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Original Article

Linkage and Association of DRD2 Gene *Taq*I Polymorphism with Schizophrenia in an Iranian Population

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Background: D2 dopamine receptor gene has been reported to be one of the most relevant candidate genes in schizophrenia. In this study, we investigated the association between *Taq*IA and *Taq*IB dopamine D2 receptor polymorphisms and psychopathology of schizophrenia.

Methods: The study subjects were 38 acutely exacerbated schizophrenic patients who were all Iranian descent. The control population consisted of 63 healthy individuals with almost the same age as patients and were also of Iranian decent. The *Taq*IA and *Taq*IB genotypes, the A1 and A2 alleles, and the B1 and B2 were determined by restriction fragment length polymorphism of the amplified DNA fragments by polymerase chain reaction .

Results: For each polymorphism (A or B) the patients were categorized according to their genotype into three groups; i.e. the patients with alleles A1/A1, A1/A2, A2/A2; B1/B1, B1/B2, and B2/B2. No significant association was found between *Taq*1A or *Taq*1B gene polymorphisms and schizophrenia in patients compared to the controls. When study subjects were stratified according to their gender, the distribution of the A1/A1 genotype did was significantly different in both men and women (patients vs. controls).

Conclusion: Our findings show that there is no genetic association between *Taq*1A and *Taq*1B gene polymorphisms and schizophrenia. Further clinical studies should be conducted to confirm and further evaluate these findings.

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Keywords: DRD2 gene • polymorphism • schizophrenia • *Taq*I

Introduction

Schizophrenia is a disorder affecting up to 1% of the world population. Extensive research efforts suggest that genetic factors play an important role in the pathogenesis of this disease. However, the search to identify mutations or disease predisposing DNA sequences involved in the etiology of schizophrenia has been inconclusive.[1,2]

The dopaminergic pathways are believed to be involved in the etiology of schizophrenia.[3–5] Therefore, the genes involved in dopaminergic pathways including the D2 dopamine receptor (DRD2) gene are considered the candidate genes closely associated with schizophrenia. In recent years, there has been increased interest in studying the relationship between the DRD2 gene and the pathogenesis of schizophrenia.[6–9] The DRD2 gene is localized on human chromosome 11 at q22–q23, extends over 270 kb, and has eight exons.[10] An uncommon *Taq*IA restriction fragment length polymorphism (RFLP) has been reported to be located in the 3' flanking region of the DRD2 gene. A second polymorphism, *Taq*IB RFLP, is closer to the regulatory and structural coding regions (5' region) of the gene.[11]

The *Taq*IA and *Taq*IB polymorphisms of DRD2 gene have been shown to be closely associated with the disease, as the allelic distribution was significantly different in schizophrenic patients and the controls.[9,12] These genetic

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variations in the DRD2 might also account for the inter-individual differences in response to drugs. Moreover, the polymorphisms in receptor protein DRD2 may affect its binding affinity for neuroleptics.[13,14]

Despite reports on the involvement of DRD2 gene in the etiology of schizophrenia, the results are still inconclusive and have not been confirmed by other investigators.[15–18] The controversial data and inter-ethnic genetic differences indicate the need to examine the association of DRD2 gene polymorphism and schizophrenia in other populations. In the present study, we investigated the possible involvement of polymorphisms of the DRD2 gene in schizophrenia in an Iranian population.

Materials and Methods

Subjects

Thirty-eight unrelated schizophrenic patients (17 men and 21 women) with a mean±SD age of 42±12 years admitted to the Psychiatric Department of Ibn-Sina Hospital, affiliated to Mashhad University of Medical Sciences, Mashhad, Iran were studied. All patients were diagnosed with chronic schizophrenia using the structured clinical interview of DSM-IV. The patients were ethnically Iranians. Sixty-three healthy subjects (24 men and 39 women) with a mean±SD age of 46±18 years were recruited as the control group. This study was approved by the Research Ethics Committee of Mashhad University of Medical Sciences, Mashhad, Iran. Approval was also obtained from the local authorities and written informed consents were given by all subjects or their next of kin.

DNA analysis

Blood samples (3 mL) were collected and genotyped. Genomic DNA was isolated from peripheral blood leukocytes using a standard protocol.[19] The *Taq*I A1/A2 and *Taq*I B1/B2 polymorphisms studies of DRD2 gene were conducted by polymerase chain reaction (PCR). The primer design and reported polymorphisms are with respect to the genomic sequence of GenBank entry AF050737. Primers MP1 (5'-GATACCCACTTCAGGAAGTC-3') and MP2 (5'-GATGTGTAGGAATTAGCCAGG-3') were used to obtain a DNA fragment of a 459-bp spanning the polymorphic *Taq1*B site. The *Taq*1A variant of the DRD2 gene was identified with primers, MP3 (5'-ACCCTTCCTGAGTGTCATCA-3') and MP4 (5'-ACGGCTGGCCAAGTTGTCTA-3').[20] For PCR reactions, 100 ng of genomic DNA was amplified in 50 μL of reaction mixture containing 2 mM $MgCl_2$, 50 mM KCl, 15 mM Tris-HCl (pH 8.4), 10 pmol of each of the forward and reverse primers, 0.2 mM of each deoxyribonucleotide triphosphate, and one unit of *Taq* polymerase (Promega). The PCR reaction was performed as follows: 95°C for three minutes; 30 cycles of 95°C for 30 sec, specific annealing temperature (Table 1) for 30 sec, and 72°C for one min. The PCR products were then digested with the appropriate restriction conditions. Digested products were separated with agarose gel electrophoresis (1.5% w/v) and visualized directly under UV lighting with ethidium bromide staining. Sequences of oligonucleotide primers, annealing temperatures, restriction fragments, and details of each polymorphism are presented in Table 1.

Statistical analysis

Analyses were performed for each polymorphism separately. Statistical analysis using Instat v3 included the χ^2 test for comparing genotype and allele frequencies. The mean values were compared between the patients and control subjects by the unpaired Student's *t*-test. $P<0.05$ was considered statistically significant.

Results

In vitro DNA amplification of the regions flanking the polymorphic sites of DRD2 gene resulted in amplification of the expected fragments

Table 1. The DNA sequences of oligonucleotide primers and reaction conditions for PCR-RFLP analysis of DRD2 polymorphism studies.

Polymorphism	Location	Primer sequences	*AT°C	Amplicon size (fragments,#bp)
*Taq*I A1/A2	3' (9.4 kb)	5'- ACCCTTCCTGAGTGTCATCA-3' 5'- ACGGCTGGCCAAGTTGTCTA-3'	58	310 (130+180)
*Taq*I B1/B2	Intron I	5'-GATACCCACTTCAGGAAGTC-3' 5'-GATGTGTAGGAATTAGCCAGG-3'	60	459 (267+192)

*AT=annealing temperature; #bp=base pairs.

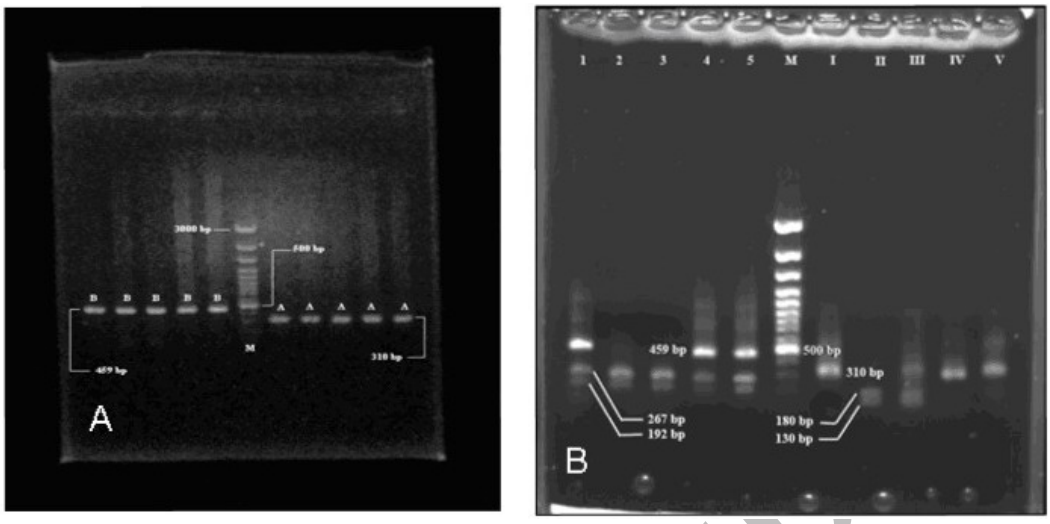

Figure 1. A) Amplification of the 310- and 459-bp fragments of human DRD2 gene. B) *Taq*IA and *Taq*IB polymorphisms of the DRD2 gene. The photo shows the representative bands 459-, 267-, and 192-bp for *Taq*IB and 310-, 180-, and 130-bp for *Taq*IA polymorphism seen on the ethidium bromide-stained gel.

of 310- or 459-bp DNA products. In *Taq*IA polymorphism study, digestion of the amplified fragments (amplicons) with *Taq*I restriction endonuclease, resulted in DNA fragments of 310-bp (A1/A1); 180-, 130-, and 310-bp (A1/A2); or 180- and 130-bp (A2/A2). Similarly, in *Taq*IB polymorphism studies, DNA fragments of 459-bp (B1/B1); 457-, 267-, and 192-bp (B1/B2), and 267- and 192-bp (B2/B2) were observed. Therefore, all the samples revealed one of the predicted electrophoretic patterns (Figure 1). Frequencies of the A1/A1, A1/A2, and A2/A2 genotypes were six, 21, and 11 in schizophrenic patients and, three, 39, and 21 in the controls, respectively. In *Taq*IB studies frequencies of the B1/B1, B1/B2, and B2/B2 genotypes were one, 13, and 24 in schizophrenic patients compared to two, 20, and 41 in the controls, respectively. Tables 2 and 3 summarize the distribution of the *Taq*IA and *Taq*IB polymorphisms of DRD2 by genotype, gender, and case-control status, respectively.

When the study subjects were categorized according to their gender, the distribution of the A1/A1 genotype did not differ significantly in both men and women (patients vs. controls) (Tables 2 and 3). When allelic frequencies for each category (gender, disease) were considered, frequency of A1 allele carriers was found higher in female patients than controls (45% vs. 32%; P=0.22). In men, this relative frequency was 41% for both controls and patients (Table 3). In similar comparisons for frequencies of A2, B1, and B2 alleles, an excess of

A2 allele in female controls vs. patients was observed. No such excesses were found in comparison for B1 and B2 alleles between the categories (Tables 2 and 3). None of the observed differences reached statistical significance.

Discussion

The DRD2 gene is one of the susceptibility genes considered to be involved in the pathogenesis of schizophrenia. This gene has been a candidate for extensive linkage and population studies on the disease.[3,9,12] Genetic studies in many ethnic populations have provided controversial data.[6,9,21-25] *Taq*IA and *Taq*IB polymorphisms of DRD2 gene create the A1, A2 and, B1 and B2 alleles,

Table 2. Frequencies of genotypes and alleles in the TaqIA study.

	Patients			Controls		
Allelic frequencies	**Male**	**Female**	**Total**	**Male**	**Female**	**Total**
A1/A1	1	5	6	1	2	3
A1/A2	12	9	21	18	21	39
A2/A2	4	7	11	5	16	21
						χ^2= 3.55, P=0.17
Allelic frequencies						
A1 allele	14 (41%)	19 (45%)	33	20 (41%)	25 (32%)	45
A2 allele	20 (59%)	23 (55%)	43	28 (58%)	53 (68%)	81
						χ^2= 0.88, P=0.35

Table 3. Frequencies of genotypes and alleles in the *TaqIB* study.

	Patients			Controls		
Genotype frequencies	**Male**	**Female**	**Total**	**Male**	**Female**	**Total**
B1/B1	0	1	1	0	2	2
B1/B2	4	9	13	7	13	20
B2/B2	13	11	24	17	24	41
						χ^2= 0.082, *P*=0.96
Allelic frequencies						
B1 allele	4 (12%)	11 (26%)	16	7 (15%)	17 (22%)	24
B2 allele	30 (88%)	31 (74%)	62	41 (85%)	61(78%)	102
						χ^2= 0.01, *P*=0.94

respectively. The *Taq*IA polymorphism has been of great concern, since the less frequent allele—the A1 allele—is reported to be associated with substance abuse.[26–28] The distribution of A1/A2 allele of this polymorphism has been shown to be significantly different in schizophrenic patients and controls.[9,12,29] However, other studies failed to show this association.[15,20,30] The distribution of B1/B2 allele of *Taq*IB polymorphism of the DRD2 gene was found to be associated with alcoholism.[31] Results of recent studies by Dubertret and co-workers in samples of French schizophrenic patients and their parents clearly demonstrates an association between *Taq*IB polymorphism of DRD2 and schizophrenia.[9,12] No data has been reported so far on the possible effect of *Taq*IA and *Taq*IB polymorphism of the DRD2 gene on the susceptibility to schizophrenia in Iranians.

We found that female subjects with A1 allele of *Taq*IA polymorphism had a higher frequency in schizophrenics compared to the controls. However, this association was not significantly high and was not observed in the male population. Although our sample size was large enough to demonstrate a significant associations, it seems that after stratification of participants (genotypes: A1/A1 and B1/B1) the size became relatively small which resulted in such inconclusive results. Therefore, these results can only be considered as a preliminary report. In terms of the observed allelic frequencies, our results seem to be consistent with the previous reports in which the A1 and B1 alleles were the less and A2 and B2 were the most frequent alleles observed in other populations.[21,22,25,32]

In summary, our data supports lack of association between *Taq*1A and *Taq*1B gene polymorphisms and schizophrenia. However, the number of participants (patients and controls) included in this study and relative frequencies of alleles (A1/B1) were probably not large enough to draw definite conclusions. Further studies on larger population samples and other ethnic groups will be required for elucidating the linkage between DRD2 polymorphism and risk of schizophrenia.

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