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Association between T-182C polymorphism of the norepinephrine transporter SLC6A2(NET) gene and major depressive disorder in Iraqi patients

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***Abstract*---**Major sympathetic nervous system neurotransmitter that is responsible for consciousness, attention, cognitive ability, as well as the function of the majority of body systems, glands, and the immune system, is called Norepinephrine (NE). Norepinephrine transporter (NET) and norepinephrine binding sites are decreased in the brains of MDD patients, showing that dysregulated synaptic NE plays a role in the pathophysiology of MDD. Indeed, different NET polymorphism combinations may be linked to different MDD sub-phenotypes. The aim of the current work is to investigate or detect if there is a relation between T-182C gene polymorphism of SLC6A2 gene with major depressive disorder (MDD) in Iraqi patients.Materials and Methods: Blood specimens were collected from 70 individuals with MDD and 20 healthy individuals as a control group (both patients and control ages ranged between 18-65 years). DNA extracted from white blood cells (WBC), and the T-182C gene was detected and amplified by the PCR-ARMS technique. Results: The distribution of TT, TC, and CC genotype ratio of MDD group was 22.9%, 27.1%, and 50.0%, respectively. While the control group show genotype distribution as 35.0% for TT, 55.0% for TC and 10.0% for CC ratio. The results exhibit that the C/C genotype ratio was more frequent in the MDD group when compared to the control group, in addition, the C allele was more frequent compared to the T allele. Conclusion: This study

revealed a significant link between the C/C genotype of SLC6A2 gene polymorphism and major depressive disorder.

***Keywords*---**norepinephrine neurotransmitter, norepinephrine receptors, ARMS-PCR, major depression disorder, SLC6A2.

Introduction

Major depressive disorder (MDD) is a heterogeneous disease responsible for the majority of disabilities worldwide, it's appeared in one of every five individuals [1]. MDD is a psychological disorder caused by several complexities, heterogenicity, and inflexibility of brain chemistry[2]. MDD is triggered or caused by several factors such as hereditary issues, stress, and pathological processes [3] [4]. Norepinephrine (NE) is a neurotransmitter synthesized, stored, and released by a neuronal system called the noradrenergic system, three structural groups in norepinephrine: catechol, amine, and amine [5] . Due to the release from the adrenal glands, norepinephrine acts primarily as a neurotransmitter with some functions as a hormone [6]. NE with the epinephrine released and bind to receptors of adrenergic (Ars) as a response to depression or anxiety, to initiate of diaphoresis, dilation of the pupils and constriction of the blood vessels, increase kidneys production to renin and inhibit the peristalsis activity [7] .

Norepinephrine role start when binding to G-protein which is coupled of α and β receptors to enhance neuronal signal transmission, but that occurs only when the concentration of NE is high [8] [9]. Alpha unit of adrenergic receptors composed of five subunits (α1A, α1B, α1C, α2A, α2B and α2C), while the Beta unit includes β1, β2, and β3 subunits [8], each type of those subunits in the site of binding G-protein with the NE neurotransmitter acts different roles effecting on memorizes, intellect, fearness and learning [6]. Norepinephrine transmitter NET belongs to Na+/Cl- dependent transporter which is coded by a gene called Solute Carrier, family 6 (SLC6) gene, the human NET carried by SCL6 gene family, member 2 (SLC6A2) which locate on chromosome 16q within 14 exons exons [10], [11]Previous studies implicated NET gene / SLC6A2 polymorphisms in the etiology of MDD. Recently, two single nucleotide polymorphisms (SNP) were detected in the SLC6A2 gene, G1287A which is located in exon 9, and T182C which is located in the promoter region, which was very related to MDD disorder[12].

Individual differences in behavior and susceptibility to depression have been linked to changes in the gene coding for NET that modify neurotransmitter release. The susceptibility of depression is affected by many factors as we mentioned previously but there are differences at individuals' levels that happened according to the behavior of an individual which is thought to be linked to gene polymorphism of NE that modifies releasing of neurotransmitters [13] . For example, the polymorphism NET-T182C gene is associated with risk increasing as a result of depression [14], [15] , while [16], found that SLC6A2 gene polymorphisms do not enroll in the etiology of MDD with suicidality.

Materials and Methods

Sample collection

The study involved 70 individuals with MDD from Imam Sadiq Hospital in Babylon Province, Iraq (50 men and 20 women), and the control group which represents healthy individuals has included 20 individuals (10 men and 10 women). Depressed patients and controls ranged in age from 18 to 65 years old. According to (DSM-IV), patients with other disorders were ruled out by meticulous clinical interview, as were those with serious medical illness. Subjects who had no history of psychiatric illnesses or previous psychiatric treatment were included in this study. All of the participants, including patients and controls, gave their consent.

Extraction and genotyping

The DNA extraction from white blood cells was perfumed by using Geneaid blood Kit (Taiwan), genotyping of NET gene polymorphism carried out by Polymerase Chain Reaction (PCR) to amplify the targeted genes using the Amplification Refractory Mutation System of PCR technique (ARMS-PCR). The primers set used in this study include (5' CCA TTT GGG GCA GGC GAA AGT 3') as a forward primer and (5' GAC GCA GGG TTC CCA GAA AAA TA 3') as a reverse primer for T/T and C/C of NET genotype, as well as the mutant-specific anti-sense primer '5-GAC GCA GGG TTC CCA GAA AAA TG-3.' This resulted in a 164 bp with either the wild or mutant anti-sense primer [17]. The reactions was performed in a total volume of 25 µL, containing 2 µl of genomic DNA, 12.5 µl of prermix, 8.5 µl of d.d H_2O and 1 µl for each forward and wild/ or mutant reverse primer. The cycling conditions were an initial denaturation at 95°C for 30 seconds, followed by 30 cycles of 95°C for 30 seconds, 61°C for 30 seconds, 72°C for 30 seconds, and a final extension at 72°C for 10 minutes.

Statistical Analyzes

Statistical analysis perfumed by SPSS software programming version 23.0, Chi-square analysis used to compare allele and genotyping frequencies of both control and patient groups, Hardy-Weinberg equilibrium was determined. The p-value of 0.05 was chosen as the crucial value.

Results

Molecular analysis

As shown in (figure 1) the electrophoresis bands of extracted genomic DNA from WBCs from both control and patient groups were appeared clearly and well extracted.

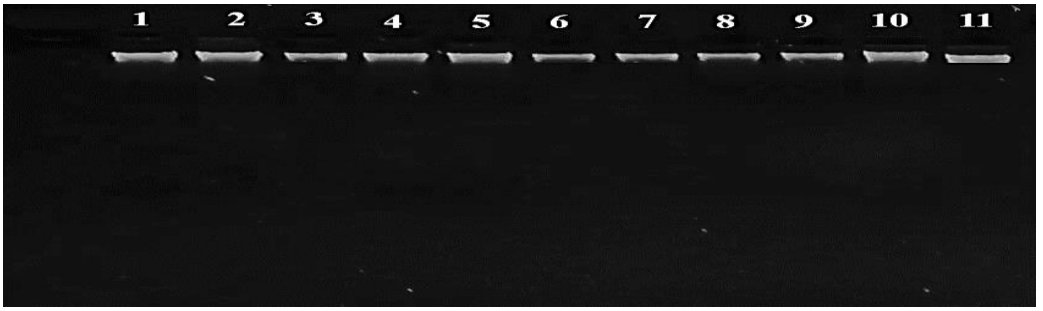

Figure 1: Electrophoresis pattern of genomic DNA extracted from white blood cells of patients with MDD (lanes 1-6) and healthy subjects' group (lanes 7-11), 0.7% agarose, 70V, 20 mA for 30 minutes.

Genotyping of NET gene polymorphism using PCR-ARMS

In both the control and patient groups, the PCR product of NET gene amplification was 164 bp (Figure 2&3). Table 2 shows the allele distribution genotype of T182C polymorphism of NE gene in MDD and the control group, and the results referred to significant differences between allele frequencies of MDD and control. In terms of NET genotype distribution, the allele ratios of TT, TC, and CC in patients with MDD were 22.9 percent, 27.1 percent, and 50 percent, respectively, while the ratios of the same alleles in the control group were 35 percent, 55 percent, and 10 percent, respectively. The frequency of alleles for the NET gene was 36.4 percent, 63.6 percent for T and C in the MDD group, while frequencies were 62.5 percent, 37.5 percent for the control group. The results showed that the C/C was the most common genotype in MDD patients (P 0.01) than (OR: 0.13, CI:0.02-0.7) in the control group, while T/C genotype in the control group was more common (OR: 0.09, CI: 0.02-0.49). The C allele was the common than T allele with OR: 0.34 and CI: 0.17-0.71 (at P-value 0.01) as shown in table 1.

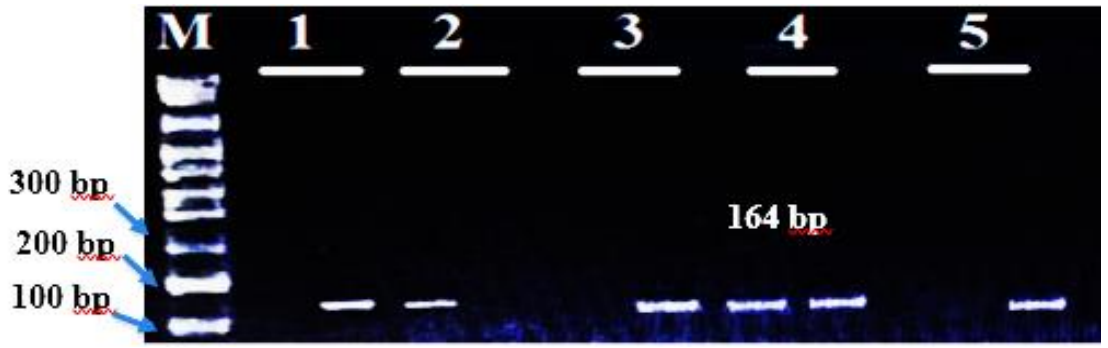

Figure 2: PCR product electrophoresis pattern of the" NET gene of five patients. Lane M: DNA ladder. Lanes: (1, 3, and 5) showed mutant type (C/C) genotype. Lane: (2) showed wild type (T/T) genotype. Lane (4) showed a (T/C) genotype. Different genotypes yielded.

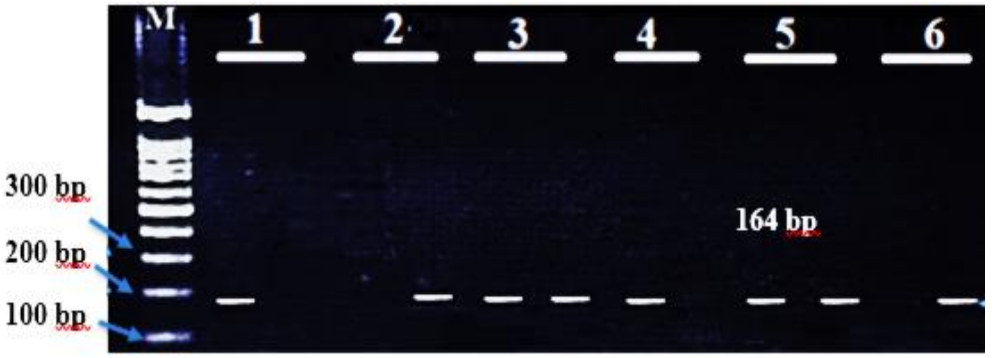

Figure 2: PCR product electrophoresis pattern of the" NET "gene in" six control subjects. Lane M: DNA ladder. Lanes: (1 and 4) showed wild type (T/T) genotype. Lanes: (2 and 6) showed mutant type (C/C) genotype. Lanes: (3 and 5) showed (T/C) genotype. Different.

Table 1: Genotype distribution and the odd ratio of norepinephrine transporter gene polymorphism of patient and control groups.

Genotype	Study Group		x2	Sig	Odds Ratio (95% CI)
	Control %	MDD %			
TT[a]	7 (35.0)	16 (22.9)	0.23	0.6	1.32 (0.42-4.21)
TC	11 (55.0)	19 (27.0)	6.97	0.008**	0.13 (0.02-0.70)
CC	2 (10.0)	35 (50.0)	10.35	0.001**	0.09 (0.02-49)
Total	20	70			
Allele Frequency					
T	25 (62.5)	51 (36.4)			
C	15 (37.5)	89 (63.6)	8.67	0.003**	0.34 (0.17-0.71)

[a]*Reference group*
***significant at P ≤ 0 .01.*

Discussion

According to the current study, there was a link between the NET gene's T-182C polymorphism and MDD. In a NET genotyping study, it was revealed significant differences between candidate MDD group with the T182C gene polymorphism (at P-value=0.01), C/C genotype was greater in patients with MDD 35 (50%) than the control group which showed 2 (10%) C/C genotype with OR: 0.13 and 95 percent CI: 0.02-0.70. Polymorphism of the T182C gene was a silent mutation which lead to no change in amino acid effect on produced protein, due to the presence of T182C in 5' end of the NET promoter region of the gene, but that’s not mean there

is no effect, because it may modulate the gene expression of a NET gene by preventing the transcription factors from promoter region binding [18].

The results of the current study concurred with those of [15] [19], they found an association between NET-CC polymorphisms and MDD in Korean and Chinese populations, whereas they conflicted with other studies that found a link between NET-TT genotype and MDD in Japanese and Chinese populations [17] [20]. Furthermore, further studies found no association between T182C polymorphisms and MDD [21-23] Norepinephrine neurotransmitter NET gene act as a target for Tricyclic anti-depressant drugs and selective norepinephrine inhibitors and because of that, the NET gene is responsible for norepinephrine reuptake by presynaptic nervous ends [24] [25] [13], on the other hand, According to [26], they suggested that the expression of receptor proteins affected by NET gene polymorphism, T/T genotype associate with high concentration of NE in plasma of healthy individuals than the C/C genotype, which may crate a link between the raised level of plasma and NE concentration at the end of synaptic fiber ends [27].

There are also an individual's behavioral differences, susceptibility to depression, and irritability all these factors may affect gene integration then neurotransmitter release is affected in its turn [14], an example, Miret, and colleagues refer that the SLC6A2 gene polymorphism strongly associate with the suicide tendency of MDD patients [28, 29], while another study suggested that the NET polymorphism in T182 C gene were not a factor or trigger for MDD susceptibility [30]. Ueda and colleagues found that the polymorphism in the T182C gene which coded for NET was very related to treatment with antidepressant drugs as a response [12].
In conclusion, when comparing the genotype of T/T, T/C, and C/C ratio, we found that MDD patients with the C/C genotype increased the risk possibility for major depression disorder.

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Abbreviation

Abbreviation	Term
NE	Norepinephrine
NET	Norepinephrine Transmitter
NA	Noradrenaline
MDD	Major Depressive Disorder
WBC	White Blood Cells
ARs	Adrenergic Receptors
SLC6	Solute Loci Carrier, Family 6
SNP	Single nucleotide Polymorphism
PCR	Polymerase Chain Reaction

ARMS-PCR	Amplification Refectory Mutation System of PCR technique

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