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Brief Research Communication

No Association of Dopamine D2 Receptor Molecular Variant Cys311 and Schizophrenia in Chinese Patients

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A serine-to-cysteine mutation of dopamine D2 receptor at codon 311 (Cys311) was found to have higher frequency in schizophrenic patients than in normal controls in Japanese by Arinami et al. [1994: Lancet 343: 703–704]. The Cys311 allele was found to be associated with patients with younger age-of-onset, positive family history, and more positive symptoms. To investigate the possible involvement of Cys311 in schizophrenia in the Chinese population, 114 unrelated Taiwanese Chinese schizophrenic patients with positive family history and 88 normal controls were genotyped for Cys311. Four patients and 5 normal controls were heterozygotes of Ser311/Cys311; no homozygotes of Cys311 were identified in either group. The allele frequencies of Cys311 in Chinese schizophrenic patients and normal controls were 2% and 3%, respectively. No significant difference was detected between the two groups. Our results do not support the argument that the Cys311 allele of DRD2 poses a genetic risk for certain types of schizophrenia in Chinese populations.

KEY WORDS: dopamine D2 receptor gene, mutation, Chinese, schizophrenia, association

INTRODUCTION

While schizophrenia is known to be a genetically heterogeneous disorder, the responsible gene(s) has yet to be identified. With advances in molecular genetics, several strategies have been attempted to clone the gene(s) responsible for schizophrenia, including linkage, case-control association, and candidate gene studies. Dopamine D2 receptor (DRD2) has been a favored candidate gene for schizophrenia, as most antipsychotics have a strong affinity to the DRD2 receptor [Seeman et al., 1975], and disturbance of dopamine neurotransmission was proposed to be associated with the etiology of schizophrenia [Carlsson, 1988]. Several lines of linkage and association studies, however, do not support the association of the DRD2 gene with schizophrenia [Coon et al., 1993; Hattori et al., 1993; Su et al., 1993]. Nonetheless, lack of linkage or association of the DRD2 locus with schizophrenia does not exclude the possible involvement of the DRD2 gene in certain subtypes of schizophrenia.

A C-to-G transversion causing a serine-to-cysteine missense mutation at codon 311 of the DRD2 gene was identified by Itokawa et al. [1993]. The Cys311 allele was found to be present more often among 156 Japanese schizophrenic patients (5.4%) than 300 normal controls (1.8%). It was also noted that Cys311 allele was associated with patients with younger age-of-onset, more positive symptoms, and positive family history. Hence, it was proposed that the Cys311 allele of the DRD2 gene may pose a genetic risk factor for certain subtypes of schizophrenia [Arinami et al., 1994]. However, this association could not be replicated by other Japanese researchers [Nanko et al., 1994]. As Cys311 was also detected in Caucasians, the association between Cys311 and schizophrenia was not confirmed [Asheron et al., 1994; Gejman et al., 1994]. An association was proposed between Cys311 and patients with unknown family history in Caucasians [Shaikh et al., 1994], but it was in contrast to what was found in the Japanese study [Arinami et al., 1994], and has not been replicated by other researchers yet.

The controversial results of experiments involving Cys311 and schizophrenia in Japanese and Caucasian patients, and the importance of the DRD2 gene in schizophrenia, prompted us to investigate the possible involvement of Cys311 with the DRD2 gene in a Chinese schizophrenic population. To address this, Cys311

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allele frequencies were determined in Taiwanese Chinese schizophrenic patients with a positive family history, and in normal Chinese controls from Taiwan.

MATERIALS AND METHODS

Subjects

One hundred and fourteen unrelated schizophrenic proband patients (mean age, 40 years; male:female ratio, 1:1) were recruited from families who had participated in the Molecular Genetic Research Project (MGRP) in Taipei, Taiwan, with informed consent. The participants comprised 24 families of parent-offspring pairs and 90 families of affected sib-pairs. The diagnosis was assessed by one of the authors (H.-G.H.) using the Psychiatrist Diagnosis Assessment Schedule [Hwu and Yeung, 1988], according to DSM-III-R diagnostic criteria [American Psychiatric Association, 1987]. Most of these patient families came from Northern Taiwan. Eighty-eight normal controls (mean age, 27.5 years; male:female ratio, 1:1.5) consisted of faculty, research assistants, and medical students of the College of Medicine, National Taiwan University, Taipei. Individuals with positive family history were excluded.

Methods

Genomic DNA was prepared from peripheral blood, using the standard method. PCR-based genotyping of Ser311/Cys311 was carried out according to method described by Arinami et al. [1994]. PCR products were digested with Sau96I restriction enzyme (an isoschizomer of Cfr13, New England Biolab, Beverly, MA), instead of Cfr13. The digested PCR products were resolved in 2% MetaPhor (FMC BioProducts, Rockland) agarose gel by electrophoresis, and visualized with ethidium bromide staining under ultraviolet light. The Ser311 allele shows bands of 126 bp, 92 bp, 53 bp, and 23 bp, whereas the Cys311 allele shows bands of 149 bp, 92 bp, and 53 bp.

RESULTS

Of the 114 schizophrenic patients tested, 4 unrelated patients were found to be Ser311/Cys311 heterozygotes, and no Cys311 homozygotes were identified. The allele frequency was 2%. Of the 88 normal controls, 5 individuals were identified as Ser311/Cys311 heterozygotes, and no Cys311 homozygotes were found. The allele frequency was 3%. The frequencies of genotypes in both patient and normal control groups did not deviate from the Hardy-Weinberg equilibrium. No significant difference of allele frequency of Cys311 was noted between patients and normal controls. All 4 Ser311/Cys311 heterozygous patients were female. No consistency in younger age-of-onset or positive symptoms were noted in these heterozygotes after reviewing their records and history.

DISCUSSION

In this study, we confirmed the presence of the Cys311 allele of the DRD2 gene in both Chinese schizophrenic patients and normal controls from Taiwan. We detected similar allele frequencies of Cys311 in both groups. In addition, we did not find association between Cys311 and younger age-of-onset and positive symptoms in our heterozygous patients. Our results are in line with several replication studies performed in Japanese and Caucasian patients, indicating no evidence to support that Cys311 may confer susceptibility to certain subtypes of schizophrenia. Our results also add further evidence to exclude the association between the DRD2 gene and schizophrenia.

In this study, we adopted a case-control association with modifications. Candidate gene and case-control association studies have more advantages than a linkage analysis in investigating complex multifactorial diseases such as schizophrenia [Cooper and Clayton, 1988]. However, they are also likely to give rise to false-positive associations because of the larger number of candidate genes, the low a priori probability, and population admixture and stratification [Kidd, 1993]. Crowe [1993] suggested several ways to decrease false-positive associations, one of which was to study candidate genes in probands from a multiplex pedigree. Once association was found, further family study could be undertaken to examine the cosegregation of mutation and schizophrenia. In our study, patient groups consisted of probands with positive family history. Such an approach increases our chances of observing the association of schizophrenia with Cys311, if the proposed association between Cys311 and patients with positive family history really exists. Such a modification would also facilitate further family study, once a positive association was detected.

The relative risk calculated from the study of Arinami et al. [1994] was 2.94, a figure belonging in the so-called "weak-to-moderate" association range [Hodge, 1994]. The presence of the associated allele in this range is neither necessary nor sufficient for the existence of schizophrenia, and is also likely to lead to false-positive results due to sampling stratification. The proposed association between Cys311 and schizophrenia in Japanese patients [Arinami et al., 1994] and in Caucasian patients [Shaikh et al., 1994] may be due to chance. The best way of proving true association between the disease and genetic markers is to replicate the original study. Our failure to replicate the positive association of Cys311 and schizophrenia in a Chinese population, as well as results of other replication studies performed in the Japanese and in Caucasians, is consistent with previous linkage and association studies [Coon et al., 1993; Hattori et al., 1993; Su et al., 1993], which showed no support for a major role played by the DRD2 mutation in the pathogenesis of schizophrenia.

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