ELECTROENCEPHALOGRAM RECORDING

Electroencephalograms (EEGs) were recorded based on the previous report from our laboratory (Sumiyoshi et al., 2006, 2009; Kawasaki et al., 2007a; Higuchi et al., 2008, 2010, 2013a,b; Itoh et al., 2011).

A 32-channel DC-amplifier (EEG-2100 version 2.22J, Nihon Kohden Corp., Tokyo, Japan) was used. Recordings were performed using an electro cap (Electrocap Inc., Eaton, OH) in a sound-attenuated room. Data were collected with a sampling rate of 500 Hz. EEG data were collected from 29 scalp electrodes (Fp1, Fp2, F3, F4, F7, F8, FC3, FC4, C3, C4, T3, T4, CP3, CP4, TP7, TP8, P3, P4, T5, T6, O1, O2, FPz, Fz, FCz, Cz, CPz, Pz, and Oz according to the extended International 10–20 system). All electrodes were referred to the average amplitude of the ear electrodes (bandwidth = 0.53–120 Hz, 60 Hz notch filter). Electrode impedance was <5 kΩ.

Measurements of dMMN/P3a/RON complex were based on our previous report (Higuchi et al., 2010). One-thousand auditory stimuli were delivered binaurally through headphones with interstimulus intervals 500 ms. Standard/target tones of 50/100 ms duration were randomly presented with the presentation probability of 0.9/0.1. All tones were 60 dB, 1000 Hz, and with a rise–fall time of 10 ms. The subjects were requested to watch silent animation movie (Tom and Jerry) and pay attention to the monitor and ignore the tones.

Averaging of ERP waves and related procedures were performed using Vital Tracer and EPLYZER II software (Kissei Comtec, Co. Ltd., Nagano, Japan). Epochs were 600 ms, including a 100 ms prestimulus baseline. Eye movement artifacts (blinks and eye movements) were manually rejected. MMN waveforms were obtained by subtract standard waveforms from target ones. MMN, P3a, and RON peaks were identified within the 150–250 ms (minus peak), 200–350 ms (plus peak), and 250–500 ms (minus peak) search windows, respectively.

STATISTICAL METHODS

Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) version 20 (SPSS Japan Inc., Tokyo, Japan). We performed comparison of age between four groups (HC, ARMS, FES, and CS) by one-way analysis of variance. Onset age and duration of illness of two schizophrenia groups (first-episode and chronic) were compared by independent *t*-test. Drug dose, SAPS, and SANS score among three groups (ARMS, FES, and CS) were analyzed by one-way ANOVA.

Event-related potential amplitudes and latencies were measured and analyzed at five electrodes; three from frontal lobe (F3, F4, and Fz), and two from midline (Cz and Pz). They are typical electrodes that commonly used on ERP studies. MMN amplitudes are generally largest at frontal electrodes, so we choose three electrodes from frontal lobe. Moreover, grand average waveforms (**Figures 1** and **3**) and scatterplots (**Figures 2** and **4**) were drawn and analyzed by Fz lead as a representative of electrodes because amplitudes ERPs of Fz were largest. Laterality of ERPs was analyzed by F3/F4 comparison as we performed in previous report (Higuchi et al., 2008), but there were no difference in this study (data not shown).

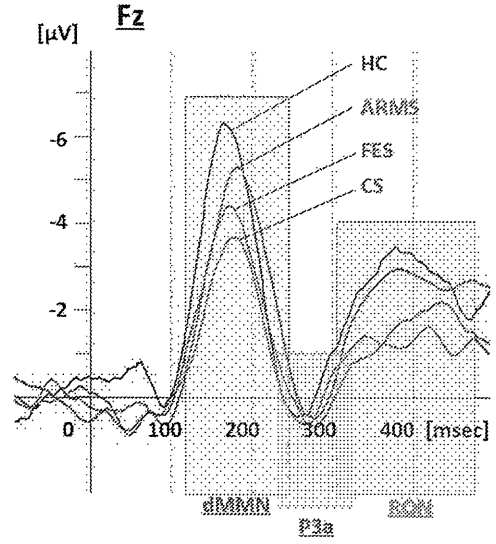

FIGURE 1 | Duration mismatch negativity (dMMN), P3a, and reorienting negativity (RON) complex waveforms at Fz for all subjects. Waveforms are presented for healthy controls (HC, blue line), at-risk mental state (ARMS, red line), first-episode schizophrenia (FES, light green line), and chronic schizophrenia (CS, dark green line).

Two-way ANOVA was conducted on amplitudes and latencies of dMMN, P3a, and RON, with "Stage" (HC, ARMS, FES, and CS) and "Lead" (F3, F4, Fz, Cz, and Pz) as fixed factors. Main effects (of Stage and Lead) were described on **Table 1B** (significant differences were seen in all leads of dMMN amplitude and F4/Fz of RON amplitude). The Stage-by-Lead interactions on amplitudes (dMMN, $F = 1.172$, $p = 0.30$; P3a, $F = 0.511$, $p = 0.90$; RON, $F = 1.024$, $p = 0.42$) and latencies (dMMN, $F = 1.254$, $p = 0.246$; P3a, $F = 1.475$, $p = 0.13$; RON, $F = 0.516$, $p = 0.904$) were not significant.

Gender difference between Conv and Non-C were analyzed by Chi-square test. Other factors (age, drug dose, SAPS, SANS, ERP amplitude, and latency) of them were calculated by independent *t*-test. All analyses of variance were corrected by Bonferroni correction.

Correlations of symptoms and ERP amplitudes were performed by Pearson product–moment correlation coefficient. SAPS scores (hallucinations, delusions, bizarre behavior, and positive formal thought disorder) and SANS scores (affective flattening/blunting, alogia, avolition-apathy, anhedonia-asociality, and attention) were used.

Raters were not informed of subjects' profiles and diagnosis.

RESULTS

SUBJECTS' PROFILE

Demographic and clinical data of participants are shown in **Tables 1A** and **2**. There was significant group difference in age [$F(3,74) = 4.94$, $p = 0.004$, ANOVA], and Conv subjects were older than Non-C in age ($p = 0.009$, *t*-test). Male/female ratio did not differ between Conv. and Non-C groups [$\chi^2 = 2.47$, $p = 0.3$, Chi-square test].

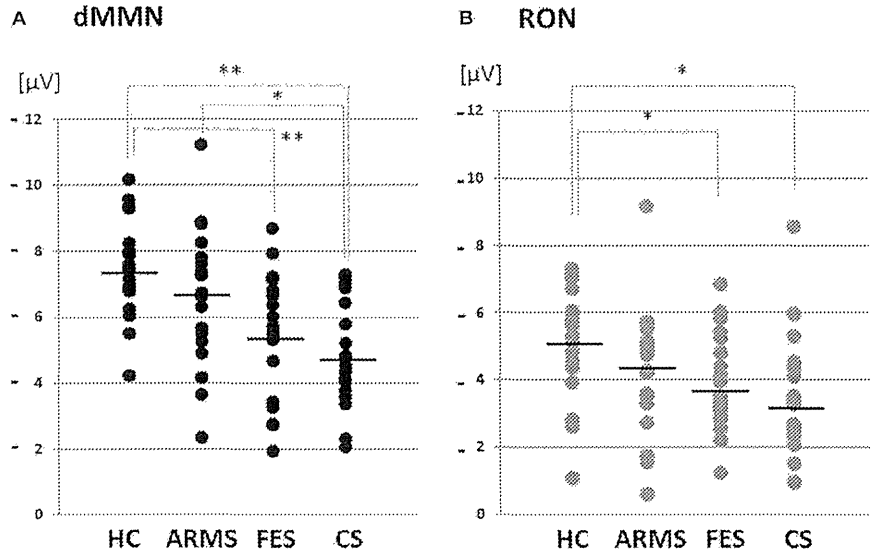

FIGURE 2 | Distribution of amplitudes of duration mismatch negativity [dMMN; (A)] and reorienting negativity [RON; (B)] at Fz for all subjects. Data are presented for healthy controls (HC), ARMS, first-episode schizophrenia (FES), and chronic schizophrenia (CS). $*p < 0.05$, $**p < 0.01$.

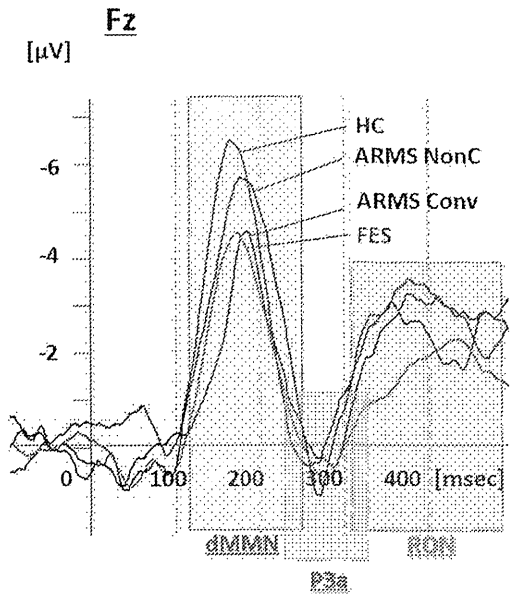

FIGURE 3 | Duration mismatch negativity (dMMN), P3a, and reorienting negativity (RON) complex waveforms at the Fz lead. Waveforms are presented for healthy controls (blue line), ARMS Converters (Conv), and non-Converter (Non-C) (black lines), and FES (green line).

Sixteen out of 19 ARMS subjects were not taking any medication, while three were prescribed a small dose of risperidone (1.5 mg/day), aripiprazole (3 mg/day), and sulpiride (150 mg/day), respectively, for acute psychosis episodes (sometimes with strong agitation), based on the criteria of International Early Psychosis Association Writing Group (2005). MMN recordings for these subjects were conducted immediately after medications were started (9, 15, and 27 days). Two out of the three subjects subsequently developed schizophrenia. Thirteen out of 19 FES patients and 15 out of 19 CS patients were taking antipsychotic medications. There were no significant differences among ARMS, FES, and CS groups in SAPS [$F(2,56) = 2.29$, $p = 0.11$, ANOVA] and SANS [$F(2,56) = 0.52$, $p = 0.59$, ANOVA] scores. Conv and Non-C groups did not differ in the SAPS and SANS scores at baseline ($p = 0.08$, 0.24, respectively, t-test).

COMPARISONS OF ERP BETWEEN HEALTHY CONTROLS VS. ARMS VS. SCHIZOPHRENIA

Grand average ERP waveforms in the Fz lead following deviant stimulation are shown in **Figure 1**. Scatterplots of dMMN and RON amplitudes at Fz lead are shown in **Figures 2A,B**, respectively. P3a did not show any statistical differences so we skipped making scatterplot of P3a. ARMS subjects showed relatively smaller dMMN amplitudes at Fz ($-6.5 \pm 2.0\,\mu V$) compared to those of healthy control subjects ($-7.4 \pm 1.4\,\mu V$), which was not statistically significant ($p = 0.13$, t-test). On the other hand, FES group showed significantly smaller dMMN amplitudes at Fz ($-5.4 \pm 1.9\,\mu V$) compared to healthy control ($p = 0.001$, t-test). Patients with CS showed greater amplitude reductions at Fz ($-4.8 \pm 1.5\,\mu V$) compared to healthy controls ($p = 0.000004$, t-test).

At-risk mental state subjects showed relatively smaller RON amplitudes at Fz ($-4.2 \pm 1.8\,\mu V$) than healthy controls ($-5.1 \pm 1.6\,\mu V$), which was not significant ($p = 0.15$, t-test). On the other hand, FES group showed significantly smaller RON amplitudes at Fz ($-3.9 \pm 1.4\,\mu V$, $p = 0.02$). Patients with CS also elicited significantly smaller RON amplitudes at Fz ($-3.4 \pm 1.7\,\mu V$) compared to healthy controls ($p = 0.005$, t-test).

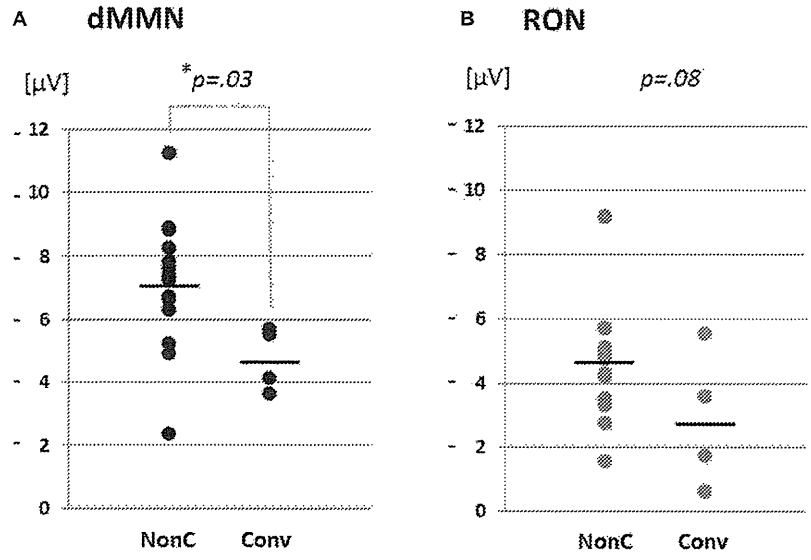

FIGURE 4 | Distribution of amplitudes of duration mismatch negativity [dMMN; (A)] and reorienting negativity [RON, (B)] at Fz for ARMS. Data are presented Converters (Conv) and Non-Converter (Non-C). $*p < 0.05$.

Latencies of dMMN, P3a, and RON at any electrodes did not differ among the four groups (see **Table 1B**).

COMPARISONS BETWEEN CONVERTERS VS. NON-CONVERTERS

Grand average ERP waveforms are shown in **Figure 3**. Scatterplots of dMMN and RON amplitudes at Fz lead are shown in **Figures 4A,B**, respectively. P3a did not show any statistical differences so we skipped making scatterplot of P3a. Waveforms of Conv group were similar to those of FES patients. By contrast, waveforms of Non-C subjects resembled to those of healthy controls. Conv subjects showed significantly smaller dMMN amplitudes at Fz and Cz electrodes compared with Non-C subjects ($p = 0.03$, 0.05 by t-test, respectively, **Table 2**). On the other hand, amplitudes of Non-C did not differ from those of HC ($p = 0.51$ at Fz, t-test, data not shown) and there was no significant difference in dMMN amplitudes between Conv and FES subjects ($p = 0.44$ at Fz, t-test, data not shown). In other electrode of Non-C vs. HC and Conv vs. FES comparisons, differences were smaller and did not reach significance.

Conv subjects tended to show smaller RON amplitudes compared to those of Non-C subjects at Fz and F4 electrodes ($p = 0.08$, $p = 0.08$ by t-test, respectively, **Table 2**). Also, HC group showed relatively larger RON amplitudes at Fz lead compared to Conv subjects, which did not reach significant level ($p = 0.08$, t-test, data not shown). No significant differences were found at any electrode between FES vs Non-C groups (data not shown).

Latencies of dMMN, P3a, and RON at any electrodes did not differ between Conv and Non-C groups (see **Table 2**).

RELATIONSHIP BETWEEN SYMPTOMS AND ERPs

We evaluated the correlations between dMMN, P3a, and RON amplitudes and symptoms (SAPS and SANS) in patients (schizophrenia and ARMS, $n = 57$).

Data are shown in **Table 3**. There were significant correlation between attention disorder score (SANS) and dMMN amplitude at Fz and F3 lead ($r = 0.317$; $p = 0.025$, $r = 0.290$, $p = 0.041$, respectively, by Pearson's correlation). Moreover, there were significant correlation between positive formal thought disorder score (SAPS) and RON amplitude at Fz and F3 lead ($r = 0.280$; $p = 0.049$, $r = 0.346$, $p = 0.014$, respectively, by Pearson's correlation). Thus, reduction of ERPs was correlated with severity of some symptoms.

DISCUSSION

Duration mismatch negativity amplitudes at frontal and central leads were reduced in ARMS subjects who later converted to overt schizophrenia in comparison with non-converters and normal subjects, consistent with previous reports (Bodatsch et al., 2011; Shaikh et al., 2012; Higuchi et al., 2013b). Specifically, the current data from gender matched subjects across groups (**Table 1**) confirmed previous observations in patients with variable demographic backgrounds (Bodatsch et al., 2011; Shaikh et al., 2012; Higuchi et al., 2013b). Importantly, this study is the first to suggest that RON provides a marker for the progression to overt schizophrenia in subjects with ARMS, based on longitudinal observations.

Three out of 4 Conv, 7 out of 15 Non-C, 7 out of 19 FES, 5 out of 19 CS, and 9 out of 19 HC subjects overlapped with subjects in our previous report (Higuchi et al., 2013b). We selected subjects for the current study, according to the following considerations; (1) ARMS subjects with a longer followed-up period, (2) gender-match between HC and schizophrenia patients, (3) younger HC and schizophrenia patients than those used in the previous study. The current one used a longer observation period, and was gender-matched across groups with less variation in age. According to a previous report (Yung et al., 2003), 10–40% of ARMS subjects

Table 2 | Comparison between converters and non-converters of ARMS subjects.

	ARMS ($n=19$)		Group comparison (p)
	Non-C ($n=15$)	Conv ($n=4$)	
Male/female	7/8	3/1	$\chi 2=2.47$, $p=0.3$
Age (years)	18.3 (2.2)	23.4 (4.9)	0.009
Drug dose[a]	0.1 (0.2)	0.4 (0.6)	0.12
SAPS	15.3 (7.0)	22.7 (5.8)	0.08
SANS	56.9 (26.3)	73.7 (9.6)	0.24
dMMN amplitude (μV)			
F3	−6.5 (2.1)	−4.9 (0.6)	0.16
F4	−7.0 (2.2)	−4.6 (0.9)	0.06
Fz	−7.0 (2.0)	−4.7 (1.0)	0.03*
Cz	−6.1 (2.1)	−3.7 (0.6)	0.05*
Pz	−3.8 (2.1)	−3.0 (0.4)	0.48
dMMN latency (ms)			
F3	169.3 (18.5)	182.5 (8.2)	0.19
F4	174.2 (20.1)	182.0 (8.1)	0.47
Fz	176.2 (13.6)	181.5 (8.2)	0.47
Cz	180.2 (19.7)	190.0 (13.3)	0.37
Pz	186.8 (25.2)	195.5 (23.2)	0.54
P3a amplitude (μV)			
F3	1.0 (1.4)	1.5 (1.1)	0.60
F4	1.2 (2.0)	1.2 (1.1)	0.96
Fz	1.6 (1.5)	2.0 (1.2)	0.67
Cz	1.9 (1.6)	2.6 (1.4)	0.47
Pz	2.0 (1.4)	0.7 (0.8)	0.10
P3a latency (ms)			
F3	264.7 (27.9)	267.0 (27.9)	0.88
F4	270.1 (31.3)	268.0 (31.3)	0.90
Fz	269.1 (32.3)	267.5 (32.3)	0.93
Cz	264.8 (28.8)	270.5 (28.8)	0.71
Pz	268.4 (29.0)	286.5 (29.0)	0.26
RON amplitude (μV)			
F3	−4.3 (1.7)	−3.1 (1.2)	0.20
F4	−4.5 (1.4)	−3.1 (1.0)	0.08
Fz	−4.6 (1.6)	−2.8 (2.1)	0.08
Cz	−4.2 (2.1)	−2.5 (1.6)	0.16
Pz	−2.7 (1.8)	−2.7 (1.1)	0.97
RON latency (ms)			
F3	388.0 (51.3)	353.5 (33.4)	0.22
F4	403.6 (51.4)	405.5 (72.0)	0.95
Fz	391.3 (44.8)	419.5 (53.8)	0.29
Cz	399.3 (48.6)	394.5 (28.4)	0.85
Pz	401.8 (51.3)	401.2 (33.4)	0.98

Values represent mean (SD).

[a]Risperidone equivalent (mg/day).

ARMS, at-risk mental state; Non-C., ARMS non-Converters; Conv., ARMS Converters; SAPS, Scale for the Assessment of Positive Symptoms; SANS, Scale for the Assessment of Negative Symptoms.

*$p < 0.05$.

later developed schizophrenia, consistent with our observations that 21.0% progressed to the illness.

ARMS subjects as a whole have been reported to demonstrate reduced dMMN amplitudes, but with a lesser degree compared to patients with overt schizophrenia (Bodatsch et al., 2011; Atkinson et al., 2012; Jahshan et al., 2012), consistent with the present results (**Figure 1**). On the other hand, the current data may be partly different from our previous observations indicating the lack of difference in dMMN amplitudes between ARMS subjects as whole and healthy controls (Higuchi et al., 2013b). One of the reasons for this discrepancy may include the difference in age and gender ratio. In fact, as previous reports indicate ERPs amplitudes gradually decrease by age, and male subjects show relatively smaller amplitudes than female because of the difference in skull thickness (Ikezawa et al., 2008; Matsubayashi et al., 2008; Naatanen et al., 2012). Another confounding factor may include the observation periods for follow-up. While our previous report (Higuchi et al., 2013b) employed a relatively short period (mean ± SD = 1.6 ± 0.8 years for non-converters), the present study used a longer period (2.2 ± 1.5 years), similar to those in the literature.

Compared to Non-C, Conv subjects elicited significantly smaller dMMN amplitudes at F4 and Fz leads (**Table 2**). These observations suggest the ability of dMMN amplitudes to differentiate between high-risk individuals who later progress to schizophrenia and those who do not, as has been suggested (Higuchi et al., 2013b; Sumiyoshi et al., 2013).

Little information has been available about the feature of RON in schizophrenia. In this study, RON amplitudes of ARMS subjects as a whole were not different from those of HC subjects, while FES and CS group showed significantly smaller RON amplitudes at Fz and F4 leads compared to the HC group. This finding is consistent with observations by Jahshan et al. (2012). As the results of the current study suggest that RON amplitudes may decrease according to progression of clinical stages of schizophrenia (**Table 1B**; **Figure 1**), they may provide an intermediate phenotype of the illness.

Importantly, RON amplitudes of Conv subjects tended to be smaller than those of Non-C at the Fz and F4 leads (**Figure 4**). The failure to reach statistical significance may be due to the fact that RON waveforms are not stable and smaller compared to dMMN waveforms. Future investigations with a larger number of subjects would be desirable to determine if the combined measurement of RON and dMMN would further facilitate early detection of schizophrenia.

P3a amplitudes were barely detectable in this study (**Figures 1** and **3**). These amplitudes have been reported to be decreased in schizophrenia and ARMS (Friedman et al., 2001; Jahshan et al., 2012; Mondragon-Maya et al., 2013; Nagai et al., 2013). Variations of P3a amplitudes may be large, due, probably, to the difference in measurement.

Limitations of this study include the small sample number, especially in ARMS ($n=19$) and Conv subjects ($n=4$). According to the power analysis, at least 26 patients are needed to obtain adequate effect size (i.e., 0.6). Investigations with a larger number of patients will make the data more satisfactory. Second, significant age difference was seen in the ARMS vs. HC and FES

Table 3 | ERP amplitudes and symptoms.

	SAPS							
	Hallucinations		Delusions		Bizarre behavior		Positive formal thought disorder	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*
dMMN amplitude (μV)								
F3	0.039	0.786	0.011	0.938	−0.122	0.398	0.086	0.552
F4	0.071	0.625	0.066	0.648	−0.131	0.365	0.076	0.599
Fz	0.021	0.884	−0.016	0.910	−0.199	0.166	0.090	0.536
Cz	−0.021	0.888	−0.036	0.805	−0.108	0.457	0.056	0.697
Pz	−0.163	0.258	−0.178	0.216	−0.225	0.117	−0.069	0.636
P3a amplitude (μV)								
F3	−0.148	0.305	−0.188	0.192	−0.188	0.191	−0.036	0.802
F4	−0.075	0.605	−0.190	0.187	−0.256	0.073	0.029	0.842
Fz	−0.191	0.185	−0.181	0.209	−0.233	0.104	−0.008	0.956
Cz	−0.149	0.302	−0.056	0.701	−0.213	0.138	0.020	0.891
Pz	0.022	0.879	0.046	0.753	−0.023	0.874	−0.117	0.417
RON amplitude (μV)								
F3	0.014	0.926	−0.131	0.363	0.067	0.646	0.280	**0.049***
F4	0.087	0.549	−0.092	0.523	−0.158	0.274	0.244	0.087
Fz	−0.024	0.869	−0.109	0.450	−0.265	0.063	0.346	**0.014***
Cz	−0.033	0.818	−0.214	0.136	−0.081	0.578	0.151	0.295
Pz	0.002	0.990	−0.257	0.071	−0.025	0.861	0.022	0.881

	SANS									
	Affective flattening		Alogia		Avolition-apathy		Anhedonia-asociality		Attention	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
dMMN amplitude (μV)										
F3	0.109	0.452	0.149	0.301	0.096	0.509	−0.102	0.483	0.317	**0.025***
F4	0.142	0.325	0.122	0.399	0.016	0.910	−0.066	0.650	0.260	0.068
Fz	0.115	0.427	0.165	0.254	−0.014	0.923	−0.081	0.576	0.290	**0.041***
Cz	0.060	0.680	0.147	0.307	0.117	0.420	0.050	0.730	0.262	0.066
Pz	−0.041	0.778	0.066	0.650	0.122	0.400	−0.063	0.666	−0.007	0.963
P3a amplitude (μV)										
F3	−0.029	0.843	−0.034	0.815	0.037	0.796	−0.037	0.796	0.130	0.368
F4	0.021	0.883	0.021	0.885	0.003	0.984	−0.102	0.480	0.148	0.306
Fz	−0.043	0.767	0.032	0.823	−0.032	0.827	−0.090	0.533	0.101	0.487
Cz	−0.066	0.649	−0.029	0.843	0.012	0.934	−0.010	0.943	0.112	0.441
Pz	0.063	0.662	−0.027	0.852	0.108	0.454	−0.046	0.753	0.032	0.827
RON amplitude (μV)										
F3	−0.112	0.438	−0.111	0.441	−0.054	0.712	−0.215	0.134	−0.055	0.704
F4	0.022	0.882	0.039	0.788	−0.089	0.539	−0.103	0.475	−0.050	0.730
Fz	−0.046	0.752	−0.017	0.905	−0.104	0.474	−0.073	0.617	−0.025	0.861
Cz	0.128	0.375	0.210	0.143	−0.040	0.781	0.002	0.988	−0.108	0.455
Pz	0.014	0.922	0.095	0.513	−0.123	0.393	−0.117	0.419	−0.242	0.090

SAPS, Scale for the Assessment of Positive Symptoms; SANS, Scale for the Assessment of Negative Symptoms.

$p < 0.05$, r = Pearson product–moment correlation coefficient.

vs. CS comparisons. Since part of ARMS subjects is regarded as prodromal state of schizophrenia, it is natural that they are mostly younger than schizophrenia patients. Therefore, adjustment of age between FES/CS and ARMS subjects may increase the number of certain type of schizophrenia, e.g., hebephrenic type. Due to an effort to make the FES/CS groups more homogeneous, patients of these groups became somewhat older than the ARMS group. Application of ANCOVA to 19 members may provide over-adjustment. Although MMN amplitudes are reduced gradually by age, the decline is not substantial (−0.056 μV/year in schizophrenia and −0.079 μV/year in healthy control) (Kiang et al., 2009). ARMS/HC subjects are about 2.5 years younger than FES/CS (**Table 1**). According to this formula, about 0.2 μV amplitude reduction may occur between these two. Differences in our data presented (at Fz lead) were 1.1 μV or greater (ARMS vs. FES groups.), which was sufficiently large. Third, some ARMS subjects and most schizophrenia patients were taking antipsychotic drugs, which may be another limitation of the current study. Fourth, in this study, we measured ERPs at baseline, and did not perform follow-up measurements. Therefore, little information is available about longitudinal data of ERPs parameters.

In conclusions, diminished amplitudes in dMMN/RON may provide a biomarker that is present before and after the development of psychosis. Our results should be interpreted with caution before applying to the at-risk population, especially to avoid over-diagnosis. Ideally, the combination with other cognitive modalities, e.g., neuropsychological tests (Higuchi et al., 2013b), brain morphology, and biochemical markers, would enhance the sensitivity and specificity for early diagnosis. These efforts are expected to help improve functional outcome in subjects with schizophrenia and vulnerable individuals as well.

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REFERENCES

Andreasen, N. C. (1990). Methods for assessing positive and negative symptoms. *Mod. Probl. Pharmacopsychiatry* 24, 73–88.

Atkinson, R. J., Michie, P. T., and Schall, U. (2012). Duration mismatch negativity and P3a in first-episode psychosis and individuals at ultra-high risk of psychosis. *Biol. Psychiatry* 71, 98–104. doi:10.1016/j.biopsych.2011.08.023

Berti, S., Roeber, U., and Schroger, E. (2004). Bottom-up influences on working memory: behavioral and electrophysiological distraction varies with distractor strength. *Exp. Psychol.* 51, 249–257. doi:10.1027/1618-3169.51.4.249

Bodatsch, M., Ruhrmann, S., Wagner, M., Muller, R., Schultze-Lutter, F., Frommann, I., et al. (2011). Prediction of psychosis by mismatch negativity. *Biol. Psychiatry* 69, 959–966. doi:10.1016/j.biopsych.2010.09.057

Broome, M. R., Woolley, J. B., Johns, L. C., Valmaggia, L. R., Tabraham, P., Gafoor, R., et al. (2005). Outreach and support in south London (OASIS): implementation of a clinical service for prodromal psychosis and the at risk mental state. *Eur. Psychiatry* 20, 372–378. doi:10.1016/j.eurpsy.2005.03.001

Bruder, G. E., Tenke, C. E., Towey, J. P., Leite, P., Fong, R., Stewart, J. E., et al. (1998). Brain ERPs of depressed patients to complex tones in an oddball task: relation of reduced P3 asymmetry to physical anhedonia. *Psychophysiology* 35, 54–63. doi:10.1111/1469-8986.3510054

Chang, W. C., Hui, C. L., Tang, J. Y., Wong, G. H., Lam, M. M., Chan, S. K., et al. (2011). Persistent negative symptoms in first-episode schizophrenia: a prospective three-year follow-up study. *Schizophr. Res.* 133, 22–28. doi:10.1016/j.schres.2011.09.006

Edwards, J., McGorry, P. D., Waddell, F. M., and Harrigan, S. M. (1999). Enduring negative symptoms in first-episode psychosis: comparison of six methods using follow-up data. *Schizophr. Res.* 40, 147–158. doi:10.1016/S0920-9964(99)00043-2

Friedman, D., Cycowicz, Y. M., and Gaeta, H. (2001). The novelty P3: an event-related brain potential (ERP) sign of the brain's evaluation of novelty. *Neurosci. Biobehav. Rev.* 25, 355–373. doi:10.1016/S0149-7634(01)00019-7

Galderisi, S., Mucci, A., Bitter, I., Libiger, J., Bucci, P., Wolfgang Fleischhacker, W., et al. (2012). Persistent negative symptoms in first episode patients with schizophrenia: results from the European First Episode Schizophrenia Trial. *Eur. Neuropsychopharmacol.* 23, 196–204. doi:10.1016/j.euroneuro.2012.04.019

Green, M. F. (1996). What are the functional consequences of neurocognitive deficits in schizophrenia? *Am. J. Psychiatry* 153, 321–330.

Harvey, P. D., Green, M. F., Keefe, R. S., and Velligan, D. I. (2004). Cognitive functioning in schizophrenia: a consensus statement on its role in the definition and evaluation of effective treatments for the illness. *J. Clin. Psychiatry* 65, 361–372. doi:10.4088/JCP.v65n0312

Heinrichs, R. W., and Zakzanis, K. K. (1998). Neurocognitive deficit in schizophrenia: a quantitative review of the evidence. *Neuropsychology* 12, 426–445. doi:10.1037/0894-4105.12.3.426

Higuchi, Y., Sumiyoshi, T., Ito, T., and Suzuki, M. (2013a). Perospirone normalized P300 and cognitive function in a case of early psychosis. *J. Clin. Psychopharmacol.* 33, 263–266. doi:10.1097/JCP.0b013e318287c527

Higuchi, Y., Sumiyoshi, T., Seo, T., Miyanishi, T., Kawasaki, Y., and Suzuki, M. (2013b). Mismatch negativity and cognitive performance for the prediction of psychosis in subjects with at-risk mental state. *PLoS ONE* 8:e54080. doi:10.1371/journal.pone.0054080

Higuchi, Y., Sumiyoshi, T., Kawasaki, Y., Ito, T., Seo, T., and Suzuki, M. (2010). Effect of tandospirone on mismatch negativity and cognitive performance in schizophrenia: a case report. *J. Clin. Psychopharmacol.* 30, 732–734. doi:10.1097/JCP.0b013e3181faa57d

Higuchi, Y., Sumiyoshi, T., Kawasaki, Y., Matsui, M., Arai, H., and Kurachi, M. (2008). Electrophysiological basis for the ability of olanzapine to improve verbal memory and functional outcome in patients with schizophrenia: a LORETA analysis of P300. *Schizophr. Res.* 101, 320–330. doi:10.1016/j.schres.2008.01.020

Horvath, J., Winkler, I., and Bendixen, A. (2008). Do N1/MMN, P3a, and RON form a strongly coupled chain reflecting the three stages of auditory distraction? *Biol. Psychol.* 79, 139–147. doi:10.1016/j.biopsycho.2008.04.001

Ikezawa, S., Nakagome, K., Mimura, M., Shinoda, J., Itoh, K., Homma, I., et al. (2008). Gender differences in lateralization of mismatch negativity in dichotic listening tasks. *Int. J. Psychophysiol.* 68, 41–50. doi:10.1016/j.ijpsycho.2008.01.006

International Early Psychosis Association Writing Group. (2005). International clinical practice guidelines for early psychosis. *Br. J. Psychiatry Suppl.* 48, s120–s124. doi:10.1192/bjp.187.48.s120

Itoh, T., Sumiyoshi, T., Higuchi, Y., Suzuki, M., and Kawasaki, Y. (2011). LORETA analysis of three-dimensional distribution of delta band activity in schizophrenia: relation to negative symptoms. *Neurosci. Res.* 70, 442–448. doi:10.1016/j.neures.2011.05.003

Jahshan, C., Cadenhead, K. S., Rissling, A. J., Kirihara, K., Braff, D. L., and Light, G. A. (2012). Automatic sensory information processing abnormalities across the illness course of schizophrenia. *Psychol. Med.* 42, 85–97. doi:10.1017/S0033291711001061

Kasai, K., Yamada, H., Kamio, S., Nakagome, K., Iwanami, A., Fukuda, M., et al. (2002). Do high or low doses of anxiolytics and hypnotics affect mismatch negativity in schizophrenic subjects? An EEG and MEG study. *Clin. Neurophysiol.* 113, 141–150. doi:10.1016/S1388-2457(01)00710-6

Kawasaki, Y., Maeda, Y., Higashima, M., Nagasawa, T., Koshino, Y., Suzuki, M., et al. (1997). Reduced auditory P300 amplitude, medial temporal volume reduction and psychopathology in schizophrenia. *Schizophr. Res.* 26, 107–115. doi:10.1016/S0920-9964(97)00055-8

Kawasaki, Y., Sumiyoshi, T., Higuchi, Y., Ito, T., Takeuchi, M., and Kurachi, M. (2007a). Voxel-based analysis of P300 electrophysiological topography associated with positive and negative symptoms of schizophrenia. *Schizophr. Res.* 94, 164–171. doi:10.1016/j.schres.2007.04.015

Kawasaki, Y., Suzuki, M., Kherif, F., Takahashi, T., Zhou, S. Y., Nakamura, K., et al. (2007b). Multivariate voxel-based morphometry successfully differentiates schizophrenia patients from healthy controls. *Neuroimage* 34, 235–242. doi:10.1016/j.neuroimage.2006.08.018

Kiang, M., Braff, D. L., Sprock, J., and Light, G. A. (2009). The relationship between preattentive sensory processing deficits and age in schizophrenia patients. *Clin. Neurophysiol.* 120, 1949–1957. doi:10.1016/j.clinph.2009.08.019

Leung, S., Croft, R. J., Baldeweg, T., and Nathan, P. J. (2007). Acute dopamine D(1) and D(2) receptor stimulation does not modulate mismatch negativity (MMN) in healthy human subjects. *Psychopharmacology (Berl.)* 194, 443–451. doi:10.1007/s00213-007-0865-1

Lin, Y. T., Liu, C. M., Chiu, M. J., Liu, C. C., Chien, Y. L., Hwang, T. J., et al. (2012). Differentiation of schizophrenia patients from healthy subjects by mismatch negativity and neuropsychological tests. *PLoS ONE* 7:e34454. doi:10.1371/journal.pone.0034454

Loebel, A. D., Lieberman, J. A., Alvir, J. M., Mayerhoff, D. I., Geisler, S. H., and Szymanski, S. R. (1992). Duration of psychosis and outcome in first-episode schizophrenia. *Am. J. Psychiatry* 149, 1183–1188.

Malla, A. K., Norman, R. M., Takhar, J., Manchanda, R., Townsend, L., Scholten, D., et al. (2004). Can patients at risk for persistent negative symptoms be identified during their first episode of psychosis? *J. Nerv. Ment. Dis.* 192, 455–463. doi:10.1097/01.nmd.0000131804.34977.c1

Matsubayashi, J., Kawakubo, Y., Suga, M., Takei, Y., Kumano, S., Fukuda, M., et al. (2008). The influence of gender and personality traits on individual difference in auditory mismatch: a magnetoencephalographic (MMNm) study. *Brain Res.* 1236, 159–165. doi:10.1016/j.brainres.2008.07.120

McGorry, P. D., Nelson, B., Amminger, G. P., Bechdolf, A., Francey, S. M., Berger, G., et al. (2009). Intervention in individuals at ultra-high risk for psychosis: a review and future directions. *J. Clin. Psychiatry* 70, 1206–1212. doi:10.4088/JCP.08r04472

Melle, I., Larsen, T. K., Haahr, U., Friis, S., Johannesen, J. O., Opjordsmoen, S., et al. (2008). Prevention of negative symptom psychopathologies in first-episode schizophrenia: two-year effects of reducing the duration of untreated psychosis. *Arch. Gen. Psychiatry* 65, 634–640. doi:10.1001/archpsyc.65.6.634

Mondragon-Maya, A., Solis-Vivanco, R., Leon-Ortiz, P., Rodriguez-Agudelo, Y., Yanez-Tellez, G., Bernal-Hernandez, J., et al. (2013). Reduced P3a amplitudes in antipsychotic naive first-episode psychosis patients and individuals at clinical high-risk for psychosis. *J. Psychiatr. Res.* 47, 755–761. doi:10.1016/j.jpsychires.2012.12.017

Naatanen, R., Kujala, T., Escera, C., Baldeweg, T., Kreegipuu, K., Carlson, S., et al. (2012). The mismatch negativity (MMN) – a unique window to disturbed central auditory processing in ageing and different clinical conditions. *Clin. Neurophysiol.* 123, 424–458. doi:10.1016/j.clinph.2011.09.020

Naatanen, R., Paavilainen, P., Rinne, T., and Alho, K. (2007). The mismatch negativity (MMN) in basic research of central auditory processing: a review. *Clin. Neurophysiol.* 118, 2544–2590. doi:10.1016/j.clinph.2007.04.026

Nagai, T., Tada, M., Kirihara, K., Yahata, N., Hashimoto, R., Araki, T., et al. (2013). Auditory mismatch negativity and P3a in response to duration and frequency changes in the early stages of psychosis. *Schizophr. Res.* 150, 547–554. doi:10.1016/j.schres.2013.08.005

Nakamura, K., Kawasaki, Y., Suzuki, M., Hagino, H., Kurokawa, K., Takahashi, T., et al. (2004). Multiple structural brain measures obtained by three-dimensional magnetic resonance imaging to distinguish between schizophrenia patients and normal subjects. *Schizophr. Bull.* 30, 393–404. doi:10.1093/oxfordjournals.schbul.a007087

Otten, L. J., Alain, C., and Picton, T. W. (2000). Effects of visual attentional load on auditory processing. *Neuroreport* 11, 875–880. doi:10.1097/00001756-200003200-00043

Ozgurdal, S., Gudlowski, Y., Witthaus, H., Kawohl, W., Uhl, I., Hauser, M., et al. (2008). Reduction of auditory event-related P300 amplitude in subjects with at-risk mental state for schizophrenia. *Schizophr. Res.* 105, 272–278. doi:10.1016/j.schres.2008.05.017

Perkins, D. O., Gu, H., Boteva, K., and Lieberman, J. A. (2005). Relationship between duration of untreated psychosis and outcome in first-episode schizophrenia: a critical review and meta-analysis. *Am. J. Psychiatry* 162, 1785–1804. doi:10.1176/appi.ajp.162.10.1785

Roth, W. T., Pfefferbaum, A., Horvath, T. B., Berger, P. A., and Kopell, B. S. (1980). P3 reduction in auditory evoked potentials of schizophrenics. *Electroencephalogr. Clin. Neurophysiol.* 49, 497–505. doi:10.1016/0013-4694(80)90392-2

Schroger, E., Giard, M. H., and Wolff, C. (2000). Auditory distraction: event-related potential and behavioral indices. *Clin. Neurophysiol.* 111, 1450–1460. doi:10.1016/S1388-2457(00)00337-0

Schroger, E., and Wolff, C. (1998). Attentional orienting and reorienting is indicated by human event-related brain potentials. *Neuroreport* 9, 3355–3358. doi:10.1097/00001756-199810260-00003

Shaikh, M., Valmaggia, L., Broome, M. R., Dutt, A., Lappin, J., Day, F., et al. (2012). Reduced mismatch negativity predates the onset of psychosis. *Schizophr. Res.* 134, 42–48. doi:10.1016/j.schres.2011.09.022

Sumiyoshi, T., Higuchi, Y., Itoh, T., Matsui, M., Arai, H., Suzuki, M., et al. (2009). Effect of perospirone on P300 electrophysiological activity and social cognition in schizophrenia: a three-dimensional analysis with sloreta. *Psychiatry Res.* 172, 180–183. doi:10.1016/j.pscychresns.2008.07.005

Sumiyoshi, T., Higuchi, Y., Kawasaki, Y., Matsui, M., Kato, K., Yuuki, H., et al. (2006). Electrical brain activity and response to olanzapine in schizophrenia: a study with LORETA images of P300. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 30, 1299–1303. doi:10.1016/j.pnpbp.2006.04.028

Sumiyoshi, T., Jayathilake, K., and Meltzer, H. Y. (2003). The effect of melperone, an atypical antipsychotic drug, on cognitive function in schizophrenia. *Schizophr. Res.* 59, 7–16. doi:10.1016/S0920-9964(01)00329-2

Sumiyoshi, T., Miyanishi, T., Seo, T., and Higuchi, Y. (2013). Electrophysiological and neuropsychological predictors of conversion to schizophrenia in at-risk subjects. *Front. Behav. Neurosci.* 7:148. doi:10.3389/fnbeh.2013.00148

Takahashi, T., Zhou, S. Y., Nakamura, K., Tanino, R., Furuichi, A., Kido, M., et al. (2011). A follow-up MRI study of the fusiform gyrus and middle and inferior temporal gyri in schizophrenia spectrum. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 35, 1957–1964. doi:10.1016/j.pnpbp.2011.07.009

Takayanagi, Y., Takahashi, T., Orikabe, L., Mozue, Y., Kawasaki, Y., Nakamura, K., et al. (2011). Classification of first-episode schizophrenia patients and healthy subjects by automated MRI measures of regional brain volume and cortical thickness. *PLoS ONE* 6:e21047. doi:10.1371/journal.pone.0021047

Umbricht, D., Javitt, D., Novak, G., Bates, J., Pollack, S., Lieberman, J., et al. (1998). Effects of clozapine on auditory event-related potentials in schizophrenia. *Biol. Psychiatry* 44, 716–725. doi:10.1016/S0006-3223(97)00524-6

Umbricht, D., and Krljes, S. (2005). Mismatch negativity in schizophrenia: a meta-analysis. *Schizophr. Res.* 76, 1–23. doi:10.1016/j.schres.2004.12.002

Yamazawa, R., Nemoto, T., Kobayashi, H., Chino, B., Kashima, H., and Mizuno, M. (2008). Association between duration of untreated psychosis, premorbid functioning, and cognitive performance and the outcome of first-episode schizophrenia in Japanese patients: prospective study. *Aust. N. Z. J. Psychiatry* 42, 159–165. doi:10.1080/00048670701787537

Yung, A. R., McGorry, P. D., McFarlane, C. A., Jackson, H. J., Patton, G. C., and Rakkar, A. (1996). Monitoring and care of young people at incipient risk of psychosis. *Schizophr. Bull.* 22, 283–303. doi:10.1093/schbul/22.2.353

Yung, A. R., Phillips, L. J., Yuen, H. P., Francey, S. M., McFarlane, C. A., Hallgren, M., et al. (2003). Psychosis prediction: 12-month follow up of a high-risk (prodromal) group. *Schizophr. Res.* 60, 21–32. doi:10.1016/S0920-9964(03)80061-0

Yung, A. R., Yuen, H. P., McGorry, P. D., Phillips, L. J., Kelly, D., Dell'Olio, M., et al. (2005). Mapping the onset of psychosis: the comprehensive assessment of at-risk mental states. *Aust. N. Z. J. Psychiatry* 39, 964–971. doi:10.1080/j.1440-1614.2005.01714.x

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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

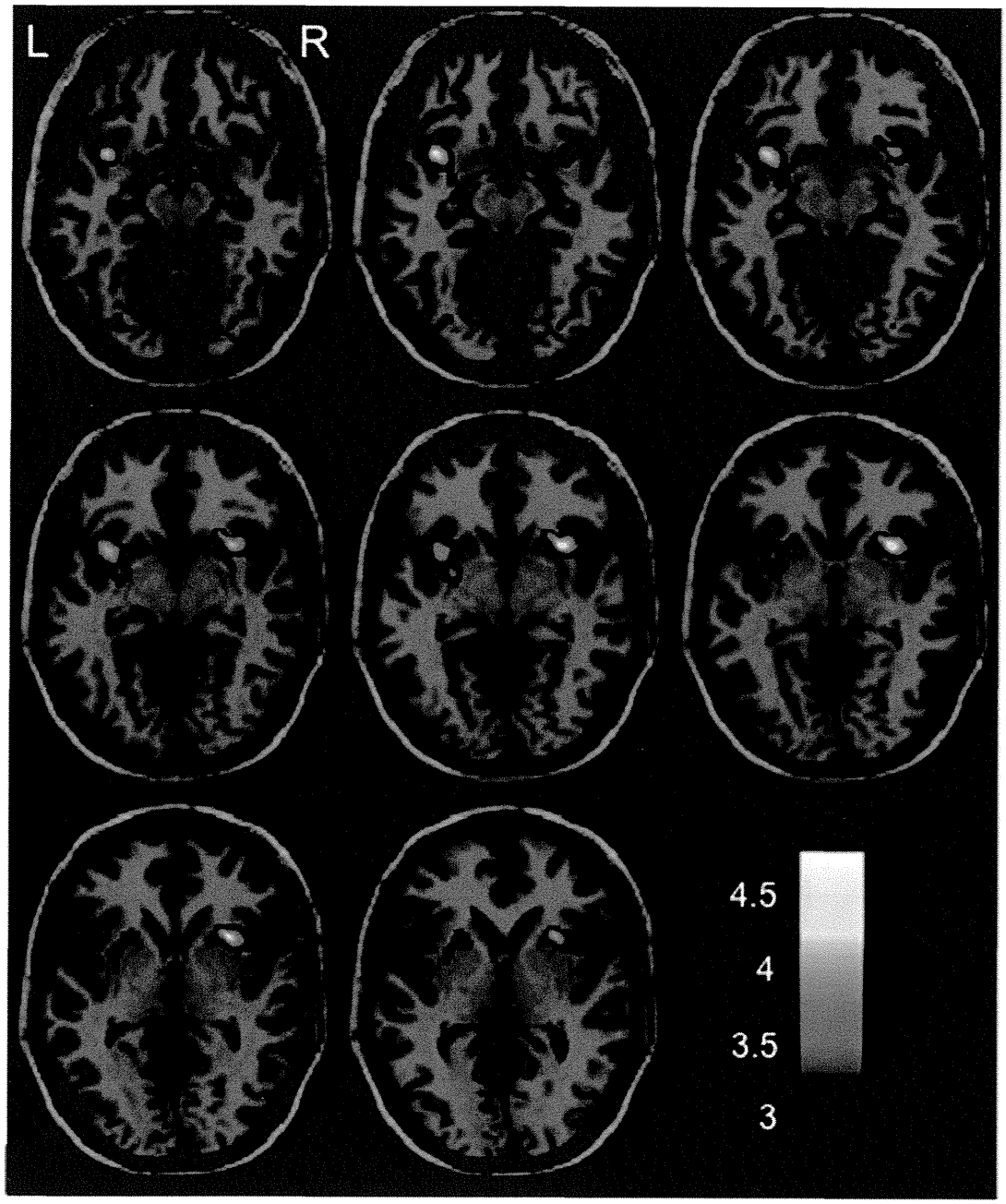

Figure 1. The *YWHAE* (*rs28365859*) genotype-by-diagnosis interaction on gray matter volume. The regions showing interaction in all subjects are displayed by a hot colormap. The color bar shows t values corresponding to the color in the figure.
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Hardy–Weinberg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

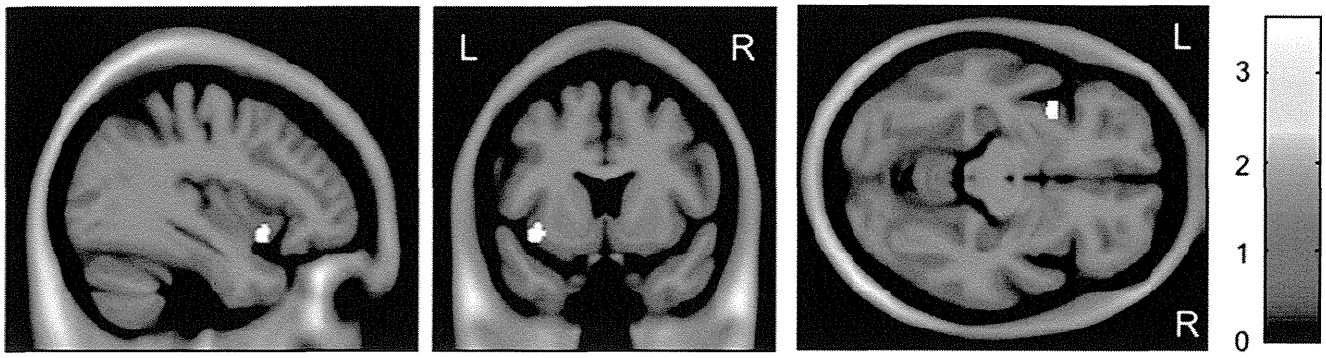

Figure 2. Impact of the *rs28365859* genotype on gray matter volume of left insula in schizophrenia. Age, sex, illness duration, and medication dose were used as covariates. The protective C allele carriers had a significantly larger left insula than the G homozygotes. Anatomical localizations are displayed on the normal template MR images in three directions. The color bar shows t values corresponding to the color in the figure.
doi:10.1371/journal.pone.0103571.g002

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.

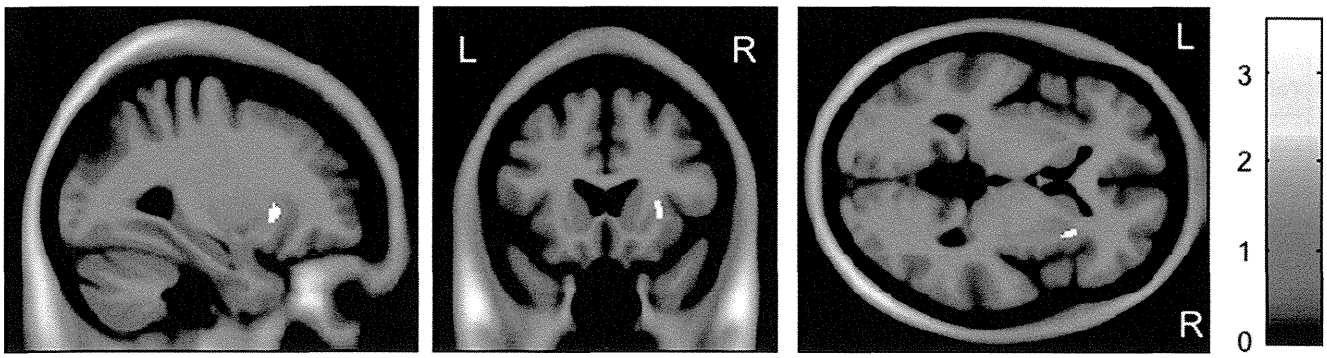

Figure 3. Impact of the *rs28365859* genotype on gray matter volume of the right putamen in healthy controls. The G allele homozygotes had a significantly larger right putamen than the C allele carriers. Anatomical localizations are displayed on the normal template MR images in three directions. The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

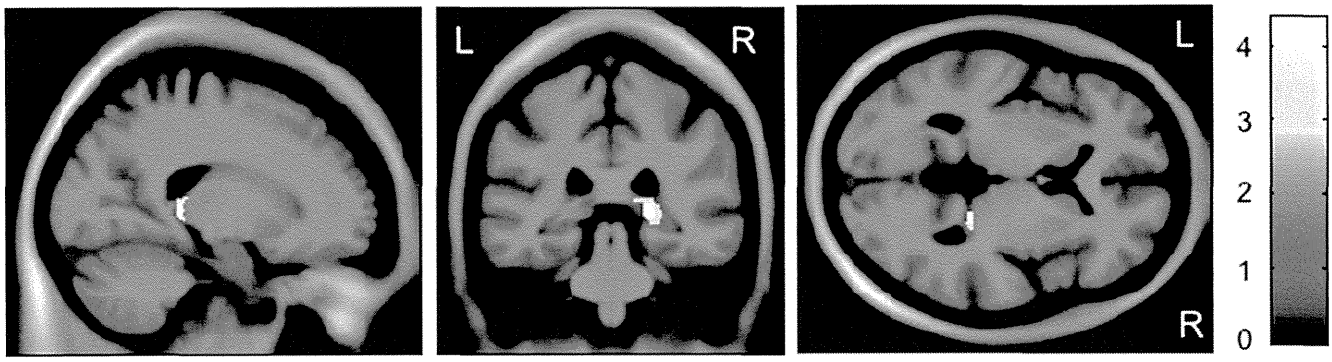

Figure 4. Impact of the *rs28365859* genotype on gray matter volume of the right hippocampus in schizophrenia. Age, sex, illness duration, and medication dose were used as covariates. The protective C allele carriers had a significantly larger right hippocampus than the G allele homozygotes. Anatomical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

References

1. Harrison PJ, Weinberger DR (2005) Schizophrenia genes, gene expression, and neuropathology: on the matter of their convergence. Mol Psychiatry 10: 40–68.
2. Sawa A, Snyder SH (2002) Schizophrenia: diverse approaches to a complex disease. Science 296: 692–695.
3. Shenton ME, Dickey CC, Frumin M, McCarley RW (2001) A review of MRI findings in schizophrenia. Schizophr Res 49: 1–52.
4. Suzuki M, Nohara S, Hagino H, Kurokawa K, Yotsutsuji T, et al. (2002) Regional changes in brain gray and white matter in patients with schizophrenia demonstrated with voxel-based analysis of MRI. Schizophr Res 55: 41–54.
5. Gottesman II, Gould TD (2003) The endophenotype concept in psychiatry: etymology and strategic intentions. Am J Psychiatry 160: 636–645.
6. Keshavan MS, Prasad KM, Pearlson G (2007) Are brain structural abnormalities useful as endophenotypes in schizophrenia? Int Rev Psychiatry 19: 397–406.
7. Millar JK, Wilson-Annan JC, Anderson S, Christie S, Taylor MS, et al. (2000) Disruption of two novel genes by a translocation co-segregating with schizophrenia. Hum Mol Genet 9: 1415–1423.
8. St Clair D, Blackwood D, Muir W, Carothers A, Walker M, et al. (1990) Association within a family of a balanced autosomal translocation with major mental illness. Lancet 336: 13–16.
9. James R, Adams RR, Christie S, Buchanan SR, Porteous DJ, et al. (2004) Disrupted in Schizophrenia 1 (DISC1) is a multicompartmentalized protein that predominantly localizes to mitochondria. Mol Cell Neurosci 26: 112–122.
10. Kamiya A, Kubo K, Tomoda T, Takaki M, Youn R, et al. (2005) A schizophrenia-associated mutation of DISC1 perturbs cerebral cortex development. Nat Cell Biol 7: 1167–1178.
11. Kirkpatrick B, Xu L, Cascella N, Ozeki Y, Sawa A, et al. (2006) DISC1 immunoreactivity at the light and ultrastructural level in the human neocortex. J Comp Neurol 497: 436–450.
12. Ozeki Y, Tomoda T, Kleiderlein J, Kamiya A, Bord L, et al. (2003) Disrupted-in-Schizophrenia-1 (DISC-1): mutant truncation prevents binding to NudE-like (NUDEL) and inhibits neurite outgrowth. Proc Natl Acad Sci U S A 100: 289–294.
13. Taya S, Shinoda T, Tsuboi D, Asaki J, Nagai K, et al. (2007) DISC1 regulates the transport of the NUDEL/LIS1/14-3-3epsilon complex through kinesin-1. J Neurosci 27: 15–26.
14. Ishizuka K, Paek M, Kamiya A, Sawa A (2006) A review of Disrupted-in-Schizophrenia-1 (DISC1): neurodevelopment, cognition, and mental conditions. Biol Psychiatry 59: 1189–1197.
15. Roberts RC (2007) Schizophrenia in translation: disrupted in schizophrenia (DISC1): integrating clinical and basic findings. Schizophr Bull 33: 11–15.
16. Callicott JH, Straub RE, Pezawas L, Egan MF, Mattay VS, et al. (2005) Variation in DISC1 affects hippocampal structure and function and increases risk for schizophrenia. Proc Natl Acad Sci U S A 102: 8627–8632.
17. Hashimoto R, Numakawa T, Ohnishi T, Kumamaru E, Yagasaki Y, et al. (2006) Impact of the DISC1 Ser704Cys polymorphism on risk for major depression, brain morphology and ERK signaling. Hum Mol Genet 15: 3024–3033.
18. Takahashi T, Suzuki M, Tsunoda M, Maeno N, Kawasaki Y, et al. (2009a) The Disrupted-in-Schizophrenia-1 Ser704Cys polymorphism and brain morphology in schizophrenia. Psychiatry Res 172: 128–135.
19. Duff BJ, Macritchie KA, Moorhead TW, Lawrie SM, Blackwood DH (2013) Human brain imaging studies of DISC1 in schizophrenia, bipolar disorder and depression: a systematic review. Schizophr Res 147: 1–13.
20. Sullivan PF (2013) Questions about DISC1 as a genetic risk factor for schizophrenia. Mol Psychiatry 18: 1050–1052.
21. Toyo-oka K, Shionoya A, Gambello MJ, Cardoso C, Leventer R, et al. (2003) 14-3-3epsilon is important for neuronal migration by binding to NUDEL: a molecular explanation for Miller-Dieker syndrome. Nat Genet 34: 274–285.
22. Ikeda M, Hikita T, Taya S, Uraguchi-Asaki J, Toyo-oka K, et al. (2008) Identification of YWHAE, a gene encoding 14-3-3epsilon, as a possible susceptibility gene for schizophrenia. Hum Mol Genet 17: 3212–2322.
23. Goldman-Rakic PS (1994) Working memory dysfunction in schizophrenia. J Neuropsychiatry Clin Neurosci 6: 348–357.
24. World Health Organization (1993) The ICD-10 classification of mental and behavioural disorders: diagnostic criteria for research. World Health Organization, Geneva.
25. Andreasen NC, Flaum M, Arndt S (1992) The Comprehensive Assessment of Symptoms and History (CASH). An instrument for assessing diagnosis and psychopathology. Arch Gen Psychiatry 49: 615–623.
26. Andreasen NC (1984a) Scale for the assessment of negative symptoms (SANS). University of Iowa, Iowa City.

27. Andreasen NC (1984b) Scale for the assessment of positive symptoms (SAPS). University of Iowa, Iowa City.
28. Ashburner J, Friston KJ (2005) Unified segmentation. NeuroImage 26: 839–851.
29. Ashburner J (2007) A fast diffeomorphic image registration algorithm. NeuroImage 38: 95–113.
30. Jones DK, Symms MR, Cercignani M, Howard RJ (2005) The effect of filter size on VBM analyses of DT-MRI data. Neuroimage 26: 546–554.
31. Salmond CH, Ashburner J, Vargha-Khadem F, Connelly A, Gadian DG, et al. (2002) Distributional assumptions in voxel-based morphometry. Neuroimage 17: 1027–1030.
32. Maldjian JA, Laurienti PJ, Kraft RA, Burdette JH (2003) An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets. Neuroimage 19: 1233–1239.
33. Tzourio-Mazoyer N, Landeau B, Papathanassiou D, Crivello F, Etard O, et al. (2002) Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. Neuroimage 15: 273–289.
34. Talairach J, Tournoux P (1988) Co-planar stereotaxic atlas of the human brain. 3-Dimensional proportional system: an approach to cerebral imaging. Stuttgart Thieme.
35. Lancaster JL, Summerln JL, Rainey L, Freitas CS, Fox PT (1997) The Talairach Daemon, a database server for Talairach Atlas Labels. NeuroImage 5: S633.
36. Lancaster JL, Woldorff MG, Parsons LM, Liotti M, Freitas CS, et al. (2000) Automated Talairach atlas labels for functional brain mapping. Hum Brain Mapp 10: 120–131.
37. Toru M (2008) Psychotropic Manual, Third Edition. Igaku-shoin, Tokyo. (in Japanese).
38. Wylie KP, Tregellas JR (2010) The role of the insula in schizophrenia. Schizophr Res 123: 93–104.
39. Augustine JR (1996) Circuitry and functional aspects of the insular lobe in primates including humans. Brain Res Brain Res Rev 22: 229–244.
40. Glahn DC, Laird AR, Ellison-Wright I, Thelen SM, Robinson JL, et al. (2008) Meta-analysis of gray matter anomalies in schizophrenia: application of anatomic likelihood estimation and network analysis. Biol Psychiatry 64: 774–781.
41. Shepherd AM, Matheson SL, Laurens KR, Carr VJ, Green MJ (2012) Systematic meta-analysis of insula volume in schizophrenia. Biol Psychiatry 72: 775–784.
42. Palaniyappan L, Liddle PF (2012) Aberrant cortical gyrification in schizophrenia: a surface-based morphometry study. J Psychiatry Neurosci 37: 399–406.
43. Jakob H, Beckmann H (1986) Prenatal developmental disturbances in the limbic allocortex in schizophrenics. J Neural Transm 65: 303–326.
44. Pennington K, Dicker P, Hudson L, Cotter DR (2008) Evidence for reduced neuronal somal size within the insular cortex in schizophrenia, but not in affective disorders. Schizophr Res 106: 164–171.
45. Borgwardt SJ, Riecher-Rössler A, Dazzan P, Chitnis X, Aston J, et al. (2007) Regional gray matter volume abnormalities in the at risk mental state. Biol Psychiatry 61: 1148–1156.
46. Takahashi T, Wood SJ, Yung AR, Phillips LJ, Soulsby B, et al. (2009b) Insular cortex gray matter changes in individuals at ultra-high-risk of developing psychosis. Schizophr Res 111: 94–102.
47. Borgwardt SJ, Picchioni MM, Ettinger U, Toulopoulou T, Murray R, et al. (2010) Regional gray matter volume in monozygotic twins concordant and discordant for schizophrenia. Biol Psychiatry 67: 956–964.
48. Chakravarty MM, Felsky D, Tampakeras M, Lerch JP, Mulsant BH, et al. (2012) DISC1 and Striatal Volume: A Potential Risk Phenotype For mental Illness. Front Psychiatry 3: 57.
49. Lipina TV, Niwa M, Jaaro-Peled H, Fletcher PJ, Seeman P, et al. (2010) Enhanced dopamine function in DISC1-L100P mutant mice: implications for schizophrenia. Genes Brain Behav 9: 777–789.
50. Ballmaier M, Schlagenhauf F, Toga AW, Gallinat J, Koslowski M, et al. (2008) Regional patterns and clinical correlates of basal ganglia morphology in non-medicated schizophrenia. Schizophr Res 106: 140–147.
51. Glenthoj A, Glenthoj BY, Mackeprang T, Pagsberg AK, Hemmingsen RP, et al. (2007) Basal ganglia volumes in drug-naive first-episode schizophrenia patients before and after short-term treatment with either a typical or an atypical antipsychotic drug. Psychiatry Res 154: 199–208.
52. Gunduz H, Wu H, Ashtari M, Bogerts B, Crandall D, et al. (2002) Basal ganglia volumes in first-episode schizophrenia and healthy comparison subjects. Biol Psychiatry 51: 801–808.
53. Li M, Chen Z, Deng W, He Z, Wang Q, et al. (2011) Volume increases in putamen associated with positive symptom reduction in previously drug-naive schizophrenia after 6 weeks antipsychotic treatment. Psychol Med 42: 1475–1483.
54. Ebdrup BH, Skimminge A, Rasmussen H, Aggernaes B, Oranje B, et al. (2011) Progressive striatal and hippocampal volume loss in initially antipsychotic-naive, first-episode schizophrenia patients treated with quetiapine: relationship to dose and symptoms. Int J Neuropsychopharmacol 14: 69–82.
55. Borgwardt S, Smieskova R, Fusar-Poli P (2012) Gray matter pathology of hippocampus - a specific endophenotype for schizophrenia? Psychiatry Res 202: 273–274.
56. Lipska BK, Peters T, Hyde TM, Halim N, Horowitz C, et al. (2006) Expression of DISC1 binding partners is reduced in schizophrenia and associated with DISC1 SNPs. Hum Mol Genet 15: 1245–1258.
57. Bis JC, DeCarli C, Smith AV, van der Lijn F, Crivello F, et al. (2012) Common variants at 12q14 and 12q24 are associated with hippocampal volume. Nat Genet 44: 545–551.
58. Stein JL, Medland SE, Vasquez AA, Hibar DP, Senstad RE, et al. (2012) Identification of common variants associated with human hippocampal and intracranial volumes. Nat Genet 44: 552–561.
59. Sekiguchi H, Iritani S, Habuchi C, Torii Y, Kuroda K, et al. (2011) Impairment of the tyrosine hydroxylase neuronal network in the orbitofrontal cortex of a genetically modified mouse model of schizophrenia. Brain Res 1392: 47–53.
60. Haijma SV, Van Haren N, Cahn W, Koolschijn PC, Hulshoff Pol HE, et al. (2013) Brain volumes in schizophrenia: a meta-analysis in over 18 000 subjects. Schizophr Bull 39: 1129–1138.
61. Andreasen NC, Liu D, Ziebell S, Vora A, Ho BC (2013) Relapse duration, treatment intensity, and brain tissue loss in schizophrenia: a prospective longitudinal MRI study. Am J Psychiatry 170: 609–615.
62. Lieberman JA, Tollefson GD, Charles C, Zipursky R, Sharma T, et al. (2005) Antipsychotic drug effects on brain morphology in first-episode psychosis. Arch Gen Psychiatry 62: 361–370.
63. Anderson D, Ardekani BA, Burdick KE, Robinson DG, John M, et al. (2013) Overlapping and distinct gray and white matter abnormalities in schizophrenia and bipolar I disorder. Bipolar Disord 15: 680–693.

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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 ($\chi^2 = 1.00$, $p = 0.317$).

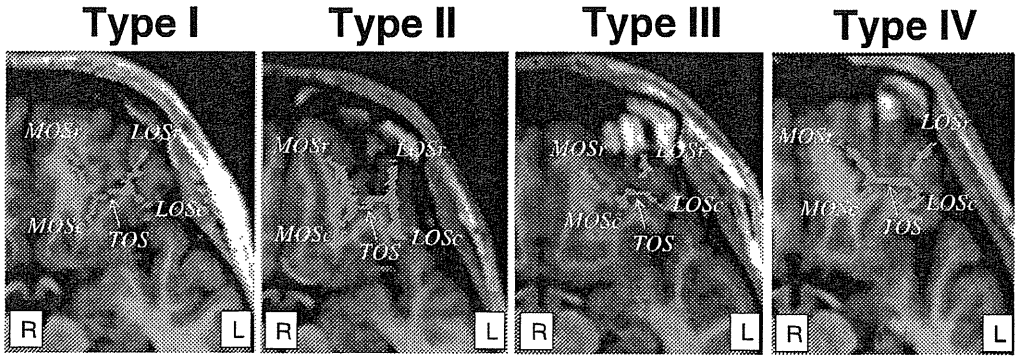

Fig. 1. Classification of the orbitofrontal sulcogyral pattern on an axial view parallel to the anterior commissure–posterior commissure line. Note that these sulci were identified using orthogonal views in three directions and colored on consecutive coronal slices. c, caudal portion; LOS, lateral orbital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