Original Research

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Association study between schizophrenia and the *DISC1* gene polymorphism.

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Abstract

Background: The disrupted-in-schizophrenia 1 (DISC1) gene, on the chromosome position 1q42, was initially identified at the breakpoint of a balanced translocation, t(1,11)(q42.1;q14.3), which segregated with major mental disorders in a large Scottish family.

Methods: Our samples included 200 unrelated patients diagnosed with Schizophrenia on the basis of DSM-IV criteria and 200 normal controls, which were gathered from Iran. The allele and genotype frequencies of the polymorphism were determined using Polymerase Chain Reaction-Restricted Fragment Length Polymorphism (PCR-RFLP) and the data were analyzed by Logistic Regression test.

Results: In this study we genotyped the rs821616 polymorphism (Serin704Cystein) located within exon 11 of the DISC1 gene. The samples were matched on the basis of sex and ethnicity. We used the case control study to determine the possible association between the ser704cys (rs821616) polymorphism and Schizophrenia. Analysis of data in the samples, revealed no association between the rs821616 polymorphism and Schizophrenia (OR= 0.697, 95% CI= 0.47-1.033, P=0.072).

Conclusion: In this study we did not find any association between the rs821616 SNP and schizophrenia.

Keywords: association study, schizophrenia, DISC1 gene, rs821616, single nucleotide polymorphism.

Introduction

Multiple lines of evidence favor a genetic predisposition to schizophrenia [1]. Several kinds of studies, including brain imaging and neuropathology suggest abnormalities in schizophrenic and cerebral cortical development that might reflect cytoskeletal disturbances [2]. Genetic studies have linked schizophrenia to multiple loci, suggesting the involvement of certain candidate genes as susceptibility factors [3,4]. Subsequent genetic studies in several independent populations, including association and linkage studies have also suggested that the Disrupted-In-Schizophrenia gene (DISC1) may be implicated in both schizophrenia and bipolar disorder [5,6,7,8].

The DISC1 was identified as the sole gene in which a mutant truncation by a balanced translocation, t (1; 11) (p42.1;q14.3) is co segregated with schizophrenia in a large Scottish family [9]. Ekelund and colleagues found a mi-

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crosatellite maker in DISC1 provided strong evidence for linkage to schizophrenia in a Finnish family [10] Using another set of samples of 70 Finnish families with multiple individuals affected with schizophrenia, they also found that a polymorphic marker and haplotypes in DISC1 showed evidence of association with schizophrenia[11]. Furthermore, a marker located near the breakpoint of a balanced translocation t (1; 11) (q42.1; q14.3) in DISC1 showed linkage to schizophrenia in Taiwanese families [12]. Recently, Cannon et al. found a haplotype incorporating 3 single-nucleotides polymorphic markers near the translocation break point of DISC1 associated with schizophrenia in their population-based twin cohort study [13]. Positive associations between DISC1 and both bipolar disorder and schizophrenia were reported in a Scottish population and a North American white population [8, 14]. Data from biochemical and cellular assays also provided evidences to support the important role of DISC1 in schizophrenia pathogenesis [15].

The DISC1 protein consists of 854 amino acids. The translocation that may cause the disease involves the deletion of the C-terminal 257 amino acids [9]. The DISC1 is developmentally regulated with highest levels in the late embryonic development of rats at the time of maximal cerebral cortical neuronal migration [16]. DISC1 gene is localized in particulate fractions and has structural characteristics resembling a cytoskeletal protein. Yeast 2-hybrid analysis reveals interactions of The DISC1 with several cytoskeletal proteins. Detailed analysis of these proteins, such as nuclear distribution gene E homolog (NUDEL) suggested a putative role of DISC1 in neuronal migration and neurite outgrowth. Currently, the functions ascribed to DISC1 are largely inferred from the known functions of the proteins that interact with the DISC1. It is therefore suggested to play a role in neuronal migration [17], neurite outgrowth [18], signal transduction, cyclic adenosine monophosphate (cAMP) signaling [19], cytoskeleton modulation, and translational regulation [17].

The evidence for DISC1 being a causal factor in major mental illness is strongly supported by a raft of independent genetic studies and by the growing evidence from biological studies. With exception of the t (1; 11), the data for explanatory functional variants in the gene and protein are still lacking. Much still needs to be understood about the biology of DISC1 and of DISC1 interacting partners, in human and other species. However, the DISC1 pathway currently offers avenues for exploring the molecular etiology of both normal and abnormal brain development. Such studies have the potential to produce new levels of understanding and possibly more effective interventions for disorders that are among the most common and debilitating of human conditions, major mental illness [17]. Our aim in the present study was to examine the association between Schizophrenia and the DISC1 gene in an Iranian population. We analyzed the rs821616 polymorphism in the DISC1 gene in a case-control study.

Methods

Subjects

In this case-control study, our samples included 200 unrelated patients with Schizophrenia (mean age 43.34±SD=11.353) diagnosed based on DSM-IV criteria. The samples were gathered from hospitals of Fars and Khuzestan provinces which are located in south and southwest of Iran, respectively. Of these, 117 were men (63 from Fars and 54 from Khuzestan) and 83 were women (48 from Fars and 35 from Khuzestan). From all participants informed consent was obtained. Also, 200 normal controls were gathered from Khuzestan and Fars blood donors from blood transfusion centers and matched on the basis of sex and ethnicity with patients. The socio-demographic characteristics of the case and control samples are presented in Table 1.

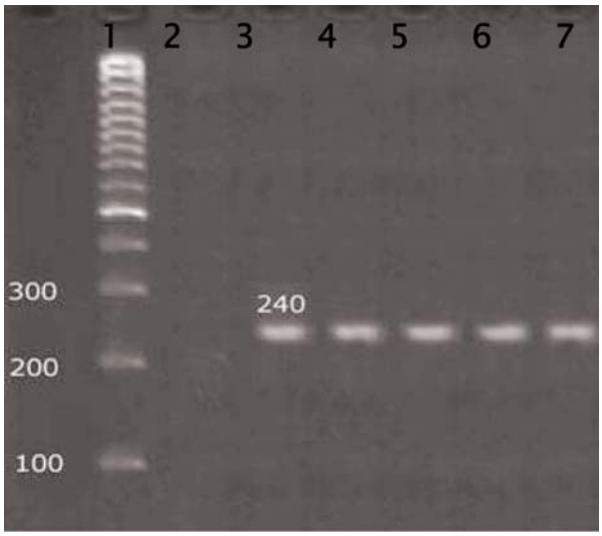

Fig. 1. The primer specific PCR yielded a 240 bp product. Lines: 1: 100 bp DNA ladder (Fermentase; Denmark); 2: negative PCR; 3 to 7: PCR products generated by sample's DNA.

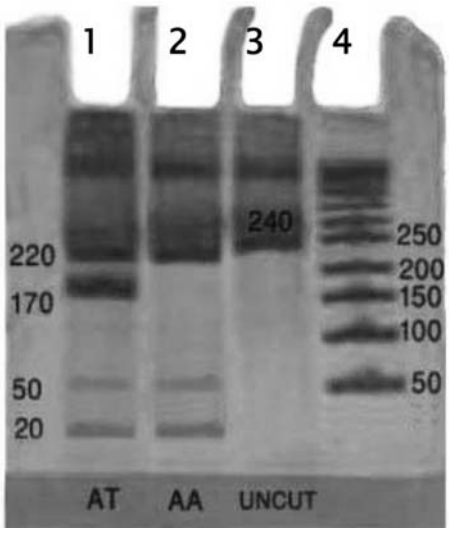

Fig. 2. Digested PCR products with the restriction enzyme TspR1 yielded 4 bands in the case of AT allele (line 1) and 3 bands in the case of AA allele (line 2), in comparison to undigested PCR product (line 3). Line 4 shows the 50 bp DNA ladder (Fermentase; Denmark).

DNA Extraction and genotyping analysis

Blood samples were collected from patient and control groups. Genomic DNA was extracted from peripheral blood leukocytes using Diatom Prep 100 DNA extraction kit (Genfanavaran, Tehran, Iran). The rs821616 polymorphism in exon 11 of the DISC1 gene was screened using PCR-RFLP method. The primers with the sequences 5′GCAGACCATGTATTTGAAAAGC 3′for forward primer and 5′ GCCAGTTTCCTCAAAATTCC 3′ for reverse primer were designed by primer3out (www.primer3.com) program and have been used to amplify the region containing the rs821616 polymorphism.

The PCR reactions were carried out in final volume of 25 µl, containing 10 ng of genomic DNA, 10 mM Tris-HCl (PH:8.3), 50Mm KCL, 2Mm MgCl2, 200 µM dNTP (Sigma Co), 0.5 Pmol of each primer (Genfanavaran, Tehran, Iran) and 0.25 unit of Taq DNA polymerase (Genfanavaran, Tehran, Iran).

The amplification thermal conditions were as following: denaturation at 94°C for 3″, followed by 35 cycles at 95°C for 30″, 57°C for 30″, 72°C for 30″, and a final extension at 72°C for 5 minutes (Fig.1).

Subsequently the PCR products, (240 bp long) were digested with 0.5 units of the TspR1 restriction endonuclease (Bio Labs, UK) overnight at 65oC and analyzed on a 12% poly Acryl amid gel (Fig.2)

Statistical analysis

Statistical differences in genotype and allele frequencies between Schizophrenics and controls were evaluated using the θ^2 test in Fars and Khuzestan provinces samples. Odd ratios (ORs) and their 95% confidence intervals (CIs) were calculated by statistical software package SPSS 13 to evaluate the effects of different genotypes.

Results

The rs821616 allelic and genotypic frequencies in cases and controls are presented in Table 2.

Comparison of Genotype frequencies between Khuzestan and Fars samples presented no differences and therefore samples from two regions were grouped together in analyses

Variables	Cases	Controls
Continuous variable		
Age	43.34±11.353	39.43±11.103
Age of onset	22.02± 9.047	
Discontinuous variables		
Sex		
Females	83	83
Males	117	117
Educational level		
Illiteracy	28	3
Primary school	113	13
High school	42	145
College	7	39
Missing data	10	
Marital status		
Single	139	43
Married	35	159
Divorced	16	1
Missing data	10	

Table 1. Socio-demographic characteristics of the case and control groups.

(OR= 0.697, 95% CI= 0.47-1.033, P<0.072) and as well as association between schizophrenia and the rs821616 polymorphism was not observed.

Discussion

We studied the possible association of the rs821616 SNP in the DISC1 gene with schizophrenia. In the recent years, many polymorphisms have been reported on the human chromosome one as susceptible loci for schizophrenia.

Positive results of linkage or association studies were found at 1q21-23 [22, 23, 24], 1q22 [25, 26], 1q31-32[27], 1q32.2-q41 [28, 29] or 1q42 [10]. However, other studies did not detect any polymorphism that was associated with the increased risk for schizophrenia [30, 31, 32]. Initially, the position 1q42 was identified as a susceptible site for schizophrenia due to a balanced translocation (1; 11) (q42.1; q14.3). The DISC1 gene was also found to be co-ssegregated with schizophrenia [10]. Afterward, linkage and association findings of DISC1 at 1q42 could be replicated in Finnish and Taiwanese families [10, 11, 12]. This finding is consistent with other evidence according a recent report that the peneterance of gene effects related to psychiatric disorders is greater at the level of brain information processing than at the level of behavior [32]. These data confirmed the DISC1 gene as a strong candidate that is considering to be closely related to schizophrenia. The observations that Serine to Cystein substitution at position 704 is a non-conservative polymorphism residing within or near an alternative splicing domain in exon 11 makes it

	Cases	Controls
Allelic frequencies		
Fars province		
A	0.770	0.752
T	0.230	0.248
Khuzestan province		
A	0.792	0.713
T	0.208	0.287
Genotypic Distribution		
Fars province		
AA	60	56
AT	51	55
TT	0	0
Khuzestan province		
AA	52	38
AT	37	51
TT	0	0

Table 2. Allelic frequencies and genotypic distribution of the rs821616 polymorphism in DISC1 gene in Fars and Khuzestan provinces.

a particularly attractive candidate as a functional polymorphism of relevance to the function of this gene.

The present case control study, which is the first to be conducted in an Iranian population, did not support any association between the rs821616 SNP gene and schizophrenia. The rs821616, a non-synonymous SNP in exon 11, is associated with schizophrenia in Caucasian population according to a recent report [5] and did not associate with schizophrenia in Han Chinese samples [15]. Also, earlier studies that reported evidence for association schizophrenia to the DISC1 gene were negative for this SNP [14, 20, 21]. The conflicting results between different investigations may be due to genetic heterogeneity, which different loci may be involved. According to reports schizophrenia is a complex and multifactorial disorder with complicated etiology which can supposed different loci may have a small and additive effects on age of onset and severity of the disorder.

Our results did not provide any evidence that can support the importance and association of rs821616 polymorphism in the DISC1 gene in etiology of schizophrenia at least in Iranian population. We suppose more investigations are needed to evaluate the role of DISC1 gene in the schizophrenia.

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