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Original Article

Association between rs1344706 Polymorphism in the ZNF804A Gene and the Risk for Schizophrenia

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Abstract

Objective: Schizophrenia is known as a severe mental disorder worldwide. Genome-wide association studies have revealed that rs1344706, located in ZNF804A, is a risk variant for schizophrenia among various populations. The current study was conducted to find correlation between rs1344706 polymorphism and schizophrenia in East of Iran.
Method: This case-control study assessed 150 schizophrenia cases as well as 150 healthy controls. The single nucleotide polymorphism (SNP) was genotyped using the Tetra-Amplification Refractory Mutation System-Polymerase Chain Reaction (Tetra-ARMS-PCR) method. Analyses based on the Chi-square test and logistic regression were calculated by SPSS.
Results: The TT, GT, and GG genotype frequencies at rs1344706 in schizophrenia cases were 48.0%, 40.0%, and 12.0%, whereas in controls, they were 49.3 %, 36.7 %, and 14.0 %, respectively. The T and G allele frequencies were 68 % and 32 % in cases and 67 % and 33 % in healthy controls. The results of logistic regression indicated that there is no association between rs1344706 alleles (P = 1.000) and genotypes (P = 0.647 for GT and P = 0.726 for GG) with susceptibility to schizophrenia.
Conclusion: Overall, there was no significant relationship between rs1344706 SNP and schizophrenia in Iran's Eastern population. However, further research focusing on more SNPs of ZNF804A and larger samples in other ethnicities is necessary to confirm these results.

Key words: *Mental Disorder; Polymorphism; Schizophrenia; ZNF804A Protein*

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Schizophrenia is a chronic neuropsychiatric disorder considered the top ten leading causes of disability (WHO) (1). Although this disease remains a significant public health problem with a global lifetime prevalence of ~1 %, only partially effective treatments are available (2). Schizophrenia is commonly recognized as a heterogeneous disorder with complex etiology. The importance of heritability in the range of 64–81 %, along with other developmental and environmental factors, has been notably demonstrated (3).

Over one hundred genetic loci have been detected by genome-wide association studies significantly linked with schizophrenia, including DISC1, Dysbindin, Neuregulin 1, D-amino acid oxidase activator, and catechol-o-methyl transferase (4). Zinc finger protein 804A (ZNF804A), which is considered an important risk gene in various psychotic disorders (5), has been robustly identified in schizophrenia samples (6). Furthermore, ZNF804A overexpression in rat neural progenitor cells can significantly deregulate schizophrenia-related genes (7). ZNF804A is located at 2q32.1, containing four exons and encoding a 1210-amino acid protein expressed in the brain (8). Zinc-finger protein 804A includes a specific DNA binding domain regulating transcription factors involved in neurodevelopment and neuronal phenotypes that may be involved in the structure and function of the nervous system (9, 10). Rs1344706 maps to a short-conserved sequence proposed to contain transcription factor binding sites. It is hypothesized that rs1344706 might increase the expression levels of ZNF804A, suggesting that a higher expression of this transcript can be a risk factor for schizophrenia (11, 12).

Reportedly, a high number of single nucleotide polymorphisms (SNPs) in the ZNF804A gene correlate with schizophrenia (8, 13). Rs1344706 is an intronic SNP in the ZNF804A gene on chromosome 2q32.1, the first associated with schizophrenia (6) and other psychiatric diseases (14). Inquiry into SNPs across varying populations has shown different results. Accordingly, this study was designed to assess the association between this SNP and risk of schizophrenia.

Materials and Methods

Study population

One hundred fifty schizophrenia cases were recruited from a Psychiatric Clinic in Birjand, South Khorasan, Iran. They were diagnosed and categorized by two psychiatrists using the Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition, Text Revision (DSM-5), and the 10th revision of the International Statistical Classification of Disease and Related Health Problems (ICD-10). The main diagnostic criterion for patients was system DSM-5, but then the diagnostic compliance with criteria ICD-10 and DSM-IV-TR was checked, so diagnostic conflict did not exist. Therefore, patients were divided according to the classification DSM-IV-TR.

The initial selection was made by referring to the hospital medical records unit and selecting patient files with a final diagnosis under schizophrenia, schizoaffective, and schizophreniform. Two psychiatrists invited patients for a final and definitive diagnosis.

Consulting psychiatrists excluded them based on their history and medical records with medical illness, substance abuse, and other psychiatric disorder.

Moreover, 150 gender- and age-matched controls without psychiatric diagnoses and family history of mental and/or neurological disorders were enrolled from the general population. Patients and people referred to the study were residents and, in fact, natives of the region and did not belong to a particular ethnic race. If any doubt existed regarding participants belonging to a specific race, such as Baluch, Turk, or Kurd, they were not included in the study.

The study procedure was approved by the Medical Research Ethics Committees of Birjand University of Medical Sciences (IR.BUMS.REC.1397.120) and was carried out following the latest version of the Declaration of Helsinki. Because the patients were psychotic, consent was obtained from the legal guardian.

DNA extraction and SNP genotyping

Genomic DNA was extracted from 2 ml peripheral blood as per the salting-out method's standard protocol, and rs1344706 genotyping was performed using the Tetra Amplification Refractory Mutation System Polymerase Chain Reaction (T-ARMS-PCR) assay. The amplification reaction condition was comprised of initial denaturation at 95 °C for 5 min, denaturation at 95 °C for 1 min, annealing at 61 °C for 1 min, extension at 72 °C for 1 min for 30 cycles, and a final extension at 72 °C for 5 min. Subsequently, PCR products were separated on 2 % agarose gel, stained, and visualized under UV light. The primers used in this project included; forward inner primer (G); 5'- AAA CAC TGA AAC AAA GAA TCA AAA GCT- 3', reverse inner primer (T); 5'- GAT AGA TAT CCA AGA AGT TGA TTC TGG TC- 3', forward outer primer; 5'- ACA TGA GTA GTG AGG TTA AGC ATC TTT T- 3', and reverse outer primer; 5'- TAG ATA CGG TAT TCT TGG TTG ACA GTT C- 3' (Figure 1).

Statistical genetic analyses

SPSS software, version 25, was used to carry out statistical analyses. Hardy-Weinberg equilibrium was evaluated between expected and observed genotype distributions. A chi-square test was used to detect allele and genotype frequencies in cases and controls. Correlations between rs1344706 and schizophrenia risk were computed by estimating the odds ratio (OR) and 95 % confidence intervals (95 % CI). Logistic regression was employed for each genotype and genetic model. P values smaller than 0.05 were considered statistically significant.

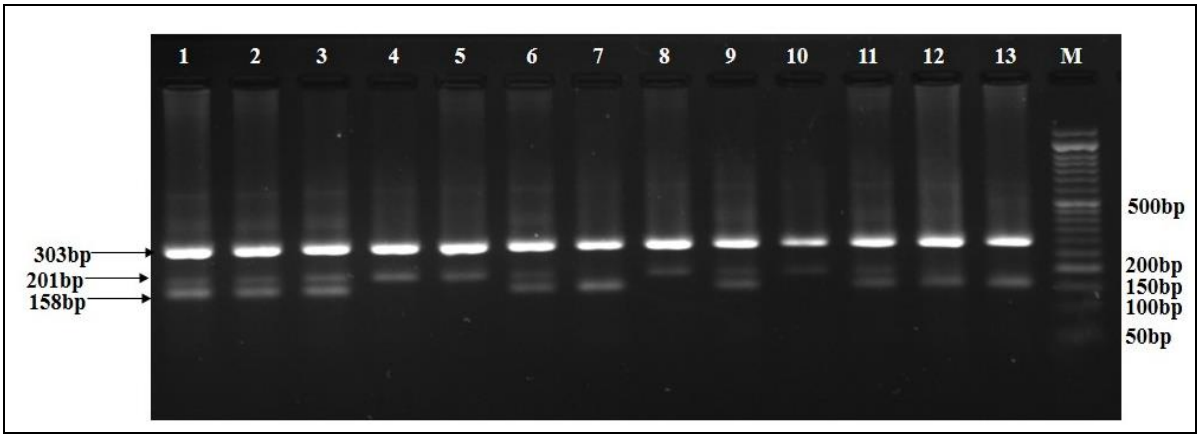

Figure1. Gel Electrophoresis Genetic Variants rs1344706. Wells 5, 8, 10, and 4 Show TT Genotype, Wells 7, 12, and 13 Show GG Genotype, Wells 2, 3, 6, 9, 11, and 1 Indicate GT Genotype and the M Well is a 50 bp Marker

Results

Demographic features demonstrated that 150 cases were included: 95 (63.3 %) males and 55 (36.7 %) females. 150 controls were selected: 86 (57.3 %) males and 64 (42.7 %) females (p = 0.288). The mean age in the case group was 39.5 ± 11.84 years and in the control group was 41.3 ± 10.99 years (p = 0.118).

The demographic characteristics of cases and controls with different genotypes are presented in Table 1. Genotype-phenotype analyses were performed for gender (P = 0.562), subtype (P = 0.450), and familial history (P = 0.183) using the Chi-square test. However, no associations were found between these variants and schizophrenia. TT, GT, and GG genotype frequencies at rs1344706 among schizophrenia cases were 72 (48.0 %), 60 (40.0 %), and 18 (12.0 %), whereas among controls, the frequencies were 74 (49.3 %), 55 (36.7 %), and 21 (14.0 %), respectively. T and G allele frequencies were 204 (68 %) and 96 (32 %) in cases, and 203 (67 %) and 97 (33 %) among controls, respectively. Hardy-Weinberg equilibrium was maintained in the case group (P = 0.766) and in the control group (P = 0.364). Schizophrenia cases were 65 (43.3 %) paranoid patients, 52 (34.7 %) undifferentiated, 16 (10.7 %) residual, 14 (9.3 %) disorganized, and 3 (2 %) catatonic patients. Genotype frequency in the different types of schizophrenia are shown in Table 2 .

Logistic regression analysis of the association between rs1344706 alleles and genotypes with schizophrenia described in Table 3. The distribution of allele and genotype frequencies were not significantly different between cases and controls. No associations were detected between rs1344706 and risk of schizophrenia. Table 4 contains the analyses of genetic models; however, none were appropriate for this SNP.

Table 1. Demographic Characteristics of Cases and Controls with Different rs1344706 Genotypes

Variables	TT	GT	GG	Total
Case				
Male, n (%)	45 (62.5 %)	39 (65.0 %)	11 (61.1 %)	95 (63.3 %)
Female, n (%)	27 (37.5 %)	21 (35.0 %)	7 (38.9 %)	55 (36.7 %)
Total	72 (48.0 %)	60 (40.0 %)	18 (12.0 %)	150 (100.0 %)
Age, years mean (SD)	40.3 (11.6)	38.1 (11.5)	41.3 (13.7)	39.5 (11.8)
Control				
Male, n (%)	42 (56.8 %)	32 (58.2 %)	12 (57.1 %)	86 (57.3 %)
Female, n (%)	32 (43.2 %)	23 (41.8 %)	9 (42.9 %)	64 (42.7 %)
Total	74 (49.3 %)	55 (36.7 %)	21 (14.0 %)	150 (100.0 %)
Age, years mean (SD)	43.0 (11.1)	40.4 (11.2)	37.3 (9.3)	41.3 (10.9)

Table 2. Frequency Distribution of rs1344706 Genotype Variants among Different Groups of Schizophrenia Patients

Types of Schizophrenia	TT N (%)	GT N (%)	GG N (%)	Total	P
Paranoid	34 (52.3)	22 (33.8)	9 (13.8)	65	
Disorganized	5 (35.7)	8 (57.1)	1 (7.1)	14	
Catatonic	1(33.3)	1 (33.3)	1 (33.3)	3	0.82
Undifferentiated	27(51.9)	19 (36.5)	6 (11.5)	52	
Others	5(31.2)	10 (62.5)	1 (6.2)	16	

Table 3. Logistic Regression Results of the rs1344706 Alleles and Genotype Distribution in Schizophrenia

Genotype and allele	Case	Control	Crude OR	95 % CI	P
TT	72 (48.0 %)	74 (49.3 %)		Reference	
GT	60 (40.0 %)	55 (36.7 %)	0.892	0.547-1.454	0.647
GG	18 (12.0 %)	21 (14.0 %)	1.135	0.559-2.305	0.726
T	204 (68.0 %)	203 (67.0 %)		Reference	
G	96 (32.0 %)	97 (33.0 %)	1.000	0.020-50.397	1.000

Table 4. Genetic Models for rs1344706 in Schizophrenia

Genetic model	Combined genotypes	Frequency of combined genotypes in case	Frequency of combined genotypes in control	Crude OR	95% CI	P
Recessive	TT/GT + GG	72/78	74/76	0.948	0.603-1.491	0.817
Dominant	TT + GT/GG	132/18	129/21	1.194	0.608-2.344	0.607
Codominant	GT/TT + GG	60/90	55/95	1.152	0.723-1.835	0.553

Discussion

Schizophrenia is one of the most critical mental disorders worldwide (1). Accumulating evidence has demonstrated that rs1344706, located in intron 4 of ZNF804A, is correlated with schizophrenia in various populations. Finding promising biomarkers and detecting the role of ZNF804A in schizophrenia pathogenesis may provide new therapeutic strategies for this disorder (15). Very few studies have been conducted on this association in the Middle East. To determine the correlation between rs1344706 and risk of schizophrenia, this study was conducted to analyze 150 schizophrenia cases and 150 healthy controls from the Iranian population .

Investigations have studied rs1344706 in ZNF804A and promoted our understanding of its contribution to schizophrenia (12, 16). The first positive correlation between rs1344706 and schizophrenia was observed in the British populations (6). This finding was later supported by other studies in the Irish and Icelandic populations (11, 13, 16), indicating the association between rs1344706 and schizophrenia in European populations. Lezheiko et al. also revealed a strong association between rs1344706 and schizophrenia in Russians (17). Moreover, a recent meta-analysis with data of 18,945 cases and 38,675 controls has demonstrated a similar correlation (12).

However, inconsistent findings came to the fore when the Han Chinese population was investigated. A study on the Han Chinese population showed that rs1344706 could be considered a risk factor for schizophrenia (18). Zhang et al. (13) and Chen et al. (19) also observed this association in two Han Chinese samples. However, other research in the same population has published no association in the Han Chinese (20-26). Sample populations in these studies were drawn from the same geographical area, suggesting that regional variation in genotype cannot be the only reason for different results (18, 20). Meanwhile, several of these studies used adequate and acceptable sample sizes to ascertain genetic differences (20, 22, 25). In the present study, rs1344706 was not correlated with schizophrenia in an Iranian population, similar to most studies in Asian populations (22, 23, 27, 28), indicating that rs1344706 cannot be a risk factor for schizophrenia in some Asian populations.

However, Li et al. observed significant differences in ZNF804A between Asians and Europeans (21). The

discrepancy between Europeans and the Han Chinese may probably be because of genetic heterogeneity often observed in the genetic association analyses for complex diseases resulting from various population histories. Other population-specific factors, such as diet, culture, or environmental exposure might contribute to this observed heterogeneity (29).

ZNF804A overexpression influences the expression of multiple genes correlated with schizophrenia (7). A short-conserved mammalian region has been observed downstream of rs1344706, which may function as a cis-acting element of the ZNF804A gene and play a critical role in the etiology of schizophrenia (8). Proteins with zinc-finger domains usually have a modulatory function and behave as transcription factors but have different interactions with different molecules, such as RNA and proteins. It has been reported that ZNF804A modulates the expression of schizophrenia-associated genes, including PDE4B, COMT, PRSS16, and DRD (7). Transcription factor-binding sites in the conserved DNA sequence around rs1344706 may interact with Myt1L zinc-finger protein and the POU3F1/Oct-6 POU domain transcription factor, contributing to oligodendrocyte differentiation and proliferation (30). Despite the sufficient statistical power of this research, the sample size is considered a limitation. Therefore, more studies with more comprehensive sample sizes are necessary to confirm these results.

Limitation

The limitations of this study were due to the referral of patients for re-evaluation and taking blood samples for research, especially from rural areas. Our sample size was relatively small. On the other hand, due to the limited number of patients, classification and matching based on other criteria such as age, treatment type, response to drugs, and drug side effects were impossible.

Conclusion

The rs1344706 SNP ZNF804A may not confer a risk variant of schizophrenia in eastern Iran. Other SNPs in this region probably, not covered in the present study, may be responsible for an association. Other genetic and environmental factors can also affect the current results. More research focusing on different SNPs in ZNF804A among various populations with larger sample sizes is required to confirm these findings.

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Conflict of Interest

None.

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