Original Research Article

Assessment the correlation between Polymorphisms of *ZNF804A* and *DISC1* Genes in Patients with Schizophrenia in Iran

Abstract

Background: Schizophrenia is a complex and chronic psychological disorder characterized by a set of symptoms including illusions, speech and behavioral disorders, and cognitive impairment. Schizophrenia is a complex multiple disorder, and the biggest risk factor for it is a positive family history. The aim of this study was to investigate the relationship between polymorphism of *ZNF804A* and DISC1 genes in patients with schizophrenia in Iran.

Materials and Methods: In this case-control study, 50 patients with schizophrenia and 50 healthy controls were evaluated. The PCR-RFLP method was used to evaluate single nucleotide polymorphism in both groups of patients and control. For enzymatic digestion of PCR products, rs1344706 and rs6675281 were amplified and digested using *MboI* and *BseLI* enzymes at 37 ° C for 16 hours, respectively.

Results: The frequency of TT, GT and GG genotypes for *ZNF804A* gene in rs1344706 was 26%, 52%, and 22%, respectively, and in healthy subjects 46%, 42%, and 8%, respectively. In the DISC1 gene, the frequency of TT, CT and CC genotypes in the rs6675281 region was 2%, 14%, and 84%, respectively, and 2%, 14%, and 80%, respectively, in healthy subjects or controls, respectively.

Conclusion: Frequency of homozygous GG and heterozygote GT genotypes was 8% and 14% higher than healthy subjects, but the frequency of homozygous TT in healthy subjects was 22% higher than those with schizophrenia for *ZNF804A* gene in rs1344706 region. However, in case of *DISC1* gene, the frequency of TT, CT and CC genotypes in the rs6675281 region was very similar in healthy and healthy subjects, and there was no significant difference between homozygous and heterozygous genotypes. Therefore, the result of our study can be a way to providing suitable information about the disease in order to prepare patients and family and to program adjusted treatment to prevent major injuries

Keywords: Schizophrenia, Polymorphism, *DISC1* gene, and *ZNF804A* gene

Introduction

Schizophrenia is a complex and chronic psychiatric disorder characterized by a set of symptoms including illusions, speech and behavioral disorders, and cognitive impairment [1]. Schizophrenia usually appears in early adulthood or adolescence. Men are more commonly than women and tend to have symptoms in their adolescence or early 20's and have a more severe form of illness with more negative symptoms, with less likelihood of full recovery, and thus it is generally worse, while women usually show symptoms in the late 20's or early 30's [2]. Schizophrenia is a complex multiple disorder, and the biggest risk factor is a positive family history. While the risk of life in the general population is less than 1%, in the first-degree relatives of patients 6.5% and in identical twins increases by more than 40% [3, 4]. Schizophrenic patients are significantly weaker in performing neuropsychological work than healthy people [5]. Schizophrenia is a severe psychiatric disorder with a great impact on the individual and society. While results may not be equally negative, more than 50% of diagnosed people have intermittent and prolonged psychiatric problems, and approximately 20% have chronic symptoms and disabilities [6, 7].

Schizophrenia is a chronic and debilitating mental illness that affects millions around the world. The study of the causes of schizophrenia is underway and it has been reported with various causes, including the psychiatric, the autoimmune and genetic disorders leading to the disease [8]. Schizophrenia appears to be a polygenic disorder with environmental and growth factors that make the person with the disease [9]. A family study of adults with schizophrenia has confirmed that genetics plays an essential role in the progression of the disease [10]. The basis of the genetics of schizophrenia has been discussed over the course of decades, and recent studies, especially last year, have confirmed genetics as a major contributor to this complicated situation [11].

The *ZNF804A* gene, a protein encoded by this gene, is bound to zinc metal. Polymorphism in this gene, in particular, rs1344706, appears to increase the sensitivity or predisposition to schizophrenia. In humans, *ZNF804A* is widely distributed throughout the brain, especially in the hippocampus, and the cortex and also in the cerebellum. *ZNF804A* binds to DNA and regulates the expression of the gene like other zinc-containing proteins [12-14].

Another gene known as *DISC1* encodes a spiral-like motif located in the nucleus, cytoplasm, and mitochondria. This protein interacts with other proteins in neuritis and cortical growth and is associated with schizophrenia and psychiatric disorders. In coordination with a widespread set of interaction factors, *DISC1* has been demonstrated to participate in regulating cell proliferation, differentiation and migration, dendritic growth and neuronal axon, mitochondrial transmission, fission, or fusion, and cell adhesion to the cell. Various studies have demonstrated that misleading expression or altered protein structure of the *DISC1* may lead to the development of schizophrenia, clinical depression, bipolar disorder, and other psychiatric conditions [15, 16]. The aim of this study was to investigate the relationship between polymorphism of *ZNF804A* and *DISC1* genes in patients with schizophrenia in Iran.

Materials and methods

Fifty patients with schizophrenia and 50 healthy controls were evaluated in this case-control study. All samples were taken after that being approved by a specialist. Blood samples were taken after a complete examination and transferred to the laboratory for extraction of genomic DNA and stored at minus 70 °C after extraction of the DNA. Genomic DNA was extracted using 6 M saturation salt. The extracted DNA quality was measured using Salting out method and gel electrophoresis and also by spectrophotometer. The PCR-RFLP

method was used to determine genotype polymorphism of *ZNF804A* and *DISC1* genes.

In this method, specific primers were used for these genes. The total volume of each 15 µl PCR reaction, contained 15 ng DNA extracted, 1.2 µmol of each primer, 1.5 µM /mM MgCl2, 2.0 Unit Taq DNA polymerase and 0.2 µm dNTP. The primers used in this study, and also the temperature of reactions used to amplify by the ingredients, are demonstrated in Tables 1, 2 and 3.

Table 1: The primers used to determine the presence of *ZNF804A* and *DISC1* genes

SNP ID	Sequence 5' to 3'	Primer
rs1344706	CCTGTTAACCTTTGATTACTTTCCA	F:*ZNF804A*
	ATGCAGGCACATGAGTAGTG	R: *ZNF804A*
	CCGAGAGGACTGCTAAGGAAATTG	F: *DISC1*
rs6675281	GCCCGTCTCTCTTCTCTGATGTTAATG	R:*DISC1*

Table 2: Temperature and time conditions used to amplify the ZNF804A gene

Stages of **FOLH1** amplification	Time	T (°C)	cycles
primary denaturation	5min	95	**1**
Secondary denaturation	30s	95	**29**
Annealing	30s	59	
Extension	20s	72	
Final extension	5min	72	**1**

Table 3: Temperature and time conditions used to amplify the *DISC1* gene

Stages of ***DISC1*** amplification	Time	T (°C)	cycles
primary denaturation	5min	95	**1**
Secondary denaturation	30s	95	**29**
Annealing	30s	62	
Extension	30s	72	
Final extension	10min	72	**1**

For enzymatic digestion of PCR products, the rs1344706 and rs6675281 sites were incubated using *MboI* and *BseLI* (Thermo Scientific) enzymes at 37 ° C for 16 hours, respectively. The MboI restriction enzyme detects and digests the ^ GATC region and the BseLI enzyme identifies and cuts in the CCNNNNN ^ NNGG region. Each enzyme was used 2 units amount for enzyme digestion. The enzyme digestion product was then taken on an acrylamide gel 12%. Applied Biosystem SimpliAmp device was used to amplify the fragments in PCR.

Results

A total of 100 samples (50 from patients and 50 from healthy subjects) were included from all blood samples of patients and healthy individuals and their genomic DNA was extracted from them. In the next step, using primers for *ZNF804A* and *DISC1* genes in regions rs1344706 and rs6675281 they were amplified. In our study, the PCR-RFLP was used to study single nucleotide polymorphism in both groups including patients and control.

Polymorphic analysis of the length of the PCR-restricted fragment of the *ZNF804A* gene in the rs1344706 region is demonstrated in Fig. 1. The polymorphism of the *ZNF804A* gene in the above region was observed in three forms. The frequency of homozygous TT gene (T wild-type allele) without

polymorphism with a fragment length was 195 bp, heterozygote GT, had polymorphism in one of the two DNA strands: 195 bp, 53 bp, and 142 bp, and homozygous GG (G mutant allele) Polymorphism was observed in both DNA sequences with 53 and 144 bp sizes in electrophoresis on 12% gel acrylamide.

For the *DISC1* gene in the rs6675281 region, the sequence polymorphism analysis of the PCR-restricted fragment is demonstrated in Fig. 2. The above residual polymorphism was also determined for the *DISC1* gene in three modes of TT, CT, and CC. The frequency of homozygote TT gene was 219 bp size, heterozygote CT polymorphism in one of two DNA strands was 219 bp, 182 bp, and 37 bp, and homozygous CC polymorphisms in both DNA sequences with two bands of bp,182bp, and 37 bp size in12% acrylamide gel electrophoresis.

The frequency of TT, GT, and GG genotypes for *ZNF804A* gene in rs1344706 was 26%, 52%, and 22%, respectively, and in healthy subjects was 48%, 44%, and 8%, respectively. In the *DISC1* gene, the frequency of TT, CT and CC genotypes in the rs6675281 region was 2%, 14%, and 84%, respectively in patients, and 2%, 14%, and 84%, in healthy subjects or controls respectively.

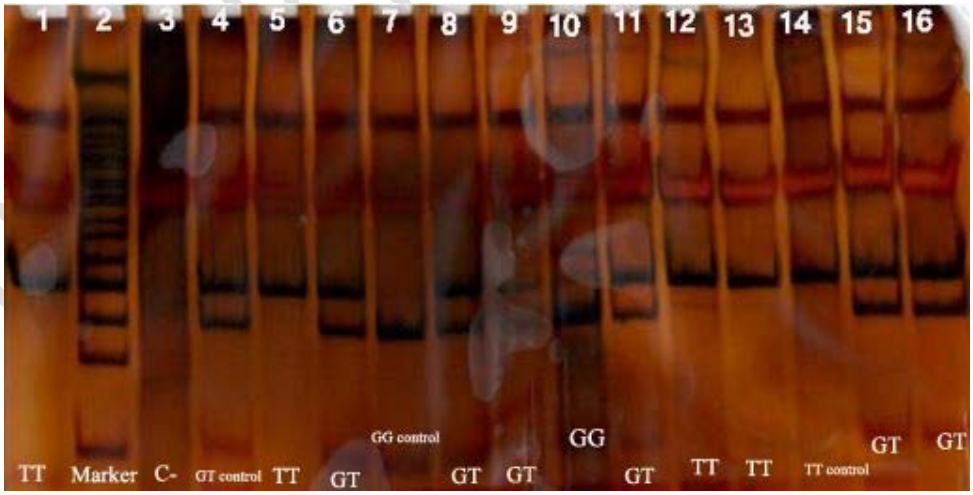

Figure 1: Sequence polymorphism analysis of a PCR-restricted fragment for *ZnF804A* gene in rs1344706 region after enzymatic digestion using *MboI* restriction enzyme, well 2: 50 bp DNA marker (Sinaclon Co.), well 3: negative control, wells 1, 5 , 12, 13 and 14

for homozygous TT and wells 4, 6, 8, 9, 11, 15 and 16 for heterozygote GT and wells 7 and 10 for homozygous GG in schizophrenic patients.

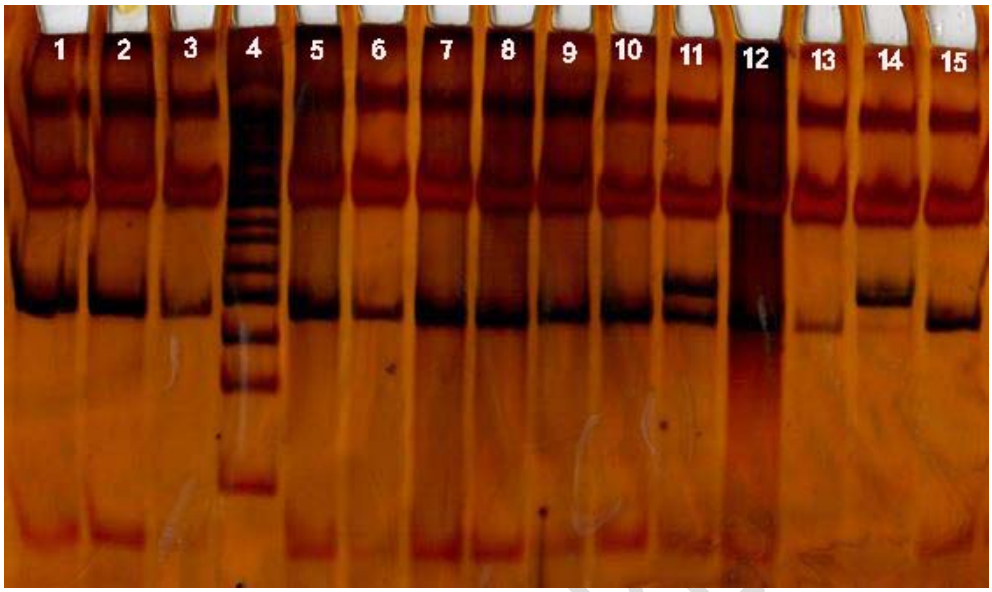

Figure 2: Sequence polymorphism analysis of a PCR-restricted fragment of the *DISC1* gene in rs6675281 region after enzymatic digestion using a *BseLI* restriction enzyme, well 4: 50 bp well (Sinaclon), wells 1 to 3, and 5 to 10 as well as wells 13 and 15 exhibit homozygote CC; wells 11 for CT heterozygote and wells 14 for homozygous TT in schizophrenic patients

Discussion

Schizophrenia is a psychological and multigenic disorder which has covered roughly one percent of the world's population. It affects one's personality and causes painful thoughts, illusions, and uncontrollable excitement. The consequences of schizophrenia are heavily influenced by family members, and the costs of not using the potential of the patient and long-term maintenance on the community are significant. The mortality rate among schizophrenic patients is twice as healthy subjects [17]. This mortality rate is probably due to the lack of recognition and treatment of disorders in these patients. The results of several studies have shown that about 50% of schizophrenic patients with concurrent indigenous illnesses which may not be known [18].

Single nucleotide polymorphism in rs1344706 in the *ZNF804A* highlights wide correlation of genome with schizophrenia and bipolar disorder. In a study conducted by Ran Tao and his colleagues in the United States in 2014 showed that rs1344706 affects the expression of *ZNF804A* and is only in embryo's brain restricted to this isoform. This may be part of a mechanism in which changes in *ZNF804A* can affect fluency [19].

ZNF804A is known as a gene for the risk of schizophrenia among different populations in the world. A single nucleotide polymorphism in the rs1344706 region is one of the strongest susceptibility variables that is of particular importance in genomic research on the prevalence of schizophrenia and has been widely studied by Chang H et al. 2017, indicating the discovery and possible role of *ZNF804A* in brain development and the pathogenesis of schizophrenia [20].

In our study results, the prevalence of homozygous genotype GG, and heterozygous GT in patients was 8% and 14% respectively higher than normal, but the frequency of homozygotes TT in healthy people was 22% higher than those with schizophrenia in the *ZNF804A* gene in rs1344706 region.

In a study conducted by Hossein Zadeh and his colleagues in the year 2015 to investigate the *DISC1* gene polymorphism on 300 schizophrenic patients, they confirmed that this gene is associated with schizophrenia [21] but was different with our study, because they studied polymorphism rs2738874 in a population in Iran. Other extensive studies by Maurice and colleagues, they found that *DISC1* is a multi-functional protein which possibly helps mutation to develop schizophrenia by interfering intracellular transmission and neuronal migration [22]. In this study, only the rs6675281 region was investigated among multiple polymorphisms of *DISC1* gene. The results showed that in our patients, there was not a significant difference between the control subjects and those with

schizophrenia in this region regarding the observed polymorphism. Given the focus of many research centers on SNPs and their relation to illnesses, it can be proper to obtain more accurate information through more advanced techniques such as microarray and sequencing.

Conclusion:

Based on the results of this study, it was found that in Iranian patients with the schizophrenic disorder, homozygous GG and heterozygote GT genotypes or polymorphisms in the *ZNF804A* gene in the rs1344706 region have high frequency compared to healthy subjects, but this frequency is low in homozygous TT polymorphism. However, there was no significant difference between homozygous and heterozygous genotypes of *DISC1* gene in the rs6675281 region in comparison with healthy subjects in our country. Therefore, the result of this research in this field can be considerably helpful in managing the diagnosis of schizophrenia genetically. Therefore, a way to providing suitable information about the disease in order to prepare patients and family and to program adjusted treatment to prevent major injuries

Ethical Disclaimer:

The study was approved by the Institutional Review Board of Central Tehran Branch, Islamic Azad University, Tehran, Iran (IRB No.1396/08) and performed in accordance with the principles of the Declaration of Helsinki and each subject signed an informed consent before participating to the study. All procedures were approved by the relevant ethics committees, and written informed consent was obtained from all participants.

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