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Dopamine D4 Receptor Variants in Chinese Sporadic and Familial Schizophrenics

Chen-Jee Hong,[1,3] Yi-Ling Lee,[2] Cho-Boon Sim,[1] and Hai-Gwo Hwu[2]*

[1]*Department of Psychiatry, Veterans General Hospital–Taipei, Taipei, Taiwan*
[2]*Department of Psychiatry, National Taiwan University Hospital, Taipei, Taiwan*
[3]*College of Medicine, National Yang-Ming University, Taipei, Taiwan*

Variation in the number of tandem repeats of a 48 base pair (bp) unit was found in the gene of the dopamine D4 receptor (DRD4). The number of repetitions of the 48bp unit was shown to influence the binding of clozapine, which suggests that different alleles may function differently in vivo and affect the pathogenesis of schizophrenia. Genotypes of DRD4 polymorphism were analyzed for 47 schizophrenic probands who had at least one living sibling with a diagnosis of schizophrenia, 35 unaffected siblings of the schizophrenic proband, 42 sporadic schizophrenic patients, and 43 healthy controls without a family history of psychosis. There was no significant difference in genotypic or allelic distributions among the four groups. Significant differences in the frequencies of two- and seven-repeats alleles between the Chinese and Caucasians controls were noted. The present study did not support that a particular allele or genotype of the 48bp-repeat of DRD4 was associated with schizophrenia. Am. J. Med. Genet. 74: 412–415, 1997. © 1997 Wiley-Liss, Inc.

KEY WORDS: dopamine D4 receptor; familial schizophrenia; sporadic schizophrenia

INTRODUCTION

Disturbances in dopamine neurotransmission and dopamine receptors have long been postulated to underlie schizophrenia [Carlsson et al., 1988]. Among the different dopamine receptors, the dopamine D4 receptor is of particular interest in schizophrenia because of its high affinity for the atypical neuroleptic clozapine [Van Tol et al., 1991]. The dopamine D4 receptor, which closely resembles the dopamine D2 and D3 receptors, belongs to a family of G-protein coupled receptors and provides a substrate to bind antipsychotics. A polymorphism consisting of variation in the number of tandem repeats of a 16 amino acid (equivalent to 48 base pairs (bp) of nucleotide sequence) unit was found in the third cytoplasmic loop of the dopamine D4 receptor. The number of repeats influenced the binding of clozapine: variants with two or four repeats had lower dissociation constants in the absence of sodium chloride than variants with seven repeats [Van Tol et al., 1992]. The apparent functional difference in the ability of the various alleles to bind neuroleptics suggests that the alleles may function differently in vivo and affect the pathogenesis of schizophrenia. Sommer et al. [1993] investigated the distribution of alleles in a large group of Caucasian schizophrenic cases and controls and found a trend toward a greater prevalence of homozygotes for the 4-repeat allele in schizophrenics, which was not supported by the study of Daniels et al. [1994].

This association study compares the allelic distribution of the 48bp repeat of dopamine D4 receptor gene (DRD4) between Chinese schizophrenic patients and controls. Because schizophrenia is considered etiologically heterogeneous [Lander, 1988], and there have been a number of studies [reviewed by Murray et al., 1985] reporting differences between familial and sporadic schizophrenia, this study further divided schizophrenic patients into familial and sporadic subgroups in order to reduce heterogeneity. The results of this study and that of Daniels et al. [1994] will also be compared to see the difference in allelic distribution of DRD4 between the Chinese and Caucasians.

METHOD

Patients

The subjects included in this study were 47 schizophrenic patients (probands) who had at least one living sibling with a diagnosis of schizophrenia, 35 unaffected

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*Correspondence to: Dr. Hai-Gwo Hwu, Department of Psychiatry, National Taiwan University Hospital, 7 Chung-Shan South Rd., Taipei, Taiwan 100, R.O.C. E-mail: haigohwu@ntumc1.mc.ntu.edu.tw

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TABLE I. Demographic Characteristics

Characteristics	Normal controls	Sporadic schizophrenics (%)	Sib-paired schizophrenics: Probands (%)	Sib-paired schizophrenics: Normal sibs (%)
Sample size	42	42	47	35
Gender:				
Male	26	21	32	20
Female	16	21	15	15
Age	28.7 ± 4.4	30.8 ± 8.0	34.0 ± 8.1	34.1 ± 9.0

The mean age of normal controls was not significantly different from that of the other three groups ($P > .05$). Gender distributions were not significant in different groups ($\chi^2 = 3.19.1$, df = 3, $P = 0.363$).

siblings of the schizophrenic probands, 42 sporadic schizophrenic patients, and 43 healthy controls without a family history of psychosis. Most of the controls were recruited from the hospital staff. All study subjects were personally interviewed by the authors using the psychiatrist diagnostic assessment schedule, which is a semi-structured interview designed to be performed by psychiatrists [Hwu and Yang, 1987; Hwu, 1991]. The medical charts of the study subjects were reviewed and these data were used for diagnostic assignment according to DSM-III-R schizophrenic criteria [American Psychiatric Association, 1987].

Using the same diagnostic instruments, all available first-degree relatives were assessed by the family study method. Unavailable relatives were diagnosed using the family history method [Andreasen, 1977]. All patients and control subjects were Chinese and had parents who were natives of northern Taiwan.

Laboratory Procedures

Genomic DNA was isolated from lymphocytes and analyzed by PCR with oligonucleotide primers according to Shaikh et al. [1993]. Polymerase chain reaction (PCR) was carried out in a 25 μl mixture per reaction, which contains 50 mM KCl, 10 mM tris-HCl pH 8.3, 0.5 mM MgCl2, 10% dimethylsulfoxide (DMSO), 200 μM each of dATP, dTTP, and dCTP, 50 μM of dGTP, and 150 μM of 7-deaza-guanosine, 0.5 μM of each primer, 100 ng template DNA, and 0.6 units of Dynazyme. The mixture was denatured at 95°C for 5 min, followed by 30 cycles of amplification (94°C, 1 min; 52°C, 1 min; 72°C, 2 min) and 5 min of elongation at 72°C. PCR products were detected by ethidium bromide-stained 2% agarose gel electrophoresis and UV-photography.

Statistical Analysis

Allele frequencies were estimated by counting alleles and calculating sample proportions. Comparisons of genotype frequencies and allele frequencies were made using the Chi-square test. The two-tailed Student's t-test was used to compare quantitative data. Allelic distributions in the Chinese and Caucasian controls were compared with the present data and the data of Daniels et al. [1994]. Statistic power was calculated with significance level = < 0.05 and a presumed odds ratio of 4.0. Computer software of Epi Info Version 5.0 [Dean et al., 1990] was used for the calculations.

RESULTS

The demographic characteristics of the subjects are presented in Table I. The mean age of the controls was not significantly different from that of the other three groups ($P > 0.05$). Gender distribution within the four groups was also not significantly different (df = 3, $P = 0.363$). Allele assignment of the polymorphism was made according to the number of 48bp repeats. The distribution of DRD4 genotypes in each group is given in Table II. The allelic frequencies in each group is shown in Table III. There was no significant difference in genotypic or allelic distributions among the four groups; the results did not change when males and females were examined separately (Table IV). There were significant differences in the frequencies of two- ($P = 0.0045$) and seven-repeat ($P < 0.0001$) alleles between the Chinese and Caucasian controls (Table V).

DISCUSSION

The results of this study show no evidence of an association between schizophrenia and repeat length variation of DRD4, which is concordant with other similar studies [Sommer et al., 1993; Daniels et al., 1994]. Although various alleles have been demonstrated to have differential neuroleptic binding properties in cell culture [Von Tol et al., 1992], the present

TABLE II. Genotypic Distribution of DRD4 48bp-Repeat in Controls, Sporadic Schizophrenics, and Familial Schizophrenics

Genotype of 48bp-repeats	Normal controls (%)	Sporadic schizophrenics (%)	Sib-paired schizophrenics: Probands (%)	Sib-paired schizophrenics: Normal sibs (%)
2/2	2 (4.8)	4 (9.5)	1 (2.1)	0 (0.0)
2/3	0 (0.0)	0 (0.0)	1 (21.)	0 (0.0)
2/4	16 (38.1)	13 (31.0)	17 (36.2)	18 (51.4)
2/5	0 (0.0)	0 (0.0)	1 (2.1)	0 (0.0)
2/8	1 (2.4)	0 (0.0)	0 (0.0)	0 (0.0)
3/4	1 (2.4)	1 (2.4)	0 (0.0)	1 (2.9)
4/4	17 (40.5)	20 (47.6)	26 (55.3)	14 (40.0)
4/5	1 (2.4)	3 (7.1)	0 (0.0)	0 (0.0)
4/6	4 (9.5)	1 (2.4)	1 (2.1)	1 (2.9)
4/7	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.9)

$\chi^2 = 31.1$, df = 27, $P = 0.268$ for comparison of genotype distribution in these four groups.

TABLE III. Allelic Distribution of DRD4 48bp-Repeats in Controls, Sporadic Schizophrenics, and Probands and Normal Sibs of Familial Schizophrenics

Number of 48bp-repeats	Normal controls (%)	Sporadic schizophrenics (%)	Sib-paired schizophrenics	
			Probands (%)	Normal sibs (%)
2	21 (25.0)	21 (25.0)	21 (22.3)	18 (25.7)
3	1 (1.2)	1 (1.2)	1 (1.1)	1 (1.4)
4	56 (66.7)	58 (69.0)	70 (74.5)	49 (70.0)
5	1 (1.2)	3 (3.6)	1 (1.1)	0 (0.0)
6	4 (4.8)	1 (1.2)	1 (1.1)	1 (1.4)
7	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.4)
8	1 (1.2)	0 (0.0)	0 (0.0)	0 (0.0)
Total alleles	84 (100)	84 (100)	94 (100)	70 (100)

$\chi^2 = 14.8$, df = 18, $P = 0.677$ for comparison of allelic distribution in these four groups.

data do not substantiate the hypothesis that particular alleles may be associated with the occurrence of schizophrenia.

Because no single genetic model can readily accommodate all of the empirical evidence from family, twin, and adoption studies, and findings have pointed to etiological heterogeneity in schizophrenia [Lander, 1988], this study sub-grouped schizophrenic patients into familial and sporadic groups to reduce heterogeneity. Those with a positive family history are classified as "familial" and are considered more likely to have the genetic form of the illness. Those with a negative family history are classified as "sporadic" and considered more likely to have an environmental form of the illness. Although the distinction between familial and sporadic schizophrenia is by no means definitively demonstrated, there have been a number of studies reporting differences between familial and sporadic schizophrenia. Familial schizophrenia has been associated with poor performance on the Continuous Performance Task [Orzack and Kornetsky, 1971; Walker and Sheye, 1982] and other attention tasks [Asarnow et al., 1978]. Sporadic schizophrenia has been associated with a higher prevalence of EEG abnormalities [Hays, 1977; Kendler and Hays, 1982] in comparison to familial schizophrenia. These findings are consistent with those of research on relatives of schizophrenics. Murray et al. [1985] reviewed 11 studies and suggested that dividing schizophrenia crudely into familial and sporadic cases was a useful first step, because there was an inverse relationship between cerebral pathology (primarily CT scan assessed ventricular brain ratio) and familial schizophrenia. The familial/sporadic distinction could refer to a situation where either 1) there are two distinct and separate "causes" of schizophrenia, or 2) there is a continuum of genetic/environmental contribution to the etiology.

In addition to no true difference, there are two possibilities that may obscure the distinction between familial and sporadic schizophrenia on the allelic distribution of 48bp repeat of DRD4. The first possibility is misclassification. The definition of familial schizophrenia in this study is based on the presence of schizophrenia in first-degree relatives; however, it would be premature to rigidly and narrowly define what an "affected" relative is. The definition of "affected" could be expanded to include putative schizophrenic spectrum disorders or non-clinical phenotypes. For example, individuals were considered to be "affected" if they had schizophrenia or abnormal smooth eye pursuit movement [Holzman et al., 1988]. Familial cases might be erroneously classified as sporadic cases if the family size was too small, and sporadic cases might be classified as familial cases if the family members were affected by common non-genetic factors. Although there is a possibility that the relatives were too young to have developed the disorder, which might be the case in the controls (mean age 28.7 ± 4.4 years) of this study, it is unlikely that more than one control will develop the illness, given a lifetime morbidity risk of approximately 1% [Slater and Cowie, 1971]. Thus, such a misclassification should not have a significant effect on this study.

The second possibility is inadequate statistical power [Lyons et al., 1989]. The sample size in this study would allow detection of a fourfold increased risk of schizophrenia associated with the 4/4 genotype with 80% power. If a true association exists but only very slightly increased risk (i.e., an odds ratio less than 4.0), then this may have been missed.

Comparing the present data and the results of Daniels et al. [1994], we found significant differences in allelic and phenotypic distributions between the Chi-

TABLE IV. Allelic Distribution of DRD4 48bp-Repeat in Different Sex of Controls, Sporadic Schizophrenics, Probands and Normal Sibs of Familial Schizophrenics

Number of 48bp-repeats	Controls		Sporadic schizophrenics		Probands		Normal sibs	
	M (%)	F (%)	M (%)	F (%)	M (%)	F (%)	M (%)	F (%)
2	14 (27)	7 (22)	8 (19)	13 (31)	15 (23)	6 (20)	11 (28)	7 (23)
3	1 (2)	0 (0)	1 (2)	0 (0)	0 (0)	1 (3)	0 (0)	1 (3)
4	33 (64)	23 (72)	31 (74)	27 (64)	47 (73)	23 (77)	28 (70)	21 (70)
5	0 (0)	1 (3)	1 (2)	2 (5)	1 (2)	0 (0)	0 (0)	0 (0)
6	4 (8)	0 (0)	1 (2)	0 (0)	1 (2)	0 (0)	1 (3)	0 (0)
7	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (3)
8	0 (0)	1 (3)	0 ()0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Total	32	32	42	42	64	30	40	30

$\chi^2 = 9.01$, df = 12, $P = 0.701$ for comparison of allelic distribution in males of these four groups.
$\chi^2 = 13.2$, df = 15, $P = 0.584$ for comparison of allelic distribution in females of these four groups.

TABLE V. Allelic Distribution of DRD4 48bp-Repeat in the Chinese and Caucasians*

	Control		Schizophrenics	
Number of 48bp-repeats	Chinese (%)	Caucasian (%)	Chinese (%)	Caucasian (%)
2	21 (25.0)[a]	27 (11.3)	21 (25.0)	24 (11.3)
3	1 (1.2)	15 (6.3)	1 (1.2)	13 (6.1)
4	56 (66.7)	151 (63.4)	58 (69.0)	122 (57.5)
5	1 (1.2)	0 (0)	3 (3.6)	0 (0)
6	4 (4.8)	0 (0)	1 (1.2)	1 (0.5)
7	0 (0.0)[b]	45 (18.9)	0 (0.0)	52 (24.5)
8	1 (1.2)	0 (0)	0 (0.0)	0 (0)
Total alleles	84 (100)	238 (100)	84 (100)	212 (100)

*From the study of Daniels et al. [1994].
[a]$P = 0.0045$ compared with the two-repeat frequency of Caucasian controls.
[b]$P < 0.0001$ compared with the seven-repeat frequency of Caucasian controls.

nese and Caucasians (Table V). This finding reminds us of the caveat of association study. If patients and controls are not carefully matched for ethnicity, spurious differences in allele frequencies between groups will be erroneously interpreted as significant association.

Although no evidence was found of an association between schizophrenia and repeat length variation of DRD4, it is premature to conclude that DRD4 is not related to the pathogenesis of schizophrenia. At least 20 haplotypes in this 48bp-repeat polymorphic region have been detected by direct sequencing [Lichter et al., 1992]. Only the alleles created by length polymorphisms were analyzed in this study. All of the sequence variants change amino acids, which could affect the structure and function of the dopamine D4 receptor. It is therefore possible that variations other than repeat length may be found to be associated with schizophrenia.

Although the familial/sporadic distinction of schizophrenia does not show an association between schizophrenia and DRD4 48bp-repeat polymorphism, there is still the possibility of an association occurring in other sub-groups of schizophrenia. This needs to be explored in a large sample, which requires pooling clinical and laboratory data among centers.

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References

American Psychiatric Association (1987): "Diagnostic and Statistical Manual," 3rd ed., revised. Washington DC: American Psychiatric Association.

Andreasen NC, Endicott J, Spitzer RL, Winokur G (1977): The family history method using diagnostic criteria. Arch Gen Psychiatry 34:1229–1235.

Asarnow RF, Cromwell RL, Rennick PM (1978): Cognitive and evoked response measures of information processing in schizophrenics with and without a family history of schizophrenia. J Nerv Ment Dis 166: 719–730.

Carlsson A (1988): The current status of the dopamine hypothesis of schizophrenia. Neuropsychopharmacology 1:179–186.

Daniels J, Williams J, Mant R, Asherson P, McGuffin P, Owen MJ (1994): Repeat length variation in the dopamine D4 receptor gene shows no evidence of association with schizophrenia. Am J Med Genet 54:256–258.

Dean J, Dean A, Burton A, Dicker R (1990): "Epi Info Version 5.0 in Public Domain Software for Epidemiology and Disease Surveillence." Centers for Disease Control Epidemiology Program Office, Atlanta, Georgia.

Hays P (1977): Electroencephalographic variants and genetic predisposition to schizophrenia. J Neurol Neurosurg Psychiatry 40:753–755.

Holzman PS, Kringlen E, Matthysse S, Flanagan SD, Lipton RB, Cramer G, Levin S, Lange K, Levy DL (1988): A single dominant gene can account for eye tracking dysfunctions and schizophrenia in offspring of discordant twins. Arch Gen Psychiatry 45:641–647.

Hwu HG (1991): Psychiatrist diagnostic assessment. In Hwu HG (ed): "Manual of Psychiatric Diagnosis." Taipei: Publication Committee, College of Medicine, National Taiwan University, pp 7–42.

Hwu HG, Yang SY (1987): Psychiatrist diagnostic assessment: Establishment and interrater reliability. Clin Psychiatry 2:267–278.

Kendler KS, Hays P (1982): Familial and sporadic schizophrenia: A symptomatic, prognostic, and EEG comparison. Am J Psychiatry 139:1557–1562.

Lander ES (1988): Splitting schizophrenia. Nature 336:105–106.

Lichter JB, Livak KJ, Rogers J, Kennedy JL, Van Tol HHM (1992): Echoes of D4 receptor repeats. Nature 360:424.

Lyons MJ, Faraone SV, Kremen WS, Tsuang MT (1989): Familial and sporadic schizophrenia: A simulation study of statistical power. Schizophr Res 2:345–353.

Murray RM, Lewis SW, Reveley AM (1985): Towards an etiological classification of schizophrenia. Lancet 1(8436):1023–1026.

Orzack MH, Kornetsky C (1971): Environmental and familial predictors of attention behaviors in chronic schizophrenics. J Psychiatr Res 9:21–29.

Shaikh S, Collier D, Kerwin RW, Pilowsky LS, Gill M, Xu WM, Thornton A (1993): Dopamine D4 receptor subtypes and response to clozapine. Lancet 341:116.

Slater E, Cowie V (1971): "The Genetics of Mental Disorders." London: Oxford University Press, pp 11–44.

Sommer SS, Lind TJ, Heston LL, Sobell JL (1993): Dopamine D4 receptor variants in unrelated schizophrenic cases and controls. Am J Med Genet 48:90–93.

Van Tol HHM, Wu CM, Guan HC, Ohara K, Bunzow JR, Civelli O, Kennedy J, Seeman P, Niznik HB, Jovanovic V (1991): Cloning of the gene for human D4 receptor with high affinity for the antipsychotic clozapine. Nature 350:610–614.

Van Tol HHM, Wu CM, Guan HC, Ohara K, Bunzow JR, Civelli O, Kennedy J, Seeman P, Niznik HB, Jovanovic V (1992): Multiple dopamine D4 receptor variants in the human population. Nature 358:149–152.

Walker E, Sheye J (1982): Familial schizophrenia: A predictor of neuromotor and attentional abnormalities in schizophrenia. Arch Gen Psychiatry 39:1153–1156.