

ORIGINAL ARTICLE

Evaluation of eNOS G/T 894 Polymorphism in Iranian Catatonic Schizophrenia Patients with Positive Response to Chlorpromazine Treatment

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ABSTRACT

Catatonic schizophrenia is a sophisticated neurodegenerative disorder with complex and Multi-factorial pathogenesis. Catatonic schizophrenia is just a mental disorder that typically occurs in late or early adulthood, though, it could appear any time in life. Heredity has been demonstrated to play a significant role in Catatonic schizophrenia pathogenesis. There are numerous common risk factors such as for instance NOS3 gene G894T polymorphism that play fundamental roles in the development of Catatonic schizophrenia disease. It's widely established that nitric oxide (NO) is associated with Catatonic schizophrenia pathogenesis. Many genetic risk factors have already been related with Catatonic schizophrenia, but no study has unraveled in regards to possible positive response to treatment with chlorpromazine in Catatonic schizophrenia patients with G894T polymorphism. In the current case control study, the eNOS polymorphisms (G894T polymorphism) have been investigated in 71 patients with Catatonic schizophrenia and 140 healthy subjects by utilizing ARMS-PCR method. Then, the info was analyzed by SPSS statistics 18 software. A substantial difference between cases and controls was found for the GG genotype in contrast to GT and TT genotypes ($P \leq 0.00$). As result NOS3 gene G894T polymorphism is actually a significant genetic predisposition factor for treatment in Iranian Catatonic schizophrenia patients.

Keywords: Catatonic schizophrenia, G894T Polymorphism, Nitric oxide Polymorphism, chlorpromazine

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INTRODUCTION

Catatonic schizophrenia is just a Multi-factorial disease that's the most frequent reason for dementia in elderly adults. Catatonic schizophrenia affects about 1% of population worldwide and characterized by severe mental symptoms, injury in social cognition, delusions, and hallucinations and decreased emotional affect that might influence patients standard of living and their own families [1-3]. Catatonic schizophrenia disease is characterized by progressive and advanced lack of memory and other Functional disorders [4, 5].

Many factors such as for instance genetics, environment, neurobiology, and psychological and social processes have already been demonstrated to be the most crucial contributory factors in the development of Schizophrenia; however, twin and family studies declare that genetic factors play an important role in this mysterious disease [6, 7]. Many eNOS polymorphisms have already been found. Three of their subjected to be related with disease pathophysiology. G/T 894 polymorphism is one of many main eNOS polymorphisms, to define a possible role for Catatonic schizophrenia patients. Nitric oxide is quite reactive molecules and its half-life is the main reason to be hidden until it discovers. In mammalian cells, nitric oxide is created by one type of NO synthetase .NOS included of three isoforms :neuronal NOS(Nnos,nos1),inducible NOS(iNOS,nos2)endothelial NOS (eNOS,nos3).all of NOS will change the L-arginine metabolism into the L-citruline and NO is another production of this technique .NADPH

and oxygen will become necessary as cofactor. NNOS exist in central neurons and peripheral neurons and several other neurons. INOS could be a reaction to lipopolysaccharide, cytokines, and a great many other factor .endothelial NOS which exist in endothelial cells and also in platelets, cardiac myocytes it discovered in a few special neurons brain [8,9, 10].

Three different isoforms of NOS produced from various genes generate NO: neuronal NOS (nNOS or NOS1), inducible NOS (iNOS or NOS2), and endothelial NOS (eNOS or NOS3) these isoforms are related in function and structure. Three isoforms of NOS have already been demonstrated: NOS, constitutive neuronal NOS, and endothelial constitutive NOS (ecNOS). There's an increasing proof that NO is associated with Catatonic schizophrenia. The oxidative stress brought on NO production in mental performance is one of much pathogenic mechanism in Catatonic schizophrenia. The NO oxidative stress in mental performance has been caused many signs related with Catatonic schizophrenia, One of many enzymes associated with NO synthesis, is eNOS that expressed at increased concentrations in glial cells from patients with Catatonic schizophrenia. Also, an amyloid protein, the major part of the senile plaques that characterize Catatonic schizophrenia, induces NO production by microglial cells and astrocytes [11, 12]. Chlorpromazine is a dopamine antagonist of the typical anti-psychotic class of medications possessing used to treat schizophrenia [13, 14]. Some evidence advocates that, with conservative dosing, the incidence of such effects for chlorpromazine might be comparable compared to that of newer agents. Chlorpromazine may deposit in ocular tissues when occupied in high dosages for long periods of time [15, 16, 17]. Consequently this study has been done to define a possible role involving the NOS3 gene G894T polymorphism in Iranian Catatonic schizophrenia patients and positive response to chlorpromazine treatment.

MATERIAL AND METHODS

Patients and controls:

All clinically confirmed, 71 Catatonic schizophrenia patients along with 140 healthy controls were selected from Hazrat-e-Abolfazl Mental Rehabilitation Center, Hamadan, Iran. The schizophrenia group consisted of 42 males and 29 females, and the control group was comprised of 106 males and 34 females. All patients were diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV). Blood samples were collected from each subject after fasting for 10-12 hours. DNA was extracted from whole blood samples and then we used Amplification Refractory Mutation System PCR (ARMS PCR) method. Formerly, the data were analyzed by SPSS software.

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. Thee NOS G/T894 polymorphism genotyping was performed base on ARMS-PCR by eNOS- READY GENE ZG Kit (Zima gene, Iran).

Genotyping of NOS3 gene:

ARMS-PCR reactions were performed using specific primers was designed by Primer3 online software. The reactions prepared in two tube labeled as normal and mutant for final 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, Denmark) .The PCR cycling conditions were carried out with an initial denaturation step for 7 min at 95 °C, followed by 35 cycles of 75 s at 94 °C, 55 s at 59°C and 30 s at 72°C and final extension step at 72°C for 5 min.

Sequence of Primers was 5'-AAGGCAGGAGACAGTGGATG-3' as forward primer,

5'-TGAAGGAAGAGTTCTGGTGGC-3' as reverse normal primer and

5'-TGAAGGAAGAGTTCTGGTGGGA-3' as reverse mutant primer. Human beta-globin gene amplified in each reactions using specific primers, 5'-ACACAAGTgTgTTCCTACTAgC-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	TGAAGGAAGAGTTCTGGTGGGA	21	58.31	47.62

Specifications primers used for internal control:

Primer	Sequence (5'->3')	Length	Tm	GC%
F	GTGTACCCACCTGCATTCT	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	1 minute and 15 seconds
	59	
	72	
thirty-six	72	5Minutes

Statistical Analysis

Statistical analyses were conducted using with the SPSS software (Statistical Package for Social Sciences) version 18. Chi-square test (χ^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

We genotyped and analyzed 71 patients with Catatonic schizophrenia (average above 65 years), and 140 healthy controls younger than 65 years (range 46 to 65), for the NOS3 and G894T polymorphism.

G894T polymorphism frequencies were in equilibrium in patients and controls. Patients showed 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 schizophrenia in 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 have similar gene frequencies, suggesting that this polymorphism is related with schizophrenia in Iranian population and these patients had better response to chlorpromazine treatment.

Table 1: Genotype Table of G894T polymorphism:
Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Genotype * Group	211	100.0%	0	.0%	211	100.0%

Genotype * Group Cross tabulation
Count

	Genotype	Group		Total
		PATIENTS	CONTROL	
	GG	21	0	21
	GT	30	85	115
	TT	20	55	75
	Total	71	140	211

Table 2: Allele Table of G894T polymorphism:

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

Table3:Chi-Square Tests table

Chi-Square Tests			
	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	45.992 ^a	2	.000
Likelihood Ratio	50.525	2	.000
Linear-by-Linear Association	19.935	1	.000
N of Valid Cases	211		

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 7.07.

Symmetric Measures

	Value	Asymp. Std. Error ^a	Approx. T ^b	Approx. Sig.
Interval by Interval Pearson's R	.308	.068	4.682	.000 ^c
Ordinal by Ordinal Spearman Correlation	.267	.071	4.011	.000 ^c
N of Valid Cases	211			

- a. Not assuming the null hypothesis.
- b. Using the asymptotic standard error assuming the null hypothesis.
- c. Based on normal approximation.

Risk Estimate

	Value
Odds Ratio for Genotype (1 / 2)	^a

a. Risk Estimate statistics cannot be computed. They are only computed for a 2*2 table without empty cells.

Table 3 is showing that there are significantly correlation between NOS3 gene G894T polymorphism and schizophrenia.

DISCUSSION

It has been shown that nitric oxide synthase (NOS) isoenzymes play several roles in Catatonic schizophrenia. Studies have also demonstrated that Patients With schizophrenia own an abnormal NOS/NO pathway [18, 19]. There is a growing evidence that the G894T polymorphism plays a part in the pathogenesis of schizophrenia [20].Several lines of evidence suggest that G894T polymorphism may play a part in Catatonic schizophrenia by modulating inflammation [21, 22]. In accordance with this, an increased frequency of the allele among patients with Catatonic schizophrenia has been seen in this study. In an attempt to discover a genetic marker to screen patients who are more susceptible to schizophrenia, NOS3 gene G894T polymorphism were investigated in this study. Our results demonstrated that above-mentioned polymorphism may be a genetic predisposing factor for Catatonic schizophrenia in Iranian population. This study analyzed different populations, and found an increased frequency of the allele. We also found an association between the allele and Catatonic schizophrenia disease in our 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. As a result NOS3 gene G894T polymorphism could be a significant genetic predisposition factor for in Iranian Catatonic schizophrenia patients. Therefore, NOS3 gene G894T polymorphism may be a genetic predisposing factor for Catatonic schizophrenia treatment with chlorpromazine in Iranian population.

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