Twelve-Nucleotide Repeat Polymorphism of D4 Dopamine Receptor Gene in Chinese Familial Schizophrenic Patients

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Background: *Disturbances in dopaminergic transmission have been implicated in the etiology of schizophrenia. Catalano et al reported an association between delusional disorder and the number of a 12-nucleotide (bp) repeat sequence in the first exon of dopamine D4 receptor gene (DRD4), which indicated a possible role of this polymorphism in the pathogenesis of psychotic disorders.*

Methods: *DNA of 42 Chinese controls, 50 sporadic schizophrenic patients, and 30 familial schizophrenic probands were collected. Genotype of the 12-bp repeat polymorphism of DRD4 was determined with polymerase chain reaction and agarose gel electrophoresis. Genotypic and allelic frequencies were compared among the three groups using the chi-square test.*

Results: *Forty-three (86%) sporadic schizophrenic patients, 25 (83.3%) familial schizophrenic probands, and 35 (83.3%) controls were A1 (two 12-bp repeat) homozygotes. One (2.0%) sporadic schizophrenic and 1 familial schizophrenic patient were A2 (one 12-bp repeat) homozygotes. There was no significant difference in allelic and genotypic distributions among the three groups.*

Conclusions: *The present data do not support an association between schizophrenia and any specific allele of the 12-bp repeat polymorphism of DRD4. Significance of familial/sporadic division of schizophrenia cannot be supported regarding this repeat polymorphism.* Biol Psychiatry 1998;43:432–435 © *1998 Society of Biological Psychiatry*

Key Words: Dopamine D4 receptor, D4 dopamine receptor gene, familial schizophrenia, polymorphism

Introduction

Disturbances in dopaminergic transmission have been implicated in the etiology of schizophrenia (Carlsson 1988). Among the different dopamine receptors, D4 receptor is of particular interest because it has high affinity for clozapine, which is an atypical antipsychotic agent with specific therapeutic effect on refractory schizophrenia (Van Tol et al 1991). There are two kinds of repeat polymorphism in D4 dopamine receptor gene (DRD4), one of which, located in the third exon, is characterized by a polymorphic 48-base-pair (bp) repeat coding for a sequence of 16 amino acids in the region of the third cytoplasmic loop. In vitro expression systems show that receptors with different repeat numbers have differing affinity for clozapine and spiperone (Van Tol et al 1992). The other polymorphism, located in the first exon of DRD4, is characterized by a 12-bp tandem repeat coding for a sequence of four amino acids in the extracellular N-terminal part of the receptor, which borders the first transmembrane domain. The 12-bp repeat occurs as a twofold repeat in the more common variant and is represented only once in the rarer variant (Catalano et al 1993). Although the 48-bp repeat polymorphism has drawn the attention of most researchers interested in DRD4, linkage studies have failed to demonstrate any multiplex family linked to this locus (Barr et al 1993; Shaikh et al 1994; Macciardi et al 1994), and association studies reject the possibility that the 48-bp polymorphism is associated with the occurrence of schizophrenia (Sommer et al 1993; Daniels et al 1994) or the response to clozapine (Shaikh et al 1993). Catalano et al (1993) reported a 12-bp repeat polymorphism in the first exon, which is located in the N-terminal, of DRD4 and found an association between this polymorphism and delusional disorder. Because the N-terminal part of a gene is a site for glycosylation, which contributes to the proper expression of membrane proteins (Kornfeld and Kornfeld 1985), and the density of dopamine D4-like receptors was found to be elevated in postmortem brain striata in schizophrenia (Seeman et al 1993; Seeman and Van Tol 1995), we believe the finding of Catalano et al (1993) indicates a significant role of the 12-bp repeat sequence in the pathogenesis of psychotic disorders.

To test the relationship between schizophrenia and polymorphism in the 12-bp repeat sequence of DRD4, an

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association study was conducted. Here we assume that schizophrenia is etiologically heterogeneous, and patients with or without family history were divided to two subgroups.

Methods and Materials

Patients

The subjects included in this study were 42 healthy controls, 50 patients with sporadic (nonfamilial) schizophrenia, and 30 patients with familial schizophrenia who had at least one first-degree relative with schizophrenia. Most of the controls were recruited from the hospital staff. All the patients had at least one admission to the Department of Psychiatry of the Veterans General Hospital–Taipei (VGH-Taipei) and were directly interviewed by two of the researchers (Dr. Hong and Dr. Chiu, both of whom are qualified psychiatric specialists). The diagnosis of schizophrenia was made according to DSM-III-R (American Psychiatric Association 1987). All the schizophrenic relatives had their diagnosis made at VGH-Taipei. 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 the polymerase chain reaction (PCR) with oligonucleotide primers specific for the D4 sequence: 5′-CGCCATGGGGAACCG-CAG-3′ and 5′-CGGCTCACCTCGGAGTAGA-3′. The 30 μL PCR mixture contained: 200 ng template DNA, 0.3 μmol/L of each primer, 200 μmol/L deoxyribonucleoside triphosphates, 1.0 mmol/L $MgCl_2$, 5% formamide, 10% dimethyl sulfoxide, and 0.6 units Dynazyme. Samples were processed in a Minicycler (MJ). After an initial cycle at 95°C for 5 min, 72°C for 1.5 min, 40 cycles were carried out at 94°C for 1 min, 57°C for 1 min, and 72°C for 1.5 min. The PCR products were electrophoresed on 3% ethidium bromide-stained agarose gel and visualized under ultraviolet.

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. The power of detecting association was calculated with SigmaStat for Windows Version 1.0. The attributable risk was calculated by subtracting the frequency of A2A2 in the normal control group from that in the schizophrenic group.

Results

Demographic information on the subjects is shown in Table 1. Allele assignment of the polymorphism was made according to the report of Catalano et al (1993). A1 represents two 12-bp repeats, and A2 represents one 12-bp repeat. Distribution of the 12-bp repeat genotype and allelic frequencies are shown in Table 2. Both the sporadic schizophrenic group and familial schizophrenic group had 1 patient with homozygous A2A2, which was not found in the controls. The difference in genotype distribution and allelic frequencies among familial schizophrenics, sporadic schizophrenics, and controls was not significant. The power of detecting association is .262, with significance level ≤.1. The attributable risk for schizophrenia due to the A2A2 homozygote is 2.5%.

Table 1. Demographic Characteristics of Controls and Schizophrenic Patients

Characteristic	Normal control	Sporadic schizophrenia	Familial schizophrenia
Sample size	42	50	30
Gender			
Male	26	24	16
Female	16	26	14
Age (years)	28.7 ± 4.4	30.2 ± 7.8	27.5 ± 4.7

The difference of mean age and sex distribution among the three groups was not significant ($p > .05$).

Discussion

Association studies offer a potentially powerful way of detecting genes of relatively minor effect. Since antidopaminergic agents have been the target of attention in the treatment of schizophrenia, intragenic variation in the sequence or structure of dopamine receptors is likely to be significant in the pathogenesis of schizophrenia. In terms of searching for genes of schizophrenia, association study does not require pedigrees with multiple ill family members and is more straightforward than linkage study. Instead of a complex linkage analysis, the investigator can simply compare the rates of marker alleles (or genotypes) in patients and controls with standard statistical tests. Furthermore, since the genetic etiology of schizophrenia

Table 2. Genotype Distribution and Allelic Frequencies of the DRD4 12-bp Repeat Polymorphism in Chinese Controls and Schizophrenic Patients

12-bp repeat polymorphism	Normal control (%)	Sporadic schizophrenia (%)	Familial schizophrenia (%)
Genotype[a]			
A1/A1	35 (83.3)	43 (86.0)	25 (83.3)
A1/A2[b]	7 (16.7)	6 (12.0)	4 (13.3)
A2/A2	0 (0.0)	1 (2.0)	1 (3.3)
Allele[c]			
A1	77 (91.7)	92 (92.0)	54 (90.0)
A2	7 (8.3)	8 (8.0)	6 (10.0)

[a] Mantel–Haenszel test for linear association $\chi^2 = 0.97$, DF = 1, $p = .324$.
[b] A1, two 12-bp repeats; A2 = one 12-bp repeat.
[c] Mantel–Haenszel test for linear association $\chi^2 = 0.102$, DF = 1, $p = .750$.

may be multifactorial or polygenic (Gottesman and Shields 1982), any single susceptibility gene contributes only a small fraction to the overall risk (liability), which would not be detected by the current linkage method, which assumes schizophrenia as a single major locus disease. Consequently, allelic variation at specific candidate genes directly evaluated as susceptibility factors using case–control association studies is an important approach to understand the pathogenesis of schizophrenia. Association between the e4 allele of apolipoprotein and Alzheimer's disease is a good example (Corder et al 1993).

This study found two A2A2 homozygotes in the schizophrenic patients, which was not found in the normal controls, but the difference did not reach statistical significance. As the frequency of A2A2 in the schizophrenic patients was low, and the size of available samples was small, the power of detecting association in this study was only .262. It was premature to conclude that A2A2 homozygote of the 12-bp repeat polymorphism in DRD4 did not play a role in the pathogenesis of schizophrenia. Although our result failed to demonstrate a difference in the 12-bp polymorphism between familial and sporadic schizophrenia, it does not mean other regulatory element or intragenic variation in the sequence or structure of dopamine receptors in DRD4 is also refuted. Gershon et al (1989) suggested comparing a large number of well individuals and a small number of ill observations to detect association for diseases with limited samples of patients. Accordingly, the power of this study would be improved to .42, .70, and .82 without increasing the number of schizophrenic patients, if the number of normal controls was increased to 200, 1000, and 10,000, respectively, assuming the frequency of A2A2 homozygote in the population is .005 and the significance level remains the same (.1).

This study found a higher frequency of A2 allele in the Chinese than in Caucasians (Catalano et al 1993); this finding held true when the Chinese schizophrenic patients were compared with the Caucasian controls. This reminds us of the caveat of association study. If patients and control groups are not carefully matched for ethnicity, spurious differences in allele frequencies between groups will erroneously be interpreted as significant association.

As to the classification of schizophrenia, it is quite common that clinicians and researchers in the schizophrenia field subdivide patients into those who have a family history of psychosis and those who have a "negative" family history. The two groups of patients are then compared on a variety of "markers," such as symptomatology, psychomotor performance, history of birth trauma, electroencephalogram, or certain biochemical variables. If any of the markers are significantly more common in the familial cases, then the claim is usually made that this marker reflects the genetic diathesis to schizophrenia. This approach has been criticized on several points (Kendler and Hays 1982; McGuffin et al 1987; Farmer et al 1990). First, genes could play a major role in the etiology of "sporadic" cases. This might occur because the family size was too small or because relatives were too young to have developed the disorder. Second, factors other than genes could produce familial cases. Third, all modes of genetic transmission, except that of a fully penetrant Mendelian dominant gene, frequently have no affected relatives. These critiques, however, are made according to the concept that schizophrenia is an etiologically homogeneous disease; familial and sporadic cases are assumed to be genetically identical. If we are not convinced of the truth of this assumption, facts against it should be looked for.

Although the present data failed to find a difference between familial and sporadic schizophrenia in the 12-bp repeat sequence of DRD4, comparisons between these subgroups can be made in an infinite number of ways. When subdividing a sample by family history, we hope that "genetic" cases will be concentrated in the familial group, but the method does not require any proof of this. The test is successful if a significant difference between the subgroups is found and the result can be replicated. Success is the only thing that counts, and success means that the homogeneity view is refuted.

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References

American Psychiatric Association (1987): *Diagnostic and Statistical Manual of Mental Disorders*, 3rd ed rev. Washington, DC: American Psychiatric Press.

Barr CL, Kennedy JL, Lichter JB, et al (1993): Alleles at the dopamine D4 receptor locus do not contribute to the genetic susceptibility to schizophrenia in a large Swedish kindred. *Am J Med Genet* 48:218–222.

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

Catalano M, Nobile M, Novelli E, Nothen MM, Smeraldi E (1993): Distribution of a novel mutation in the first exon of the human dopamine D4 receptor gene in psychotic patients. *Biol Psychiatry* 34:459–464.

Corder EH, Saunders AM, Strittmatter WJ, et al (1993): Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science* 261:921–923.

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.

Farmer A, McGuffin P, Gottesman II (1990): Problems and pitfalls of the family history positive and negative dichotomy: Response to Dalen. *Schizophr Bull* 16:367–370.

Gershon ES, Martinez M, Goldin L, Gelernter J, Silver J (1989): Detection of marker association with a dominant disease gene in genetically complex and heterogeneous diseases. *Am J Hum Genet* 45:578–585.

Gottesman II, Shields J (1982): *Schizophrenia, the Epigenetic Puzzle.* Cambridge: Cambridge University Press.

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

Kornfeld R, Kornfeld S (1985): Assembly of asparagine-linked oligosaccharides. *Annu Rev Biochem* 54:631–64.

Macciardi F, Petronic A, Van Tol HHM, et al (1994): Analysis of the D4 dopamine receptor gene variant in an Italian schizophrenia kindred. *Arch Gen Psychiatry* 51:288–293.

McGuffin P, Farmer A, Gottesman II (1987): Is there really a split in schizophrenia? The genetic evidence. *Br J Psychiatry* 150:581–592.

Seeman P, Van Tol HH (1995): Dopamine D4-like receptor elevation in schizophrenia: Cloned D2 and D4 receptors cannot be discriminated by raclopride competition against [3H]nemonapride. *J Neurochem* 64:1413–1415.

Seeman P, Guan HC, Van Tol HH (1993): Dopamine D4 receptors elevated in schizophrenia. *Nature* 365:441–445.

Shaikh S, Collier D, Kerwin RW, et al (1993): Dopamine D4 receptor subtype and response to clozapine. *Lancet* 341:116.

Shaikh S, Gill M, Owen M, et al (1994): Failure to find linkage between a functional polymorphism in the dopamine receptor gene and schizophrenia. *Am J Med Genet* 54:8–11.

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, et al (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, et al (1992): Multiple dopamine D4 receptor variants in the human population. *Nature* 358:149–152.