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ORIGINAL ARTICLE

Evaluation of NOS3 gene rs1800779 Polymorphism in Iranian Patients affected by Alzheimer and Normal individuals

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ABSTRACT
Alzheimer's is the most typical kind of dementia, a general word for memory loss and other intellectual abilities serious enough to interfere with everyday life. Alzheimer's disorder accounts for 60 to 80 percent of dementia cases. Evidence suggests that NOS3 might have a role in this disorder; as a result we studied NOS3 gene rs1800779 polymorphism in Iranian patients affected by Alzheimer and normal individuals. In the present case-control study, the polymorphism of NOS3 gene rs1800779 has been investigated in 70 patients and 100 healthy subjects by using ARMS-PCR method. Then, the information was analyzed by SPSS software. The results of this research showed considerable association NOS3 gene rs1800779 polymorphism in Iranian patients affected by Alzheimer and normal individuals.
Key words: NOS3, gene rs1800779, Alzheimer, Iranian

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INTRODUCTION

Alzheimer's disease (AD) is the most typical type of dementia among aged people. Dementia is just a brain disease that dangerously touches a person's ability to perform everyday activities [1].

AD begins gradually. It first involves the areas of the brain that organize thinking, memory and language. People who have AD might have trouble remembering issues that occurred lately or names of men and women they were acquainted with [2]. A related problem, mild cognitive impairment (MCI), makes more memory problems than normal for individuals of exactly the same age. Many, but not absolutely all, individuals with MCI will develop AD [2, 3].

In AD, as time passes, symptoms become worse [4]. Individuals might not recognize the others or have problem in speaking, reading or writing. They might forget just how to brush their teeth or comb their hair [5]. Afterwards, they could become nervous, or get lost from residence. Finally, they require full care. This could cause huge pressure for family members who must take care for them [4, 6].

AD usually starts after age 60. The risk goes up as you grow older. Your risk can also be higher if a member of family has already established the ailment [7].

NOS3 (Nitric Oxide Synthase 3 (Endothelial Cell)) is just a Protein Coding gene [8]. Endothelial NOS is just a nitric oxide syntheses generating NO in blood vessels and is a part of regulating vascular tone by inhibiting smooth muscle contraction and platelet aggregation [9]. Variations in this gene are connected with susceptibility to coronary spasm. A relationship between a polymorphism in the gene and late-onset Alzheimer's disease has been reported [10].

As a result, we studied NOS3 gene rs1800779 polymorphism in Iranian patients affected by Alzheimer and normal individuals.

MATERIAL AND METHODS

This research was performed on 70 patients with Alzheimer and 100 healthy controls. The patient's samples were casually extracted from Hazrat-e-Abolfazl Mental Rehabilitation Center, Hamadan, Iran. The control group was selected from random participants whose health was established by medical diagnostic.

DNA extraction and PCR Reaction

Genomic DNA from venous blood samples were isolated using DNA Extraction Kit PGS (Model: PGS0051) in accordance with manufacturer's instructions. DNA were quantified with the NanoDrop technology (Thermo Scientific / NANODROP 1000 Spectrophotometer).The NOS3 gene rs1800779 polymorphism genotyping was performed base on the amplification-refractory mutation sequencing (ARMS) assay. The Thermal cycling conditions for ARMS-PCR were the following. Figure1 Utilizing the BIOER TECHNOLLOGY CO .LTD. (Model: TC-24/H.b) For The PCR We Used 20 µL Sample: 1 µL Forward Primer, 1 µL Reverse Primer, 6 µL Diluents' Water, 2 µL DNA 50 ng/ml, 10 µL Master Mix Sequence of Primers was 5'- GTT CCT TTC CCC AGC AGT G -3' ´as forward primer,5´-5'-AGA ATG CAT GTC ACG CTC T -3'as reverse normal primer and 5'-AGA ATG CAT GTC ACG CTC C -3'as reverse mutant primer.

PCR program used for NOS3 gene rs1800779 polymorphism:

cycle	Temperature (Celsius)	Time
first	95	Minutes 7
Two to thirty-five	94 59 72	1 minute and15seconds 55Seconds 30seconds
thirty-six	72	5Minutes

Gel Electrophoresis

The electrophoresis was carried out using 1% Gel Red stained agarose gel, at 80V for 35 min We Use Horizontal Electrophoresis Cell (Model: JY-SPAT) with TBE Buffer (PH=8.3) , Ladder Were Used 50bp DNA Ladder (JenaBioscience) After electrophoresis, the amplified PCR products were Perceive under U. V. light.

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 check the association between two categorical variables or even to detect difference between several proportions. Pearson chi-square was used to investigate the connection involving the NOS3 gene rs1800779 polymorphism and Alzheimer.

RESULTS

We analyzed 70 patients with Alzheimer genotype, and 100 healthy controls, for the NOS3 gene rs1800779 polymorphism.

Rs3738401 polymorphism frequencies were in equilibrium in patients and controls. Patients showed an extensively increased frequency of the rs3738401 polymorphism allele compared with controls. Thus the rs1800779 polymorphism allele would confer a slightly increased risk of developing late onset Alzheimer.

Table1: Genotype Table of NOS3 gene rs1800779 polymorphism:

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Genotype * Group	170	100.0%	0	.0%	170	100.0%

Genotype * Group Cross tabulation

Count

		Group		
		1	2	Total
Genotype	1	22	93	115
	2	38	6	44
	3	10	1	11
Total		70	100	170

The results of genotyping are depicted in Table1: The following genotypes were identified for NOS3 gene rs1800779 polymorphism.

Table2: ALLEL Table of NOS3 gene rs1800779 polymorphism:

Allele * Group Cross tabulation

Count

		Group		
		CASE	CONTROL	Total
Allele	G	82	192	274
	T	58	8	66
Total		140	200	340

Table 2 showed that there were significantly correlation between NOS3 gene rs1800779 polymorphism and Alzheimer. Therefore, NOS3 gene rs1800779 polymorphism may be a genetic predisposing factor for Alzheimer in Iranian population.

Table3: Chi- square test ($\chi 2$) for analyzing NOS3 gene rs1800779 polymorphism:

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	71.401[a]	2	.000
Likelihood Ratio	76.330	2	.000
Linear-by-Linear Association	62.515	1	.000
N of Valid Cases	170		

a. 1 cells (16.7%) have expected count less than 5.
The minimum expected count is 4.53.

DISCUSSION

There is growing evidence that the NOS3 gene rs1800779 polymorphism plays a part in Iranian patients affected by Alzheimer. In accordance with this, an increased frequency of the allele among patients with Alzheimer has been seen in this study.

This study analyzed different populations, and found an increased frequency of the allele. We also found an association between the allele and Alzheimer's 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 NOS3 gene rs1800779 polymorphism is truly involved in Iranian patients affected by Alzheimer.

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