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ORIGINAL ARTICLE

Evaluation of DISC1 rs3738401 polymorphism in Iranian patients affected by Alzheimer and normal individuals

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ABSTRACT
Alzheimer's disease (AD) is the most frequent form of dementia among older people. AD begins slowly. It first involves the elements of the mind that control thought, memory and language. People who suffer AD could have trouble remembering issues that occurred recently or names of men and women they know. In this study, the evaluation of the DISC1 rs3738401 polymorphism in Iranian patients affected by Alzheimer and individuals was investigated. In the present case-control study, the polymorphism of DISC1 rs3738401 has been investigated in 60 Alzheimer patients and 100 healthy subjects by using ARMS-PCR method. Then, the data were evaluated by SPSS software. In summary, the end result of present study shows considerable relation between DISC1 rs3738401 polymorphism in Iranian patients affected by Alzheimer and individuals. It could be a significant genetic predisposition factor.
Keywords:*DISC1,rs3738401,polymorphism,Alzheimer*

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INTRODUCTION

Alzheimer's disease is named after Dr. Alois Alzheimer. In 1906, Dr. Alzheimer detected changes in the brain tissue of a lady who had died of a strange mental disease [1]. Her symptoms included memory loss, language difficulty, and unpredictable performance [2]. After her death, Dr. Alois Alzheimer checked up her brain and found many nonstandard clumps which now is called amyloid plaques and tangled bundles of fibers [3].

Alzheimer's disease is definitely an irreversible, progressive brain illness that slowly destroys memory and thinking ability and eventually even the capacity to carry out the simplest tasks [4]. Generally in most people who have Alzheimer's, symptoms first come into view after age 65. Estimates differ, but specialists claim that up to 5 million Americans age 65 and older might have Alzheimer's disorder [5]. Alzheimer's disorder is the most frequent cause of dementia between adult people. Dementia is the increasing loss of cognitive functioning-thinking, remembering, and reasoning-and behavioral abilities, to this kind of extent that it inhibits a person's everyday life and activities [6].

Although we still don't know how the Alzheimer's disease process starts, it looks likely that damage to the brain begins 10 years or maybe more before troubles become obvious [7]. Through the preclinical phase of Alzheimer's disorder, individuals are free from any symptoms but toxic changes are going on in the brain [8].

Alzheimer's disease is generally diagnosed on the basis of the person's medical history, history from family members, and behavioral observations [9]. The clear presence of characteristic neurological

and neuropsychological features and the lack of alternative conditions are supportive. The diagnosis could be confirmed with quite high accuracy post-mortem when brain material can be acquired and can be examined histological [10].

Disrupted in schizophrenia 1 is just a protein which is determined by the DISC1 gene in humans. Several investigations have revealed that unregulated expression or distorted protein structure of DISC1 may predispose persons to the development of schizophrenia, clinical depression, bipolar disorder, and other psychiatric conditions [11]. The cellular functions which can be disrupted by permutations in DISC1which direct to the development of those diseases, have yet to be obviously defined and are the main topic of present ongoing study. In harmonization with a wide selection of interacting partners, DISC1 has been publicized to be involved in the regulation of cell proliferation, differentiation, migration, neuronal axon and dendrite result, mitochondrial movement, and cell-to-cell adhesion [12, 13].

The DISC1 gene is placed at chromosome 1q42.1 and overlies with DISC2 open reading frame.Multiple DISC1 isoforms have been recognized at the RNA level, including a TSNAX-DISC1 trans gene splice variant, and at the protein rank. Of the isolated RNA isomers, 4 have been confirmed to be translated that is extended form (L), long variant isoform (Lv), tiny isoform (S), and particularly tiny isoform (Es) [14]. Human being DISC1 is transcribed as two major splice variants, L shape and Lv isoform. The L and Lv transcripts use distal and proximal join sites, correspondingly, in exon 11. The L and Lv protein isoforms differ by only 22 amino acids within the C-terminus [15].

The present study was done including a number of 60 Iranian patients suffering from Alzheimer and 100 normal subjects by using ARMS-PCR technique. In conclusion, the facts acknowledged from this investigation were analyzed by SPSS software. In a word, the end outcome of this study demonstrates considerable relation between DISC1 gene rs3738401 polymorphism in Iranian patients affected by Alzheimerand individuals. It could be a significant genetic predisposition factor.

MATERIAL AND METHODS

This research was performed on 60 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 samples of both case and control group were extracted using proteinase K digestion predicated on manufacturer's instructions. Nanodrop was used to judge the purity and concentration of genomic DNA. The reactions prepared in two tube containing 1 ng/ml forward primers, 1 ng/ml reverse primers, 6ml distilled water and 12.5 µlTaq DNA Polymerase 2x Master Mix Red. DISC1 gene rs3738401 polymorphism was used as primer gene. The principle supply of gene sequence information was extracted from NCBI website. { The first denaturation step was carried out for 15 min at 94 °C, followed closely by second denaturation step at exactly the same temperature for 20 seconds. The PCR cycling conditions was prepared for 45 sec at 45 °C, accompanied by 30 cycles of 45 sec at 72 °C. PCR product was operate on a 2% Arose gel in 0.5× TBE buffer and visualized on a Gel Documentation System using Gel Red dye.

Fifty healthy blood donors were used as controls. Genomic DNA was amplified by polymerase chain reaction (PCR) with congruous primers. Primers were designed based on a Primer Blast program at NCBI .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. Human beta-globin gene amplified in each reactions using specific primers, 5'-ACACAACTGTGTTCACTAGC-3' as forward and 5'-CAACTTCATCCACGTTCACC-3' ,.The DISC1 gene rs3738401 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. The electrophoresis was carried out using 1%Gel Redstained 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 DISC1 gene rs3738401 polymorphism of the endothelial nitric oxide synthase gene and Alzheimer.

RESULTS

We analyzed 60 patient's genotyped of Alzheimer, and 100 healthy controls younger than 65 years, for the DISC1 gene rs3738401 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 rs3738401 polymorphism allele would confer a slightly increased risk of developing late onset Alzheimer.

Table1: Genotype Table of DISC1 gene rs3738401 polymorphism:

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Genotype * Group	160	100.0%	0	.0%	160	100.0%

Genotype * Group Cross tabulation

		Group		
		Case	Control	Total
Genotype	GG	18	87	105
	GT	36	11	47
	TT	6	2	8
Total		60	100	160

The results of genotyping are depicted in Table1: The following genotypes were identified for DISC1 gene rs3738401 polymorphism.

Table1: Chi- square test ($\chi 2$) for analyzing DISC1 gene rs3738401 polymorphism:

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	99.622[a]	2	.000
Likelihood Ratio	105.255	2	.000
Linear-by-Linear Association	90.536	1	.000
N of Valid Cases	160		

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 5.60.

Symmetric Measures

		Value	Asymp. Std. Error [a]	Approx. T[b]	Approx. Sig.
Interval by Interval	Pearson's R	-.603	.045	-11.903	.000[c]
Ordinal by Ordinal	Spearman Correlation	-.629	.048	-12.756	.000[c]
N of Valid Cases		160			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

c. Based on normal approximation.

DISCUSSION

The data shown in the piece of writing confirms that DISC1 rs3738401 polymorphism plays an important partin Alzheimer of Iranian patient. According to this,an increased frequency of the allele among patients with Alzheimer has been observed. By analyzing a crowd of Iranian patients, it is found that the DISC1 gene rs3738401 has been associated with Alzheimer's a result DISC1 gene rs3738401 polymorphism is in

fact a noteworthy genetic tendency factor for in Iranian Alzheimer patients. Hence, DISC1 rs3738401 polymorphism may be a genetic predisposing factor for Alzheimer treatment in Iranian population.

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