American Journal of Medical Genetics (Neuropsychiatric Genetics) 67:483–484 (1996)

Brief Research Communication

Dopamine DRD2/Cys311 Is Not Associated With Chronic Schizophrenia

Fiona Crawford, Jonathan Hoyne, Xingang Cai, Aaron Osborne, Devin Poston, Jose Zaglul, Nabil Dajani, Shirley Walsh, Richard Bradley, Richard Solomon, and Michael Mullan

Roskamp Laboratories, Institute for Research in Psychiatry, University of South Florida, Tampa (F.C., J.H., X.C., A.O., D.P., J.Z., N.D., M.M.); and Medical Executive's Department, G. Pierce Wood Memorial Hospital, Arcadia, Florida (S.W., R.B., R.S.)

A mutation in the DRD2 receptor gene has been reported in association with schizophrenia in Japanese and Caucasian populations. The variation, Ser to Cys at codon 311, occurs in the third intracellular loop of the receptor and is therefore putatively functional. We report the results of screening US Caucasian schizophrenic and nonschizophrenic populations. We detected the occurrence of the DRD2 Cys311 variant in both schizophrenics and controls. Our data demonstrates no significant difference between the frequency of Cys311 in Caucasian schizophrenic and non-schizophrenic populations, indicating no association with schizophrenia.

KEY WORDS: schizophrenia, genetic association, DRD2 receptor, Ser/Cys 311 variant

INTRODUCTION

Arinami et al. [1994] examined the occurrence of a rare polymorphism in the dopamine DRD2 receptor gene and found an association with schizophrenia in a Japanese population. The variation, which substitutes cysteine for serine at codon 311 in the third intracellular loop of the receptor, may produce a functional change. DRD2/Cys311 had been previously detected, but with no association to schizophrenia, in Caucasian populations [Gejman et al., 1994; Laurent et al., 1994]. The DRD2 receptor gene has previously been suggested as a candidate gene for schizophrenia largely because of the ability of antipsychotic drugs to block dopamine receptors. Japanese results suggested the possible association of DRD2/Cys311 with schizophrenia or with the Japanese schizophrenic population in particular. This prompted a study by Shaikh et al. [1994], who examined a population of British Caucasian schizophrenics with unknown family history, and demonstrated a significant association between Cys311 and schizophrenia ($P = 0.017$). However, in another British Caucasian population, Asherson et al. [1994] detected no difference in the frequency of the variant between schizophrenics and controls.

Therefore we examined the DRD2 gene for the presence of this variant in 84 chronic schizophrenics from a Florida State Mental Hospital, and in 81 nonschizophrenic controls. Both populations were Caucasian. Entry criteria for the schizophrenics were a DSMIII-R diagnosis, of schizophrenia. Controls were unrelated, nonschizophrenic individuals unrelated to schizophrenic probands, recruited for other studies, who had lived through the mean age of risk for schizophrenia. We used a PCR-based assay adapted from Arinami et al. [1994] using primers D2A 5′ ACC AGC TGA CTC TCC CCG ACC GGT 3′ and D2B 5′ GGA AGG ACA TGG CAG GGA ATG GGA C 3′. The PCR reaction used 2 mM $MgCl_2$, and 50 pmol of each primer. PCR conditions were 94°C, 10 min; 35 cycles of 94°C, 1 min; 61°C, 1 min; 72°C, 1 min; and 72°C, 5 min. This was followed by a 4-hr digestion at 37°C with Sau96I and electrophoresis on a 1.5% Metaphor agarose, 1.5% regular agarose gel. We confirmed the source of the digestion polymorphism by direct sequencing of DRD2 exon 7 from the PCR product, amplified using primers DRD27A 5′ ATG GGT GGC TGA TGC CTG GG 3′, and DRD27B 5′ TGG CAG GGA ATG GGA CC 3′ (Fig. 1). This demonstrated that the polymorphism observed was due to variation at codon 311 alone, rather than by a novel variation at codons 310 and 311, which might result in the creation of a restriction site for Sau96I.

We identified 5 schizophrenics heterozygous for the variant, and one homozygous for the variant (allele frequency of 0.042 in the total schizophrenic population). However, we also found three heterozygotes in our con-

Received for publication June 9, 1994; revision received April 29, 1996.

Address reprint requests to Fiona Crawford, Roskamp Laboratories, Institute for Research in Psychiatry, University of South Florida, Tampa, FL 33613.

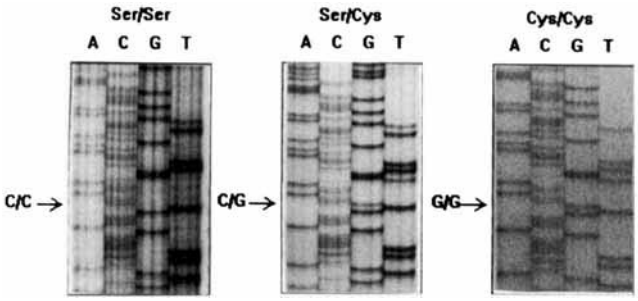

Fig. 1. Autoradiograph showing direct sequencing of DRD2 exon 7 at codon 311 in three schizophrenic samples: Ser/Ser, Ser/Cys, and Cys/Cys.

trol population (allele frequency of 0.018). These allele frequencies do not differ by χ^2 analysis ($P = 0.367$, Table I). Using our sample size with the desired difference in proportions detected by Arinami et al. [1994], we had 56% power to detect a difference in either direction, or 84% power to detect difference in the expected direction (i.e., an increase in the Cys311 allele frequency in the schizophrenic population).

We made a preliminary comparison of the clinical data of the schizophrenics in our data set. Of the Cys311 cases, all were Caucasian with no reported family history of schizophrenia; the homozygote (male) had a diagnosis of paranoid schizophrenia with no other diagnoses, while the heterozygotes (3 male, 2 female) were chronic undifferentiated schizophrenics with other diagnoses of alcohol abuse and bipolar disorder. All 6 were modest responders to the antipsychotic medication clozapine, with the case homozygotic for the variant showing the best response. Monthly assessments determine response to clozapine by the decline in scores on the Brief Psychiatric Rating Scale (BPRS) [Overall et al., 1962] and Positive and Negative Symptom Scale (PANSS) [Kay et al., 1987] from the original baseline, with a more dramatic decline indicating good response to treatment. As comparison of the schizophrenics with and without the variant revealed a number of nonvariant cases with very similar clinical pictures to those of the variant cases, we conclude that there are no gross clinical features associated with the Cys311 variant.

TABLE I. χ^2 Analysis of Cys311 Variant Frequency in Schizophrenics and Controls*

Allele frequency	Controls	Schizophrenics
Serine 311	159	161
Cysteine 311	3	7

*χ^2 ($df = 1$) = 0.814, $P = 0.367$ (with Yates correction for small cell size, as in Armitage and Berry [1987]).

Therefore, in contrast to Arinami et al. [1994] and Shaikh et al. [1994], we find that this rare variant does occur at similar frequency in both schizophrenic and nonschizophrenic controls. This suggests that Cys311 is a rare but normal variant and that the results of both Arinami et al. [1994] and Shaikh et al. [1994] are anomalous. As all reported individuals homozygotic for this variant have schizophrenia (three reported by Arinami, one reported here), it remains possible, but statistically not significant, that homozygosity of this variant is associated with schizophrenia.

ACKNOWLEDGMENTS

This study was supported by the staff of the G. Pierce Wood Mental Hospital and with funds from Mr. R. Roskamp, G. Pierce Wood, and the University of South Florida President's Council.

REFERENCES

Arinami T, Itowaka M, Enguchi H, Tagaya H, Yano S, Shimuzu H, Hamaguchi H, Toru M (1994): Association of dopamine D2 receptor molecular variant with schizophrenia. Lancet 343:703–704.

Armitage P, Berry G (1987): "Statistical Methods in Medical Research," 2nd ed. Oxford: Blackwell Scientific Publications, pp 129–130.

Asherson P, Williams N, Roberts E, McGuffin P, Owen M (1994): DRD2 Ser 311 polymorphism and schizophrenia. Lancet 343:1045.

Gejman P, Ram A, Gelernter J, Friedman E, Cao Q, Pickar D, Blum K, Noble E, Kranzler H, O'Malley S, Hamer D, Whitsitt F, Rao P, DeLisi L, Virkkunen M, Linnoila M, Goldman D, Gershon E (1994): No structural mutation in the dopamine D2 receptor gene in alcoholism or schizophrenia. JAMA 271:204–208.

Kay SR, Fiszbein A, Opler L (1987): The positive and negative syndrome scale for schizophrenia. Schizophrenia Bulletin 13:261–276.

Laurent C, Bodeau-Pean S, Campion D, d'Amato T, Jay M, et al. (1994): No major role for the dopamine D2 receptor Ser to Sys311 mutation in schizophrenia. Psychiatr Genet 4:229–230.

Overall J, Gorham G (1962): The brief psychiatric rating scale. Psychological Reports 10:799–812.

Shaikh S, Collier D, Arranz M, Ball D, Gill M, Kerwin R (1994): DRD2 Ser311 polymorphism and schizophrenia. Lancet 343:1045–1046.