allele homozygotes. Anatomical localizations are displayed on the normal template MR images in three directions. The color bar shows t values corresponding to the color in the figure.
doi:10.1371/journal.pone.0103571.g004

We also found significant *rs28365859* genotype-by-diagnosis interaction on the right putamen, with the C allele carriers having a smaller putamen volume only for healthy subjects. This finding might have some association with a previous MRI study that demonstrated the relationship between functional *DISC1* genotype and striatal volume [48]. Taken together with animal data that the *DISC1* gene influences striatal dopamine receptor levels [49], Chakravarty et al. [48] hypothesized that a key risk pathway for schizophrenia might be conferred via *DISC1*'s effects on the striatum. MRI findings of the putamen in schizophrenia have been highly controversial; smaller [50] or normal [51,52] volume was reported in first-episode antipsychotic-naïve patients, with both volume expansion [51,53] and decrease [54] following antipsychotic treatment. We did not find a significant effect of the genetic variation of 14-3-3epsilon, a *DISC1*-interacting molecule, on the basal ganglia in our sample of chronically medicated schizophrenia patients. However, the possible role of genetic variation of *DISC1* and its interacting molecules on brain morphology in schizophrenia should be examined in future, ideally using a larger antipsychotic-naïve sample.

In this study, as hypothesized, we also demonstrated that the subjects with the protective C allele of *rs28365859* had a larger hippocampal volume, especially for schizophrenia patients. Hippocampal GM volume is thought to represent an endophenotype associated with the clinical expression of schizophrenia [55]. Brain imaging studies suggest that variants in the *DISC1* gene may influence normal neurodevelopment, brain structure, function, and neurochemistry, but the association of the common *DISC1* SNPs with hippocampal regions has been inconsistent for both schizophrenia and healthy subjects (reviewed by Duff et al. [19]). However, the expression of *DISC1*-binding partners such as *NUDEL* and *LIS1*, which form a complex with 14-3-3epsilon [13,21], is reduced in the hippocampus of postmortem schizophrenia brains [56]. More specifically, animal studies using genetically modified 14-3-3epsilon-deficient mice showed developmental defects of hippocampal neurons [21] as well as behavioral changes related to clinical features of schizophrenia (i.e., anxiety-like behavior, working memory deficits) [22]. Schizophrenia is a complex disorder with a variety of pathologies and risk factor genes, and the variation of a single gene could explain only a part of its clinical expression. We found no direct interaction between the *YWHAE* (*rs28365859*) and *DISC1* (*rs821616*) SNPs on gray matter volume in schizophrenia in this study. Nevertheless, the present and previous basic studies suggest the possibility that genetically defined impairment of *DISC1* and/or 14-3-3epsilon could cause neuronal developmental defects in brain regions including the hippocampus, which result in the increased risk of developing schizophrenia.

There are several confounding factors in the present study. First, in contrast to recent large multinational consortium genome-wide association studies [57,58], this study examined the effect of the *YWHAE* genotype only in a relatively small Japanese sample. Our whole-brain analysis found a specific *YWHAE* genotype effect only on the left insula in schizophrenia, but the current study was potentially underpowered to detect significant genotype effects on other brain regions owing to the small sample size. For example, the relation between the protective C allele of *rs28365859* and larger hippocampal volume in all subjects (but more robust in

Table 3. Effect of *rs28365859* genotype on right hippocampal gray matter volume.

Contrast[a]	Covariates	Talairach coordinate			Cluster size	FWE *p*
		x	y	z		
C+>C−	age, sex	24	−35	0	120	0.009
SzC+>SzC−	age, sex	20	−33	3	78	0.009
	age, sex, doi, med	20	−33	3	120	0.002

C+, subjects with C allele; C−, subjects without C allele; doi, duration of illness; FWE, family-wise error; med, daily medication dose; SzC+, schizophrenia patients with C allele; SzC−, schizophrenia patients without C allele.
[a]There were no suprathreshold clusters for other contrasts.
doi:10.1371/journal.pone.0103571.t003

schizophrenia patients) was detectable only by the hypothesis-driven ROI analysis, which is thought to be more sensitive than whole-brain analysis. Furthermore, an animal study by Sekiguchi et al. [59] suggested a relationship between the defect of 14-3-3epsilon and axon elongation abnormality in the prefrontal cortex. As we also found mild diagnosis-by-genotype interaction in frontal regions when we used a significance level of uncorrected $p<0.001$ in exploratory whole-brain analysis (data not shown), future studies on a larger sample of schizophrenia might detect other *YWHAE* genotype effects on brain morphology including the frontal regions. Second, we examined schizophrenia patients with an illness duration of approximately 5 years in this study. Illness chronicity [60] and medication with antipsychotics [61,62] could significantly affect brain morphology. Although there was no difference in these variables between the patients with and without the C allele of *rs28365859* (Table 1) and we statistically controlled these factors, the present findings should be replicated using patients at early illness stages. Third, the current study cannot address the disease specificity of our *YWHAE* findings. There are overlapping GM structural abnormalities in the neurobiology of schizophrenia and bipolar disorder [63] and there are several susceptibility genes (e.g., *DISC1*) for both of these disorders [19]. Finally, considering that we examined only four selected SNPs in the present study, more comprehensive assessment would be required to clarify the role of genetic variation of *DISC1* and its interacting molecules in the pathophysiology of schizophrenia.

In conclusion, we found that the C allele of *YWHAE* (*rs28365859*) is related to preserved GM volume of the insula and hippocampus in schizophrenia, major brain regions related to the illness, in a Japanese sample. These findings are likely to provide neurobiological support for previous genetic and expression studies suggesting that this SNP reduces the risk of schizophrenia [22].

Supporting Information

Figure S1 Diagnosis effect on gray matter volume in all subjects analyzed by using the SPM8 full factorial model. Age and sex were used as covariates. Healthy controls had a larger gray matter volume compared with schizophrenia patients predominantly in fronto-temporo-limbic regions (family-wise error-corrected $p<0.05$). Anatomical localizations are displayed on the normal template MR images in three directions. The color bar shows t values corresponding to the color in the figure.
(TIFF)

Table S1 Diagnosis effect on gray matter volume in all subjects. Each region was defined using the Automated Anatomical Atlas (AAL) atlas [33].
(DOCX)

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Author Contributions

Conceived and designed the experiments: NO KK NI MS. Performed the experiments: MK Yukako Nakamura K. Nemoto BA MI. Analyzed the data: MK Yukako Nakamura K. Nemoto. Contributed reagents/materials/analysis tools: TT Yumiko Nakamura AF MK K. Noguchi. Wrote the paper: MK Yukako Nakamura TT K. Nemoto MS BA NO.

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The polymorphism of *YWHAE*, a gene encoding 14-3-3epsilon, and orbitofrontal sulcogyral pattern in patients with schizophrenia and healthy subjects

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ABSTRACT

An altered sulcogyral pattern in the orbitofrontal cortex (OFC) has been implicated in schizophrenia as a possible marker of abnormal neurodevelopment, while its genetic mechanism remains unknown. This magnetic resonance imaging study investigated the relationship between the polymorphism of *YWHAE* (*rs28365859*), a gene encoding 14-3-3epsilon that is a Disrupted-in-Schizophrenia 1 (*DISC1*)-interacting molecule associated with neuronal development, and the OFC subtypes of the 'H-shaped' sulcus (Types I, II, and III) in a Japanese sample of 72 schizophrenia patients and 86 healthy controls. The schizophrenia patients had significantly increased Type III ($p = 0.004$) and decreased Type I ($p = 0.013$) expression on the right hemisphere compared to the controls. The subjects carrying the protective C allele showed a decrease in Type III ($p = 0.005$) and an increase in Type I ($p = 0.017$) compared to the G allele homozygotes, especially for the healthy subjects in the left hemisphere. These results suggest a possible role for the *YWHAE* genotype in the early development of the OFC sulcogyral pattern, but its effect alone is not likely to explain the altered sulcogyral pattern in schizophrenia.

1. Introduction

Altered gross cortical folding patterns, which are formed during neurodevelopment (Armstrong et al., 1995; Chi et al., 1977), have been reported in schizophrenia (Fujiwara et al., 2007; Palaniyappan et al., 2013; Yücel et al., 2002), as well as in genetic high-risk individuals (Chakirova et al., 2010; Harris et al., 2004, 2007; Jou et al., 2005). These observations support the possible role of genetic mechanisms related to brain gyrification (Bartley et al., 1997; Kippenhan et al., 2005) in the neurodevelopmental pathology of schizophrenia (Fatemi and Folsom, 2009; Weinberger, 1987). Although not consistently replicated (e.g., Bartholomeusz et al., 2013), several magnetic resonance imaging (MRI) studies of schizophrenia have investigated variations in the orbitofrontal cortex (OFC) 'H-shaped' sulcus [Types I, II, and III; defined by Chiavaras and Petrides (2000)] and demonstrated increased Type III and decreased Type I expression on the right hemisphere in schizophrenia (Chakirova et al., 2010; Nakamura et al., 2007; Takayanagi et al., 2010). These altered OFC sulcogyral patterns could be a possible endophenotypic risk marker of schizophrenia (Bartholomeusz et al., 2013), but the genetic mechanism underlying such gross morphologic changes remains largely unknown.

YWHAE is a gene encoding 14-3-3epsilon, one of the Disrupted-in-Schizophrenia 1 (*DISC1*)-interacting molecules associated with neuronal development (Taya et al., 2007; Toyo-oka et al., 2003), and is a possible susceptibility gene for schizophrenia (Ikeda et al., 2008). Genetic and expression evidence indicated that a functional single-nucleotide polymorphism (SNP) in the 5′ flanking region (*rs28365859*) was associated with schizophrenia, with subjects with the C allele having a

Abbreviations: ANOVA, analysis of variance; CASH, Comprehensive Assessment of Symptoms and History; *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.

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reduced risk of the illness (Ikeda et al., 2008). In addition, recent animal studies using genetically modified 14-3-3epsilon heterozygous knockout mice revealed impairment of axon elongation in the OFC (Sekiguchi et al., 2011), as well as a working memory deficit (Ikeda et al., 2008), which is one of the prominent features related to prefrontal dysfunction in schizophrenia (Goldman-Rakic, 1994). Despite these observations supporting the significant role of *YWHAE* especially in the prefrontal neurodevelopmental pathology, it remains largely unknown whether its genotype variation is related to brain morphologic changes, such as altered OFC sulcogyral pattern, in schizophrenia.

In this MRI study, we investigated the effects of *YWHAE* SNP (*rs28365859*) on OFC sulcogyral pattern in a Japanese sample of schizophrenia patients and matched healthy controls. Based on the potential role of *YWHAE* in the neuronal development of OFC (Sekiguchi et al., 2011), as well as previous MRI findings of altered OFC sulcogyral patterns in schizophrenia (Nakamura et al., 2007), we predicted that variation in the *YWHAE* genotype in the present sample could be related to the OFC subtypes of the H-shaped sulcus, especially in schizophrenia.

2. Methods

2.1. Subjects

Seventy-two patients with schizophrenia (39 males and 33 females; mean age = 27.5 years, SD = 6.0) 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; Andreasen, 1984) and the Scale for the Assessment of Positive Symptoms (SAPS; Andreasen, 1984). Sixty-eight patients were right-handed and four patients were mixed-handed.

The control subjects consisted of 86 right-handed healthy volunteers (45 males and 41 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.

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 by neuroradiologists. The Committee 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 promoter SNP in *YWHAE* (*rs28365859*) 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 1.5 T 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. 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.

2.4. OFC sulcogyral pattern classification

The images were processed on a Linux PC (Fujitsu Limited, Tokyo, Japan) using Dr. View software (AJS, Tokyo, Japan). 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. The medial orbital sulcus (MOS), lateral orbital sulcus (LOS), and transverse orbital sulcus (TOS) were highlighted on consecutive 1-mm coronal slices, and then viewed in axial plane for the OFC pattern classification based on the definition by Chiavaras and Petrides (2000). Briefly, the OFC sulcogyral patterns were classified according to the continuity of the 'H-shaped' sulcus consisting of the MOS, TOS, and LOS; for Type I the MOS is disconnected while the LOS is intact, for Type II both the MOS and LOS are continuous, and for Type III both the MOS and LOS are disconnected (Fig. 1. Also, see Bartholomeusz et al., 2013). In rare instances where the MOS was continuous, but the LOS was disconnected, this pattern was classified as Type IV (Chakirova et al., 2010).

The OFC sulcogyral pattern classification was performed by one rater (TT), who was blind to the subjects' identity. Intra- and inter-rater (TT and YN) reliabilities (Cronbach's α) in a subset of 20 randomly selected brains (40 hemispheres) were 0.97 and 0.81, respectively.

2.5. Statistical analysis

Demographic and clinical differences between groups were examined by using a χ^2 test or one-way analysis of variance (ANOVA). Genotypes were tested for Hardy–Weinberg equilibrium (HWE) using the χ^2 goodness-of-fit test. Since the number of subjects with C allele homozygosity was quite small (3 schizophrenia patients and 4 control subjects), and on the basis of a previous report on lymphocytes of healthy control subjects (Ikeda et al., 2008), the study participants were categorized into C allele carriers (protective allele group) or G allele homozygotes. Group differences in the OFC sulcogyral pattern distribution were evaluated using the χ^2 test. The relationships between the sulcogyral pattern and clinical/demographic variables were analyzed for each hemisphere using ANOVA with the OFC sulcogyral pattern (Types I–III) as a between-subject factor. The subjects with the Type IV pattern ($N = 2$) were excluded from the ANOVAs. Post-hoc Spjotvoll and Stoline tests were used to follow up significant main effects or interactions. Statistical significance was defined as $p < 0.05$.

3. Results

3.1. Sample characteristics and genotyping results

Groups were matched for age, sex, and parental education, but the controls had attained a higher level of education than the schizophrenia patients. There was no significant difference in clinical or demographic data between the C allele carriers and the G allele homozygotes in the schizophrenia and control groups (Table 1). The observed genotype frequency of SNP was within the distribution expected according to the HWE. The patients with schizophrenia and healthy comparisons did not differ significantly in genotype distributions ($\chi^2 = 1.62$, $p = 0.204$) or allele frequencies ($\chi^2 = 1.00$, $p = 0.317$).

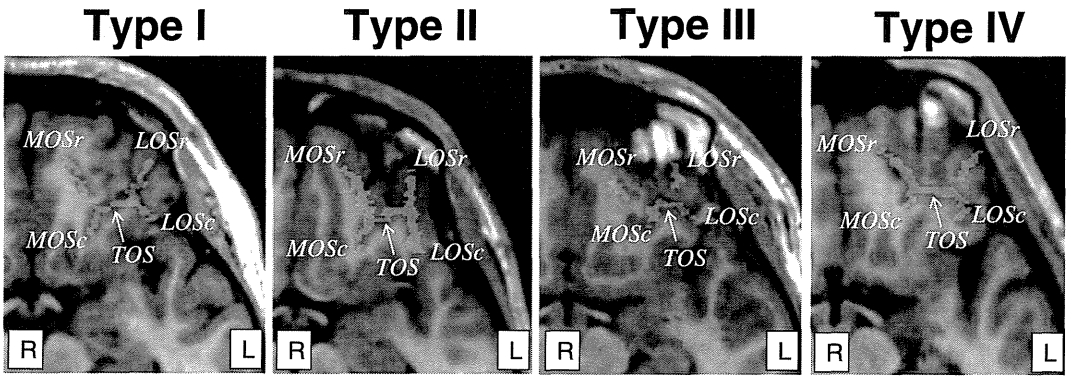

Fig. 1. Classification of the orbitofrontal sulcogyral pattern on an axial view parallel to the anterior commissure–posterior commissure line. Note that these sulci were identified using orthogonal views in three directions and colored on consecutive coronal slices. c, caudal portion; LOS, lateral orbital sulcus; MOS, medial orbital sulcus; r, rostral portion; TOS, transverse orbital sulcus.

3.2. Diagnosis effect on the OFC pattern distribution

The OFC sulcogyral patterns were significantly different between the schizophrenia patients and controls in the right hemisphere (Table 2), with the patients having increased Type III ($\chi^2 = 8.24$, $p = 0.004$) and decreased Type I ($\chi^2 = 6.20$, $p = 0.013$) expression.

3.3. Genotype effect on the OFC pattern distribution

The protective C allele carriers had a decrease in Type III ($\chi^2 = 8.01$, $p = 0.005$) and an increase in Type I ($\chi^2 = 5.73$, $p = 0.017$) compared to the G allele homozygotes in the left hemisphere (Table 3).

For the analyses in each diagnostic group, such an effect of the *YWHAE* genotype on the left OFC pattern was significant only in healthy subjects (overall distribution, $\chi^2 = 10.94$, $p = 0.012$; Type I distribution, $\chi^2 = 6.75$, $p = 0.009$; and Type III distribution, $\chi^2 = 8.70$, $p = 0.003$) (Fig. 2).

3.4. OFC pattern and clinical/demographic variables

ANOVAs with post-hoc tests revealed no significant effects of the OFC pattern on demographic (age, education, and parental education) or clinical (onset age, illness duration, medication, and symptom severity in the schizophrenia patients) variables.

4. Discussion

To our knowledge, this is the first MRI study to report the relationship between the functional polymorphism of *YWHAE*, a gene encoding 14-3-3epsilon, and the OFC sulcogyral pattern in schizophrenia and healthy controls. We found in total subjects that the C allele carriers (protective allele group) exhibited a decrease in Type III expression and an increase in Type I expression of the left OFC pattern compared to the G allele homozygotes. Contrary to our prior prediction, however, such a *YWHAE* genotype effect on the OFC was significant only in the healthy subjects. We also replicated previous MRI findings of altered distribution of the OFC subtypes in schizophrenia (e.g., Nakamura et al., 2007). Our results thus suggest that the genotype variation of 14-3-3epsilon is related to cortical folding during early neurodevelopment, but that the altered OFC sulcogyral pattern in schizophrenia may also be associated with other genetic and/or environmental factors.

Regarding the OFC pattern in schizophrenia, our results are consistent with previous MRI findings of increased Type III and decreased Type I expression on the right hemisphere (Chakirova et al., 2010; Nakamura et al., 2007; Takayanagi et al., 2010), although we failed to replicate the relation between the OFC Type III and symptom severity (Nakamura et al., 2007; Uehara-Aoyama et al., 2011), possibly due to the chronically medicated nature of our samples. Our controls, as well as those of Bartholomeusz et al. (2013) (left Type II, 17.8%; right Type II, 11.0%), had a somewhat lower prevalence of Type II compared to previous reports (see Table 2), but such a difference may be attributable to different sample characteristics (Bartholomeusz et al., 2013), as well as different OFC pattern classification methods between the studies; we and Bartholomeusz et al. (2013) traced the main sulci on consecutive coronal slices, which could detect subtle sulcus disconnection, whereas some other studies (Nakamura et al., 2007; Takayanagi et al., 2010; Uehara-Aoyama et al., 2011) defined the OFC patterns predominantly by surface analyses in axial slices. Taken together, the present results

Table 1
Clinical description of schizophrenia patients and healthy controls with and without the *YWHAE C allele*.

	Schizophrenia patients		Controls		Group comparisons
	C allele carriers	G homozygotes	C allele carriers	G homozygotes	
	($N = 34$)	($N = 38$)	($N = 32$)	($N = 54$)	
Male/female	14/20	25/13	19/13	26/28	$\chi^2 = 3.95$, $p = 0.27$
Age (years)	27.2 ± 5.9	27.9 ± 6.2	25.5 ± 6.6	27.0 ± 6.6	$F(3,154) = 0.85$, $p = 0.47$
Height (cm)	162.3 ± 8.7	166.4 ± 8.1	166.9 ± 9.6	164.5 ± 7.4	$F(3,154) = 2.22$, $p = 0.09$
Education (years)	13.9 ± 1.7	13.6 ± 2.1	16.0 ± 2.2	15.9 ± 2.3	$F(3,153) = 13.79$, $p < 0.01$; Con > Sz
Parental education (years)	13.0 ± 1.8	12.4 ± 2.5	13.2 ± 2.5	13.3 ± 2.4	$F(3,153) = 1.22$, $p = 0.30$
Age of onset (years)	21.7 ± 4.1	23.3 ± 5.1	–	–	$F(1,70) = 2.21$, $p = 0.14$
Duration of illness (years)	5.4 ± 5.8	4.4 ± 4.6	–	–	$F(1,70) = 0.64$, $p = 0.43$
Duration of medication (years)	2.9 ± 3.9	3.2 ± 3.7	–	–	$F(1,70) = 0.11$, $p = 0.75$
Drug dose (haloperidol equivalent, mg/day)[a]	8.2 ± 7.2	9.3 ± 8.3	–	–	$F(1,70) = 0.37$, $p = 0.55$
Total SAPS score[b]	32.3 ± 26.3	28.3 ± 26.6	–	–	$F(1,69) = 0.40$, $p = 0.53$
Total SANS score[b]	53.1 ± 24.1	52.2 ± 20.6	–	–	$F(1,69) = 0.03$, $p = 0.87$

Values represent means ± SDs. Con, controls; SANS, Scale for the Assessment of Negative Symptoms; SAPS, Scale for the Assessment of Positive Symptoms; Sz, schizophrenia.
[a] The different typical and atypical antipsychotic dosages were converted into haloperidol equivalents according to the guideline by Toru (2008).
[b] Data missing for one patient.