may indicate that decreased activation of the prefrontal cortex underlies neural malfunction related to social cognition and behavior.

Although patients with ED with extremely low body weight often pose a serious clinical problem, there are few studies of this population. In addition, patients with ED require careful physical management and behavioral suppression in the acute phase of therapy. The portability, compactness, and non-invasive features of near-infrared spectroscopy (NIRS) make it an ideal tool with which to study functional brain activity in patients with ED. NIRS allows the measurement of functional brain activity under near-natural conditions [7]. It is based on the principle that near-infrared light is preferentially absorbed by oxygenated hemoglobin (oxy-Hb) and de-oxygenated hemoglobin (deoxy-Hb) compared with other body tissues [8]; furthermore, it quantifies regional oxy- and deoxy-Hb concentrations with a high time resolution. Patients with depression, schizophrenia, and bipolar disorder have been studied using NIRS, and their characteristic time courses of oxy-Hb changes in the frontal lobe have been investigated [8-10]. Four NIRS studies in patients with ED have been conducted [11-14], three of which reported lower oxy-Hb concentrations in the frontal cortex during the letter fluency task (LFT) in patients with ED than in healthy CTLs [11-13]. However, the patient population and methodology varied across these studies. For example, Uehara et al. (2007) did not evaluate the relevance of clinical symptoms [11], Suda et al. (2010) excluded patients with body mass index (BMI) less than 14.5 kg/m^2 to exclude the effect of malnutrition [12], Nagamitsu et al. (2011) studied children [13], and Sutoh et al. (2013) studied patients with AN with relatively high BMI (mean ± SD, 17.0 ± 3.1 kg/m^2) [14].

Some patients with ED experience feelings of social self-doubt and unhappiness, which may have implications for treatment [15]. Many studies have reported that social skills and interpersonal difficulties are strongly associated with the psychopathology of patients with ED [16,17]. It is speculated that patients with ED tend to have interpersonal sensitivity, low self-esteem, social anxiety, poor emotional support, and social inhibition, all of which are associated with ED psychopathology [16]. A recent study reported that patients with AN showed impaired cognitive flexibility as well as hypoactivity in the ventrolateral prefrontal cortex [18]. Furthermore, patients with ED had alterations in the frontal cortex that contribute to reward and anxiety processing [19]. Moreover, it was shown that the frontal cortex is involved in reward-guided learning and decision-making [20], while the reward system is associated with prosocial behavior (i.e., helping, sharing, donating, cooperating, and volunteering) [21]. For these reasons, it is hypothesized that patients with ED, especially ones with severe weight loss, have a neural abnormality that influences social cognition and behavior and prevents them from adapting well to society.

In summary, there are several studies showing that patients with AN have both feelings of social insecurity (SI) and functional abnormalities in the frontal cortex that are associated with prosocial behavior. Therefore, the correlation between SI and frontal activity may be altered in patients with AN compared to CTLs. Although a previous study showed that the correlation between frontal cortex oxy-Hb concentrations during the LFT and Eating Attitudes Test scores differed between the ED and CTL groups [13], no neuroimaging studies have directly examined the relation between frontal cortex activity and SI in subjects with ED and CTLs. The aim of the present study was to investigate brain activity and its association with social relationships in patients with ED with extremely low body weight. We tested the hypothesis that frontal cortex oxy-Hb concentrations during the LFT would be lower in patients with ED than in healthy subjects, and that the correlation between frontal cortex oxy-Hb concentrations during the LFT and Eating Disorder Inventory-2 (EDI-2) score, which includes the SI subscale, would differ between groups.

Methods

Participants

Twenty patients with ED and 31 healthy CTLs were included in this study (Table 1). Patients with ED were primarily recruited from November 2010 to May 2012 from among inpatients at Nagoya University Hospital. They were diagnosed in accordance with the criteria of the Diagnostic and Statistical Manual of Mental Disorders, 4th edition, Text Revision (DSM-IV-TR, American Psychiatric Association, 2000). The Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I), module H, was used to define subtypes of ED. All patients with ED were treated with medication, supportive psychotherapy, and behavioral therapy during their regular hospitalization. We excluded subjects who were left-handed, male, diagnosed with bulimia nervosa (BN), and younger than 17 years, as well those who did not consent to study participation or those who did not meet the diagnostic criteria for ED as shown by findings on SCID-I. All patients with ED had food intake between 400 and 800 kcal within 4 hours before NIRS measurements.

CTL subjects were all female and were interviewed using the SCID-I to confirm the absence of psychiatric disorders. All participants were right-handed as indicated by the Edinburgh Handedness Inventory score [22].

The ethics review committees at Nagoya University Graduate School of Medicine and Nagoya University

Table 1 Demographic characteristics of study participants

	ED ($n = 20$)	CTL ($n = 31$)	p	Cohen's d
Age (years)	28.7 ± 7.5	29.2 ± 7.8	0.824	0.06
Education (years)	14.4 ± 2.0	15.6 ± 1.8	0.025	0.66
Number of hospitalizations	3.6 ± 4.8	N/A	N/A	N/A
Number of days of hospitalization (days)	20.6 ± 17.2	N/A	N/A	N/A
Cumulative number of days of hospitalization (days)	123.3 ± 191.5	N/A	N/A	N/A
Current BMI (kg/m^2)	14.0 ± 2.3	21.8 ± 3.7	< 0.001	2.45
BDI score	24.1 ± 10.5	4.3 ± 4.8	< 0.001	2.58
Task performance of LFT	15.5 ± 5.2	12.6 ± 3.5	0.024	0.66

Data are expressed as mean ± standard deviation.
ED, eating disorder; CTL, control; N/A, not applicable; BMI, body mass index; BDI, Beck Depression Inventory; LFT, letter fluency task.

Hospital approved the study protocol, and written, informed consent was obtained from all participants prior to enrollment.

Assessment of clinical symptoms: eating disorder inventory-2

ED symptoms were assessed in all participants using the Japanese version of the EDI-2 [23]. The EDI-2 is one of the most frequently used, self-reported assessment instruments [24]. The Japanese version of the EDI-2 is validated and reliable tool for the evaluation of the psychopathology of ED [23]. It consists of 91 items with 11 subscales and is designed to assess attitudinal and behavioral dimensions relevant to both AN and BN. Three subscales (drive for thinness, bulimia, and body dissatisfaction) relate to the diagnosis of ED, and eight subscales (ineffectiveness, perfectionism, interpersonal distrust, interceptive awareness, maturity fears, asceticism, impulse regulation, and SI) relate to the general psychopathology of ED. The SI subscale was added to the Eating Disorder Inventory (EDI-1) [25] when it was revised to the EDI-2 [26]. SI assesses the beliefs that social relationships are tense, insecure, disappointing, unrewarding, and generally of poor quality [26].

Assessment of clinical symptoms: beck depression inventory

Depressive symptoms were also assessed using the Japanese version of the Beck Depression Inventory (BDI) [27]. BDI scores were used to control for the role that depressive symptomatology may have on the relation between NIRS measures and SI scores.

Activation task

The LFT was performed during NIRS measurements. LFT generally activates frontal brain areas [28], and has been used in the study of many psychiatric disorders, including ED [11-14]. Participants sat opposite a video monitor in a comfortable chair with their eyes open in a moderately lit room. Cued by instructions on the monitor, they attempted to verbally generate as many words as possible with the initial Japanese syllable of either 'a', 'ki', or 'ha' in 20 s. Duplications and proper nouns were not permitted. The three initial syllables were used consecutively in the above-mentioned order, and the total duration of the LFT was 60 s. The number of words generated was recorded as a measure of LFT performance. The task was performed in a block design that consisted of a 30 s pre-task baseline, 60 s LFT, and 70 s post-task baseline. During the pre- and post-task baseline periods, participants were instructed to repeat the syllables 'a', 'i', 'u', 'e', and 'o' [12]. Participants were able to practice the task until they fully understood the instructions before NIRS measurements began.

NIRS measurements

Relative concentrations of oxy- and deoxy-Hb were measured at three wavelengths (780, 805, and 830 nm) by a functional NIRS system (FOIRE-3000; Shimadzu Corporation, Kyoto, Japan). Light emitters and detectors were arranged in an NIRS shell in a 3 × 5 array with 3-cm inter-probe distance (Figure 1). The relative concentrations of oxy- and deoxy-Hb were measured at 22 points at a depth of 2–3 cm [29] from the scalp, over a 9 × 15 cm area (Figure 1). The NIRS shell was placed over the frontal region, which was determined according to the International 10–20 system used in electroencephalography, with the lowest probes positioned along the Fp1-2 line [30]. Although the NIRS system is able to measure three kinds of hemoglobin (oxy-, deoxy-, and total-Hb), the change in oxy-Hb concentration was selected for analysis because it is the best indicator of the change in regional cerebral blood flow [31,32]. As the Hb concentration associated with changes in regional cerebral blood flow reflects changes in neuronal activity in real time [31], we used the words "high (or low) brain activity" interchangeably with "high (or low) oxy-Hb concentration". The correspondence between the NIRS channels and the measurement points in the cerebral cortex was confirmed using virtual registration [33] based on probabilistic registration [34].

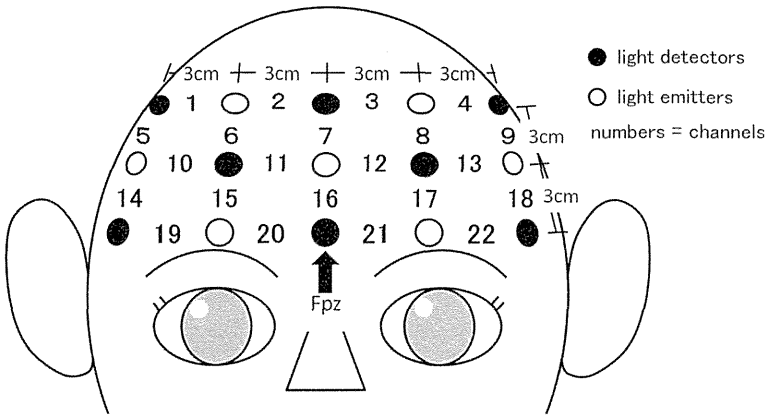

Figure 1 Placement of near-infrared spectroscopy probes and channels. The location of the light detector between channels 20 and 21 (indicated by black arrow) corresponds to FPZ of the electroencephalography 10–20 system.

The anatomical label of the cerebral cortex was identified according to the Brodmann area [35].

Data analysis and statistics

Oxy- and deoxy-Hb concentrations were measured in all 22 NIRS channels with a time resolution of 0.1 s. Motion artifacts were determined by close observation of the participant and the oxy-Hb waveform and were excluded from further analysis. Data were filtered with a low-pass filter at 0.1 Hz to remove short-term motion artifacts. Pre-task baseline oxy-Hb concentration was quantified as the arithmetic mean oxy-Hb concentration over the final 10 s of the 30 s pre-task baseline period. The baseline value was subtracted from the mean oxy-Hb concentration over the 60-s task period in order to determine the mean change in oxy-Hb concentration during the LFT. Also, the baseline value was subtracted from the mean oxy-Hb concentration over the 70-s post-task period to determine the mean change in oxy-Hb concentration during the post-task period.

The mean change in oxy-Hb concentration was calculated in this manner for each channel, and then compared between the ED and CTL groups using independent *t*-tests. At that time, significance levels were corrected for multiple comparisons using the false discovery rate method [36] in which the number of comparisons were 22, and effect sizes were calculated using Cohen's *d* [37]. The mean change in oxy-Hb concentration in each channel during the LFT was correlated with the score of each subscale of the EDI-2 using Pearson product–moment correlation coefficient (r). Guilford (1956) [38] describes correlations of 0.20 > to 0.40 as "weak correlations", correlations of 0.40 to 0.70 as "moderate correlations", and 0.70 to 0.90 as "strong correlations, marked relationship". Values of $p < 0.05$ were considered statistically significant for all analyses. As oxy-Hb concentrations measurements yield 22 results based on readings from 22 NIRS channels, we applied the false discovery rate method to avoid a multiple testing problem. A nominal p value was adjusted so that the adjusted p values could be compared to the same significance level of 0.05. Pearson product–moment correlation coefficient was also used to evaluate the relationship between the mean change in oxy-Hb concentration in each channel during the LFT, and age, years of education, BMI, BDI score, and LFT performance. After adjusting for variables with a significant Pearson product–moment correlation coefficient correlation, a partial correlation analysis adjusting for age, years of education, BMI, and BDI score was performed to examine the relationship between the mean change in oxy-Hb during the LFT and the score of each subscale of the EDI-2. Then, significance levels were also corrected for multiple comparisons using the false discovery rate method. All statistical analyses were performed using SPSS-20 software (SPSS Inc., Chicago, Illinois).

Results

Patient characteristics

All patients with ED were female and diagnosed with: anorexia nervosa binge-eating/purging type ED (13 patients), anorexia nervosa restricting type ED (4 patients), or ED not otherwise specified (EDNOS, 3 patients). All three patients with EDNOS had BMI <14.0 kg/m^2, and did not meet the criteria of "Intense fear of gaining weight or becoming fat, even though underweight" or other criteria for BN. They corresponded to a variant termed "non-fat-phobic AN" (NFP-AN), but NFP-AN has not been recognized as a distinct diagnosis [39]. BMI at the time of NIRS measurements was significantly lower in the ED group than in the CTL group (Table 1). The number of years

of education was significantly higher in the CTL group than in the ED group (Table 1). BMI at the time of NIRS measurements was significantly lower in the ED group than in the CTL group (Table 1). Information about comorbidities and medications received by patients with ED are described in an additional table (see Additional file 1).

LFT performance, BDI, and EDI-2 score

LFT performance was significantly better in the ED group than in the CTL group (Table 1). BDI scores and the scores on all subscales of the EDI-2, including the SI subscale, were significantly higher in the ED group than in the CTL group (Table 2).

Mean change in oxy-Hb during the LFT and the post-task period

Figure 2 shows the grand averaged waveforms of oxy-Hb during the LFT in each of the 22 NIRS channels. Although the oxy-Hb concentration increased as soon as the task began and decreased after the task was over in both groups, the change in oxy-Hb concentration was larger in the CTL group than in the ED group (Figure 2). The mean change in oxy-Hb concentration during the LFT was significantly greater in the CTL group than in the ED group in nine channels (Channel 9–11, 14–16, 18, 21, and 22, all $p < 0.05$, Cohen's $d = 0.59$ to 0.96), and remained significantly greater after significance levels were corrected by the false discovery rate method in six channels (Channel 10, 11, 14, 16, 18 and 22, all $p < 0.01$, Cohen's $d = 0.77$ to 0.96). An additional table shows the p values without and with correction by the false discovery rate method (see Additional file 2). According to Tsuzuki et al. (2007) [33], NIRS channels 11, 12, 15–17 and 19–22 include the bilateral orbitofrontal cortex (OFC), We found that the mean changes in oxy-Hb concentration during the LFT in NIRS channels 11, 16, 18, and 22, which include the bilateral OFC, were significantly smaller in the ED group than in the CTL group even after correction of significance levels by the false discovery rate method (all $p < 0.05$, Cohen's $d = 0.77$ to 0.96,). Differences between the ED group and the CTL group in NIRS channels 12, 15, 17, and 19–21, which include the bilateral OFC, were not significant but did present a medium-to-large effect size (all $p > 0.05$, Cohen's $d = 0.50$ to 0.70) (see Additional file 2).

The mean change in oxy-Hb concentration during the post-task period was significantly greater in the CTL group than in the ED group in four channels (Channel 10, 11, 16, and 21, all $p < 0.05$, Cohen's $d = 0.66$ to 0.78), but this difference did not remain significant after correction of significance levels by the false discovery rate method (all $p > 0.05$). Differences between the ED group and the CTL group in NIRS channels 17, 19, 20, and 22 that include the bilateral OFC did present a small effect size (Cohen's $d = 0.02$ to 0.32) (see Additional file 3).

Correlation between oxy-Hb concentration and demographic characteristics

In the ED group, the mean change in oxy-Hb concentration during the LFT in channels 1–3, 5, 6, 10, 13, and 17–20 significantly correlated with age ($r = -0.46$ to -0.75, all $p < 0.05$), the mean change in channel 13 significantly correlated with LFT performance ($r = 0.47$, $p < 0.05$), and the mean change in channels 3 and 5 significantly correlated with BDI score ($r = -0.50$ and -0.59, $p < 0.05$ and $p < 0.01$, respectively). Years of education and BMI did not correlate with the mean change in oxy-Hb concentration during the LFT in any channel. Only age remained significantly correlated with the mean change

Table 2 Participant scores on each subscale of the eating disorder inventory-2

	ED ($n = 20$)	CTL ($n = 31$)	p	Cohen's d
Drive for thinness	9.0 ± 5.7	3.7 ± 4.0	< 0.001	1.09
Bulimia	5.7 ± 6.5	1.1 ± 1.7	0.006	1.06
Body dissatisfaction	13.8 ± 5.3	8.9 ± 6.5	0.007	0.81
Ineffectiveness	14.2 ± 6.7	4.4 ± 3.1	< 0.001	1.99
Perfectionism	5.8 ± 3.8	1.1 ± 1.7	< 0.001	1.72
Interpersonal distrust	8.3 ± 4.6	3.4 ± 2.9	< 0.001	1.31
Interoceptive awareness	11.9 ± 7.7	1.4 ± 2.3	< 0.001	2.00
Maturity fears	10.4 ± 5.0	3.5 ± 2.8	< 0.001	1.78
Asceticism	7.6 ± 5.3	3.6 ± 2.2	0.008	1.05
Impulse regulation	10.3 ± 7.5	1.3 ± 2.3	< 0.001	1.74
Social insecurity	11.3 ± 4.3	5.0 ± 3.2	< 0.001	1.69

Data are expressed as mean ± standard deviation.
ED, eating disorder; CTL, control.

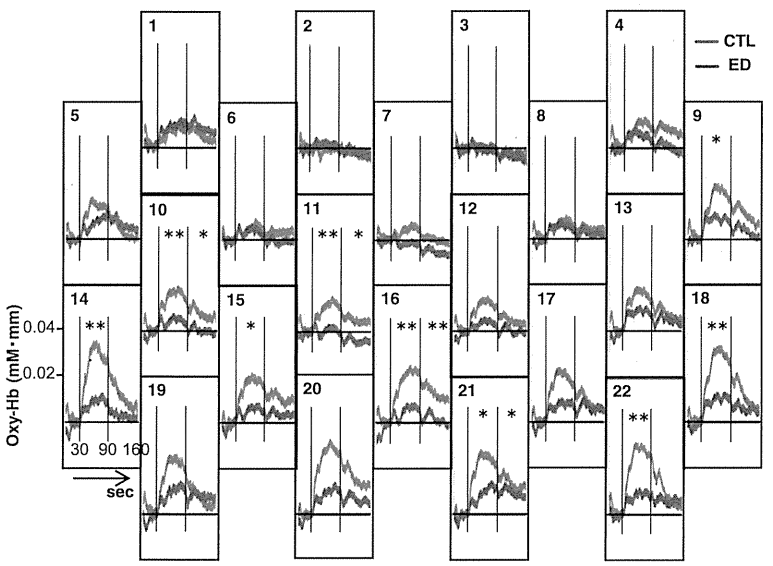

Figure 2 Grand averaged waveforms of oxygenated hemoglobin concentration (Oxy-Hb) during the 60-s letter fluency task. Blue lines represent the control group (CTL) and red lines represent patients with eating disorders (ED). Vertical lines indicate the beginning and end of the letter fluency task. *$p < 0.05$ and **$p < 0.01$ between Oxy-Hb in the ED group and Oxy-Hb in the CTL group in the task period or the post-task period, as assessed by independent *t*-test and before correction by the false discovery rate method.

in oxy-Hb concentration during the LFT in channels 1 and 5 after significance levels were corrected by the false discovery rate method ($r = -0.64$ and -0.75, $p < 0.05$ and $p < 0.01$, respectively). More detailed information is shown in an additional table [see Additional file 4].

Correlation between oxy-Hb concentration and clinical characteristics

In the CTL group, the mean change in channels 2–4 and 7 significantly correlated with BDI score ($r = -0.39$ to -0.43, $p < 0.05$). Age, years of education, BMI, and LFT performance did not correlate with the mean change in oxy-Hb concentration during the LFT in any channel. There were no significant correlations in oxy-Hb concentration during the LFT with BDI score after significance levels were corrected by the false discovery rate method (all $p > 0.05$). An additional table shows this in more detail [see Additional file 5].

In both the ED group and the CTL group, the mean change in oxy-Hb concentration during the LFT was significantly correlated with the score on the SI subscale of the EDI-2 before significance levels were corrected by the false discovery rate method (Figures 3 and 4); however, the direction of the relation was opposite in the two groups. In the ED group, the mean change in oxy-Hb concentration during the LFT in channels 12, 16, 17, and 19–22 was positively correlated with SI score ($r = 0.47$ to 0.70, all $p < 0.05$), whereas in the CTL group, the mean change in oxy-Hb concentration during the LFT in channels 1, 5, 10, 13, 17, 18, and 22 was negatively correlated with SI score ($r = -0.38$ to -0.56, all $p < 0.05$). Additional tables show these findings in more detail (see Additional files 6 and 7).

In the ED group, the mean change in oxy-Hb concentration during the LFT in channel 18 was significantly correlated with the score on the drive for thinness subscale of the EDI-2 ($r = 0.67$, $p < 0.01$), and the mean change in channel 12 was significantly correlated with the score on the bulimia subscale of the EDI-2 ($r = 0.56$, $p < 0.05$). In the CTL group, the mean change in oxy-Hb concentration during the LFT in channel 9 was significantly correlated with the score on the drive for thinness subscale of the EDI-2 ($r = -0.39$, $p < 0.05$), and the mean change in channel 4 was significantly correlated with the score on the bulimia subscale of the EDI-2 ($r = -0.46$, $p < 0.05$).

In the ED group, the correlation between the mean change in oxy-Hb concentration during the LFT and the SI score remained significant after the partial correlation analysis in channels 16, 17, and 19–22 (partial correlation coefficient = 0.62 to 0.88, $p < 0.05$), but was not significant in channel 12 ($p > 0.05$). Then significance levels were corrected by the false discovery rate method, and the correlation between the mean change in oxy-Hb concentration during the LFT and the SI score remained significant only in channel 20 and 21 (partial correlation coefficient = 0.84 and 0.88, $p < 0.05$ and $p < 0.01$, respectively). An additional table shows this in more detail (see Additional file 6). The correlation between the mean change in oxy-Hb concentration during the LFT and the

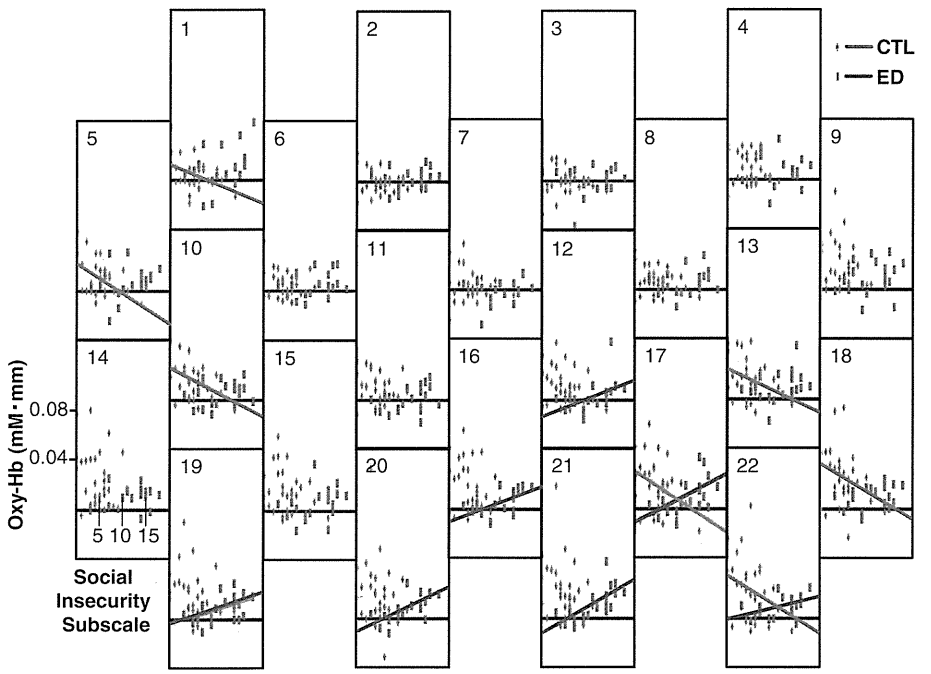

Figure 3 Relation between the social insecurity subscale and the change in oxygenated hemoglobin concentration (Oxy-Hb). Oxy-Hb during the letter fluency task was measured by each near-infrared spectroscopy channel for the control group (CTL; blue) and patients with eating disorders (ED; red). Blue and red lines represent significant correlations in the CTL and ED groups respectively.

drive for thinness subscale and the bulimia subscale score were not significant after the partial correlation analysis in channels 18 and 12 respectively.

In the CTL group, the correlation between the mean change in oxy-Hb concentration during the LFT and SI score remained significant after the partial correlation analysis in channels 5 and 10 (partial correlation coefficient = −0.61 and −0.44 respectively, $p < 0.05$), but was not significant in channels 18 and 20–22 ($p > 0.05$).

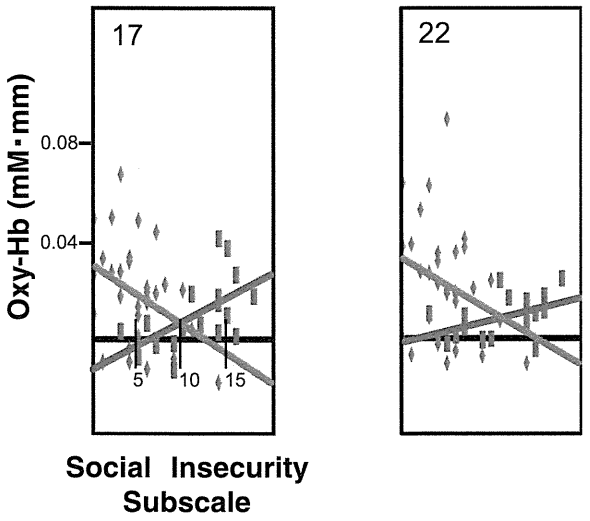

Figure 4 Enlarged graphs for channels 17 and 22.

After correction of significance levels by the false discovery rate method, the correlation between the mean change in oxy-Hb concentration during the LFT and SI score in the CTL group was not significant (all $p > 0.05$) (see Additional file 7).

Discussion

Mean change in oxy-Hb during the LFT and the post-task period

In the present study, we examined frontal cortex activity in patients with ED with extremely low body weight using hemodynamic changes measured by NIRS during an LFT. Although mean change in oxy-Hb concentration during LFT in several channels, including the bilateral OFC, did not show significant differences between the ED group and the CTL group, their effect sizes were medium-to-large. This could be due to a low statistical power to detect an effect when one is present (type II error), which is in part explained by adjustment for multiple testing and small sample size. This result suggests that the oxy-Hb concentration during LFT in the bilateral OFC tended to be lower in the ED group than in the CTL group. The result that oxy-Hb concentration during LFT in the bilateral OFC tended to be lower in the ED group than in the CTL group is consistent with our first hypothesis and previous reports [11-13].

Nagamitsu et al. [13] suggested that impairment of regional cerebrovascular reactivity might be caused by

prolonged starvation or abnormal eating behavior during the illness, and that the unchanged or less fluctuating response pattern of oxy-Hb in the prefrontal area might indicate abnormal cortical processing during cognitive activation. In the following sections, we focus on the relevance of the activity of the OFC and LFT performance and EDI-2 score to discuss whether this suggestion is consistent with our results or not.

In terms of the post-task period, we cannot compare our results with a previous report directly because the previous report use different methods for data processing called linear fitting [13]. In our study, there were no significant differences in the mean change in oxy-Hb concentration during the post-task period between the ED group and the CTL group after correction of significance levels by the false discovery rate method, and their effect sizes were small to large. It is difficult to evaluate these results because of a lack of robustness.

LFT performance, BDI, and EDI-2 score

All subscales of the EDI-2 were significantly higher in the ED group than in the CTL group, which is consistent with a previous report except for the subscale of Body Dissatisfaction [40]. Our finding that the ED group showed significantly higher scores on the BDI than the CTL group is also consistent with a previous report [41]. Several studies reported that LFT performance was significantly positively related to level of education [42-47], and that the performance of LFT fell in subjects with damage to the frontal lobe [48,49]. It is conflicting that the ED group in our study showed significantly higher LFT performance than the CTL group despite the fact that years of education were significantly lower, and oxy-Hb concentration of the bilateral OFC during the LFT tended to be smaller in the ED group than in the CTL group.

Previous NIRS reports that adopted a similar LFT protocol to our study showed that the ED group had lower frontal oxy-Hb concentration but showed almost exactly same performance on the LFT [11-13]. Nagamitsu et al. [13] mentioned that the specific patterns of oxygenation changes might indicate less supply and less demand of cerebral blood volume. A meta-analysis reported that patients with AN performed better on the LFT than CTL subjects [50]. One author suggested that this finding may be because patients with AN patients showed a higher intelligence quotient (IQ) than the CTL group [51], and LFT has been shown to have a strong relationship with IQ [52]. Even so, we cannot explain the reason that the ED group showed better performance than the CTL group on the LFT yet had a lower oxy-Hb concentration during the LFT.

Two hypotheses may help explain these findings. One hypothesis is that as a result of a malfunction in the OFC, patients with ED might have partial overactivity in other cortical brain regions such as the thalamus, parietal lobes, or temporal lobes, which are also reported to be activated during the LFT [28]. Another hypothesis is that as a result of a malfunction of the mechanisms that coordinate work and energy supply, so called "neurovascular coupling" may occur [53], and neural overactivity might occur despite low blood perfusion in the OFC in patients with ED. It was reported that in patients with ED, OFC volume was higher compared to CTL, which, in general, is supposed to reflect anxiety and high frontal activation in patients with ED [19].

Altogether, we hypothesize that overactivity in other cortical brain areas as a result of a malfunction in the OFC or neural overactivity despite low blood perfusion in the OFC might be related to high performance on the LFT and inadequate feelings of SI, for example, over-optimistic expectations. To gain evidence of the hypofrontality and better performance in patients with ED, a further study enabling measurements of the entire cortex or of neuroimaging signals of the OFC is required.

Correlation between oxy-Hb concentration and demographic characteristics

The mean BMI of patients with ED in the present study was equivalent to the lowest BMI of patients with ED included in previous NIRS studies [11-14], and lower than the BMI of patients with ED included in many studies using fMRI [54-64] or PET [65-72]. To the best of our knowledge, this study represents the first report of both brain activity and clinical features of patients with ED with extremely low body weight. As such, the results may be influenced by malnutrition. However, the BMI of patients with ED was not significantly correlated with oxy-Hb concentration during the LFT of the bilateral dorsolateral prefrontal cortex and bilateral frontopolar areas, and the mean change in oxy-Hb concentration during the LFT was significantly correlated with SI score, even after adjusting for BMI and after correction of significance levels by the false discovery rate method. These results suggest that the BMI of patients with ED may not affect the frontal activity and SI score. Further study of recovered patients with ED is needed to examine whether the observed correlations are a trait of patients with ED that remains after recovery or are associated with the state of malnutrition.

Correlation between oxy-Hb concentration and clinical characteristics

The mean change in oxy-Hb concentration during the LFT in channels 20 and 21, which include the bilateral OFC, had a strong, positive correlation with SI score in the ED group even after adjusting for age, years of education, BMI, and BDI score as well as correction of

significance levels by the false discovery rate method. In contrast, the mean change in oxy-Hb concentration during the LFT in channels 17 and 22, which includes the left OFC, had a weak negative correlation with SI score in the CTL group before adjusting for age, years of education, BMI, and BDI score, but lacked significance after adjusting for these variables. Consistent with our hypothesis, oxy-Hb concentration during the LFT of the OFC in the ED group was lower than in the CTL group, and correlations between oxy-Hb concentration during the LFT in OFC and SI were different in the ED and CTL groups.

Recent work has emphasized the role of the OFC both in value-based decision-making [73] and in signaling outcome expectancies that are crucial for changing established behavior in the face of unexpected outcomes [74]. Signaling expected outcomes could be considered a general property of the OFC [73]. The reason that oxy-Hb concentration during the LFT in OFC correlated with only SI but not with other EDI-2 subscales may be that, in our opinion, SI is directly related to expectations and other subscales are not. The CTL group had high oxy-Hb concentration in the OFC during the LFT, and there is a tendency that the higher the oxy-Hb concentration of the OFC, the lower the SI score.

We propose both that the CTL group, which showed increased oxy-Hb concentration in the OFC during the LFT, behave adaptively and exhibit value-based decision-making in the face of unexpected outcomes in complex human relationships, and that this adaptive behavior may enable them to solve problems and form good human relationships, thus enabling them to integrate in society; as a result, their SI score becomes lower.

In contrast, the ED group had low oxy-Hb concentration in the OFC during the LFT and a high SI score, and the lower the oxy-Hb concentration of the OFC, the lower the SI score. In other words, the patients with ED who have low oxy-Hb concentration of the OFC during the LFT tend not to feel SI. We propose that low oxy-Hb concentration during the LFT of the OFC means that patients with ED neither behave adaptively nor exhibit value-based decision-making in the face of unexpected outcomes in complex human relationships, and that this maladaptive behavior may inhibit the formation of human relationships, thus isolating patients with ED from society. In support of this proposal, it has been shown that patients with ED have a non-assertive interpersonal style, greater social skill difficulties, less socially effective behavior, a smaller social support network, and more difficulties using this network than CTL subjects [16]. Furthermore, OFC malfunction may mean that patients with ED are not aware of their isolation. This is supported by a report finding that although AN patients had significantly less social support than BN patients, they were satisfied with the support they received [75]. This phenomenon may relate to denial of illness that most patients with AN have, which is also associated with resistance to treatments observed in these patients [76].

A solid therapeutic relationship is recommended to overcome treatment resistance [76,77], and it might be also recommended for patients with ED who have low oxy-Hb concentration of the OFC during the LFT. Interpersonal psychotherapy that improves interpersonal functioning by enhancing communication skills in significant relationships has been reported to be an effective therapy for AN [78].

Therefore, malfunction of the OFC may underlie the maladaptive behavior of patients with ED and may represent a biological cause of the psychopathological factors of ED. To support this hypothesis, methodological improvements that can investigate relations between the function of the OFC and performance of tasks that directly induce SI are needed in future studies. In addition, a comparison of AN and BN using the same method would be of interest because there is evidence that there are functional and structural cerebral differences between BN and AN [79].

Regional hemodynamic changes in the left dorsolateral prefrontal cortex (Channel 18) were positively correlated with the drive for thinness score, and regional hemodynamic changes in the left frontopolar area (Channel 12) were positively correlated with the bulimia score. This is inconsistent with a previous study using the Japanese version of the Eating Attitude Test (EAT-26) [80,81], which reported that regional hemodynamic changes in the right frontotemporal regions negatively correlated with dieting tendency scores on the EAT-26, and regional hemodynamic changes in the left OFC negatively correlated with binge eating scores in patients with ED [12]. These discrepancies may be due to differences in the methods used to evaluate ED symptoms and/or differences in ED patient characteristics such as BMI. Further study is needed to test these possibilities. In the present study, the higher the drive for thinness score, the larger the increase in oxy-Hb concentration in the left prefrontal cortex during LFT in the ED group. However, a previous PET study reported that [18 F]-altanserin binding potential in several cortical regions, including the prefrontal cortex, was negatively related to the drive for thinness in patients with AN [82]. It is interesting that oxy-Hb concentration increased in the left prefrontal cortex of patients with ED during LFT, whereas metabolism in the same region was decreased.

Study limitations

This study has several limitations. First, the number of participants was small, and further study with more participants is required to increase the statistical power.

There was heterogeneity of ED subtypes, but each subtype had too few participants to analyze intra-group differences. Additional subjects are needed for future studies. Second, the ED group was not homogeneous in terms of comorbidity, psychotherapy, and medications, and this may have influenced the results. Third, there may be a selection bias in the CTL group such as years of education, because they were recruited from the hospital staff. Fourth, NIRS has several methodological limitations. The exact measurement point over the cortex differs across subjects according to the size of the skull and brain; therefore, the point of measurement can only be determined in a probabilistic manner. In addition, determining the exact distance of the near-infrared light emitters from light detectors that is required to calculate the change in oxy-Hb remains difficult. As brain atrophy has been seen in patients with AN [2,83-85], data from the NIRS was possibly affected due to path length factors of near-infrared light. Moreover, evaluating deep structures of the brain is not possible. The validity of NIRS measured on the forehead as a measure of functional brain activity is unknown; however, according to Takahashi et al. (2011), NIRS signals measured on the forehead during the LFT would reflect task-related changes in subcutaneous blood flow [86].

Conclusions

In conclusion, the present NIRS study is, to the best of our knowledge, the first report of the relationship between SI and brain activity in patients with ED with extremely low body weight. More frontal reactivity was associated with lower SI scores in the CTL group and less frontal reactivity was associated with lower SI scores in patients with ED with extremely low body weight. These results indicate that patients with ED with extremely low body weight had OFC malfunction and higher SI scores, which may underlie their lack of insight and social isolation. Further studies targeting larger samples of patients with ED, including those who have recovered, are necessary.

Additional files

Additional file 1: Information about comorbidities and medications received by patients with eating disorders.

Additional file 2: Mean change in oxygenated hemoglobin concentration during the letter fluency task.

Additional file 3: Mean change in oxygenated hemoglobin concentration during the post-task period.

Additional file 4: Correlation between mean change in oxygenated hemoglobin concentration during the letter fluency task and letter fluency task performance, age, Beck Depression Inventory score, years of education, and body mass index in the eating disorders group.

Additional file 5: Correlation between mean change in oxygenated hemoglobin concentration during the letter fluency task an letter fluency task performance, age, Beck Depression Inventory score, years of education, and body mass index in the control group.

Additional file 6: Correlation between the mean change in oxygenated hemoglobin concentration during the letter fluency task and the social insecurity score in the eating disorder group.

Additional file 7: Correlation between the mean change in oxygenated hemoglobin concentration during the letter fluency task and the social insecurity score in the control group.

Competing interests

The authors declare that they have no conflicts of interest.

Authors' contributions

HK, ST, and NO conceived and designed the experiments. HK, KK, ST, MI, NK, and KN performed the experiments. HK, KK, NK, MA, TI, and NO analyzed the data. YN contributed reagents/materials/analysis tools. HK, KK, NK, BA, TI, and NO wrote the paper. All authors read and approved the final manuscript.

Acknowledgements

We wish to offer our sincere thanks to both the patients and the healthy volunteers who participated in our study. This work was done at Department of Psychiatry, Nagoya University Graduate School of Medicine. The summary of this study was presented at the 11th World Congress of Biological Psychiatry, in 2013, Japan. Funding for this study was provided by research grants from the Ministry of Education, Culture, Sports, Science and Technology of Japan (Grant-in-Aid for Scientific Research on Innovative Areas 23118004 "Adolescent Mind & Self-Regulation"); JSPS KAKENHI Grant Number 22591283; the Ministry of Health, Labor and Welfare of Japan and The Academic Frontier Project for Private Universities, Comparative Cognitive Science Institutes, Meijo University.

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Received: 24 December 2013 Accepted: 5 June 2014
Published: 12 June 2014

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doi:10.1186/1471-244X-14-173
Cite this article as: Katayama *et al.*: Social insecurity in relation to orbitofrontal activity in patients with eating disorders: a near-infrared spectroscopy study. *BMC Psychiatry* 2014 14:173.

Progress in Neuro-Psychopharmacology & Biological Psychiatry 56 (2015) 11–17

Contents lists available at ScienceDirect

Progress in Neuro-Psychopharmacology & Biological Psychiatry

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

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ARTICLE INFO

Article history:
Received 4 June 2014
Received in revised form 10 July 2014
Accepted 14 July 2014
Available online 1 August 2014

Keywords:
Cavum septi pellucidi
DISC1
Neurodevelopment
Orbitofrontal cortex
Schizophrenia

ABSTRACT

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

1. Introduction

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

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

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

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http://dx.doi.org/10.1016/j.pnpbp.2014.07.005
0278-5846/

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

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

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

2. Methods

2.1. Subjects

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

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

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

2.2. SNP genotyping

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

2.3. MRI procedures

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

2.4. Assessment of the neurodevelopmental markers

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

2.4.1. Midline brain structures

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

2.4.2. Olfactory sulcus depth

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

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

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

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

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

2.4.3. OFC sulcogyral pattern classification

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

2.5. Statistical analysis

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

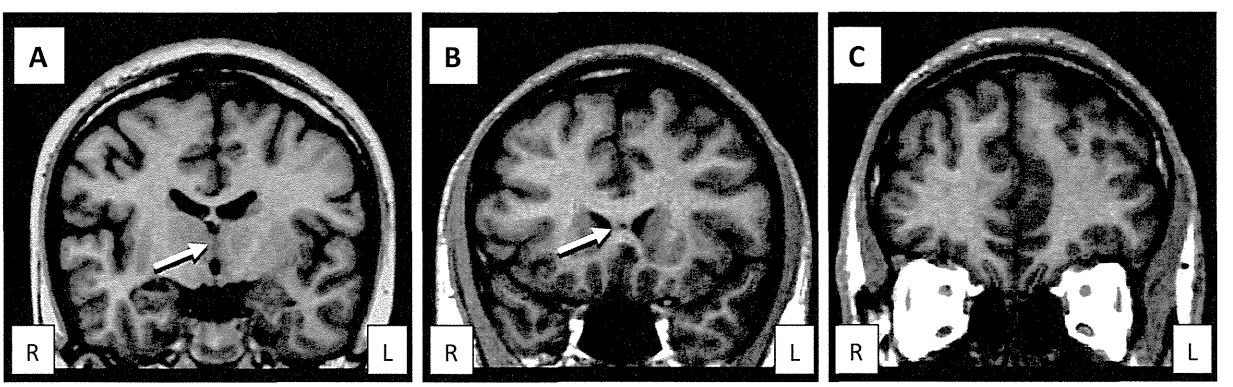

Fig. 1. Sample coronal slices showing the adhesio interthalamica (A, arrow), cavum septum pellucidum (B, arrow), and olfactory sulcus (C, colored in red). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

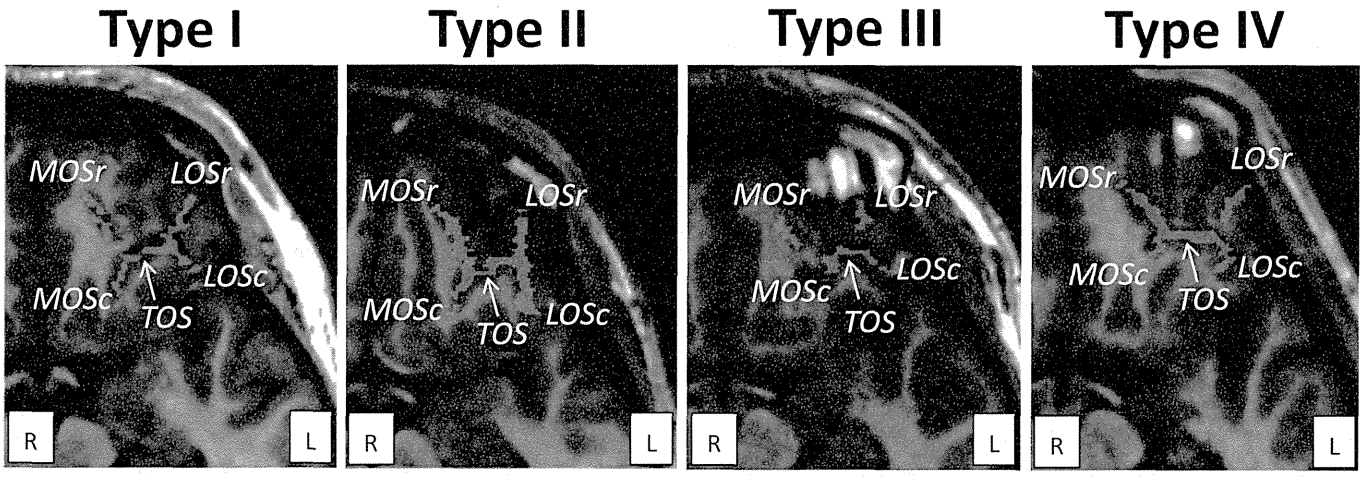

Fig. 2. Classification of the orbitofrontal sulcogyral pattern on an axial view. Note that these sulci were identified using orthogonal views in three directions and colored on consecutive coronal slices. c, caudal portion; LOS, lateral orbital sulcus; MOS, medial orbital sulcus; r, rostral portion;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

3. Results

3.1. Sample characteristics and genotyping results

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

3.2. Diagnosis effect on the neurodevelopmental markers

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

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

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

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

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

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

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

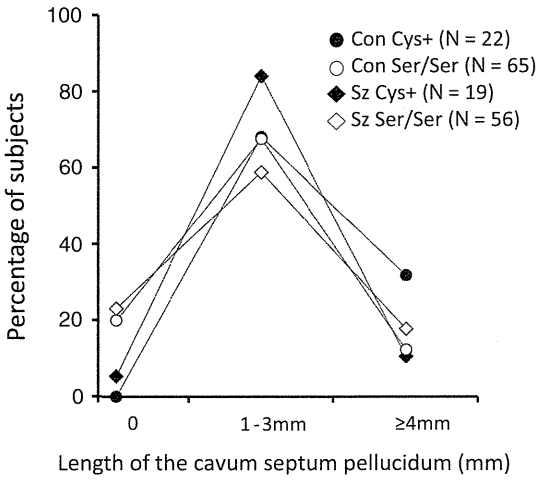

Fig. 3. Length of the cavum septum pellucidum (CSP) in the study participants. Con, controls; Cys+, Cys carriers; Ser/Ser, Ser homozygotes; Sz, schizophrenia.

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

3.4. Neurodevelopmental markers and clinical variables in schizophrenia

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

3.5. Possible relation between neurodevelopmental markers in schizophrenia

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

3.6. Possible interaction between DISC1 and YWHAE on neurodevelopmental markers

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

4. Discussion

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

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

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

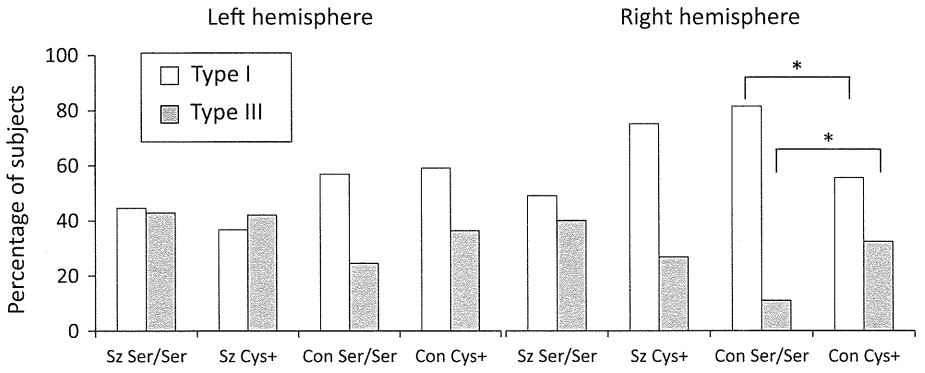

Fig. 4. Distribution of the orbitofrontal sulcogyral pattern (Type I *vs* Type III) in each diagnostic group. Con, controls; Cys+, Cys carriers; Ser/Ser, Ser homozygotes; Sz, schizophrenia. *$p < 0.05$.

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

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

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

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

5. Conclusion

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

Acknowledgments

This research was supported in part by Grants-in-Aid for Scientific Research (C) (Nos. 22591275, 24591699, and 26461739) and Grant-in-Aid for Scientific Research (B) (No. 24390281) from the Japanese Society for the Promotion of Science, Health and Labour Sciences Research Grants (Comprehensive Research on Disability, Health and Welfare, H23-Seishin-Ippan-002 and H23-Seishin-Ippan-009), a Research Grant from the JSPS Asian Core Program, and a Grant from Research Group for Schizophrenia, Japan. It was also supported by a Grant-in-Aid for "Integrated research on neuropsychiatric disorders" carried out under the Strategic Research Program for Brain Sciences by the Ministry of Education, Culture, Sports, Science and Technology of Japan and Grant-in-Aid for Scientific Research on Innovative Areas, "Glial assembly: a new regulatory machinery of brain function and disorders". We would like to thank Ms. Hiroko Itoh, who assisted in the genomic DNA extraction for all the participants in this study.

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