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Polymorphism of Exon 1 of Dopamine Receptor D3 (DRD3) gene and Exon 2 of 5-hydroxytryptamine type 2a (HTR2A) in Schizophrenia Baghdad Patients Sample

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Abstract: The aim of this study is to investigate the association between polymorphisms Exon1 of Dopamine Receptor D3 (DRD3) receptor and Exon 2 of 5-hydroxytryptamine type 2a (HTR2A) genes and schizophrenia patients from Baghdad Iraq. To achieve this goal, blood samples were collected from 50 patients with schizophrenia (25 samples of male and 25 samples of female) and 25 samples of healthy individual, DNA was isolated and the DRD3 receptor and HTR2A genes were amplified by using specific primers for exon1 and exon 2 of this genes, then sequencing of nucleic acid of genes was performed by machine is AB13730XL, (Applied Biosystem, Macro gen company, USA). The DNA sequencing results of flank sense of DRD3 receptor and HTR2A genes from healthy individual was found to be compatible 100% with wild type of Homo sapiens from the Gene Bank, the DNA sequencing results of flank sense of DRD3 receptor from 50 cases schizophrenia patients was found to be compatible 99% and score 398 and expect 0.0 with the wild type sequences of gene bank, and the differences may be attributed to one transition mutations (G/A), at position 1804 of exon 1. It is a missense mutation that leads to changes in amino acid from Serine (S) to Glysine (G). Our results showed that the incidence of G/A mutation was highly significant ($X^2=100$, $P<0.01$). In total, 8% of Shizophreina patients had two transition mutation +1804 G/A and +1830 G/A single nucleotide polymorphism, and compatibility 99%, score 397 and expect 0.0 with the wild type sequences of gene bank. The +1830 G/A SNP was silent mutation which result change of codon from GCG to GCA (Alanine to Alanine). However, the DNA sequencing results of flank sense HTR2A genes from 50 cases schizophrenia patients was found to be compatible 99% and score 259 and expect 5e-132 with the wild type sequences of gene bank, there was no significant correlation between shizopherina and incidence of transition mutation G/A (Serine to Serine) at nucleotide 156 of exon 2 HTR2A gene ($X^2=0.055$, $P>0.05$). In conclusion, our case study suggests that the 1804 A/G SNP of the DRD3 gene is strongly associated with genetic susceptibility to schizophrenia in the Baghdad / Iraqi population.

Key words: Schizophrenia, Expect Value, Score, and Exon.

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تعدد الأشكال في منطقه التشفير الاولى لمستقبلات الدوبامين ومنطقه التشفير الثانية لجين HTR2A في عينة من مرضى فصام الشخصية قي بغداد / العراق

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الخلاصة: الهدف من هذا البحث هو دراسة العلاقة بين الأشكال المتعددة في منطقة التشفير الاول لجين DRD3 ومنطقة التشفير الثاني لجين HTR2A لمرضى فصام الشخصية في بغداد / العراق . ولتحقيق هذا الهدف، تم جمع عينات دم من 50 مريضا يعانون من انفصام الشخصية (25 عينة من الذكور و 25 عينة من الإناث) و25 عينة من اشخاص اصحاء، تم عزل الحامض النووي وتضخيم DRD3 مستقبلات الجينات و HTR2A باستخدام بادئات محددة لمنطقه التشفير الاولى ومنطقه التشفير الثانيه على التوالي واجراء تسلسل الاحماض النووية للجينات بواسطة آستخدام الجهاز AB13730XL، من قبل شركة ماكروجين في الولايات المتحدة الأمريكية باستخدام برنامج BLAST في الموقع الالكتروني www.ncbi.nlm.nih.go وبرنامج BioEdit . أظهرت نتائج تتابع الحامض النووي الدنا للجين DRD3 و HTR2A عند أفراد السيطرة والذي تطابق بنسبة 100% مع الحامض النووي الدنا في البنك الجيني العالمي. ونتائج تسلسل الحامض النووي DRD3 مستقبلات ل 50 عينة من فصام الشخصية حيث كانت نسبة التطابق 99٪ ونقطة النجاح 398 وقيمة التوقع 0.0 مع تسلسل الحامض النووي للنوع البري من بنك الجينات، ويمكن أن يعزى الاختلاف الى وجود الطفره (G/A)، في الموقع 1804 من اكسون 1 ، وهي طفرة missense التي تودي الى تغير الحامض الأميني سيرين إلى كلايسين. وقد اظهر التحليل الإحصائي لهذه الطفرة G/A انها ذات تأثير عالي المعنوية X2 = 100, P< 0.01 . وكان 8٪ من مرضى فصام الشخصيه لهم طفرتين في الموقع +1804 A / G و A / 1830+ G ومقدار التطابق 99٪، ونقطه النجاح 397 وقيمة التوقع 0.0 مع تسلسل النوع البري من بنك الجينات، بينما الطفره +1830 G / هي من الطفرات الصامته التي تؤدي الى تغيير الشفرة الوراثية GCA الى GCC واللتان تشفران لنفس الحامض الاميني الأدنين . ومع ذلك، تم العثور على نتائج تسلسل الحامض النووي لجين HTR2A ل50 عينة لمرضى انفصام الشخصية لنسبة تطابق 99٪ ونقطه نجاح 259 و قيمة التوقع 5e-132 مع تسلسل النوع البري من بنك الجينات، لم يكن هناك ارتباط كبير بين انفصام الشخصية وظهور الطفره في الموقع 156 من منطقه التشفير الثانية لجين HTR2A (سيرين الى سيرين) ضمن المعنوية P> 0.05 و X2 = 0.055. وتشير الدراسة ان 1804الطفرة A / G SNP من الجين DRD3 يرتبط بقوة مع ظهور مرض فصام الشخصية لدى عينة من المواطنين الموجودين في بغداد .

Introduction

Schizophrenia is a clinical syndrome of the brain that manifests with multiple signs and symptoms thought perception, emotion, movement and behavior. These manifestations show considerable diversity among patients and the final prognosis of the disorder is severe and usually long lasting (1). The dopamine D3 receptor gene DRD3 was mapped to chromosome 3q13.3 and its polymorphic site in exon 1 with a serine to glycine substitution has been postulated as useful investigating tool in psychiatric disorder (2, 3). The dopamine transporter (DAT) seems to play an important role in the regulation of dopamine levels and neurotransmission by mediating the re uptake of synaptic dopamine into the neurons (4,5). DRD3 has a more restricted area of expression in the brain, which is known to be associated with cognitive and emotional function. In particular, the suggestion that the dopamine D3 receptor gene (DRD3) is a candidate for increased susceptibility to the disease comes from the high affinity of D3 receptors for neuroleptic drugs (6,7). Higher levels of DRD3 binding have been found in the mesolimbic system of schizophrenics (8). A common variant of a single nucleotide polymorphism (SNP) of A/G at position 25 of the DRD3 coding

sequence has been identified (2). Gene encodes the D3 subtype of the five (D1-D5) dopamine receptors. The activity of the D3 subtype receptor is mediated by G proteins which inhibit adenylyl cyclase. This receptor is localized to the limbic areas of the brain, which are associated with cognitive, emotional, and endocrine functions (9). A common A206G transition in the sequence of the DRD3 gene that leads to a Ser9Gly amino acid substitution in the N-terminal extracellular domain of the receptor (A206C, Ser9Gly, rs6280) has been the most extensively investigated variant in connection with Schizophrenia (10). 5-hydroxytryptamine type 2a (HTR2A) encodes one of the receptors for serotonin, a neurotransmitter. Serotonin (5-hydroxytryptamine; 5-HT) is a neurotransmitter that occupies a uniquely important place in neurobiology because of its role in many physiologic processes such as sleep, appetite, thermoregulation, pain perception, hormone secretion, and sexual behavior. Serotonin dysfunction has been implicated in the pathogenesis of schizophrenia. Previous studies have shown an association between the T102C polymorphism of the 5-hydroxytryptamine receptor 2A (HTR2A) gene and schizophrenia (11). Mutations in this gene are associated with susceptibility to schizophrenia and obsessive-compulsive disorder, and are also associated with response to the antidepressant citalopram in patients with major depressive disorder (MDD) (12). This study provides evidence that a genetic variant in the DRD3 and HTR2A genes are associated with schizophrenia.

Materials and Methods

Samples and DNA extraction

Schizophrenia were diagnosed by the consultant medical staff at Rasheed Teaching Hospital. Collection of Whole blood from 50 patients of Baghdad / Iraqi (25 male and 25 female, age ranged 18-62 years) and also obtained from 25 healthy individuals used as a control group. In total, 4 ml whole blood was collected into an EDTA-tube. DNA was extracted by DNA extraction kit (QIAamp DNA Blood Mini Kit, Number cod: 51104, USA) according to the manufacturer's protocol in the Medical and Molecular Biotechnology dept., Biotechnology Research Center, Al-Nahrain University, Baghdad, Iraq.

Amplification of exon 1 of Dopamine Receptor D3 (DRD3) gene and exon 2 of 5-hydroxytryptamine type 2a (HTR2A) gene

Detection of DRD3 and HTR2A gene was conducted by using primers for amplification of for DRD3 and HTR2A gene (13). A fragment 463 bp of DRD3 and 342 bp of HTR2A were amplified using a forward primer of DRD3: 5′-GCTCTATCTCCAACTCTCACA-3′ and a reverse primer of DRD3: 5′-AAGTCTACTCACCAGGTA-3′, and forward primer of HTR2A: 5′-TCTGCTACAAGTTCTGGCTT-3′ and a reverse primer of HTR2A: 5′-CTGCAGCTTTTTCTCTAGGG-3′ respectively, Primers set supplied by IDT (Integrated DNA Technologies) Company, USA. The PCR amplification was performed in a total volume of 25µl containing 1.5µl DNA, 5 µl Taq PCR PreMix (Bioneer, Korea), 1µl of each primer (10 pmol) then add distilled

water into Taq PCR PreMix tube to a total volume of 20µl, do not calculate any volume for the dried pellet. The thermal cycling conditions were done of DRD3 and HTR2A as follows: Denaturation at 94 °C for 7min, followed by 35 cycles of 94 °C for 35s, 62°C for 45s, 54°C for 35s respectively and 72 °C for 35s with final incubation at 72°C for 10 min using a thermal Cycler (Gene Amp, PCR system 9700; Applied Biosystem). Sequencing of exon 1 of DRD3 and exon 2 of HTR2A gene were performed by machine is AB13730XL, Applied Biosystem, Macrogen company, USA. Homology search was conducted using Basic Local Alignment Search Tool (BLAST) program which is available at the National Center Biotechnology Information (NCBI) online at (http:// www.ncbi.nlm.nih.gov) and BioEdit program.

Results

The results shown in figure (1) indicated that a yield of single band of the desired product with a molecular weight about 463 bp for exon 1 DRD3 gene and 432 bp for exon 2 HTR2A gene was obtained.

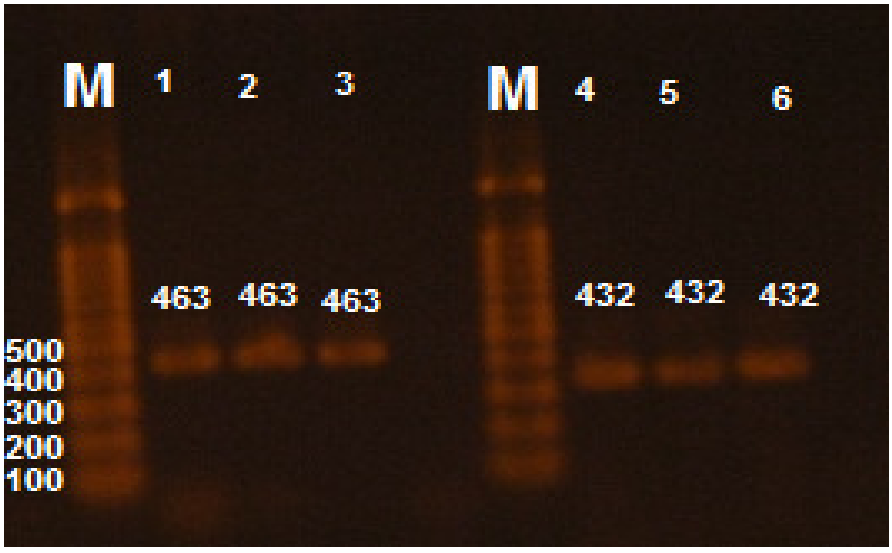

Figure 1: Agarose gel electrophoresis for amplified *DRD3* and *HTR2A* gene of schizophrenia patients and healthy individual. Bands were fractionated by electrophoresis on a 1.5 % agarose gel (2 h., 5V/cm^2, 0.5X TBE buffer) and visualized under U.V. light after staining with ethidium bromide staining. Lane: M:100bp ladder; Lane: 1,2,3, product for exon 1 *DRD3* gene (463 bp) and Lane: 4,5,6, product for exon 2 *HTR2A* gene (432bp).

Sequencing of coding regions of the amplified product of DRD3 and HTR2A of Exon 1 and Exon 2 for these samples were done seeking for detection of any polymorphism 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 and HTR2A gene of all groups (Healthy and Patients) with data published for known sequence seeking for enough homology. A homology with DRD3 gene of Homo sapiens from the Gene Bank was done using the BioEdit software. 100% compatibility of that gene was found with DRD3 and HTR2A genes from healthy with standard DRD3 and HTR2A genes of Gene Bank results as shown in figure (2). The polymorphism A1804G that we observed within the exon1 of DRD3 receptor gene are shown in figure (2), appear all sample of patient have 99% identities with wild type of Homo sapiens from the Gene Bank, this polymorphism G/A, at position 1804 of exon 1 change cod from AGC> GGC, It is a missense mutation that leads to changes in amino acid from Serine (S) to Glysine (G), that have a significant association is clearly determined between DRD3 genetic polymorphism and the presence of Schizophrenia disorder shown Figure (3) and Table (1) appear types of mutations detected in partial DRD3 (Exon1) gene of schizophrenia patients. Score of healthy sample compare with wild type of Homo sapiens have 400, however low score of patient to 398 and 397 score compare with wild type are shown in figure (2). In total, 8% of Schizophrenia had two transition mutations +1804 G/A and +1830 G/A single nucleotide polymorphism, the +1830 G/A SNP was silent mutation which result change of codon from GCG to GCA (Alanine to Alanine), and compatibility 99%, score 397 with the wild type of gene bank. However, the polymorphism of HTR2A genes from 50 cases schizophrenia patients was found to be compatible 99% and score 259 compare with wild type (259 score) with the wild type sequences of gene bank, there was no significant correlation between Schizophrenia and incidence of transition mutation C/T at nucleotide 156 of exon 2 HTR2A gene ($X2=0.055$, $P>0.05$), as shown in Figure (4,5) and Table (1).

A : *Homo sapiens* dopamine D3 receptor (DRD3) gene, partial cds

Score	Expect	Identities	Gaps	Strand
736 bits(398)	0.0	400/401(99%)	0/401(0%)	Plus/Plus

```
Query  157   TGGCATCTCTGAGCCAGCTGAGTGGCCACCTGAACTACACCTGTGGGGCAGAGAACTCCA  216
             ||||||||||||||||||||||| ||||||||||||||||||||||||||||||||||
Sbjct  1780  TGGCATCTCTGAGCCAGCTGAGTAGCCACCTGAACTACACCTGTGGGGCAGAGAACTCCA  1839
```

B: *Homo sapiens* dopamine D3 receptor (DRD3) gene, partial cds

Score	Expect	Identities	Gaps	Strand
734 bits(397)	0.0	401/403(99%)	0/403(0%)	Plus/Plus

```
Query  155   TATGGCATCTCTGAGCCAGCTGAGTGGCCACCTGAACTACACCTGTGGGGCGGAGAACTC  214
             ||||||||||||||||||||||| ||||||||||||||||||||||| ||||||||
Sbjct  1778  TATGGCATCTCTGAGCCAGCTGAGTAGCCACCTGAACTACACCTGTGGGGCAGAGAACTC  1837
```

C: Homo sapiens dopamine D3 receptor (DRD3) gene, partial cds

Score	Expect	Identities	Gaps	Strand
745 bits(403)	0.0	403/403(100%)	0/403(0%)	Plus/Plus

```
Query  155   TATGGCATCTCTGAGCCAGCTGAGTAGCCACCTGAACTACACCTGTGGGGCAGAGAACTC  214
             |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1778  TATGGCATCTCTGAGCCAGCTGAGTAGCCACCTGAACTACACCTGTGGGGCAGAGAACTC  1837
```

Figure (2): A: Sequencing of sense flanking the partial *DRD3* (Exon1) gene for 50 cases schizophrenia patients. B: Sequencing of sense flanking the partial *DRD3* (Exon1) gene for 8 cases schizophrenia C: Sequencing of sense flanking the partial *DRD3* (Exon1) gene for healthy as compared with standard *DRD3* obtained from Gene Bank.

Query represent of sample; Sbject represent of database of National Center Biotechnology Information (NCBI). The bit Score: Statistical measure of the moral similarity and the higher value indicates that the high degree of similarity, and if dropped from the class of 50 points, the sense that there is no similarity mention. Expectation value: Give an estimate of the number of times the expected to get the same similarity coincidental and the lower the value of E whenever this indicates that the degree of similarity high between sequences which gives greater confidence that this relay views already follow under study, as the value of a very close to zero means that these sequences are identical.

Score	Expect	Method	Identities	Positives	Gaps
159 bits(402)	2e-48	Compositional matrix adjust.	80/81(99%)	80/81(98%)	0/81(0%)

```
Query  1   MASLSQLSGHLNYTCGAENSTGASQARPHAYYALSYCALILAIVFGNGLVCMAVLKERAL  60
Wild   1   MASLSQLSSHLNYTCGAENSTGASQARPHAYYALSYCALILAIVFGNGLVCMAVLKERAL  60

Query  61  QTTTNYLVVSLAVADLLVATL  81
Sbjct  61  QTTTNYLVVSLAVADLLVATL  81
```

Figure (3): Amino acid sequence of the translated partial *DRD3* (*Exon 1*) of Schizophrenia patients, the character in group is the site of mutation with change from Seine to Glycine.

Table (1): Types of mutations detected in partial *DRD3* (Exon 1) and *HTR2A* (Exon 2) gene of schizophrenia patients. Query represent of sample; Sbject represent of database of National Center Biotechnology Information (NCBI)

Type of mutation	Predicted effect	Amino acid change	No. of sample	Nucleotide change	Location of gene bank	Name of gene
Transition	Missense1804	Serine (S) / Glycine (G)	50	AGC> GGC	601	*DRD3*
Transition	Silent 1830	Alanine (A) / Alanine (A)	10	GCG> GCA	610	*DRD3*
Transition	Silent 156	Serine (S) / Serine (S)	50	TCC >TCT	52	*HTR2A*

A: Homo sapiens serotonin (HTR2A) gene, HTR2A-C allele, partial cds

Score	Expect	Identities	Gaps	Strand	Frame
497 bits (269)	2e-137()	269/269 (100%)	0/269 (0%)	Plus/Minus	

```
Query  121  AGTCGACTGTCCAGTTAAATGCATCAGAAGTGTTAGCTTCTCCGGAGTTAAAGTCATTAC  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  184  AGTCGACTGTCCAGTTAAATGCATCAGAAGTGTTAGCTTCTCCGGAGTTAAAGTCATTAC  125
```

B: Homo sapiens serotonin (HTR2A) gene, HTR2A-C allele, partial cds

Score	Expect	Identities	Gaps	Strand	Frame
479 bits (259)	5e-132 ()	261/262 (99%)	0/262 (0%)	Plus/Minus	

Features:

```
Query  121  TGTCCAGTTAAATGCATCAGAAGTGTTAGCTTCTCCAGAGTTAAAGTCATTACTGTAGAG  180
            ||||||||||||||||||||||||||||||||||| ||||||||||||||||||||||
Sbjct  177  TGTCCAGTTAAATGCATCAGAAGTGTTAGCTTCTCCGGAGTTAAAGTCATTACTGTAGAG  118
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Figure (4): A: Sequencing of sense flanking the partial *HTR2A* (Exon2) gene for healthy and B: Sequencing of sense flanking the partial *HTR2A* (Exon2) gene for 50 schizophrenia patients as compared with standard *HTR2A* obtained from Gene Bank.

5-hydroxytryptamine (serotonin) receptor 2A [*Homo sapiens*]

Score	Expect	Method	Identities	Positives	Gaps
178 bits(451)	3e-55	Compositional matrix adjust.	88/88(100%)	88/88(100%)	0/88(0%)

```
Query  1   MDILCEENTSLSSTTNSLMQLNDDTRLYSNDFNSGEANTSDAFNWTVDSENRTNLSCEGC  60
Sbjct  1   MDILCEENTSLSSTTNSLMQLNDDTRLYSNDFNSGEANTSDAFNWTVDSENRTNLSCEGC  60

Query  61  LSPSCLSLLHLQEKNWSALLTAVVIILT  88
Sbjct  61  LSPSCLSLLHLQEKNWSALLTAVVIILT  88
```

Figure (5): Amino acid sequence of the translated partial *HTR2A* (*Exon 2*) gene of Schizophrenia patients, Query represent of sample; Sbject represent of database of National Center Biotechnology Information (NCBI).

Discussion

Genetic factors are important possibly in all cases, but it is not clear which genes are deviant and how they contribute to pathophysiology of the disease. Pharmacological profiles have divided dopamine receptors into DRD1, DRD2, DRD3, DRD4, and DRD5 subtype. DRD2 has been extensively studied a sit shows high affinity for neuroleptics binding (14), DRD3 is similar to DRD2 in that it shares common intronic sequences, cellular signaling pathways and has a similar affinity for typical neuroleptics (15 ,16). Shi *et al*. (17) showed that the DRD3 gene was not among the top seven candidates for predisposition to Schizophrenia. Utsunomiya *et al*. (18) suggest that no association between the DRD3 Ser9Gly polymorphism in Schizophrenia and Tardive dyskinesis (TD), in east Asian populations (Japanese, Chinese, and Korean). Molhotra *et al*. (19), shown allelic variation in DRD3 may not play a role in the pathophysiology of Schizophrenia or in clozapine response and Tee *et al*. (20), found no significant association of DRD3 Ser9Gly polymorphisms and COMT (rs16565) with Schizophrenia in Malays. Some studies suggested a significant association between the DRD3 Ser9Gly polymorphism and Schizophrenia (21). A common A206G transition in the sequence of the DRD3

gene that leads to a Ser9Gly amino acid substitution in the N-terminal extracellular domain of the receptor (A206G, Ser9Gly, rs6280), has been the most extensively variant in connection with Schizophrenia (10). Shaikh *et al*. (22) have reported a significant association between the DRD3 Ser9 allele and the Ser9/Ser9 genotype with Schizophrenia in 133 Caucasians. The DRD3 Ser9Gly polymorphism may be a contributing factor to the performance of eye movements used as a phenotypic marker of Schizophrenia (3). Sivagnonasundaram *et al.* (23), appeared Ser9Gly variant of dopamine D3 receptor gene and the corresponding coding changes may exert a combined or synergistic effect on susceptibility to Schizophrenia. Saiz, *et al*., (24) shown that genetic variant in the DRD2 gene and possible interaction between DRD3 and SLC6A3 genes are associated with Schizophrenia in a large Spanish sample. Zhang *et al*., (25) suggest that DRD3 a gene possibly under natural selection, might be involved in vulnerability to Schizophrenia in the Han Chinese population. The silent mutation T102C (Ser34 Ser) within the coding region of the 5-hydroxytryptamine type 2a receptor gene as well as the A206G transition in the sequence of the dopamine type 3 (DRD3) receptor gene are genetic polymorphisms previously implicated to confer susceptibility to psychiatric disorders (26 , 27). Zhang *et al.,* (11) shown T102C polymorphism of HTR2A of schizophrenic patients in two southern Chinese populations, No significant positive association with all schizophrenics. HTR2C and HTR2A gene polymorphisms seem to be associated with the occurrence of metabolic abnormalities in schizophrenics patients treated with olanzapine or clozapine (28). Melkersson*, et al*. (29) showed the -1438A/G, 102T/C and His452Tyr polymorphisms of the HTR2A gene are connected with a constitutive cellular change that causes susceptibility to schizophrenia. Baritaki, *et al.* (13) shown T102C transitions in the 5HTR2a a significant association with Schizophrenia, and manifested as increased risk of Schizophrenia for carriers of the T102 allele. The conclusion, A1804G transition in the sequence of the DRD3 gene that leads to a Ser9Gly amino acid substitution in the receptor has been the most extensively variant in connection with Schizophrenia, however T102C polymorphism of HTR2A of schizophrenic patients in Iraq populations, no significant positive association with Schizophrenics.

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