Schizophrenia Research 143 (2013) 116–124

Contents lists available at SciVerse ScienceDirect

Schizophrenia Research

journal homepage: www.elsevier.com/locate/schres

A multimodal approach to investigate biomarkers for psychosis in a clinical setting: The integrative neuroimaging studies in schizophrenia targeting for early intervention and prevention (IN-STEP) project

Shinsuke Koike [a,b,*], Yosuke Takano [a], Norichika Iwashiro [a], Yoshihiro Satomura [a], Motomu Suga [a], Tatsuya Nagai [a], Tatsunobu Natsubori [a], Mariko Tada [a], Yukika Nishimura [a], Syudo Yamasaki [c,d], Ryu Takizawa [a], Noriaki Yahata [a], Tsuyoshi Araki [d], Hidenori Yamasue [a], Kiyoto Kasai [a,e]

[a] Department of Neuropsychiatry, Graduate School of Medicine, the University of Tokyo, Bunkyo-ku, Tokyo 113-8655, Japan
[b] Office for Mental Health Support, Division for Counseling and Support, the University of Tokyo, Bunkyo-ku, Tokyo 113-0033, Japan
[c] Department of Psychiatry and Behavioral Science, Tokyo Metropolitan Institute of Medical Science, Setagaya-ku 156-8506, Japan
[d] Department of Youth Mental Health, Graduate School of Medicine, the University of Tokyo, Bunkyo-ku, Tokyo 113-8655, Japan
[e] JST, National Bioscience Database Center (NBDC), 5-3, Yonbancho, Chiyoda-ku, Tokyo 102-0081, Japan

ARTICLE INFO

Article history:
Received 9 August 2012
Received in revised form 29 October 2012
Accepted 12 November 2012
Available online 4 December 2012

Keywords:
Schizophrenia
Neuroimaging
Biological markers
Early intervention
Clinical outcome

ABSTRACT

Longitudinal clinical investigations and biological measurements have determined not only progressive brain volumetric and functional changes especially around the onset of psychosis but also the abnormality of developmental pathways based on gene–environment interaction model. However, these studies have contributed little to clinical decisions on their diagnosis and therapeutic choices because of subtle differences between patients and healthy controls. A multi-modal approach may resolve this limitation and is favorable to explore the pathophysiology of psychosis. The integrative neuroimaging studies for schizophrenia targeting early intervention and prevention (IN-STEP) is a research project aimed at exploring the pathophysiological features of the onset of psychosis and investigating possible predictive biomarkers for the clinical treatment of psychosis. Since 2008, we have adopted blood sampling, neurocognitive batteries, neurophysiological assessment, structural imaging, and functional imaging longitudinally for help-seeking ultra-high-risk (UHR) individuals and patients with first-episode psychosis (FEP). Here, we intend to introduce the IN-STEP research study protocol and present preliminary clinical findings. Thirty-seven UHR individuals and 30 patients with FEP participated in this study. Six months later, there was no difference in objective and subjective scores between the groups, which suggests that young people having symptoms and functional deficits should be cared for regardless of their history of psychosis according to their clinical stages. The rate of transition to psychosis was 7.1%, 8.0%, and 35.3% (at 6, 12, and 24 months, respectively). Through this research project, we expect to clarify the pathophysiological features around the onset of psychosis and improve the prognosis of psychosis through clinical application.

1. Introduction

Longitudinal clinical investigations and neuropsychological, neurophysiological, and neuroimaging measurements have helped to elucidate the pathophysiological features of schizophrenia (Insel, 2010). Recent studies have shown the abnormality of developmental pathways based on gene–environment interaction model and progressive brain volumetric and functional changes especially around the onset (Insel, 2010; van Os et al., 2010). However, these studies have contributed little to clinical decisions on their diagnosis and therapeutic choices until now (Borgwardt and Fusar-Poli, 2012). One possible problem is that most of the methods and instruments applied have substantial limitations and cannot clearly illustrate detailed and schematic brain changes, because differences between patients and healthy controls are subtle. Replication studies and more sophisticated methods are required to provide good reliability and validity prior to clinical application.

Multi-modal approaches may resolve these limitations and reveal the specific pathophysiology of psychosis (Salisbury et al., 2007; Prata et al., 2009; Takizawa et al., 2009; Fusar-Poli et al., 2010, 2011b). For example, several imaging genetic studies have suggested that variations in the catechol-*O*-methyltransferase genotype have different effects on the frontal cortical function in patients with schizophrenia and healthy controls (Prata et al., 2009; Takizawa et al., 2009). A previous longitudinal multi-modal imaging study indicated that

* Corresponding author at: Office for Mental Health Support, Division for Counseling and Support, the University of Tokyo, Yasuda-Auditorium, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan. Tel.: +81 3 5841 2578; fax: +81 3 5800 6894.
E-mail address: skoike-tky@umin.ac.jp (S. Koike).

0920-9964/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.schres.2012.11.012

progressive deficits of auditory mismatch negativity (MMN) occur concurrently with progressive brain volume reduction in the temporal cortex (Salisbury et al., 2007). Longitudinal and multi-modal studies are required to elucidate the pathophysiology of psychosis, to develop biomarkers that are easy to use in clinical settings, and finally to help with patients better prognoses (Borgwardt and Fusar-Poli, 2012).

We previously reported several neurocognitive (Suga et al., 2011; Yoshida et al., 2011), neurophysiological (Kawakubo and Kasai, 2006; Kawakubo et al., 2007; Salisbury et al., 2007), and neuroimaging studies (Kasai et al., 2003a, 2003b; Takizawa et al., 2008, 2009; Suga et al., 2010; Yamasaki et al., 2010; Koike et al., 2011b). However, most of the studies were cross-sectional and evaluated patients with chronic schizophrenia using a single modality. Since 2008, we have longitudinally evaluated ultra-high-risk (UHR) individuals and patients with first-episode psychosis (FEP) in a multi-modal fashion, and we already reported cross-sectional data from these studies (Koike et al., 2011b; Iwashiro et al., 2012). In this article, we introduce our research project, the Integrative Neuroimaging Studies in Schizophrenia Targeting for Early Intervention and Prevention (IN-STEP), aimed at exploring the pathophysiological features of the onset of psychosis and investigating possible predictive biomarkers for the clinical treatment of psychosis. We also introduce preliminary clinical assessments at baseline and at a 6-month follow-up until July 2012.

2. Methods

2.1. Research design

This research project was designed as a prospective observational cohort study to explore the pathophysiological features of psychosis, especially toward the onset, and to investigate possible predictive biomarkers of clinical outcome; therapeutic choice; early detection; and finally, the prediction of psychosis in clinical settings. The measurement protocol is illustrated in Table 1.

Table 1
The timeline of clinical assessment and measurement in the study.

		0 m	3 m	6 m	12 m	18 m	24 m	36 m
Clinical assessment	SOPS	x	x	x	x	x	x	x
	PANSS	x	x	x	x	x	x	x
	GAF	x	x	x	x	x	x	x
	SES	x			x		x	x
Subjective assessment	CAPE	x	x	x	x	x	x	x
	CES-D	x	x	x	x	x	x	x
	WHO-BREF	x	x	x	x	x	x	x
Pharmacological assessment	DIEPSS	x	x	x	x	x	x	x
	DAI-10	x	x	x	x	x	x	x
	BEMIB	x	x	x	x	x	x	x
Blood sampling		x		x	x		x	x
Neuropsychological battery	BACS-J	x		x	x		x	x
	UPSA-B	x		x	x		x	x
MRI	Structural	x			x		x	x
	Functional	x			x		x	x
ERP	MMN	x			x		x	x
	ASSR	x			x		x	x
NIRS	LFT	x		x	x		x	x
Clinical outcomes	Remission		x	x	x	x	x	x
	Recovery				x		x	x

Abbreviations: SOPS, the Scale of Prodromal Symptoms; PANSS, the Positive and Negative Syndrome Scale; GAF, the Global Assessment of Functioning; SES, socioeconomic status; CAPE, the Community Assessment of Psychic Experiences; CES-D, the Center for Epidemiologic Studies Depression Scale; WHO-BREF, the 26-item brief version of the WHO Quality of Life Scale; DIEPSS, the Drug-induced Extrapyramidal Symptoms Scale; DAI-10, the 10-item version of Drug Attitude Inventory; BEMIB, the Brief Evaluation of Medication Influences and Beliefs; BACS-J, the Brief Assessment of Cognition in Schizophrenia Japanese version; UPSA-B, the Brief UCSD Performance-Based Skills Assessment; MRI, magnetic resonance imaging; ERP, event related potential; MMN, mismatch negativity; ASSR; auditory steady state response; NIRS, near-infrared spectroscopy; LFT, letter version of verbal fluency task.

The target sample size is 100 help-seeking UHR individuals and 100 patients with FEP for the clinical evaluations. The primary outcomes are the number of transitions to psychosis in the UHR group and the number of symptomatic and functional remissions in the FEP group every year from registration. Concurrently, we collected peripheral blood in fasting to sample their genes and plasma. We also applied the Brief Assessment of Cognition in Schizophrenia Japanese Version (BACS-J) and the Brief UCSD Performance-Based Skills Assessment (UPSA-B) as neurocognitive measures, event-related potential (ERP) as brain neurophysiological measure, structural magnetic resonance imaging (sMRI) to obtain brain morphometric data, and functional MRI (fMRI) and near-infrared spectroscopy (NIRS) to measure brain activity, subject to the participants' condition. This study was approved by the ethics committee of the University of Tokyo Hospital (approval no. 2226-2) in accordance with the Declaration of Helsinki, registered in the University Hospital Medical Information Network Clinical Trials Registry of the International Committee of Medical Journal Editors (no. UMIN000008660), and written in line with the Strengthening the Reporting of Observational Studies in Epidemiology statement (von Elm et al., 2007).

2.2. Participants

The participants are help-seekers recruited from the outpatient and inpatient units of the University of Tokyo Hospital, University of Tokyo Health Service Center, psychiatry clinics, and internet referrals. All eligible participants are assessed using the Structured Interview for Prodromal Symptoms (SIPS) and evaluated using the UHR or psychosis criteria. Psychosis in SIPS criteria is the same as psychotic disorders in the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) (American Psychiatric Association (APA), 1997).

The eligibility criteria are summarized in Table 2. The inclusion criteria are ages of 15 to 30 years for UHR and 15 to 40 years for FEP, no antipsychotic medications for psychosis for more than 16 cumulative weeks, and continuous psychotic symptoms within the past 60 months (Lieberman et al., 2005).

The exclusion criteria are 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 addiction. Illegal substance use (e.g., cannabis) occurs among young people all over the world, and has a crucial effect on the transition to psychosis (Cannon et al., 2008; van Os et al., 2010) and poor symptomatic and functional outcomes (Harrison et al., 2008; Schimmelmann et al., 2008). However, the incidence rate of experienced drug use in young individuals is still relatively low in Japan (Degenhardt et al., 2008). Therefore, we adopted previous continuous substance use as an exclusion criterion in this study.

Table 2
Summary of the eligibility criteria. Target condition: individuals diagnosed as ultra-high-risk or having already psychosis using the Structured Interview for Prodromal Symptoms (SIPS) criteria.

Inclusion criteria
1. Age, 15–40 years (individuals at UHR, 15–30 years)
2. No history of antipsychotic medications for psychosis for more than 16 cumulative weeks
3. Continuous psychotic symptoms within the past 60 months

Exclusion criteria
1. Neurological illness
2. Previous traumatic brain injury with any known cognitive consequences or loss of consciousness for more than 5 min
3. History of electroconvulsive therapy
4. Low premorbid IQ (below 70)
5. Previous alcohol addiction
6. Previous continuous substance use
7. Clearly diagnosed with an autistic disorder according to the DSM-IV criteria

In clinical assessment, the symptoms seen in pervasive developmental disorder (PDD) and dissociative disorders sometimes appear similar to the symptoms seen in schizophrenia. However, our recent findings have suggested that the differences between these disorders with respect to the development of the prefrontal cortex may clarify the pathogenesis of psychosis (Suga et al., 2010; Yamasaki et al., 2010). Therefore, we excluded participants who were clearly diagnosed with PDD and dissociative disorders according to the DSM-IV criteria to explore the specific pathophysiological features of psychosis, particularly schizophrenia.

All the participants provide written informed consent after they are given a complete explanation of the study. The registration and measurement began on July 1, 2008, and the new study protocol described here was implemented on December 1, 2011. We present the preliminary findings of the clinical assessments in the Results section.

2.3. Sample size

One-hundred help-seeking UHR individuals are considered for the clinical evaluation study, which was determined based on previous studies on the transition to psychosis with similar recruitment backgrounds (Yung et al., 2006; Woods et al., 2009) as well as the feasibility of the study, given our resources. One-hundred patients with FEP are also considered for the evaluation of clinical prognosis. The effect sizes of the rate of transition to psychosis for 6 months and 30 months were 0.32 (Yung et al., 2006) and 0.60 (Woods et al., 2009), respectively, and the estimated sample sizes (alpha error, 0.05; beta error, 0.2) were 129 and 36, respectively, as determined using G*Power 3.1.2 (Erdfelder et al., 1996).

2.4. Clinical assessment

All the clinical assessments and measurement points are summarized in Table 1. We assess the severity of symptoms and functioning using the Global Assessment of Functioning (GAF) (American Psychiatric Association (APA), 1994), Social and Occupational Functioning Assessment Scale (SOFAS), and Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987) at each measurement point. The participants in the UHR group are also assessed with regard to subthreshold symptoms using the positive subscale in the Scale of Prodromal Symptoms at each measurement point. We assess the subjective depressive symptoms using the Center for Epidemiologic Studies Depression Scale (CES-D) (Radloff, 1977) and the quality of life using the 26-item brief version of the WHO Quality of Life Scale (WHO-BREF) (Tazaki and Nakane, 1997; The World Health Organization Quality of Life Group (WHOQOL Group), 1998) at each measurement point.

To evaluate symptomatic remission, we adopt a proposal from the Remission in Schizophrenia Working Group (Andreasen et al., 2005), which defined the symptomatic remission of illness as the corresponding 8 PANSS subscores (P1, P2, P3, N1, N4, N6, G5, and G9) indicating "mild" or less on all items for at least 6 months. To evaluate functional recovery, we assess 3 major domains: independent residence, productive activity, and social relationship (Harvey and Bellack, 2009; Emsley et al., 2011).

If the patients take any antipsychotic, anxiolytic, and/or antiparkinsonian agents, we calculate the chlorpromazine, diazepam, and biperiden equivalent doses, respectively. Blood sampling is performed not only for gene and plasma sampling but also for assessing the general health condition.

2.5. Data reliability of clinical assessment

Data collection by multiple raters is affected by methodological problems that result in reduced inter-rater reliability. To control the quality of data assessment, we use video interviews to ensure the scoring of PANSS, GAF, and SOFAS scores more than once a year. We also calculate the inter-rater reliability of the PANSS, GAF, and SOFAS scores provided by the raters in the video interviews and provided feedback based on the results to maintain the quality of the assessment (Cronbach alpha = 0.857).

2.6. Measurement of candidate biomarkers

We use blood sampling, BACS-J, UPSA-B, ERP, sMRI, fMRI, and NIRS as objective biomarkers, as described in detail in the following sections and summarized in Table 1. Before every measurement, we consider the participants' condition and respected their voluntary cooperation.

2.6.1. Neurocognitive batteries

Cognitive impairment is a core feature of schizophrenia and a key determinant of functional outcome; therefore, the assessment of cognitive function is important for the evaluation of patients with schizophrenia (Green et al., 2004; Keefe et al., 2004). The BACS-J is an important and sensitive tool to assess cognitive domains, especially for domains that are severely impaired in patients with schizophrenia such as verbal memory, working memory, motor speed, verbal fluency, attention, and executive function (Keefe et al., 2004; Kaneda et al., 2007). Recent neurocognitive studies suggested that individuals with UHR have relatively worse performance in verbal memory and processing speed than healthy controls; however, they show similar working memory and executive function (Seidman et al., 2010; Carrion et al., 2011). Furthermore, the severity of verbal memory impairment may predict the onset of psychosis (Brewer et al., 2005; Lencz et al., 2006; Seidman et al., 2010). The BACS-J battery is less time-consuming and easy to use in clinical settings, and it has already been used as a valid cognitive assessment tool for participants with UHR and FEP.

In the measurement of functional outcomes, the UPSA is used to assess performance in 5 real-world domains: planning/organization, finances, communication, travel, and household (Patterson, 2010; Sumiyoshi et al., 2011). The UPSA does not require specialized qualifications for administration, and it has high reliability and validity in patients with schizophrenia (Harvey et al., 2007). The UPSA-B is a brief version that consists of 2 of the 5 domains mentioned above (finances and communication). The UPSA-B requires only 10–15 min to administer and has easy instructions and high validity for the residential status of patients with schizophrenia (Mausbach et al., 2007).

2.6.2. Structural and functional MRI

MRI is well recognized for its excellent spatial resolution, lack of radioactivity, and noninvasiveness. MRI has recently made investigation of the neural correlates of pathophysiology possible in schizophrenia. Previous MRI studies in patients with schizophrenia provided quantitative evidence of structural abnormalities. Longitudinal sMRI studies demonstrated the progression of the loss of neocortical gray matter volume characteristically after the onset of psychosis (Kasai et al., 2003a, 2003b; Olabi et al., 2011). Previous longitudinal investigations of UHR individuals who later developed psychosis showed that the brain changes during the period of transition to psychosis especially in the lateral and medial temporal cortex (Pantelis et al., 2003; Velakoulis et al., 2006; Takahashi et al., 2009). These results suggest that brain morphological changes occur around the onset and sMRI is a possible biomarker for predicting the onset and identifying clinical stages of psychosis (Koutsouleris et al., 2009; Fusar-Poli et al., 2011a).

fMRI allows the imaging of brain function with high spatial resolution. Previous studies that used fMRI in UHR individuals reported significant abnormalities in the neural correlates of working memory, visual attention, and emotional processing (Fusar-Poli, 2011). Moreover, neural correlates of disrupted interpersonal cognition were reported in the previous literature (Brune et al., 2011). Patients with schizophrenia show deficits in interpersonal cognition, including abnormal performance in tasks targeting the "theory of mind"

or perspective-taking and empathy. Although impaired interpersonal cognition is considered as one of the core contributors to the poor social functioning of patients with schizophrenia (Pinkham and Penn, 2006), how and when such psychological disruptions emerge and progress have not been fully investigated. We investigated the neural correlates of disrupted interpersonal cognition in patients with schizophrenia by using a perspective-taking task and facial imitation task that were adapted from previous studies (Baron-Cohen et al., 1985; Carr et al., 2003; Lee et al., 2006; Vollm et al., 2006). The present studies may contribute to a better understanding of the pathophysiological features of schizophrenia and the development of effective and objective biomarkers for the early detection and prediction of psychosis.

2.6.3. Event-related potential

The ERP is a suitable method for investigating the neurophysiology of the brain with a high temporal resolution. We use the duration MMN, the frequency MMN, and the auditory steady-state response (ASSR) measured by a 64-channel net station in patients at different clinical stages to identify clinically useful biological markers.

To evaluate the cognitive dysfunction in schizophrenia, a promising approach involves the use of auditory MMN, which is one of the ERP components and is thought to reflect the pre-attentive deviance detection process in the early auditory function (Naatanen and Kahkonen, 2009). Primary generators for MMN have been localized to the auditory cortex (Naatanen and Kahkonen, 2009). MMN amplitude reduction observed upon the direct application of *N*-methyl-D-aspartate (NMDA) receptor antagonists suggests that the MMN reflects current influx in NMDA receptors in the primary auditory cortex (Javitt et al., 1996). Patients with chronic schizophrenia often exhibit reduced MMN amplitude. MMN deficits are specific for patients with schizophrenia, and MMN is one of the most widely replicated biomarkers of cognitive dysfunction in schizophrenia (Kawakubo and Kasai, 2006; Salisbury et al., 2007; Naatanen and Kahkonen, 2009). Several studies have suggested that deficits of MMN occur in different clinical stages according to the type of deviant stimulus. The duration MMN amplitude was reduced before the onset of psychosis (Brockhaus-Dumke et al., 2005; Bodatsch et al., 2011; Atkinson et al., 2012; Shaikh et al., 2012), whereas the frequency MMN amplitude progressively decreased after the onset (Salisbury et al., 2007; Magno et al., 2008).

Another approach to evaluate the cognitive dysfunction in schizophrenia is the measurement of the ASSR, which is one of the standard methods for the evaluation of gamma band oscillation. Cortical gamma oscillation may be associated with high-order cognitive functions such as working memory, and require inhibitory inputs to pyramidal neurons from the parvalbumin basket cell class of GABAergic neurons (Lewis et al., 2012). Several studies reported that patients with schizophrenia exhibit reduced power and coherence in response to 40-Hz click trains. This selective disturbance of ASSRs in the gamma-range frequency in schizophrenia may be caused by specific cellular neuropathological changes in gamma-aminobutyric acid-mediated neural networks in the auditory cortex (Brenner et al., 2009). Only one study showed an abnormal response in patients with FEP (Spencer et al., 2008).

2.6.4. Near-infrared spectroscopy

NIRS is a relatively new method that can measure changes in the hemodynamic oxygenated and deoxygenated hemoglobin concentrations in the cerebral cortex. NIRS offers advantages of noninvasiveness, an easy setup, minimal constraints, compactness, and quietness. Because of these advantages, NIRS can be used to measure the cortical function of patients noninvasively and repetitively in clinical settings (Takizawa et al., 2008; Koike et al., 2011b). Our multichannel NIRS study using a letter version of a verbal fluency task showed reduced activation over the prefrontal regions that showed significant positive correlations with lower GAF scores in the schizophrenia group (Takizawa et al., 2008; Koike et al., 2011b). We also found different impairment patterns in UHR, FEP, and chronic schizophrenia (Koike et al., 2011b). The verbal fluency task requires various cognitive domains such as memory recall, verbal learning and memory, inhibition, and executive function. This task is suitable because most patients can perform the task, and the impairment of verbal manipulation may be a core element for the onset of psychosis (Brewer et al., 2005; Lencz et al., 2006; Seidman et al., 2010; Carrion et al., 2011). Our major aim in this project is to determine whether these changes reflect present symptoms and/or functions and predict the transition to psychosis or symptomatic and functional outcomes.

2.6.5. Statistical analysis for clinical assessment until July 2012

In this article, we introduce the preliminary data for the clinical assessment at baseline and 6-month follow-up until July 2012 and for the transition to psychosis in the UHR group until Oct 2012. We analyzed the data by using Mann–Whitney *U*-test and Wilcoxon signed-rank test using SPSS 17.0J software (SPSS Inc., Chicago, IL, USA).

3. Results

In total, 159 individuals were assessed using SIPS, and 104 individuals fulfilled the UHR or FEP criteria (Fig. 1). Thirty-seven UHR individuals and 30 patients with FEP participated in this study. The number of diagnoses in the UHR individuals according to SIPS and in the FEP patients according to the DSM-IV is summarized in Table 3. At baseline, the GAF score was significantly different between the groups, but the other scores were not significantly different (Table 4).

Six months later, we assessed the clinical characteristics for 23 UHR individuals and 21 patients with FEP. The reasons for the lack of follow-up are summarized in Fig. 1. Although demographic characteristics of patients with FEP were not significantly different whether follow-up or not, the participants who were able to be followed-up in the UHR group were more likely to be young and have mild symptoms in the baseline assessment (age: not follow-up, 22.9 [3.6]; follow-up, 20.4 [3.3]; $p=0.032$; GAF score: 42.2 [7.6], 49.5 [11.5], $p=0.027$; PANSS general score: 42.2 [7.6], 49.5 [11.5], $p=0.027$; WHO-BREF Physical health: 2.2 [0.6], 2.8 [0.6], $p=0.009$; Psychological health: 2.0 [0.6], 2.6 [0.6], $p=0.013$; Environment: 2.8 [0.6], 3.4 [0.5], $p=0.008$; and CES-D score: 35.7 [11.7], 23.7 [10.6], $p=0.003$; respectively). The PANSS positive, general pathology, and GAF scores in the FEP group significantly changed from the baseline to the 6-month follow-up, whereas the PANSS positive and GAF scores, and the WHOQOL-BREF social relationship score significantly changed in the UHR group (Table 4). The follow-up assessment did not demonstrate significant differences between the UHR and FEP groups. Two (7.1%) of the 27 UHR individuals within 6 months, 2 (8.0%) of 24 within 12 months, and 6 (35.3%) of 17 within 24 months transitioned to psychosis according to the SIPS criteria.

4. Discussion

The goal of our research project is to explore the pathophysiological features with regard to the onset of psychosis using a longitudinal and multi-modal measurement approach and to investigate the possible predictive biomarkers of clinical outcome, therapy choice, early detection, and prediction of psychosis in clinical settings. We adopted gene, biochemical agents, neurophysiological data, brain images, and clinical features as objective biomarkers (Table 1). We have already reported cross-sectional results and now evaluate the longitudinal data (Koike et al., 2011b; Iwashiro et al., 2012).

Recent longitudinal neuroimaging investigations for UHR and FEP involved early intervention services based on community mental health services, for example, the Orygen Youth Health at Melbourne University (Pantelis et al., 2003; Brewer et al., 2005; Velakoulis et al., 2006; Takahashi et al., 2009) and LEO and OASIS at King's

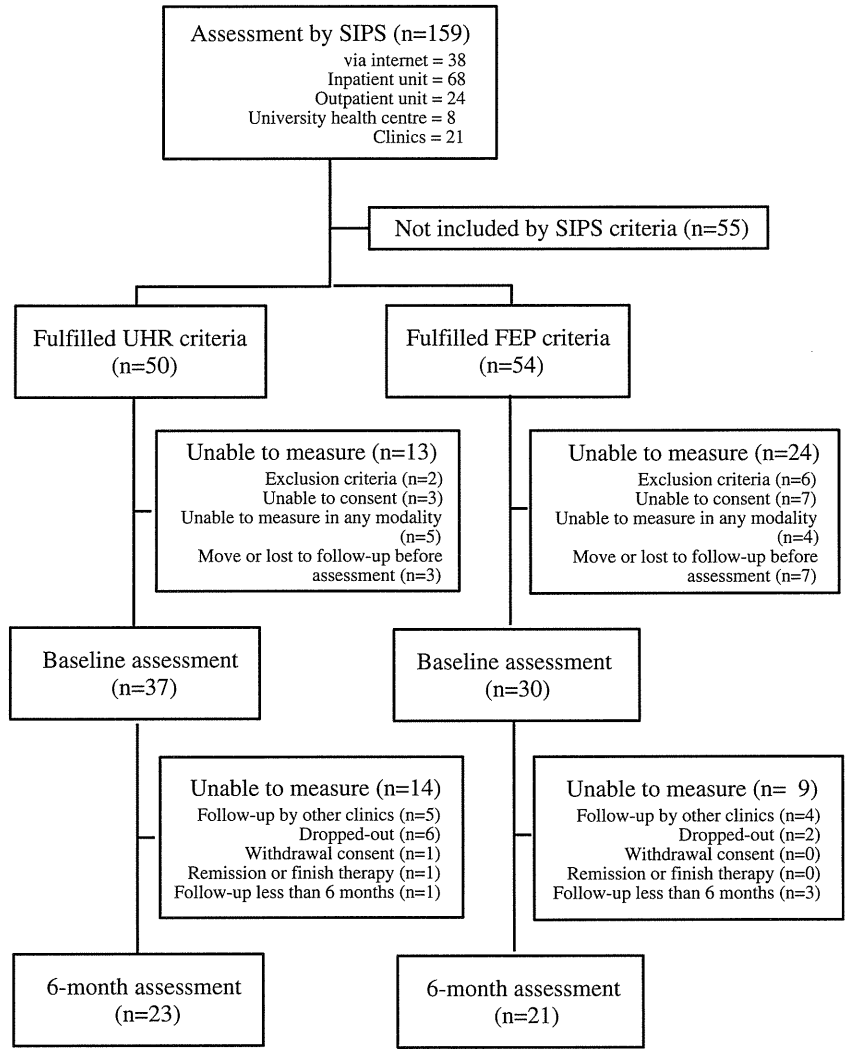

Fig. 1. Eligible individuals and participants flow in this study.

College London (Fusar-Poli et al., 2010, 2011b; Shaikh et al., 2012). In particular, several novel multi-modal approaches for UHR and FEP have been introduced by the group at King's College London (Fusar-Poli et al., 2010, 2011b). In contrast, a multi-site study with many participants in U.S., the North American Prodrome Longitudinal Study (NAPLS), especially addressed UHR individuals and proposed possible predictive clinical findings for the transition to psychosis (Cannon et al., 2008; Woods et al., 2009; Seidman et al., 2010). Recently the NAPLS-2 project was conducted to replicate possible predictors, and it also used blood sampling as well as neurocognitive batteries, ERP, sMRI, and fMRI to explore possible objective biomarkers (Addington et al., 2012). A multi-site study with many participants in Germany, the German Research Network on Schizophrenia, has conducted several pharmacological and/or psychosocial interventions for both UHR and FEP (Wölwer et al., 2006) and suggested possible biomarkers using MMN (Bodatsch et al., 2011). Differences among these research projects and the IN-STEP project include objectives, inclusion criteria, and recruitment background. The main purpose of our project was to investigate possible predictive biomarkers for the transition to psychosis as well as symptomatic and functional outcomes by using multi-level objective measurements for both UHR and FEP. Therefore, we excluded several conditions that could have affected the results such as drug abuse, PTSD, and PDD. Various genetic studies have identified substantial common risk genes between schizophrenia and autism (Stefansson et al., 2008). Neuroimaging studies have also suggested that the anatomic abnormality of the brain seen in autism also occurs in schizophrenia (Cheung et al., 2010). However, the typical clinical features and long-term outcomes are different, and also the neural basis is

Table 3
Number of diagnoses in UHR individuals according to SIPS and patients with FEP and ChSZ according to the DSM-IV.

Diagnosis	Number
UHR	
BIPS	1
APS	24
GRDS	4
BIPS + APS	2
APS + GRDS	6
FEP	
295.1 Schizophrenia, disorganized type	7
295.3 Schizophrenia, paranoid type	13
295.4 Schizophreniform disorder	7
297.1 Delusional disorder	1
298.9 Psychotic disorder not otherwise specified	2

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

Table 4
Demographic characteristics of study participants at baseline measurement until 31 July 2012.

	UHR					FEP					Group differences	
	Baseline		6 months			Baseline		6 months			Baseline	6 months
	Ave.	SD	Ave.	SD	P value	Ave.	SD	Ave.	SD	P value	P value	P value
Participants (male)	37 (20)		23 (13)		NA	30 (18)		21 (14)		NA	NA	NA
Living with family (%)	26 (70.2)		NA		NA	23 (76.7)		NA		NA	.096	NA
Employment or Education (%)	27 (73.0)		NA		NA	20 (66.7)		NA		NA	.086	NA
Symptomatic remission (%)	NA		NA		NA	6 (20.0)		10 (47.6)		NA	NA	NA
Age (year)	21.3	3.6	NA	NA	NA	23.7	6.0	NA	NA	NA	.066	NA
Education (year)	13.2	2.3	NA	NA	NA	13.2	2.6	NA	NA	NA	.943	NA
Age at onset of illness (year)	19.1	4.1	NA	NA	NA	21.2	6.2	NA	NA	NA	.108	NA
Age at onset of psychosis (year)	NA	NA	NA	NA	NA	22.9	6.1	NA	NA	NA	NA	NA
DUP (week)	NA	NA	NA	NA	NA	37.5	71.3	NA	NA	NA	NA	NA
Premorbid IQ	106.3	9.4	NA	NA	NA	103.9	10.5	NA	NA	NA	.337	NA
GAF	46.7	10.7	55.4	15.9	**.014**	37.3	10.8	47.3	12.7	**.039**	**.001**	.069
PANSS												
Positive	14.2	3.6	12.0	3.4	**.026**	15.7	4.8	11.7	5.2	**.044**	.143	.800
Negative	18.9	6.32	16.6	6.2	.260	20.5	7.9	16.6	8.2	.061	.206	.996
General pathology	34.6	7.9	31.6	7.6	.559	36.2	9.1	27.3	9.1	**.003**	.433	.097
WHOQOL-BREF												
Physical health	2.57	0.66	2.89	0.72	.635	2.59	0.78	2.70	0.78	.202	.923	.429
Psychological health	2.36	0.70	2.68	0.75	.402	2.48	0.85	2.58	0.66	.066	.546	.693
Social relationships	2.84	0.67	3.22	0.77	**.018**	2.60	1.01	2.76	0.68	.256	.290	.061
Environment	3.13	0.60	3.51	0.51	.231	3.18	0.53	3.25	0.50	.097	.762	.125
Overall	2.21	0.85	2.53	0.75	.150	2.56	0.99	2.25	1.07	.369	.171	.363
CES-D	28.0	12.3	20.5	13.5	.488	28.4	12.1	25.8	13.5	.083	.908	.233

Abbreviation: UHR, ultra-high risk; FEP; first-episode psychosis; Ave., average; SD, standard deviation; DUI, duration of illness; DUP, duration of untreated psychosis; IQ, intelligent quotient; GAF, the Global Assessment of Functioning; SOPS, the Scale of Prodromal Symptoms; PANSS, the Positive and Negative Syndrome Scale; WHO-BREF, the 26-item brief version of the WHO Quality of Life Scale; CES-D, the Center for Epidemiologic Studies Depression Scale.
Because of minor change of measurement design, WHO-BREF (7 ARMS and 4 FEP) and CES-D (1 ARMS and 6 FEP) were not assessed at baseline.

different in genetic and neuroimaging studies among these disorders. The brain volume in patients with autism at the age of 2 to 3 years is larger than that in children with normal development at the same age and becomes smaller when these patients become older (Courchesne et al., 2001). In contrast, the brain volume of patients with schizophrenia before the onset is smaller than that in healthy controls, and a further progressive loss of brain volume then occurs (Kasai et al., 2003a, 2003b; Pantelis et al., 2003; Velakoulis et al., 2006; Salisbury et al., 2007; Takahashi et al., 2009). Through structural brain imaging studies, we have also reported that the abnormalities in schizophrenia are partially similar to but different from those in PDD and PTSD in some aspects (Kasai et al., 2003a, 2003b; Yamasue et al., 2003; Araki et al., 2005; Kasai et al., 2008; Rogers et al., 2009; Suga et al., 2010; Yamasaki et al., 2010). Our studies have shown different brain volumes in the inferior frontal gyrus between FEP and PDD, which implied that the brain functionalization related to verbal manipulation and mirror neuron system within the inferior frontal gyrus manifested the difference of characteristic symptoms between the diseases (Suga et al., 2010; Yamasaki et al., 2010; Iwashiro et al., 2012). Although patients with PDD and chronic schizophrenia showed volume reduction in the pars opercularis (corresponding to Brodmann area 44) and pars triangularis (Brodmann area 45), PDD was associated with relatively smaller volume in the pars opercularis and chronic schizophrenia in the pars triangularis. Patients with UHR and FEP have volume reduction only in the pars triangularis (Iwashiro et al., 2012). Recent studies have also suggested that brain maturation in the prefrontal cortex occurs from the caudal to rostral areas in accordance with the human evolution of verbal communication and social interaction (Badre and D'Esposito, 2009). Our study criteria may therefore be used to identify differences among several psychiatric disorders in terms of neurodevelopmental trajectory and brain maturation.

In the baseline assessment, the PANSS score in the FEP group was similar to those in the UHR group. The reason was that the clinical assessments at baseline relied on feasibility of neurocognitive batteries and/or neuroimaging instruments. The baseline assessment was performed when patients with FEP were in a relatively stable condition and therefore had similar scores on the symptom scales when compared with individuals with UHR (Koike et al., 2011b). At the 6-month follow-up, the clinical difference between the groups disappeared. It has been suggested that outcomes in schizophrenia can be roughly divided into the following three categories: symptomatic remission with little functional deficit, continuous symptoms and/or functional deficit, and severe continuous symptoms and functional deficit (van Os and Kapur, 2009). As observed for individuals at risk for psychosis, recent studies have also suggested that outcomes in UHR individuals may be classified into the following three categories: symptomatic remission and functional recovery, sustained symptoms and/or functional deficit, and transition to psychosis (Addington et al., 2011; Simon et al., 2011). These results suggest that regardless of their history of psychosis, their symptoms and functional deficits should be addressed according to the clinical stages (McGorry et al., 2006). We also plan to perform a psychological intervention for patients with FEP and to scale up intensive care for young people (Koike et al., 2011a). With regard to the transition to psychosis in the UHR group, the transition rate was similar to the previous high-risk studies, although the mean age of the participants was relatively higher than that in previous studies (Fusar-Poli et al., 2012).

Our project has possible limitations regarding the study design because our observations were mainly naturalistic and some participants have entered other psychosocial clinical trials (Koike et al., 2011a). Therefore, the number of participants who entered into these trials and their distribution must be considered. Second, selection bias must be considered as participants were recruited mainly from the university units. In addition, we have to consider participants' feasibility of measurements when registering because our project was based on neuroimaging studies. Therefore, potential selection bias according to participants' symptoms and severities also has to be considered. Third, and our naturalistic design allowed the psychiatrists to prescribe medications to the participants prior to entry. Because of free access to the medical system in Japan, it is relatively easy for patients to receive medications including

antipsychotics and benzodiazepines. Although we minimized medication and determined whether all the participants received proper medication under the guidelines for FEP after entry, it was difficult to collect blood samples and perform imaging under medication-free conditions.

In conclusion, we have introduced the research protocol for our prospective observational cohort study to explore the pathophysiological features with regard to the onset of psychosis using a multi-modal measurement approach. Our aim is also to investigate possible predictive biomarkers of clinical outcome, therapy choice, early detection, and the prediction of psychosis in clinical settings. In this research project, we expect to improve patients' prognoses applying these findings to clinical use.

Role of funding source

This study was supported by grants from the Ministry of Health, Labour, and Welfare (Health and Labour Sciences Research Grants, Research on Psychiatric and Neurological Diseases and Mental Health, H22-seishin-ippan-015 to KK; Health and Labour Science Research Grants for Comprehensive Research on Disability Health and Welfare, H23-seishin-ippan-002 to RT and YN), from Grant-in-Aid for Scientific Research on Innovative Areas (Comprehensive Brain Science Network and Adolescent Mind & Self-Regulation (23118001 and 23118004)) to KK; KAKENHI No. 23791309 to RT), from National Bioscience Database Center (NBDC) of Japan Science and Technology Agency (JST), and from the Intramural Research Grant (23-10) for Neurological and Psychiatric Disorders of NCNP (to RT and YN). A part of this study was also from the "Development of biomarker candidates for social behavior" project of the Strategic Research Program for Brain Sciences sponsored by MEXT.

Contributors

SK, YT, MS, SY, RT, TA, and KK designed the protocol. SK wrote the entire manuscript and undertook the statistical analysis in this article. MS and SY wrote the "Neurocognitive battery" section; YT, NI, T Natsubori, NY, and HY, the "MRI" section; T Nagai, MT, and TA, the "Event-related potential" section; and SK, YS, and YN, the "Near-infrared spectroscopy" section. SK, YT, NI, YS, T Nagai, T Natsubori, MT, MS, and SY contributed to the implementation of this project. SK, MS, SY, RT, TA, and KK contributed financial resources to this project. All authors have approved the final version of the manuscript.

Conflict of interest

None.

Acknowledgment

We thank all the participants for voluntary cooperation in this study. We also thank Chie Shimojyo for database management.

References

Addington, J., Cornblatt, B.A., Cadenhead, K.S., Cannon, T.D., McGlashan, T.H., Perkins, D.O., Seidman, L.J., Tsuang, M.T., Walker, E.F., Woods, S.W., Heinssen, R., 2011. At clinical high risk for psychosis: outcome for nonconverters. Am. J. Psychiatry 168 (8), 800–805.

Addington, J., Cadenhead, K.S., Cornblatt, B., Mathalon, D.H., McGlashan, T.H., Perkins, D.O., Seidman, L.J., Tsuang, M., Walker, E.F., Woods, S.W., Addington, J.A., Cannon, T.D., 2012. North American Prodrome Longitudinal Study (NAPLS 2): overview and recruitment. Schizophr. Res. 142 (1), 77–82.

American Psychiatric Association (APA), 1994. Diagnostic and Statistical Manual of Mental Disorders, 4th ed. American Psychiatric Press, Washington, D.C. U.S.A.

American Psychiatric Association (APA), 1997. Practice guideline for the treatment of patients with schizophrenia. Am. J. Psychiatry 154 (4 Suppl.), 1–63.

Andreasen, N.C., Carpenter Jr., W.T., Kane, J.M., Lasser, R.A., Marder, S.R., Weinberger, D.R., 2005. Remission in schizophrenia: proposed criteria and rationale for consensus. Am. J. Psychiatry 162 (3), 441–449.

Araki, T., Kasai, K., Yamasue, H., Kato, N., Kudo, N., Ohtani, T., Nakagome, K., Kirihara, K., Yamada, H., Abe, O., Iwanami, A., 2005. Association between lower P300 amplitude and smaller anterior cingulate cortex volume in patients with posttraumatic stress disorder: a study of victims of Tokyo subway sarin attack. Neuroimage 25 (1), 43–50.

Atkinson, R.J., Michie, P.T., Schall, U., 2012. Duration mismatch negativity and P3a in first-episode psychosis and individuals at ultra-high risk of psychosis. Biol. Psychiatry 71 (2), 98–104.

Badre, D., D'Esposito, M., 2009. Is the rostro-caudal axis of the frontal lobe hierarchical? Nat. Rev. Neurosci. 10, 659–669.

Baron-Cohen, S., Leslie, A.M., Frith, U., 1985. Does the autistic child have a "theory of mind"? Cognition 21 (1), 37–46.

Bodatsch, M., Ruhrmann, S., Wagner, M., Muller, R., Schultze-Lutter, F., Frommann, I., Brinkmeyer, J., Gaebel, W., Maier, W., Klosterkotter, J., Brockhaus-Dumke, A., 2011. Prediction of psychosis by mismatch negativity. Biol. Psychiatry 69 (10), 959–966.

Borgwardt, S., Fusar-Poli, P., 2012. Third-generation neuroimaging in early schizophrenia: translating research evidence into clinical utility. Br. J. Psychiatry 200 (4), 270–272.

Brenner, C.A., Krishnan, G.P., Vohs, J.L., Ahn, W.Y., Hetrick, W.P., Morzorati, S.L., O'Donnell, B.F., 2009. Steady state responses: electrophysiological assessment of sensory function in schizophrenia. Schizophr. Bull. 35 (6), 1065–1077.

Brewer, W.J., Francey, S.M., Wood, S.J., Jackson, H.J., Pantelis, C., Phillips, L.J., Yung, A.R., Anderson, V.A., McGorry, P.D., 2005. Memory impairments identified in people at ultra-high risk for psychosis who later develop first-episode psychosis. Am. J. Psychiatry 162 (1), 71–78.

Brockhaus-Dumke, A., Tendolkar, I., Pukrop, R., Schultze-Lutter, F., Klosterkötter, J., Ruhrmann, S., 2005. Impaired mismatch negativity generation in prodromal subjects and patients with schizophrenia. Schizophr. Res. 73 (2–3), 297–310.

Brune, M., Ozgurdal, S., Ansorge, N., von Reventlow, H.G., Peters, S., Nicolas, V., Tegenthoff, M., Juckel, G., Lissek, S., 2011. An fMRI study of "theory of mind" in at-risk states of psychosis: comparison with manifest schizophrenia and healthy controls. Neuroimage 55 (1), 329–337.

Cannon, T.D., Cadenhead, K., Cornblatt, B., Woods, S.W., Addington, J., Walker, E., Seidman, L.J., Perkins, D., Tsuang, M., McGlashan, T., Heinssen, R., 2008. Prediction of psychosis in youth at high clinical risk: a multisite longitudinal study in North America. Arch. Gen. Psychiatry 65 (1), 28–37.

Carr, L., Iacoboni, M., Dubeau, M., Mazziotta, J.C., Lenzi, G.L., 2003. Neural mechanisms of empathy in humans: a relay from neural systems for imitation to limbic areas. PNAS 100 (9), 5497–5502.

Carrion, R.E., Goldberg, T.E., McLaughlin, D., Auther, A.M., Correll, C.U., Cornblatt, B.A., 2011. Impact of neurocognition on social and role functioning in individuals at clinical high risk for psychosis. Am. J. Psychiatry 168 (8), 806–813.

Cheung, C., Yu, K., Fung, G., Leung, M., Wong, C., Li, Q., Sham, P., Chua, S., McAlonan, G., 2010. Autistic disorders and schizophrenia: related or remote? An anatomical likelihood estimation. PLoS One 5 (8), e12233.

Courchesne, E., Karns, C.M., Davis, H.R., Ziccardi, R., Carper, R.A., Tigue, Z.D., Chisum, H.J., Moses, P., Pierce, K., Lord, C., Lincoln, A.J., Pizzo, S., Schreibman, L., Haas, R.H., Akshoomoff, N.A., Courchesne, R.Y., 2001. Unusual brain growth patterns in early life in patients with autistic disorder: an MRI study. Neurology 57 (2), 245–254.

Degenhardt, L., Chiu, W.T., Sampson, N., Kessler, R.C., Anthony, J.C., Angermeyer, M., Bruffaerts, R., de Girolamo, G., Gureje, O., Huang, Y., Karam, A., Kostyuchenko, S., Lepine, J.P., Mora, M.E., Neumark, Y., Ormel, J.H., Pinto-Meza, A., Posada-Villa, J., Stein, D.J., Takeshima, T., Wells, J.E., 2008. Toward a global view of alcohol, tobacco, cannabis, and cocaine use: findings from the WHO World Mental Health Surveys. PLoS Med. 5 (7), e141.

Emsley, R., Chiliza, B., Asmal, L., Lehloenya, K., 2011. The concepts of remission and recovery in schizophrenia. Curr. Opin. Psychiatry 24 (2), 114–121.

Erdfelder, E., Faul, F., Buchner, A., 1996. GPOWER: a general power analysis program. Behav. Res. Methods Instrum. Comput. 28, 1–11.

Fusar-Poli, P., 2011. Voxel-wise meta-analysis of fMRI studies in patients at clinical high risk for psychosis. J. Psychiatry Neurosci. 36 (6), 110021.

Fusar-Poli, P., Howes, O.D., Allen, P., Broome, M., Valli, I., Asselin, M.C., Grasby, P.M., McGuire, P.K., 2010. Abnormal frontostriatal interactions in people with prodromal signs of psychosis: a multimodal imaging study. Arch. Gen. Psychiatry 67 (7), 683–691.

Fusar-Poli, P., Borgwardt, S., Crescini, A., Deste, G., Kempton, M.J., Lawrie, S., Mc Guire, P., Sacchetti, E., 2011a. Neuroanatomy of vulnerability to psychosis: a voxel-based meta-analysis. Neurosci. Biobehav. Rev. 35 (5), 1175–1185.

Fusar-Poli, P., Stone, J.M., Broome, M.R., Valli, I., Mechelli, A., McLean, M.A., Lythgoe, D.J., O'Gorman, R.L., Barker, G.J., McGuire, P.K., 2011b. Thalamic glutamate levels as a predictor of cortical response during executive functioning in subjects at high risk for psychosis. Arch. Gen. Psychiatry 68 (9), 881–890.

Fusar-Poli, P., Bonoldi, I., Yung, A.R., Borgwardt, S., Kempton, M.J., Valmaggia, L., Barale, F., Caverzasi, E., McGuire, P., 2012. Predicting psychosis: meta-analysis of transition outcomes in individuals at high clinical risk. Arch. Gen. Psychiatry 69 (3), 220–229.

Green, M.F., Nuechterlein, K.H., Gold, J.M., Barch, D.M., Cohen, J., Essock, S., Fenton, W.S., Frese, F., Goldberg, T.E., Heaton, R.K., Keefe, R.S., Kern, R.S., Kraemer, H., Stover, E., Weinberger, D.R., Zalcman, S., Marder, S.R., 2004. Approaching a consensus cognitive battery for clinical trials in schizophrenia: the NIMH-MATRICS conference to select cognitive domains and test criteria. Biol. Psychiatry 56 (5), 301–307.

Harrison, I., Joyce, E.M., Mutsatsa, S.H., Hutton, S.B., Huddy, V., Kapasi, M., Barnes, T.R., 2008. Naturalistic follow-up of co-morbid substance use in schizophrenia: the West London first-episode study. Psychol. Med. 38 (1), 79–88.

Harvey, P.D., Bellack, A.S., 2009. Toward a terminology for functional recovery in schizophrenia: is functional remission a viable concept? Schizophr. Bull. 35 (2), 300–306.

Harvey, P.D., Velligan, D.I., Bellack, A.S., 2007. Performance-based measures of functional skills: usefulness in clinical treatment studies. Schizophr. Bull. 33 (5), 1138–1148.

Insel, T.R., 2010. Rethinking schizophrenia. Nature 468 (7321), 187–193.

Iwashiro, N., Suga, M., Takano, Y., Inoue, H., Natsubori, T., Satomura, Y., Koike, S., Yahata, N., Murakami, M., Katsura, M., Gonoi, W., Sasaki, H., Takao, H., Abe, O., Kasai, K., Yamasue, H., 2012. Localized gray matter volume reductions in the pars triangularis of the inferior frontal gyrus in individuals at clinical high-risk for psychosis and first episode for schizophrenia. Schizophr. Res. 137 (1), 124–131.

Javitt, D.C., Steinschneider, M., Schroeder, C.E., Arezzo, J.C., 1996. Role of cortical N-methyl-D-aspartate receptors in auditory sensory memory and mismatch negativity generation: implications for schizophrenia. Proc. Natl. Acad. Sci. U. S. A. 93 (21), 11962–11967.

Kaneda, Y., Sumiyoshi, T., Keefe, R., Ishimoto, Y., Numata, S., Ohmori, T., 2007. Brief assessment of cognition in schizophrenia: validation of the Japanese version. Psychiatry Clin. Neurosci. 61 (6), 602–609.

Kasai, K., Shenton, M.E., Salisbury, D.F., Hirayasu, Y., Lee, C.U., Ciszewski, A.A., Yurgelun-Todd, D., Kikinis, R., Jolesz, F.A., McCarley, R.W., 2003a. Progressive decrease of left superior temporal gyrus gray matter volume in patients with first-episode schizophrenia. Am. J. Psychiatry 160 (1), 156–164.

Kasai, K., Shenton, M.E., Salisbury, D.F., Hirayasu, Y., Onitsuka, T., Spencer, M.H., Yurgelun-Todd, D.A., Kikinis, R., Jolesz, F.A., McCarley, R.W., 2003b. Progressive decrease of left Heschl gyrus and planum temporale gray matter volume in first-episode schizophrenia: a longitudinal magnetic resonance imaging study. Arch. Gen. Psychiatry 60 (8), 766–775.
Kasai, K., Yamasue, H., Gilbertson, M.W., Shenton, M.E., Rauch, S.L., Pitman, R.K., 2008. Evidence for acquired pregenual anterior cingulate gray matter loss from a twin study of combat-related posttraumatic stress disorder. Biol. Psychiatry 63 (6), 550–556.
Kawakubo, Y., Kasai, K., 2006. Support for an association between mismatch negativity and social functioning in schizophrenia. Prog. Neuropsychopharmacol. Biol. Psychiatry 30 (7), 1367–1368.
Kawakubo, Y., Kamio, S., Nose, T., Iwanami, A., Nakagome, K., Fukuda, M., Kato, N., Rogers, M.A., Kasai, K., 2007. Phonetic mismatch negativity predicts social skills acquisition in schizophrenia. Psychiatry Res. 152 (2–3), 261–265.
Kay, S.R., Fiszbein, A., Opler, L.A., 1987. The positive and negative syndrome scale (PANSS) for schizophrenia. Schizophr. Bull. 13 (2), 261–276.
Keefe, R.S., Goldberg, T.E., Harvey, P.D., Gold, J.M., Poe, M.P., Coughenour, L., 2004. The Brief Assessment of Cognition in Schizophrenia: reliability, sensitivity, and comparison with a standard neurocognitive battery. Schizophr. Res. 68 (2–3), 283–297.
Koike, S., Nishida, A., Yamasaki, S., Ichihashi, K., Maegawa, S., Natsubori, T., Harima, H., Kasai, K., Fujita, I., Harada, M., Okazaki, Y., 2011a. Comprehensive early intervention for patients with first-episode psychosis in Japan (J-CAP): study protocol for a randomised controlled trial. Trials 12, 156.
Koike, S., Takizawa, R., Nishimura, Y., Takano, Y., Takayanagi, Y., Kinou, M., Araki, T., Harima, H., Fukuda, M., Okazaki, Y., Kasai, K., 2011b. Different hemodynamic response patterns in the prefrontal cortical sub-regions according to the clinical stages of psychosis. Schizophr. Res. 132 (1), 54–61.
Koutsouleris, N., Meisenzahl, E.M., Davatzikos, C., Bottlender, R., Frodl, T., Scheuerecker, J., Schmitt, G., Zetzsche, T., Decker, P., Reiser, M., Moller, H.J., Gaser, C., 2009. Use of neuroanatomical pattern classification to identify subjects in at-risk mental states of psychosis and predict disease transition. Arch. Gen. Psychiatry 66 (7), 700–712.
Lee, T., Josephs, O., Dolan, R.J., Critchley, H.D., 2006. Imitating expressions: emotion-specific neural substrates in facial mimicry. Soc. Cogn. Affect. Neurosci. 1 (2), 122–135.
Lencz, T., Smith, C.W., McLaughlin, D., Auther, A., Nakayama, E., Hovey, L., Cornblatt, B.A., 2006. Generalized and specific neurocognitive deficits in prodromal schizophrenia. Biol. Psychiatry 59 (9), 863–871.
Lewis, D.A., Curley, A.A., Glausie, J.R., Volk, D.W., 2012. Cortical parvalbumin interneurons and cognitive dysfunction in schizophrenia. Trends Neurosci. 35 (1), 57–67.
Lieberman, J.A., Tollefson, G.D., Charles, C., Zipursky, R., Sharma, T., Kahn, R.S., Keefe, R.S., Green, A.I., Gur, R.E., McEvoy, J., Perkins, D., Hamer, R.M., Gu, H., Tohen, M., 2005. Antipsychotic drug effects on brain morphology in first-episode psychosis. Arch. Gen. Psychiatry 62 (4), 361–370.
Magno, E., Yeap, S., Thakore, J.H., Garavan, H., De Sanctis, P., Foxe, J.J., 2008. Are auditory-evoked frequency and duration mismatch negativity deficits endophenotypic for schizophrenia? High-density electrical mapping in clinically unaffected first-degree relatives and first-episode and chronic schizophrenia. Biol. Psychiatry 64 (5), 385–391.
Mausbach, B.T., Harvey, P.D., Goldman, S.R., Jeste, D.V., Patterson, T.L., 2007. Development of a brief scale of everyday functioning in persons with serious mental illness. Schizophr. Bull. 33 (6), 1364–1372.
McGorry, P.D., Hickie, I.B., Yung, A.R., Pantelis, C., Jackson, H.J., 2006. Clinical staging of psychiatric disorders: a heuristic framework for choosing earlier, safer and more effective interventions. Aust. N. Z. J. Psychiatry 40 (8), 616–622.
Naatanen, R., Kahkonen, S., 2009. Central auditory dysfunction in schizophrenia as revealed by the mismatch negativity (MMN) and its magnetic equivalent MMNm: a review. Int. J. Neuropsychopharmacol. 12 (1), 125–135.
Olabi, B., Ellison-Wright, I., McIntosh, A.M., Wood, S.J., Bullmore, E., Lawrie, S.M., 2011. Are there progressive brain changes in schizophrenia? A meta-analysis of structural magnetic resonance imaging studies. Biol. Psychiatry 70 (1), 88–96.
Pantelis, C., Velakoulis, D., McGorry, P.D., Wood, S.J., Suckling, J., Phillips, L.J., Yung, A.R., Bullmore, E.T., Brewer, W., Soulsby, B., Desmond, P., McGuire, P.K., 2003. Neuroanatomical abnormalities before and after onset of psychosis: a cross-sectional and longitudinal MRI comparison. Lancet 361 (9354), 281–288.
Patterson, P., 2010. UCSD Performance-based Skills Assessment – Brief (UPSA-B), International Version: Administration and Scoring Manual.
Pinkham, A.E., Penn, D.L., 2006. Neurocognitive and social cognitive predictors of interpersonal skill in schizophrenia. Psychiatry Res. 143 (2–3), 167–178.
Prata, D.P., Mechelli, A., Fu, C.H., Picchioni, M., Kane, F., Kalidindi, S., McDonald, C., Howes, O., Kravariti, E., Demjaha, A., Toulopoulou, T., Diforti, M., Murray, R.M., Collier, D.A., McGuire, P.K., 2009. Opposite effects of catechol-O-methyltransferase Val158Met on cortical function in healthy subjects and patients with schizophrenia. Biol. Psychiatry 65 (6), 473–480.
Radloff, L.S., 1977. The CES-D Scale: a self-report depression scale for research in the general population. Appl. Psychol. Meas. 1 (3), 385–401.
Rogers, M.A., Yamasue, H., Abe, O., Yamada, H., Ohtani, T., Iwanami, A., Aoki, S., Kato, N., Kasai, K., 2009. Smaller amygdala volume and reduced anterior cingulate gray matter density associated with history of post-traumatic stress disorder. Psychiatry Res. 174 (3), 210–216.
Salisbury, D.F., Kuroki, N., Kasai, K., Shenton, M.E., McCarley, R.W., 2007. Progressive and interrelated functional and structural evidence of post-onset brain reduction in schizophrenia. Arch. Gen. Psychiatry 64 (5), 521–529.
Schimmelmann, B.G., Huber, C.G., Lambert, M., Cotton, S., McGorry, P.D., Conus, P., 2008. Impact of duration of untreated psychosis on pre-treatment, baseline, and outcome characteristics in an epidemiological first-episode psychosis cohort. J. Psychiatr. Res. 42 (12), 982–990.
Seidman, L.J., Giuliano, A.J., Meyer, E.C., Addington, J., Cadenhead, K.S., Cannon, T.D., McGlashan, T.H., Perkins, D.O., Tsuang, M.T., Walker, E.F., Woods, S.W., Bearden, C.E., Christensen, B.K., Hawkins, K., Heaton, R., Keefe, R.S., Heinssen, R., Cornblatt, B.A., 2010. Neuropsychology of the prodrome to psychosis in the NAPLS consortium: relationship to family history and conversion to psychosis. Arch. Gen. Psychiatry 67 (6), 578–588.
Shaikh, M., Valmaggia, L., Broome, M.R., Dutt, A., Lappin, J., Day, F., Woolley, J., Tabraham, P., Walshe, M., Johns, L., Fusar-Poli, P., Howes, O., Murray, R.M., McGuire, P., Bramon, E., 2012. Reduced mismatch negativity predates the onset of psychosis. Schizophr. Res. 134 (1), 42–48.
Simon, A.E., Velthorst, E., Nieman, D.H., Linszen, D., Umbricht, D., de Haan, L., 2011. Ultra high-risk state for psychosis and non-transition: a systematic review. Schizophr. Res. 132 (1), 8–17.
Spencer, K.M., Salisbury, D.F., Shenton, M.E., McCarley, R.W., 2008. Gamma-band auditory steady-state responses are impaired in first episode psychosis. Biol. Psychiatry 64 (5), 369–375.
Stefansson, H., Rujescu, D., Cichon, S., Pietilainen, O.P., Ingason, A., Steinberg, S., Fossdal, R., Sigurdsson, E., Sigmundsson, T., Buizer-Voskamp, J.E., Hansen, T., Jakobsen, K.D., Muglia, P., Francks, C., Matthews, P.M., Gylfason, A., Halldorsson, B.V., Gudbjartsson, D., Thorgeirsson, T.E., Sigurdsson, A., Jonasdottir, A., Bjornsson, A., Mattiasdottir, S., Blondal, T., Haraldsson, M., Magnusdottir, B.B., Giegling, I., Moller, H.J., Hartmann, A., Shianna, K.V., Ge, D., Need, A.C., Crombie, C., Fraser, G., Walker, N., Lonnqvist, J., Suvisaari, J., Tuulio-Henriksson, A., Paunio, T., Toulopoulou, T., Bramon, E., Di Forti, M., Murray, R., Ruggeri, M., Vassos, E., Tosato, S., Walshe, M., Li, T., Vasilescu, C., Muhleisen, T.W., Wang, A.G., Ullum, H., Djurovic, S., Melle, I., Olesen, J., Kiemeney, L.A., Franke, B., Sabatti, C., Freimer, N.B., Gulcher, J.R., Thorsteinsdottir, U., Kong, A., Andreassen, O.A., Ophoff, R.A., Georgi, A., Rietschel, M., Werge, T., Petursson, H., Goldstein, D.B., Nothen, M.M., Peltonen, L., Collier, D.A., St Clair, D., Stefansson, K., Kahn, R.S., Linszen, D.H., van Os, J., Wiersma, D., Bruggeman, R., Cahn, W., de Haan, L., Krabbendam, L., Myin-Germeys, I., 2008. Large recurrent microdeletions associated with schizophrenia. Nature 455 (7210), 232–236.
Suga, M., Yamasue, H., Abe, O., Yamasaki, S., Yamada, H., Inoue, H., Takei, K., Aoki, S., Kasai, K., 2010. Reduced gray matter volume of Brodmann's area 45 is associated with severe psychotic symptoms in patients with schizophrenia. Eur. Arch. Psychiatry Clin. Neurosci. 260 (6), 465–473.
Suga, M., Uetsuki, M., Takizawa, R., Araki, T., Kasai, K., 2011. Phonological fluency is uniquely impaired in Japanese-speaking schizophrenia patients: confirmation study. Psychiatry Clin. Neurosci. 65 (7), 672–675.
Sumiyoshi, T., Sumiyoshi, C., Hemmi, C., 2011. UCSD Performance-based Skills Assessment – Brief (UPSA-B) Japanese Version: Administration and Scoring Manual.
Takahashi, T., Wood, S.J., Yung, A.R., Soulsby, B., McGorry, P.D., Suzuki, M., Kawasaki, Y., Phillips, L.J., Velakoulis, D., Pantelis, C., 2009. Progressive gray matter reduction of the superior temporal gyrus during transition to psychosis. Arch. Gen. Psychiatry 66 (4), 366–376.
Takizawa, R., Kasai, K., Kawakubo, Y., Marumo, K., Kawasaki, S., Yamasue, H., Fukuda, M., 2008. Reduced frontopolar activation during verbal fluency task in schizophrenia: a multi-channel near-infrared spectroscopy study. Schizophr. Res. 99 (1–3), 250–262.
Takizawa, R., Tochigi, M., Kawakubo, Y., Marumo, K., Sasaki, T., Fukuda, M., Kasai, K., 2009. Association between catechol-O-methyltransferase Val108/158Met genotype and prefrontal hemodynamic response in schizophrenia. PLoS One 4 (5), e5495.
Tazaki, M., Nakane, Y., 1997. WHOQOL 26 Japanese Version. Kaneko Shobo Press, Tokyo.
The World Health Organization Quality of Life Group (WHOQOL Group), 1998. Development of the World Health Organization WHOQOL-BREF quality of life assessment. The WHOQOL Group. Psychol. Med. 28 (3), 551–558.
van Os, J., Kapur, S., 2009. Schizophrenia. Lancet 374 (9690), 635–645.
van Os, J., Kenis, G., Rutten, B.P., 2010. The environment and schizophrenia. Nature 468 (7321), 203–212.
Velakoulis, D., Wood, S.J., Wong, M.T., McGorry, P.D., Yung, A., Phillips, L., Smith, D., Brewer, W., Proffitt, T., Desmond, P., Pantelis, C., 2006. Hippocampal and amygdala volumes according to psychosis stage and diagnosis: a magnetic resonance imaging study of chronic schizophrenia, first-episode psychosis, and ultra-high-risk individuals. Arch. Gen. Psychiatry 63 (2), 139–149.
Vollm, B.A., Richardson, P.C., McKie, S., Deakin, J.F.W., Elliott, R., Anderson, I., 2006. Serotonergic modulation of neuronal responses to behavioural inhibition and reinforcing stimuli: an fMRI study in healthy volunteers. Eur. J. Neurosci. 23, 552–560.
von Elm, E., Altman, D.G., Egger, M., Pocock, S.J., Gotzsche, P.C., Vandenbroucke, J.P., 2007. The strengthening the reporting of observational studies in epidemiology (STROBE) statement: guidelines for reporting observational studies. PLoS Med. 4 (10), e296.
Wölwer, W., Baumann, A., Bechdolf, A., Buchkremer, G., Häfner, H., Janssen, B., Klosterkötter, J., Maier, W., Möller, H., Ruhrmann, S., Gaebel, W., 2006. The German Research Network on Schizophrenia – impact on the management of schizophrenia. Dialogues Clin. Neurosci. 8, 115–121.
Woods, S.W., Addington, J., Cadenhead, K.S., Cannon, T.D., Cornblatt, B.A., Heinssen, R., Perkins, D.O., Seidman, L.J., Tsuang, M.T., Walker, E.F., McGlashan, T.H., 2009. Validity of the prodromal risk syndrome for first psychosis: findings from the North American Prodrome Longitudinal Study. Schizophr. Bull. 35 (5), 894–908.
Yamasaki, S., Yamasue, H., Abe, O., Suga, M., Yamada, H., Inoue, H., Kuwabara, H., Kawakubo, Y., Yahata, N., Aoki, S., Kano, Y., Kato, N., Kasai, K., 2010. Reduced gray matter volume of pars opercularis is associated with impaired social communication in high-functioning autism spectrum disorders. Biol. Psychiatry 68 (12), 1141–1147.
Yamasue, H., Kasai, K., Iwanami, A., Ohtani, T., Yamada, H., Abe, O., Kuroki, N., Fukuda, R., Tochigi, M., Furukawa, S., Sadamatsu, M., Sasaki, T., Aoki, S., Ohtomo, K., Asukai, N., Kato, N., 2003. Voxel-based analysis of MRI reveals anterior cingulate gray-matter

volume reduction in posttraumatic stress disorder due to terrorism. Proc. Natl. Acad. Sci. U. S. A. 100 (15), 9039–9043.
Yoshida, T., Suga, M., Arima, K., Muranaka, Y., Tanaka, T., Eguchi, S., Lin, C., Yoshida, S., Ishikawa, M., Higuchi, Y., Seo, T., Ueoka, Y., Tomotake, M., Kaneda, Y., Darby, D., Maruff, P., Iyo, M., Kasai, K., Higuchi, T., Sumiyoshi, T., Ohmori, T., Takahashi, K., Hashimoto, K., 2011. Criterion and construct validity of the CogState Schizophrenia Battery in Japanese patients with schizophrenia. PLoS One 6 (5), e20469.
Yung, A.R., Stanford, C., Cosgrave, E., Killackey, E., Phillips, L., Nelson, B., McGorry, P.D., 2006. Testing the Ultra High Risk (prodromal) criteria for the prediction of psychosis in a clinical sample of young people. Schizophr. Res. 84 (1), 57–66.

NeuroImage xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

NeuroImage

journal homepage: www.elsevier.com/locate/ynimg

Genetic influences on prefrontal activation during a verbal fluency task in adults: A twin study based on multichannel near-infrared spectroscopy

Eisuke Sakakibara [a,b,*,1], Ryu Takizawa [a,c,**,1], Yukika Nishimura [a,d], Shingo Kawasaki [e], Yoshihiro Satomura [a], Akihide Kinoshita [a], Shinsuke Koike [f], Kohei Marumo [a], Masaru Kinou [a], Mamoru Tochigi [a], Nao Nishida [g], Katsushi Tokunaga [g], Satoshi Eguchi [h], Syudo Yamasaki [i], Tatsunobu Natsubori [a], Norichika Iwashiro [a], Hideyuki Inoue [a], Yosuke Takano [a], Kunio Takei [f], Motomu Suga [a], Hidenori Yamasue [a], Junko Matsubayashi [a], Kenji Kohata [a], Chie Shimojo [a], Shiho Okuhata [j,k], Toshiaki Kono [l], Hitoshi Kuwabara [m], Ayaka Ishii-Takahashi [a], Yuki Kawakubo [m], Kiyoto Kasai [a]

[a] *Department of Neuropsychiatry, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan* [2]
[b] *Department of Psychiatry, National Center Hospital, National Center of Neurology and Psychiatry, Tokyo, Japan*
[c] *MRC Social, Genetic and Developmental Psychiatry Centre, Institute of Psychiatry, King's College London, London, UK*
[d] *Department of Youth Mental Health, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan*
[e] *Hitachi Medical Corporation, Chiba, Japan*
[f] *Office for Mental Health Support, Division for Counseling and Support, The University of Tokyo, Tokyo, Japan*
[g] *Department of Human Genetics, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan*
[h] *Day Hospital, Department of Rehabilitation, The University of Tokyo Hospital, Tokyo, Japan*
[i] *Department of Psychiatry and Behavioral Sciences, Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan*
[j] *Department of Electrical Engineering, Graduate School of Engineering, Kyoto University, Kyoto, Japan*
[k] *Japan Society for the Promotion of Science, Tokyo, Japan*
[l] *Department of Mental Health Policy and Evaluation, National Institute of Mental Health, National Center of Neurology and Psychiatry, Tokyo, Japan*
[m] *Department of Child Neuropsychiatry, Graduate School of Medicine, The University of Tokyo Hospital, Tokyo, Japan*

ARTICLE INFO

Article history:
Accepted 13 March 2013
Available online xxxx

Keywords:
Endophenotype
Twin study
Heritability
Verbal fluency task
Near-infrared spectroscopy
Prefrontal hemodynamic response

ABSTRACT

Near-infrared spectroscopy (NIRS) studies have reported that prefrontal hemodynamic dysfunction during executive function tasks may be a promising biomarker of psychiatric disorders, because its portability and noninvasiveness allow easy measurements in clinical settings. Here, we investigated the degree to which prefrontal NIRS signals are genetically determined. Using a 52-channel NIRS system, we monitored the oxy-hemoglobin (oxy-Hb) signal changes in 38 adult pairs of right-handed monozygotic (MZ) twins and 13 pairs of same-sex right-handed dizygotic (DZ) twins during a letter version of the verbal fluency task. Heritability was estimated based on a classical twin paradigm using structured equation modeling. Significant genetic influences were estimated in the right dorsolateral prefrontal cortex and left frontal pole. The degrees of heritability were 66% and 75% in the variances, respectively. This implies that the prefrontal hemodynamic dysfunction observed during an executive function task measured by NIRS may be an efficient endophenotype for large-scale imaging genetic studies in psychiatric disorders.

Abbreviations: DZ, dizygotic; FIQ, full-scale intelligence quotient; LFT, Letter Fluency Task; MZ, monozygotic; NIRS, near-infrared spectroscopy; rCBV, regional cerebral blood volume; SES, socioeconomic status; SNP, single nucleotide polymorphism; WAIS-R, Wechsler Adult Intelligence Scale-Revised; [oxy-Hb], concentration of oxyhemoglobin; [deoxy-Hb], concentration of deoxyhemoglobin.

* Correspondence to: E. Sakakibara, Department of Neuropsychiatry, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan. Fax: +81 3 5800 9162.
** Correspondence to: R. Takizawa, MRC Social, Genetic and Developmental Psychiatry Centre, Institute of Psychiatry, King's College London, London, UK. Fax: +81 3 5800 9162.
E-mail address: sakakibara-tky@umin.ac.jp (E. Sakakibara).

[1] These authors equally contributed to this work.
[2] Study site.

1053-8119/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.neuroimage.2013.03.052

Introduction

It is well known that there is a substantial genetic influence in major psychiatric disorders, such as schizophrenia and bipolar disorder. The identification of endophenotypes in psychiatric disorders is becoming an increasingly pressing matter in the elucidation of their genetic underpinnings (Gottesman and Gould, 2003). An endophenotype is a type of biomarker that is both associated with a specific psychiatric disorder and is genetically influenced. Endophenotypes are thought to link complicated pathways from genotypes to phenotypes.

Event-related potentials, such as P50, P300, and mismatch negativity, have been investigated as candidate endophenotypes of schizophrenia and other psychiatric conditions (Bramon et al., 2004; Hall et al., 2006; Umbricht and Krljes, 2005). Working memory and executive performance are also thought to contribute to the psychopathology of schizophrenia. Abnormal functioning of the dorsolateral prefrontal cortex was found not only in patients with schizophrenia, but also in their unaffected siblings (Callicott et al., 2003). More recently, several functional magnetic resonance imaging (fMRI) studies revealed a genetic contribution to prefrontal blood oxygenation level-dependent (BOLD) signal changes in response to a working memory task (Blokland et al., 2008, 2011; Koten et al., 2009). These findings support the contention that such characteristics in cognitive neuroscience might serve as an endophenotype of schizophrenia.

Multichannel near-infrared spectroscopy (NIRS) is a functional neuroimaging modality that enables the noninvasive detection of the concentrations of oxyhemoglobin ([oxy-Hb]) and deoxyhemoglobin ([deoxy-Hb]), which are assumed to reflect the regional cerebral blood volume (rCBV). NIRS is suitable for clinical application, particularly in psychiatric disorders, because it has a relatively low cost, is easy to set up, the subject can be examined in a natural sitting position, and its measurements are relatively insensitive to motion artifacts (Takizawa et al., 2008).

NIRS studies using the letter version of the verbal fluency task (LFT) as a cognitive activation task have revealed an LFT-related increase in prefrontal [oxy-Hb] among healthy subjects (Herrmann et al., 2003; Kameyama et al., 2004). Frontal-task-related NIRS signals are being vigorously investigated as potential clinically applicable biomarkers. Decreased or abnormal LFT-related brain activation was found among patients with a variety of psychiatric disorders, including schizophrenia (Kubota et al., 2005; Suto et al., 2004; Takizawa et al., 2008) and mood disorders (Herrmann et al., 2004; Kameyama et al., 2006; Matsuo et al., 2000). Individuals with pervasive developmental disorders (Kuwabara et al., 2006) and their unaffected siblings (Kawakubo et al., 2009) also showed a decreased hemodynamic response compared with individuals with typical development.

Several previous studies also reported that polymorphisms in the catechol-*O*-methyltransferase (*COMT*) and sigma-1 receptor genes were associated with the variations in prefrontal hemodynamic response observed among patients with psychiatric disorders, such as schizophrenia (Takizawa et al., 2009a,b) and panic disorder (Tanii et al., 2009). Those results imply that there are genetic influences on prefrontal activation as measured by NIRS.

In the present study, we investigated the heritability of LFT-related prefrontal hemodynamic responses, as measured by NIRS, in healthy twins using a conventional twin study paradigm. To our knowledge, this is the first twin NIRS study to further our understanding of the genetic contribution to the variation in brain function, which might deepen our interpretation of individual differences in brain processing and vulnerability to brain disorders.

Materials and methods

Participants

This study was performed as a part of a large-scale neuroimaging study on healthy twins (Todai-TWIN). Fifty-one same-sex twin pairs who had been reared together (102 participants) were recruited via newspaper advertisements and participated in the study. All participants were right-handed according to the Edinburgh Inventory (Oldfield, 1971) and were native Japanese speakers. Twins were screened for significant medical conditions, traumatic brain injuries with loss of consciousness for more than 5 min, current use of medication that was likely to affect cognition, history of neurological and psychiatric disorders, history of alcohol and illicit drug abuse, and family history of axis I psychiatric disorders in their first-degree relatives. The zygosity of 76 twin pairs (74.5%) was confirmed genetically. To do this, DNA extracted from peripheral leukocytes was genotyped using the Genome-Wide Human SNP Array 6.0 (Affymetrix, Santa Clara, CA). The zygosity of the remaining twin pairs was determined using a questionnaire composed of 3 questions that can be used to diagnose zygosity with more than 90% accuracy (Ooki et al., 1990). These analyses revealed that 38 pairs were monozygotic (MZ) (35 females and 3 males) and 13 pairs were dizygotic (DZ) (12 females and 1 male).

Socioeconomic status (SES) was assessed using the Hollingshead scale (Hollingshead, 1957). Full-scale intelligent quotient (FIQ) was estimated using the short version of the Japanese Wechsler Adult Intelligence Scale-Revised (WAIS-R) (Misawa et al., 1993; Wechsler, 1981). The distance between T3 and T4 along the scalp was measured and considered as the index of head size. The demographic data are summarized in Table 1. All twins were scanned on the same day as their cotwins. The ethics committee of the Faculty of Medicine, the University of Tokyo, approved this study (No. 630-(6) & 2450-(2)).

Activation task

Participants were asked to seat themselves on a chair with their eyes open and to minimize bodily movements throughout the NIRS measurements. The procedure of cognitive activation was the same as that used in previous studies (Nishimura et al., 2009; Takizawa et al., 2008), which included a 30-s pre-task baseline, a 60-s verbal fluency task (letter version), and a 70-s post-task baseline (Fig. 1). Prepared vocal instructions were given to the participants during the procedure. During the pre-task and post-task periods, the subjects were instructed to repeat a train of Japanese vowels (/a/, /i/, /u/, /e/, and /o/). This was intended to prevent task-unrelated contemplation and to record brain activities due to vocalization. During the verbal fluency task period, participants were instructed to generate as many Japanese words beginning with a given syllable as possible. The initial syllables were changed every 20 s during the 60-s task period, to reduce wordless time. The responses generated by the participants were assessed, and the number of correct words generated was defined as a measure of task performance.

NIRS measurements

Relative changes in [oxy-Hb] and [deoxy-Hb] were measured using 695 nm and 830 nm wavelengths of near-infrared light. The 52-channel NIRS machine (ETG-4000, Hitachi Medical Co.) used in this study included 16 emitter probes and 15 detector probes that were fixed alternately with thermoplastic 3 × 11 shells, which constitutes 52 adjacent emitter–detector probe pairs separated by 3.0 cm (henceforth termed "channel (Ch)"). These probes were placed over a subject's bilateral prefrontal regions so that the lowest 11 probes were located along the Fp1–Fp2 line according to the international 10–20 system in electroencephalography. This arrangement of the probes can measure [oxy-Hb] and [deoxy-Hb] from bilateral prefrontal and superior temporal cortical surface regions (Fig. 2). For the purpose of estimating the cortical localization of each channel, the use of the virtual registration method (Tsuzuki et al., 2012; Tzourio-Mazoyer et al., 2002) enabled the probabilistic registration of NIRS data onto the Montreal Neurological Institute (MNI) coordinate space without measurement of probe positions or the use of MRI (Fig. 2). The placement of the emitter and detector probes on the scalp at a distance of 3.0 cm from each other enables the detection of [Hb]

Please cite this article as: Sakakibara, E., et al., Genetic influences on prefrontal activation during a verbal fluency task in adults: A twin study based on multichannel near-infrared spectroscopy, NeuroImage (2013), http://dx.doi.org/10.1016/j.neuroimage.2013.03.052

Table 1
Demographic data of participants and influences of the genetic component and common environmental component on the variance observed for each item. The first 2 columns show the mean (± SD) of each item. The *r* values and 95% confidence intervals of the correlation between monozygotic and dizygotic cotwins for each item are shown in the third and the fourth columns. The right-most 3 columns show the estimated percentage of influence by additive genetic (A), common environmental (C), and unique environmental (E) components on the variance observed for each item from the most efficient model. Only genetic influence onto FIQ reached significance ($p = 0.004$, asterisk). Note that the homoscedasticity requirement was unsatisfied as for task performance. MZ, monozygotic twins; DZ, dizygotic twins; SES, socioeconomic status.

	Means (SD)		Correlations (95% CIs)		A, C, and E (%) estimates		
	MZ (N = 76)	DZ (N = 26)	MZ	DZ	A	C	E
Age (years)	35.3 (10.7)	30.6 (8.5)					
SES	2.3 (0.7)	2.0 (0.6)	0.49 (0.20, 0.70)	0.07 (−0.50, 0.60)	46	0	54
Education (years)	14.6 (1.8)	15.3 (1.5)	0.56 (0.29, 0.75)	0.43 (−0.16, 0.79)	0	53	47
Task performance	14.5 (4.0)	13.7 (3.3)	0.51 (0.23, 0.72)	−0.24 (−0.70, 0.36)	(46)	(0)	(54)
FIQ	105 (12)	100 (15)	0.69 (0.47, 0.83)	0.15 (−0.44, 0.65)	72*	0	28
T3–T4 interval (cm)	29.1 (1.3)	28.7 (1.1)	0.77 (0.60, 0.88)	0.29 (−0.31, 0.73)	74	0	26

changes on the surface of the cerebral cortex beneath the probes (Hock et al., 1997; Okada and Delpy, 2003; Toronov et al., 2001).

The sampling rate of the data was 0.1 s. During the analysis of task-related relative [Hb] changes, a first-order correction was performed to exclude task-unrelated changes. The mean value recorded across the last 10 s of the pre-task period and the mean value recorded across the last 10 s of the post-task period were defined as the pre- and post-task baselines, respectively: a linear fitting was applied to the data between the two baselines. Subsequently, a moving averaging with a 5-s window was performed to remove short-term motion artifacts. Because moving averaging cannot smooth all the artifacts, the automatic algorithm described in the Supplementary Material of another of our articles (Takizawa et al., 2008) was used to reject quantitatively artifact-contaminated data for each channel. The average number of rejected data in each channel was 9.0 (range, 1–32).

Statistical analyses

The assignment of the twins as number 1 or 2 was performed according to the birth order, if available, indicated in their maternal and child health handbooks (the official birth record in Japan) (92%). When birth order was unavailable, the assignment was performed according to their declaration.

As cognitive-task-related [oxy-Hb] changes are correlated more strongly with blood oxygenation level-dependent signal measured by fMRI than are [deoxy-Hb] changes (Strangman et al., 2002), we focused on [oxy-Hb] here. Because the verbal fluency task draws on a blocked design paradigm, mean [oxy-Hb] changes during the task period were calculated for each channel compared with the pre- and post-task baselines; this mean [oxy-Hb] change was considered as the task-related brain activation (Fig. 1).

First, we calculated the means and variances of demographic variables and task-related brain activations in each channel for 4 groups (MZ twin1, MZ twin2, DZ twin1, and DZ twin2). The equality of means and homoscedasticity across groups, which is the presupposition of genetic modeling, was tested using one-way analysis of variance (ANOVA) and Levene's test. Subsequently, we calculated the correlations of observed data among MZ pairs and DZ pairs to compare the similarity between MZ cotwins with that between DZ cotwins.

Genetic modeling was performed according to classical structured equation modeling in twin studies (Neale and Cardon, 1992). An observed phenotypic value P is decomposed into a linear sum of an underlying additive genetic component (A), a genetic dominance component (D), a common environmental component (C), and a unique environmental component (E). It is known that the contributions of common environmental and genetic dominance cannot be estimated at the same time when data from twins who were reared apart are unavailable (Neale and Cardon, 1992). Therefore, we decided to adopt models containing A, C, and E components (ACE model). This may underestimate, but not overestimate, the overall genetic contribution, which is the sum of additive and dominant genetic components.

Assuming all variables are scaled as deviations from zero, we obtain:

$$P = aA + cC + eE.$$

Such decomposition is possible using structured equation modeling with the following assumptions: the observed correlation between data

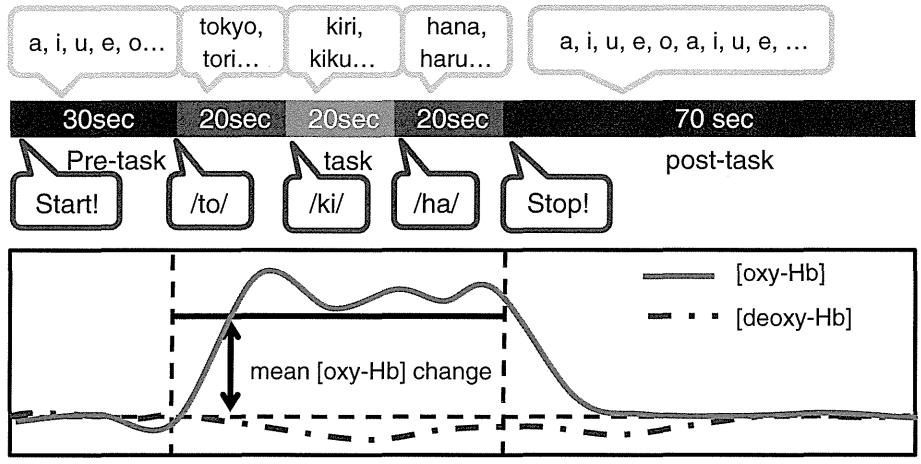

Fig. 1. Design of the Letter Fluency Task (LFT). The LFT is a Japanese version of the verbal fluency task, which includes a 30-s pre-task baseline, a 60-s verbal fluency task, and a 70-s post-task baseline. In the pre- and post-task baseline periods, the subjects were instructed to repeat Japanese vowels (/a/, /i/, /u/, /e/, and /o/) aloud. During the task period, they were instructed to generate as many Japanese words beginning with a designated syllable as possible. LFT-related [oxy-Hb] changes were calculated as the mean relative [oxy-Hb] change during the 60 s of LFT task period compared with the [oxy-Hb] at the pre- and post-task baselines.

Please cite this article as: Sakakibara, E., et al., Genetic influences on prefrontal activation during a verbal fluency task in adults: A twin study based on multichannel near-infrared spectroscopy, NeuroImage (2013), http://dx.doi.org/10.1016/j.neuroimage.2013.03.052

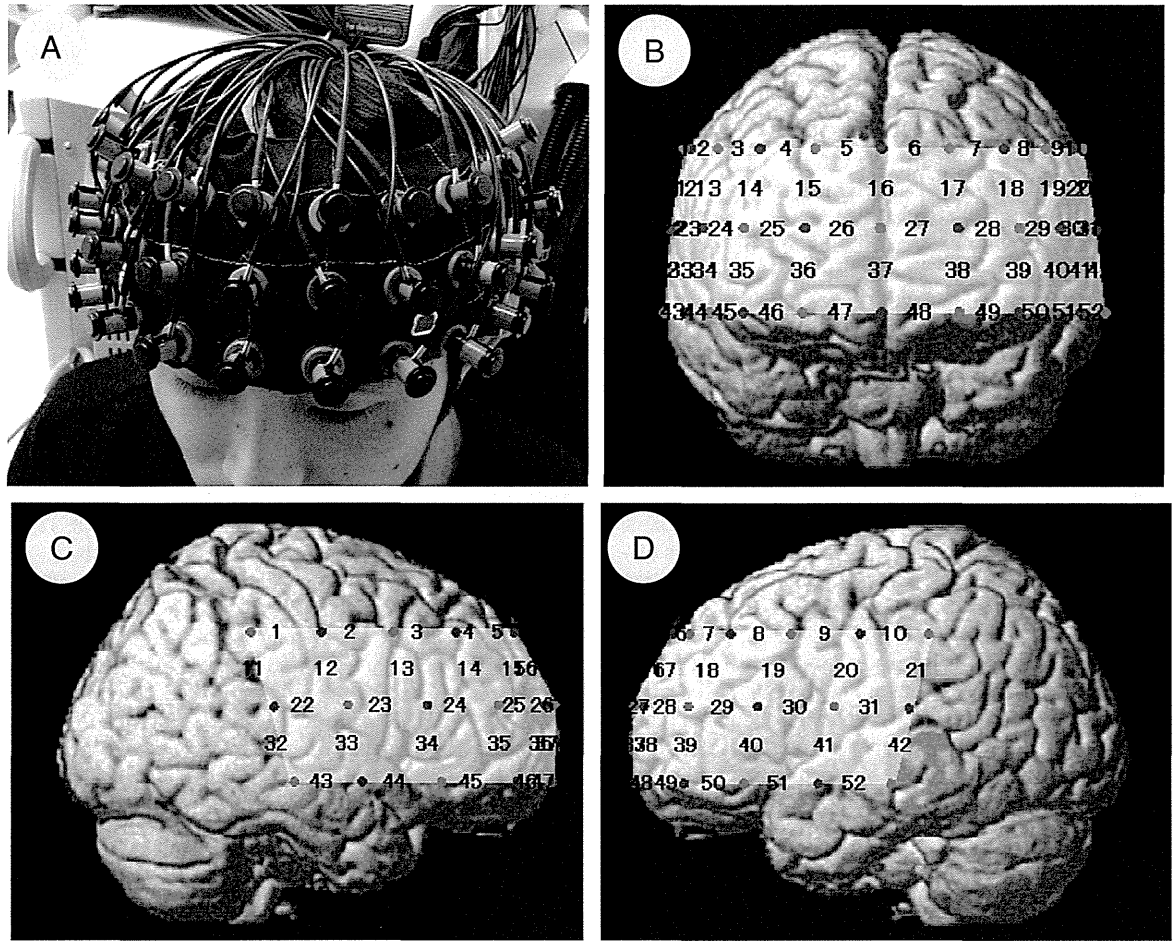

Fig. 2. Probe setting and measurement points of 52-channel near-infrared spectroscopy (NIRS). (A) Placement of the NIRS system over a subject's bilateral frontal regions. (B–D) The 52 measuring points (channels) of the NIRS system are superimposed on a 3D-reconstructed cerebral cortical surface from the Montreal Neurological Institute (MNI) average MRI image (B, frontal view; C, right view; D, left view). Channels are numbered from 1 to 52 from the top-right to bottom-left positions. The red and blue spots represent the placement of emitter and detector probes, respectively.

from twin pairs is caused by their shared genes and/or their common environment. The correlations of common environmental components between cotwins, including socioeconomic status, the rearing style of their parents, and living environment during childhood were fixed as 1 in both MZ and DZ pairs, as such factors are fully shared by twin pairs as long as they are reared together. The correlation of additive genetic factors between cotwins was 1 for MZ pairs, as they share 100% of their genes, and 0.5 for DZ twin pairs, as they share, on average, 50% of their genes. Unique environmental factors are defined as being uncorrelated between cotwins. Subsequently, genetic influences were estimated in cases in which MZ twins were significantly more similar with each other than were DZ twins, because such differences are unexplainable based only on common environmental factors. The path diagram of the ACE model is shown in Fig. 3.

The variances of the latent variables A, C, and E are standardized as unity, and the path coefficients a, c, and e are free parameters, to be estimated. Let Var(X) stand for the variance of X and Cov(X, Y) stand for the covariance between X and Y. In addition, let P_{MZ1}, P_{MZ2}, P_{DZ1}, and P_{DZ2} be the observed phenotypic measures in MZ twin1, MZ twin2, DZ twin1, and DZ twin2. The following equations are derived from the path diagram:

$$\mathrm{Var}(P_{MZ1}) = \mathrm{Var}(P_{MZ2}) = \mathrm{Var}(P_{DZ1}) = \mathrm{Var}(P_{DZ2}) = a^2 + c^2 + e^2,$$

$$\mathrm{Cov}(P_{MZ1}, P_{MZ2}) = a^2 + c^2, \text{ and}$$

$$\mathrm{Cov}(P_{DZ1}, P_{DZ2}) = 0.5a^2 + c^2.$$

Using the equations provided above, parameters a, c, and e are estimated by fitting them to observed variances and covariances of phenotypic values by using a maximum likelihood method. The significance of genetic effects was tested by assessing whether dropping factor A from the ACE model (i.e., assuming $a = 0$, called CE model) resulted in a significant increase in the goodness-of-fit chi-squared value. The significance of common environmental effects was tested similarly by dropping factor C from the ACE model (i.e., assuming $c = 0$, called AE model). The efficiency of the full ACE model relative to that of its submodels (i.e., AE model, CE model, and E model, where $a = c = 0$ is assumed) was compared according to the Akaike information criterion (AIC) (Akaike, 1973), and parameters that were calculated from the most efficient model were considered to be the final estimates. Heritability is defined as the rate of the variance of genetic origin among the total variance in observed phenotypic values. Thus, we obtain:

$$\text{heritability} = \frac{a^2}{a^2 + c^2 + e^2}.$$

Similarly, the rates of the contribution from common environmental and unique environmental components are defined as c^2 and e^2 divided by $a^2 + c^2 + e^2$, respectively. The observed phenotypic measures of the ACE model included (1) demographic data (years of education, FIQ,

Please cite this article as: Sakakibara, E., et al., Genetic influences on prefrontal activation during a verbal fluency task in adults: A twin study based on multichannel near-infrared spectroscopy, NeuroImage (2013), http://dx.doi.org/10.1016/j.neuroimage.2013.03.052

SES, T3–T4 interval, and task performance) and (2) task-related brain activation in each channel.

In addition, to further corroborate the genetic contribution, we performed 3 confirmatory analyses using the data from the channels from which a significant genetic influence was estimated. First, there were disproportionately more female participants than male participants in this study. Hemodynamic activation in response to cognitive tasks has been reported previously as being greater in males than in females (Kameyama et al., 2004). Therefore, we recalculated the genetic contribution to the brain activation in those channels using data that excluded male participants. Second, to exclude potential confounding factors, we controlled for possible effects of age, years of education, FIQ, SES, head size, and task performance on brain activation in female participants by including their data as covariates. Stepwise multiple linear regressions were performed with a probability of F for conservative entries and removal criteria of 0.05 and 0.10, respectively. In addition, genetic influences were estimated as for the residual values after removing the effect from those potential confounding factors. Third, to rule out the possibility that the results were affected by the existence of outliers, we performed outlier detection using a generalized Mahalanobis distance (Mahalanobis, 1936) and re-estimated genetic contribution using female data that excluded outliers.

For those channels in which genetic influences were confirmed using the procedure described above, we calculated the correlation of the [oxy-Hb] signals at each sampling point during the 60-s task period (601 points at 0.1-s intervals) between monozygotic and dizygotic cotwins. A genetic modeling analysis was applied to estimate the time course of genetic influences on the [oxy-Hb] change during the task period. The statistical analyses were performed using the software package SPSS Amos, Ver. 20.0 (IBM Corp.).

Results

Demographic data

No significant differences in the mean values of all demographic variables (age at measurement, years of education, FIQ, SES, head size as measured by the T3–T4 interval, and task performance) were found across the 4 groups (MZ twin1, MZ twin2, DZ twin1, and DZ twin2). Homoscedasticity was maintained in all demographic variables, with the exception of task performance ($p = 0.03$). Greater correlations were found between MZ cotwins than between DZ cotwins regarding FIQ, SES, T3–T4 interval, and task performance. In structured equation modeling, genetic influences on those demographic variables were estimated. However, dropping the genetic factor from the ACE model resulted in a significant increase in the goodness-of-fit chi-squared value only for FIQ ($p = 0.003$). Because moderate correlations were found both in MZ and DZ cotwins, the insignificant ($p = 0.29$) influence of the common environmental component was estimated based on years of education.

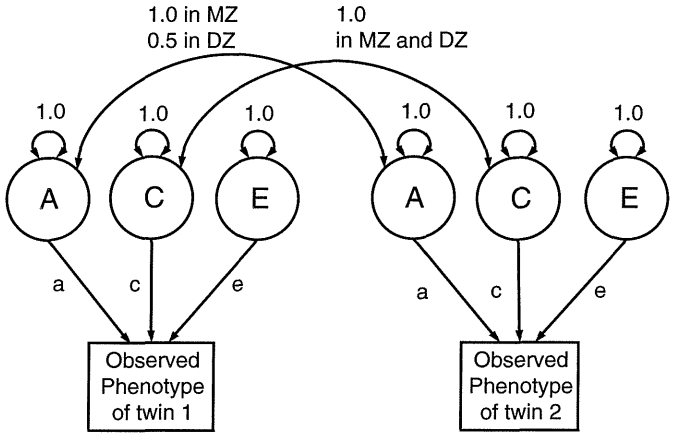

Fig. 3. Path diagram of the ACE model. According to the convention, circles represent latent variables, rectangles represent observed variables, two-way arrows represent variances and covariances, and one-way arrows represent causal influences. MZ, monozygotic twins; DZ, dizygotic twins; A, additive genetic factor; C, common environmental factor; E, unique environmental factor.

NIRS results

Grand average waveforms of [oxy-Hb] during cognitive tasks were similar between the groups (Fig. 4). No significant differences in the mean values for all 52 channels across the 4 groups were found regarding task-related brain activation. Homoscedasticity in task-related activation across groups was achieved in most channels, with the exception of Ch9 ($p = 0.04$) and Ch30 ($p = 0.01$).

As illustrated in Fig. 5, a moderate-to-strong correlation of task-related brain activation was found between MZ cotwins in channels covering the bilateral frontal poles and the right dorsolateral prefrontal cortex. Correlations reached significance for Ch1, 10, 12, 22, 24–26, 28, 33–37, 39, 40, 42, 44–50, and 52 (median p value is 0.007). Moreover, the observed correlations between DZ cotwins fluctuated because of smaller sample size. Although the correlations were weaker between DZ cotwins in most prefrontal regions, they were stronger than the correlations between MZ cotwins in some channels, including those corresponding to the ventrolateral PFC (cf. Broca's area) and right superior temporal gyrus, among which only Ch11, 20, and 22 reached significance (the median p value was 0.03). The variance observed in task-related brain activation was best explained by models containing additive genetic components (i.e., the ACE model or the AE model) in 18 channels (Ch 1, 8, 14, 16, 21, 24, 27, 33, 34, 37, 39, 41, 42, 44, 47, 48, 49, and 50). As shown in Fig. 6, the estimated heritability in those channels ranged from 23 to 75% (mean, 43%). Among those channels, significant genetic influences were found in 2 channels corresponding to the right dorsolateral prefrontal cortex (Ch24, $p = 0.02$) and the left frontal pole (Ch48, $p < 0.01$), respectively. Genetic contribution to the observed variance in task-related brain activation was estimated as being 66% and 75% of the variances calculated for these 2 channels, respectively. None of the channels were significantly influenced by common environmental components.

We performed additional confirmatory analyses. First, the significant genetic contribution observed in the 2 channels remained almost unchanged when data from male participants were excluded (62% of the variance in Ch24, $p = 0.03$; 75% of the variance in Ch48, $p < 0.01$). Second, a stepwise multiple regression analysis revealed that 5.4% and 5.8% of total variance in brain activation in Ch 24 and Ch 48 were explained by age ($\beta = -0.23$, $p = 0.03$ for Ch24; $\beta = -0.24$, $p = 0.03$ for Ch48). After removing the effect of age, the significant genetic contributions were retained (59% of the variance in Ch24, $p = 0.04$; 73% of the variance in Ch48, $p < 0.01$). Other demographic factors did not exhibit significant correlations with brain activation in those channels. Third, a Mahalanobis outlier analysis detected 1 significant outlier in MZ twin pairs in each channel. The estimation of correlations using data that excluded outliers revealed that genetic contributions were essentially unchanged and remained significant (68% of the variance in Ch24, $p < 0.01$; 66% of the variance in Ch48, $p = 0.01$).

The [oxy-Hb] signals in each sampling point during the 60-s task period at 0.1-s intervals in Ch24 and Ch48 were analyzed further. The time course of correlation coefficients of [oxy-Hb] signals during the task period in Ch24 and 48 between monozygotic and dizygotic twin pairs is shown in Fig. 7. Throughout the task period, the correlation was greater among MZ twins compared with DZ twins. A genetic modeling analysis revealed that genetic factors accounted for 40–80% of the total variance in the [oxy-Hb] signals during most of the task period. Regarding Ch48, which recorded the strongest genetic influence, the time course of the estimated heritability of [oxy-Hb] signals during the task period is illustrated in Fig. 8.

Please cite this article as: Sakakibara, E., et al., Genetic influences on prefrontal activation during a verbal fluency task in adults: A twin study based on multichannel near-infrared spectroscopy, NeuroImage (2013), http://dx.doi.org/10.1016/j.neuroimage.2013.03.052

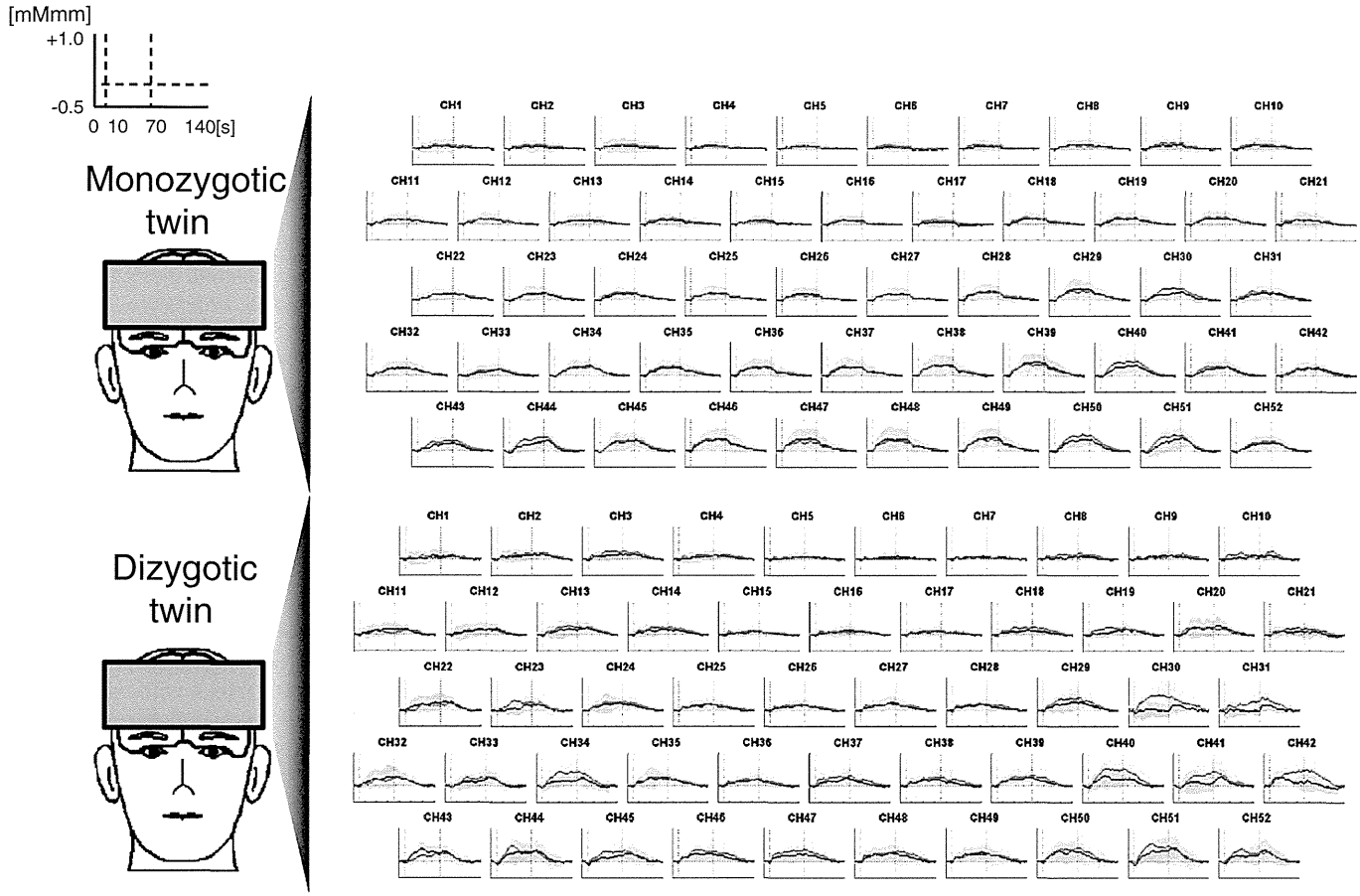

Fig. 4. Grand average waveforms in monozygotic and dizygotic twins. [oxy-Hb] changes during cognitive activation are presented as grand average waveforms with their standard deviation in 52 channels in twin1 (blue lines with light blue bands) and twin2 (red lines with pink bands).

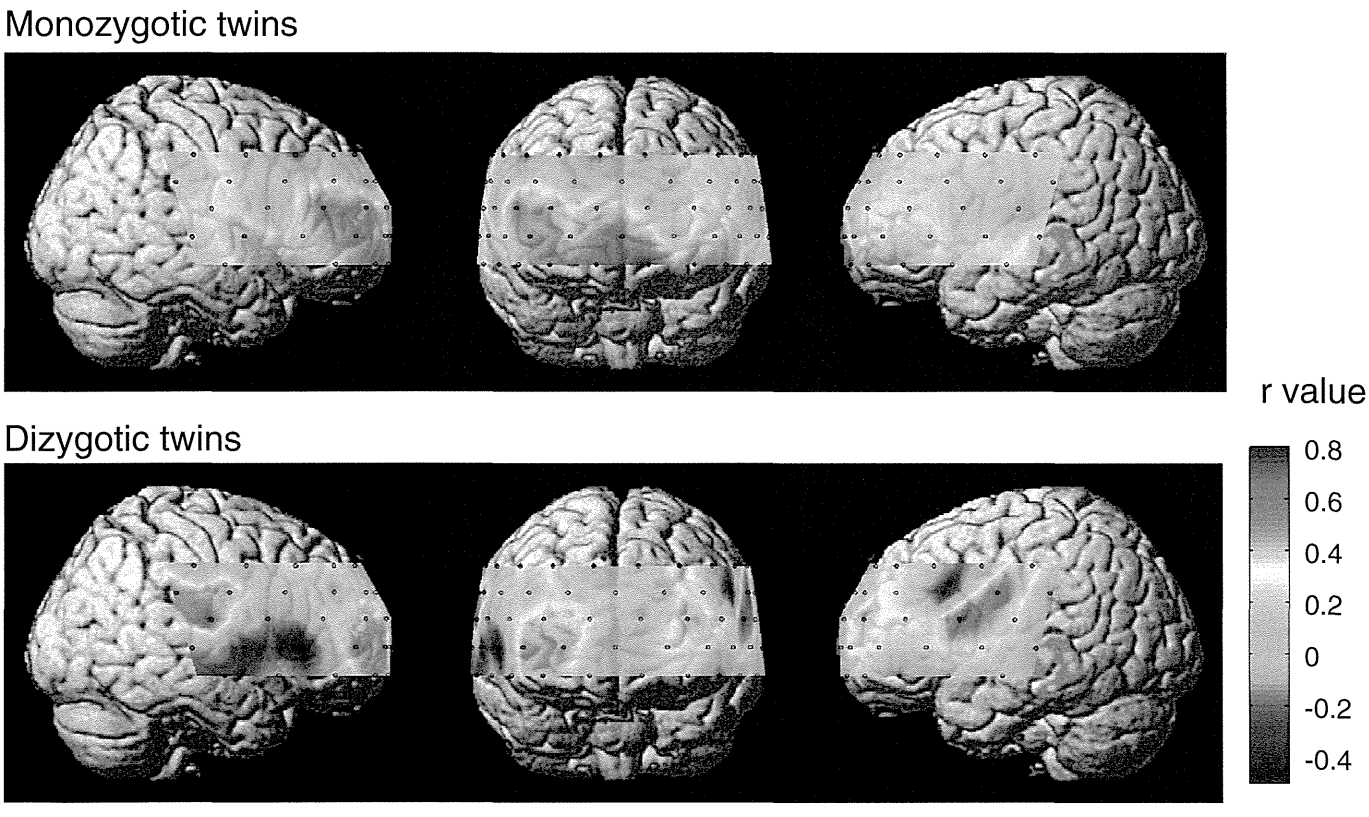

Fig. 5. Correlation of letter fluency test (LFT)-related brain activation between monozygotic twin pairs and dizygotic twin pairs.

Please cite this article as: Sakakibara, E., et al., Genetic influences on prefrontal activation during a verbal fluency task in adults: A twin study based on multichannel near-infrared spectroscopy, NeuroImage (2013), http://dx.doi.org/10.1016/j.neuroimage.2013.03.052

Discussion

This is the first twin NIRS study to investigate the heritability of prefrontal activation during a verbal fluency task. A significant genetic influence on prefrontal NIRS signals was detected even after controlling for potential confounding factors. In particular, the frontopolar and dorsolateral prefrontal subregions were more influenced by genetic factors. The "imaging genetics" approach has been used to identify susceptibility genes for heterogeneous psychiatric disorders (Meyer-Lindenberg, 2012). For this purpose, the sample size of the data should be very large. As NIRS is portable and its measurement is easier compared with other neuroimaging modalities, such as MRI, NIRS may serve as an efficient endophenotype for large-scale imaging genetic studies in psychiatric disorders.

Heritability of LFT performance and task-related brain activation

The LFT is a version of the verbal fluency task in which participants generate as many words beginning with given phonological cues as possible. The LFT is supposed to recruit executive function, including self-initiated retrieval of words from long-term memory storage, working memory capacity to keep track of the aforementioned items, and inhibition of the habitual behavior of treating words according to their meaning (Henry and Crawford, 2004; Perret, 1974). Earlier neuropsychological studies have shown that about 50% of individual variance in the number of words generated during this task is accounted for by genetic factors (Bratko, 1996; Vandenberg, 1962), which is comparable to the estimated heritability of LFT task performance (46% of the variance) calculated in this study.

The LFT is thought to reflect prefrontal function, particularly that of the dorsolateral prefrontal cortex (DLPFC), as assessed in lesion studies (Henry and Crawford, 2004) and in functional imaging studies (Frith et al., 1991; Phelps et al., 1997). Previous multichannel NIRS studies have replicated the results of brain activation measured based on the [oxy-Hb] increase detected in a broad prefrontal area, including both the right and left DLPFC and the frontal pole (Brodmann area 10) (Herrmann et al., 2006; Kameyama et al., 2004; Takizawa et al., 2008).

The results of the present study suggest that a substantial portion of LFT-related [oxy-Hb] changes in the frontal pole and the right dorsolateral prefrontal cortex are genetically determined. Time course analysis indicated sustained genetic influence on the [oxy-Hb] changes throughout the task period. The genetic influence on brain activation might not be mediated by task performance because most NIRS reports, including the present study, found no significant correlation between task performance and the brain activation in those channels (Herrmann et al., 2003; Kono et al., 2007; Pu et al., 2008). In contrast, the r values of the correlation of brain activation between MZ twins in the ventrolateral PFC were not high. This suggests that the brain activation in that area is substantially influenced by environmental (i.e., nongenetic) factors. Further investigations of the environmental contributions to these brain activations are needed.

Two fMRI studies reported the genetic contribution of BOLD signal changes using a working memory task. The first group used a digit memory task with arithmetic distraction in 10 families with male MZ twins and an additional non-twin brother, and genetic influences were implicated in regions including the inferior frontal gyrus and the anterior cingulate cortex (Koten et al., 2009). The second group used an N-back task in 319 healthy MZ and DZ twins and found that 40–65% of the

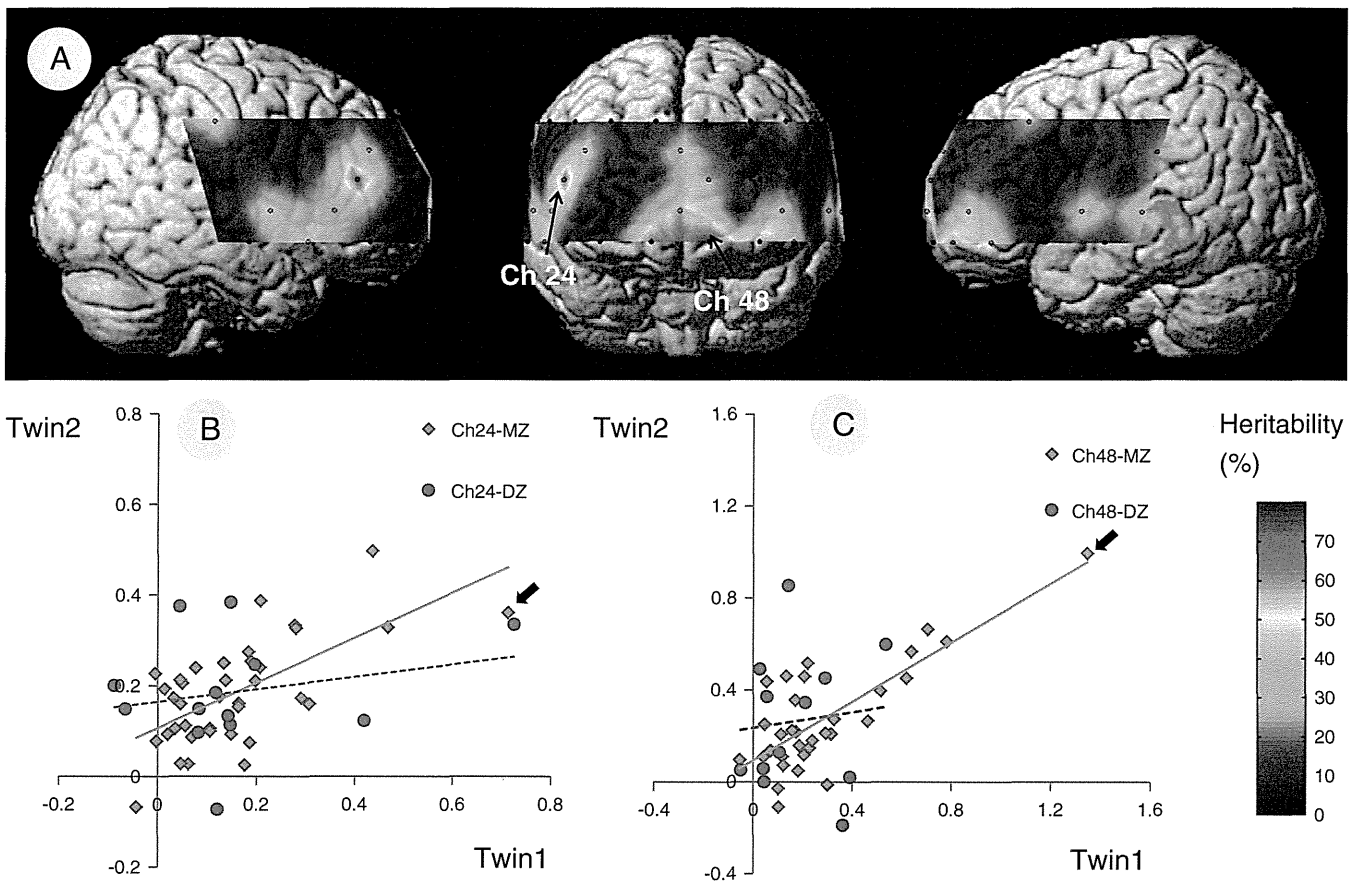

Fig. 6. Estimated genetic influences on LFT-related mean [oxy-Hb] change during the LFT. (A) Topographical map of estimated heritability. Channel 24 (right DLPFC) and Ch 48 (left frontal pole) were significantly influenced by genetic factors ($p < 0.05$). (B) and (C) Scatter plot of LFT-related mean [oxy-Hb] change in each twin pair in Ch 24 and Ch 48. Blue squares represent monozygotic (MZ) data and red circles represent dizygotic (DZ) data. Blue and red lines are linear regression lines of MZ and DZ data, respectively. Black arrows designate twin pairs that were judged as outliers in the Mahalanobis outlier analysis.

Please cite this article as: Sakakibara, E., et al., Genetic influences on prefrontal activation during a verbal fluency task in adults: A twin study based on multichannel near-infrared spectroscopy, NeuroImage (2013), http://dx.doi.org/10.1016/j.neuroimage.2013.03.052

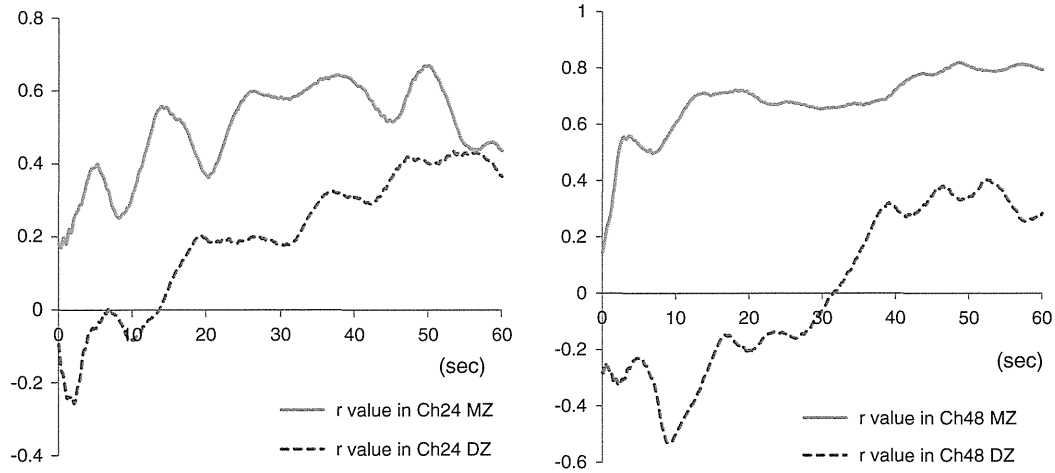

Fig. 7. Time course of the *r* values of correlation coefficients of [oxy-Hb] signals during the 60-s task period in Ch24 and Ch48 between monozygotic and dizygotic twin pairs. Blue solid lines and red dotted lines represent *r* values in monozygotic and dizygotic twins, respectively.

variance in the task-related BOLD signals was accounted by genetic factors in the regions including the bilateral inferior, middle, and superior frontal gyri (Blokland et al., 2008, 2011). The results of the present study further support the contention that the prefrontal brain activities underlying executive function are partially heritable.

Prefrontal NIRS signals activated by LFT as a candidate endophenotype of psychiatric disorders

Verbal fluency impairment is associated with various psychiatric disorders, including schizophrenia (Bokat and Goldberg, 2003), bipolar disorder (Martinez-Aran et al., 2004), and unipolar depression (Henry and Crawford, 2005). Altered brain function has been detected in several groups of patients with schizophrenia during an LFT; however, the findings of regions are inconsistent. In comparison with healthy controls, patients with schizophrenia have been reported to display reduced brain activation in the left DLPFC (Curtis et al., 1998), loss of deactivation in the superior temporal cortex (Frith et al., 1995), deactivation of the precuneus (Spence et al., 2000), and loss of left frontal dominance (Weiss et al., 2004). This might be due to the small size of the samples included in each study. NIRS studies consistently reported reduced or abnormal prefrontal brain activation in the bilateral DLPFC and in the frontal pole in patients with depression and schizophrenia compared with healthy controls (Suto et al., 2004; Takizawa et al., 2008), even if their task performances were matched. These neurophysiological disturbances might be the underlying neural basis of the executive-function impairment observed in psychiatric disorders.

In addition, it has been reported that executive performance as assessed by LFT is associated with two genetic polymorphisms that are known to increase the risk of developing schizophrenia. One such example is the Val^{158}Met polymorphism located in the *COMT* gene. Carriers of the Val allele (higher enzymatic activity) compared with carriers of the Met/Met genotype (low enzymatic activity) reportedly exhibit low performance in various executive and visuospatial tasks, including the LFT (de Frias et al., 2005). Another example is the A allele of the SNP4 polymorphism located in the metabotropic glutamate receptor-modulating synaptic glutamate gene, which is a potential candidate gene for schizophrenia and is associated with lower performance in the LFT (Egan et al., 2004).

Based on those evidences and on the heritability of brain activation during an LFT observed in this study, the prefrontal hemodynamic abnormality reported among patients with schizophrenia and mood disorder in previous NIRS studies can be considered as a candidate endophenotype for those disorders. However, caution should be taken, because the variance among the healthy population and differences between psychiatric patients and healthy controls might not necessarily have the same etiology. To confirm that the differences in neural correlates during an LFT observed between healthy controls and psychiatric patients are also genetically determined, evidence from family studies of individuals with psychiatric disorders and their unaffected siblings should be gathered.

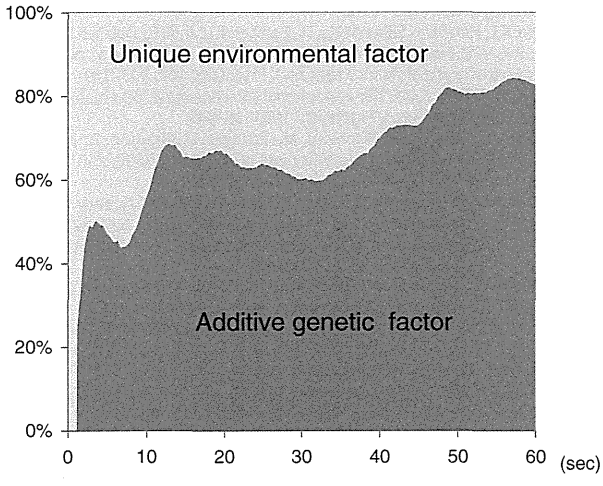

Fig. 8. Time course of the estimated heritability of the [oxy-Hb] signals during the task period in Ch48.

Limitations

First, before definite implications can be drawn from this study, the present results should be confirmed in a future twin NIRS study using a larger and more balanced sample. In addition, the number of female participants was disproportionately larger than that of male participants. Although the genetic influences on brain activation observed in the whole sample were reproduced using data that excluded male participants, we should be careful when extending the results of the present study to the estimation of the heritability of brain activation in male participants.

Second, the brain areas that are activated by the LFT have been shown to be highly heritable in twin studies investigating brain structure (Schmitt et al., 2007; Thompson et al., 2001). This imposes special consideration for twin studies employing the NIRS system. Our NIRS system calculated the product of hemoglobin concentration changes and the differential pathlength factor (DPF) (ΔC^*L) as a solution to the simultaneous equations based on the modified Beer–Lambert law (Yamashita

Please cite this article as: Sakakibara, E., et al., Genetic influences on prefrontal activation during a verbal fluency task in adults: A twin study based on multichannel near-infrared spectroscopy, NeuroImage (2013), http://dx.doi.org/10.1016/j.neuroimage.2013.03.052

et al., 1996). However, DPF might be influenced by the regional brain volume and the morphology of the areas through which the near-infrared light passes. Therefore, part of the estimated genetic influences on the task-related [oxy-Hb] changes in the prefrontal area was confounded by the heritability of the brain volume and morphology of that area. Although head size (as measured by the T3–T4 interval) was not correlated with task-related brain activation, this result is insufficient to rule out the possibility stated above. Therefore, the technology used for the real-time measurement of the estimated DPF at each channel, to determine the proportion of changes in hemoglobin concentration that are unaffected by DPF, should be evaluated as a separate issue in future NIRS studies.

Third, one recent study indicated that the major part of [Oyx-Hb] signals from forehead probes of NIRS are explained by skin blood flow change (Takahashi et al., 2011). This may imply that the correlation of LFT-related [oxy-Hb] change in the left frontal pole between cotwins might mainly be due to their similarity in task-related skin blood flow fluctuations. If that is the case, the estimated heritability in the left frontal polar brain activation should be reinterpreted as the heritability of the skin blood flow change of the forehead. In contrast, the correlation of brain activations between MZ twin pairs was stronger in the right hemisphere than in the left hemisphere. As a result, genetic influence was estimated in a channel corresponding to the right dorsolateral prefrontal area. Such laterality and inhomogeneous distribution are difficult to explain solely by the effect of skin blood flow. These findings suggest that most NIRS [oxy-Hb] signals cannot be exclusively explained by skin blood flow.

Conclusions

The results of the present study suggest that hemodynamic activation in the prefrontal cortex during an executive task, as measured by functional NIRS, is a genetically influenced trait. These findings indicate that prefrontal NIRS signals induced by cognitive activation may be a promising endophenotype for large-scale imaging genetic studies aimed at disentangling the genetic background of heterogeneous psychiatric disorders.

Funding and role of the funding source

This work was supported in part by a Grant-in-Aid for Scientific Research (innovative areas nos. 23118001 & 23118004 [Adolescent Mind & Self-Regulation] to KK; no. 23791309 to RT) and by a grant from the "Development of Biomarker Candidates for Social Behavior" study carried out under the Strategic Research Program for Brain Sciences (to KK) by the MEXT. This study was also supported in part by Health and Labor Sciences Research Grants for Comprehensive Research on Disability Health and Welfare (H23-seishin-ippan-002 to RT&YN); an Intramural Research Grant for Neurological and Psychiatric Disorders of NCNP (no. 23-10 to RT&YN); grants from the Takeda Science Foundation (to YN); and by a grant from the Japan Research Foundation for Clinical Pharmacology (to RT). The sponsors had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all data in this study and had the final responsibility in the decision to submit for publication.

Contributors

RT, YK, and KK were involved in the conception, design, and management of the study. ES, RT, YN, and KK analyzed and interpreted the data and wrote the first draft of the paper. RT, YN, KM, MK, SE, SY, HI, YT, KT, MS, HY, TK, and KK conducted data acquisition. The other contributors revised the first draft critically for important intellectual content. All contributors have approved the final version of the manuscript.

Acknowledgments

We acknowledge the study participants for their help. We also thank the members of the Todai-TWIN project for their dedication, hard work, and insights.

Conflict of interest

Regarding all financial and material support for the present study, Dr. Kasai has a potential conflict of interest (see below for details). All other authors have no relevant conflicts of interest.

Beginning July 31, 2003 and continuing through to present, the University of Tokyo and the Hitachi Group (Advanced Research Laboratory, Hitachi Ltd. and The Research and Developmental Center, Hitachi Medical Corporation) have had an official contract for a collaborative study on the clinical application of near-infrared spectroscopy (NIRS) in psychiatric disorders, which has been approved by the Research Promotion Office, University of Tokyo Hospital. The principal investigator of this study is Kiyoto Kasai. For this study, the Hitachi Medical Corporation provided a project grant (JPY 300,000 per year).

References

Akaike, H., 1973. Information theory and an extension of the maximum likelihood principle. International Symposium on Information Theory, 2nd, Tsahkadsor, Armenian SSR, pp. 267–281.
Blokland, G.A.M., McMahon, K.L., Hoffman, J., Zhu, G., Meredith, M., Martin, N.G., Thompson, P.M., De Zubicaray, G.I., Wright, M.J., 2008. Quantifying the heritability of task-related brain activation and performance during the N-back working memory task: a twin fMRI study. Biol. Psychol. 79, 70–79.
Blokland, G.A.M., McMahon, K.L., Thompson, P.M., Martin, N.G., de Zubicaray, G.I., Wright, M.J., 2011. Heritability of working memory brain activation. J. Neurosci. 31, 10882–10890.
Bokat, C.E., Goldberg, T.E., 2003. Letter and category fluency in schizophrenic patients: a meta-analysis. Schizophr. Res. 64, 73–78.
Bramon, E., Rabe-Hesketh, S., Sham, P., Murray, R.M., Frangou, S., 2004. Meta-analysis of the P300 and P50 waveforms in schizophrenia. Schizophr. Res. 70, 315–329.
Bratko, D., 1996. Twin study of verbal and spatial abilities. Pers. Individ. Differ. 21, 621–624.
Callicott, J.H., Egan, M.F., Mattay, V.S., Bertolino, A., Bone, A.D., Verchinksi, B., Weinberger, D.R., 2003. Abnormal fMRI response of the dorsolateral prefrontal cortex in cognitively intact siblings of patients with schizophrenia. Am. J. Psychiatry 160, 709–719.
Curtis, V.A., Bullmore, E.T., Brammer, M.J., Wright, I.C., Williams, S.C., Morris, R.G., Sharma, T.S., Murray, R.M., McGuire, P.K., 1998. Attenuated frontal activation during a verbal fluency task in patients with schizophrenia. Am. J. Psychiatry 155, 1056–1063.
de Frias, C.M., Annerbrink, K., Westberg, L., Eriksson, E., Adolfsson, R., Nilsson, L.G., 2005. Catechol O-methyltransferase Val158Met polymorphism is associated with cognitive performance in nondemented adults. J. Cogn. Neurosci. 17, 1018–1025.
Egan, M.F., Straub, R.E., Goldberg, T.E., Yakub, I., Callicott, J.H., Hariri, A.R., Mattay, V.S., Bertolino, A., Hyde, T.M., Shannon-Weickert, C., Akil, M., Crook, J., Vakkalanka, R.K., Balkissoon, R., Gibbs, R.A., Kleinman, J.E., Weinberger, D.R., 2004. Variation in GRM3 affects cognition, prefrontal glutamate, and risk for schizophrenia. Proc. Natl. Acad. Sci. U. S. A. 101, 12604–12609.
Frith, C., Friston, K., Liddle, P., Frackowiak, R., 1991. A PET study of word finding. Neuropsychologia 29, 1137–1148.
Frith, C.D., Friston, K.J., Herold, S., Silbersweig, D., Fletcher, P., Cahill, C., Dolan, R.J., Frackowiak, R.S., Liddle, P.F., 1995. Regional brain activity in chronic schizophrenic patients during the performance of a verbal fluency task. Br. J. Psychiatry 167, 343–349.
Gottesman, I.I., Gould, T.D., 2003. The endophenotype concept in psychiatry: etymology and strategic intentions. Am. J. Psychiatry 160, 636–645.
Hall, M.H., Schulze, K., Rijsdijk, F., Picchioni, M., Ettinger, U., Bramon, E., Freedman, R., Murray, R.M., Sham, P., 2006. Heritability and reliability of P300, P50 and duration mismatch negativity. Behav. Genet. 36, 845–857.
Henry, J.D., Crawford, J.R., 2004. A meta-analytic review of verbal fluency performance in patients with traumatic brain injury. Neuropsychology 18, 621–628.
Henry, J., Crawford, J.R., 2005. A meta-analytic review of verbal fluency deficits in depression. J. Clin. Exp. Neuropsychol. 27, 78–101.
Herrmann, M., Ehlis, A.C., Fallgatter, A., 2003. Frontal activation during a verbal-fluency task as measured by near-infrared spectroscopy. Brain Res. Bull. 61, 51–56.
Herrmann, M., Ehlis, A.C., Fallgatter, A., 2004. Bilaterally reduced frontal activation during a verbal fluency task in depressed patients as measured by near-infrared spectroscopy. J. Neuropsychiatry Clin. Neurosci. 16, 170–175.
Herrmann, M., Walter, A., Ehlis, A.C., Fallgatter, A., 2006. Cerebral oxygenation changes in the prefrontal cortex: effects of age and gender. Neurobiol. Aging 27, 888–894.
Hock, C., Villringer, K., Muller-Spahn, F., Wenzel, R., Heekeren, H., Schuh-Hofer, S., Hofmann, M., Minoshima, S., Schwaiger, M., Dirnagl, U., Villringer, A., 1997. Decrease in parietal cerebral hemoglobin oxygenation during performance of a verbal fluency task in patients with Alzheimer's disease monitored by means of near-infrared spectroscopy (NIRS)—correlation with simultaneous rCBF-PET measurements. Brain Res. 755, 293–303.
Hollingshead, A.B., 1957. Two Factor Index of Social Position. Yale University Press, New Haven.

Please cite this article as: Sakakibara, E., et al., Genetic influences on prefrontal activation during a verbal fluency task in adults: A twin study based on multichannel near-infrared spectroscopy, NeuroImage (2013), http://dx.doi.org/10.1016/j.neuroimage.2013.03.052

Kameyama, M., Fukuda, M., Uehara, T., Mikuni, M., 2004. Sex and age dependencies of cerebral blood volume changes during cognitive activation: a multichannel near-infrared spectroscopy study. Neuroimage 22, 1715–1721.
Kameyama, M., Fukuda, M., Yamagishi, Y., Sato, T., Uehara, T., Ito, M., Suto, T., Mikuni, M., 2006. Frontal lobe function in bipolar disorder: a multichannel near-infrared spectroscopy study. Neuroimage 29, 172–184.
Kawakubo, Y., Kuwabara, H., Watanabe, K., Minowa, M., Someya, T., Minowa, I., Kono, T., Nishida, H., Sugiyama, T., Kato, N., Kasai, K., 2009. Impaired prefrontal hemodynamic maturation in autism and unaffected siblings. PLoS One 4, e6881.
Kono, T., Matsuo, K., Tsunashima, K., Kasai, K., Takizawa, R., Rogers, M.A., Yamasue, H., Yano, T., Taketani, Y., Kato, N., 2007. Multiple-time replicability of near-infrared spectroscopy recording during prefrontal activation task in healthy men. Neurosci. Res. 57, 504–512.
Koten Jr., J.W., Wood, G., Hagoort, P., Goebel, R., Propping, P., Willmes, K., Boomsma, D.I., 2009. Genetic contribution to variation in cognitive function: an fMRI study in twins. Science 323, 1737–1740.
Kubota, Y., Toichi, M., Shimizu, M., Mason, R.A., Coconcea, C.M., Findling, R.L., Yamamoto, K., Calabrese, J.R., 2005. Prefrontal activation during verbal fluency tests in schizophrenia—a near-infrared spectroscopy (NIRS) study. Schizophr. Res. 77, 65–73.
Kuwabara, H., Kasai, K., Takizawa, R., Kawakubo, Y., Yamasue, H., Rogers, M.A., Ishijima, M., Watanabe, K., Kato, N., 2006. Decreased prefrontal activation during letter fluency task in adults with pervasive developmental disorders: a near-infrared spectroscopy study. Behav. Brain Res. 172, 272–277.
Mahalanobis, P.C., 1936. On the generalized distance in statistics. Proceedings of the National Institute of Sciences of India. New Delhi, pp. 49–55.
Martinez-Aran, A., Vieta, E., Reinares, M., Colom, F., Torrent, C., Sanchez-Moreno, J., Benabarre, A., Goikolea, J.M., Comes, M., Salamero, M., 2004. Cognitive function across manic or hypomanic, depressed, and euthymic states in bipolar disorder. Am. J. Psychiatry 161, 262–270.
Matsuo, K., Kato, T., Fukuda, M., Kato, N., 2000. Alteration of hemoglobin oxygenation in the frontal region in elderly depressed patients as measured by near-infrared spectroscopy. J. Neuropsychiatry Clin. Neurosci. 12, 465–471.
Meyer-Lindenberg, A., 2012. The future of fMRI and genetics research. Neuroimage 62, 1286–1292.
Misawa, G., Kobayashi, S., Fujita, K., Maekawa, H., Dairoku, H., 1993. Japanese Wechsler Adult Intelligence Scale-Revised Short Forms. Nihon Bunka Kagakusha Co. Ltd., Tokyo.
Neale, M., Cardon, L.R., 1992. Methodology for Genetic Studies of Twins and Families. Springer.
Nishimura, Y., Tanii, H., Hara, N., Inoue, K., Kaiya, H., Nishida, A., Okada, M., Okazaki, Y., 2009. Relationship between the prefrontal function during a cognitive task and the severity of the symptoms in patients with panic disorder: a multi-channel NIRS study. Psychiatry Research: Neuroimaging 172, 168–172.
Okada, E., Delpy, D.T., 2003. Near-infrared light propagation in an adult head model. II. Effect of superficial tissue thickness on the sensitivity of the near-infrared spectroscopy signal. Appl. Opt. 42, 2915–2922.
Oldfield, R.C., 1971. The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia 9, 97–113.
Ooki, S., Yamada, K., Asaka, A., Hayakawa, K., 1990. Zygosity diagnosis of twins by questionnaire. Acta Genet. Med. Gemellol. (Roma) 39, 109–115.
Perret, E., 1974. The left frontal lobe of man and the suppression of habitual responses in verbal categorical behaviour. Neuropsychologia 12, 323–330.
Phelps, E.A., Hyder, F., Blamire, A.M., Shulman, R.G., 1997. FMRI of the prefrontal cortex during overt verbal fluency. Neuroreport 8, 561–565.
Pu, S., Matsumura, H., Yamada, T., Ikezawa, S., Mitani, H., Adachi, A., Nakagome, K., 2008. Reduced frontopolar activation during verbal fluency task associated with poor social functioning in late-onset major depression: multi-channel near-infrared spectroscopy study. Psychiatry Clin. Neurosci. 62, 728–737.
Schmitt, J.E., Eyler, L.T., Giedd, J.N., Kremen, W.S., Kendler, K.S., Neale, M.C., 2007. Review of twin and family studies on neuroanatomic phenotypes and typical neurodevelopment. Twin Res. Hum. Genet. 10, 683–694.
Spence, S.A., Liddle, P.F., Stefan, M.D., Hellewell, J.S., Sharma, T., Friston, K.J., Hirsch, S.R., Frith, C.D., Murray, R.M., Deakin, J.F., Grasby, P.M., 2000. Functional anatomy of verbal fluency in people with schizophrenia and those at genetic risk. Focal dysfunction and distributed disconnectivity reappraised. Br. J. Psychiatry 176, 52–60.
Strangman, G., Culver, J.P., Thompson, J.H., Boas, D.A., 2002. A quantitative comparison of simultaneous BOLD fMRI and NIRS recordings during functional brain activation. Neuroimage 17, 719–731.
Suto, T., Fukuda, M., Ito, M., Uehara, T., Mikuni, M., 2004. Multichannel near-infrared spectroscopy in depression and schizophrenia: cognitive brain activation study. Biol. Psychiatry 55, 501–511.
Takahashi, T., Takikawa, Y., Kawagoe, R., Shibuya, S., Iwano, T., Kitazawa, S., 2011. Influence of skin blood flow on near-infrared spectroscopy signals measured on the forehead during a verbal fluency task. Neuroimage 57, 991–1002.
Takizawa, R., Kasai, K., Kawakubo, Y., Marumo, K., Kawasaki, S., Yamasue, H., Fukuda, M., 2008. Reduced frontopolar activation during verbal fluency task in schizophrenia: a multi-channel near-infrared spectroscopy study. Schizophr. Res. 99, 250–262.
Takizawa, R., Hashimoto, K., Tochigi, M., Kawakubo, Y., Marumo, K., Sasaki, T., Fukuda, M., Kasai, K., 2009a. Association between sigma-1 receptor gene polymorphism and prefrontal hemodynamic response induced by cognitive activation in schizophrenia. Prog. Neuropsychopharmacol. Biol. Psychiatry 33, 491–498.
Takizawa, R., Tochigi, M., Kawakubo, Y., Marumo, K., Sasaki, T., Fukuda, M., Kasai, K., 2009b. Association between catechol-O-methyltransferase Val108/158Met genotype and prefrontal hemodynamic response in schizophrenia. PLoS One 4, e5495.
Tanii, H., Nishimura, Y., Inoue, K., Koshimizu, H., Matsumoto, R., Takami, T., Hara, N., Nishida, A., Okada, M., Kaiya, H., 2009. Asymmetry of prefrontal cortex activities and catechol-O-methyltransferase Val158Met genotype in patients with panic disorder during a verbal fluency task: near-infrared spectroscopy study. Neurosci. Lett. 452, 63.
Thompson, P.M., Cannon, T.D., Narr, K.L., Van Erp, T., Poutanen, V., Huttunen, M., Lonnqvist, J., Standertskjold-Nordenstam, C., Kaprio, J., Khaledy, M., 2001. Genetic influences on brain structure. Nat. Neurosci. 4, 1253–1258.
Toronov, V., Webb, A., Choi, J.H., Wolf, M., Michalos, A., Gratton, E., Hueber, D., 2001. Investigation of human brain hemodynamics by simultaneous near-infrared spectroscopy and functional magnetic resonance imaging. Med. Phys. 28, 521–527.
Tsuzuki, D., Cai, D.S., Dan, H., Kyutoku, Y., Fujita, A., Watanabe, E., Dan, I., 2012. Stable and convenient spatial registration of stand-alone NIRS data through anchor-based probabilistic registration. Neurosci. Res. 72, 163–171.
Tzourio-Mazoyer, N., Landeau, B., Papathanassiou, D., Crivello, F., Etard, O., Delcroix, N., Mazoyer, B., Joliot, M., 2002. Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. Neuroimage 15, 273–289.
Umbricht, D., Krljes, S., 2005. Mismatch negativity in schizophrenia: a meta-analysis. Schizophr. Res. 76, 1–23.
Vandenberg, S.G., 1962. The hereditary abilities study: hereditary components in a psychological test battery. Am. J. Hum. Genet. 14, 220–237.
Wechsler, D., 1981. Wechsler Adult Intelligence Scale, rev. ed. Psychological Corporation, New York.
Weiss, E.M., Hofer, A., Golaszewski, S., Siedentopf, C., Brinkhoff, C., Kremser, C., Felber, S., Fleischhacker, W.W., 2004. Brain activation patterns during a verbal fluency test-a functional MRI study in healthy volunteers and patients with schizophrenia. Schizophr. Res. 70, 287–291.
Yamashita, Y., Maki, A., Ito, Y., Watanabe, E., Mayanagi, Y., Koizumi, H., 1996. Noninvasive near-infrared topography of human brain activity using intensity modulation spectroscopy. Opt. Eng. 35, 1046–1049.

Please cite this article as: Sakakibara, E., et al., Genetic influences on prefrontal activation during a verbal fluency task in adults: A twin study based on multichannel near-infrared spectroscopy, NeuroImage (2013), http://dx.doi.org/10.1016/j.neuroimage.2013.03.052

NeuroImage 62 (2012) 1999–2006

Contents lists available at SciVerse ScienceDirect

NeuroImage

journal homepage: www.elsevier.com/locate/ynimg

Validating atlas-guided DOT: A comparison of diffuse optical tomography informed by atlas and subject-specific anatomies

Robert J. Cooper [a,*,1], Matteo Caffini [b,g,1], Jay Dubb [a], Qianqian Fang [a], Anna Custo [c], Daisuke Tsuzuki [d], Bruce Fischl [a], William Wells III [e,f], Ippeita Dan [d], David A. Boas [a]

[a] Athinoula A. Martinos Center for Biomedical Imaging, Department of Radiology, Massachusetts General Hospital, Harvard Medical School, Charlestown, MA, USA
[b] Department of Health Sciences, University of L'Aquila, L'Aquila, Italy
[c] Centre Medical Universitaire, Functional Brain Mapping Laboratory University of Geneva, Geneva, Switzerland
[d] Functional Brain Science Laboratory, Center for Development of Advanced Medical Technology, Jichi Medical University, Shimotsuke, Tochigi 329-0498, Japan
[e] Department of Radiology, Harvard Medical School, Boston, MA, USA
[f] Brigham and Women's Hospital, Boston, MA, USA
[g] Dipartimento di Fisica - Politecnico di Milano, Milan, Italy

ARTICLE INFO

Article history:
Accepted 15 May 2012
Available online 23 May 2012

Keywords:
Diffuse optical tomography
NIRS
MRI
Anatomical atlas
Registration

ABSTRACT

We describe the validation of an anatomical brain atlas approach to the analysis of diffuse optical tomography (DOT). Using MRI data from 32 subjects, we compare the diffuse optical images of simulated cortical activation reconstructed using a registered atlas with those obtained using a subject's true anatomy. The error in localization of the simulated cortical activations when using a registered atlas is due to a combination of imperfect registration, anatomical differences between atlas and subject anatomies and the localization error associated with diffuse optical image reconstruction. When using a subject-specific MRI, any localization error is due to diffuse optical image reconstruction only. In this study we determine that using a registered anatomical brain atlas results in an average localization error of approximately 18 mm in Euclidean space. The corresponding error when the subject's own MRI is employed is 9.1 mm. In general, the cost of using atlas-guided DOT in place of subject-specific MRI-guided DOT is a doubling of the localization error. Our results show that despite this increase in error, reasonable anatomical localization is achievable even in cases where the subject-specific anatomy is unavailable.

Introduction

Near-infrared spectroscopy (NIRS) provides functional information about the oxygenation status of tissue by measuring optical signals which reflect changes in the concentrations of oxygenated-hemoglobin (HbO) and deoxygenated-hemoglobin (HbR) (Jöbsis, 1977). Diffuse optical tomography (DOT) is a multichannel NIRS approach, whereby numerous near-infrared sources and detectors coupled to the skin enable depth-resolved images of the spatio-temporal variations in hemoglobin concentrations to be reconstructed (Bluestone et al., 2001; Culver et al., 2003; Gibson et al., 2005; Zeff et al., 2007). Both NIRS and DOT have been widely applied to investigate brain function over the last 15 years (Durduran et al., 2010; Gibson et al., 2005; Lloyd-Fox et al., 2010). Recently, DOT has been used to map the visual cortex and investigate functional connectivity and motor–visual coordination with millimeter-order spatial resolution (White et al., 2009; Zeff et al., 2007). Whole-head, three-dimensional image reconstruction of regional blood volume and oxygenation has also been demonstrated in healthy and neurologically damaged infants (Austin et al., 2006; Gibson et al., 2006).

Numerous approaches have been investigated for improving DOT image sensitivity, resolution and accuracy (Boas et al., 2004; Gibson et al., 2005; Zeff et al., 2007). Employing a large number of sources and detectors (optodes), densely packed so as to provide spatially overlapping measurements, is essential for accurate DOT image reconstruction (Culver et al., 2003; Durduran et al., 2010; Zeff et al., 2007). The importance of including source–detector pairs with a relatively short separation (of 10 mm or less) has also been confirmed for both NIRS (Gagnon et al., 2011) and DOT (Gregg et al., 2010). Short-separation channels are sensitive to superficial tissues only. Such measurements not only allow the confounding effects of scalp hemodynamics to be removed from standard-separation signals in NIRS studies, but also improve the separation of superficial and cortical signals inherent to depth-resolved DOT.

Despite these advances, the most significant drawback of traditional DOT approaches is the absence of corresponding images of brain structure. Knowledge of the specific brain anatomy not only allows registration of DOT images to the cerebral cortex, but can also significantly improve the images themselves by restraining the

* Corresponding author.
E-mail address: rcooper@nmr.mgh.harvard.edu (R.J. Cooper).
[1] These authors contributed equally to this paper.

1053-8119/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.neuroimage.2012.05.031