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ORIGINAL ARTICLE

Evaluation of eNOS G/T 894 Polymorphism in Iranian patients affected by Catatonic Schizophrenia with type 2 Diabetes

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ABSTRACT
Catatonic schizophrenia is one of several subtypes of schizophrenia, a psychological disorder that usually emerges in late adolescence or early adulthood, however, it can appear at any time in life. Several studies have shown that Nitric oxide (NO) has several important roles in schizophrenia with type 2 Diabetes. Several studies have demonstrated that that eNOS might play a role in schizophrenia and type 2 Diabetes .We conducted study including a clinically well-defined group of 67 schizophrenia with type 2Diabetespatients and 60 healthy case to test the association between NOS3 G894T polymorphism and schizophrenia and type 2 Diabetes in Iranian population. So the NOS3 gene G894T polymorphism were carried out using ARMS-PCR method and then, the data were analyzed by SPSS software.The results of this study showed considerable association between Catatonic Schizophrenia with type 2 Diabetes disease and NOS3 gene G894T polymorphism in Iranian population.
Keywords:*Endothelial nitric oxide synthase, Schizophrenia, NOS3, Polymorphism, G/T894, type 2 Diabetes*

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INTRODUCTION

There are many common risk factors that eNOS G/T 894 polymorphism can play key roles in development of Catatonic schizophrenia patients with type 2Diabetes .Schizophrenia is a Multi-factorial disease that is the most common cause of dementia in elderly adults. Schizophrenia disease is characterized by progressive and advanced loss of memory and other Functional disorders. Heredity has been shown to play a significant role in schizophrenia pathogenesis. Many factors such as genetics, environment, neurobiology, and psychological and social processes have been demonstrated to be the most important contributory factors in the development of Schizophrenia, however, twin and family studies suggest that genetic factors play a major role in this mysterious disease.[1][2]

NO molecule contributes in cell signaling importantly. In the presence of oxygen, L-arginine, is converted to L- citrulline and release NO. The responsible enzyme for catalyzing this reaction is Nitric oxide synthase (NOS), and it occurs in three isoforms. [3][4]

NO is one of the simplest of biologically active molecules with exclusive chemical properties that constructed by three nitric oxide synthase (NOS) isoenzymes , including endothelial NOS (eNOS) , neuronal NOS (nNOS) and inducible NOS(iNOS).[5][6]Nitric oxide (NO) as a candidate molecule shows significant association in Schizophrenia. The NO increased expression levels' in plasma, serum and brain of patients affected by Schizophrenia emphasis on abnormal NO synthase pathway.[7][8]NO is one of the simplest of biologically active molecules with unique chemical properties that produced by three nitric oxide synthase (NOS) isoenzymes , including endothelial NOS (eNOS) , neuronal NOS (nNOS) and inducible NOS(iNOS).NO is constitutively expressed and produced by eNOS and nNOS whereas iNOS is often induced by a variety of factors related to inflammation.[9][10]

It has extensively established that nitric oxide (NO) is involved in Schizophrenia pathogenesis. Numerous genetic risk factors have been related with Schizophrenia, but no study has unraveled a possible association between Catatonic schizophrenia patients with type 2 Diabetes and G894T polymorphism.
Although association between nNOS genes Polymorphisms and schizophrenia was showed by some studies but there is lack of knowledge about association between eNOS and iNOS gene related polymorphisms and schizophrenia. [11][12]
Several studies have suggested that eNOS may interfere with schizophrenia. As a result, we studied eNOS G/T894 polymorphism in Iranian patient affected by Catatonic Schizophrenia with type 2Diabetes. We genotyped and analyzed 67 patients with Catatonic schizophrenia, and 60 healthy controls to define a possible role for the NOS3 gene G894T polymorphism in Iranian patients with Catatonic Schizophrenia with type 2Diabetes.
This study analyzed different populations, and found an increased frequency of the allele. We also found an association between the allele and Catatonic schizophrenia patients with type 2Diabetesand G894T polymorphism in our population.

MATERIAL AND METHODS

Patients and controls

All clinically confirmed, 67 schizophrenia patients along with 60 healthy controls were selected from Hazrat-e-Abolfazl Mental Rehabilitation Center, Hamadan, Iran. The schizophrenia group consisted of 49 males and 18 females, and the control group was comprised of 51 males and 9 females. All patients were diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV).

DNA Extraction:

Case and control blood samples were collected in EDTA treated tubes and total Genomic DNA was isolated from these samples using Quick-gDNA™ Blood MicroPrep Kit (Zymo Research, U.S.A.) according to manufacturer's instructions. Purity and concentration of genomic DNA was evaluated using Nanodrop and prepared a concentration 50 ng/µl as working tubes.

Genotyping of NOS3 gene:

ARMS-PCR reactions were performed using specific primers was designed by Primer3 online software. The reactions preparedin two tube labeled as normal and mutant forfinal volume 25 µl containing 30-120 ng total DNA from the patient, 10 pmol of each primers , 7.5µl distilled water and 12.5 µlTaq DNA Polymerase 2x Master Mix Red (Ampliqon, Danmark) .The PCR cycling conditions were carried out with an initial denaturation step for 7 min at 95 ºC, followed by 35 cycles of 75 s at 94 ºC, 55 s at 59ºC and 30 s at 72ºC and final extension step at 72ºC for 5 min.
Sequence of Primers was5´-AAGGCAGGAGACAGTGGATG-3´as forward primer,
5´-TGAAGGAAGAGTTCTGGTGGC-3´ as reverse normal primer and
5´-TGAAGGAAGAGTTCTGGTGGA-3´ as reverse mutant primer. Human beta-globin gene amplified in each reactions using specific primers, 5'-ACACAACTgTgTTCACTAgC-3' as forward and 5'-CAACTTCATCCACgTTCACC-3' , as internal control as well and the PCR product was run on a 2% Arose gel in 0.5× TBE buffer and visualized on a Gel Documentation System using Gel Red dye.

This polymorphism was genotyped by ARMS-PCR, with the primer pairs:

Primer	Sequence (5'->3')	Length	Tm	GC%
F_C	AAGGCAGGAGACAGTGGATG	20	59.38	55.00
R_N	TGAAGGAAGAGTTCTGGTGGC	21	59.93	52.38
R_M	TGAAGGAAGAGTTCTGGTGGA	21	58.31	47.62

Specifications primers used for internal control:

Primer	Sequence (5'->3')	Length	Tm	GC%
F	GTGTACCCCACCTGCATTCT	20	59.67	55.00
R	CCCAGCAAGGATGTAGTGAC	20	57.97	55.00

PCR program used for ARMS-PCR polymorphism G-T894:

cycle	temperature(Celsius)	Time
first	95	7Minutes
Two to thirty-five	94 59 72	1minute and 15 seconds 55Seconds 30seconds
thirty-six	72	5Minutes

Statistical Analysis

Statistical analyses were conducted using with the SPSS software (Statistical Package for Social Sciences) version18. Chi- square test ($\chi 2$), was used to test the association between two categorical variables or to detect difference between Catatonic schizophrenia patients with type 2 Diabetes and NOS3 gene G894T polymorphism.

RESULTS

The case and control include 67 and 60 individuals respectively. The average age forCatatonic schizophrenia patients with type 2 Diabetes individuals were 51±1.9 years and 47.4± 2.3 years for control group. In order to investigate the association between Catatonic Schizophrenia with type 2 Diabetes disease and NOS3 gene G894T polymorphism we genotyped and analyzed 67 patients with Catatonic schizophrenia and type 2 Diabetes and 60 healthy controls for the NOS3 and G894T polymorphism.

G894T polymorphism frequencies were in equilibrium in patients and controls. Patients did not show an extensively increased frequency of the G894T allele compared with controls. Thus the G894T allele would confer a slightly increased risk of developing late onset Catatonic schizophrenia patients with type 2 Diabetesin Iranian population.

Carriers of the G894T were at a slightly but significantly increased frequency in patients compared with controls. Both groups of healthy controls, older than 85 and younger than 65 years, did not had similar gene frequencies, suggesting that this polymorphism is related with Catatonic schizophrenia patients with type 2 Diabetes in Iranian population (table).

Table1:Genotype Table of G894T polymorphism:

		Genotype						Total
		GG		GT		TT		
GROUP	Case	22	36.6%	32	53.3 %	6	10%	60
	Control	76	92.7%	5	6.1%	1	1.2%	82
Total		98		37		7		142

The results of genotyping are depicted in Table1: The following genotypes were identified for NOS3 gene G894T polymorphism.

Table2: Allele Table of G894T polymorphism:

		Allele				Total
		G		T		
GROUP	Case	76	63%	44	37%	120
	Control	157	92%	7	8%	164
Total		233		51		284

Table 2 showed that there were significantly correlation between NOS3 gene G894T polymorphism and Catatonic schizophrenia patients with type 2. Therefore, NOS3 gene G894T polymorphism may be a genetic predisposing factor for schizophrenia in Iranian population.

Table3: Chi- square test ($\chi 2$) for analyzing G894T polymorphism:

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Genotype * Group	100	100.0%	0	.0%	100	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	24.233[a]	2	.000
Likelihood Ratio	24.689	2	.000
Linear-by-Linear Association	21.920	1	.000
N of Valid Cases	100		

DISCUSSION

Several lines of evidence suggest that G894T polymorphism may play a part in Catatonic schizophrenia disease by modulating inflammation. In accordance with this, an increased frequency of the allele among patients with Catatonic schizophrenia and type 2 Diabetes has been seen in this study.

The G894T polymorphism has been associated with brain disorders. To define a possible role of this polymorphism in brain disorders as a consequence of a decreased risk of developing Catatonic schizophrenia patients with type 2 Diabetes, we genotyped healthy controls. Gene frequencies were almost identical between controls, indicating that the G894T polymorphism is related with Catatonic schizophrenia patients with type 2 Diabetes in Iranian population.

This study analyzed different populations, and found an increased frequency of the allele. We also found no association between the allele and Catatonic schizophrenia in Iranian population. However, the difference between patients and controls was significant in Iranian population, and the fact that a similar result has been found in different populations suggests that the G894T polymorphism is truly involved in the development of Catatonic schizophrenia patients with type 2 Diabetes in Iranian population.

CONCLUSION

It is important to define a possible role of gene G894T polymorphism in brain disorders as a consequence of a decreased risk of developing Catatonic schizophrenia patients with type 2 Diabetes in Iranian population. As a result we studied eNOSG/T894 polymorphism in Iranian patient affected by Catatonic schizophrenia and type 2 Diabetes.

The results of this study showed considerable association between Catatonic schizophrenia patients with type 2 Diabetes and NOS3 gene G894T polymorphism in Iranian population.

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