Linkage and Association of DRD2 Gene TaqI Polymorphism with Schizophrenia in an Iranian Population

Javad Behravan PhD*, **, Mehdi Hemayatkar PharmD*, Hamid Toufani MD***, Ebrahim Abdollahian MD***

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 TaqIA and TaqIB 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 TaqIA and TaqIB 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 TaqIA or TaqIB 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 TaqIA and TaqIB gene polymorphisms and schizophrenia. Further clinical studies should be conducted to confirm and further evaluate these findings.

Keywords: DRD2 gene • polymorphism • schizophrenia • TaqI

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 TaqIA restriction fragment length polymorphism (RFLP) has been reported to be located in the 3’ flanking region of the DRD2 gene. A second polymorphism, TaqIB RFLP, is closer to the regulatory and structural coding regions (5’ region) of the gene.11

The TaqIA and TaqIB 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
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 TaqI A1/A2 and TaqI 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'-GATACCCAC-TTCAGGAAGTC-3') and MP2 (5'-GATGTG-TAGGAATTAGCCAGG-3') were used to obtain a DNA fragment of a 459-bp spanning the polymorphic Tag/B site. The Taq1A variant of the DRD2 gene was identified with primers, MP3 (5'-ACCTCTCCTGAGTGTCATCA-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 MgCl2, 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 $\chi^2$ 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.
of 310- or 459-bp DNA products. In TaqIA polymorphism study, digestion of the amplified fragments (amplicons) with TaqI 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 TaqIB 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 TaqIB 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 TaqIA and TaqIB 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. Genetic studies in many ethnic populations have provided controversial data. TaqIA and TaqIB polymorphisms of DRD2 gene create the A1, A2 and, B1 and B2 alleles.

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

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<th>Allelic frequencies</th>
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<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Total</td>
<td>Male</td>
<td>Female</td>
<td>Total</td>
</tr>
<tr>
<td>A1/A1</td>
<td>1</td>
<td>5</td>
<td>6</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>A1/A2</td>
<td>12</td>
<td>9</td>
<td>21</td>
<td>18</td>
<td>21</td>
<td>39</td>
</tr>
<tr>
<td>A2/A2</td>
<td>4</td>
<td>7</td>
<td>11</td>
<td>5</td>
<td>16</td>
<td>21</td>
</tr>
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\[\chi^2 = 3.55, P=0.17\]

<table>
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<tr>
<th>Allelic frequencies</th>
<th>Patients</th>
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<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Total</td>
<td>Male</td>
<td>Female</td>
<td>Total</td>
</tr>
<tr>
<td>A1 allele</td>
<td>14 (41%)</td>
<td>19 (45%)</td>
<td>33</td>
<td>20 (41%)</td>
<td>25 (32%)</td>
<td>45</td>
</tr>
<tr>
<td>A2 allele</td>
<td>20 (59%)</td>
<td>23 (55%)</td>
<td>43</td>
<td>28 (58%)</td>
<td>53 (68%)</td>
<td>81</td>
</tr>
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\[\chi^2 = 0.88, P=0.35\]
The Taq1A 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 is reported to be associated with the less frequent allele—the A2 allele—and B2 were the most frequent alleles observed in other populations.21,22,25,32

In summary, our data supports lack of association between Taq1A and Taq1B 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.

Acknowledgment

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References

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