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Effect of metabotropic glutamate receptor-3 variants on prefrontal brain activity in schizophrenia: An imaging genetics study using multi-channel near-infrared spectroscopy

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ABSTRACT

Background: The glutamatergic system is essential for learning and memory through its crucial role in neural development and synaptic plasticity. Genes associated with the glutamatergic system, including metabotropic glutamate receptor (mGluR or GRM) genes, have been implicated in the pathophysiology of schizophrenia. Few studies, however, have investigated a relationship between polymorphism of glutamate-related genes and cortical function in vivo in patients with schizophrenia. We thus explored an association between genetic variations in *GRM3* and brain activation driven by a cognitive task in the prefrontal cortex in patients with schizophrenia.
Materials and Methods: Thirty-one outpatients with schizophrenia and 48 healthy controls participated in this study. We measured four candidate single nucleotide polymorphisms (rs274622, rs2299225, rs1468412, and rs6465084) of *GRM3*, and activity in the prefrontal and temporal cortices during a category version of a verbal fluency task, using a 52-channel near-infrared spectroscopy instrument.
Results and Discussion: The rs274622 C carriers with schizophrenia were associated with significantly smaller prefrontal activation than patients with TT genotype. This between-genotype difference tended to be confined to the patient group. *GRM3* polymorphisms are associated with prefrontal activation during cognitive task in schizophrenia.

1. Introduction

Glutamate is the major excitatory neurotransmitter in the mammalian central nervous system (Fairman and Amara, 1999), and is essential for learning and memory through its fundamental role in neural development and synaptic plasticity (Suzuki et al., 2006). Regulation of glutamatergic neurotransmission is critical for these neural activities because excessively high synaptic glutamate levels and glutamate receptor activity trigger apoptosis, which is considered the major mechanism of cell death in many central nervous system diseases (Wang and Qin, 2010). Recent studies have identified genetic risk variants associated with glutamatergic function in schizophrenia (Cherlyn et al., 2010).

Metabotropic glutamate receptors (mGluRs or GRMs) belong to the class of seven-transmembrane domain receptors and can be classified into eight subtypes (GRM1–8) (Holscher et al., 1999; Ichise et al., 2000; Nakanishi, 1994). Of these, *GRM3* is differentially distributed among the presynaptic, postsynaptic, and glial compartments, and it

Abbreviations: NIRS, Near-infrared spectroscopy; fMRI, functional magnetic resonance image; BOLD, blood oxygenation level-dependent; MMN, mismatch negativity; CFT, category version of the verbal fluency task; [oxy-Hb], concentrations of oxygenated hemoglobin; [deoxy-Hb], concentrations of deoxygenated hemoglobin; SCID, Structured Clinical Interview for DSM-IV Axis I Disorders; PANSS, the positive and negative syndrome scale; GAF, global assessment of functioning scale; IQ, intelligence quotient; NA, not applicable; mGluR or GRM, metabotropic glutamate receptor; NMDA, N-methyl-D-aspartate; COMT, catechol-O-methyltransferase; EGR3, early growth-response-3; SNPs, single nucleotide polymorphisms; MTG, middle temporal gyrus; DLPFC, dorsolateral prefrontal cortex; VLPFC, ventrolateral prefrontal cortex; FPC, fronto-polar prefrontal cortex; STG, superior temporal gyrus; ANOVA, analysis of variance; ANCOVA, analysis of covariance; FDR, false discovery rate.

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has effects on the modulation of NMDA receptor function and local microcirculation (Ghose et al., 2009). The *GRM3* agonist LY354740 could improve anxiety symptoms (Dunayevich et al., 2007; Michelson et al., 2005) and cognitive impairment (Krystal et al., 2005). Genetic association studies of *GRM3* have reported inconclusive results (positive: (Bishop et al., 2005; Chen et al., 2005; Egan et al., 2004; Fujii et al., 2003; Mossner et al., 2008); negative: (Albalushi et al., 2008; Norton et al., 2005; Schwab et al., 2008; Tochigi et al., 2006)); however, a recent large-scale genome-wide association study has confirmed the significant association of *GRM3* with schizophrenia (Schizophrenia Working Group of the Psychiatric Genomics C, 2014). Postmortem studies have also shown inconsistent results of altered *GRM3* expression in the prefrontal cortex in terms of its effect on schizophrenia risk. (Corti et al., 2007; Crook et al., 2001; Ghose et al., 2009; Gupta et al., 2005). Because *GRM3* distribution is substantially different among brain regions (Ghose et al., 2009), variation of altered *GRM3* distribution and protein expression were also reported across brain regions in schizophrenia (Corti et al., 2007; Crook et al., 2001; Ghose et al., 2009). Because several studies have reported that *GRM3* polymorphisms have substantial effects on cognitive function (Egan et al., 2004), neuronal activity in the temporal cortex (Kawakubo et al., 2011), and function and metabolism in the prefrontal cortex (Egan et al., 2004), *GRM3* polymorphisms may affect prefrontal activity during cognitive demands.

The "imaging genetics" technique is a powerful approach to explore relationship between genetic variations conferring risk for psychiatric disorders and brain structure and function as measured by in vivo neuroimaging (Meyer-Lindenberg and Weinberger, 2006). Although functional magnetic resonance imaging (fMRI) has been extensively used as an analysis tool of intermediate phenotype, fMRI may have some limitations for that purpose. The fMRI measurement requires constraint to participants, which makes it differentially difficult for patients to undergo measurement procedures, resulting in possible selection bias in imaging genetics studies. It may be also important to assess cortical function in more natural settings. Near-infrared spectroscopy (NIRS), a fully non-invasive neuroimaging technology measuring hemoglobin concentration changes from cortical surface areas under a natural condition, may resolve these issues, although it has lower spatial resolution than fMRI. Using a multi-channel NIRS, we have previously found impaired prefrontal function in patients with schizophrenia (Koike et al., 2011, 2013b; Marumo et al., 2014; Takizawa et al., 2008). The NIRS signals show high correlations with the BOLD signals (Sato et al., 2013). Moreover, our twin study of healthy adults indicated significant heritability of prefrontal activity as measured by NIRS (Sakakibara et al., 2014). Thus, NIRS may serve as a reliable intermediate phenotype for imaging genetics studies in schizophrenia. In fact, through imaging genetics approaches, we have reported that, in patients with schizophrenia, catechol-*O*-methyltransferase (*COMT*), Sigma-1 receptor, and early growth-response-3 (*EGR3*) gene polymorphisms are associated with prefrontal activity as measured by NIRS (Nishimura et al., 2014; Takizawa et al., 2009a, 2009b). In line with possible modulation of prefrontal activity during a cognitive task by *GRM3* genotype (Egan et al., 2004), we hypothesized that the *GRM3* genotype also would be associated with prefrontal function in schizophrenia. Therefore, we explored the association of several single nucleotide polymorphisms (SNPs) related to *GRM3* with brain activity during a category version of the verbal fluency task (CFT), using a multi-channel NIRS instrument.

2. Methods

2.1. Study participants

Thirty-one outpatients with schizophrenia and 48 healthy controls matched for age and gender were enrolled in this study (Table 1). All participants were native Japanese speakers and right-handed according to Oldfield's Edinburgh Inventory (Oldfield, 1971). All patients were medicated and recruited in their stable condition at the outpatient unit of the University of Tokyo Hospital. Schizophrenia was diagnosed on the basis of the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID) by experienced psychiatrists (R.T., K.M., K.K.) (First et al., 2002). The exclusion criteria for both groups were neurological illness, traumatic brain injury with any known cognitive consequences or loss of consciousness for more than 5 min, a history of electroconvulsive therapy, and alcohol/substance abuse or addiction. The healthy control group had an additional exclusion criterion, which was any history of psychiatric disease or any such history in a first-degree relative. On the same day as the NIRS experiment, psychiatric symptoms were evaluated by trained psychiatrists (R.T., K.M., K.K.) using the Positive and Negative Syndrome Scale (PANSS) without knowledge of the NIRS data (Kay et al., 1987). At the time of the study, all patients with schizophrenia were on medication. Therefore, equivalent-doses-as-chlorpromazine were calculated (Inagaki and Inada, 2008, 2010). Premorbid IQs were estimated using the Japanese version of the National Adult Reading Test (Matsuoka et al., 2006). The study protocol was approved by the ethics committee of the University of Tokyo Hospital (approval No.630-[8], 639-[30]) in accordance with the Declaration of Helsinki.

Table 1
Demographic characteristics of study participants for rs274622.

	Healthy controls (n = 48)							Patients with Schizophrenia (n = 31)							Group difference
	Total		TT		C carriers		*t*-test [b]	Total		TT		C carriers		*t*-test [b]	*t*-test [c]
	Mean	SD	Mean	SD	Mean	SD	p value	Mean	SD	Mean	SD	Mean	SD	p value	p value
Participants (male: female)[a]	48 (33:15)		29 (20:9)		19 (13:6)		.97	31 (15:16)		20 (10:10)		11 (5:6)		.81	.81
Age, year	33.0	6.0	32.8	6.1	33.1	6.3	.89	35.7	7.6	35.9	7.4	35.6	8.4	.92	.075
Premorbid IQ	109.8	6.7	109.3	7.5	108.6	7.2	.73	103.4	12.2	102.8	11.5	104.6	13.8	.70	**.012**
Task performance, words	22.8	3.7	23.3	3.3	22.2	4.2	.31	19.9	4.5	19.4	4.8	20.8	4.0	.41	**.0030**
Age at onset, year	NA	NA	NA	NA	NA	NA	NA	26.2	8.3	26.6	8.6	27.1	9.7	.87	NA
GAF	NA	NA	NA	NA	NA	NA	NA	51.1	10.7	51.7	10.6	46.5	13.2	.26	NA
PANSS															
Positive	NA	NA	NA	NA	NA	NA	NA	15.2	5.0	16.1	4.9	13.3	4.9	.14	NA
Negative	NA	NA	NA	NA	NA	NA	NA	19.3	6.1	21.5	5.4	17.2	7.4	.096	NA
General psychopathology	NA	NA	NA	NA	NA	NA	NA	35.9	7.5	37.2	7.6	35.8	8.6	.62	NA
Chlorpromazine equivalent dose, mg/day	NA	NA	NA	NA	NA	NA	NA	633.7	456.5	626.6	503.0	646.6	379.8	.91	NA

Abbreviations: IQ, intelligence quotient; GAF, global assessment of functioning; PANSS, Positive and Negative Symptom Scale; NA, not applicable
[a] chi-Square test was used for testing group difference.
[b] Group differences between TT and C carriers subgroup in each group. $p < 0.05$ was considered significant.
[c] Group differences between healthy controls and patients with schizophrenia. $p < 0.05$ was considered significant.