Original Research Article

Investigation of the association of *FOLH1* rs61886494 and *DISC1* rs12133766 loci with schizophrenic in Iran

Abstract

Background: The aim of this study was to determine the relation between *FOLH1* and *DISC1* genes polymorphisms in patients with schizophrenia in Iran.

Materials and Methods: In this case-control study, 50 patients with schizophrenia and 50 healthy controls were evaluated. PCR-RFLP method for *FOLH1* gene and Tetra-ARMS for *DISC1* gene was used to study single nucleotide polymorphism in both of patients and control groups.

Results: The frequency of CC, CT, and TT genotypes for FOLH1 gene in rs61886494 locus in schizophrenic patients was 92%, 8%, and 0%, respectively, and in healthy subjects, 94%, 0%, and 6%, respectively. In the DISC1 gene, the frequency of GG, GA, and AA genotypes in rs12133766 locus in patients group was 84%, 8%, and 8%, respectively, and in healthy individuals was 82%, 18%, and 0%, respectively.

Conclusion: For *FOLH1* gene in rs61886494 locus, the frequency of CC and TT genotypes in patients was 2% and 6% lower in healthy people, while CT genotype in patients was 8% higher in healthy people. Interestingly, TT genotype was not observed in patients and CT genotype in healthy people was not observed. Regarding the DISC1 gene, the results showed that the frequency of homozygous GG and GA homozygote genotypes in the patients was higher in the rs12133766 locus, while the heterozygote GA was high in healthy subjects and was not observed in patients. Therefore, the result of this study in our country can provide significant assistance in managing the diagnosis of the disease genetically and provide a suitable treatmentPURELY OF . ;NOY CLINICALLU USEFUL -PRELIMINARY INTEREST

Keywords: Schizophrenia, Polymorphism, *DISC1* gene, *FOLH1* gene

Introduction

Schizophrenia is a condition developing with at least two of the following symptoms: Illness, imagination, maladaptive speech, abnormal behavior or negative symptoms that occur over a period of one month and with continuous problems with a period of more than 6 months [1]. Significant symptoms in schizophrenia include positive symptoms (such as hearing impairment, mental disorder, and illusions) and negative symptoms such as self-neglect and reduced feelings that should be present to detect at least one of these symptoms. The onset of a disease is usually early in adulthood and the suicidal tendency is one of the most dangerous complications of the disease. The risk of suicide is about 5%, and its risk is greater than the onset of the disease. The average lifetime mortality risk associated with schizophrenia is 7.2 per 1,000 people [2]. Men's risk ratio is 1.4/1 compared to women [3, 4].

The prevalence and incidence of schizophrenia appear to vary according to race and geographical location [5]. Age is usually less than 25 years for men and 35 years for women. Those who are most affected are born in the winter and spring or summer, but this data is controversial [6, 7]. In addition, there is a higher incidence of disease in urban and low-income communities compared to rural and high-income populations [3]. The incidence and prevalence of the disease increase over time [8]. A higher incidence has been reported in the immigrant population, which apparently goes on to the second generation [9, 10].

Scientists have been trying to determine which genes increase the heredity of schizophrenia. Unfortunately, they estimate that there are between 100 and 10,000 genes with damaged brain mutations and more than 280 genes have now been identified being associated with schizophrenia. "Schizophrenia does not seem to be a disease but is the end point of 10,000 different disorders in the subtle architecture of the human brain" [11].

The *FOLH1* gene encodes a type 2 membrane glycoprotein belonging to the M28peptidase family. This protein acts as a carboxy-peptidase glutamate on various substrates, including folate and n-acetyl-L-aspartate L-glutamate neuropeptidase, and is expressed in a number of tissues such as the prostate, the central and the peripheral nervous system, and the kidney. Mutations in this gene may be associated with poor intestinal absorption of dietary folate, which results in a decrease in blood folate levels and thus hyperhomocysteinemia [12].

Expression of this protein in the brain may be involved in a number of pathological conditions associated with stimulation of glutamate toxicity. In the prostate, protein is regulated in cancer cells and is used as an indicator of the diagnosis and prognosis of prostate cancer [13-15]. *FOLH1* is also expressed in the brain by disrupting NAAG (n-acetyl-aspartyl glutamate) into NAA and glutamate in disorders of the nervous system, such as multiple sclerosis, sclerosis, Alzheimer's disease and schizophrenia due to impairs in *FOLH1* at various levels [16, 17].

DISC1 gene was first identified as a risk factor for mental illness in a Scottish family where a balanced transmission between chromosomes 1 and 11 associated with schizophrenia, bipolar disorder and depression was identified [18]. Since 2000, a number of additional research efforts have been launched to illustrate the importance and relevance of *DISC1* for psychiatric illness. Genetic studies have later confirmed that *DISC* locus is involved in several psychiatric disorders and cognitive functions in several populations around the world [19].

The aim of this study was to investigate the relation between *FOLH1* and *DISC1* genes polymorphisms in the rs61886494 and rs12133766 loci among schizophrenic patients in IranTo . onschozohrenic n and cpolymorphisms between schizophreni fferentincidence of di compare .populationx

Materials and methods

Recruitment – how were patients recruited?

Sample size calculation – how was sample size determined?

In this case-control study, 50 samples from schizophrenic patients and 50 from healthy individuals (as a control group) were used. All samples were collected after approval by a physician specialist. The specimens were collected from the blood after a full clinical examination and transferred to the laboratory for extraction of genomic DNA and next stored at -70 °C conditions after extraction of DNA. Genomic DNA extraction from samples was performed using 6 molar saturation salt. The quality of extracted DNA by Salting Out method was measured by agarose gel electrophoresis and spectrophotometer (Nanodrop).

To determine the *FOLH1* gene polymorphism, PCR-RFLP method was used to determine the genotype of *DISC1* gene polymorphism using Tetra-ARMS-PCR and using internal and external primers. The genotypes and alleles of a mutation or polymorphism using Tetra-ARMS-PCR technique and determining the risk level of individuals with these mutations were used to identify human traits such as the incidence of schizophrenia. This method is based on the design of specific primer pairs and the amplification of the desired allele in a PCR reaction. The number of primers pairs, depending on the design, can be 3 or 4 pairs. In our method, using 4 primers (Tetra primer ARMS-PCR) to detect a specific mutation, two pairs of control primers around the mutation site were used to ensure the accuracy of PCR reaction. To amplify the region containing the mutation, two primer pairs were designed, which were for a mutation allele and a natural allele, respectively.

In this method, specific primers for these genes were used. The total volume of PCR reaction was 15 µl containing 20 ng of extracted DNA, 1 pmol of each primer (2 primers pairs), 1.5 mM of MgCl2, 0.2 Unit of Taq DNA polymerase and 0.2 µm of dNTP. The primers used in this study, along with the thermal profile for amplification of products, are shown in Tables 1, 2 and 3.

Table 1: The primers used to determine the presence of *FOLH1* and *DISC1* genes

SNP ID	Sequence 5' to 3'	Primer
rs61886494	CTAGGTCACCTCTCAAAATCT	**F: *FOLH1***
	GAGCCAAGGATAAAAGAGAGAG	**R: *FOLH1***
rs12133766	AGATCATTAACAACAGAGAGAGAAGGGATG	**F (Outer): *DISC1***
	AACAGCTTGCTGAGGGAGTCCCGCT	**R (Outer) :*DISC1***
	TGCTGCTAGATCTTCCATGTGTGTGGAT	**F (Inner): *DISC1***
	ATCATCAATATCTTGCCGGGGAACAGTT	**R (Inner) :*DISC1***

Table 2: Temperature and time conditions used to amplify the *FOLH1* gene

Stages of *FOLH1* amplification	Time	T (°C)	cycles
primary denaturation	5min	95	**1**
Secondary denaturation	30s	95	**29**
Annealing	30s	60	
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	1min	95	**29**
Annealing	1min	60	
Extension	1min	72	
Final extension	10min	72	**1**

For digestion of PCR products of the rs61886494 and rs12133766 loci, the tubes were incubated for 37 hours at 16 °C using MseI (Tru1I) and BseLI (Thermo Scientific) enzymes, respectively. The MseI restriction enzyme detects and digests the T ^ TAA region and the BseLI enzyme digests in the CCNNNNN ^ NNGG region. Each enzyme was used 2 units for enzyme digestion. The enzyme digestion product was then taken on an polyacrylamide gel 12%. Applied Biosystem SimpliAmp was used for the amplification of the fragments in PCR.

Ethical process

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 **(PLEASE ATTACH)** before participating to the study. All procedures were approved by the relevant ethics committees, and written informed consent was obtained from all participants.

Results

A total of 100 samples (50 patients' samples and 50 healthy individuals' samples) were collected in this study from all blood samples and their genomic DNA was extracted. In the next step, the primers for *FOLH1* and *DISC1* genes were amplified in loci rs61886494 and rs12133766, respectively. In this study, PCR-RFLP and Tetra-ARMS were used to study the single-nucleotide polymorphism in two groups of patients and control.

The sequence polymorphism analysis of a PCR-restricted fragment for the *FOLH1* gene in the rs61886494 locus is shown in Fig. 1. The polymorphism of the *FOLH1* gene was observed in three forms. Frequency of homozygote gene CC (wild-type C allele) without polymorphism with a fragment length of 235 bp, heterozygote CT has polymorphism in one of two DNA strands with 235 bp, 100 bp, and 135 bp, and homozygous TT (T mutant allele) Polymorphism was observed in both DNA sequences with two products of 100 bp and 135 bp in electrophoresis on 12% polyacrylamide gel.

For the DISC1 gene in the rs12133766 locus, the sequence polymorphism analysis of the Tetra-Arms restricted segment is shown in Fig. 2. This product polymorphism was observed for the DISC1 gene in three modes of GG, GA, and AA. The frequency of the homozygous GG gene has a band of 189 bp, heterozygote GA had a polymorphism in one of two DNA sequences with 189 bp and 168 bp, and a homozygote of AA had a product of 168 bp in electrophoresis on 12% acrylamide gel.

The frequency of CC, CT, and TT genotypes for *FOLH1* gene in rs61886494 locus in patients was 92%, 8%, and 0%, respectively, and in healthy individuals was 94%, 0%, and 6%, respectively. In the *DISC1* gene, the frequency of GG, GA, and AA genotypes in the rs12133766 locus in patients was 84%, 8%, and 8%, respectively, and in the healthy control group was 82%, 18%, and 0%, respectively.

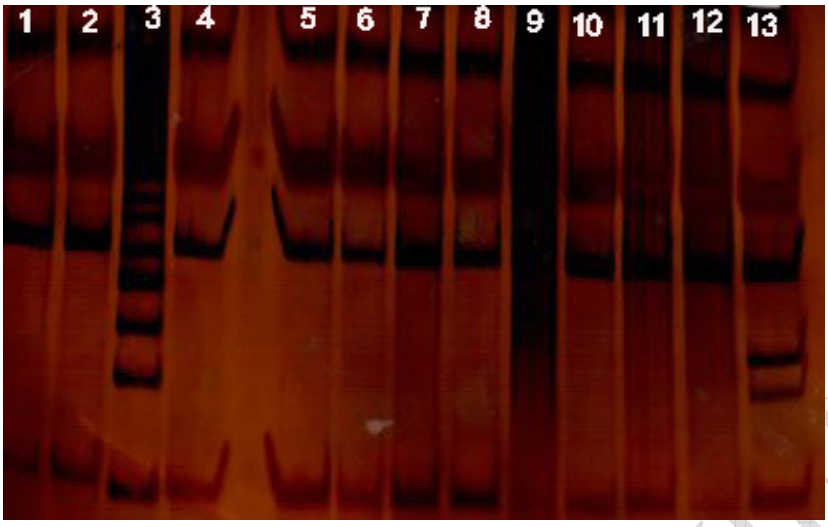

Fig. 1: Sequence polymorphism analysis of a PCR-restricted fragment of the *FOLH1* gene in rs61886494 locus after enzymatic digestion using a MseI restriction enzyme, well 3: 50 bp DNA marker, (Sinaclon Co.), well 13: the heterozygote CT and the rest of the wells (wells 1-12) associated with homozygous CC in schizophrenic patients

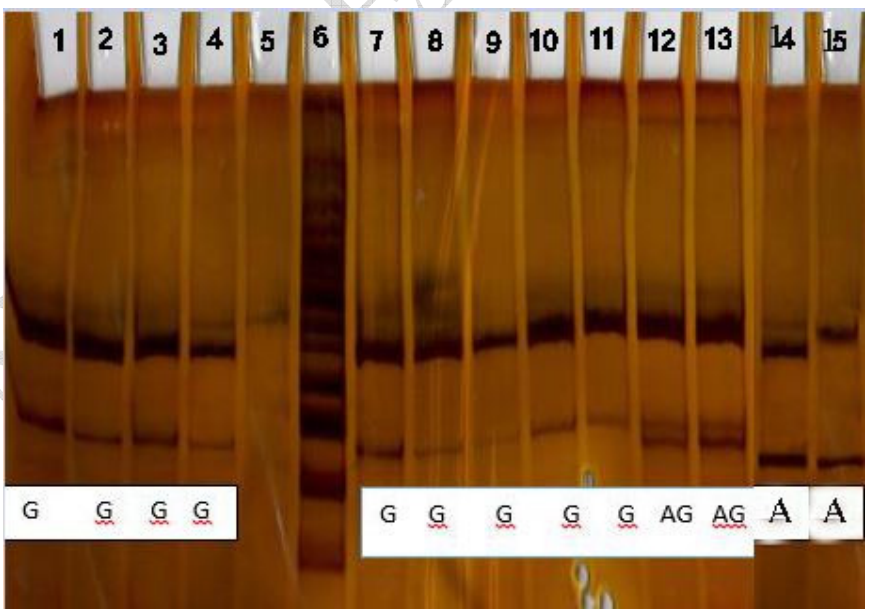

Figure 2: Sequence polymorphism analysis of a Tetra-ARMS-restricted fragment of the *DISC1* gene in the rs12133766 locus after enzymatic digestion using a BseLI restriction enzyme, 6: 50 bp

DNA marker (Sinaclon), well 5: negative control, wells 1 to 4 and 7 to 11: homozygous GG and wells 12 and 13 indicate heterozygote AG and Wells 14 and 15 indicate homozygous of AA related to schizophrenic patients

STATTISTICAL ANALYSIS IS MISSING

Discussion

[Schizophrenia is a psychiatric disorder that usually manifests itself in the late adolescence or early adulthood. The disease can often be a lifelong struggle characterized by illusions and other psychological problems.

Schizophrenia is the most injured between the ages of 16 to 30, and men show signs at a lower age than women. In many cases, the disorder slowly develops in such a way that one does not know that it has been the disease for many years. However, in some cases, it can also develop rapidly and promptly. Schizophrenia affects about 1 percent of adults in the world. Scientific research suggests that schizophrenia may be due to inverse neuronal progression in the fetal brain, which later occurs in life as a complete illness [20, 21].

Experts believe that several factors are involved in the development of the disease; evidence suggests that genetic and environmental factors interact with each other in the development of schizophrenia. It is defined as an inherited agent, but environmental stimuli also significantly affect it. If there is no history of schizophrenia in a family, its possibility of developing is less than 1%. However, if the parent is diagnosed as a patient, the risk increases by 10% [22, 23]. A large number of genes, including *FOLH1, PRODH, COMT, ZDHHC8, DNTBP1, CAPON* and DISC1, have been investigated in relation to schizophrenia and their association with the disease has been documented. In this study, we investigated polymorphisms of two *FOLH1* and *DISC1* genes in rs61886494 and rs12133766 loci using molecular techniques.A LL THIS BELONGS IN THE INTRO]

DISCUSSION

THIS IS A PILOT STUDY PN A SMALL SAMPEL TO DETERMINE WHETHER THE METHOD WORKS, WHETHER PEOPLE (WITH SCHIZOPHRENIA AND WTHOUT) ARE AMENABLE TO GENE TESTING. BECAUSE OF THE HIGH REATE OFCONSANGUINOUS MARRIAGES IN IRAN, GENETIC TESTING IN THIS COUNTERY COULD YIELD IMPORTANT RESULTS. ….We found that for the *FOLH1* gene in the rs61886494 locus frequency of TT and CC genotypes was lower in patients compared to controls, while heterozygous CT genotype was higher than control subjects and not observed in the healthy group. Interestingly, TT genotype was not observed in patients and healthy. Regarding the *DISC1* gene, the results showed that the frequency of GG and AA homozygote genotypes in the patients was higher in the rs12133766 locus when the heterozygote GA was high in healthy subjects and was not observed in patients with this heterozygote. The final conclusion is that the *FOLH1* and *DISC1* genes can be considered as an important candidate in the population as a factor in the incidence of schizophrenia.

In our country, there have been no extensive studies on *FOLH1* and *DISC1* genes, but in studies related to the analysis of polymorphism in relation to the incidence of schizophrenia, Rahmanzadeh et al. (2012) studied the *PRODH* gene at the site and showed that there is a significant relationship between the incidence of this nucleotide mutation and its frequency among patients. Based on the statistical data for this genotype, there was a significant difference between the control group and the patient for the mutated allele C frequency. The allele frequency of this mutated allele was significantly higher in the patient group than in the control group, suggesting that the T1945C polymorphism in this population could be associated with the incidence of schizophrenia. In other words, the marker C> T SNP1945 showed a significant

relationship with schizophrenia in the studied population [24]. In our studies, there were changes in the genotypes between patient and control, which was the highest change in CT genotype in *FOLH1* gene and homozygote genotypes in *DISC1* gene.

In a similar study by Guoqin HU that examined the *DISC1* gene polymorphism among Han Chinese people, there was a significant correlation between polymorphism in this gene and schizophrenia [25]. In a similar study in South Korean schizophrenia by Kim HJ, the *DISC1* gene was introduced as a prone gene for identifying these patients, which was consistent with the studies [26]. Although many genes in schizophrenia have been discussed, the *FOLH1* gene has been proposed as an important goal in the pathophysiology of schizophrenia. In our study, *FOLH1* gene was more frequent in CC and CT genotypes than in normal individuals, and the importance of this gene in Iranian patients was presented. A study by Joshua L. Roffman in Boston showed that genetic changes and folate metabolic pathway influences negative symptom severity in Schizophrenia patients [27].

Conclusion

Based on the results of this pilot study, our results suggestthat in Iranian patients with schizophrenic disorder homozygous CC and CT genotypes in the rs61886494 locus have a high frequency in comparison with healthy subjects, but the homozygous TT is infrequent. However, in homozygous and heterozygous genotypes of *DISC1* gene in the rs12133766 locus, the results showed high homozygote AA and homozygous GG genotypes in comparison of healthy and schizophrenia groups. **THIS WAS A PILOT STUDY IN A SMALL SAMPLE BUT SHOWED HAT OUR LABORATORY IS EQUIPPRF TO DO THIS WORK AD THAT PEOPLE ARE WILLING TO COME IN FOR TESTING,**

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