Dopamine Receptor Gene D1 Reveals No Significant Association with Delusional Disorder on the Basis of SSP Analysis

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ABSTRACT Dysregulation of dopaminergic neurotransmission has been implicated in the etiology of major psychoses. The dopamine D1 receptor (DRD1) plays a role in some brain functions and mechanisms of psychotropic drugs. Therefore, the DRD1 gene makes a good candidate gene for molecular genetic study in delusional disorder. In the present investigation, the association has been studied between DRD1 gene and delusional disorder patients. No association was found between the DRD1 gene and delusional disorder, either in the whole group of patients or in subgroups divided by disease type or predominance of DRD1 positive or negative patients. Moreover, there were no significant differences observed between the delusional disorder patients and normal healthy controls when they were compared for different clinical and demographic variables. These findings suggest that this gene may not be involved in the pathogenesis of delusional disorder.

INTRODUCTION

Delusional disorder is characterized by monosymptomatic paranoid symptoms. In contemporary classifications of mental disorders, delusions are considered as cornerstone symptoms for the diagnosis of psychotic disorders. Since the beginning of psychiatry, delusional disorder has been a central subject of attention and continues to engender controversy right up to the present time. Delusional formation is a fascinating and enigmatic psychic process that has been the subject of numerous scientific debates and theoretical models. However, surprisingly only few empirical studies have been done so far (Berrios1991; Butler and Braff 1991).

Delusions are understood to mean inter subjectivity disconcerting convictions, with a tendency toward subjective certainty, that lose their disconcerting character when made the object of psychiatric analysis. Delusions involve thought contents and, as such, tend to be idiosyncratic and richly varied. It comprises an uncommon and probably heterogeneous group of illness; it is complicated by more than 100 conditions and agents, including neurologic disorders, metabolic and endocrine disorders, infections, pharmacologic agents, alcohol and other substances and psychiatric disorders (Manchreck 1999). Although its prevalence is low, delusional disorder is not rare(Manchreck 1996). Recent studies have revealed that delusional disorder is mostly under diagnosed and thereby results in poor anticipation of its implications (Ulzen and Carpentier 1997).

In recent communications, our laboratory has emphasized the association of HLA-A*03 allele with delusional disorder in Indian Bengali population(Debnath et al. 2005). Since delusional disorder is characterized by monosymptomatic paranoid symptoms, several investigators have suggested that delusional disorder is a naturally occurring model psychosis based on abnormalities of the dopaminergic temporolimbic system (Munro 1994). Molecular genetic evidence for this dopamine hypothesis of delusional disorder has been supported by a few studies on D2 receptor variation (Serretti et al. 1999) and D4 receptor Exon 3 variation (Serretti et al.2001).

But no data has been available till to date on the association between the D1 receptor with the delusional disorder. Considering the uniqueness of delusional disorder and the above

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noted advances in the arena of molecular psychiatry, we were stimulated to examine the possible role of D1 receptor in delusional disorder.

MATERIALS AND METHODS

Subjects

30 patients with delusional disorder, classified according to DSM-IV and ICD-10 criteria, attended the psychiatric out- patient department (OPD) of North Bengal Medical College and Hospital were studied. On an average of 1500 new patients with various psychiatric illness and about 4000 recurrent follow-up cases attend the OPD every year. Initially, about 68 unrelated patients were recruited with the symptoms of delusions. All subjects were screened independently by two psychiatrists using the structured clinical interview (SCID) for DSM-IV to determine the diagnosis of delusional disorder (American Psychiatric Association 1994). After longitudinal follow-up, 30 patients represented genuine cases of delusional disorder of various types have been considered. The distribution of delusional disorder subtypes as defined by DSM-IV criteria was as follows: 56.66% Jealous, 26.66% Persecutory, Erotomanic 3.33%, Paranoid 3.33%, Somatic 3.33%, Grandiose 3.33% and Mixed 3.33%. Most patients were clustered between the ages of 25-55 years. The male/female ratio was 1:2.

The patients having any one of the following conditions were excluded from the study like: 1) substance abuse during the past year; 2) any history of other general medical illness or treatment with anti-inflammatory or immune-suppressive medication; 3) any past history of psychotic illness and 4) any history of co morbidity. Further, the delusional patients and the family members were made to answer a questionnaire included self reported age, sex, caste, medical history, age of onset, age of severity, pedigree, disease duration among patients.

Written informed consent was obtained from each subject after a complete description of the study. The study was conducted as per the norms of the "World Medical Association Declaration of Helsinki, Ethical Principles for Medical Research Involving Human Subjects".

30 age and sex matched healthy individuals with no past history of psychiatric illness belonging to the same ethnic group as the patients were used as control subjects. The subjects were mostly from middle-class urban and semirural society and belonged to a nuclear family.

Genotyping

DNA Isolation: 5ml of blood samples were collected by vein puncture from each individual and stored in 10mM EDTA until further processing. Genomic DNA was isolated from blood lymphocytes using high salt extraction method (Miller et al.1988) and were quantified by OD260 measurement as well as agarose gel electrophoresis.

PCR Amplification with Sequence Specific Primer (SSP): Polymerase chain reaction was performed using 10ng of DNA, 250.M dNTP, 1.5mM MgCl2, 5.l 10X buffer (Sigma-Aldrich Pvt. Ltd.), 1.5 units of Taq polymerase (Sigma-Aldrich Pvt. Ltd.) and 0.1.M each of the primer (Sigma-Aldrich Pvt. Ltd.) in a total volume of 50¼l. 5'-CAG TCC ACG CCA AGA ATT GCC-3' and 5'-ATT GCA CTC CTT GGA GAT GGA GCC-3' primers were used for the amplification of D1 receptor gene. Samples were amplified for an initial cycle of 5 mins at 94.C followed by 40 cycles. After the initial denaturation, each of first 20 cycles has denaturing of 1min at 94.C, annealing of 1min at 60.C and extension of 1min 30 sec at 72.C followed by second 20 cycles, each of which has denaturing of 1min at 94.C, annealing of1min at 66.C and extension of 1min 30 sec at 72.C. A final extension step was added at 72.C for 7mins. The electrophoresis was performed with 2% agarose gel PCR products.

Statistical Analysis

Phenotypic frequency was calculated by direct count. The frequency of the allele of the D1 receptor gene is compared in the patient group as a whole with that of the control population using χ^2 test. Relative risk was also been estimated. Means, standard deviations of age of onset, age of severity, disease duration have been calculated for (a) all patients, (b) among the DRD1 positive as well as DRD1 negative patients, (c) disease type wise. The correlation coefficient between the DRD1 receptor gene and the demographic variables (age of onset, age of severity and disease duration) were calculated

by using t-test. Two orthogonal contrasts (age of onset vs age of severity) were used to examine the significance of differences of means among the DRD1 positive group of patients by using t-test. In this analysis, the correlation between age of onset and the age of severity was also measured among the jealous and persecutory type of DRD1positive patients.

Paired t tests were used to examine the significant differences of age of onset and the age of severity in cases of (a) DRD1 positive and DRD1 negative patients, (b) disease types (Jealous and Persecutory) and (c) Jealous and Persecutory separately in cases of DRD1 positive as well as for DRD1 negative patients. Two-way frequency table has been prepared for DRD1 type X sex. Chi-square (χ^2) test has been done to examine the presence of associations in the above mentioned two-way table. Data analysis has been done using SPSS package.

RESULTS

There was no significant association found between the D1 receptor gene and delusional disorder patients. The patients and control subjects were found to be homogeneous when two-way frequency table have been calculated for both D1 positive and D1 negative cases between the patients and the control groups (χ^2 =1.714, $d.f.$=1, p= 0.19 and Relative Risk=1.952).

When frequency distribution of D1 positive/ negative patients was observed in case of the male and female subsets, no such association or gender biasness has been observed among the D1 positive/negative patients (χ^2=0.817, $d.f.$=1, p= 0.37). Mean and standard deviations (SD) of the age of onset, age of severity and disease duration have been calculated for the D1 positive patients (Table 1). Only the significant difference has been found between the age of onset and the age of severity in case of D1 positive patients {(t value=3.304, df=19, p=<0.01), Table 2}. No significant difference has been found when mean, SD, t value, and p value were calculated between the Jealous and the persecutory type of patients

Table 2: Average differences between age of severity and age of onset among jealous and persecutory type of patients

Disease type	Difference (year) (age of severity –age of onset)	d.f.	t-value
Jealous	3.47	16	3.094*
Persecutory	7.56	8	2.827**

*P<0.01
**P<0.05

Table 3: Comparison of clinical characteristics between D1 positive/negative patients among Jealous type

Clinical characteristics	D1 positive patients N=13		D1 negative patients N=4	
	Mean	SD	Mean	SD
Age of onset	31.92	9.38	35.25	13.05
Age of severity	36.08	9.47	36.50	13.30
Disease duration	5.77	4.73	3.50	0.58

Table 4: Mean and SD values of age of onset, age of severity and disease duration between D1 positive/ negative patients among persecutory type

Clinical characteristics	D1 positive patients N=5		D1 negative patients N=4	
	Mean	SD	Mean	SD
Age of onset	35.40	10.38	46.50	31.76
Age of severity	44.20	9.01	52.50	29.78
Disease duration	10.60	10.14	13.00	17.26

(Tables 3, 4).When Correlation coefficients between age of onset, age of severity and disease durations are estimated, it shows significant values only between age of onset and age of severity, but those between disease duration and age of onset or age of severity are insignificant in almost all cases (Table 5).

DISCUSSION AND CONCLUSION

Dysregulation of dopaminergic neurotransmission has some implications in the etiology of major psychoses(Dmitrzak-Weglarz et al. 2006). The dopamine D1 receptor (DRD1) plays a role in some brain functions and mechanisms of

Table 1: Comparison of demographic variables among D1 positive patients

D1 positive patients N=20	Mean	SD	t	p	Significance
Age of onset	34.50	12.49	-0.266	0.792	NS
Age of severity	39.50	12.25	-0.242	0.811	NS
Disease duration	6.65	6.52	-0.621	0.540	NS

Table 5: Comparison of correlation coefficients among jealous and persecutory type of patients.

	D1 present and Disease type: Jealous			D1 absent and Disease type: Jealous			D1 present and Disease type: Persecutory			D1 absent and Disease type: Persecutory		
	Age of onset	Age of severity	Disease duration	Age of onset	Age of severity	Disease duration	Age of onset	Age of severity	Disease duration	Age of onset	Age of severity	Disease duration
Age of onset	1.000	0.855**	-.292	1.000	0.994**	-0.288	1.000	0.413	-0.561	1.000	0.993**	-0.413
Age of severity		1.000	0.207		1.000	-0.391		1.000	0.510		1.000	-0.496
Disease duration			1.000			1.000			1.000			1.000

**P<0.05
Note: The necessary corrections are made in red.

psychotropic drugs (Tauscher et al. 2004). Therefore, the DRD1 gene makes a good candidate gene for molecular genetic studies in delusional disorder. Paranoid (delusional) disorders are usually thought to overlap with schizophrenic disorders, and a continuum may exist (Munro 1988). To our knowledge, the present investigation is the first report on possible association between delusional disorder and D1 dopamine receptor gene (DRD1). In the present investigation, no association was found between the DRD1 gene and delusional disorder, either in the whole group of patients or in subgroups divided by disease type or predominance of DRD1 positive or negative patients.

However, several studies have been done on the association of DRD1 and schizophrenia. In one study (Dmitrzak-Weglarz et al. 2006) showed that there is no association found between the–48A/G polymorphism of the DRD1 gene with schizophrenia when compared either in the whole group of patients or in their subgroups (Dmitrzak-Weglarz et al. 2006). In another study on the Japanese population, it has been suggested that the DRD1 gene might not be involved in the pathogenesis of schizophrenia (Kojima et al. 1999). Linkage analysis between the D1 receptor RFLPs and schizophrenia shows that the inheritability of this gene is unlikely to be involved in the pathogenesis of schizophrenia (Cloninger 1994).

In this respect the present study suggests that the idea of a singularly powerful constellation of DRD1 gene being responsible for the pathogenesis of delusional disorder seems to be too beautiful to be true. This finding concurs with other similar association studied (Kojima et al. 1999; Dmitrzak-Weglarz et al. 2006) with schizophrenia and may suggest that the DRD1 gene does not play a major role in conferring susceptibility to delusional disorder.

CONCLUSION AND RECOMMENDATIONS

In conclusion, we suggest that D1 receptor gene may not be involved in the pathogenesis of delusional disorder. Our result is preliminary and, so far, is not correlated with the drug efficacy in DRD1 positive and DRD1 negative patients. However, further studies may be justified on a large cohort of samples to assess a possible association between DRD1 gene and the diagnostic subdivisions of delusional disorder.

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