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Association study between *Disrupted-in-Schizophrenia-1* (*DISC1*) and Japanese patients with treatment-resistant schizophrenia (TRS)

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ABSTRACT

Treating the 20–30% of patients with schizophrenia whose symptoms are resistant to antipsychotic treatment, a condition known as treatment-resistant schizophrenia (TRS), can be problematic. Recently, an association between *Disrupted-in-Schizophrenia-1* (*DISC1*), a candidate susceptibility gene for schizophrenia, and TRS was reported. Associations between three missense SNPs, rs3738401 (Q264R), rs6675281 (L607F), and rs821616 (S704C) in *DISC1*, especially rs3738401, showed strong significance. Thus, the main aim of our current study was to examine if the reported possible functional polymorphisms in *DISC1* were related to Japanese TRS. First, *DISC1* was re-investigated in 485 Japanese patients with schizophrenia and 660 healthy controls with a case–control study using four candidate SNPs, rs751229, rs3738401, rs821597, and rs821616. *DISC1* was not associated with schizophrenia in the Japanese population. Second, we investigated whether these SNPs contributed to TRS in 127 inpatients with schizophrenia (35 patients; TRS and 92 patients; non-TRS). The genotypic distributions of these four SNPs were not significantly different between TRS and non-TRS in either genotypic or recessive models of minor alleles. In addition, clinical variables, such as improvement in clinical symptoms, duration of hospitalization, and total antipsychotics dose amounts, were not different among the genotypes of these SNPs. Taken together, results showed that *DISC1* had no apparent degree of association with Japanese patients with schizophrenia as a candidate susceptibility gene for disease per se or TRS.

1. Introduction

Schizophrenia is a major, serious psychiatric disorder with complex genetic, environmental, and psychological etiologies. Although antipsychotics provide symptom improvements to large groups of patients, treating the 20–30% of people with schizophrenia whose symptoms are resistant to antipsychotic treatment, a condition known as treatment-resistant schizophrenia (TRS) (Hosak and Libiger, 2002; Meltzer, 1992), can be problematic (Premkumar and Pick, 2006). Although a new antipsychotic, clozapine, can be used to treat TRS as a last resort, this agent has actions on glutamatergic neurotransmission. Disruption of glutamatergic neurotransmission is considered to be a partial cause of negative symptoms and cognitive dysfunction resulting in TRS (Heresco-Levy et al., 2005; Tsai et al., 1998; Tsai et al., 1999). Patients with TRS who are treated with clozapine but who show no improvements in the clinical symptoms (stringent criteria: patients who experience no clinical, social, and/or occupational remission despite treatment with clozapine and at least two periods of treatment with distinct conventional or atypical antipsychotics) are said to have ultra-resistant schizophrenia (URS) (Demily and Franck, 2008; Mouaffak et al., 2010). Thus, TRS and URS are important subtype in schizophrenia. Several genetic studies have focused on the association between TRS and candidate genes, and genotypic–phenotypic association studies have shown some positive findings, especially involving genes related to serotonin receptors (Ji et al., 2008a,b), cytochrome families (Kawanishi et al., 2002; Kohlrausch et al., 2008), and dopamine receptors (Inada et al., 2003; Kohlrausch et al., 2008; Potkin et al., 2003). However, not all results were reproducible. *Disrupted-in-Schizophrenia-1* (*DISC1*) was identified by cytogenetic approaches as a genetic risk factor. Sequencing showed a translocation breakpoint in a region on chromosome 1q42 (Millar et al., 2000) that had been identified in a large Scottish family with mental and/or behavioral disorders in a pedigree with familial psychosis (St Clair et al., 1990). Thus, *DISC1* is considered a candidate susceptibility gene for schizophrenia. Several studies have shown 1) genetic risk factors for schizophrenia in different ethnic populations (Callicott et al., 2005; Kim et al., 2008; Lepagnol-Bestel et al., 2010; Nakata et al., 2009; Qu et al., 2007; Zhang et al., 2005) and

Abbreviations: BPRS, Brief Psychiatric Rating Scale; CP, chlorpromazine; CGI-S, Clinical Global Impressions-Severity scale; CGI-I, Clinical Global Impressions-Improvement scale; DSM-IV, Diagnostic and Statistical Manual of Mental Disorders-IV; DISC1, Disrupted-in-Schizophrenia-1; GAF, Global Assessment of Functioning; TRS, treatment-resistant schizophrenia; HWE, Hardy-Weinberg equilibrium; LD, linkage disequilibrium; URS, ultra-resistant schizophrenia; SNPs, single-nucleotide polymorphisms; SCID-IV, Structured Clinical Interview for DSM-IV.

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2) association with some specific pathophysiological phenotypes of schizophrenia such as brain morphology in schizophrenia (Callicott et al., 2005; Takahashi et al., 2009), disrupted cognitive function (Thomson et al., 2005), and an association with some clinical symptoms (Kim et al., 2008; Lepagnol-Bestel et al., 2010). For the past quarter century, these phenotypes of schizophrenia, such as brain morphological abnormalities and negative symptoms, have seemed to be clinically involved with TRS (Crow, 1985). Accordingly, an association between *DISC1* and TRS should be considered. Associations between three missense single-nucleotide polymorphisms (SNPs) [rs3738401 (Q264R), rs6675281 (L607F), and rs821616 (S704C)] in *DISC1* and URS were recently reported, and the association with rs3738401 was especially strong (Mouaffak et al., 2010). Thus, in our current study, we first performed a genetic case–control study for candidate functional SNPs in *DISC1* to reinvestigate whether these may be genetic risk factors for schizophrenia in the Japanese population. Second, we investigated whether these SNPs were involved in TRS as a first trial with Japanese samples.

2. Materials and methods

2.1. Participants

The case–control genetic association study included 485 Japanese patients with unrelated schizophrenia (266 males and 219 females; mean age 39.5 years, S.D. ± 14.1) that met the Diagnostic and Statistical Manual of Mental Disorders-IV (DSM-IV) diagnosis of schizophrenia according to structured clinical interviews (Structured Clinical Interview for DSM-IV [SCID-IV]) (First et al., 1995), and 660 healthy controls (305 males and 355 females; mean age 47.5 years, S.D. ± 17.5). Healthy controls did not meet the current or past criteria for any Axis I disorder. All participants met the following criteria: 1) no systemic or neurological disease, 2) no prior head trauma with loss of consciousness, and 3) no lifetime history of alcohol or substance dependency. Patients and controls were recruited from two geographic regions of Japan: Saitama and Tokyo. The mean age of the schizophrenic group was significantly different from that of the control group (Student's *t*-test, $t = 8.28$, $P < 0.001$). The gender distribution between the two groups was also significantly different ($\chi^2 = 9.904$, $P = 0.007$). The Ethics Committee of the Juntendo University School of Medicine approved this study. All individuals gave written informed consent prior to participating in the study.

Among the 485 patients with schizophrenia, 127 patients (64 males and 63 females, age 33.9 ± 11.7 years) were admitted to the Juntendo Koshigaya Hospital due to worsening of their symptoms and were thus available for detailed evaluation of clinical symptoms, in addition to genetic analysis. The clinical symptoms of the patients with schizophrenia were evaluated using the Clinical Global Impressions-Severity scale (CGI-S), the CGI-Improvement scale (CGI-I), and the Brief Psychiatric Rating Scale (BPRS) by experienced psychiatrists (YH, TO, RH, HM, TH, YT, MH, and MK). The mean values (± SD) of the clinical variables for the 127 schizophrenic patients were: age, 33.8 ± 12.1 years; age of onset, 24.2 ± 8.5 years; duration of illness, 9.7 ± 8.4 years; untreated period, 25.3 ± 37.3 months; duration of hospitalization, 144.6 ± 163.1 days; CGI-S, 5.6 ± 1.1; Global Assessment of Functioning (GAF), 32.6 ± 14.0; and total BPRS score, 49.8 ± 13.9.

Because all 127 patients were inpatients, complete medication compliance was achieved. Strictly speaking, patients who met the definition of URS were not included in our current study, because at the time we started this study, clozapine was not available in Japan. Accordingly, none of the patients in this study had been treated with clozapine. Because clozapine is indicated as a treatment for TRS, we used the indications of clozapine for the definition of TRS, which was almost similar to the definition of TRS described by Kane et al. [14]: a sufficiently long treatment with at least two different antipsychotics and a sufficient daily dose that failed to satisfactorily provide improvement in symptoms (GAF was never higher than 41 points). In detail, for the antipsychotics used, not only typical antipsychotics but also at least one atypical antipsychotic must have been used. The highest daily chlorpromazine (CP) equivalent dose of antipsychotic must have been >600 mg. The duration of medication with typical antipsychotics must have been more than 1 year, and the duration of atypical antipsychotics must have been at least 4 weeks. Considering these clozapine indications, 35 patients (14 males and 21 females; mean age 32 ± 11.0) met the criteria of TRS, and 92 patients (50 males and 42 females; mean age 33.9 ± 11.8) met the criteria of non-TRS.

2.2. Genotyping

Genomic DNA was extracted from peripheral white blood cells using a QIAamp® DNA Blood Maxi kit (Qiagen, Courtaboeuf, France). All SNPs were typed using TaqMan® technology (Assay-by-Design™) on an ABI7500 system (Applied Biosystems, Foster City, CA, USA). All probes and primers were designed by the Assay-by-Design™ service of Applied Biosystems. Standard PCR reactions were carried out using the TaqMan® Universal PCR Master Mix reagent kit in a 5-μl volume.

Although the main aim of this study was reinvestigation of a previously identified relationship between SNPs rs3738401, rs6675281, and rs821616 in *DISC1* and TRS (Mouaffak et al., 2010), the minor allele frequency (MAF) of rs6675281 in the Japanese population was null according to the HapMap data. Therefore, this SNP was excluded from the current study. Instead, two possibly important SNPs (rs751229 and rs821597) that also may be associated with the pathophysiology of schizophrenia were added to our analysis. SNPs in *DISC1* investigated in our current study were chosen according to the following criteria: 1) SNPs reported to be associated with TRS (Mouaffak et al., 2010); 2) possible functional polymorphisms, showing associations with some phenotypes in schizophrenic pathophysiology (Callicott et al., 2005; Takahashi et al., 2009; Thomson et al., 2005); 3) SNPs associated with Asian or other ethnic patients with schizophrenia as genetic risk factors (Kim et al., 2008; Lepagnol-Bestel et al., 2010; Nakata et al., 2009; Qu et al., 2007; Zhang et al., 2005); and 4) a MAF>0.05 in the Japanese population from HapMap data. Thus, the following four SNPs were ultimately chosen: rs751229, rs3738401 (Q264R), rs821597, and rs821616 (S704C) ("rs" number of each SNP is the ID of the US National Center for Biotechnology Information SNP cluster from the dbSNP database; http:/www.ncbi.nlm.nih.gov/SNP/).

2.3. Statistics

For the case–control association study, genetic statistical analysis, Hardy–Weinberg equilibrium (HWE) testing, and differences in genotypic/allelic frequencies were all done using SNPAlyse V7.0 Pro (Dynacom, Yokohama, Japan). Linkage disequilibrium (LD), denoted as D', was calculated from the haplotype frequency using the expectation-maximization algorithm. The LD block was also identified using SNPAlyse software when D' was greater than 0.75. Case–control haplotype analysis was also performed using SNPAlyse software. Permutation analysis was used to determine empirical significance and to calculate the *P*-values based on 10,000 replications. The global *P*-values represent the overall significance using the χ^2-test when the observed versus expected frequencies of all haplotypes are considered together. All *P*-values reported are two-tailed. Statistical significance was defined as $P<0.05$. We performed power calculations using the Power Calculator for Two Stage Association Studies (http://www.sph.umich.edu/csg/abecasis/CaTS/). Power was calculated under prevalence of 0.01 using an additive or a multiplicative model, based on allelic frequencies of the associated markers ranging from 0.14 (rs821616) to 0.43 (rs821597) and odds ratios ranging from 1.28 (rs751229) to 1.71 (rs821616) for the SNPs investigated in the