Advances in Bioresearch
Adv. Biores., Vol 6 (2) March 2015: 95-98
©2015 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
ICV 7.20 [Poland]

Advances in Bioresearch

ORIGINAL ARTICLE

Relation between DISC1 gene rs3738401 Polymorphism in Iranian Patients affected by Bipolar Disorder

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ABSTRACT
Bipolar disorder, also known as bipolar affective disorder is a mental illness characterized by periods of elevated mood and periods of depression. Bipolar disorder is very severe and may cause dangerous behavior, even suicidal tendencies. The cause is not obviously understood, but both genetic and environmental factors play an important role. In this study, the association of the DISC1 gene rs3738401 polymorphism in Iranian patients affected by Bipolar disorders and individuals was investigated. The present study was conducted including a total number of 100 Iranian patients suffering from Bipolar disorders and 150 normal subjects by utilizing ARMS-PCR method. Finally, the data received from this investigation was analyzed by SPSS software. In summary, the end result of present study shows considerable relation between DISC1 gene rs3738401 polymorphism in Iranian patients affected by Bipolar disorder and individuals. It could be a significant genetic predisposition factor.
Keywords: DISC1 gene rs3738401 polymorphism, Bipolar disorder,

Received 24/12/2014 Accepted 09/02/2015

How to cite this article:
Ehsan J, Aleme M, Ali M S L , Hossein T M, Bahar R, Zahra F. Relation between DISC1 gene rs3738401 Polymorphism in Iranian Patients affected by Bipolar Disorder. Adv. Biores., Vol 6 [2] March 2015: 95-98**.** DOI: 10.15515/abr.0976-4585.6.2.9598

INTRODUCTION

Bipolar disorder, also referred to as manic-depressive disease, is just a brain disorder that creates abnormal shifts in mood, energy, activity levels, and the capability to carry out day-to-day tasks. They are distinctive from the ordinary ups and downs that everyone experiences from time to time [1]. Symptoms of bipolar disorder are severe. Bipolar disorder symptoms may result in spoiled relationships, job or school, and even suicide. But bipolar disorder could be treated, and patients with this illness can lead productive lives [2,3].

Bipolar disorder is difficult to identify when it starts. Some individuals suffer for a long time before they're properly diagnosed and treated. Like diabetes or cardiovascular illness bipolar disorder is actually a long-term illness that have to be carefully managed during your life.

Bipolar disorder regularly appears in the late teens or early adult years. At least 50% of all cases start before age 25. Some individuals have their first symptoms throughout childhood, while others may develop symptoms late.

Disrupted in schizophrenia 1 is a protein that is determined by the *DISC1* gene in humans [4]. A number of 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 [5]. The cellular functions that are disrupted by permutations in DISC1,which direct to the development of these illnesses, have yet to be evidently defined and are the subject of

present ongoing study. In coordination with a broad range of interacting partners, DISC1 has been publicized to participate in the regulation of cell proliferation, differentiation, migration, neuronal axon and dendrite result, mitochondrial movement, and cell-to-cell adhesion [6].

The DISC1 gene is located at chromosome 1q42.1 and overlies with DISC2 open reading frame [7].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 isextended form (L), long variant isoform (Lv), tiny isoform (S), and particularlyminiature isoform (Es). Human DISC1 is transcribed as two main 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 [8].

Bipolar disorder, Schizophrenia and schizoaffective disorder are typical psychiatric disorders with high heritability and variable phenotypes. The Disrupted in Schizophrenia 1 ***(***DISC1***)*** gene, on chromosome 1q42, was initially discovered and connected to schizophrenia in Scottish kindred carrying a balanced translocation that disrupts DISC1 and DISC2***.*** In recent times DISC1 was associated with Bipolar Disorders.

The present study was done including a number of 100 Iranian patients suffering from bipolar disease and 150 normal subjects by utilizing ARMS-PCR method. Finally, the facts received from this investigation were analyzed by SPSS software. In summary, the end result of present study shows considerable relation between DISC1 gene rs3738401 polymorphism in Iranian patients affected by Bipolar disorder and individuals. It could be a significant genetic predisposition factor.

MATERIAL AND METHODS

This research was performed on 100 patients with Bipolar disorder and 150 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

DNA samples of both case and control group were extracted using proteinase K digestion predicated on manufacturer's instructions. Nano drop 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 ona 2% Arose gel in 0.5× TBE buffer and visualized on a Gel Documentation System using Gel Red dye.

Patients and controls:

This study included 50 patients with Bipolar disorder diagnosed by neurologist From Abalfazl mental institution of Hamadan. Blood sampling was performed after an educated consent either at the diagnosis.

Fifty healthy blood donors were used as controls. Genomic DNA was amplified by polymerase chain reaction (PCR) with congruous primers

Primer

Primers were designed based ona 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' ,

DNA extraction

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)

PCR

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

Gel Electrophoresis

The electrophoresis was carried out using 1%GelRedstained 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 (Jena Bioscience) 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 Bipolar disorder

RESULTS

We analyzed 100 patients genotyped with Bipolar disorder, and 150 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 Bipolar disorder.

Table1: Genotype Table of DISC1 gene rs3738401 polymorphism:

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Genotype * Group	250	100.0%	0	.0%	250	100.0%

Genotype * Group Cross tabulation

Count

		Group		
		Case	Control	Total
Genotype	GG	31	137	168
	GT	56	12	68
	TT	13	1	14
Total		100	150	250

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	250		

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		250			

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]

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	250		

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

DISCUSSION

The evidence shown in the article proves that DISC1 gene rs3738401 polymorphism has an important role Bipolar disorders of Iranian patients. According to this, an increased frequency of the allele among patients with Bipolar Disorders has been seen.

By analyzing a group of Iranian patients, it is found that the DISC1 gene rs373401 has been associated with Bipolar disorder. Consequently DISC1 gene rs3738401 polymorphism is actually a noteworthy genetic tendency factor for in Iranian Bipolar disorder patients. Therefore, DISC1 gene rs3738401 polymorphism may be a genetic predisposing factor for Bipolar disorder treatment in Iranian population.

ACKNOWLEDGMENT

We thank Dr. Ali Reza Mousa Mayali for Improving the reporting of the findings and Suggesting some relevant references

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