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The *SIGMAR1* gene is associated with a risk of schizophrenia and activation of the prefrontal cortex

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ABSTRACT

Several studies have identified the possible involvement of sigma non-opioid intracellular receptor 1 (*SIGMAR1*) in the pathogenesis of schizophrenia. The Gln2Pro polymorphism in the *SIGMAR1* gene has been extensively examined for an association with schizophrenia. However, findings across multiple studies have been inconsistent. We performed a meta-analysis of the association between the functional Gln2Pro polymorphism and schizophrenia using combined samples (1254 patients with schizophrenia and 1574 healthy controls) from previously published studies and our own additional samples (478 patients and 631 controls). We then used near-infrared spectroscopy to analyze the effects of the Gln2Pro genotype, a schizophrenia diagnosis and the interaction between genotype and diagnosis on activation of the prefrontal cortex (PFC) during a verbal fluency task (127 patients and 216 controls). The meta-analysis provided evidence of an association between Gln2Pro and schizophrenia without heterogeneity across studies (odds ratio = 1.12, $p = 0.047$). Consistent with previous studies, patients with schizophrenia showed lower bilateral activation of the PFC when compared to controls ($p < 0.05$). We provide evidence that Pro carriers, who are more common among patients with schizophrenia, have significantly lower activation of the right PFC compared to subjects with the Gln/Gln genotype ($p = 0.013$). These data suggest that the *SIGMAR1* polymorphism is associated with an increased risk of schizophrenia and differential activation of the PFC.

1. Introduction

Schizophrenia is a common and complex psychiatric disease with strong genetic components. Schizophrenia has an estimated heritability of approximately 80% (Cardno and Gottesman, 2000; Tsuang, 2000) and many genes have been implicated in the pathogenesis of schizophrenia (Sun et al., 2008).

Sigma-1 receptors are relatively small single transmembrane proteins located on the plasma and subcellular membranes, particularly in the endoplasmic reticulum; the protein plays a role in modulating intracellular calcium signaling (van Waarde et al., 2010). Sigma-1 receptors are also involved in modulating the activity of some ion channels and in several neurotransmitter systems such as glutamatergic and dopaminergic neurotransmission (Hayashi and Su, 2004). Several drugs targeted to the central nervous system, including antipsychotics (haloperidol, chlorpromazine and nemonapride), selective serotonin reuptake inhibitors (fluvoxamine and sertraline) and acetylcholinesterase inhibitors (donepezil), show high to moderate affinities for sigma-1 receptors (Cobos et al., 2008). Of the antipsychotics, only haloperidol is known to act as an antagonist for sigma-1 receptor (Cobos et al., 2008). This affinity between antipsychotic drugs and sigma-1 receptors suggests that the receptors play a substantial role in the pathogenesis of schizophrenia. Sigma-1 antagonists improve the behavior of animals in models based on the

Abbreviations: SIGMAR1, sigma non-opioid intracellular receptor 1; PFC, prefrontal cortex; NIRS, near-infrared spectroscopy; SNP, single nucleotide polymorphism; VFT-letter, letter version of the verbal fluency test; oxyHb, oxygenated hemoglobin; OR, odds ratio; ANCOVA, analysis of covariance.

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motor effects of dopaminergic stimulants or NMDA antagonists (Cobos et al., 2008). In clinical trials, putative selective antagonists of sigma receptors showed antipsychotic effects for schizophrenia (Cobos et al., 2008).

The sigma non-opioid intracellular receptor 1 (*SIGMAR1*) gene is located on chromosome 9p13. This gene contains cytokine and steroid responsive elements. In genetic association studies, Ishiguro et al. (1998) detected associations between schizophrenia and two functional polymorphisms of the *SIGMAR1* gene, Gln2Pro and GC-241-240TT. These two polymorphisms were in near complete linkage disequilibrium with each other and resulted in two haplotypes, Pro2/TT241-240 and Gln2/GC-241-240 (Ishiguro et al., 1998). The transcriptional activity of the TT-241-240 haplotype, which was in near complete linkage disequilibrium with Pro2 in *SIGMAR1* gene, was significantly reduced compared with that of the GC-241-240 haplotype (Miyatake et al., 2004). The Gln2Pro polymorphism is part of the N-terminus amino acid sequence motif MQWAVGRR, which is a putative endoplasmic reticulum retention signal (Schutze et al., 1994). The functional polymorphism has been extensively examined for an association with schizophrenia. However, the findings of multiple studies have been inconsistent (Ohmori et al., 2000; Satoh et al., 2004; Uchida et al., 2003). A meta-analysis of Gln2Pro in *SIGMAR1* has found no evidence for a significant association between the genetic variant and schizophrenia, although the Pro allele was marginally more frequent in schizophrenia patients (32%) than in controls (29%) ($p=0.06$) (Uchida et al., 2003). The lack of association identified in the meta-analysis may be the result of a type II error stemming from a small sample size (779 patients with schizophrenia and 636 healthy controls).

Many attempts have been made to minimize clinical and genetic heterogeneity for schizophrenia. A strategy for gene discovery proposes using quantitative neurobiological traits as intermediate phenotypes instead of the diagnosis of schizophrenia (Meyer-Lindenberg and Weinberger, 2006; Tan et al., 2008). This strategy has the potential to reduce clinical and genetic heterogeneity by applying intermediate phenotypes that reflect underlying genetic vulnerability better than diagnostic categorization. Combined imaging and genetic studies have shown that brain function, as assessed by neuroimaging techniques, is a sensitive intermediate phenotype that bridges the gap between genotype and diagnostic categorization (Weinberger et al., 2001). Near-infrared spectroscopy (NIRS) is a functional neuroimaging technology used to noninvasively assess changes in cerebral blood volume. Verbal fluency, a classic test of executive function, is the most reliable task currently used to induce prominent and wide-spread frontotemporal activation in normal subjects that can be well-differentiated from that of patients with schizophrenia (Ikezawa et al., 2009; Takizawa et al., 2008). Structural and functional abnormalities of the prefrontal cortex (PFC) are well known to exist in patients with schizophrenia (Ragland et al., 2009; Segall et al., 2009). Sigma-1 receptors are widely expressed in the mammalian brain tissues (Kekuda et al., 1996; Kitaichi et al., 2000). The chronic administration of the preferential sigma-1 receptor ligand is able to modify levels of several glutamate subunits in the rat PFC (Guitart et al., 2000). Postmortem study comparing normal controls to patients with schizophrenia revealed that schizophrenics have a reduced density of sigma binding sites in the frontal cerebral cortex (Simpson et al., 1991). There is evidence that activation of the PFC during the verbal fluency task, as assessed using multi-channel NIRS, is significantly lower in Pro carriers of the *SIGMAR1* gene than in individuals with the Gln/Gln genotype (Takizawa et al., 2009a). We hypothesized that the lower *SIGMAR1* expression modulated by the Gln2Pro polymorphism might be related to hypoactivation of the PFC in schizophrenia via impaired regulation of NMDA receptor-mediated glutamatergic neurotransmission.

In this study, we first attempted to replicate the association between a functional single nucleotide polymorphism (SNP) in the *SIGMAR1* gene and schizophrenia in our samples; we then added our samples to the available samples from previous studies and performed a meta-analysis. We next examined the influence of the Gln2Pro polymorphism on prefrontal hemodynamic activation during a verbal fluency task using a noninvasive neuroimaging technique, two-channel NIRS, in patients with schizophrenia and in healthy volunteers.

2. Methods

2.1. Subjects

Subjects for the genetic association study included 478 unrelated patients with schizophrenia [48.5% males (232 males/246 females), mean age ± SD: 48.3 ± 15.7 years] and 631 unrelated healthy controls [46.9% males (296/335), mean age ± SD: 58.7 ± 21.4 years]. The sex ratio did not differ significantly between cases and controls ($p=0.56$), while the mean age of schizophrenia patients was significantly lower than that of controls ($p<0.001$). Cases were recruited from both outpatients and inpatients at Osaka University Hospital and the psychiatric hospitals. Each subject with schizophrenia had been diagnosed by at least two trained psychiatrists based on an unstructured clinical interview; diagnoses were made based on the criteria of the fourth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV). Controls were recruited through local advertisements. Psychiatrically healthy controls were evaluated using unstructured interviews to exclude individuals who had current or past contact with psychiatric services.

The subjects who underwent NIRS analysis were 127 unrelated patients with schizophrenia [55.1% males (70/57), mean age ± SD: 36.9 ± 12.3 years] and 216 unrelated healthy controls [44.4% males (96/120), mean age ± SD: 36.8 ± 11.6 years]. These subjects were included in the genetic association study and agreed to receive the examination using NIRS. These subjects in the present NIRS analysis included subjects in our two previous NIRS studies (Azechi et al., 2010; Ikezawa et al., 2009). All subjects were biologically unrelated Japanese. Subjects were excluded from this analysis if they had neurological or medical conditions that could potentially affect the central nervous system, such as atypical headache, head trauma with loss of consciousness, chronic lung disease, kidney disease, chronic hepatic disease, thyroid disease, cancer with active stage, cerebrovascular disease, epilepsy, seizures, substance-related disorders or mental retardation. Cases were recruited from both outpatients and inpatients at Osaka University Hospital. Each patient with schizophrenia had been diagnosed by a trained psychiatrist according to the DSM-IV criteria based on Structured Clinical Interview for DSM-IV (SCID). Controls were recruited through local advertisements at Osaka University. Psychiatrically, medically and neurologically healthy controls were evaluated using the SCID-Non-Patient version to exclude individuals who had current or past contact with psychiatric services or had received psychiatric medication (Hashimoto et al., 2010; Ohi et al., 2009). Current symptoms of schizophrenia were evaluated using the five syndrome models of the positive and negative syndrome scale (PANSS) (Lindenmayer et al., 1994). There were 15 patients taking haloperidol, which has a high affinity and acts as an antagonist for sigma-1 receptor (Cobos et al., 2008), at the NIRS measurement [Pro carrier $N=10$, mean chlorpromazine equivalents (CPZeq) of haloperidol ± SD: 398.8 ± 338.2 mg/day, Gln/Gln $N=5$, 500.0 ± 326.0 mg/day]. We found no differences in CPZeq of haloperidol in subjects taking haloperidol between the genotype groups ($p=0.46$). The sex ratio and mean age did not differ significantly between cases and controls ($p>0.06$), while years of education, estimated premorbid intelligence quotient (IQ) and performance score on the letter version of the verbal fluency test (VFT-letter) during the NIRS measurement were significantly lower in patients with schizophrenia than in controls ($p<0.001$) (Table 1). When the genotype groups were compared, we found no differences in demographic variables, age, sex, years of education, estimated premorbid IQ, performance score on VFT-letter, CPZeq of total antipsychotics, ratio of subjects taking haloperidol, age at onset of