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Brief Research Communication

Lack of Association Between Dopamine D2 Receptor Gene Cys311 Variant and Schizophrenia

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Itokawa et al. [1993] reported identifying one missense nucleotide mutation from C to G resulting in a substitution of serine with cysteine at codon 311 in the third intracellular loop of the dopamine D2 receptor in schizophrenics. Arinami et al. [1994] reported finding a positive association between the Cys311 variant and schizophrenia. In response to the report by Arinami et al. [1994] we examined 106 unrelated Japanese schizophrenics and 106 normal controls to determine if there is any association of the Cys311 variant with schizophrenia. However, we found no statistically significant differences in allelic frequencies of Cys311 between schizophrenia and normal controls. The present results as well as those of all previous studies except for that of Arinami et al. [1994] indicated that an association between the dopamine D2 receptor gene and schizophrenia is unlikely to exist.

KEY WORDS: schizophrenia in the Japanese population, dopamine D2 receptor Cys311 variant

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 unclear.

Numerous studies have suggested that disturbances in dopaminergic transmission are involved in the etiology of schizophrenia. Much of the evidence for the hypothesis of dopamine (DA) overactivity in schizophrenia is based on the fact that neuroleptic drugs block DA 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 affinities for binding to DA receptors, especially to the DAD2 receptor [Seeman et al., 1976]. Additional evidence for a dopaminergic basis of schizophrenia was provided by the observation of exacerbating effects of DA-mimetic drugs [Janowsky et al., 1976], producing schizophrenia-like psychosis in normal individuals [Snyder, 1973], and the elevation of DAD2 receptors in the postmortem brains of schizophrenic patients [Lee et al., 1978].

Based on the above findings, the DAD2 receptor gene has been suggested as a candidate gene for schizophrenia. Previous linkage studies revealed no significant correlation between the DAD2 receptor gene and schizophrenia [Moises et al., 1991; Gill et al., 1993; Coon et al., 1993]. Itokawa et al. [1993] reported identifying one missense nucleotide mutation from C to G resulting in a substitution of serine with cysteine at codon 311 in the third intracellular loop of the DAD2 receptor in schizophrenics. Arinami et al. [1994] reported observing a positive association between the Cys311 variant and schizophrenia. They reported that the allele frequency of Cys311 was significantly higher in patients with onset before age 25, and in those with a family history of schizophrenia than in the controls. They also reported that the patients with the Cys311 allele showed significantly less severe thought disorder and negative symptoms of schizophrenia than those without the Cys311 allele. At the same time, a lack of association between the Cys311 allele and schizophrenia, determined based on mutational analysis of the complete coding sequences of the DAD2 receptor gene in 106 unrelated schizophrenics, was reported [Gejman et al., 1994].

In response to the report by Arinami et al. [1994], several studies were performed in some countries other than Japan [Asherson et al., 1994; Shaikh et al., 1994; Nöthen et al., 1994; Sobell et al., 1994; Laurent et al., 1994]; however, in none of these was the association reported by Arinami et al. [1994] observed. In the Japanese population, there are two reports [Nanko et al., 1994; Hattori et al., 1994] in response to the study of Arinami et al. [1994] and both presented negative results. We referred only to the data of the latter study in

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the Japanese population in response to the data of Arinami et al. [1994] because these two reports were performed in the same institute and the latter was more reliable than the former in molecular biological techniques [Hattori et al., 1994]. The data of Hattori et al. [1994] could not reject the positive association because the effect of the Cys311 allele reported by Arinami et al. [1994] was small and Hattori et al.'s [1994] study only had a power of about 65% to detect an effect of this size [Cohen, 1977]. Against this background, we examined 106 unrelated Japanese schizophrenics and 106 normal controls, and analyzed our data combining those of Hattori et al.'s [1994] report to determine if there is any association of the Cys311 variant with schizophrenia in the Japanese population.

One hundred and six in- and outpatients (55 men and 51 women) aged 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 experienced psychiatrists and was based on clinical interviews. Family history of schizophrenia was defined as follows: at least one first-degree or second-degree relative had received treatment for schizophrenia according to hospital records, clinical interviews with patients, or information from the hospital staff. The Manchester scale [Krawiecka et al., 1977] was used to assess the severity of the symptoms. Our control group consisted of 106 university students, hospital staff, and former volunteers (63 men and 43 women) aged 22–86 years. There was no evidence that the controls were suffering from psychiatric diseases. The mean (±S.D.) age of the patients was 44.7 (±11.8) years, and that of the controls was 45.8 (±25.0) years. All patients and controls were unrelated and from the Japanese population. The patients provided written informed consent prior to the study.

The Ser311 → Cys variant was typed with the polymerase chain reaction assay method of Arinami et al. [1994]. The products were digested with 15 U of *Cfr* 13 I (isoschizomer of *Asu* I; Takara, Kyoto, Japan) and then electrophoresed through agarose gel (3.0% NuSieve agarose [FMC Bioproducts, Rockland, ME]).

The allele and genotype frequencies were compared for controls versus schizophrenics as well as clinical subtypes with regard to the age at onset, familial loading, and severity of symptoms as assessed with the Manchester scale using a chi-square test, Fisher's exact probability method and Student's t-test.

Figure 1 shows results of polymerase chain reaction restriction fragment length polymorphism analysis of exon 3 of the DAD2 receptor gene. We detected two genotypes, a heterozygous one for the Cys311 allele and a homozygous one for the Ser311 allele. No individuals with homozygous genotype for the Cys311 allele were found among either schizophrenics or controls. Genotypic and allelic distributions of schizophrenics and controls are shown in Table I. Neither allele ($\chi^2 = .06$, d.f. = 1, $P = .79$) nor genotype ($\chi^2 = .06$, d.f. = 1, $P = .79$) frequencies in schizophrenics were significantly different from those of controls. The genotypes of the patients and controls were in Hardy-Weinberg equilibrium (patients, $\chi^2 = .25$, controls, $\chi^2 = .16$, d.f. = 1, $P > .05$). Then the patients were divided into three groups based on familial loading, age at onset, and severity of symp-

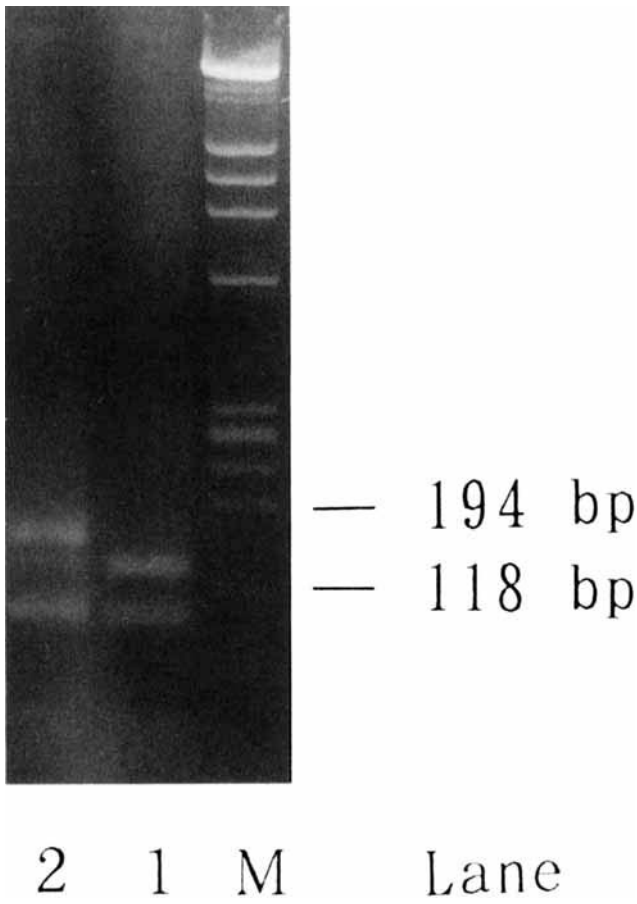

Fig. 1. Direct analysis of Polymerase chain reaction PCR product in dopamine D2 receptor Cys311 variant. **Lane 1:** Cysteine 311/serine 311. **Lane 2:** Serine 311/serine 311. Lane M: λ/*Hind* III-ϕX174/*Hae* III size standard.

TABLE I. Dopamine D2 Receptor Genotype and Allele Frequencies for Ser311-Cys31 Variance

	Genotype			
	Ser311/Ser311[a]	Ser311/Cys311[b]	Cys311/Cys311	Allele frequency of Cys311
Control (n = 106)	98	8	0	0.038
Schizophrenia (n = 106)	97	9	0	0.042

[a]Ser311: Serine 311.
[b]Cys311: Cysteine 311.

toms. Neither allele nor genotype frequencies in each clinical subtype were significantly different from those of controls (data not shown). The present results as well as all previous studies except for that of Arinami et al. [1994] indicate that the association between the DAD2 receptor gene and schizophrenia is unlikely to exist and that the Cys311 variant is not associated with clinical features of schizophrenia.

The following is one possible explanation for the apparent discrepancy between our results and those of Arinami et al. [1994]. As mentioned above, the effect of the Cys311 allele reported by Arinami et al. [1994] was small and our study only had a power of about 65% to detect an effect of this size [Cohen, 1977]. Hattori et al.'s [1994] study had almost the same power as ours. By combining our results with those of Hattori et al. [1994], which also suggested a lack of association between schizophrenia and the Cys311 allele in the Japanese population, the statistical power was increased to almost 90%. Of 206 controls, 14 were heterozygous and 1 was homozygous for the Cys311 allele (allele frequency = .039) based on the above-mentioned combined results, whereas of 300 controls, 11 were heterozygous and none were homozygous for the same allele (allele frequency = .018) in the samples in Arinami et al. 's [1994] study. There is a significantly higher Cys311 allele frequency in the controls of the former two studies than in those of Arinami et al.'s [1994] study ($\chi^2 = 3.95$, d.f. = 1, $P = .04$). On the other hand, of the 206 patients in the former two studies, 16 were heterozygous and none were homozygous for the Cys311 allele (allele frequency = .039); in contrast to the 156 patients in Arinami et al.'s [1994] study, 11 were heterozygous and 3 were homozygous for the same allele (allele frequency = .054) in the report of Arinami et al. [1994] No significant differences in Cys311 allele frequencies were observed between the patients in the former 2 studies and those in Arinami et al.'s [1994] study ($\chi^2 = 1.0$, d.f. = 1, $P = .32$). In sampling the controls from the Japanese population, we found that data obtained in Arinami et al.'s [1994] study were affected by a different bias from that exhibited by our study and that of Hattori et al. [1994] This unknown bias may account for the discrepancy among the results of studies on Japanese populations. Combining the data of these three studies in the Japanese population, we found that 27 of the 362 patients were heterozygous for the Cys311 allele and only 3, who were reported by Arinami et al. [1994], were homozygous for it (allele frequency = .046); whereas 25 of the 506 controls were heterozygous and 1 was homozygous for it (allele frequency = .027). Significant differences in the Cys311 allele frequencies were observed between patients and controls ($\chi^2 = 4.52$, d.f. = 1, $P = .03$), although results of our study and those of Hattori et al. [1994] suggested a lack of association between the Cys311 allele and schizophrenia. This discrepancy is presumably due to the heterogeneity of controls in each study as mentioned above.

On the other hand, combining all the data obtained in several studies involving patients from several countries, including data obtained in our study, of 1,359 patients, 60 were heterozygous for the Cys311 allele and only 3, included among the patients of Arinami et al. [1994], were homozygous for it (allele frequency = .024), whereas of 2,914 controls, 103 were heterozygous and 2, included among the patients of Sobell et al. [1994] and Hattori et al. [1994], were homozygous for it (allele frequency = .019). No significant differences in Cys311 allele frequencies were observed between the patients and controls ($\chi^2 = 2.88$, d.f. = 1, $P = .09$). These results suggest that only in Japanese, particularly in those included in Arinami et al.'s [1994] study, does a positive association exist between schizophrenia and the Cys311 variant. However, this association was not observed in the combined data of 2 other studies of Japanese subjects, including ours. Further studies, including correctly assessed Japanese controls, are needed to confirm this association.

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