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Association Study Between Schizophrenia and Dopamine D3 Receptor Gene Polymorphism

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Crocq et al. [1992: J Med Genet 29:858–860] reported the existence of an association between schizophrenia and homozygosity of a *BalI* polymorphism in the first exon of the dopamine D3 receptor (DRD3) gene. In response to this report, further studies were conducted; however, these studies yielded conflicting results. In the present study, we examined 100 unrelated Japanese schizophrenics and 100 normal controls to determine any association between this polymorphism and schizophrenia. Results suggest that neither allele nor genotype frequencies of the DRD3 gene in the schizophrenics as a whole are significantly different from those of the controls. Further, we found no association between any allele or genotype and any clinical subtype based on family history of schizophrenia and age-at-onset. A significantly high frequency of homozygosity of a dopamine D3 receptor gene allele was not observed in the schizophrenics as a whole, or in clinical subtypes. Our results suggest that an association between the dopamine D3 receptor gene and schizophrenia is unlikely to exist.

KEY WORDS: schizophrenia, genetics, association study, D3 dopamine receptor gene alleles

INTRODUCTION

Evidence from family, twin, and adoption studies suggests that there is an important genetic contribution to the etiology of schizophrenia [Gottesman and Shields, 1982; McGuffin, 1988]. The exact mode of inheritance, however, remains unknown.

Dopamine receptors have been thought to play a major etiological role in schizophrenia. The hypothesis of dopamine overactivity in schizophrenia is supported mainly by two lines of evidence. First, neuroleptic drugs block dopamine receptors in vivo [Andén et al., 1970] and in vitro [Seeman et al., 1974]. The clinical antipsychotic potencies of various neuroleptic agents are directly related to their binding potencies to dopamine receptors. Dopamine D3 receptor (DRD3) may play an important role in mediating antipsychotic drug action [Sokoloff et al., 1992]. Second, evidence for a dopaminergic basis of schizophrenia comes from the observation of exacerbating effects of dopamine-mimetic drugs in schizophrenics [Janowsky and Davis, 1976]; these drugs produce schizophrenia-like psychosis in normal individuals [Snyder, 1973]. An additional hypothesis regarding the etiology of schizophrenia involves a limbic brain system abnormality [Torrey and Peterson, 1974]. This region has been implicated in several emotional and cognitive behaviors. DRD3 messenger RNA is expressed almost exclusively in the limbic system [Sokoloff et al., 1990; Bouthenet et al., 1991]. Based on these findings, the DRD3 gene is an important candidate gene for schizophrenia, since it enters into the dopamine receptor hypothesis and limbic system hypothesis of schizophrenia.

The DRD3 gene was cloned [Sokoloff et al., 1990; Giros et al., 1990] and found to contain a polymorphic site in the first exon that gives rise to a glycine (allele 2) to serine (allele 1) substitution in the N-terminal extracellular domain. This results in the creation of a *Bal*I restriction endonuclease recognition site [Lanfelt et al., 1992]. Crocq et al. [1992] reported that significantly more patients than controls were homozygous for the *Bal*I polymorphism. In response to this report, additional studies were conducted. Results of linkage studies suggested that there is no major etiologic involvement of this gene in the pathogenesis of schizophrenia [Wiese et al., 1993; Sabate et al., 1994; Nanko et al., 1994]. On the other hand, results of association studies were conflicting [Nöthen et al., 1993; Jönsson et al., 1993; Nanko et al., 1993; Nimgaonkar et al., 1993; Yang et al., 1993; Sabate et al., 1994; Mant et al., 1994; DiBella et al., 1994; Kennedy et al., 1995]. Mant et al.

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[1994] and Kennedy et al. [1995] reported finding a positive association between schizophrenia as a whole and homozygosity of the DRD3 gene allele. Mant et al. [1994] also reported a significant excess of the 1:1 genotype (homozygote of allele 1) in schizophrenics as a whole in comparison to controls. Furthermore, the results of some studies suggest that an excess homozygosity occurs in those patients with a family history of schizophrenia [Nimgaonkar et al., 1993; Mant et al., 1994] or in those who respond well to neuroleptic treatment [Jönsson et al., 1993; Mant et al., 1994]. In the Japanese population, Nanko et al. [1993] reported obtaining negative results. Their subjects, however, included only outpatients, and patients with severe symptoms might not have been examined. In light of these findings, we investigated allele frequencies of the *Msc*I (isoschizomer of *Bal*I; Takara, Kyoto, Japan) polymorphism of the DRD3 gene in Japanese controls and schizophrenics, including inpatients, with regard to clinical subtypes, based on age-of-onset and family history of schizophrenia.

One hundred in- and outpatients (52 men and 48 women, 74 inpatients and 26 outpatients), age 15–70 years, fulfilling the DSM-III-R criteria for schizophrenia [American Psychiatric Association, 1987], were included in this study. Demographic and clinical information was collected by psychiatrists. Diagnostic assessment was performed by two psychiatrists and based on clinical interviews. Family history of schizophrenia was defined as follows: at least one first-degree relative had received treatment for schizophrenia according to hospital records, clinical interviews with patients, or information from the hospital staff. The early-onset group was defined as patients whose schizophrenia developed before the average age-of-onset (23 years) in our data sets. Our control group consisted of 100 subjects, including 44 university students, 25 hospital staff, and 31 elderly volunteers (71 men and 29 women in total), ranging in age from 22–86 years. One of our research team members interviewed the controls and confirmed that none were under psychiatric treatment. The mean (±SD) age of the patients was 44.2 (±12.0) years, and that of the controls was 42.7 (±23.7) years. All patients and controls were unrelated and from the Japanese population. The patients provided written informed consent prior to the study, according to the research protocol approved by the Ethics Committee of the Niigata University School of Medicine.

Analysis of the *Msc*I polymorphism of the DRD3 gene was performed as described by Crocq et al. [1992]. The polymorphism corresponding to the point mutation in the extracellular N-terminal part of the DRD3 protein resulting in the substitution of glycine (allele 2) for serine (allele 1) at position 9 was detected. Results of the examinations are shown in Table I. Neither allele nor genotype frequencies in the schizophrenics as a whole were significantly different from those of controls. The patients were then divided into two subgroups based on familial loading and age-of-onset. Neither allele nor genotype frequencies in each clinical subtype were significantly different from those of controls. A significantly high frequency of homozygotes was observed neither among the schizophrenics as a whole, nor when they were divided into the two subgroups, in comparison with controls. The genotypes of the patients and controls were in Hardy-Weinberg equilibrium (schizophrenics as a whole, $\chi^2 = 0.13$, $df = 1$, $P = 0.72$; patients with family history, $\chi^2 = 0.32$, $df = 1$, $P = 0.57$; early-onset patients, $\chi^2 = 0.51$, $df = 1$, $P = 0.48$; controls, $\chi^2 = 1.15$, $df = 1$, $P = 0.28$).

Our findings showed a lack of association between the DRD3 *Msc*I polymorphism and schizophrenia in the Japanese individuals examined. In response to the study by Crocq et al. [1992], some studies have been conducted, and almost all reports showed no association between schizophrenia as a whole and homozygosity of the *Bal*I polymorphism in the first exon of the DRD3 gene [Nöthen et al., 1993; Jönsson et al., 1993; Nanko et al., 1993; Nimgaoukar et al., 1993; Yang et al., 1993; Sabate et al., 1994]. On the other hand, Mant et al. [1994] and Kennedy et al. [1995] reported finding a positive association between schizophrenia as a whole and homozygosity of the DRD3 gene allele. Mant et al. [1994] also reported a significant excess of the 1:1 genotype in schizophrenics as a whole, in comparison to controls. The discrepancies between our results and those of Crocq et al. [1992] are, however, not likely due to the statistical power of our study. To detect an excess homozygosity (1:1 genotype + 2:2 genotype) in the schizophrenics as a whole, our sample size (100 patients and 100 controls) adequately had a power of 90% [Cohen, 1988], with α set at 0.05 (two-tailed significance, $P < 0.050$. This power was estimated using the same Cardiff samples used in the study by Crocq et al. [1992]. To examine the excess homozygosity of the 1:1 genotype

TABLE I. Allele Frequencies and Genotype Counts for DRD3 Gene *Msc*I Polymorphism in Patients and Controls*

		Allele frequencies		Genotype counts			
	N	1	2	1-1	1-2	2-2	(1-1) + (2-2)
Controls	100	0.74	0.26	52 (54.8)	43 (38.5)	5 (6.8)	57
Schizophrenics							
As a whole	100	0.73	0.27	54 (53.3)	38 (39.4)	8 (7.3)	62
With family history	43	0.69	0.31	21 (20.5)	17 (18.4)	5 (4.1)	26
Age-at-onset <23 years	47	0.74	0.26	27 (25.7)	16 (18.1)	4 (3.2)	31

* Parentheses indicate H-W expected; nos. with each genotype expected according to Hardy-Weinberg equilibrium.

in the schizophrenics as a whole, our sample size had a power of >80%, which was also adequate to the examination. Other possible reasons for these discrepancies include chance variation in allele frequencies, heterogeneity among patient groups, and the effect of racial stratification among subjects. Though the causes of these discrepancies are unclear, results of the present study confirmed a lack of association between the DRD3 polymorphism and schizophrenia in the Japanese individuals examined.

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