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Original article

No association between the SNP rs1625579 in miR-137 gene and schizophrenia in Iranian population

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ABSTRACT

Background: Schizophrenia is a common and severe neuropsychiatric disorder with symptoms such as hallucination, delusion and mental disease. Among candidate genes for schizophrenia, miR-137 gene has been recently suggested to contribute to schizophrenia by genome-wide association study (GWAS) and a single nucleotide polymorphism (SNP) rs1625579 (G>T) as a presumed risk allele within an intron of miR-137 gene has been contributed by schizophrenia. Since mir-137 is a considerable gene in the performance of neural systems, the present study dealt with the association between SNP rs1625579 in miR-137 gene and schizophrenia in Iranian patients.

Aim of study: This study aimed to evaluate the association between SNP rs1625579 in miR-137 gene and schizophrenia in Iranian patients.

Methods: Hoping to identify a single-nucleotide polymorphism as a possible locus for schizophrenia, we carried out this case-control study on 80 blood samples collected from individuals suffering from schizophrenia and 48 healthy controls. DNA was extracted from the samples, and the frequency of the polymorphisms was analyzed using ARMS-PCR method. Finally, the products were detected on 1.5% agarose gel electrophoresis.

Results: The analysis on the data showed that 43.75% of the patients and 37.5% of the controls were mutant homozygous and 56.25% of the patients and 62.5% of controls were heterozygous. In addition, 0.0% of the patients and 0.0% of the controls were normal homozygous. Both the genotype ($p = .48 > .05$) and allele ($p = .5 > .05$) distribution of the rs1625579 SNP has no significant difference between patients and controls.

Conclusion: There was no significant relationship between rs1625579 and the incidence of schizophrenia. To the best of our knowledge, this is first study in Iran that assesses the frequency of the polymorphism among Iranian patients. However, further studies with more samples are necessary.

1. Introduction

Schizophrenia is a common, complex and debilitating mental disorder, which has significant morbidity, numerous heritability and wide genetic heterogeneity [1]. Its incidence is about 1% of the worldwide population [2]. The disorder is specified by positive psychotic symptoms, including hallucinations, delusions and disorganized behavior, and negative symptoms, such as social withdrawal and apathy [3]. It is due to multiple genes and environmental factors and is a highly polygenic disease. However genetic mechanisms are still unknown [2].

In the nervous system, miRNAs have cascaded influence on gene expression and functional pathways by regulating many biological processes such as embryonic and adult neurogenesis, axon guidance, dendritic protein synthesis and synaptic development. Therefore, they play a major role in a variety of diseases. The dysregulation of miRNA expression leads to some abnormalities in neural functions and structures [4,5] and causes psychiatric disorders [6]. In 2011, Genome Wide Association Study (GWAS) has provided evidence to suggest that MIR-137 is at the top of new associations [7]. MIR-137 is located on chromosome 1p22 in

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human, and it seems to play a role as a tumor suppressor in several cancer types [8]. Mir-137 is involved in multiple biological processes is highly expressed in brain tissue and has a regulatory role in both neurogenesis and synaptic plasticity and by recent study, which has been confirmed as an association gene with schizophrenia. According to Guan et al. and Egawa et al. there have been many studies to confirm the association between many single nucleotide polymorphism (SNP) loci of miR-137 (rs4634961, rs9324387, rs2391905, rs4950117, rs1938568, rs4950101, rs2802535, rs2660304, g.98511534 G>C, g.98511769 G>T, and g.98511780 T>C) and schizophrenia not including the rs1625579 genotype. The location of rs1625579 was identified at an 8 kb region downstream of host gene MIR137HG. This locus has a great correlation with schizophrenia ($p = 1.6 \times 10^{-11}$) in a large-scale genome-wide association study (GWAS) of the disorder. There have been some correlation studies on population; they demonstrated that rs1625579 influence many phenotypes of schizophrenia (Table 1); Population consists of the UK, Australia, China, the USA, and Canada. Lette et al. indicated that the time of beginning of disease in patients who are with the TT genotype at this SNP was so earlier than the patient who carried the G allele ($p = 3.1 \times 10^{-5}$). Some research showed that white matter density in the brain was diminished by the TT genotype. As a result of imaging examinations, the TT genotype contributed to reduce hippocampal volume and expanded lateral ventricles. These abnormalities may be a consequence of the unusual development of neural growth by dysregulation of miR-137 expression. Van Erp et al. signified that TT genotype in rs1625579 was associated with poor working memory, episodic memory and attention. The studies of Cummings et al. on patients determined that the patients who have been with T allele showed a few mental symptoms and more cognitive deficits [9].

Despite these studies, we want to show whether this SNP associates with schizophrenia in Iranian population or not. Therefore to provide a more conclusive answer on rs1625579 as a risk of schizophrenia in the present study, considering the importance of this SNP using a simple rapid and cost-effective technique, we made Tetra-Primer Amplification Refractory Mutation System PCR (T-ARMS-PCR) for genotyping SNP rs1625579 of MIR137HG gene. Using T-ARMS-PCR to confirm the association of the SNP rs1625579 with schizophrenia, we conducted a case-control study in a province of Iranian population. We considered the case-control study involving 80 patients and 48 controls. The purpose of this study was to evaluate the association between the rs1625579 and the clinical characteristics of schizophrenia as a diagnostic biomarker.

2. Subjects and methods

2.1. Case-control sample

In this case-control study, we represented our study sample, which includes 80 schizophrenia patients and 48 unrelated healthy controls from Golestan province of Iran. The diagnosis of schizophrenia was affirmed by some experienced psychiatrists for the patients. It used the structured clinical interview based on the diagnostic and statical Manual of Mental Disorders IV (DSM-IV-IR) and interview for negative and positive symptom scale (PANSS) was carried out. Control subjects selected from the same province and their hospitalization history for psychiatric disorders was perused individually and they did not have any unhealthy backgrounds. After obtaining informed consent from patients, a dose of 5 ml blood was taken in an EDTA-containing vial. The vials were transferred to the Milad Medical Genetic Laboratory by maintaining the cold chain and kept at 4 °C until the next process.

2.2. DNA extraction

DNA was extracted from 800 μL of peripheral blood samples leukocytes of case and control subjects by standard phenol-chloroform method. First, lysis solution was used to lyse blood cells. Then, DNA was precipitated by absolute ethanol. Last, it was washed by 70% ethanol and dissolved in double distilled water. After evaluating the purify, quality and concentration of extracted DNA by spectrophotometer and electrophoresis in 0.8 agarose gel; DNA samples were stored at −20 °C for genotype analysis.

2.3. SNP genotyping

The genotype of SNP rs1625579 was determined using Tetra_ ARMS PCR. Four primers were designed using Primer3 software (table 2.). Then, using Blast and Gene Runner software, we made sure that they were proprietary and specific. To detect a specific mutant, two primers were designed for T allele (mutant) and G allele (normal). PCR was performed in a 25 μL reaction composed of 12.5 μL of Amplicon (taq polymerase, dNTPs, Mgcl2, PCR buffer) (cinnagen, Iran), 3 μL of template DNA, 1.4 μL of primers C, A and 1.4 μL of PCR primers. Amplification was performed with the following program:

5 min at 95 °C, followed by 35 cycles of 95 °C for 30 s, 63 °C for 30 s, 72 °C for 30 s and ending with a final extension step at 72 °C

Table 1
Summary of human genetic studies linking the miR-137 rs1625579 polymorphism to schizophrenia and endophenotypes [9].

Population	Number of participants	Genotype frequencies (%) TT	GT	GG	*p*-value	Correlated phenotypes	*p*-value
American, Canadian (Lett et al.)	510 SZ	67.45	29.8		2.75	age-at-onset and white matter integrity	3.1×10^{-5}*
	121 CTR	62.81	33.06	4.13	>.05*		3.88×10^{-8}*
Australian (Green et al.)	491 SZ	64.97	31.16	3.87	.85*	cognitive deficits in combination with greater	.017*
	328 CTR	62.5	33.23	4.27			
Scottish (Whalley et al.)	44 SZ	59.09	38.64	2.27	>.05	activation in the posterior right medial	<.001
	81 CTR	61.73	35.8	2.47		frontal gyrus, BA 6	
American (Theo et al.)	48 SZ	81.25	18.75	0	.47*	left DLPFC activation	.02*
	63 CTR	73.02	23.81	3.17			
Chinese (Guan et al.)	1430 SZ	77.7	20.8	1.5	.023		
	1570 CTR	74	23.6	2.4			

Abbreviations: SZ, schizophrenia cases; CTR, healthy controls; BA, Brodmann area; DLPFC, Dorsal Lateral Prefrontal Cortex.
* Statistical analyses compared TT with GT/GG genotypes.

Table 2
The sequences of primers used to genotype SNP rs1625579 by Tetra-Primer ARMS PCR.

Name Primer	Sequence	PCR Production Length	Allele Product
F common	5′-GTGAATGTATCAACAGATTCCAAAGG-3′	512 bp	control fragment
FC	5′-ACAAGGGAAATGTTAATCACAATTAC-3′	351 bp	C
FA	5′-ACAAGGGAAATGTTAATCACAATTAA-3′	351 bp	Allele
R com order	5′-AATAGCACAACAGAAGTGAACAGGG-3′	512 bp	A Allele control fragment

for 7 min. As in summary, for each sample reaction was made in two vials containing normal primers and mutant primers separately and used a pair of primers as internal controls in the samples. In order to evaluate the accuracy of PCR productions and genotyping, electrophoresis used in 1.5% agarose gel. PCR product created in length of 512 bp was considered as a control fragment for PCR reactions and a 351 bp fragment for the T allele and C allele (Fig. 1). In order to confirm the accuracy of the method for determining the mutation and specifying definitively the type of mutation; sequencing was performed (Fig. 2). The PCR productions were selected randomly then after sequencing the genotypes were compared by PCR productions.

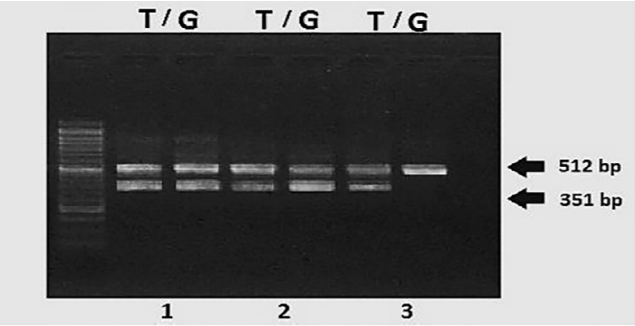

Fig. 1. The results of Tetra-Primers-ARMS PCR for genotyping of SNP rs1625579. The 512 bp band represented the control fragment and the 351 bp fragment amplification indicates the presence of T and G alleles at SNP site.

3. Statistical analysis

Hardy-Weinberg equilibrium (HWE) examined using the Chi-square test. Genotype and allele frequencies calculated in patients and healthy controls. The odd ratio (OR) with 95% confidence intervals (CI) calculated for the alleles and P-value $\leq .05$ considered significant. Statistical analyzes performed using SPSS version 24.0 software.

4. Result

For the association between rs1625579 polymorphism in miR-137 and we analyzed data from 80 schizophrenia patients and 48 healthy controls. Tetra-primer ARMS-PCR was used for genotyping the SNP rs1625579 in MIR137HG. After determining of the genotypes of both groups, Hardy-Weinberg equilibrium was tested in both the case and control populations. In both patients group and controls group by Chi-square test, the allelic frequencies' distributions were not in Hardy-Weinberg equilibrium what is its significance for female (Table 3) the genotype frequencies in the patients' group were GG 0.0%; GT 56.25%; TT 43.75% vs. the genotype frequencies in the control group: GG 0.0%; GT 62.5%; TT 37.5%. So the T allele was not significantly associated with schizophrenia risk ($p > .05$) in Golestan province of Iran (Table 4). As the genotypic distribution between the two groups was compared,

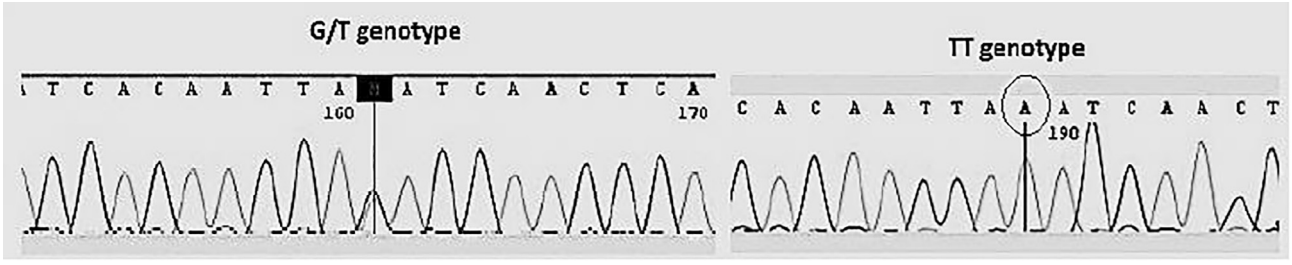

Fig. 2. The result of sequencing was consistent with determined genotype by T-ARMS-PCR. The presence of two peaks at SNP site indicates the T allele as a mutant and G allele as a normal nucleotide in the samples.

Table 3
Hardy-Weinberg equilibrium.

	N	χ^2	df	P
Total				
Patients	80	12.249	1	0.000466
Controls	48	9.9174		0.00163
Male				
Patients	70	10.437		0.00123
Controls	37	7.52		0.0061
Female				
Patients	10	1.836		0.175
Controls	11	2.39		0.122

$P < .05$; df: degree of freedom; χ^2: Chi square.

Table 4
Genotype and allele frequencies of the SNP rs1625579 in schizophrenic patients and controls.

	N	Genotype n (%)				Allele n (%)				OR	95% CI
		TT	GT + GG	χ^2	P	T	G	χ^2	P		
Total											
Patients	80	35 (43.75)	45 (56.25)	0.483	0.488	115 (71.87)	45 (28.12)	0.59	0.282	1.2963[a]	0.6231–2.6967
Controls	48	18 (37.5)	30 (62.5)			66 (68.75)	30 (31.25)			1.1616[b]	0.6687–2.0180
Male											
Patients	70	31 (44.28)	39 (55.71)	0.412	0.52	101 (72.14)	39 (27.85)	0.244	0.62	1.3059[a]	0.5782–2.9495
Controls	37	14 (37.83)	23 (62.16)			51 (68.91)	23 (31.08)			1.1679[b]	0.6311–2.1614
Female											
Patients	10	4 (40)	6 (60)			14 (70)	6 (30)			1.1667[a]	0.2000–6.8084
Controls	11	4 (36.36)	7 (63.63)	0.029	0.86	15 (68.18)	7 (31.81)	0.016	0.89	1.0889[b]	0.2934–4.0410

OR: odd ratio; 95% CI: 95% confidence interval.
[a] TT versus GT+GG.
[b] T versus G; P < .05.

we identified that T allele of SNP rs1625579, might not be a risk locus, which has not been associated with schizophrenia. Our analyses showed that there was not a significant difference between the TT genotype versus GT+GG genotypes in the case population ($\chi^2 = 0.483$, df = 1, p = .488) and the control population ($\chi^2 = 0.412$, df = 1, p = .5). Table 4 shows the summarized results from statistical analyses.

5. Discussion

The latest GWAS in 2014 [2], the miR-137 gene was highly associated with schizophrenia [10]. In this study, we investigated the association of rs1625579 in Iran for the first time. Our study did not confirm the association of SNP rs1625579 with schizophrenia in the population of Iran. The major problem in genetic studies in schizophrenia is that if one research confirmed a risk polymorphism in one population usually it cannot be approved in another population [11]. The association of rs1625579 has been confirmed in European populations as a risk SNP for schizophrenia. Previous reports on SNP rs1625579 indicate that in Chinese the T allele and TT genotype were risk factors and significant differences in the genotype or allele distribution were identified between patients and controls ($\chi^2 = 4.409$, p = .036 and $\chi^2 = 4.971$, p = .026, respectively) [12]. These results demonstrated that rs1625579 is a promising risk SNP under a recessive model, which is the T allele. This data is in agreement with European GWAS results [13–14]. In comparison with some studies, the frequency of GG genotype in patients and controls was low (0.33% in the cases and 0.80% in the controls) in Chinese Han's population, and a lower frequency of G allele was detected (5.16% in the cases and 7.32% in the controls) in southern China. These results were lower than the results in American and Canadian studies (17.65% in the cases and 20.66% in the controls) which were observed [15]. Much more significant differences of G allele and GG genotype frequencies between some Ethnicities were 20.37% and 2.47% in healthy Scottish controls [16] and 20.83% and 4.27% in Australians [17] populations. Therefore, according to these data, there was a risk of SNP rs1625579 for schizophrenia in European populations and there were inconsistent results in Asian populations. These studies suggest that there is an ethnic heterogeneity in distribution of SNP rs1625579 [12]. In our results, we failed to find the association of miR-137 SNP with schizophrenia. Many reasons must be involved for these results and maybe one of them is that the participants of this study were from the Golestan province of Iran. In many researches of Chinese's population, data showed that the T allele frequencies of SNP rs1625579 were different from different geographical areas. Therefore, the small population must be a limitation in this study, which may cause negative findings [18].

6. Conclusions

The results of our case-control study could not confirm the association of MIR137HG rs1625579 with schizophrenia in this sample size of Iranian populations. By this study, we try to examine the relationship between genetic variants of MIR137HG with schizophrenia pathogenesis, and we hope this data will be helpful on this way. Future studies in large-scale are needed to confirm the exact association of MIR137HG gene with schizophrenia disease in the Iranian population.

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