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Association of promoter variants of human dopamine transporter gene with schizophrenia in Han Chinese

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ABSTRACT

Objective: Although dopamine was implicated in the etiology of schizophrenia, the human dopamine transporter gene (*DAT1*; *SLC6A3*) has not consistently been associated with schizophrenia. The purpose of this study was to examine whether six polymorphisms within the *DAT1* gene are associated with schizophrenia.
Methods: Six polymorphisms of the *DAT1* gene (3 SNPs [*rs6413429*, *rs2652511*, and *rs2975226*] in the promoter region, one SNP [*rs6347*] in exon 9, and one SNP [*rs27072*]/one variable number tandem repeat [VNTR] in exon 15) were analyzed in 352 Chinese patients with schizophrenia and in 311 healthy controls. Pretreatment psychopathology was assessed using the Positive and Negative Syndrome Scale in a subset of 160 hospitalized schizophrenia patients who were drug-free or drug-naïve.
Results: A statistically significant difference in two polymorphisms (*rs2652511* and *rs2975226*) and a promoter region haplotype (*rs2652511*, *rs2975226*, and *rs6413429*) was found between patients and healthy controls. No association with schizophrenia was found for other polymorphisms and another haplotype (3′ region). Symptoms severity (PANSS global, positive, negative and general symptoms scores) was similar regardless of *DAT1* polymorphism.
Conclusion: The promoter region of the *DAT1* gene may play a role in increasing susceptibility to schizophrenia, but does not affect the severity of psychotic symptoms in Han Chinese.

1. Introduction

Family, twin, and adoption studies have provided evidence for the contribution of genetic factors to schizophrenia susceptibility and a wide range of phenotypic expressions (Birkett et al., 2008; Cardno et al., 2002; Toulopoulou et al., 2007). Dysfunction of the central dopamine neurotransmission evidently plays a key role in the pathogenesis of schizophrenia (Davis et al., 1991; Howes et al., 2009). First, psychotomimetic agents, such as cocaine and amphetamine, inhibit dopamine reuptake and increase dopaminergic activity (Borowski and Kokkinidis, 1998; Breier et al., 1997). Second, the efficacy and the potency of most antipsychotic drugs are correlated with their ability to act as antagonists of the dopamine D2 receptor (Goldstein and Deutch, 1992; Hjerde et al., 2005). Furthermore, the dopamine transporter not only plays a vital role in dopaminergic neurotransmission by mediating the active

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reuptake of dopamine from the synaptic terminal (Giros and Caron, 1993), but it also regulates synaptic dopamine concentrations and the duration of dopamine activity (Carvelli et al., 2008). Therefore, the dopamine transporter gene is a promising candidate for genetic association studies.

The human dopamine transporter gene (*DAT1*; *SLC6A3*) is located on chromosome 5p15.3 and consists of 15 exons, spanning ~65 kb. Several studies have examined whether the 40-bp variable number tandem repeat (VTNR) polymorphism in exon 15 of the *DAT1* gene is associated with schizophrenia. Although the results of these studies are controversial, a meta-analysis failed to find such an association (Gamma et al., 2005). Khodayari et al. (2004) used the *T-67A* (rs2975226) polymorphism in the promoter region of the *DAT1* gene as a candidate locus for schizophrenia. They found that the T allele of the rs2975226 polymorphism was associated with schizophrenia in a sample of Iranian males. In addition, Stober et al. (2006) studied 5 single nucleotide polymorphism (SNP) markers in the promoter region of *DAT1* gene in 82 German Caucasian index cases with schizophrenia and their biological parents. Their finding of significant overrepresentation of 5 distinct loci in subjects with schizophrenia compared to parental controls supported the involvement of *DAT1* genotypes in schizophrenia. However, only a single drug response study (Zhang et al., 2007) and no genetic studies have examined the association between the promoter polymorphisms of *DAT1* gene and schizophrenia in Han Chinese. Therefore, this study was conducted to evaluate the association of five SNP markers (three in the promoter region, one within exon 9, and one within exon 15) and a VNTR polymorphism within the *DAT1* gene with schizophrenia in Han Chinese.

Furthermore, the genes affecting susceptibility to a given psychiatric illness may also affect the symptoms of the illness, so that studying symptomatological factors may help elucidate genetic influences on psychopathological traits (Risch, 1990). Therefore, we subsequently investigated whether the six polymorphisms (rs6413429, rs2652511, rs2975226, rs6347, rs27072, or *3′VNTR*) were associated with severity of clinical symptoms in a subset of schizophrenic patients who were drug-free or drug-naïve.

2. Experimental procedures

2.1. Samples collection

This study was conducted at the inpatient and outpatient units of the Tri-Service General Hospital (TSGH), a medical teaching hospital belonging to the National Defense Medical Center in Taipei, Taiwan. The protocol was approved by the Ethics Committee of TSGH for the Protection of Human Subjects. Written informed consent was obtained from all participants, after the procedures of the study were fully explained. To minimize the effect of ethnic differences in gene frequencies, all participants were unrelated Han Chinese born and living in Taiwan, and all their biological grandparents were of Han Chinese ancestry. Individuals with a history of substance dependence, severe medical illness, organic brain disease, or any concomitant major psychiatric disorders were excluded.

The patient group consisted of 352 schizophrenic patients who were recruited from various clinical settings. Patients were assessed for schizophrenia using the criteria of the Diagnostic and Statistical Manual of mental disorders, fourth edition (DSM-IV) (1994). Each patient was initially examined by one experienced attending psychiatrist (S.Y.H) and then interviewed by a well-trained psychologist using the Chinese Version of the Modified Schedule of Affective Disorder and Schizophrenia-Life Time (SADS-L) (Endicott and Spitzer, 1978; Merikangas et al., 1998). The inter-rater reliability *k* values for the SADS-L were as follows: major depression, 0.79; bipolar disorder, 0.71; anxiety disorder, 0.86; schizophrenia, 0.95; and substance abuse and dependence, 0.82 (Huang et al., 2004). In addition, since antipsychotic drugs can alter psychopathology status and regulate dopamine concentration, it is important to avoid their potential confounding effects. Therefore, all recruited inpatients were evaluated by a well-trained psychiatrist using the Positive and Negative Symptom Scale (PANSS) (Kay et al., 1987) on the day of admission. Patients ($n = 160$) who were drug-naïve or drug-free for at least one month before hospitalization and had a minimum baseline PANSS score of 70 were selected for further analysis. The healthy control group ($n = 311$) were volunteers from the community. The Chinese version of the modified SADS-L was used to screen out those in the control group who had psychiatric conditions. This group was free of past or present major or minor mental illnesses. In addition, there was no family history of psychiatric disorders in the first-degree relatives of the control subjects.

2.2. Blood sampling and genotyping

A peripheral vein blood sample from each subject was drawn into a vacutainer tube containing the anticoagulant ethylene-diaminetetracetic acid (EDTA). Genomic DNA was extracted from the leukocytes using standard techniques. Polymorphisms (rs6413429, rs2652511, rs2975226, rs6347, rs27072, and 3′VNTR) of the *DAT1* gene were detected using modified polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) methods (Huang et al., 2007).

PCR was carried out in a total reaction mixture volume of 25 μl containing of 50 ng genomic DNA, 20 pmol of each primer, 2 mM of each dNTP, 1.5 mM $MgCl_2$, 50 mM Tris–HCl (PH 8.3 at 25 °C) and 1 U of Tag DNA polymerase (Life Technologies, Carlsbad, CA). The cycling protocols carried out on a Perkin Elmer 9700 (Boston, MA, USA) included an initial denaturation at 94 °C for 5 min, amplification (30 cycles of denaturation at 94 °C for 30 s, annealing at 60 °C for 30 s, extension at 72 °C for 30 s), and a final extension at 72 °C for 10 min. The PCR protocol depended on the polymorphism. Moreover, the PCR products of each polymorphism were digested with appropriate restriction enzymes (New England Biolabs, Ipswich, MA) for appropriate reaction times. To detect the alleles, the fragments were electrophoresed on 2–3% agarose gel (in most cases) and visualized by ethidium bromide staining under UV light. The MspI polymorphism (rs2652511) was detected on 12% polyacrylamide gels. The SNP position, primers' design, PCR products, restriction enzymes used, DNA variant, and allele sizes are listed in Table 1. For quality control, genotyping accuracy was confirmed by bidirectional direct sequencing of 50 random