ASSOCIATION BETWEEN SCHIZOPHRENIA AND DRD3 RECEPTOR GENE VARIANTS OF IRAQI POPULATION

Najwa Sh. Ahmed1, Hayba Q. Younan2, Mushtaq Talib Hashim3 and Haneen M. Ismaeel2

1Biotechnology Research Center, Al-Nahrain University, Baghdad, Iraq.
2University of Baghdad, College of Science, Biotechnology dept., Baghdad, Iraq.
3University of Baghdad, college of Medicine, Psychiatry. Baghdad, Iraq.

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ABSTRACT

This study aimed to detect mutations in DRD3 receptor genes for schizophrenia patients in Iraq. To achieve this goal, blood samples were collected from 50 patients with schizophrenia (25 samples of male and 25 samples of female) and 10 samples of healthy, DNA was isolated and the DRD3 receptor gene (4147C-T and 712G-C) were amplified by using specific primers for exon1 of this gene, and then found the sequence of this region. The DNA sequencing results of flank sense of DRD3 (4147C-T) receptor gene from 25 cases schizophrenia patients and healthy was found to be compatible 100% with DNA sequence of gene bank, while 98% compatibility were found for that gene in the flank sense from 10 cases schizophrenia patients and differences may be attributed to two transversion mutations (C/G) and one transition mutation (G/A), however, 99% compatibility was found for that gene in the flank sense of another 5 cases schizophrenia patients and differences may be attributed to one transversion mutation (G/T), and 99% compatibility was found for that gene in the flank sense of another 5 cases schizophrenia patients and differences may be attributed to two transversion mutations (G/T and T/G) and one transition mutation (C/T). However, 100% compatibility was found flank sequences DNA sense of DRD3 (712G-C) receptor gene from healthy and 50 cases patients with DNA sequence of gene bank. There is a significant correlation ship between schizophrenia disease and incidence of mutations (C/G; G/A; and G/T) position in exon 1 of DRD3 receptor gene with value (X²=3.662, P>0.05), however, there is lower significant correlation ship between schizophrenia and incidence of mutations (G/T and T/G) with value (X²=1.089, P>0.05). The conclusion is that there is enough evidence to support the claim that there is a relationship between the appearance of mutations and the occurrence of schizophrenia disease in Iraqi population.

Key word: DRD3 receptor gene, Schizophrenia, transition and transversion mutation.

INTRODUCTION

Schizophrenia is a syndrome embrace advance range of disturbances of perception thought emotion and motor activity its an illness in which episodes of florid disturbance are usually set against a background of sustained disability, the level of chronic disability ranges
from a mild decrease in the ability to cope with stress to a profound difficulty in initiating and organizing activity that can render patients unable to care for themselves [9]. A wide range of genetic studies strongly suggests a genetic component to the inheritance of schizophrenia showed that a person is likely to have schizophrenia when other members of the family have the disorder and that the likelihood of the persons having schizophrenia is correlated with the closeness of the relationship [3]. Many associations between chromosomal sites and schizophrenia have been reported since the application of the techniques of molecular biology became widespread. In molecular genetics that of association studies takes a gene that is suspected of involvement in pathogenesis of the disorder a gene involved in dopamine metabolism and then compares the frequency of its various alleles in a series of individuals with schizophrenia as opposed to a control group, some candidate gene studies imply a weak effect of the D3 dopamine receptor gene [4]. The distribution of schizophrenia in families and populations is consistent with a substantial genetic basis for the disorder [8]. Several lines of evidence suggest that the dopamine D3 receptor is involved in the pathophysiology of schizophrenia. The D3 receptor has a restricted pattern of expression in brain limbic areas associated with cognitive function and motivated behavior [11]. The D3 dopamine receptor gene has been implicated by association studies as a possible candidate for a number of neuropsychiatric disorders and phenotypes including schizophrenia, bipolar disorder, tourette syndrome, and substance abuse [9]. The phenotype most intensively investigated in connection with DRD3 is schizophrenia, and since the original report of association [4], there have been more than 24 independent follow-up studies of a non-synonymous A/G polymorphism (Ser9Gly) in exon 1. The present study aimed to investigate correlation between polymorphism in exon 1 of DRD3 receptor gene and increasing schizophrenia in Iraqi population.

**MATERIALS AND METHODS**

**Subjects & DNA extraction**

Whole blood samples was obtained from 50 Iraqi patients affected by schizophrenia (25 male and 25 female, age ranged 18-62 years) and also obtained from healthy men used as a control group, the disease were diagnosed by the consultant medical staff at Rasheed Teaching Hospital. Whole blood was collected (4ml) into an EDTA- tube; the samples were stored at -20°C until further processing. DNA was extracted from the samples by wizard genomic (DNA purification kit, Promega, USA) according to the isolating genomic DNA from whole blood protocol. DNA extracted from 300 µl whole blood in each case. The volume of the extracted DNA solution was usually 100 µl were stored at -20°C.

**Detection of Gene DRD3 by Using PCR**

A 281 bp fragment containing exon 1 of DRD3 (4147C-T) was amplified using a forward primer (DRD3 4147C-T: 5′-CGTCAACTTCCATGCTGCTAT-3′) and a reverse primer (DRD3 4147C-T: 5′-TAAAAAGGCGAGGAACAGA-3′) and 262bp fragment containing exon 1 of DRD3 (712G-C) was amplified using a forward primer (DRD3 712G-C: 5′-TTGGGCCTCAGCCTGGCTAAAAGTCG-3′) and a reverse primer (DRD3 712 G-C: 5′-GGAAAGGAGTGACAAACTTGG-3′) (Primers set supplied by alpha DNA Company, Canada). The PCR amplification was performed in a total volume of 25µl containing 2µl DNA (conc. 100 ng/µl), 12.5 µl Go Taq green master mix 2X (green maschuerit mix is a premixed ready to use solution containing Taq DNA polymerase, dNTPs, MgCl₂ and reaction buffers at optimal concentrations)
concentrations for efficient amplification of DNA template by PCR supplied by promega (Promega corporation, USA), 1µl of each primer (10 pmol/µL) and up to 25µl with nuclease free water. The thermal cycling was as follows: Denaturation at 94 °C for 7 min, followed by 33 cycles of 94 °C for 1min, 58°C for 1 min, and 72 °C for 1min, with final incubation at 72 °C for 7 min [1] using a thermal Cycler (Multigene TM Gradient Thermal Cycler, Labnet International, Korea). The PCR products were separated by 1.5% agarose gel electrophoresis and visualized by exposure to ultraviolet light (302nm) after ethidium bromide staining.

Sequencing
Sequencing of exon 1 of DRD3 gene was done by Macro gen company/USA for sequencing of products through used individual up and downstream primer was used in each sequencing reactions.

Sequence Alignment
Homology searches were conducted between the sequence of standard gene BLAST program which is available at the national center biotechnology information (NCBI) online at (http://www.ncbi.nlm.nih.gov) and using BioEdit program and ExPASY program for amino acid sequence.

Statistical analysis
The statistical analysis is a very important final step in the research to analyse and evaluate the obtained results. Medical statistics of this study was conducted via computer based statistical program which was: X² for Windows computer package. The statistical analysis tests which used in this were as follows: P value <0.05 is considered a significant correlation.

RESULTS AND DISCUSSION
The genomic DNA from 50 patient were extracted using wizard genomic DNA promega, DRD3 (4147C-T and 712G-C) gene from genomic DNA were amplified by using specific PCR primers for exon 1, results shown in figure (1) indicated that a yield of single band of the desired product with a molecular weight about 281 bp for exon 1 DRD3 (4147C-T) gene and 262 bp for exon 1 DRD3 (712G-C) gene was obtained. Sequencing of coding regions of the amplified product (Exon 1) for these samples were done seeking for detection of any mutation within these sequences related to schizophrenia development as shown in figure (1). The results were compared with data obtained from Gene Bank published BLAST program which is available at the NCBI online at www.ncbi.nlm.nih.gov and using BioEdit program. Alignment of DRD3 (4147C-T) gene of all groups (Healthy and patient) with data published for known sequence seeking for enough homology. A homology with DRD3 (4147C-T) gene of Homo sapiens from the Gene Bank was done using the BioEdit software. 100% compatibility of that gene was found with DRD3 (4147C-T) gene from healthy and 25 cases from schizophrenia patients with standard DRD3 (4147C-T) of Gene Bank results as shown in figure (2). The DRD3 (4147C-T) gene from 10 schizophrenia patients shows 98% compatibility with standard DRD3 (4147C-T) gene of Gene Bank, and there are two transversion mutations (C/G) lead to change translate amino acid from Histidine to Aspartic acid; from Cystein to Tryptophan respectively and one transition mutation (G/A) lead to change translate amino acid from Valine to Isoleucine, as shown in figure (3) and table (1) shown type of mutation and predicted effect.

While there are one transversion mutation (G/T) in the flank DNA sense of DRD3 (4147C-T) gene from 10 schizophrenia patients shows 99% compatibility with standard DRD3 (4147C-T) gene of Gene Bank,
lead to change translate amino acid from serine to isoleucine. As shown in figure (4) and table (1).

However, there are one transversion mutation (G/T) and lead to change translate amino acid from Serine to Isoleucin, and one transition mutation (C/T) in the flank DNA sense of DRD3 (4147C-T) gene (silent) and one transversion mutation (T/G) lead to change amino acid from Cysteine to Glisine from 5 schizophrenia patients shows 99% compatibility with standard DRD3 (4147C-T) gene of Gene Bank as shown in figure (5) and table (1).

The results were compared with data obtained from Gene Bank published BLAST program which is available at the NCBI online at www.ncbi.nlm.nih.gov and using BioEdit program. Alignment of DRD3 (712 G/C) gene of all groups (Healthy and patient) with data published for known sequence seeking for enough homology. A homology with DRD3 (712G/C) gene of Homo sapiens from the Gene Bank was done using the BioEdit software. 100% compatibility of that gene was found with DRD3 (712G/C) gene from healthy and all cases from schizophrenia patients with standard DRD3 (721G/C) of Gene Bank results as shown in figure (6).

The A206G transition in the sequence of the dopamine type 3 DRD3 receptor gene that leads to a Ser9Gly amino-acid substitution in the N terminal extracellular domain of the receptor are genetic polymorphisms previously implicated to confer susceptibility to psychiatric disorders [2,15]. European multicentre studies indicate significant association between schizophrenia and C-102 variant of the T-102C polymorphism [12,14] as well as an increased homozygosity of either allele of the DRD3 polymorphism [6]. However, negative associations have widely been reported, especially for the DRD3 Ser9Gly transition [16,1]. DRD3 receptor gene polymorphisms in Greek samples, in order to investigate the distribution of the allelic variants within the population and to examine their putative correlation with schizophrenia. A common variant of a single nucleotide polymorphism (SNP) of A/G at position 25 of the DRD3 coding sequence has been identified [4] and Sivagnanasundaram, refer to association between schizophrenia and the Ser9Gly variant of the dopamine D3 receptor gene has been the subject of numerous studies and suggested that these SNPs and the corresponding coding changes may exerta combined or synergistic effect on susceptibility to schizophrenia [10] and Talkowski, refer to association SNP of DRD3 receptor gene with risk for schizophrenia [13]. An excess frequency of homozygotes for both alleles was originally reported in schizophrenic patients [7]. The DRD3 cDNA was believed to consist of six exons (total length 1.2 kb) and five introns (total length_45 kb). However, neither the extent of the 5_UTR nor the location of the promoter (s) were known. An additional 1724 bp of 5_ flanking sequence has been recently reported but no gene structure has been attributed to this [10]. Ishiguro et al., refer to the 59 region of the DRD3 gene and found three novel polymorphisms: 712G/C, 205A/G, and Ala38Thr, case-control comparisons in 153 Japanese schizophrenia patients and 122 Japanese controls did not suggest an association between Ala38Thr and schizophrenia and Indicates unknown variant in linkage disequilibrium with the DRD3 haplotypes associated with schizophrenia [5]. There is study a significant correlation ship between schizophrenia and incidence of mutations (C/G; G/A; and G/T) position in exon 1 of DRD3 (4147C-T) receptor gene (X2=3.662, P>0.05), however, there is lower significant correlation ship between schizophrenia and incidence of mutations (G/T
and T/G) position in exon 1 of DRD3 (4147C-T) receptor gene ($\chi^2=1.089$, $P>0.05$), the conclusion is that there is enough evidence to support the claim that schizophrenia is related to these mutations.

**REFERENCE**


Figures and Tables:

Figure 1: Agarose gel electrophoresis for amplified DRD3 gene of schizophrenia patients and healthy. Bands were fractionated by electrophoresis on a 1.5 % agarose gel (2 h., 5V/cm, 0.5X TBE buffer) and visualized under U.V. light after staining with ethidium bromide staining. Lane: 12 (M:100bp ladder); Lane: N (negative control); Lane: 1,2,3,4,5,6,7,8,9,10 product for exon 1 DRD3 (4147C-T) gene; and Lane: 11,12,13,14,15,16,17,18,19,20 product for exon 1 DRD3 (712G-C) gene, Lane A, B: Healthy.

Homo sapiens chromosome 3 genomic contig, Features flanking this part of subject sequence: D (3) dopamine receptor isoform a

Score = 374 bits (202), Expect = 4e-101, Identities = 202/202 (100%), Gaps = 0/202 (0%) Strand=Plus/Minus

Query 1

| CAGATGTAAGTGTCTCTACTTCTCGAGAGACAAATATTTAAA | 60 |

Sbjct 20390225

| CAGATGTAAGTGTCTCTACTTCTCGAGAGACAAATATTTAAA | 20390166 |

Query 61

| CTCTGTAAGTCTTAATGAGGTGCTAAGGAGGAACCCCAGAATGTTTCAGGAGACTTGT | 120 |

Sbjct 20390165

| CTCTGTAAGTCTTAATGAGGTGCTAAGGAGGAACCCCAGAATGTTTCAGGAGACTTGT | 20390106 |

Query 121

| ATTCAGCACTGAGGGATTGAACATCAGCAAAGCA GGACAAATGTCATAACTGATGGGGAC | 180 |

Sbjct 20390105

| ATTCAGCACTGAGGGATTGAACATCAGCAAAGCA GGACAAATGTCATAACTGATGGGGAC | 20390046 |

Query 181

| CTGACAACTCTCTGGTTCCCCTG | 202 |

Sbjct 20390045

| CTGACAACTCTCTGGTTCCCCTG | 20390024 |

Figure 2: Sequencing of sense flanking the partial DRD3 (4147C-T) gene for healthy as compared with standard DRD3 (4147C-T) obtained from Gene Bank.
Figure (3): Sequencing of sense flanking the partial DRD3 (4147C-T) gene for 10 cases schizophrenia as compared with standard DRD3 (4147C-T) obtained from Gene Bank.

Figure (4): Sequencing of sense flanking the partial DRD3 (4147C-T) gene for 10 cases schizophrenia patients as compared with standard DRD3 (4147C-T) obtained from Gene Bank.

Figure (5): Sequencing of sense flanking the partial DRD3 (4147C-T) gene for 5 cases schizophrenia patient as compared with standard DRD3 (4147C-T) obtained from Gene Bank.
**Table (1):** Types of mutations detected in partial *DRD3* (4147C-T) gene of schizophrenia patients.

<table>
<thead>
<tr>
<th>No.</th>
<th>location of gene bank</th>
<th>Nucleotide change</th>
<th>No. of sample</th>
<th>Amino acid change</th>
<th>Predicted effect</th>
<th>Type of mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C/G 2+</td>
<td>CAT/ GAT</td>
<td>10</td>
<td>Histidine/ Aspartic acid</td>
<td>Missense</td>
<td>Transversion</td>
</tr>
<tr>
<td>2</td>
<td>C/G 4+</td>
<td>TGC/ TGG</td>
<td>10</td>
<td>Cysteine/ Tryptophan</td>
<td>Missense</td>
<td>Transversion</td>
</tr>
<tr>
<td>3</td>
<td>G/A 8+</td>
<td>GTA/ ATA</td>
<td>10</td>
<td>Valine/ Isoleucine</td>
<td>Missense</td>
<td>Transition</td>
</tr>
<tr>
<td>4</td>
<td>G/T 2+</td>
<td>AGT/ ATT</td>
<td>10</td>
<td>Serine/ Isoleucin</td>
<td>Missense</td>
<td>Transversion</td>
</tr>
<tr>
<td>5</td>
<td>G/T 3+</td>
<td>AGT/ ATT</td>
<td>5</td>
<td>Serine/ Isoleucin</td>
<td>Missense</td>
<td>Transversion</td>
</tr>
<tr>
<td>6</td>
<td>C/T 71+</td>
<td>CCC/CCT</td>
<td>5</td>
<td>Proline/ Proline</td>
<td>Silent</td>
<td>Transition</td>
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<tr>
<td>7</td>
<td>T/G 72+</td>
<td>TGC/GGC</td>
<td>5</td>
<td>Cysteine/ Glisine</td>
<td>Missense</td>
<td>Transversion</td>
</tr>
</tbody>
</table>

Homo sapiens chromosome 3 genomic contig, Features flanking this part of subject sequence: D(3) dopamine receptor isoform a.

Score = 292 bits (158), Expect = 8e-77, Identities = 158/158 (100%), Gaps = 0/158 (0%) Strand=Plus/Minus

**Figure (6):** Sequencing of sense flanking the partial *DRD3* (712G/C) gene for healthy and all cases schizophrenia patient as compared with standard *DRD3* (712G/C) obtained from Gene Bank.