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D3 as a Possible Marker Based on D1-D4 Dopamine Receptors Expression in Paranoid Schizophrenia Patients

Sorour Ahmadian[1], Ghazal Delavari[2], Donya Ghanbari[2] and Davood Zaeifi[1]*

[1]*Department of Biology, Tonekabon branch, Islamic Azad university of Tonekabon,Tonekabon, Iran*
[2]*Institute of Molecular diagnostic and Microbial research center of BioBarcode, Tehran, Iran*

Abstract

Paranoid schizophrenia is one of several types of schizophrenia, a chronic mental illness in which a person loses touch with reality. The classic features of paranoid schizophrenia are having delusions and hearing things that aren't real. Based on Numerous studies about dopamine and schizophrenia, it suggested that changes in the dopamine systems are in related with schizophrenia, but still there is no clear direct evidence for dopamine hypothesis in schizophrenia.

In terminated examination, 20 paranoid schizophrenia patients mRNA from white blood cells extracted, then cDNA were synthesized. After Quantitive Real-time PCR examination with the related primaries for D1-D4 receptors were terminated and the compared consequences in abundance of genes expression with the normal samples reveal that D1-D4 dopamine receptors were expressed in all samples. Abundance of normal individuals were D1 100%, D2 6.6%, D3 40%, D4 86.6% and for patients were D1 100%, D2 26.6%, D3 33.3%, D4 73.3%. The results of this study reveals significant differences between D3 receptor apply to others. Therefore D3 has a possible clinical significance for using as rapid diagnosis of people who suspicious of paranoid schizophrenia.

Keywords: Dopamine receptors; Gene expression; Paranoid schizophrenia; Real-time PCR

Introduction

Paranoid schizophrenia is one of several types of schizophrenia, a chronic mental illness in which a person loses touch with reality. The classic features of paranoid schizophrenia are having delusions and hearing things that aren't real. Recent progress in molecular biology and imaging techniques has enabled new insight for schizophrenia research, but these methods are still limited by their availability and often reveal inconsistent results. Dopamine (DA) is a Catecholamine neurotransmitter, moreover is the most abundant neurotransmitters in CNS of animals. Dopamine, together with other catecholamine such as norepinephrine, is also a critical transmitter in sympato adrenergic terminals. Such terminals lie in close contact with immune cells in lymphoid organs and there is increasing evidence which points to the ability of dopamine to affect immune cell function [1]. Dopamine receptors are integral membrane proteins that interact with G proteins to transduce dopamine stimulation into intracellular responses. Dopaminergic neurons in the human central nervous system are involved in the control of motor activity and in emotional and cognitive processes [2]. The human genome is known to contain five genes encoding the functional dopamine receptors, DRD1, DRD2, DRD3, DRD4, DRD5 and two genes highly homologous to the DRD5 encoding the pseudogenes [3-5]. The expression of the dopamine receptors is well characterized in the brain but little work has been done to examine the expression in other tissue organs. Human Peripheral Blood Lymphocytes (PBL) expresses dopamine receptors and dopamine transporters and synthesizes endogenous dopamine and the related catecholamine norepinephrine and epinephrine through tyrosine hydroxylase dependent pathway [5]. Interestingly, dysfunction of dopaminergic pathways in PBL has been reported in neurological disorders characterized by dysfunctional central dopaminergic neurotransmission such as peripheral dopaminergic [6].

Although, it is yet unclear whether they simply mirror dysfunctional dopaminergic mechanisms or primarily reflect a dynamic interaction between the central nervous system and circulating immune cells. It has been proposed that neurotransmitter expression in peripheral immune cells reflects expression of these receptors in the brain. The purposes of this study were to examine if the mRNA of peripheral dopamine receptor is statically changed in paranoid schizophrenia, and whether or not these receptors have some value as a potential peripheral marker reflecting central one in paranoid schizophrenia.

Materials and Methods

Study design

The total number of subjects was 87 paranoid schizophrenia patients. 22 of them were chronic who had been taking antipsychotic drugs for more than 3 years (drug-med patients), 15 were drug-free who had not taken antipsychotic drugs for more than 3 months (drug-free patients), and 5 were drug-naïve who had never taken antipsychotic drugs (drug-naïve patients). For the controls, age and sex matched 20 healthy persons were enrolled. All patients fulfilled the DSM-IV criteria and the patients with a previous history of neurologic, neuropsychologic, medical, and surgical disease were excluded.

Quantitation of dopamine receptor RNA

Preparation of blood lymphocyte: 2 ml of peripheral blood samples were placed in 200 µl EDTA 0.05M falcon gradients and centrifuged in 2500 rpm for 20minutes. Upper layer moved in new falcon and washed by v/v PBS; then centrifuged in 1500 rpm for 10 minutes for two times. Separation of lymphocytes did not done laterthan one hour after drawing blood [7].

***Corresponding author:** Davood Zaeifi, Department of Biology, Tonekabon branch, Islamic Azad university of Tonekabon, Tonekabon, Iran, Tel:984271104; E-mail: gene_d.zaeifi@hotmail.com

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Extraction of total RNA and cDNA synthesis: Total RNAs were extracted from lymphocytes by High Pure RNA Isolation kit (Roche, Germany). 2 µg of total extracted RNA from lymphocytes were reverse transcribed into first-strand cDNA with Revert AID™ First Strand cDNA synthesis kit (Thermo Scientific, USA).

Oligonucleotide primer used for PCR amplification: The oligonucleotide primers used for PCR amplification of D1, D2, D3 and D4 dopamine receptor and beta-actin (βA) as internal controls were synthesized using a nucleotide synthesizer. Their sequences are shown at the Table 1.

Quantitative Real Time-PCR

PCR amplification was performed from 75 ng cDNA with 5 µl PCR buffer (pH 8.5, 2.5 mM $MgCl_2$), 4 µl 1.25 mM dNTP, 0.2 µl (5 U/µl) *Taq DNA polymerase*, 9.8 µl distilled water, 1 µl forward and reverse D1/D4primers in 10 µl final volume. PCR was carried out in alightCycler (Roche, Germany) after first denaturation at 95°C for 10minutes, and each cycle consisted of denaturation at 95°C for 5 seconds, annealing at 64°C for 10 seconds, and extension at 72°C for 30 seconds. The number of total PCR cycle was 35. PCR for βA was carried out for 35 cycles and each cycle was consisted of denaturation at 94°C for 5 seconds, annealing at 63°C for 10 seconds, and extension at 72°C for 9 seconds. For quantitative analysis, each 10 µl of PCR products were examined by electrophoresis on 1.5% agarose gel containing 0.5 µg/ml ethidium bromide [8].

Statistical test

Statistical tests were performed with SPSS software version 11.5. One-way ANOVA test and repeated measures of ANOVA were used to assess the association between drug-free, treated patients and healthy controls with beta-actin expression. P-values<0.05 were considered to indicate a statistically significant result.

Results

After performing tests on D1-D4 samples on healthy, naïve and treated patients and comparison of their expression to β-actin, and P value calculation, the following results were obtained (Figures 1-5).

Target	Primer	Primer sequence
βA	F	5'-TGA AGT GTC ACG TGG ACA TCC G- 3'
	R	5'- GCT GTC ACC TTC ACC GTT CCA G- 3'
DRD1	F	5'-AAA CCC ACA AGA CCC TCT GAT G- 3'
	R	5'- GAT GAA TTA GCC CAC CCA AAC C- 3'
DRD2	F	5-GCG GAC AGA CCC CAC TAC AA- 3'
	R	5'- AAG GGC ACG TAG AAG GAG AC- 3'
DRD3	F	5'-GGA GAC GGA AAA GGA TCC TCA CTC G- 3'
	R	5'- TCA GCA AGA CAG GAT CTT GAG GAA GG- 3'
DRD4	F	5'-CGGGATCCCACCCCAGACTCCACC - 3'
	R	5'- CGGAATTCCGTTGCGGAACTCGGC- 3'

Table 1: Primer used to amplify the dopamine receptors and beta-actin.

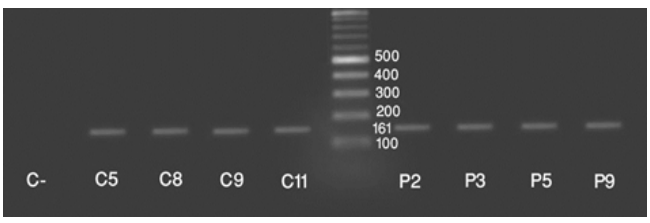

Figure 1: Examples of quantitative RT-PCR of β-actin mRNA in patients.

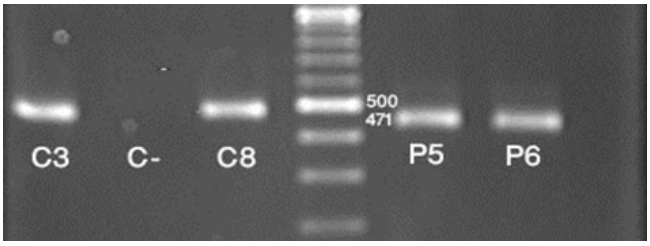

Figure 2: Examples of quantitative RT-PCR of D1 mRNA in patient.

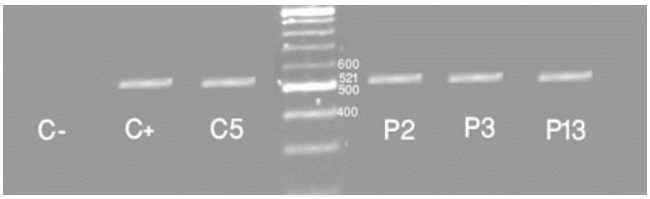

Figure 3: Examples of quantitative RT-PCR of D2 mRNA in patients.

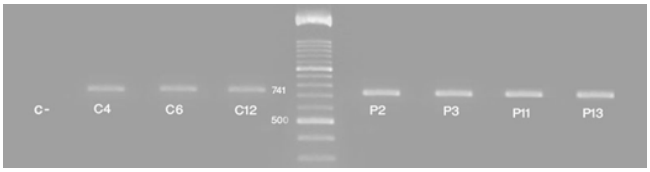

Figure 4: Examples of quantitative RT-PCR of D3 mRNA in patients.

Figure 5: Examples of quantitative RT-PCR of D4 mRNA in patients.

D1 receptor

In all three groups of healthy control, drug-naïve and treated expressed. However, statistically significant differences between paranoid schizophrenia patients with healthy controls and drug-naïve patients and healthy controls were not found. In both cases, comparison of P-value was >0.05.

D2 receptor

Expression state of this receptor in healthy controls was 6.66%, in drug-free patients were 40% and in treated ones were 20%. Statistical comparison between these three groups shows no significant differences in these groups, P-value>0.05.

D3 receptor

Expression state of this receptor in healthy controls was 40%, in drug-free patients were 60% and in treated ones were 20%. Statistical comparison between naïve patient and healthy controls reveal significant differences in these groups, P-value>0.05 and reveals that the D3 expression in drug-free patients has been increased in compare

with controls; but statistical comparison of healthy controls and treated patient did not show any significant difference.

D4 receptor

Expression state of this receptor in healthy controls was 86.6%, in drug-free patients were 60% and in treated ones were 80%. Statistical comparison between these three groups shows no significant differences in these groups, P-value>0.05.

Discussion

Dopamine and Dopaminergic neurons are localized incertain parts of the central nervous system and involvedin the pathophysiology of schizophrenia. However, thereis no direct evidence of dysfunction of brain dopaminergic systems in schizophrenic patients because no pathogenomic change in the dopaminergic system has been found at autopsy and the direct assessment of brain dopaminergic systems *in vivo* is almost impossible at present. In addition, abnormal function of the dopamine system results not only from the dopaminergic neuron, but from dopamine receptors and these dopamine receptors can be changed secondly by dopamine. Therefore, the importance of the physiological and pathologicalroles of dopamine receptors is emerging.

Done progress in molecular biology has revealed 5 distinct subtypes of dopamine receptors, and their structure and physiological functions have been identified. Owing to this advance, receptors other than D2 dopamine receptors, which are traditionally considered to be important in pathophysiology of schizophrenia, have been issued in relation to schizophrenia [9-11]. Among these other dopamine receptors, the D3 dopamine receptor are primarily localized in the limbic area has a high affinity for antipsychotics, and it has been found to beelevated in postmortem study of schizophrenic patients [12]. As a result, the D3 dopamine receptor is considered to be important in the pathophysiology of schizophrenia. Therefore, the notion that the effect of antipsychotic medication is more closely related to D3 dopamine receptors, while extrapyramidal drug side effects are related to the D2 dopamine receptor, is one of the modified dopamine hypotheses [13,14].

Our findings suggest that D3-receptor mRNA levels in PBLs may function as convenient and reliable peripheral markers for paranoid schizophrenia and thus assist in the early diagnosis and possible follow up of the illness. Early diagnosis and treatment of paranoid schizophrenia may have prognostic significance, because many consider that more optimal management at an early stage of the illness may alter its course [15]. In this manner, our observations would be of significant clinical and practical relevance. In addition to serving potentially as a possible marker, these changes in the D3-receptor subtype may further indicate its involvement centrally in the pathophysiology of paranoid schizophrenia and thus may potentially play a role in the development of medication suitable for management of the chronic disorder. Further studies, clearly warranted to test these observations, are now under way.

Conclusion

The results of this study reveals significant differences between D_3 receptor apply to others. The difference which either in P-value<0.05 and gel electrophoresis bond visual is observable and also reduces mutual mistakes.

Therefore this factor (D_3) has a possible clinical significance for using as rapid diagnosis of people who suspicious of paranoid schizophrenia.

Acknowledgment

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