speaking countries. One of which is the new Tanaka B Intelligence Scale [22]. Testing may be conducted simultaneously on groups, with no special equipment required, and only writing materials, test paper, and a short time (only 40–45 minutes) frame are required. This is convenient for assessing overall intellectual development. This test seems to be suitable for individuals with varied educational backgrounds, in varied linguistic environments, and over a wide range of linguistic levels, because it does not need complex instructions and is easily understood. This test was originally developed by Kanichi Tanaka in 1936 and has repeatedly been revised and restandardized since the 1930s. The test was most recently restandardized in 2001–2002 and has very high split-half correlations ($r = 0.89$ to 0.96) and high test-retest reliability ($r = 0.73$ to 0.79). There is high validity ($r = 0.69$ to 0.78) with overall scholastic ability, including Japanese language, mathematics, science, social studies, and English [22].

However, academic performance is influenced not only by intellectual development, but also by various environmental factors, including educational background. To the best of our knowledge, the correlation between the new Tanaka B Intelligence Scale and other intelligence tests has not yet been investigated. Therefore, more information about the reliability and validity of this test is needed along with a standardized test to assess individual intellectual development.

Thus, the present study examined the reliability of the new Tanaka B Intelligence Scale and its concurrent validity with the Wechsler Intelligence Scale for Children-Third Edition (WISC-III), which is one of the most-used tests to assess the intelligence quotient of individuals. Moreover, the clinical utility of the new Tanaka B Intelligence Scale as a screening scale for individuals who have a deficit in intellectual function [WISC-III full intelligence quotient (FIQ) less than 70] was evaluated. If the new Tanaka B Intelligence Scale is standardized, intellectual assessment becomes possible even in settings where the number of cases who can receive individual assessment has previously been limited, such as schools and correctional facilities. This will contribute to setting goals for those individuals and planning strategies to achieve those goals.

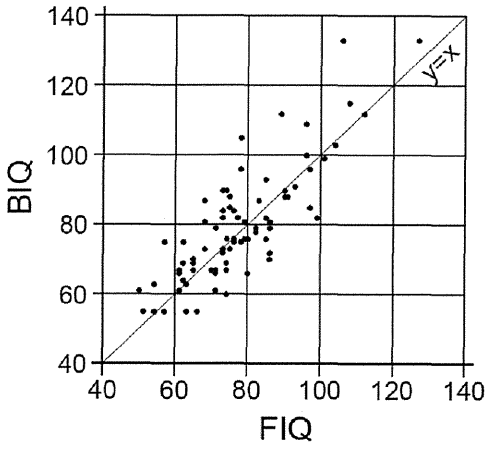

Figure 1. Distribution of BIQ and FIQ scores. Notes: The straight line represents the diagonal line y = x.
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Materials and Methods

New Tanaka B Intelligence Scale

The new Tanaka B Intelligence Scale [22,23] uses diagrams, pictures, and symbols (such as numbers), and has no tasks or problems presented in story form. Diagrams and symbols are used for responses, so testing is not readily affected by learning differences, such as reading or writing, or by linguistic or cultural influences. There are seven subtest items, including mazes, calculating cubes, replacing figures and numbers, difference discrimination of character strings, completing a number series, erasing figures, and completing figures. Testing is divided into five parts depending on the subject's age, including testing for ages 6–8 years old (early elementary school), ages 8–10 years old (middle elementary school), ages 10–12 years old (late elementary school), ages 12–14 years (junior high school years 1 and 2), and age 14-adult (junior high school year 3 and high school and up). Testing was performed for subjects aged ≥14 years in the present study.

Procedures and Subjects

Of the 81 children/adolescents in a juvenile detention home between January 1, 2009 and December 31, 2010, all took both the new Tanaka B Intelligence Scale and the WISC-III. One juvenile detention home is generally located in each prefecture. These are public facilities where children/adolescents from age 12 to less than 20 years who have committed a criminal act in a prefecture are detained for a maximum of eight weeks until a court hearing and for the purpose of evaluating the individual and deciding a future educational plan. The homes are administered by the Correction Bureau of the Ministry of Justice in Japan.

Individuals who are detained at any juvenile detention home in Japan take a test battery which is prescribed by the Correction Bureau to assess their abilities and needs within three days when they enter a home. If it is determined that additional tests need to be performed, each home can perform them at its discretion. The new Tanaka B Intelligence Scale, some personality tests and so on are contained in the battery. It is conducted in a party of three to fifteen people. The home in question performs the Wechsler Intelligence Scale in addition to the battery at its own discretion, because most tests in the battery have not been standardized, yet. The WISC-III was performed individually by a psychologist who was not same tester who examined the new Tanaka B Intelligence Scale between the day after the group test and a judgment. Motivations of the cases for all the tests (group tests and individual tests) were high, because their attitude during the tests is reflected in their court.

Although some subjects had multiple admissions to the juvenile detention home, testing was not performed on the second admission or thereafter. In other words, no subject was enrolled in the study more than once.

The mean age of the subjects was 15.2 (SD 0.7) years, with a range of 14.0 years to 16.8 years; 77 (95.1%) subjects were male, and four (4.9%) were female. Regarding intellectual development, the mean WISC-III FIQ was 76.5 (SD 15.0), with a range from 51 to 127. There were 58 individuals who had an FIQ<85 (71.6%), of which 26 individuals had an FIQ<70 (32.1%). However, no subject had been detected having a deficit in intellectual function prior to testing. There were absolutely no subjects who received special services to aid intellectual development. Moreover, as well as 26 out of 81 cases having ID (FIQ less than 70), 5 cases had *Attention Deficit/Hyperactivity Disorder*, and 1 each respectively exhibited *Pervasive Developmental Disorder - Not Otherwise Specified*, *Conduct Disorder*, and *Somatoform Disorder*. There were no individuals who had more than two diagnoses. These diagnoses were

Table 1. Mean IQs and Intraclass correlation coefficients between BIQ and each of the WISC-III IQs.

	Mean (S.D.)	ICC between BIQ and each IQ
FIQ	76.5 (15.0)	0.83
VIQ	79.0 (14.2)	0.72
PIQ	78.8 (15.7)	0.81
BIQ	78.5 (16.9)	–

Notes. ICC, intraclass correlation coefficient; BIQ = The new Tanaka B Intelligence Scale IQ; FIQ = Full IQ; VIQ = Verbal IQ; PIQ = Performance IQ.
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determined based on the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, by experienced child psychiatrists.

Statistical Analysis

Power calculation. Power analysis was performed to establish the power needed to interpret the results for the present study. A SD of 15 was estimated for both the new Tanaka B Intelligence Scale IQ (BIQ) and the FIQ. Power was calculated using a 95% confidence interval (±5) of the difference between the two intelligence tests.

Internal consistency. To assess the internal consistency of the new Tanaka B Intelligence Scale, Cronbach's alpha coefficient was calculated for the seven subtest items, including mazes, calculating cubes, replacing figures and numbers, difference discrimination, completing a number series, erasing figures, and completing figures.

Accuracy of the BIQ score for the FIQ score. The BIQ and FIQ scores were plotted, and the differences between them were determined. To assess deviation and accuracy of the BIQ for the FIQ, mean percentage error (MPE) and root mean squared error (RMSE) were calculated [24].

Validity and clinical utility. To assess concurrent validity of the new Tanaka B Intelligence Scale with the WISC-III, the IQ scores on both tests were examined using a one-way analysis of variance intraclass correlation coefficient (ANOVA ICC).

Next, the performance of the new Tanaka B Intelligence Scale as a screening scale was evaluated using receiver operating characteristic (ROC) curve analysis. Areas under the ROC curve (AUC) and their 95% confidence intervals (95% CIs) were calculated using the parametric method. In addition, the likelihood and post-test probability for detection of FIQ<70 for each BIQ stratum were calculated using the stratum-specific likelihood ratio (SSLR) [25].

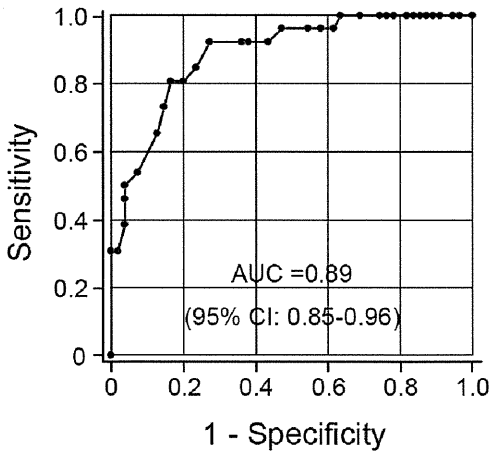

Figure 2. Receiver operating characteristic curves for BIQ for intellectual disability according to the WISC-III. Notes: AUC, area under the curve; 95% CI, 95% confidence interval.
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The SSLR indicates the odds ratio and is calculated as the "proportion of persons with a positive test among those with a disorder" divided by the "proportion of persons with a negative test among those without the disorder." The SSLR for each stratum is calculated as follows: $SSLR = (n_{1\,g}/N_1)/(n_{0\,g}/N_0)$, where $n_{1\,g}$ is the weighted number of subjects with the disorder in the g^{th} stratum, N_1 is the weighted total number of subjects with the disorder, $n_{0\,g}$ is the weighted number of subjects without the disorder in the g^{th} stratum, and N_0 is the weighted total number of subjects without the disorder. The post-test probability is a function of the SSLR, pretest odds, and post-test odds and is calculated as follows: pretest odds × SSLR = post-test odds, and post-test probability = (post-test odds)/(1+ post-test odds) [26]. Therefore, if the SSLR = 1, then the discrimination accuracy of the test is equal to chance probability. The closer the SSLR is to greater than one, the higher the likelihood of having a disorder. The closer the SSLR is to less than one, the lower the likelihood of having a disorder.

The validity of a test has traditionally been assessed using a single cut-off point approach in terms of sensitivity and specificity. A drawback in this case is that values not meeting the cut-off point, even values of results obtained as continuous variables, are treated uniformly regardless of magnitude. When using the SSLR for values of results obtained as continuous variables, the values of the results are stratified, and the probability of a disorder within each stratum can be calculated.

Ethical Considerations

The protocol of this study was approved by the Ethics Committees of the Japanese Association of Correctional Medicine and the Nagoya University Graduate School of Medicine, and the study itself was conducted in conformity with the established ethical standards of all institutions. All cases involved in this study had come out of the home. Furthermore, all of the data which was used in this study was clinical data obtained conventionally during the course of considering diagnosis and treatment, and we used it secondarily and retrospectively. Therefore, the requirement for informed consent was waived. Cooperation in the study placed no burden on individual cases. Personal information regarding subjects in this study and the resulting data were rendered anonymous, and analyses were performed using only quantitative data that could not be linked to any particular subject.

Table 2. Stratum-specific likelihood ratios and posttest probabilities of the BIQ for FIQ<70.

BIQ Stratum	Subjects		SSLR (95% CI)	Posttest probability
	FIQ≥70	FIQ<70		
51–60	1	8	16.9 (3.2–90.2)	0.89
61–65	1	5	10.6 (1.9–60.6)	0.83
66–70	7	8	2.4 (1.0–5.8)	0.53
71–75	6	3	1.1 (0.3–3.6)	0.33
76–85	19	1	0.1 (0.02–0.6)	0.05
≥86	21	1	0.02 (0.02–0.5)	0.05

Notes. SSLR, Stratum-specific likelihood rations; 95% CI, 95% confidence interval; BIQ = The new Tanaka B Intelligence Scale IQ; FIQ = Full IQ.
doi:10.1371/journal.pone.0100262.t002

Results

Power Calculation and Internal Consistency

The power for the present study including the 81 subjects was 0.85. Cronbach's alpha coefficient for the seven BIQ subtest items was $\alpha = 0.86$, indicating high internal consistency.

Accuracy of the BIQ Score for the FIQ Score

Figure 1 shows the distributions of the FIQ and BIQ scores. The mean FIQ was 76.5 (SD 15.0), while the mean BIQ was 78.5 (SD 16.9). The mean difference was 2.0 (SE 1.0, 95% CI −4.1–0.1). The MPE ± SE was 0.03±0.01, and the RMSE was 0.13. There was little deviation of the BIQ from the FIQ.

Validity and Clinical Utility

ANOVA ICC. The ANOVA ICC between the BIQ and FIQ was very high (0.83). Additionally, the ICC between BIQ with both the WISC-III verbal IQ (VIQ) and the performance IQ (PIQ) were also high (correlation coefficients: ICC = 0.72 and 0.81, respectively). Neither the VIQ alone nor the PIQ alone was predominantly reflected (see Table 1).

ROC analysis and the SSLRs. ROC analysis showed that the BIQ enabled screening of FIQ<70 with a high discrimination ability for FIQ<70 (area under the curve (AUC) = 0.89, 95% CI: 0.85–0.96) (see Fig. 2).

Next, the SSLRs were calculated. For the BIQ 51–60 stratum and the BIQ 61–65 stratum, the SSLRs were ≥10 (post-test probability for each stratum: 89%, 83%, respectively). Thus, individuals who have a deficit in intellectual function could be identified as possible. In addition, for the BIQ 76–85 stratum and the BIQ≥86 stratum, the SSLRs were approximately 0.1 (post-test probability of both strata: 5%). Thus, individuals who have a deficit in intellectual function could also be ruled out. For the BIQ 66–70 and 71–75 strata, the SSLRs were 2.4 and 1.1, respectively; the post-test probabilities were 33% and 53%, respectively (see Table 2). In the BIQ≤65 group overall, the SSLR was 13.8 (95% CI: 3.9–48.9, post-test probability: 87%); in the BIQ≥76 group overall, the SSLR was 0.11 (95% CI: 0.03–0.4, post-test probability: 5%).

Discussion

The new Tanaka B Intelligence Scale, an intelligence test that can be administered on groups, has been shown to have high split-half correlations, test-retest reliability, and concurrent validity with academic performance. However, there has not been enough information about this test for use as a standardized intelligence test. To standardize the new Tanaka B Intelligence Scale, the present study examined the reliability of the test and its concurrent validity with the WISC-III, an already established and standardized test for individual testing. Additionally, the clinical utility of the new Tanaka B Intelligence Scale was considered. Using the new Tanaka B Intelligence Scale in subjects aged ≥14 years old, there was high internal consistency and concurrent validity with the WISC-III. This demonstrated that, even in settings where performing individual intelligence tests (e.g. the WISC-III) is difficult, the new Tanaka B Intelligence Scale, a group intelligence test can be easily performed, can assess overall intellectual development and become one of the alternative to an individual test such as the WISC-III.

With an IQ score of the new Tanaka B Intelligence Scale≤65, the SSLR was ≥10 (post-test probability: 87%), and in the BIQ≥76 strata, the SSLRs were approximately 0.1 (post-test probability: 5%). Therefore, individuals who have FIQ<70 could be ruled in or out. In other words, ID can be efficiently diagnosed using detailed intelligence tests in individuals with a BIQ range of 66–75. Thus, this may be a useful test to easily screen for ID in the future.

The new Tanaka B Intelligence Scale can be administered on groups within a short period of time, with no special equipment or training required. Therefore, it can be performed in a variety of settings, enabling expanded assessment of intellectual development, even in locations where administering the WISC-III has previously been difficult. Furthermore, the verbal exchanges are simple instructions, and no complex interaction is required. Additionally, testing can be conducted on individuals with various linguistic backgrounds and verbal levels. In the present study, aside from a single cut-off point, by calculating SSLRs, predicted post-test probability for the results obtained could be determined. This point is important and significant in terms of clinical usefulness.

The SD of the FIQ for subjects in the present study was 15.0, so that the overall variation was normal. The range in intelligence was an FIQ of 51–127, thus covering the approximate strata for the general intelligence category and the category requiring an estimate of deficit in intellectual function. Furthermore, there was little work-up bias or spectrum bias in the juvenile detention home. However, the mean IQ was low, at 76.5±15.0, and 32.1% of the sample had an FIQ<70. The mean IQ of residents in juvenile correctional systems is lower than the IQ in the general public [27–33], which probably had an effect. However, none of the subjects had moderate to severe deficit in intellectual function, such as an FIQ≤50. Therefore, although there was some sample bias, many subjects had an IQ near the borderline for deficit in intellectual function, which was also an advantage in this study. Future studies should include a broader range of subjects. In

addition, 95.1% of the subjects were male, therefore future studies may also want to investigate the influence of sex. However, previous studies have reported that sex differences in VIQ, PIQ, and FIQ were negligible in Japanese and American samples [34].

In this study, the ratios of individuals who were diagnosed as having psychiatric disorders other than ID; such as Attention Deficit/Hyperactivity Disorder, were not significantly high compared with prevalence of these disorders in the general population. Therefore, sample bias on this point might be negligible. On the other hand, in terms of delinquent behavior, there might be sample bias, because most cases in this study conducted, or were entangled in, a delinquent act.

In conclusion, this study demonstrated sufficient reliability and concurrent validity of the new Tanaka B Intelligence Scale, a group intelligence test. In addition, the clinical utility of the scale in screening for individuals who have a deficit in intellectual function was also demonstrated. The validity of this test should be further evaluated within a broader setting including a wider range of subjects, for example, using a randomized sample of the general population. Additionally, the new Tanaka B Intelligence Scale may be performed on many different cultures, since it is easy to conduct, has simple instructions, and is not influenced by strong barriers to language. It is hoped that the present study's results contribute to the proper assessment of intellectual development as well as specialized and effective care and services based on the current findings.

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Author Contributions

Conceived and designed the experiments: YU HM YI. Performed the experiments: YU HM YI. Analyzed the data: YU RY MA NO. Contributed reagents/materials/analysis tools: YU HM YI. Wrote the paper: YU HM RY YI MA 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 ($\chi^2 = 1.00$, $p = 0.317$).

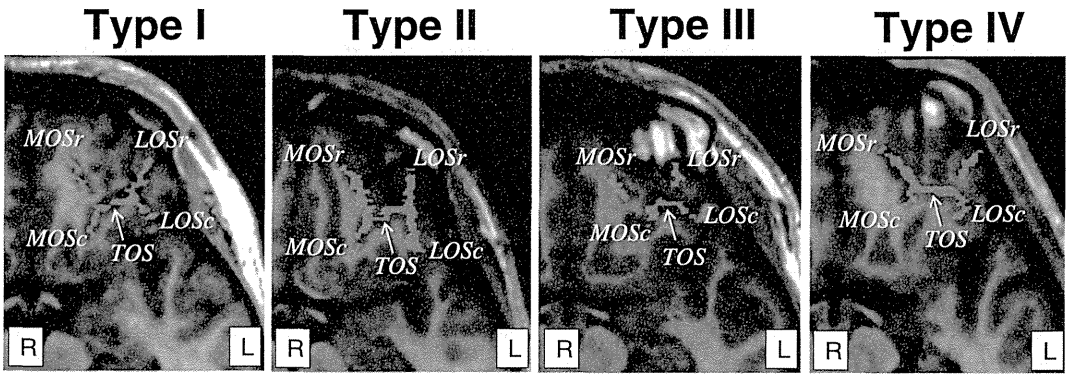


Fig. 1. Classification of the orbitofrontal sulcogyral pattern on an axial view parallel to the anterior commissure–posterior commissure line. Note that these sulci were identified using orthogonal views in three directions and colored on consecutive coronal slices. c, caudal portion; LOS, lateral orbital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

Table 2
Distribution of OFC the sulcogyral pattern in schizophrenia patients and healthy controls.

	Schizophrenia (N = 72)	Controls (N = 86)	Past controls[a] (N = 100)	χ^2	p
	N (%)	N (%)	N (%)		
Left hemisphere				6.12	0.106
Type I	29 (40.3)	49 (57.0)	47 (47.0)		
Type II	11 (15.3)	12 (14.0)	35 (35.0)		
Type III	32 (44.4)	24 (28.0)	18 (18.0)		
Type IV	0 (0)	1 (1.2)	0 (0.0)		
Right hemisphere				9.76	0.021
Type I	40 (55.6)	64 (74.4)	63 (63.0)		
Type II	6 (8.3)	9 (10.5)	27 (27.0)		
Type III	25 (34.7)	13 (15.1)	10 (10.0)		
Type IV	1 (1.4)	0 (0)	0 (0.0)		

[a] Distribution of OFC pattern in previously-reported healthy controls [combined sample of Chiavaras and Petrides (2000) and Nakamura et al. (2007)] is shown here for reference.

replicated inter-individual variability in the OFC sulcogyral pattern in healthy subjects and alteration in its distribution in schizophrenia. Given that the OFC H-shaped sulcus develops predominantly during the gestational period from 28 to 44 weeks (Chi et al., 1977; Kringelbach and Rolls, 2004), the present and previous MRI findings suggest neurodevelopmental insults, such as impairment of axon elongation in the OFC (Sekiguchi et al., 2011), occur during the mid-late gestational period in schizophrenia. It is hypothesized that such an early neurodevelopmental lesion renders the brain vulnerable to anomalous post-pubertal neurodevelopmental processes, as indicated by evidence for accelerated gray matter loss and aberrant connectivity particularly in prefrontal regions, and that these anomalous neurodevelopmental processes interact with other causative factors associated with the onset of psychosis (e.g., stress or other environmental factors) (Pantelis et al., 2005).

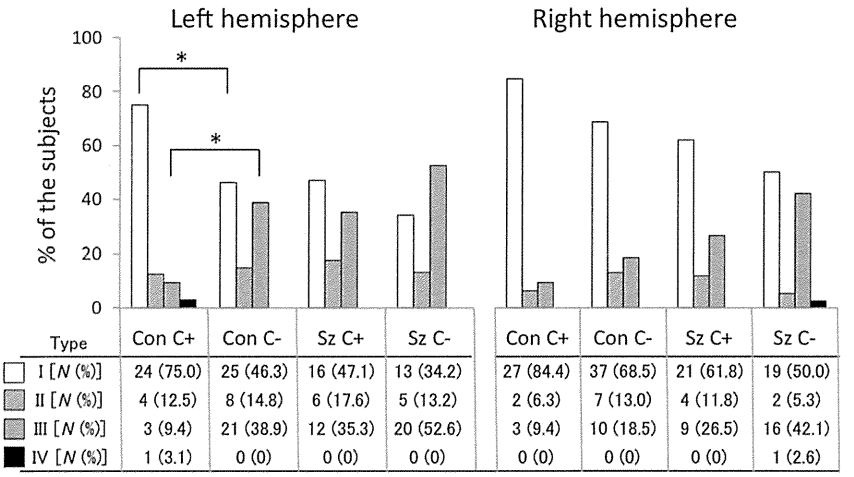
One major finding of this study was the significant effect of the *YWHAE* genotype on the left OFC sulcogyral pattern, especially for healthy subjects. *YWHAE* is a gene encoding 14-3-3epsilon, one of the *DISC1*-interacting molecules that play a crucial role in neuronal development via transport of the NudE-like (*NUDEL*)/lissencephaly-1 (*LIS1*) complex (Taya et al., 2007; Toyo-oka et al., 2003). The exact mechanism of development of the OFC sulcogyral pattern remains unclear, but the gross cortical folding pattern 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). Several MRI studies in mono- and dizygotic twins support the notion that cortical folding is also influenced by non-genetic factors (Hasan et al., 2011; Zilles et al., 2013). However, taken together with animal data that genetically modified 14-3-3epsilon-deficient mice showed decreased dendritic spine density and impairment of the local neuronal network in the OFC (Sekiguchi et al., 2011), our results suggest that the genotype variation of 14-3-3epsilon could significantly affect the processes involved in neuronal development related to cortical folding patterns in the orbitofrontal region. Furthermore, the significant relation between the Type I pattern and protective C allele of *YWHAE* in this study may partly support the hypothesis by Bartholomeusz et al. (2013) that the Type I pattern is associated with more efficient neural organization in the OFC, and this may potentially be linked to better axonal connectivity with other brain regions and more efficient processing.

Table 3
Distribution of the OFC sulcogyral pattern in subjects with and without the *YWHAE C allele*.

	C allele carriers (N = 66)	G homozygotes (N = 92)	χ^2	p
	N (%)	N (%)		
Left hemisphere			9.49	0.024
Type I	40 (60.6)	38 (41.3)		
Type II	10 (15.2)	13 (14.1)		
Type III	15 (22.7)	41 (44.6)		
Type IV	1 (1.5)	0 (0)		
Right hemisphere			3.18	0.365
Type I	48 (72.7)	56 (60.9)		
Type II	6 (9.1)	9 (9.8)		
Type III	12 (18.2)	26 (28.3)		
Type IV	0 (0)	1 (1.1)		

On the other hand, we did not find a genotype effect of *YWHAE* on the OFC pattern specific to schizophrenia, although genetic and expression evidence (Ikeda et al., 2008), as well as animal studies (Ikeda et al., 2008; Sekiguchi et al., 2011), have implicated its role as a susceptibility gene related to the prefrontal pathology of schizophrenia (Goldman-Rakic, 1994). Several MRI studies have demonstrated that individuals at increased genetic risk of schizophrenia at least partly share abnormal frontal cortical folding, including an altered OFC pattern (Chakirova et al., 2010), with patients with schizophrenia (Falkai et al., 2007; Harris et al., 2004, 2007; Jou et al., 2005). Furthermore, the structural stability of cortical folding is generally archived soon after birth (Armstrong et al., 1995) and is independent of regional volumetric changes (Nakamura et al., 2008; Takayanagi et al., 2010), whereas dynamic brain changes, including excessive cortical thinning (van Haren et al., 2011) or gray matter reduction (Mane et al., 2009) over time in the frontal area, may occur during early phases of schizophrenia (Pantelis et al., 2007). All of this neuroimaging evidence implies that disturbed frontal gyrification may represent a static endophenotypic risk marker of schizophrenia. The current findings suggest that the *YWHAE* genotype effect alone is not likely to explain the altered OFC sulcogyral pattern in schizophrenia. However, 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, 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 polymorphism in one of the *DISC1*-interacting molecules in a relatively small sample. Although we found a significant *YWHAE* genotype effect only on the left OFC sulcogyral pattern in healthy subjects, a non-significant but similar effect of the protective C allele (increased Type I and decreased Type III expression) was also observed in schizophrenia (Fig. 2). Thus, the potential role of genetic variation in *DISC1*-interacting molecules and their interaction with other genetic/non-genetic factors should be further tested in larger cohorts. Second, the current study cannot address the disease specificity of our OFC findings. An altered orbitofrontal sulcogyral pattern (increase of Type III) has been also reported in autism spectrum disorders (Watanabe et al., in press) and a genome-wide analysis has shown that specific SNPs are associated with a range of psychiatric disorders (Cross-Disorder Group of the Psychiatric Genomics Consortium and Genetic Risk Outcome of Psychosis, GROUP Consortium, 2013). Finally, we examined schizophrenia patients with an



Type	Con C+	Con C-	Sz C+	Sz C-	Con C+	Con C-	Sz C+	Sz C-
I [N (%)]	24 (75.0)	25 (46.3)	16 (47.1)	13 (34.2)	27 (84.4)	37 (68.5)	21 (61.8)	19 (50.0)
II [N (%)]	4 (12.5)	8 (14.8)	6 (17.6)	5 (13.2)	2 (6.3)	7 (13.0)	4 (11.8)	2 (5.3)
III [N (%)]	3 (9.4)	21 (38.9)	12 (35.3)	20 (52.6)	3 (9.4)	10 (18.5)	9 (26.5)	16 (42.1)
IV [N (%)]	1 (3.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2.6)

Fig. 2. Distribution of the orbitofrontal sulcogyral pattern in each diagnostic group. C+, subjects with C allele; C−, subjects without C allele; Con, controls; Sz, schizophrenia. *$p < 0.01$.

illness duration of approximately 5 years in this study. Illness chronicity (Haijma et al., 2013) and medication with antipsychotics (Andreasen et al., 2013; Lieberman et al., 2005; Moncrieff and Leo, 2010) can significantly affect brain morphology. Although there was no difference in these variables between the patients with and without the C allele (Table 1) and gross cortical folding patterns remain rather stable throughout life in healthy subjects (Armstrong et al., 1995; Magnotta et al., 1999), the present findings should be replicated using patients at early illness stages and in un-medicated patients.

5. Conclusion

The present study replicated an altered sulcogyral pattern of the OFC in schizophrenia and further suggested that genotype variation in *YWHAE* may be related to the development of cortical folding patterns in the orbitofrontal region. Although we did not observe a genotype effect of *YWHAE* on the OFC pattern specific to schizophrenia, our findings support the possible role of the OFC sulcogyral pattern as an endophenotype for future genetic studies of schizophrenia.

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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

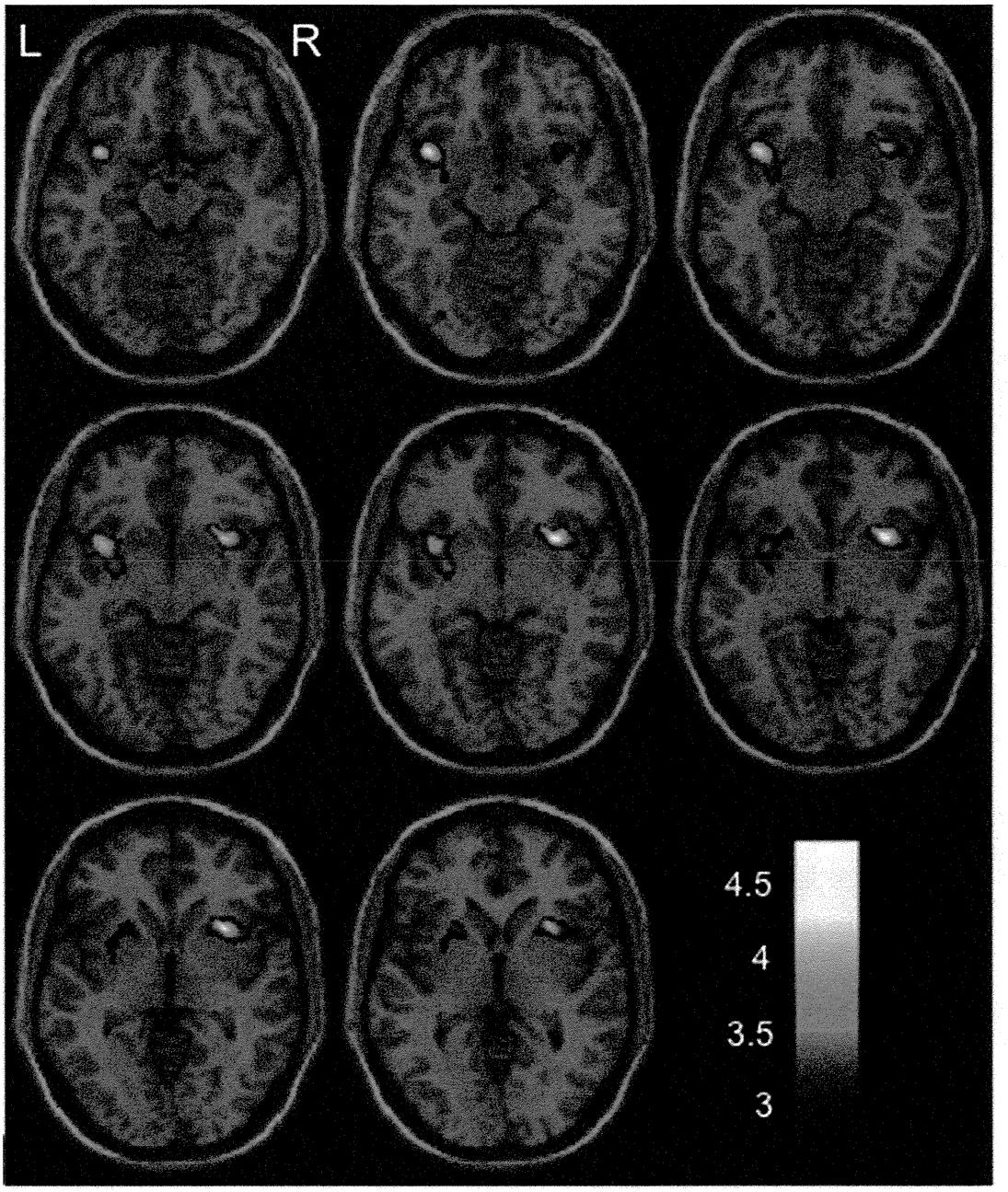

Figure 1. The *YWHAE* (*rs28365859*) genotype-by-diagnosis interaction on gray matter volume. The regions showing interaction in all subjects are displayed by a hot colormap. The color bar shows t values corresponding to the color in the figure.
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Hardy–Weinberg 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

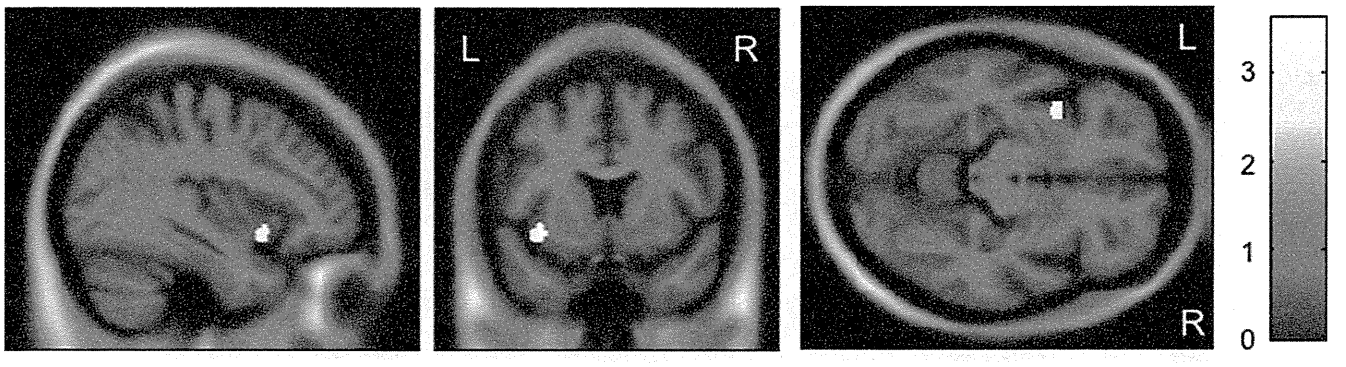

Figure 2. Impact of the *rs28365859* genotype on gray matter volume of left insula in schizophrenia. Age, sex, illness duration, and medication dose were used as covariates. The protective C allele carriers had a significantly larger left insula than the G homozygotes. Anatomical localizations are displayed 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.

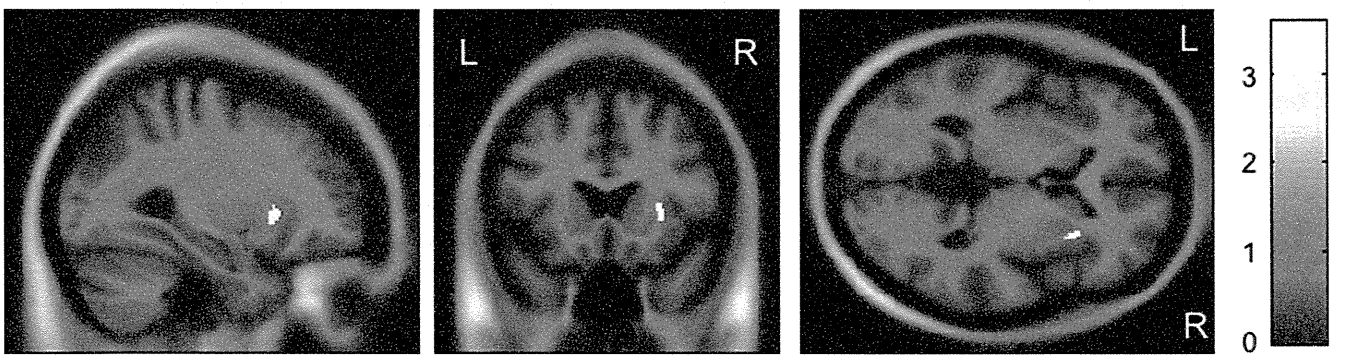

Figure 3. Impact of the *rs28365859* genotype on gray matter volume of the right putamen in healthy controls. The G allele homozygotes had a significantly larger right putamen than the C allele carriers. Anatomical localizations are displayed on the normal template MR images in three directions. The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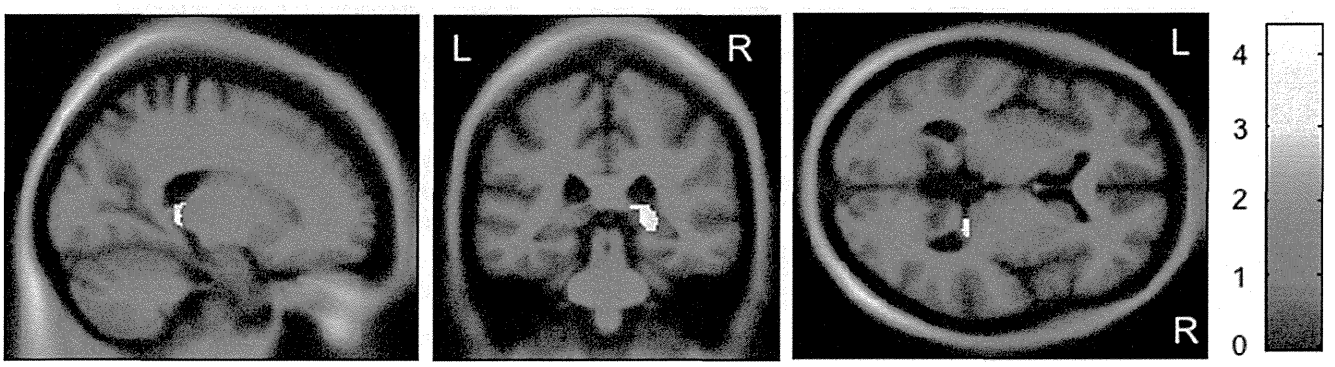

Figure 4. Impact of the *rs28365859* genotype on gray matter volume of the right hippocampus in schizophrenia. Age, sex, illness duration, and medication dose were used as covariates. The protective C allele carriers had a significantly larger right hippocampus than the G allele homozygotes. Anatomical 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.
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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.
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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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RESEARCH ARTICLE Open Access

Social insecurity in relation to orbitofrontal activity in patients with eating disorders: a near-infrared spectroscopy study

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Abstract

Background: Functional neuroimaging techniques are widely used to elucidate changes in brain activity, and various questionnaires are used to investigate psychopathological features in patients with eating disorders (ED). It is well known that social skills and interpersonal difficulties are strongly associated with the psychopathology of patients with ED. However, few studies have examined the association between brain activity and social relationships in patients with ED, particularly in patients with extremely low body weight.

Methods: In this study, 22-channel near-infrared spectroscopy was used to quantify regional hemodynamic changes during a letter fluency task (LFT) in 20 female patients with ED with a mean body mass index of 14.0 kg/m^{2}and 31 female controls (CTLs). Symptoms were assessed using the Eating Disorder Inventory-2 and Beck Depression Inventory. We hypothesized that frontal activity in patients with ED would be lower than in CTLs and would show different correlations with psychopathological features compared with CTLs.

Results: The LFT performance and score on the social insecurity subscale of the Eating Disorder Inventory-2 were significantly higher in the ED group than in the CTL group. The mean change in oxygenated hemoglobin (oxy-Hb) in bilateral frontal regions during the LFT was significantly smaller in the ED group than in the CTL group. Social insecurity score was positively correlated with the concentration of oxy-Hb in the bilateral orbitofrontal cortex in the ED group but not in the CTL group.

Conclusions: These results suggest that activity of the orbitofrontal cortex is associated with social insecurity and disturbed in patients with ED. Therefore, disturbed orbitofrontal cortex activity may underlie the lack of insight and social isolation that is characteristic of patients with ED.

Keywords: Anorexia nervosa, Extremely low body weight, Near-infrared spectroscopy, Social isolation

Background

Anorexia nervosa (AN) is an eating disorder (ED) characterized by food restriction, inappropriate eating habits, obsession with having a thin figure, an irrational fear of weight gain, and a distorted body self-perception [1]. AN is increasingly recognized as a serious disease that affects many young individuals. However, its etiology is complex and treatment effect is limited [1]. Problems with homeostasis, drive, and self-regulation are biological factors that are known to be associated with AN [1]. Although findings of magnetic resonance imaging studies of subjects with AN are inconsistent [2], other functional neuroimaging modalities, including single-photon emission tomography, positron emission tomography (PET), and functional magnetic resonance imaging (fMRI), suggest that patients with AN exhibit functional abnormalities in the frontal, parietal, and cingulate cortices [3-5]. In addition, a recent fMRI study in an adolescent population reported that activation of the medial prefrontal cortex during performance of a theory of mind task was lower in patients with AN than in controls (CTLs) [6]. These results

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