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Assessment of Association of D3 Dopamine Receptor *Msc*I Polymorphism With Schizophrenia: Analysis of Symptom Ratings, Family History, Age at Onset, and Movement Disorders

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Several studies have reported an association between schizophrenia and homozygosity for the *Msc*I restriction site in exon 1 of the D3 dopamine receptor gene, but other studies have failed to find this association. Recent reports have suggested that the association is most salient in male patients with a family history of schizophrenia. We examined this restriction site in a group of schizophrenic patients (n = 84) and in normal controls (n = 77). Patients were subdivided according to demographic and clinical features, particular attention being paid to movement disorders. No significant difference in allelic or genotypic distribution was seen between the two groups. No association was seen between homozygosity and a positive family history, age at onset of illness, clinical subtype, negative symptom score, or movement disorder scores.

KEY WORDS: genetics, candidate gene, DRD3, movement disorder, negative symptoms

INTRODUCTION

The dopamine hypothesis of schizophrenia gained new life from the cloning, sequencing, and characterization of multiple dopamine receptors [Grandy et al., 1989; Dearry et al., 1990; Giros et al., 1990; Sunahara et al., 1991; VanTol et al., 1991]. In particular, the gene for the D3 receptor is a candidate gene for vulnerability to schizophrenia, since D3 receptors have a high affinity for neuroleptic drugs [Sokoloff et al., 1992], and since the D3 receptor is strongly expressed in the limbic system [Sokoloff et al., 1990], a region implicated in schizophrenia by structural and functional imaging studies [Bogerts et al., 1993; Chua and McKenna, 1995; Liddle et al., 1992].

The human D3 dopamine receptor gene (DRD3) contains a polymorphic site in the first exon, giving rise to a glycine-to-serine substitution in the N-terminal region of the molecule [Lannfelt et al., 1993]. This substitution produces an additional *Msc*I (or *Bal*I) restriction enzyme site, the presence of which can be detected using PCR followed by restriction digest. This polymorphism, like other D3 locus markers, has not shown linkage to schizophrenia [Coon et al., 1993; Wiese et al., 1993; Nanko et al., 1994; Sabate et al., 1994], and thus an abnormality of D3 is unlikely to be a major predisposing factor that accounts for a large proportion of cases of schizophrenia. However, Crocq et al. [1992] found patients with schizophrenia to have an excess of homozygosity at this site. Their finding has been replicated in different populations of patients and has been found to be particularly marked in patients with strong family loading, male gender, and good response to neuroleptics [Nimgaonkar et al., 1993; Mant et al., 1994]. However, other studies have not found any excess of homozygosity in schizophrenics in different populations [Jonsson et al., 1993; Nothen et al., 1993; Yang et al., 1993; Laurent et al., 1994], although Jonsson et al. [1993] found an association of homozygosity with good response to neuroleptics.

In the present study we asked whether *Msc*I homozygosity was associated with particular subgroups of patients. Factor analysis has previously shown that three principal groups of symptoms can be defined, replacing the traditional positive-negative dichotomy [Liddle, 1987, 1992; Thompson and Meltzer, 1993]. The classical "negative symptoms" do indeed tend to cluster and can be regarded as a single factor. "Positive symp-

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toms" of delusions and hallucinations also tend to cluster and are regarded by Liddle [1987] as a "reality distortion" factor. The third factor can be viewed as "disorganization" and contains features that were previously split between "positive" and "negative" symptoms, such as incoherence of speech and incongruity of affect. The presence or absence of disorganization and reality distortion can be assessed by the Present State Examination (PSE) [Wing et al., 1974].

Since D3 receptors are found in nonlimbic parts of the neostriatum and substantia nigra [Herroelen et al., 1994], and since the D3 receptor seems to have an inhibitory role in motor behavior [Griffon et al., 1995], we paid especial attention to disorders of movement. Tardive dyskinesia is the most important of these. It is generally a drug side effect, but it is now accepted that it can be part of the illness itself [Rogers, 1992]. Other movement disorders, e.g., catatonia, can also be seen as part of the disease process. These can be difficult to distinguish from tardive dyskinesia and may possibly be on a continuum with it [Lund et al., 1991; McKenna et al., 1991]. A recently-devised scale allows semiquantitative ratings of catatonic and dyskinetic movement disorders without assumptions about etiology.

MATERIALS AND METHODS

Subjects

Eighty-four unrelated patients with schizophrenia were recruited through the psychiatric service based at Fulbourn Hospital, Cambridge. They were seen at the outpatient clozapine-monitoring clinic, on the wards, and at the day center of the rehabilitation service. After informed consent was obtained, patients were interviewed and further information was gathered from staff, relatives, and case notes.

All subjects except 3 were Caucasian. There was one subject each of Afro-Caribbean, Middle Eastern, and Vietnamese origin. Forty-nine patients were taking clozapine at the time of the study. Patients were categorized clinically as responding or not responding to clozapine. Psychopathology was assessed using the PSE [Wing et al., 1974], and negative symptoms were rated using the High Royds Evaluation of Negativity [Mortimer et al., 1989]. The mean negative symptom score was 12.4 ± 4.2, with a range from 0–21.

Patients were also assessed for the presence and severity of movement disorders using the Modified Rogers Scale [Lund et al., 1991]. This scale rates movement disorders phenomenologically, giving a global rating which includes both dyskinetic and catatonic phenomena. The global scores of our patients had a mean of 3.7 ± 3.6, with a range from 0–17. Catatonia scores ranged from 0–10, with a mean of 1.7 ± 2.3. Tardive dyskinesia scores (on a separate subscale) ranged from 0–40, with a mean score of 6.4 ± 6.7. Parkinsonian features were also assessed, the mean score being 1.0 ± 2.1, and the range 0–13.

Age-at-onset of illness was defined as the age at which psychotic symptoms were first documented or the age of first psychiatric admission, whichever was earlier. The mean age-at-onset was 23.5 ± 6.4 years, with a range from age 16–49 years.

A positive family history was defined for the purposes of analysis as the presence of a first- or second-degree relative with schizophrenia or schizoaffective disorder, as in previous positive reports [e.g., Nimgaonkar et al., 1993]. By this definition, 17 patients had a positive family history.

Patients were considered to have disorganization syndrome if they displayed any of the following: incoherence of speech, poverty of content of speech, distractibility, or incongruous affect. Reality distortion syndrome was considered present if the patient had any of the following: delusions of reference, delusions of persecution, grandiose delusions, passivity phenomena, or third-person auditory hallucinations. Forty-four of the 84 patients showed disorganization syndrome, and 57 showed reality distortion syndrome.

Controls

Seventy-nine control subjects were recruited from the oral surgery and ophthalmology wards and clinics in Addenbrooke's Hospital, Cambridge. All were Caucasian except for one, who was of Asian origin. A brief interview was administered to detect a personal or family history of major depression, bipolar affective disorder, schizophrenia, or schizoaffective disorder. Two subjects were excluded on the above grounds. No controls showed any overt movement disorder. Informed consent was obtained.

Isolation and Analysis of DNA

Venous blood was drawn from each subject. Genomic DNA was extracted from whole blood using the Nucleon II kit (Scotlab Inc., Shelton, CT). A 462-bp fragment, including the first part of exon 1, was amplified using the polymerase chain reaction (PCR), using the primer sequences published by Lannfelt et al. [1993]. The forward primer sequence was 5′ GCTCTATCTCAACTCTCACA 3′, and the reverse primer sequence was 5′ AAGTCTACTCACCTCCAGGTA 3′. Products were digested with *Msc*I restriction enzyme at 37°C for 1 hr, run on 2% agarose gels, and stained with ethidium bromide.

Constant bands at 111 and 47 bp were seen. The presence of a 304-bp band indicated a person who was either homozygous (or hemizygous) for allele 1, which lacks the *Msc*I site. Bands of 206 and 98 bp indicated a person homozygous (or hemizygous) for allele 2, which contains the *Msc*I site. Bands of all three sizes indicated a heterozygote.

Statistical Analysis

The chi-square test was used to compare patients with controls and to compare subgroups of patients. The robust rank order test (which makes no assumptions about distribution of values) was used to assess ordinal data for association with genotype. Student's t-test was used to compare age distributions of the patient and control samples.

RESULTS

Demographic characteristics of the sample population are shown in Table I. The mean age of controls (41.7 years) did not differ significantly from that of pa-

TABLE I. Demographic Characteristics of Patients and Controls

	Patients	Controls	Statistical test
Sample size	84	77	
Male	46	40	$\chi^2 = 0.04$
Female	38	37	$P > 0.75$
Age (mean years ± SD)	44.3 ± 14.8	41.7 ± 19.0	t = 1.18 $P > 0.1$

tients (43.8 years). There was no significant difference in gender distribution.

Genotypes and allele frequencies for patients and controls in this study are shown in Table II, with the expected values calculated for the Hardy-Weinberg equilibrium assuming random mating. Allele frequencies did not differ significantly between patients and controls ($P > 0.75$). The distribution of genotypes did not differ significantly from the expected values at the Hardy-Weinberg equilibrium for either controls or patients (patients, $P > 0.1$; controls, $P > 0.75$).

Distributions of homozygosity, allele frequency, and occurrence of the 1-1 genotype were also analyzed according to the clinical features discussed above: the chi-square test was used to assess any association with gender, response to clozapine, family history of schizophrenia or schizoaffective disorder, presence of disorganization syndrome, or presence of reality-distortion syndrome. No significant association of any of these factors was seen with homozygosity, either allele 1 or allele 2, or any genotype.

Patients taking clozapine did not differ significantly in their frequency of homozygosity, allele distribution, or occurrence of any genotype from those patients not taking clozapine. No significant association was found between genotype and movement-disorder scores, negative symptom scores, or age-at-onset using the robust rank order test.

An excess of allele 1 has been found among patients with a family history of psychiatric illness [Nimgaonkar et al., 1993, 1995]. In the sample studied here, there was no excess of allele 1 in patients with a first- or second-degree relative with schizophrenia or schizoaffective disorder when compared with patients without such a history ($P > 0.75$).

TABLE II. Allele and Genotype Frequencies for Patients and Controls

	Allele frequency,	Frequency of *Msc*I genotype		
	allele 1	1–1	1–2	2–2
Patients				
Observed	0.67	34	45	5
Expected		38.0	37.0	9.0
Controls				
Observed	0.66	34	33	10
Expected		33.1	34.8	9.1

DISCUSSION

We have assessed in patients and controls the distribution of the known *Msc*I polymorphism in the D3 dopamine receptor gene, and have not found the excess of homozygotes that has been reported in some groups of schizophrenics. Several previous studies have also failed to find excess homozygosity in schizophrenic populations [Jonsson et al., 1993; Nimgaonkar et al., 1993; Laurent et al., 1994]. One possibility for the discrepant findings is that schizophrenia is a heterogeneous disease, and different studies have drawn predominantly on different subpopulations. Thus, it has been suggested that excess homozygosity [Mant et al., 1994], or an excess of allele 1 [Nimgaonkar et al., 1993, 1995], is seen in patients with a positive family history of severe mental illness. The sample described here contained only 22 patients with a history of severe mental illness in a first-degree relative, and may therefore be drawn from a different population from those reported previously.

Another possible manifestation of genetic heterogeneity is variation in response to psychotropic drugs. Jonsson et al. [1993] and Mant et al. [1994] have observed that patients with good neuroleptic response show excess homozygosity. In this context, it is interesting that our own population was predominantly drawn from a clozapine clinic and so was necessarily composed largely of patients who have not responded to conventional neuroleptic drugs. Clozapine has its greatest affinity for the D4 receptor, whereas many classical neuroleptics bind more readily to D3. Hence, it could be hypothesized that there exists a subgroup of schizophrenics for whom the D3 receptor has a role in the etiology of the disorder, whereas this receptor is not relevant in the case of other patients, who fail to respond to classical neuroleptics.

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