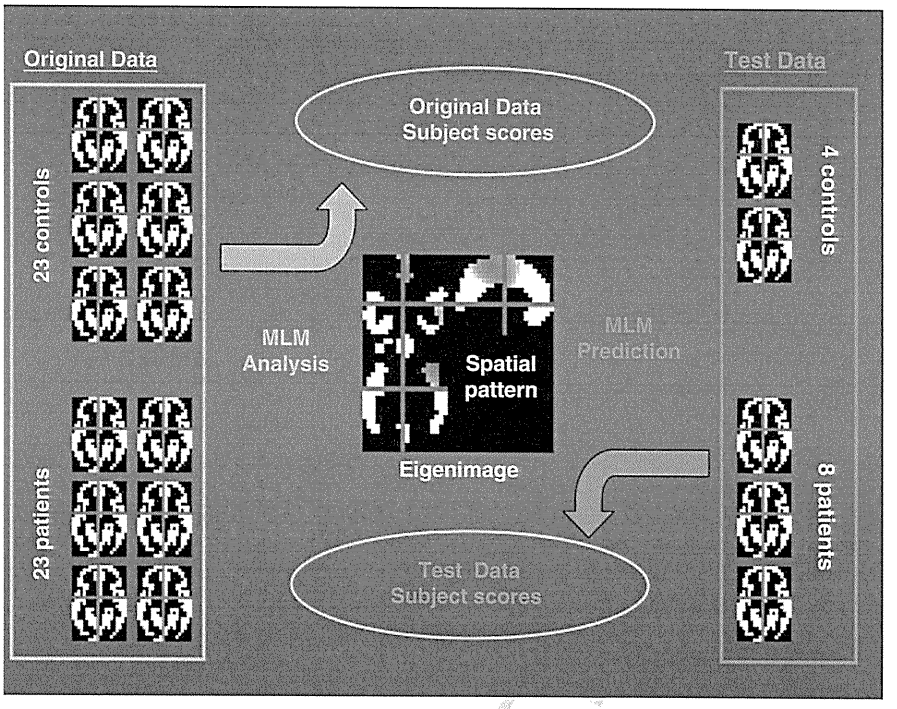

Fig. 7.10 The general scheme of discriminant function analysis of multivariate linear model (MLM) *using the* statistical parametric mapping [57]

treatment status [57], application of this method to drug-naïve subjects with first episode schizophrenia and those at the prodromal stage is likely to facilitate early intervention into the illness.

In conclusion, the utilization of neuroimaging methods enhances spatial resolution of electrophysiological evaluation, e.g. ERPs, which would provide feasible and reliable biomarkers, objective assessments of psychosis and cognition, and predictive measures of treatment response, and facilitate early diagnosis and intervention of schizophrenia.

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Different hemodynamic response patterns in the prefrontal cortical sub-regions according to the clinical stages of psychosis

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ABSTRACT

Background: Symptomatic and functional outcomes in schizophrenia are associated with the duration of untreated psychosis. However, no candidate biomarkers have been adopted in clinical settings. Multichannel near-infrared spectroscopy (NIRS), which can easily and noninvasively measure hemodynamics over the prefrontal cortex, is a candidate instrument for clinical use.
Aims: We intended to explore prefrontal dysfunction among individuals at different clinical stages, including ultra-high-risk (UHR), first-episode psychosis (FEP), and chronic schizophrenia (ChSZ), compared to healthy subjects.
Method: Twenty-two UHR subjects, 27 patients with FEP, 38 patients with ChSZ, and 30 healthy subjects participated. We measured hemodynamic changes during a block-designed letter fluency task using multichannel NIRS instruments.
Results: We found that the activations of the bilateral ventrolateral prefrontal cortex, and the fronto-polar and anterior parts of the temporal cortical regions in the UHR group were lower than those of the controls, but similar to those of the FEP and ChSZ groups. However, the activations in the bilateral dorsolateral prefrontal cortex regions decrease with advancing clinical stage.
Conclusions: To the best of our knowledge, this is the first study directly comparing differences in hemodynamic changes with respect to the 3 clinical stages of psychosis. Furthermore, this study also demonstrates different patterns of impairment according to the progression of clinical stages using NIRS instruments. NIRS measurements for UHR and FEP individuals may be candidate biomarkers for the early detection of the clinical stages of psychosis.
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1. Introduction

Retrospective studies suggest that patients with schizophrenia have prodromal symptoms before psychotic symptoms such as depression, anxiety, attenuated psychotic symptoms, and functional decline fully emerge (Yung et al., 1998; Hafner, 2000; Klosterkotter et al., 2001). A recent review suggests that patients with short durations of untreated psychosis (DUP) have better symptomatic and functional outcomes throughout their lives (Perkins et al., 2005). Therefore, supportive services and programs have been developed toward early detection and intervention for people exhibiting prodromal symptoms to reduce DUP and prevent the transition to psychosis (French et al., 2007; Yung and McGorry, 2007; Joa et al., 2008). To screen for high-risk individuals before developing psychosis, called ultra-high-risk (UHR) individuals, various clinical diagnostic tools have been developed in the last 20 years (Yung et al., 1998; Miller et al., 1999; Klosterkotter et al., 2001; Yung et al., 2004; Yung and McGorry, 2007; Cannon et al., 2008). The rate of transition to psychosis is about 20–30% per year for help seekers who meet the UHR criteria according to these assessment tools (Yung et al., 2004; Cannon et al., 2008). These clinical assessments are helpful but insufficient for early detection and intervention; therefore, more objective tools for detection are needed to help high-risk individuals. Several possible biomarkers for improving the predictive value for developing psychosis are reported in structural MRI studies (Fornito et al., 2008; Koutsouleris et al., 2009); however, no candidate biomarkers have been adopted in clinical settings. MRI instruments have an advantage in spatial resolution; this advantage has substantially contributed to the anatomical and functional clarification of psychiatric disorders. However, routine and repetitive MRI use for patients with psychiatric disorders presents difficulties due to increased costs, greater noise, and the restricted position required during testing.

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Near-infrared spectroscopy (NIRS), which can easily and noninvasively measure hemodynamics over the surface of the prefrontal cortex, is a candidate instrument for clinical use. NIRS instruments are also small and convenient such that they can be easily moved almost everywhere, including schools and care units. Our previous NIRS studies suggest that patients with chronic schizophrenia (ChSZ) have impaired activity and characteristic waveform patterns over the prefrontal cortical regions during a letter fluency task (LFT) (Suto et al., 2004; Takizawa et al., 2008). On the basis of these results, the Health, Labour, and Welfare Ministry in Japan approved the NIRS instrument as a diagnostic support system for patients with schizophrenia, major depression, and bipolar disorder. However, little is known about how the NIRS signals of pre- and postpsychotic individuals change; thus, we assumed that the activities in the prefrontal cortex might be different at each clinical stage. If we identify these differences, the NIRS system may be a potential candidate biomarker for objectively detecting and evaluating young people experiencing various symptoms and impaired functions.

The aim of this study is to explore the prefrontal dysfunction of UHR help seekers and patients with first-episode psychosis (FEP) compared to controls during a block-designed LFT, using multichannel NIRS instruments. In addition, if we succeed in early detection and intervention for young patients, they would be able to preserve their functions during the chronic phase. Therefore, we compared patients with ChSZ matched for age-at-onset and DUP to patients with FEP.

2. Method and materials

2.1. Participants

A total of 117 Japanese individuals participated in this study: 22 subjects who fulfilled the UHR criteria, 27 patients with FEP, 38 patients with ChSZ, and 30 healthy subjects (HC group) as controls (Table 1). All participants were recruited from the University of Tokyo Hospital and the Tokyo Metropolitan Matsuzawa Hospital. All UHR individuals and most patients with FEP were help seekers registered at the outpatient unit specialized for early intervention in the University of Tokyo Hospital. The route of participation was via internet homepage (http://plaza.umin.ac.jp/arms-ut), usual outpatient and inpatient units, the University of Tokyo Health Service Center, and introductions from other psychiatry clinics. All subjects gave written informed consent to the ethical committee of the Faculty of Medicine, University of Tokyo (approval No. 630-5, 2226-1), and the ethical committee of the Tokyo Metropolitan Matsuzawa Hospital (approval No. 20) after a complete explanation of this study and in accordance with the Declaration of Helsinki.

Upon entry, the UHR individuals were between 15 and 30 years of age. We used the Structured Interview for Prodromal Symptoms (SIPS) as the UHR criteria, which consists of 3 criteria: attenuated psychotic symptoms (APS), brief intermittent psychotic symptoms (BIPS), or genetic risk and deterioration (GRD) (Miller et al., 1999; Kobayashi et al., 2007). APS correspond to individuals who exhibited onset or worsened subthreshold psychotic symptoms within 12 months but not psychotic severity. BIPS correspond to individuals who had psychotic symptoms within 3 months but with a limited duration and frequency such that they were not at all or only slightly influenced by their symptoms and did not meet the psychotic episode criteria according to the DSM-IV criteria (American Psychiatric Association, 1997). GRD corresponds to individuals whose functioning had deteriorated in the previous 12 months as defined by a 30% or more decrease in the GAF score (American Psychiatric Association, 1994) as well as those who also had one or more first-degree relatives diagnosed with psychosis and/or schizotypal personality disorder according to the DSM-IV criteria.

We defined the first episode as follows: age 15–40 years, no history of antipsychotic drug treatments for more than 16 cumulative weeks, and continuous psychotic symptoms within the past 60 months. The exclusion criteria for all groups were neurological illness, traumatic brain injury with any known cognitive consequences or loss of consciousness for more than 5 min, a history of electroconvulsive therapy, low premorbid IQ (below 70), and previous alcohol abuse or addiction. For the HC group, we used the Mini-International Neuropsychiatric Interview (Sheehan et al., 1998) to rule out psychiatric disorders and also excluded participants with first-degree relative(s) with psychotic disorders.

The problem of the use of illegal substances (e.g., cannabis) among young people shows that there are still a small number of young individuals who have experienced using such drugs in Japan. Therefore, we adopted previous continuous substance use as an exclusion criterion in this study. Other research in various domains suggests a relationship between schizophrenia and pervasive developmental disorder. Therefore, we also intended to develop biomarkers related to early detection and prediction for psychosis and focused on the pathophysiological differences between these disorders. Although the basis of pervasive developmental disorder comprises a spectrum from normality to disorder and because some cases are hard to diagnose, we also excluded

Table 1
Demographic characteristics of study participants.

	Controls		UHR		FEP		ChSZ		*p* value	*F* value
		SD		SD		SD		SD		
n (male:female)[a]	30 (17:13)		22 (13:9)		27 (18:9)		38 (22:16)		=0.815	
Age (y) [b]	24.3	4.8	21.6	3.7	25.2	7.0	31.3	6.1	<0.001[c]	17.0
Premorbid IQ[b]	107.6	8.6	106.6	10.1	106.5	9.5	103.4	10.6	=0.311	1.20
Letter numbers[b]	16.2	4.8	14.6	6.1	12.7	5.2	14.3	4.1	=0.083	2.28
PANSS Positive[b]	N.A.	N.A.	14.8	3.8	15.3	4.7	14.8	5.2	=0.891	0.116
Negative[b]	N.A.	N.A.	19.3	6.5	19.8	6.1	18.7	6.3	=0.777	0.253
General[b]	N.A.	N.A.	35.8	9.0	33.4	8.7	35.8	7.8	=0.494	0.711
GAF[b]	N.A.	N.A.	44.9	12.2	41.3	12.4	49.1	11.0	=0.034[d]	3.52
Age at onset (y)	N.A.	N.A.	N.A.	N.A.	24.4	6.2	23.5	6.4	=0.906	
DUP (w)	N.A.	N.A.	N.A.	N.A.	28.9	52.4	27.0	41.1	=0.794	
DOM (m)	N.A.	N.A.	N.A.	N.A.	1.8	1.2	94.6	60.2	<0.001[d]	
CP (mg)[b]	N.A.	N.A.	77	179	630	501	667	563	<0.001[e]	12.1
Diazepam (mg)[b]	N.A.	N.A.	3.6	5.3	12.7	10.3	13.5	19.0	=0.027[e]	3.77
Biperiden (mg)[b]	N.A.	N.A.	0.1	0.4	2.4	3.9	3.1	2.2	<0.001[e]	9.43

Significant group differences are shown to the right. [a] Chi-square test, and [b] one-way ANOVA and post hoc Tukey–Welsch tests were used for testing group differences. Otherwise, *t*-tests were used. $p<0.05$ was considered significant. [c] HC, UHR, FEP<ChSZ, [d] FEP<ChSZ, [e] UHR<FEP, ChSZ.
Abbreviations: IQ, intelligence quotient; PANSS, positive and negative symptom scale; GAF, global assessment of functioning; DUP, duration of untreated psychosis; DOM, duration of medication for psychosis; CP, chlorpromazine; pt., participant.

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participants who were clearly diagnosed with autistic disorder according to the DSM-IV criteria. Well-practiced psychiatrists (S.K. and Y.T.) took detailed clinical histories from the subjects themselves and their family members, and diagnosed UHR individuals according to the SIPS criteria as well as first-episode psychosis and schizophrenia according to the DSM-IV criteria during measurement (Table 2).

The participants in the HC group were matched by age to the UHR and FEP groups, and by sex to the other 3 groups in this study. The participants in the ChSZ group were matched by premorbid IQ (Matsuoka et al., 2006; Matsuoka and Kim, 2006), age at onset, and DUP to the FEP group to estimate the long-term outcomes of the effectiveness of early detection and intervention (average duration of medication for psychosis [DOM]: 7.9 years).

The participants, except for the ChSZ group, had never been analyzed before. However, 22 out of the 38 patients with ChSZ also participated in our previous studies (Takizawa et al., 2008, 2009).

The participants in the UHR, FEP, and ChSZ groups were assessed for their functioning and symptoms, using the Global Assessment of Functioning (GAF) (American Psychiatric Association, 1994) and the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987) on the same day as the NIRS measurements. If they took any antipsychotics, anxiolytics, and/or antiparkinsonian agents, we calculated the chlorpromazine, diazepam, and biperiden equivalent doses, respectively.

2.2. Letter fluency task

We used the 160-s block-designed LFT, which is well adapted to NIRS measurement as an activation task (Takizawa et al., 2008, 2009). Briefly, in the 60-s task period, a participant was instructed to say aloud as many words that started with a phonological syllable given from a computer as possible when he/she heard it. The task period was split into 3 subperiods, and the instructed syllables consisted of 9 syllables (first, /to/, /a/, or /na/; second, /i/, /ki/, or /se/; third, /ta/, /o/, or /ha/) and changed every 20 s so that the participant avoided being silent. In the 30-s pretask and 70-s posttask periods, the participant was instructed to say Japanese vowels (/a/, /i/, /u/, /e/, and /o/) aloud repeatedly to control and remove task-related motion artifacts when he/she heard a start command from the computer. We recorded the total number of correct words the participant generated during the task period as the task performance.

Table 2
Number of diagnoses in UHR individuals according to the SIPS as well as patients with FEP and ChSZ according to the DSM-IV at their measurement points.

Diagnosis	Number
UHR	
BIPS	1
APS	15
GRDS	1
BIPS + APS	1
APS + GRDS	4
FEP	
295.1 Schizophrenia, disorganized type	2
295.2 Schizophrenia, catatonic type	0
295.3 Schizophrenia, paranoid type	15
295.4 Schizophreniform disorder	6
295.7 Schizoaffective disorder	0
295.9 Schizophrenia, undifferentiated type	2
297.1 Delusional disorder	1
298.8 Brief psychotic disorder	1
298.9 Psychotic disorder not otherwise specified	0
ChSZ	
295.1 Schizophrenia, disorganized type	7
295.2 Schizophrenia, catatonic type	2
295.3 Schizophrenia, paranoid type	15
295.6 Schizophrenia, Residual type	4
295.7 Schizoaffective disorder	1
295.9 Schizophrenia, undifferentiated type	9

Abbreviations: BIPS, brief intermittent psychotic symptoms; APS, attenuated psychotic symptoms; GRDS genetic risk and deterioration.

2.3. NIRS instrument

We used a 52-channel NIRS instrument (ETG-4000; Hitachi Medical Co., Tokyo, Japan) for measuring hemoglobin changes. The same instrument was used in our previous studies (Suto et al., 2004; Takizawa et al., 2008, 2009) where the NIRS probe attachments were thermoplastic 3 × 11 shells set with 52 fixed channels (Fig. 1); the lowest probe line was set along the Fp1–Fp2 line defined by the international 10–20 system used in electroencephalography. This probe arrangement can measure hemoglobin changes in the approximate surface regions bilaterally in the dorsolateral prefrontal cortex (DLPFC; Brodmann's area [BA] 9 and 46), ventrolateral prefrontal cortex (VLPFC; BA 44, 45, and 47), fronto-polar (BA 10), and anterior part of the temporal cortex (aTC; BA 21 and 22) (Fig. 1). The 52 measuring areas are labeled from the right-superior (ch1) to the left-inferior (ch52).

Participants only needed to sit on a chair in a relaxed state with her/his eyes open and the cap with thermoplastic attachments of the NIRS probes on her/his head. To minimize motion artifacts, we instructed them to refrain from physical movement such as head motions and strong biting during the measurement.

The theoretical methodology regarding hemoglobin concentration measurement by NIRS instruments is described in detail elsewhere (Takizawa et al., 2008). In brief, the NIRS instrument measures relative changes in [oxy-Hb] and [deoxy-Hb] using 2 wavelengths (695 and 830 nm) of infrared light (indicated as mM) on the basis of the Beer–Lambert law. We could not measure the path length because each participant had a different path length from the scalp to the cerebral cortex; therefore, we recorded the relative values of hemoglobin concentrations indicated by mM·mm.

The distance between pairs of source-detector probes was set at 3.0 cm. We defined each measurement area between pairs of source-detector probes as one "channel," which was enough to measure the points at a depth of 20–30 mm from the scalp, which corresponded to the surface of the cerebral cortex. Because the NIRS signal was occasionally unstable at the start of measurements due to technical issues and/or a participant's tense state, the acquired mean values across the last 10 s of the pretask period and the last 5 s of the posttask period were determined as the pre- and posttask baselines, respectively; a linear fitting was performed on the basis of the data between the 2 baselines. Next, although the time resolution of the NIRS signal was 0.1 s, we set the moving average window at 5 s to remove short-term artifacts.

Regardless of these artifact rejection methods, visible artifact waveforms sometimes remained. Thus, we used a newer version of the same computer program used in our previous studies that rejects a channel when it has a visible artifact waveform. Because we excluded the rejected channels from further analysis, the number of available channels varied among individuals (HC: number of channels, 37–52 [mean, 49.8; SD, 3.0]; UHR: number of channels, 43–52 [mean, 49.7; SD, 2.3]; FEP: number of channels, 43–52 [mean, 50.7; SD, 2.5]; ChSZ: number of channels, 40–52 [mean, 50.2; SD, 3.3]; n.s.).

Finally, we localized the estimated cortical regions at each channel by using a virtual registration method shown in Fig. 1 and at http://brain.job.affrc.go.jp/wordpress/ (Tsuzuki et al., 2007; Shattuck et al., 2008).

2.4. Statistical analysis

First, we tested the difference from the pretask baseline to the task period in the controls using *t*-tests for every channel. Since we performed 52 *t*-tests for each channel, we adopted the false discovery rate (FDR) method to correct multiple comparisons. We set the value specifying the maximum FDR to 0.05 so that there were no more than 5% false positives on average (Singh and Dan, 2006). We then tested the

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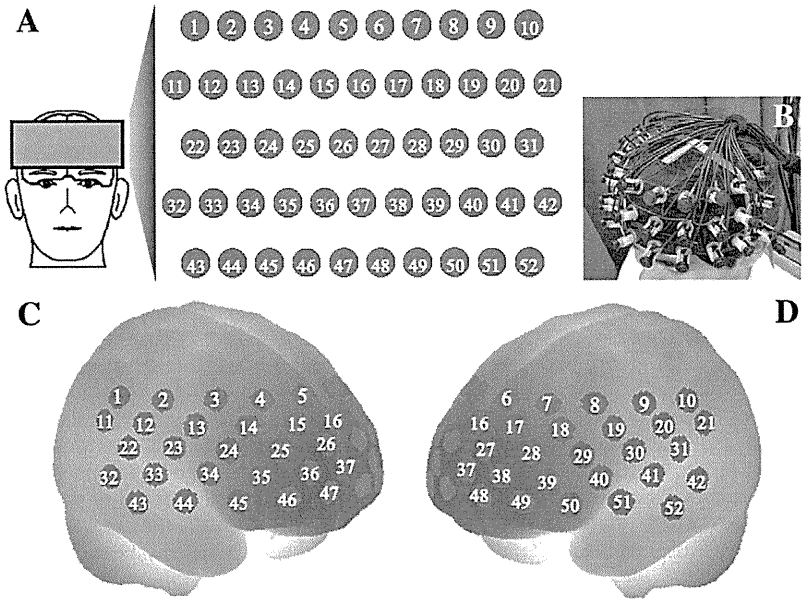

Fig. 1. Probe settings and estimated cortical regions of the 52-channel near-infrared spectroscopy (NIRS). A: 2-D topography with all channel numbers. B: probe settings with 3 × 11 thermoplastic shells in a left-anterior view. C, D: estimated cortical regions using the virtual registration method in right-anterior (C) and left-anterior (D) views. The channel numbers are indicated above the estimated cortical regions.

difference in task performance among the 4 groups by one-way analysis of variance (ANOVA); if the task performances were significantly different among the groups, we analyzed [oxy-Hb] changes using task performances as a covariate. We tested the average [oxy-Hb] changes in the task period among the 4 groups by one-way ANOVA for each channel, adopting the FDR method. Post hoc Tukey–Welsch tests were performed on these significant channels. We also investigated correlations between [oxy-Hb] changes, premorbid IQ, GAF, and PANSS scores, and task performance for each group by Pearson's correlation coefficient on whole channels, adopting the FDR method. All analyses in this study were conducted using SPSS 17.0 J (SPSS Inc., Chicago, IL, USA).

3. Results

Among the UHR, FEP, and ChSZ groups, there were no differences in premorbid IQ, GAF, and PANSS total scores or in PANSS positive, negative, and general subscores (Table 1). Sixteen UHR individuals and 2 patients with FEP were antipsychotics naïve, and 8 UHR individuals and 1 FEP patient were FEP drug naïve at their measurement points. The chlorpromazine, diazepam, and biperiden equivalent doses of the FEP group did not differ from those of the ChSZ group. Letter numbers as task performances were not significantly different among the 4 groups ($F=2.28$, $p=0.083$).

3.1. Mean [oxy-Hb] changes during the task period

Time courses of [oxy-Hb] changes are shown in Fig. 2. From the pretask baseline to the task period, the controls showed significantly increased activation in all 52 channels (FDR-corrected $p=0.001$ to 0.0044). Among all groups, we found significant main effects for [oxy-Hb] changes in 50 channels (except ch2 and 42; $F=2.76$ to 11.55, FDR-corrected $p=0.001$ to 0.046). Post hoc Tukey–Welsch tests revealed that the UHR, FEP, and ChSZ groups had significantly smaller activations than the HC group at 18 channels (ch11, 14, 17, 21, 22, 24, 31–33, 37, 40, 41, 43, 45, 48, 49, 51, and 52; Fig. 3, top) that formed a cluster of channels approximately located at the bilateral VLPFC, bilateral fronto-polar, and bilateral aTC regions. On the other hand, the activations in the bilateral DLPFC and right VLPFC regions became smaller with advancing clinical stage (Fig. 3, middle and bottom). Although we adopted the artifact rejection method for each channel, the demographic characteristics were still matched in all channels.

3.2. Correlation between [oxy-Hb] changes and demographic characteristics

In the FEP group, we found significant positive correlations between mean [oxy-Hb] changes during the task period and PANSS positive scores at ch43 (FDR-corrected $p=0.001$, Pearson's $r=0.615$), and marginally significant correlations at ch32 ($p=0.0058$, $r=0.546$). We also found positive correlations between [oxy-Hb] changes and PANSS negative scores at ch33 and 43 (FDR-corrected $p=0.001$ to 0.002; $r=0.606$ and 0.759, respectively), and marginally significant correlations at ch8 ($p=0.0033$, $r=0.575$) and ch32 ($p=0.0037$, $r=0.570$). Ch32, 33, and 43 formed a cluster of channels that was located approximately at the right temporal cortex region. We also found marginally significant positive correlations between [oxy-Hb] changes and PANSS general scores at ch32 ($p=0.0012$, $r=0.621$) and ch43 ($p=0.0012$, $r=0.602$).

In our previous study, we found significant positive correlations between [oxy-Hb] changes and GAF scores (Takizawa et al., 2008). In this study, we replicated a similar trend at ch26 ($p=0.047$, $r=0.325$) and ch37 ($p=0.035$, $r=0.344$) in the ChSZ group.

In the UHR group, we found no significant channel between antipsychotics-naïve and medicated individuals, and no channel between drug-naïve and medicated individuals.

4. Discussion

Our results show that the task-related hemoglobin changes over the prefrontal cortical surface areas and the anterior part of the temporal cortex regions gradually decrease with advancing clinical stages of psychosis (i.e., UHR, FEP, and ChSZ). The activation in the UHR group was lower than that of the controls but not significantly different when compared to the reduced activations in the bilateral VLPFC, bilateral fronto-polar, and bilateral aTC regions in the FEP and ChSZ groups. On the contrary, the activations in the bilateral DLPFC regions decreased with advancing clinical stages of psychosis. Correlational analyses show that the activations in the right temporal cortex region are greater with severe

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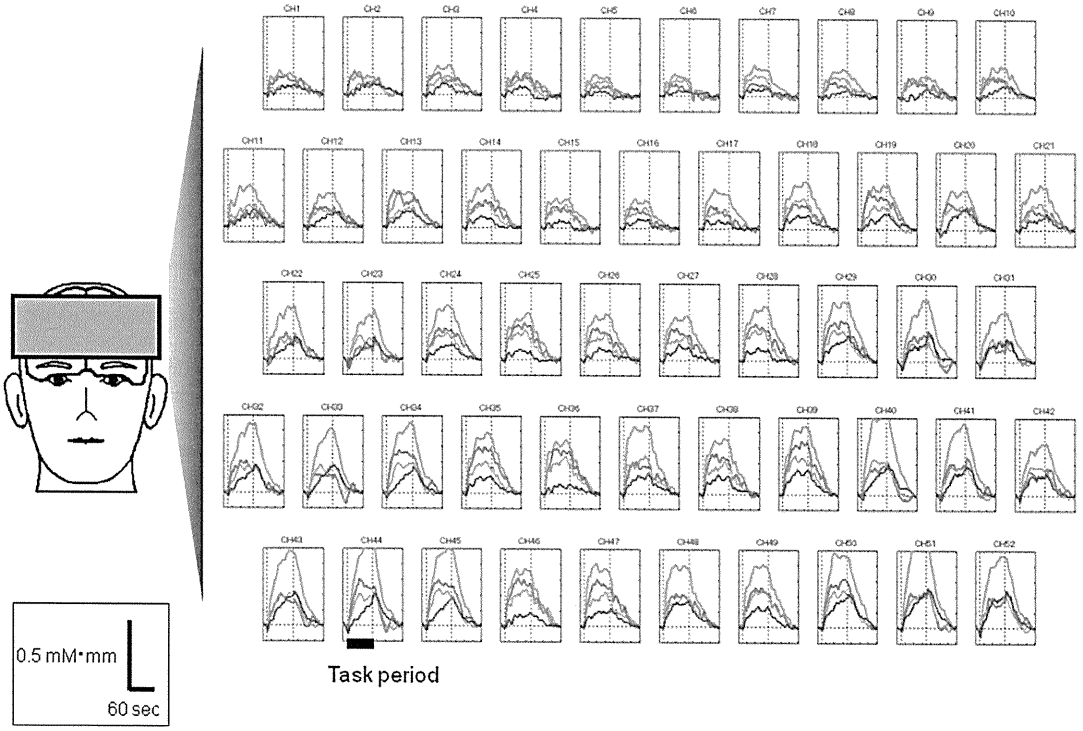

Fig. 2. Time-course [oxy-Hb] changes in the control, UHR, FEP, and ChSZ groups (light green, red, orange, and blue, respectively). The 52 measurement areas are labeled ch1–52 from the right-superior to the left-inferior. The task period is shown between the vertical dash lines (also indicated by a black bar).

negative symptoms in the FEP group. To the best of our knowledge, this is the first study that directly compares differences in hemodynamic activation changes with respect to the 3 clinical stages of psychosis and healthy controls and demonstrates different patterns of impairment according to the progression of clinical stages using multichannel NIRS instruments.

4.1. Different hemodynamic response patterns in the prefrontal cortical subregion

Our results replicate previous results in which controls exhibit a task-related hemoglobin increase over the prefrontal cortical surface areas and in the bilateral aTC regions (Suto et al., 2004; Takizawa et al.,

	Channels
Controls > UHR, FEP, ChSZ	11,14,17,21,22,24, 31-33,37,40,41,43,45, 48,49,51,52
Controls > UHR, ChSZ	7,23
Controls >FEP, ChSZ	26,27,30,44,50
Controls > UHR, FEP, ChSZ FEP > ChSZ	18
Controls > UHR, FEP, ChSZ UHR, FEP > ChSZ	28
Controls > FEP, ChSZ UHR > ChSZ	13,19,29,34,35,38,39
Controls, FEP > ChSZ	8,9
HC, UHR, FEP > ChSZ	25,36,46

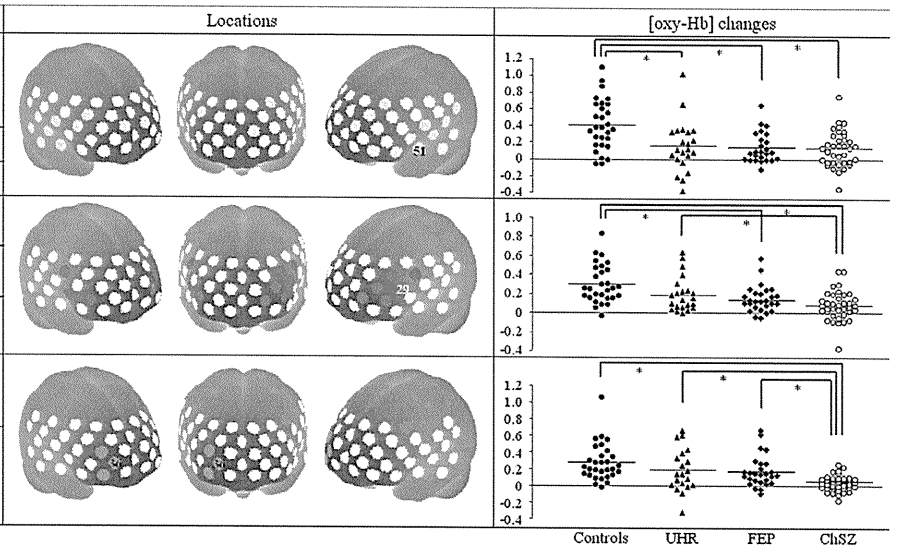

Fig. 3. 3D topographic maps of different hemodynamic response patterns in the prefrontal cortical sub-regions according to the clinical stages of psychosis. Impairment even in the UHR group, gradual impairment, and impairment only in the ChSZ group were illustrated with yellow, red, and green, respectively. Left: The channels showing significant mean [oxy-Hb] changes during the task period by post hoc Tukey–Welsch tests. Center: 3D topographic maps in which clusters of significant channels are divided into 3 different patterns: impairment in the UHR group but unchanged in the ChSZ group (top, yellow), gradual impairment according to progression of clinical stages (middle, red), impairment in the ChSZ stage but statistically unchanged until the FEP stage (bottom, green). Right: Dot plots of the mean [oxy-Hb] changes at typical channels (top, ch51; middle, ch29; bottom, ch36) during the task period. Bars show the averages of the [oxy-Hb] changes and asterisks show significant results between groups by post hoc Tukey–Welsch tests ($p<0.05$).

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2008, 2009). A LFT requires retrieval from long-term memory, verbal working memory, executive function, and inhibition of repetitive and inappropriate responses, which are assumed to be major functions of the prefrontal cortex.

Our study shows the different impairment patterns among the clinical stages of psychosis according to the cortical region (Fig. 3). These results were still significant when age was considered a covariate. The hemodynamic changes in the bilateral VLPFC, bilateral fronto-polar, and bilateral aTC regions were significantly smaller in the UHR group than in the controls but not than the FEP and ChSZ groups (Fig. 3, top). Although there are few functional neuroimaging studies that directly compare psychotic groups at 3 or more clinical stages, a functional MRI study during an odd-ball task shows that the UHR group has intermediate BOLD signal changes and lower activation in the inferior frontal gyrus than controls, but higher than those of the FEP and ChSZ groups (Morey et al., 2005). Another functional MRI study using an event-related LFT task also shows intermediate BOLD patterns in the left-inferior frontal gyrus (Broome et al., 2009). However, the results of hypo-/hyperactivation are inconsistent, owing to variable characteristics of the study participants and/or task settings (Keshavan et al., 2008; Minzenberg et al., 2009). A functional MRI and PET combination study using the same event-related LFT task shows that UHR individuals have greater activation in the left-inferior frontal gyrus than controls; furthermore, these activations are positively correlated with striatal F-Dopa uptake (Fusar-Poli et al., 2009). Our results replicate decreased hemodynamic activation patterns in the bilateral VLPFC regions and particularly, a larger cluster of significant channels in the left VLPFC region. The reason why our results show lower activation in the UHR group but similar activations in the FEP and ChSZ groups may be due to differences in modalities and tasks. However, there are few studies that directly compare various clinical stages. More studies are needed to clarify these mechanisms of hypo-/hyperactivation with respect to the characteristics of participants and tasks.

In contrast, the activations in the bilateral DLPFC as well as part of the right VLPFC region decreased with the advancing clinical stages (Fig. 3, middle and bottom). Neuroimaging studies suggest that the DLPFC plays a central role in the working memory system (D'Esposito et al., 1995, 1999) and shows reduced working memory capacity in schizophrenics (Callicott et al., 2003; Manoach, 2003; Jansma et al., 2004). However, the hypo-/hyperactivations in the DLPFC also depend on tasks (Schneider et al., 2007; Keshavan et al., 2008). A functional MRI study using an n-back task shows that UHR individuals have smaller BOLD signal changes in the middle frontal gyrus than controls (Fusar-Poli et al., 2010). Another study that compared 3 clinical stages and controls shows that the BOLD signals in the middle frontal gyrus decrease with advancing clinical stage (Morey et al., 2005). This indicates that our results are in line with those of previous UHR studies.

Our results of the activation pattern in the fronto-polar region are similar to those in the VLPFC regions. There are few functional neuroimaging studies regarding the relationship between this region and psychosis; however, it is critical to consider the relationship between the functions in this region and the progression and prognosis of psychosis. Previous studies suggest that the fronto-polar region plays a crucial role in high-level coordination with other brain functions and that these functions are required in social interactions such as self-perception, person perception, and mentalizing in humans (Frith and Frith, 1999; Kampe et al., 2001; Northoff et al., 2006; Badre and D'Esposito, 2009; Suda et al., 2010). Impairment in this region may directly reflect social dysfunction in daily life. Our previous study found a positive correlation between activation in the fronto-polar region and GAF scores in schizophrenics (Takizawa et al., 2008). Our results also show a trend-level correlation between activation in this region and GAF scores in the ChSZ group, even in those matched for age-at-onset and DUP. The reason why these correlations are absent in the UHR and FEP groups may be because those participants became ill recently and have discrepancies between the one-time assessment of GAF scores and potential capacities regarding social interaction. This effect regarding the timing of clinical assessment is assumed to reflect similar PANSS scores among the groups, because the patients in the FEP and ChSZ groups were measured in their relatively stable conditions after onset although the UHR individuals were measured when they suffered from sub-threshold psychotic symptoms and functional declines. Activation in the fronto-polar region may not reflect their present states and may rather predict their social outcomes in future prognoses.

4.2. Correlation between [oxy-Hb] changes and symptoms in the FEP group

Our results show unexpected findings in that the patients with FEP exhibited significant positive correlations between [oxy-Hb] change in the right aTC region, and PANSS positive and negative scores as well as marginally significant PANSS general scores; this indicates that the activation is greater when the symptoms are worse.

One of the major functions of the aTC, specifically the superior temporal gyrus, is assumed to be acoustic perception and comprehension (Szycik et al., 2009; Warren et al., 2009), especially for integrating meaningful verbal information in the dominant region and processing nonverbal acoustic information in the nondominant region (Kriegstein and Giraud, 2004; Warren et al., 2009). In a functional connectivity study, activities integrated bilaterally in these regions may be associated with speech perception and comprehension, and play a major role in social cognition (Warren et al., 2009). Patients with schizophrenia have difficulty in acoustic perception and communication related to social interactive functions such as the mirror neuron system, A functional MRI study shows that patients with schizophrenia have less activation in the right superior temporal gyrus, pars opercularis, and middle frontal sulcus during incongruent audiovisual speech stimuli (Szycik et al., 2009).

In this study, we found that the patients with FEP exhibited smaller activation in the right aTC region than controls; in group comparison, they showed larger activation when they had severe symptoms. The reason for this unexpected finding may be a compensatory response or overactivation of brain dysfunction related to the onset of psychosis. Although the patients in the FEP group were in their relatively stable conditions at measurement, they were still in the acute phase (DOM = 1.8 month), which might have possibly altered their PANSS scores for a short duration. Although we have to investigate the changes of this correlation coefficient longitudinally, it is possible that this finding can be applied to determine therapeutic efficiency in clinical settings. Further investigations are required to determine why these correlations are observed only in the FEP stage and are obscured in the chronic phase.

4.3. Limitations

Our results have some methodological limitations. First, as there were small subject numbers especially in the UHR group, we cannot fully detect differences among groups; studies with larger samples and more observation details are needed. Second, because this study had a cross-sectional design, we could not examine the participants' longitudinal prognoses, especially regarding the prediction for psychosis. We are currently conducting 3-month repetitive measurements for UHR and FEP individuals in a longitudinal fashion, exploiting the easy setting and noninvasive characteristics of the NIRS instruments. Furthermore, as we carry out long-term clinical follow-up and NIRS measurement on these individuals every 3 months, we will be able to demonstrate the predictive biomarkers related to the onset of psychosis as well as symptomatic and functional outcomes. Finally, as this study was naturalistic and therefore not controlled with respect to drug usage, we cannot rule out the effects of drugs. However, we found no differences in the [oxy-Hb] changes with respect to antipsychotics or

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other drug usage. In clinical settings, although it is hard to control for drug usage, we aim to examine the responses and effects of medication.

5. Conclusion

In conclusion, we found that hemodynamic changes decrease with advancing clinical stages of psychosis (i.e., at-risk mental state, first-episode psychosis, and chronic stage of schizophrenia) over the prefrontal cortical surface areas and in the bilateral anterior part of the temporal cortex regions using multichannel NIRS instruments. Although this study was cross-sectional, future studies are needed to carry out longitudinal clinical follow-up and NIRS measurements for UHR and FEP individuals to develop candidate biomarkers that can detect the clinical stages of psychosis and predict the onset of psychosis.

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Contributions

SK contributed to project management and wrote the manuscript. RT and YN conducted NIRS measurements and data analysis. Y. Takano contributed to project management, and clinical assessment and management. YN, Y. Takayanagi, HH, and YO contributed to NIRS measurements and assessment of clinical information in the Tokyo Metropolitan Matsuzawa Hospital. MK contributed to NIRS measurements. TA, MF, and KK coordinated the entire research design and took responsibility for the management of this study. All authors contributed to the critical revision and final approval of the manuscript.

Conflict of interest

The principal investigators of each site (Masato Fukuda of Gunma University, Yuji Okazaki of Tokyo Metropolitan Matsuzawa Hospital, and Kiyoto Kasai of The University of Tokyo) have potential conflicts of interest. Each site and the Hitachi Group (Advanced Research Laboratory, Hitachi Ltd. and The Research and Developmental Center, Hitachi Medical Corporation) have had official contracts for a collaborative study on the clinical application of near-infrared spectroscopy in psychiatric disorders. For this study, the Hitachi Group provided project grants (MF: JPY 1,000,000 per year since April 1, 2002; YO: JPY 500,000 per year since April 1, 2003; KK: JPY 300,000 per year since July 31, 2003) and material support (temporary rental of a near-infrared spectroscopy [Optical Topography] system, ETG-4000) for each site. The material support for KK of The University of Tokyo ended in 2009. The other authors have no relevant conflicts of interest.

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Impact of the Genome Wide Supported *NRGN* Gene on Anterior Cingulate Morphology in Schizophrenia

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Abstract

Background: The rs12807809 single-nucleotide polymorphism in *NRGN* is a genetic risk variant with genome-wide significance for schizophrenia. The frequency of the T allele of rs12807809 is higher in individuals with schizophrenia than in those without the disorder. Reduced immunoreactivity of *NRGN*, which is expressed exclusively in the brain, has been observed in Brodmann areas (BA) 9 and 32 of the prefrontal cortex in postmortem brains from patients with schizophrenia compared with those in controls.

Methods: Genotype effects of rs12807809 were investigated on gray matter (GM) and white matter (WM) volumes using magnetic resonance imaging (MRI) with a voxel-based morphometry (VBM) technique in a sample of 99 Japanese patients with schizophrenia and 263 healthy controls.

Results: Although significant genotype-diagnosis interaction either on GM or WM volume was not observed, there was a trend of genotype-diagnosis interaction on GM volume in the left anterior cingulate cortex (ACC). Thus, the effects of *NRGN* genotype on GM volume of patients with schizophrenia and healthy controls were separately investigated. In patients with schizophrenia, carriers of the risk T allele had a smaller GM volume in the left ACC (BA32) than did carriers of the non-risk C allele. Significant genotype effect on other regions of the GM or WM was not observed for either the patients or controls.

Conclusions: Our findings suggest that the genome-wide associated genetic risk variant in the *NRGN* gene may be related to a small GM volume in the ACC in the left hemisphere in patients with schizophrenia.

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Introduction

Schizophrenia is a common and complex psychiatric disorder that has a strong genetic component; the estimated heritability is 81% [1]. Many genes have been implicated in the pathogenesis of schizophrenia [2].

A genome-wide association study (GWAS) of single-nucleotide polymorphisms (SNPs) conducted by accessing thousands of DNA samples from patients and controls can be a powerful tool for identifying common risk factors for such a complex disease. Stefansson et al. examined a combined sample of 12,945 patients with schizophrenia and 34,591 controls from three large GWASs (the SGENE-plus, the International Schizophrenia Consortium and the Molecular Genetics of Schizophrenia) and a follow-up with 4,999 patients and 15,555 controls from four additional sample sets from various areas of Europe (including the Netherlands, Denmark, Germany, Hungary, Norway, Russia, Sweden, Finland and Spain) [3]. The researchers identified several

significant association signals. Seven markers gave p values smaller than the genome-wide significance threshold of approximately 1.6×10^{-7} in the combined samples. Five of these markers—rs6913660, rs13219354, rs6932590, rs13211507 and rs3131296—span the major histocompatibility complex (MHC) region on chromosome 6p21.3–22.1; one marker, rs12807809, is located 3,457 bases upstream from the neurogranin (*NRGN*) gene on 11q24.2; one additional marker, rs9960767, is located in intron four of the transcription factor 4 (*TCF4*) gene on 18q21.2. Of these seven SNPs, four SNPs, rs6913660, rs13219354, rs13211507 and rs9960767, were not polymorphic in samples from the HapMap Japanese in Tokyo (JPT) project. Minor allele frequencies (MAF) of two SNPs, rs6932590 and rs3131296, were under 5%. Because only one marker, rs12807809 in *NRGN*, was a common SNP in HapMap JPT samples (MAF>5%), we focused on this SNP in the present study.

NRGN is the human homolog of the neuron-specific rat gene RC3/neurogranin. *NRGN* encodes a postsynaptic protein kinase substrate that binds to calmodulin (CaM) in the absence of calcium [4]. The *NRGN* gene spans 7.3 kb of genomic DNA and contains four exons that transcribe a protein of 78 amino acids [5]. Exons 1 and 2 encode the protein, and exons 3 and 4 contain untranslated sequences. NRGN plays an important role in the Ca^{2+}–CaM signaling pathway [6]. A Ca^{2+} influx-induced oxidation of NRGN leads to postsynaptic activation of CaM-dependent protein kinase II (CaMKII) by CaM, which is associated with strengthened *N*-methyl-D-aspartate (NMDA) receptor signaling [7]. Altered NRGN activity may therefore mediate the effects of the NMDA hypofunction implicated in the pathophysiology of schizophrenia.

Many attempts have been made to minimize clinical and genetic heterogeneity in studies of schizophrenia. One strategy for gene discovery uses neurobiological quantitative traits (QT) as intermediate phenotypes rather than the diagnosis of schizophrenia [8,9]. This strategy has the potential to reduce clinical and genetic heterogeneity by examining intermediate phenotypes that reflect underlying genetic vulnerability better than diagnostic categorization [10]. Structural brain phenotypes are QT that show considerable variation in human populations [11]. A voxel-wise meta-analysis of gray matter (GM) alterations in patients with schizophrenia indicated that they had a reduced GM density in the bilateral insular cortex, anterior cingulate, left parahippocampal gyrus, left middle frontal gyrus, postcentral gyrus, and thalamus and had an increased GM density in the striatal regions relative to the control subjects [12]. A voxel-wise meta-analysis of white matter (WM) alterations in patients with schizophrenia indicated that these patients had a decreased WM volume in the frontal regions and internal capsule relative to control subjects [13]. Heritability estimates indicate a moderate (40–70%) to high (70–95%) genetic influence on brain structure volumes in the frontal and temporal brain regions, such as the middle frontal and the anterior cingulate cortices [11,14]. Some studies have shown that abnormalities in brain structure are intermediate phenotypes that bridge the gap between the genotype and diagnostic categorization [10,15,16]. Our research group has a long-standing interest in the effects of genetic variants on brain structure (i.e., *COMT*, *DISC1*, *PACAP*, *BDNF*, *APOE* and *AKT1*) [17,18, 19,20,21,22] and on prefrontal activity as measured by near-infrared spectroscopy (NIRS) (*TBP* and *SIGMAR1*) in psychiatric disorders [23,24]. *NRGN* is expressed exclusively in the brain, especially in the dendritic spines. Reduced NRGN immunoreactivity has been observed in prefrontal areas 9 and 32 of post-mortem schizophrenic brains [25]. To date, no study has investigated the effects of the *NRGN* polymorphism and the genotype-diagnosis interaction on brain morphology at the whole brain level. In this study, we examined the impacts of the *NRGN* polymorphism and the genotype-diagnosis interaction on GM volumes and WM volumes in patients with schizophrenia and in healthy volunteers.

Materials and Methods

Ethics statement

Written informed consent was obtained from all subjects after the procedures had been fully explained. This study was carried out in accordance with the World Medical Association's Declaration of Helsinki and approved by the Research Ethical Committee of Osaka University.

Subjects

Voxel-based morphometry (VBM) analyses were conducted on 99 patients with schizophrenia [52.5% males (52 males and 47 females); mean age ± SD, 38.4±12.9 years] and 263 healthy controls [44.5% males (117 males and 146 females); mean age ± SD, 36.7±11.6 years]. All subjects were biologically unrelated within the second-degree of relationship and of Japanese descent [23,26]. The subjects were excluded if they had neurological or medical conditions that could potentially affect the central nervous system, such as atypical headache, head trauma with loss of consciousness, chronic lung disease, kidney disease, chronic hepatic disease, thyroid disease, active cancer, cerebrovascular disease, epilepsy, seizures, substance-related disorders or mental retardation. Cases were recruited from the university hospital. Each patient with schizophrenia had been diagnosed by at least two trained psychiatrists according to the criteria of the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* (DSM-IV) based on the Structured Clinical Interview for DSM-IV (SCID). Controls were recruited through local advertisements at Osaka University. Psychiatrically, medically and neurologically healthy controls were evaluated using the non-patient version of the SCID to exclude individuals who had current or past contact with psychiatric services or who had received psychiatric medication. Current symptoms of schizophrenia were evaluated using the positive and negative syndrome scale (PANSS) [27]. Mean age, sex ratio and handedness did not differ significantly between cases and controls ($p>0.17$), while the years of education, estimated premorbid intelligence quotient (IQ) and GM volumes were significantly lower in the patients with schizophrenia than in the controls ($p<0.001$) (Table S1). When the genotype groups were compared, we found no differences in the demographic variables, except for years of education and duration of illness in patients with schizophrenia (Table S1).

SNP selection and SNP genotyping

We selected rs12807809 in the *NRGN* gene as described in the introduction. This polymorphism is reported as T/C and was previously described in the GWAS [3]. Venous blood was collected from the subjects, and genomic DNA was extracted from whole blood according to standard procedures. The SNP was genotyped using the TaqMan 5′-exonuclease allelic discrimination assay (Assay ID: C__32029000_20, Applied Biosystems, Foster City, California, USA) as previously described [18,19]. Detailed information on the PCR conditions is available upon request. No deviation from Hardy-Weinberg equilibrium (HWE) in the examined SNP was detected in the patients or in the controls ($p>0.05$).

Magnetic resonance imaging procedure

All magnetic resonance (MR) studies were performed on a 1.5T GE Sigma EXCITE system. A three-dimensional volumetric acquisition of a T1-weighted gradient echo sequence produced a

gapless series of 124 sagittal sections using a spoiled gradient recalled acquisition in the steady state (SPGR) sequence (TE/TR, 4.2/12.6 ms; flip angle, 15°; acquisition matrix, 256×256; 1NEX, FOV, 24×24 cm; slice thickness, 1.4 mm). MR images were processed using optimized VBM in Statistical Parametric Mapping 5 (SPM5) running on MATLAB R2010b (MathWorks, Natick, MA) according to the VBM5.1-Manual (http://dbm.neuro.uni-jena.de/vbm/vbm5-for-spm5/manual/) and as previously described [28,29]. We screened all scans and found no gross abnormalities, such as infarcts, hemorrhages or brain tumors, in any of the subjects. Each image was visually examined to eliminate images with motion or metal artifacts, and then the anterior commissure-posterior commissure line was adjusted. The normalized segmented images were modulated by multiplication with Jacobian determinants of the spatial normalization function to encode the deformation field for each subject as tissue volume changes in the normal space. Finally, images were smoothed with a 12-mm full-width, half-maximum isotropic Gaussian kernel.

Statistical analyses were performed with SPM8 software (http://www.fil.ion.ucl.ac.uk/spm/software/spm8/). First, we performed whole brain searches to explore the effects of the *NRGN* genotype and the genotype-diagnosis interaction on GM or WM volume in total subjects. Second, we performed separate whole brain searches to explore the effect of the *NRGN* genotype on GM or WM volume in patients with schizophrenia and in controls. The genotype effect on GM or WM volume was assessed statistically using a multiple regression model in SPM8. We contrasted GM or WM volume between the genotype groups (coded as the number of rs12807809 risk T alleles: 0, 1, or 2); GM or WM volumes were correlated with the number of risk T alleles, either positively (CC<CT<TT) or negatively (TT<CT<CC). The genotype-diagnosis interaction on GM or WM volumes was assessed full factorial model with diagnosis as a factor and genotype status as a covariate interacted with the diagnosis in SPM8. Age, sex and years of education were included as covariates of no interest into all analyses to control for confounding variables. Non-sphericity was estimated. These analyses yielded statistical parametric maps {SPM (t)} based on a voxel-level height threshold of $p<0.001$ (uncorrected for multiple comparisons). Clusters of more than 100 contiguous voxels were considered in the analyses. Family-wise error (*FWE*) correction was applied for multiple testing to avoid type I errors. The significance level was set at $p<0.05$ (*FWE* corrected). Anatomic localization was performed according to both MNI coordinates and Talairach coordinates, which were obtained from M. Brett's transformations (http://www.mrccbu.cam.ac.uk/Imaging/Common/mnispace.shtml) and presented as Talairach coordinates.

Statistical analyses

The presence of Hardy-Weinberg equilibrium was examined by the χ^2 test for goodness-of-fit using SNPAlyze V5.1.1 Pro software (DYNACOM, Yokohama, Japan). Statistical analyses of demographic variables were performed using PASW Statistics 18.0 software (SPSS Japan Inc., Tokyo, Japan). Differences in clinical characteristics between patients and controls or between genotypes were analyzed using χ^2 tests for categorical variables and the Mann-Whitney *U*-test or Kruskal-Wallis test for continuous variables. The significance level for all statistical tests was set at two-tailed $p<0.05$.

Results

Effects of the genotype and diagnosis-genotype interaction on GM or WM regions in total subjects

First, we investigated the effects of genotype and diagnosis-genotype interaction on GM or WM volumes in the whole brain analyses of total subjects. We found significant effects of the risk T allele on decreased GM volume in the right fusiform gyrus (uncorrected $p<0.001$, Table 1 and blue regions in Figure 1), and on increased WM volume in the inferior parietal lobule among total subjects (uncorrected $p<0.001$, Table 1). We also found significant genotype-diagnosis interaction on GM volume in the left anterior cingulate gyrus and the bilateral precuneus (uncorrected $p<0.001$, Table 1 and red regions in Figure 1). However, the effects of genotype and genotype-diagnosis interaction on these GM or WM regions did not survive after the *FWE*-correction for multiple tests (*FWE*-corrected $p>0.05$). There was no significant effect of the risk T allele on increased GM volumes, the risk T allele on decreased WM volumes, or genotype-diagnosis interaction on WM volume among total subjects (uncorrected $p>0.001$).

Effect of the risk T allele on decreased GM regions (TT<CT<CC)

Second, we separately investigated the effects of genotype on GM or WM volumes in the whole brain analyses of patients with schizophrenia and healthy controls. We found significant effects of the *NRGN* genotype on GM volume in the left anterior cingulate gyrus, the bilateral middle temporal gyrus and the left inferior frontal gyrus among the patients with schizophrenia (uncorrected $p<0.001$, Table 2 and red regions in Figure S1). We found significant effect of the *NRGN* genotype on GM volume in the right fusiform gyrus among the healthy controls (uncorrected $p<0.001$, Table 2 and blue regions in Figure S1). The genotype effect on the left anterior cingulate gyrus (BA32) in the patients with schizophrenia remained significant even after the *FWE*-correction for multiple tests at the whole brain level ($T_{94}=5.63$, *FWE*-corrected $p=0.0042$, Table 2); genotype effects on other regions did not survive the *FWE*-correction (*FWE*-corrected $p>0.05$). In patients with schizophrenia, the risk T carriers had a smaller GM volume in the left anterior cingulate gyrus than did the non-risk C carriers (Figure 2).

Researchers have suggested that the volume reduction of the anterior cingulate cortex (ACC) is associated with the duration of the illness (the length of time the patient has had schizophrenia) [30]. In our samples, the duration of illness differed significantly among the genotype groups in patients with schizophrenia (Table S1). Thus, we corrected for the duration of illness. The genotype effect on the left anterior cingulate gyrus remained significant even after controlling for the duration of illness ($T_{93}=5.86$, *FWE*-corrected $p=0.0017$).

Effect of the risk T allele on increased GM regions (CC<CT<TT)

We found significant effects of the *NRGN* genotype on GM volume in the bilateral precuneus among the patients with schizophrenia (uncorrected $p<0.001$, Table 2 and red region in Figure S2); however, the genotype effects on these regions did not survive after the *FWE*-correction for multiple tests (*FWE*-corrected $p>0.05$). There was no significant effect of the *NRGN* genotype on GM volume among the healthy controls (uncorrected $p>0.001$).

Effects of the risk T allele on WM regions

We found no significant effect of the risk T allele on any decreased WM regions (TT<CT<CC) for either the patients or controls (uncorrected $p<0.001$). On the other hand, we found significant effects of the risk T allele on increased WM region (CC<CT<TT) in the bilateral insula and middle frontal gyrus

Table 1. Effects of *NRGN* genotype and genotype-diagnosis interaction on GM and WM volumes in total subjects.

						p values		Talairach coordinates		
	Brain regions	**R/L**	**BA**	**CS**	***T***	**Uncorrected**	***FWE***	***x***	***y***	***z***
GM	***NRGN* genotipe-diagnosis interaction**									
	Limbic Lobe									
	Anterior Cingulate	L	32	219	4.17	<0.001	0.33	−12	40	−10
	Occipital Lobe									
	Precuneus	R	31	118	3.63	<0.001	0.90	15	−64	20
	Precuneus	L	31	165	3.54	<0.001	0.95	−7	−72	25
GM	**Total subjects; TT<CT<CC (higher risk<lower risk)**									
	Temporal Lobe									
	Fusiform Gyrus	R	20	290	4.28	<0.001	0.25	45	−30	−23
GM	**Total subjects; TT>CT>CC (higher risk>lower risk)**									
	no suprathreshold clusters									
WM	***NRGN* genotipe-diagnosis interaction**									
	no suprathreshold clusters									
WM	**Total subjects; TT<CT<CC (higher risk<lower risk)**									
	no suprathreshold clusters									
WM	**Total subjects; TT>CT>CC (higher risk>lower risk)**									
	Parietal Lobe									
	Inferior Parietal Lobule	R		616	3.72	<0.001	0.34	44	−41	25

GM: gray matter, WM: white matter, R: right, L: left, BA: Brodmann area, CS: Cluster size, FWE: family-wise error.
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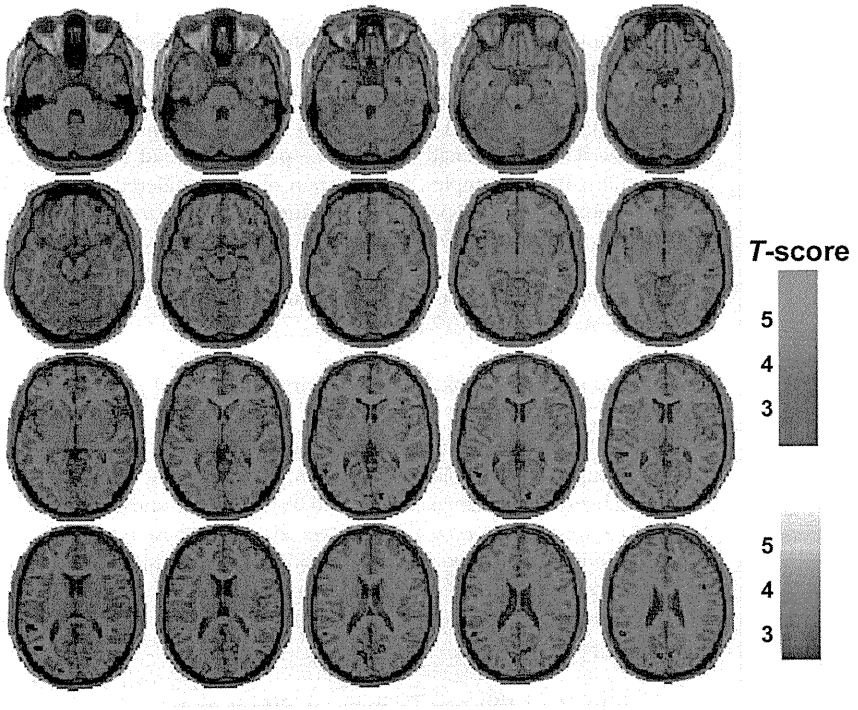

Figure 1. Effects of the risk-T-allele on decreased GM regions and diagnosis-*NRGN* genotype interaction on GM regions. Effects of the risk T allele on decreased GM regions (TT<CT<CC) in total subjects were shown by whinter colormap (blue areas). Diagnosis-*NRGN* genotype interaction on GM regions was shown by hot colormap (red areas). There was no significant effect of the risk T allele on increased GM regions (CC<CT<TT) among the total subjects. Each colormap shows *t* values corresponding to the color in the figure.
doi:10.1371/journal.pone.0029780.g001

Table 2. Effects of *NRGN* genotype on GM volumes in patients with schizophrenia and in healthy controls.

					p values		Talairach coordinates		
Brain regions	**R/L**	**BA**	**CS**	***T***	**Uncorrected**	***FWE***	***x***	***y***	***z***
SZ; TT<CT<CC (higher risk<lower risk)									
Limbic Lobe									
Anterior Cingulate	L	32	525	5.63	<0.001	**0.0042**	−12	42	−9
Temporal Lobe									
Middle Temporal Gyrus	L	21	143	3.87	<0.001	0.80	−66	−19	−5
Middle Temporal Gyrus	R	21	106	3.69	<0.001	0.93	59	−24	−6
Frontal Lobe									
Inferior Frontal Gyrus	L	10	102	3.88	<0.001	0.80	−36	45	4
HC; TT<CT<CC (higher risk<lower risk)									
Temporal Lobe									
Fusiform Gyrus	R	20	334	4.4	<0.001	0.19	45	−31	−23
SZ; TT>CT>CC (higher risk>lower risk)									
Parietal Lobe									
Precuneus	L	7	182	4	<0.001	0.68	−15	−64	38
Occipital Lobe									
Precuneus	R	31	143	3.81	<0.001	0.86	15	−64	19
HC; TT>CT>CC (higher risk>lower risk)									
no suprathreshold clusters									

GM: gray matter, R: right, L: left, BA: Brodmann area, CS: Cluster size, FWE: family-wise error, SZ: patients with schizophrenia, HC: healthy controls. Significant results [$p<0.05$ (*FWE* corrected)] are shown as bold face and underline.

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among the patients with schizophrenia (uncorrected $p<0.001$, Table S2 and red regions in Figure S3). However, the genotype effects on these regions did not survive after the *FWE*-correction (*FWE*-corrected $p>0.05$). There was no significant genotype effect on any increased WM region for the controls (uncorrected $p<0.001$). These findings suggest that *NRGN* may not play a major role in the morphology of WM.

Discussion

This is the first study to identify brain morphology associated with genome-wide significant risk variants in *NRGN* for schizophrenia at the whole brain level. Genotype-diagnosis interaction on GM volume in the left ACC was found, even though the effect did not survive after the *FWE*-correction. When we separately

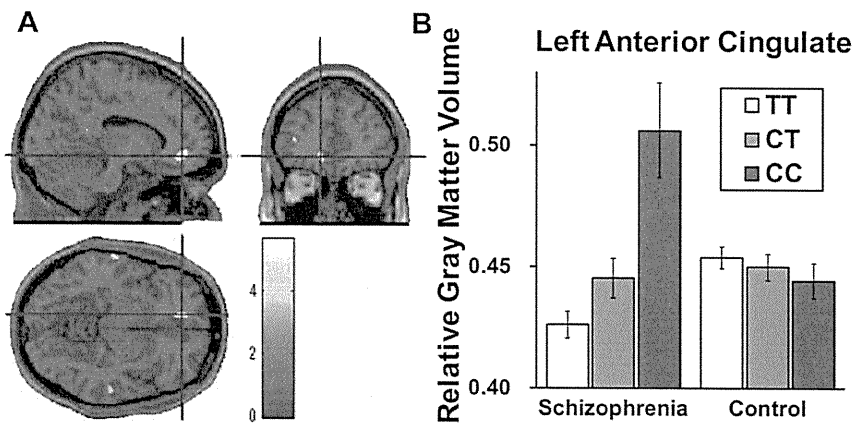

Figure 2. Impact of the *NRGN* genotype on GM volume of left anterior cingulate gyrus in schizophrenia. (**A**) Anatomical localizations are displayed on coronal, sagittal, and axial sections of a normal MRI spatially normalized into the Montreal Neurological Institute template (uncorrected $p<0.001$, cluster size>100). A significant cluster of the genotype effect was in the left anterior cingulate gyrus in the patients with schizophrenia, after controlling for differences in the duration of illness among genotypes. The region is shown as cross-hairline. The color bars show *t* values corresponding to the color in the figure. (**B**) Each column shows relative gray matter volumes extracted from the left anterior cingulate gyrus (Talairach coordinates; −12, 42, −9). We extracted a sphere with a 10 mm volume-of-interest (VOI) radius from the significant region to compare the effects of the genotype in both the patients with schizophrenia and healthy subjects. Error bars represent the standard error.

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investigated the effects of the interaction on GM volume of patients with schizophrenia and healthy controls, carrying the risk T allele of rs12807809 was associated with reduced GM volume in the left ACC in patients with schizophrenia. The genotype effect survived a correction for multiple comparisons at the whole brain level. This finding applies to the patients with schizophrenia but not to the healthy controls, and it is present even after controlling for differences in the duration of illness among genotypes. Significant difference on WM volume between genotypes was not observed for any region in patients or controls.

The ACC is a functionally heterogeneous region involved in diverse cognitive processes [30]. The functional diversity of the ACC encompasses executive, attention, social cognitive, affective and skeleton- and viscera-motor functions. Most MRI studies suggest that patients with schizophrenia show reduced GM in the ACC [30]. These reductions extend across the dorsal and rostral divisions of the limbic and paralimbic regions of the ACC. Some studies suggest that relatives of schizophrenia patients also show bilateral reductions in GM volume or thickness in the ACC [31,32]. Post-mortem findings indicate that these imaging-related changes are accompanied by reductions in neuronal, synaptic, and dendritic density as well as increased afferent input [30]. These findings suggest that the GM differences observed with MRI arise from alterations in both neuronal and non-neuronal tissue compartments.

The GM reductions in the ACC precede the onset of psychosis in some categories of high-risk individuals. Cross-sectional and longitudinal studies suggest that the earliest ACC changes in schizophrenia appear in the rostral paralimbic regions of the ACC prior to the onset of psychosis, extend across the paralimbic regions of the ACC during the transition to a first episode psychosis, and spread to engulf the limbic regions of the ACC with continued illness [30]. The regions of the genotype effect in the present study were the paralimbic regions of the ACC. A mean duration of illness in patients included in this study was 13.0±10.4 years; these patients are considered to have established schizophrenia. As the duration of illness has been related to the degree of reduction of the ACC and because it significantly differed among the genotype groups in our subjects, we ascertained whether the genotype effect in the ACC is affected by variation in the duration of illness. However, the genotype effect in the left ACC was robust even after controlling for the duration of illness. These findings suggest that part of the paralimbic regions of the ACC may be attributed to the effects of the genome-wide supported variant of *NRGN* in patients with schizophrenia, regardless of the duration of illness.

NRGN is especially enriched in CA1 pyramidal neurons in the hippocampus [33]. *NRGN* produced severe deficits in hippocampus-dependent tasks in knock-out mice [34,35]. This evidence suggests that *NRGN* may be important in neurocognitive tasks such as learning and memory and in the morphology and function of the hippocampus. Based on this hypothesis, Donohoe et al. tested the relationship between schizophrenia associated with the *NRGN* variant rs12807809 and cognition in Irish and German case-control samples [36]. They did not find a significant association between the *NRGN* variant and cognition in the samples. Pohlack et al. found that homozygous T carriers had decreased activation of the left hippocampus during contextual fear conditioning but did not find the same result in the hippocampal structure of Caucasian healthy volunteers [37]. We did not find a significant association between the *NRGN* variant and hippocampal volume, consistent with recent study using the ROI approach [37]. These findings suggest that *NRGN* may play an important role in hippocampal activity but not play a major role in the neurocognition of learning and memory or in the morphology of the hippocampus.

There were several limitations to this study. A false-positive association could not be excluded from our study despite the precautions for ethnic matching and corrections for multiple testing. It is necessary to conduct further investigations to confirm our findings in other samples with much larger sample sizes and/or with different ethnicities and/or in relatives with schizophrenia. A false-negative association could not be excluded in our study because we applied a strict correction for multiple comparisons at the whole brain level (*FWE*-corrected $p<0.05$). The regions shown in the Supporting Information (uncorrected $p<0.001$) might be helpful in further studies. It is still unclear whether this genetic variant of the *NRGN* gene is associated with the expression, transcription, splicing or translation of the gene. The lack of a clear association makes it difficult to determine whether our results are directly linked to the *NRGN* polymorphism rs12807809, to other polymorphisms in linkage disequilibrium with this variant, or to interaction between this genetic variant of the *NRGN* and other polymorphism. As with other risk variants for schizophrenia, clarifying the biological role of this variant through *in vitro* and *in vivo* studies is important to improve the understanding of the pathophysiology of schizophrenia. In addition, an extensive search for other functional variants at this locus is needed to determine whether rs12807809 is the most strongly associated variant for schizophrenia in this gene.

In conclusion, we found that a genome-wide supported variant of *NRGN* may be associated with brain morphological vulnerability of the left ACC in patients with schizophrenia. Abnormalities in ACC may partly explain the disturbances in cognitive and emotional integration in patients with schizophrenia. Further research will be required to clarify the function of the risk *NRGN* variant on the pathophysiology of schizophrenia.

Supporting Information

Figure S1 Effect of risk-T-allele on decreased GM regions in patients with schizophrenia and in healthy controls. Effect of the risk T allele on decreased GM regions (TT<CT<CC) in the patients with schizophrenia was shown by hot colormap (red areas), while effect of the T allele on decreased GM regions in the healthy controls was shown by winter colormap (blue areas).
(TIF)

Figure S2 Effect of the risk-T-allele on increased GM regions in the patients with schizophrenia. Effect of the risk T allele on increased GM regions (CC<CT<TT) in the patients with schizophrenia was shown by hot colormap (red areas). There was no significant effect of the *NRGN* genotype on GM volume among the healthy controls.
(TIF)

Figure S3 Effect of the risk-T-allele on increased WM regions in the patients with schizophrenia. Effect of the risk T allele on increased WM regions (CC<CT<TT) in the patients with schizophrenia was shown by hot colormap (red areas). There was no significant effect of the *NRGN* genotype on WM volume among the healthy controls.
(TIF)

Table S1 Demographic information for patients with schizophrenia and healthy controls included in the VBM analysis.
(DOC)

Table S2 Effects of the *NRGN* genotype on WM volumes in patients with schizophrenia and healthy controls.
(DOC)

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Author Contributions

Conceived and designed the experiments: KO RH. Performed the experiments: HY SU TOakada KN TOhnishi. Analyzed the data: KO RH KN TOhnishi HY SU TOakada. Contributed reagents/materials/analysis tools: YY MF MI HK MT. Wrote the paper: KO RH YY MF MI HK MT.

Developmental Changes of Prefrontal Activation in Humans: A Near-Infrared Spectroscopy Study of Preschool Children and Adults

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Abstract

Previous morphological studies indicated that development of the human prefrontal cortex (PFC) appears to continue into late adolescence. Although functional brain imaging studies have sought to determine the time course of functional development of the PFC, it is unclear whether the developmental change occurs after adolescence to adulthood and when it achieves a peak because of the narrow or discontinuous range in the participant's age. Moreover, previous functional studies have not focused on the anterior frontal region, that is, the frontopolar regions (BA9/10). Thus, the present study investigated the developmental change in frontopolar PFC activation associated with letter fluency task by using near-infrared spectroscopy (NIRS), in subjects from preschool children to adults. We analyzed the relative concentration of hemoglobin (ΔHb) in the prefrontal cortex measured during the activation task in 48 typically-developing children and adolescents and 22 healthy adults. Consistent with prior morphological studies, we found developmental change with age in the children/adolescents. Moreover, the average Δoxy-Hb in adult males was significantly larger than that in child/adolescent males, but was not true for females. These data suggested that functional development of the PFC continues into late adolescence. Although the developmental change of the frontopolar PFC was independent of gender from childhood to adolescence, in adulthood a gender difference was shown.

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Introduction

The pattern of development and maturation of the human prefrontal cortex (PFC) from childhood through early adulthood is an important research question in neuroscience. The activity of the catechol-o-methyltransferase (COMT) enzyme that modulates dopamine levels in the PFC increases from the neonate through to adulthood [1], consistent with the critical role of dopamine in modulating normal PFC function [2]. Previous morphological studies have used postmortem brains and MRI to indicate that development appears to continue into late adolescence in terms of synaptic density [3], gray matter volume [4,5] and cortical thickness [6].

Functional brain imaging studies have also sought to determine the time course of functional development of the PFC, although the findings have been equivocal. A positron emission tomography (PET) study showed that glucose metabolism at 4 years and 9–10 years was at a high plateau and after 9–10 years began to decline and gradually reaches adult values by 16–18 years [7]. Some functional MRI (fMRI) studies showed that the activation of the dorsolateral PFC (DLPFC) increased with age during the declarative memory task for 8–24 year-olds [8], and that the greater activation in adults than in adolescents during the Stroop task for 7–22 year-olds [9]. Others indicated that DLPFC was more active in children (9–12 year-olds) than in adults (20–30 year-olds) in the go/no-go task [10], and that adolescents (14–17 year-olds) showed greater activation than children (8–13 year-olds) and adults (18–30 year-olds) in the saccade task [11]. The ventrolateral PFC (VLPFC) was activated in adults only during the go/no-go task, but not in children (8–12 year-olds) [12], while children (8–13 year-olds) demonstrated greater activation than adults (19–48 year-olds) in the verbal fluency task [13]. In a near-infrared spectroscopy (NIRS) study, both adults and preschool children (5–6 year-olds) increased oxyhemoglobin (oxyHb) in the lateral PFC (LPFC) during the working memory task and the activation of LPFC was larger and broader in children than in adults, although children were not directly compared with adults [14]. Another study using the Stroop task, however, showed that the oxyHb responses in the young adults were greater and faster than those in children (7–13 year-olds), and reported that the DLPFC activation increased with age [15]. To summarize the above findings, previous studies have been mixed regarding in which life stage (childhood, adolescence, adulthood) the LPFC activation becomes largest.