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Dopamine Transporter Gene Polymorphism and Psychiatric Symptoms Seen in Schizophrenic Patients at Their First Episode

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To investigate the possible role of the dopamine transporter (DAT) gene in determining the phenotype in human subjects, allele frequencies for the 40-bp variable number of tandem repeats (VNTR) polymorphism at this site were compared between 117 Japanese normal controls and 118 schizophrenic patients, including six subgroups: early-onset, those with a family history, and those suffering from one of the following psychiatric symptoms at their first episode: delusion and hallucination; disorganization; bizarre behavior; and negative symptoms. No significant differences were observed between the group as a whole or any subgroup of schizophrenic patients and controls. The results indicate that VNTR polymorphism in the DAT gene is unlikely to be a major contributor to any of the psychiatric parameters examined in the present population of schizophrenic subjects. © 1996 Wiley-Liss, Inc.

KEY WORDS: negative symptoms, disorganization, bizarre behavior, delusion, hallucination

INTRODUCTION

Evidence obtained from clinical psychopharmacological studies of schizophrenic patients indicates that the central dopaminergic systems are involved in the pathophysiology of this disease. In addition, genetic factors have also been suggested in the etiology of schizophrenia based on family, twin, and adoption studies. Based on these facts, variations found in the gene loci related to central dopaminergic transmission have been extensively examined as candidate loci for this disease. Unfortunately, the genetic variations examined previously did not consistently show a positive association with schizophrenia.

The 40-bp variable number of tandem repeats (VNTR) polymorphism found in the 3′ untranslated region of the human dopamine transporter (DAT) gene [Vandenbergh et al., 1992; Sano et al., 1993] has also been investigated as one of these genetic variations. The main role of the dopamine transporter site at the synaptic cleft is thought to be regulation of extracellular catecholamine levels through uptake of released catecholamine. Psychostimulants, such as cocaine and methamphetamine, are known to block this site and increase the levels of synaptic dopamine [Inada et al., 1992; Iyo et al., 1995]. Recently, a positive association was reported between DAT genotype and cocaine-induced paranoia in white populations [Gelernter et al., 1994]. However, the exact effect of VNTR on DAT function remains to be elucidated.

We have been studying the roles of variations found in the gene loci related to central dopaminergic transmission under the hypothesis that these roles are not directly associated with the disease itself, but possibly with the more subtle variables related to psychotic conditions, such as a paranoid state. In the present study, the allele frequencies of the 40-bp VNTR polymorphism in the DAT gene were examined in a whole group and in subgroups of schizophrenic patients showing particular characteristics, in comparison with control subjects, to determine the possible role of the polymorphism at this site, and in particular to examine whether the allelic association is also evident in schizophrenic patients showing a paranoid state at their first episode.

SUBJECTS AND METHODS

Ethical Considerations

The present research was initiated after receiving approval from the Ethical Committee of the Kohnodai

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area, National Center of Neurology and Psychiatry, Chiba, Japan. Written informed consent was obtained from all participating patients.

Subjects

Schizophrenic subjects, who were diagnosed according to the criteria of DSM-III-R, were recruited from seven psychiatric facilities located around the Tokyo area. Control subjects were mostly medical staff with no history of psychosis, who volunteered to participate in the study. All subjects were of Japanese descent.

Psychiatric Parameters

Initially, schizophrenic patients were divided into four subgroups according to the presence or absence of the following particular psychiatric symptoms during their first episode: 1) delusion and hallucination, 2) disorganization, 3) bizarre behavior, and 4) negative symptoms. This classification was based on the report of Peralta et al. [1992]. Either presence or absence of these individual symptoms was determined basically from the description of clinical records, referring to the scale for assessment of negative symptoms (SANS) [Andreasen, 1982] and of positive symptoms (SAPS) [Andreasen, 1984]. In addition to these symptomatic parameters, two other subgroups were defined: patients whose age at onset was 21 years or younger were identified as early-onset patients, and those who had a first-degree relative with some form of psychosis were identified as patients with a family history.

Genetic Procedure

Genomic DNA was extracted from leukocytes in venous blood samples. The target segment was amplified by the polymerase chain reaction (PCR) method, using the primers described by Sano et al. [1993], in a total volume of 100 μl containing 200 ng of genomic DNA, 1.0 μM each primer, 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 2.0 mM $MgCl_2$, 200 μM each of dATP, dGTP, dTTC, and dCTP, and 2.5 U of Taq polymerase. PCR involved initial denaturation at 94°C for 5 min, followed by 30 cycles of 94°C for 30 sec and 71°C for 3 min, with a final polymerization at 72°C for 5 min. PCR products were fractionated in 2% agarose gel, stained with ethidium bromide, and visualized under ultraviolet light.

Statistics

Comparisons of allele and genotype frequencies were performed using the chi-square test. $P < 0.05$ was considered significant.

RESULTS

A total of 118 schizophrenic patients (54 males and 64 females) and 117 normal controls (55 males and 62 females) participated. Mean age was 59 years (range, 22–86 years) for schizophrenic patients and 46 years (range, 20–80 years) for controls. Six genotypes and five different-sized alleles were observed among the total samples (Fig. 1). Allele frequencies for the whole group or subgroups of schizophrenic patients and normal controls are shown in Table I. No significant differences in allele frequencies were observed between the whole group or any of the subgroups of schizophrenic patients and controls.

DISCUSSION

Consistent with previous reports [Byerley et al., 1993; Li et al., 1993; Persico et al., 1995], the present results did not show any allelic association between the 40-bp VNTR polymorphism at DAT and schizophrenic patients. Moreover, no allelic association with any of the psychiatric parameters examined in the present study was detected, indicating that the 40-bp VNTR polymorphism at DAT is unlikely to be a major contributor to any of the psychiatric parameters examined in our schizophrenic subjects.

Because this VNTR polymorphic site lies outside the translated region of the DAT gene, no clear molecular-biological function can be ascribed to it. In addition, VNTR polymorphism has been reported to differ widely among normal control subjects of different races, as shown in Table II. Therefore, it may be rather difficult to interpret the results properly, even if positive find-

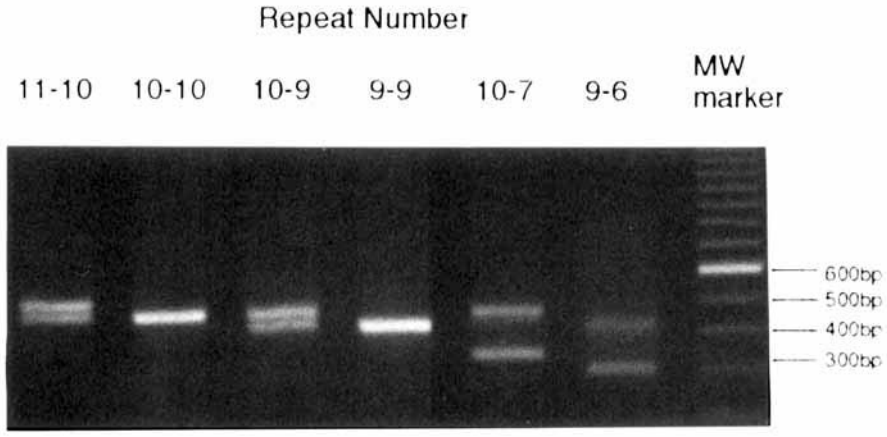

Fig. 1. PCR analysis of dopamine transporter (DAT) gene repeat polymorphism. The six genotypes (11/10, 10/10, 10/9, 9/9, 10/7, and 9/6) and the five size alleles (11, 10, 9, 7, and 6) are shown.

TABLE I. Allele Frequencies of Repeat Variation in Dopamine Transporter Gene*

	Repeat number				
	6	7	9	10	11
Controls (n = 117)	0.0% (0)	0.4% (1)	7.7% (18)	90.2% (211)	1.7% (4)
Schizophrenia (n = 118)	0.4% (1)	2.1% (5)	3.4% (8)	93.6% (221)	0.4% (1)
Delusions and hallucinations (n = 61)	0.0% (0)	4.1% (5)	3.3% (4)	91.8% (112)	0.8% (1)
Bizarre behavior (n = 70)	0.0% (0)	2.9% (4)	3.6% (5)	92.9% (130)	0.7% (1)
Disorganization (n = 47)	0.0% (0)	5.3% (5)	2.1% (2)	91.5% (86)	1.1% (1)
Negative symptoms (n = 67)	0.0% (0)	3.0% (4)	3.0% (4)	93.3% (125)	0.7% (1)
Onset at age 21 years or younger (n = 49)	0.0% (0)	2.0% (2)	5.1% (5)	91.8% (90)	1.0% (1)
With a family history (n = 23)	0.0% (0)	2.2% (1)	4.3% (2)	93.5% (43)	0.0% (0)

* Values are expressed as percentages. Numbers of alleles observed are in parentheses.

TABLE II. Previous Reports of Dopamine Transporter Gene VNTR Frequencies in Control Subjects*

	Repeat number				
References	<9	9	10	>10	Origin of subjects
Vandenbergh et al. [1992] (n = 129)	4.7% (12)	23.6% (61)	70.2% (181)	1.6% (4)	USA (black and white)
Persico et al. [1993] (n = 48)	0.0% (0)	28.1% (27)	71.9% (69)	0.0% (0)	USA
Sano et al. [1993] (n = 107)	0.9% (2)	4.2% (9)	93.0% (199)	1.9% (4)	Japanese
Li et al. [1993] (n = 98)	0.5% (1)	5.6% (11)	92.9% (182)	1.0% (2)	Chinese
Gelernter et al. [1994] (n = 103)	0.9% (1)	27.6% (32)	70.7% (82)	0.9% (1)	USA (white)
	5.6% (5)	17.8% (16)	64.4% (68)	1.1% (1)	USA (black)

* Values are expressed as percentages. Numbers of alleles observed are in parentheses.

ings are detected. However, absence of association between normal controls and schizophrenic subjects with a delusional hallucinatory state at their first episode could be taken as circumstantial evidence that the etiology of the paranoid state seen in schizophrenic patients is different from that induced by cocaine abuse.

Considering the close relationship between disturbance of central dopaminergic transmission and the development of psychiatric symptoms, the possibility of an association with other kinds of psychiatric parameters cannot be ruled out. The criteria used to define psychiatric parameters may be one of the most important factors for clarifying the exact role of the polymorphic sites found at gene loci related to central dopaminergic transmission.

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REFERENCES

Andreasen NC (1982): Negative symptoms in schizophrenia: definition and reliability. Arch Gen Psychiatry 39:784–788.

Andreasen NC (1984): Scale for the assessment of positive symptoms (SAPS). Iowa City, IA: University of Iowa.

Byerley W, Coon H, Hoff M, Holik J, Waldo M, Freedman R, Caron MG, Giros B (1993): Human dopamine transporter gene not linked to schizophrenia in multigenerational pedigrees. Hum Hered 43:319–322.

Gelernter J, Kranzler HR, Satel SL, Rao PA (1994): Genetic association between dopamine transporter protein alleles and cocaine-induced paranoia. Neuropsychopharmacology 11:195–200.

Inada T, Polk K, Purser C, Hume A, Hoskins B, Ho IK, Rockhold RW (1992): Behavioral and neurochemical effects of continuous infusion of cocaine in rats. Neuropharmacology 31:701–708.

Iyo M, Maeda Y, Inada T, Kitao Y, Sasaki H, Fukui S (1995): The effects of a selective cAMP phosphodiesterase inhibitor, rolipram, on methamphetamine-induced behavior. Neuropsychopharmacology 13:33–39.

Li T, Yang L, Wiese C, Xu CT, Zeng Z, Giros B, Caron MG, Moises HW, Liu X (1993): No association between alleles or genotypes at the dopamine transporter gene and schizophrenia. Psychiatry Res 52: 17–23.

Peralta V, de Leon J, Cuesta MJ (1992): Are there more than two syndromes in schizophrenia? A critique of the positive-negative dichotomy. Br J Psychiatry 161:335–343.

Persico AM, Vandenbergh DJ, Smith SS, Uhl GR (1993): Dopamine transporter gene polymorphisms are not associated with polysubstance abuse. Biol Psychiatry 34:265–267.

Persico AM, Wang ZW, Black DW, Andreasen NC, Uhl GR, Crowe RR (1995): Exclusion of close linkage of the dopamine transporter gene with schizophrenia spectrum disorders. Am J Psychiatry 152: 134–136.

Sano A, Kondoh K, Kakimoto Y, Kondo I (1993): A 40-nucleotide repeat polymorphism in the human dopamine transporter gene. Hum Genet 91:405–406.

Vandenbergh DJ, Persico AM, Hawkins AL, Griffin CA, Li X, Jabs EW, Uhl GR (1992): Human dopamine transporter gene (DAT1) maps to chromosome 5p15.3 and displays a VNTR. Genomics 14: 1104–1106.