Correlating Schizophrenia with DRD3 Ser9Gly or HTR2 Receptor Gene Variants by using RFLP Method

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Abstract

Genetic polymorphism in many candidates associated with psychiatric disorder and Schizophrenia (SCZ). The current research is directed to see if the polymorphisms of Dopamine Receptor D3 (DRD3) or of the 5-Hydroxytryptamine Type 2A (HTR2A) genes is related to SCZ patients. From those admitted to Al-Rasheed Hospital, fifty patients with SCZ (half of them are males and the other half are females), in addition to control group (25 healthy individuals) were included in this study. Blood samples from each individual in both, healthy and control groups were collected, DNA was extracted. Specific primers for exon1 and another for exon 2 have been used to amplify the genes of DRD3 receptor and HTR2A. The data generated in this study indicated significant positive association between T102C polymorphism of HTR2A A1804G transition and DRD3 gene sequence, that resulted in the substitution of Ser9Gly amino acid Iraqi SCZ patients.

Keywords: *T102C polymorphism; HTR2A; RFLP*

Introduction

Schizophrenia (SCZ) is considered as a neuropsychiatric disease with different etiologies like genetics [(1)]. It is linked to different risks during the life of the patients of about 1% [(2)]. There are a lot of studies took in consideration the SCZ in the members of families, between twins and even those had been adopted, and the results confirmed that the is a strong relation between disease incidence and genetic factors [(3)].

It has been found that each of the silent mutation T-102C (Ser34Ser) and the A-206G transition (which brought about the substitution of a Ser9Gly amino-acid in the N-terminal of the receptor extracellular domain) in the genetic sequences of 5-HTR2A receptors and of DRD3 receptors; respectively, are genetic polymorphisms used to establish possibility of the psychological disorders [(4,5)]. Furthermore, other studies approved that there is a strong relation between schizophrenia and the variants of C-102 in the polymorphism of the T-102C [(6)] and also with the increment of homozygosity of the DRD3 polymorphism alleles [(7)]. Oppositely, some studied found there is no correlation, specifically with the Transition of DRD3 Ser9Gly [(1,8)]. The current research examined the connection of HTR2 gene with the genetic susceptibility to SCZ, and the relationship between DRD3 receptor gene and phenotypic expression of SCZ disorder within the Iraqi people.

Material and Results

Sample collection

Approximately 50 blood samples were collected from SCZ patients of both genders (25 female and 25 males with mean age 50.3-39.1) hospitalized at Al-Rasheed Teaching Hospital in Baghdad province by the consultant medical staff. Fifty healthy individuals (25 male and 25 females with mean age 48.2-36.6 years) donored blood samples which served as control group. Samples were preserved at 4C◦ till working on it.

Genes amplification

From the whole blood, genomic DNA has been extracted by using (QIAamp DNA Blood Mini Kit, USA), the DNA concentration and purification were conducted by using the NanoDrop ND-1000 spectrophotometer (NanoDrop Technology) in the Medical and Molecular Biotechnology dept,

Biotechnology Research Center, Al-Nahrian University, Baghdad, Iraq. Conventional PCR was used to amplify the region including the polymorphisms. By using both of F 5'-GCTCTATCTCCAACTCTCACA-3' primer and R 5'-AAGTCTACTCACCTCCAGGTA-3' primer, a 463 bp fragment of DRD3 exon 1 were amplified according to (Spurlock *et al.* 1998) [7]. A 342 bp fragment of HTR2 of from exon 1, which carries the T102C polymorphic site was amplified with F 5'-TCTGCTACAAGTTCTGGCTT-3', R5'-CTGCAGCTTTTTCTCTAGGG-3' according to (Williams *et al*.1996) [6]. The PCR amplification master mix /1 reaction was performed by using 25µl volume containing DNA (1.5 µl) ; Taq PCR PreMix (5 µl) (Bioneer, Korea); 10 Pmol (1µl) from each one the primers used; then, by using nuclease free water, the volume was completed to 25 µl. Regarding thermal cycling conditions of DRD3 and HTR2A, they were done as: Denaturation (94 °C ; 7min), then, 35 cycles of secondary denaturation (94 °C ; 35sec), annealing (62°C ; 45sec) & (54°C ; 35sec) respectively & (72 °C ; 35sec) with the final extension (72°C ; 10 min) by using the thermal Cycler (Gene Amp, PCR system 9700; Applied Biosystem).

After that, polyacrylamide gel (30%) were used for the electrophoreses of PCR products, the gel was already stained by using Ethidium bromide to enable the visualization of the product clearly under UV light.

RFLP analysis

The genotypes for the T-102C; and also, for A-206G transitions in the genes of 5-HTR2A and DRD3 receptor have been assessed according to (Spurlock *et al*.1998) [7]. The digestion reactions were done (50 µl) volume; (5 µl of 10X CutSmart , 5 µl of 200 ng/ µl amplicons 342 bp, or 463 bp, 0.5 µl of 2.5 U *MspI* or MscI) (New England Biolabs, USA), incubated for (4hr : 37C) .The *MscI* able to recognize, then to cleave the TGG↓CCA in blunt ends. *MspI* able to cleave the C↓CGG and leaves 5' protruding ends. Cleavage of the PCR amplicon (342 bp) by *MspI* gives a (342 bp) product of the wild-type allele T/T (T-102), & (126 bp & 216 bp) products for the mutant allele T/C (C-102).on the other hand, cleavage of the amplicon (463 bp) by *MscI* resulted in three different fragments size (47bp, 111bp & 305bp) as the products of the wild-type allele A/A Alanine to Alanine (A-206), and four different fragments size (47bp, 99bp, 111bp & 206bp) for mutant allele A/G (G-206). The polymorphisms of the different digested fragments of the DNA have been separated by using a 10% poly acrylamide and 2% Agarose stained with Ethidium Bromide.

Statistical analysis of the present study was achieved by using different statistical programs, X2 test was applied. The tests were considered significant when P value <0.01, compare between control and patients' group in % of mutation, Chi-square –X2 test was also used to compare the significance between percentage as shown in table 1.

Results and Discussion

The HTR2 receptor gene polymorphism results in both healthy and schizophrenia patients

In recent study the conventional PCR amplification resulted two amplicons indicated the *DRD3* partial gene (342 bp) and *HTR2* (463bp) amplicons gene of SCZ patients, bands are shown in figure 1.

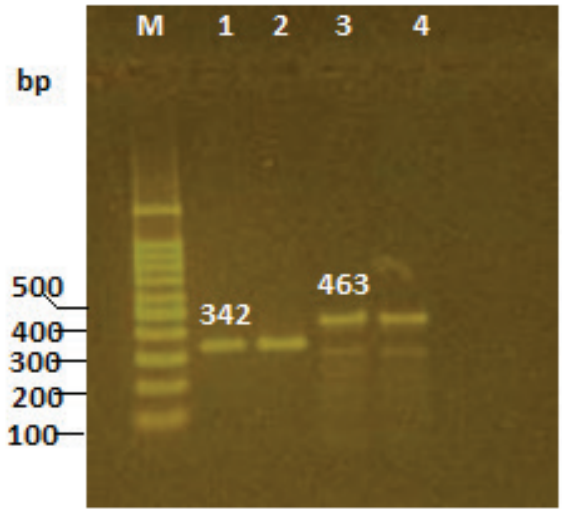

Figure (1): Electrophoresis (agarose gel) of *DRD3* amplicon (342 bp) and *HTR2* (463bp) amplicon of SCZ. The electrophoretic separation of different bands was achieved on a 2% agarose gel (2 h., 5 V / cm, 1X TBE) and then examined by U.V. light after been stained with ethidium bromide. Lane: 1 (M : 100 bp ladder), Lan1 and 2 *DRD3* bands sized (342bp). lan3, and 4 HTR2 bands sized (463bp)

The HTR2 receptor partial gene 342 bp fragments found on the chromosome-13 (long arm) was cleaved with *MapI* restriction endonuclease enzyme (RE) for detecting the frequencies of allele and their genotypes according to the transition of T-102C in the gene of HTR2. Out of 25 healthy participant 20 (80%) were showed two bands sized (216,126)bp respectively, indicated CC wild type homozygote genotype, and 5(20%) were showed three bands were sized (342,216,126)bp respectively,

indicated TC heterozygote genotype with significant relation between disorder and the allele T-102 (P<0.0001, odds ratio (OR 1.561>1), also the mutant TT genotypes was absent. (Figure 2) in compared with schizophrenia patient's genotypes according to the digestion result that showed one band sized 342 indicated higher percentage of TT genotypes 36 (72%), in comparison with TC heterozygote genotype 14(28%) that showed three bands (342,216,126) with OR 1.175 and (P<0.0001) less than the control OR considered significant. The allele frequency of C allele in healthy group (0.90) is higher than it in SCZ patient (0.14) but the allele frequency of T allele was found to be more frequent in group of patients (0.86) compare to healthy group (0.10). Table 1 shows the percentages of the frequencies of genotype and alleles of the polymorphism of HTR2 (T-102C).

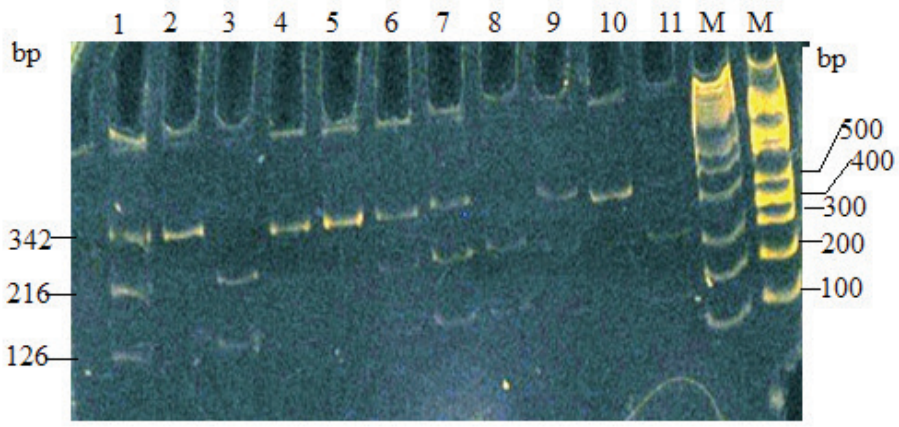

Figure (2): Polyacrylamide gel electrophoresis for amplified *HTR2A* gene (342 bp) of SCZ. The electrophoretic separation of different bands was achieved on 10% gel (3 h., 5 V / cm, 1 X TBE) and then examined by U.V. light after been stained with ethidium bromide.

Lane: 1,7,9,(342,216,26 bp fragment represent T-102/C-102 Heterozygote); Lane: 3, 11, (216,and 126 bp fragment represent C - 102 / C - 102 mutant-type homozygote); Lane : 2, 4, 5, 10(342 bp fragment represent T - 102 / T - 102 wild-type homozygote), Lane M (100bp ladder).

Table1. Genotype and allele frequency of HTR gene between the patients' group and control group.

Genotype of HTR	Control		Patients		Chi-Square (P-value)	O.R.
	No.	%	No.	%		
CC (Two band) 216, 126	20	80.00	0	0.00	13.055 ** (0.0001)	1.561
TC (three band), 342,216,126	5	20.00	14	28.00	4.007 * (0.0493)	0.628
TT (One band) 342	0	0.00	36	72.00	12.792 ** (0.0001)	1.175
Total No.	25	100%	50	100%	---	---
Chi-Square (P-value)	13.627 ** (0.0001)		11.944 ** (0.0001)		----	
Allele frequency						
C	0.90		0.14		----	
T	0.10		0.86			
* (P<0.05), ** (P<0.01).						

The results of polymorphism of the gene of DRD3 receptor gene in both healthy & schizophrenia patients

The nucleotide polymorphism (SNP) A-206G transition, which appears in the exon1 of DRD3 receptor was detected by cleaving site of *MscI* RE of the 463bp amplicon Figure 3. The allele frequency and genotypes are shown in Table 2. It has been observed that; all the control group individuals; 25 subjects (100%), showed three bands (305, 111, 47) bp indicated the AA wild homozygote genotype in comparison with zero of the patient subjects (0%), in addition to that, the appearance of four band sized (206,111,99,47)bp in 35 patient subjects (70%) refers to the higher percentage of the G-206/G-206 mutant-type homozygote, as well as, A-206G genotype heterozygotes in 15 patient subjects (30%). So, all the current findings refer to that there is a strong statically significant association between the G-206/G-206 genotype and schizophrenia (P>0.0001, OR 1.326). Higher A allele frequency 1in healthy group in compare with patient subjects 0.15 .on the other hand the G allele frequency are rising 0.85 in patients subject study, all data shown in Table 2.

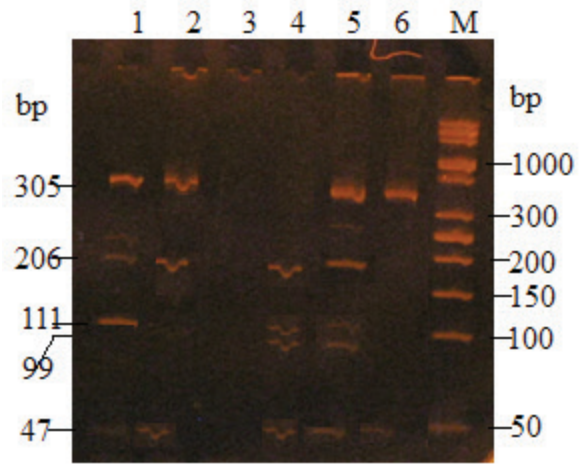

Figure (3): Polyacrylamide gel electrophoresis pattern for amplified *DRD3* gene (463 bp) digested with *MscI* of SCZ. The electrophoretic separation of different bands was achieved on 30% gel (2 h., 5 V / cm, 1 X TBE) and then examined by U.V. light after been stained with ethidium bromide. Lane: 1 and 5 (DNA band pattern of an A – 206 / G - 206 heterozygote); Lane: 2 (DNA band pattern of an A – 206 / A -206 wild - type homozygote). Lane 4 (DNA band pattern of a G - 206 / G-206 mutant - type homozygote).

Table2. Genotype and allele frequency of DRD3 gene between the patients' group and control group.

Genotype of DRD3	Control		Patients		Chi-Square (P-value)	O.R.
	No.	%	No.	%		
AA (Three band) 305,111,47	25	100	0	0.00	15.00 ** (0.0001)	2.00
AG (Five band), 305,206,111,99,47	0	0.00	15	30.00	8.750 ** (0.0026)	1.152
GG (Four band) 206,111,99,47	0	0.00	35	70.00	11.250 ** (0.0001)	1.326
Total No.	25	100%	50	100%	---	---
Chi-Square (P-value)	15.00 ** (0.0001)		11.250 ** (0.0001)		----	
Allele frequency						
A	1 (100%)		0.15		----	
G	0 (0.00%)		0.85			
** (P<0.01).						

Discussion

Many candidate genes in the brain, may interact with each other from the monoaminergic pathway, and are related to schizophrenia. Genus, *et al*., (2009) referred to that 1438 A / G, 102 T / C & His 452 Tyr polymorphisms of the gene of the HTR 2 A were liked with a different cellular change, which resulted in SCZ [9].

The present study indicated a significant positive connection between T102C polymorphism of HTR2A & SCZ. Zhang *et al,* (2004) found that there was a T102C polymorphism of HTR2A in two Chinese SCZ patients; but no significant positive correlation with all SCZ [10]. Polymorphisms of genes of HTR2C and HTR2A are thought to be the result of abnormal metabolism in patients with SCZ who were on olanzapine or clozapine [9]. Baritaki, *et al,* (2004) found that the T102C transitions in the 5HTR2A had a significant association with SCZ, and appeared as an increment in the risk of SCZ for those who carry the T102 allele [11]. Najwa Sh, (2014) assumed that the T102C polymorphism of HTR2A is not significantly correlated with SCZ in Iraq patients [12]. The findings of the current study conduct that the 1804 A/G SNP of the DRD3 gene is strongly related to the genetic tendency of the incidence SCZ in Iraqi people.

There are conflicting views in the DRD3 Ser9Gly polymorphism gene relationship with the appearance of symptoms of SCZ, as it has been shown in the case control studies which have been gave different results. Some of these results revealed a significant relation between them. Shi *et al.* (2008) conclude that the DRD3 gene was not nominated to the top seven candidates for predisposition to SCZ [13]. Other population study in East Asian (Chinese, Japanese and Korean) suggested that the polymorphism of DRD3 Ser9Gly is not connected to SCZ and Tardive dyskinesis [14]. Also, Tee *et al.* (2001) conducted in their results that DRD3 Ser9Gly polymorphisms and Catechol-O-methyl transferase (COMT) (rs16565) have no significant connection between with SCZ in Malays [15]. Furthermore, a study done on Han Chinese population concluded that the polymorphism has no effects on the genetic involvement for SCZ but refers to that variation may play role in exacerbating the symptoms SCZ [16].

Moreover, Talkowski *et al.*(2006) suggested that the DRD3 Ser9Gly polymorphism has a significant relation with SCZ [17], and Siaz,(2010) reveled that the genetic interaction of SLC6A3 with DRD3, and the DRD2 genetic variations are definitely connected with SCZ [18]. Also, Shaikh with his colleagues studied 133 schizophrenic Caucasians patients, and they found that the Ser9/Ser genotype is joined significantly with the allele of DRD3 Ser9 [19].

Conclusion

The present data indicated significant positive association between T102C polymorphism of HTR2A A1804G transition and the genetic sequence of the DRD3, which in turn causes the substitution of a Ser9Gly amino acid in the Iraqi SCZ patients.

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Conflict of Interest: Nil.

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